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ABSTRACT BOOK

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RISING STARS IN IMMUNOLOGY

Divergent tissue adaptation of DC2 and DC3 shapes antigen presentation in lung tumors

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Conventional type 2 dendritic cells (cDC2) modulate immunity in NSCLC and have been associated with patient outcome. However, cDC2 subsets DC2 and DC3 (often considered inflammatory) may undergo distinct tumor-driven adaptation with functional consequences for T-cell responses. Here, we investigated how spatial localization and tissue adaptation of DC2/DC3 relate to antigen presentation in lung tumors.

In human NSCLC tissue microarrays, multiparametric imaging revealed accumulation of DC3 within tumor niches. Reanalysis of NSCLC single-cell transcriptomic datasets showed increased DC3 abundance in both early and late disease and a stronger remodeling toward metabolic and immunoregulatory programs (including IL10) compared with DC2, suggesting subset-specific adaptation to the tumor microenvironment.

To interrogate these dynamics in vivo, we performed CITE-seq and flow cytometry in the murine LL2 lung tumor model, leveraging intravenous CD45 labeling to resolve vascular vs tissue localization. Both DC2 and DC3 accumulated during tumor growth and were detected within the tumor mass by multiplex imaging. Across compartments (blood → peritumoral tissue → tumor), DC states progressively shifted toward a more differentiated and metabolically active profile; a Hif1a-associated signature emerged predominantly in DC3. Spatial mapping further indicated that DC subsets with the highest T-cell proximity were enriched outside the tumor core.

Given these differences, we focused on antigen presentation. DC2 displayed features consistent with more efficient MHC-II peptide editing (e.g., higher H2-DMb2) than DC3. Using a fixation-based assay quantifying DC–OT-I/OT-II conjugates and interaction avidity, preliminary data suggest that, despite robust antigen uptake, DC3 are less efficient at mediating antigen presentation than DC2.

Overall, our results highlight that the spatial context in which antigen presentation occurs in lung tumors depends on the distinct adaptation of DC2 and DC3. These findings argue for careful spatial and temporal consideration when designing DC-targeting strategies, and caution against assuming that DC3 mirrors cDC2 functions in the TME.

Intestinal Epithelial Smad7 drives purine metabolic dysregulation and ileal inflammation

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Defects of the gut epithelial barrier and counter-regulatory mechanisms are a prerequisite for the onset of intestinal inflammation in patients with inflammatory bowel diseases (IBD). Elevated levels of Smad7, an inhibitor of TGF-β1, in both epithelial and immune cells have been associated with the pathological processes observed in IBD. We aim to determine the relevance of Smad7 expression in the epithelial compartment.

We generated mice overexpressing Smad7 in the gut epithelium (Smad7TgCre$^+$) and monitored pathology development. Inflammatory cell infiltration was assessed using multiplex immunofluorescence and flow cytometry. To investigate the molecular mechanisms underlying ileal damage, we performed spatial transcriptomics on frozen ileal samples and metabolomics on intestinal epithelial cells (IECs) from Smad7TgCre$^+$ and control mice. The impact of adenosine on Mucin-2 expression was evaluated through both in vitro and in vivo approaches. Finally, we evaluated the protein expression of CD73 and Smad7 in ileal tissue from Crohn's disease (CD) patients.

Our results showed that Smad7TgCre$^+$ mice developed spontaneous terminal ileitis, which was characterized by villus shortening and widening, mucus depletion, increased intestinal permeability, and infiltration of CD8$^+$ T cells. By integrating spatial transcriptomics and metabolomics data, we demonstrated that Smad7TgCre$^+$ mice exhibited altered expression of enzymes involved in purine metabolism, particularly CD73, the key enzyme responsible for adenosine production. Consequently, these mice produced lower levels of adenosine and oral adenosine administration restored mucus production and alleviated the ileal pathology. Lastly, we observed that in the inflamed ileum of CD patients, elevated Smad7 levels were correlated with decreased CD73 expression.

Our data indicate that Smad7 overexpression in IECs is sufficient to promote ileal mucosal damage, which is linked to the impairment of purine metabolism.

A chimeric Human/Dog CSPG4 DNA vaccine for overcoming chemoresistance and improving osteosarcoma treatment

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Osteosarcoma (OSA) is a rare pediatric tumor characterized by high aggressiveness and limited response to conventional treatments. The outcome of patients with metastatic OSA is poor; therefore, alternative therapies are required. Chondroitin Sulfate Proteoglycan (CSPG)4 is highly expressed in many solid tumors, where it plays a fundamental role in supporting pro-tumoral functions, while being nearly absent in healthy tissues. Hence, CSPG4 is an attractive immunotherapeutic target.

We demonstrated CSPG4 overexpression in OSA and its correlation with poorer patient survival. Its transient downregulation significantly impairs tumorigenic behavior of human OSA cells *in vitro*. We developed a chimeric human (Hu)/dog (Do)-CSPG4 DNA vaccine and tested it in both human OSA xenograft mouse models and client-owned OSA-affected dogs within an adjuvant setting. Vaccine-induced $CD8^+$ T cells and antibodies delayed tumor progression in mice. In dogs, HuDo-CSPG4 breaks immune tolerance, inducing a specific immune response which resulted clinically effective. Vaccinated canine patients displayed prolonged overall survival compared to controls. Interestingly, HuDo-CSPG4 induced a cytotoxic response in a human surrogate setting *in vitro*.

Based on these findings, and considering the rapid development of chemoresistance, we began investigating the synergy between anti-CSPG4 immunotherapy and chemotherapy in murine models and canine patients. CSPG4 is upregulated during chemoresistance acquisition in a murine OSA model, and its transient silencing restores chemosensitivity *in vitro*. In syngeneic mouse models, HuDo-CSPG4 effectively hampers both sensitive and resistant cell growth when combined with chemotherapy. Early results in canine OSA patients immunized with HuDo-CSPG4 in combination with chemotherapy are promising, showing extended survival and long-term protection from metastasis.

This approach may help slow disease progression by preventing drug resistance, providing the basis for a novel treatment for OSA patients. Given the strong translational value of pet dogs as cancer models, these studies could also speed up clinical translation and finally improve patient outcomes.

Interleukin-1 Receptor 8 acts as an immune checkpoint in NK cells and $CD8^+$ T cells, limiting cytokine-induced anti-tumor immune responses

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IL-1R8 is an atypical Interleukin-1 receptor (ILR) family member that curtails inflammation by dampening ILR and Toll-like receptor (TLR) downstream pathways. This receptor was shown to inhibit IL-18-dependent activation of Natural Killer (NK) cells in preclinical models of liver carcinogenesis and metastasis, acting as an immune checkpoint. To assess its translational relevance, we dissected the tumor immune microenvironment in patients with colorectal cancer liver metastases (CRLMs). We demonstrated that IL-1R8 expression is increased in patients and involved in the control of a tissue-resident intrahepatic CD56bright NK cell population endowed with cytotoxic activity, suggesting its role as immune checkpoint in human NK cells, like in mouse. We generated an original monoclonal antibody targeting IL-1R8 showing that its blockade restored IL-18-driven signaling and induced robust Interferon γ (IFNγ) and Tumor Necrosis Factor (TNF) production, together with enhanced cytotoxicity against a colorectal cancer cell line. Given the central role of IL-18 in shaping cytotoxic immune responses and anti-tumor polarization, we next examined whether IL-1R8 also constrains cytokine-driven immune resistance beyond NK cells. IL-1R8-deficient mice were protected from primary colorectal cancer lesion, with a substantial contribution of $CD8^+$ T cells. As shown in NK cells, this protection was

mediated by unleashed IL-18 signaling. Unexpectedly, IL-1R8 deficiency further enhanced responsiveness to IL-2 and antigen-specific responses in a cell-intrinsic manner, while also synergizing with anti-PD-1. In human, IL-1R8 blockade increased IFNγ production and proliferation in polyclonally activated $CD8^+$ T cells following cytokine stimulation and boosted Chimeric Antigen Receptor (CAR) T cell cytotoxic activity. Collectively, these findings identify IL-1R8 as a negative regulator with broad effects on cytokine-dependent immune signaling, positioning it as a novel target in cancer immunotherapy.

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METABOLIC CIRCUITS OF INFLAMMATION AND IMMUNITY

Luteinizing hormone modulation shapes sex-specific metabolic programs during hematopoietic stem cell regeneration

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Biological sex shapes hematopoietic output and regeneration. Males and females display intrinsic differences in steady-state hematopoiesis and mount distinct regenerative responses to stress, including chemotherapy and irradiation. Hematopoietic stem cell (HSC) fate is governed by metabolism, with quiescent cells relying on glycolysis to preserve stemness, while activation and differentiation shift to oxidative phosphorylation (OxPhos). We previously identified luteinizing hormone (LH) as an unexpected regulator of hematopoiesis, but whether LH signaling rewires HSC metabolism to influence fate decisions remains unclear. Here, we uncover a sex-biased regulation of metabolic programs in HSCs that controls lineage commitment during stress-induced regeneration.

C57BL/6 male and female mice were subjected to sublethal total body irradiation (SL-TBI). LH signaling was inhibited using a luteinizing hormone-releasing hormone (LHRH) antagonist. HSPCs were characterized by flow cytometry and single-cell RNA sequencing (scRNA-seq). Metabolic dependencies were assessed using SCENITH and validated by ex vivo metabolic profiling. LHCGR expression was significantly higher in female LT-HSCs, indicating sex-specific sensitivity to LH signaling. Following stress, LH suppression enhanced megakaryopoiesis selectively in females. scRNA-seq revealed that LHRH antagonism increased the proportion of megakaryocyte-primed HSCs and enriched transcriptional programs linked to megakaryocyte differentiation and platelet production. Gene set enrichment analysis showed reduced OxPhos signatures in LT-HSCs upon LH inhibition, which were restored by exogenous LH. Consistently, ex vivo metabolic assays confirmed that LHRH antagonism induces a metabolic shift in LT-HSCs and MkP cells, characterized by decreased OxPhos and increased glycolysis.

Our findings identify LH as a sex-dependent metabolic regulator of HSC fate during stresshematopoiesis. By modulating oxidative metabolism, LH signaling constrains megakaryocyte-lineage commitment. These results provide a mechanistic framework linking sex hormones to HSC metabolic programming and suggest that transient hormonal suppression may represent a therapeutic strategy to enhance platelet recovery and mitigate treatment-related cytopenias.

Immune reconstitution in transplanted survivors of childhood acute lymphoblastic leukemia associates with an altered immuno-metabolic profile and vascular dysfunction

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Background and aims: ALL is the most common childhood blood cancer, is treated with chemotherapy and, for high-risk or relapsed patients, total body irradiation followed by stem cell transplantation (TBI-HSCT). Although survival now exceeds 90%, ALL survivors show increased cardiovascular and metabolic risk. This project aims to investigate metabolic and inflammatory sequelae after TBI-HSCT and how these changes affect mature immune cells derived from transplanted stem cells.
Methods: A cohort of 14 disease-free cALL survivors who received TBI-HSCT (median age at HSCT: 13 years) (recipients) about 10 years prior to the enrolment, along with 14 matched sibling donors with an average age of 23.7 were recruited. Clinical profiling, blood sampling for immunophenotyping, transcriptomics, proteomics and telomere analysis, and ultrasound assessment of supra-aortic trunks for carotid intima-media thickness (IMT) were performed.
Results: HSCT in recipients showed accelerated telomere shortening in leukocytes (slope −0.009 vs −0.0008), reduced HSCs ($CD34^+$), and increased B cells ($CD19^+$) compared with donors. scRNA-seq revealed inflammation and impaired B-cell functions. IgM levels were lower in recipients (58,62 SE±4,062 vs 49,23 SE±3,188), while IgG remained similar (329.7 SE±16.43 vs 350.6 SE±14.49). Recipients also had higher cholesterol (171.9 SE±10.43 vs 157.5 SE±9.19), triglycerides (105.0 SE±11.31 vs 65.57 SE±5.22), insulin (15.23 SE±2.95 vs 8.46 SE±1.87), and HOMA index (2.98 SE±0.62 vs 1.59 SE±0.37), despite comparable anthropometrics. Proteomics showed increased acute-phase proteins (CRP, APS) and dyslipidaemia-related proteins (APOB, APOC4-APOC2). Clinically, recipients showed accelerated carotid IMT progression (0.0083 vs 0.0034) and a 14.3% prevalence of metabolic syndrome.
Conclusions: TBI-HSCT is associated with an impaired B-cell phenotype and metabolic profile that could increase the long-term cardiovascular risk of cALL survivors. Analysis of a cohort of ALL survivors, who were treated with chemotherapy instead of TBI before HSCT, is ongoing to evaluate the impact of radiation conditioning on the reported phenotype.

Obesity as a driver of premature immune senescence

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Background: Global life expectancy has increased over recent decades; however, it has been accompanied by a rise in age-related conditions, including cancer, type 2 diabetes, and obesity. Although cellular senescence is hallmark of chronological aging and contributes to immune remodeling, whether obesity can trigger similar senescence-associated T cell alterations independently of age remains unclear.
Method: We investigated whether obesity promotes or accelerates immune senescence in T cells from young and elderly individuals. We isolated peripheral blood mononuclear cells from young and elderly lean subjects, as well as from young and elderly individuals with obesity to characterize senescence-associated markers (SA-β-gal, CD57, KLRG1, CD27, CD28) in T cell subsets by flow cytometry; also, we performed molecular and DNA methylation analyses.
Results: We found that aging associates with increased $CD4^+$ and decreased $CD8^+$ T cell frequency, with senescent features in both subsets, further exacerbated by obesity. In contrast, young obese individuals show a reduction of $CD4^+$ and an expansion of $CD8^+$ T cells. Notably, these $CD4^+$ cells display a more pronounced senescent phenotype, along with Treg and Th1 cell expansion, thus indicating premature immune remodeling. At molecular level, obesity is associated with induction of the p53-p21 axis in young individuals and increased p21 expression in the elderly. Furthermore, KLRG1 hypomethylation was observed specifically in the young. Finally, exposure of naïve T cells to obese or elderly sera induces a senescent phenotype, predominantly observed in Th1 cells treated with elderly sera and in Th17 cells exposed to obese serum. Ongoing senescence-associated secretory phenotype profiling and DNA methylation analyses aim to further elucidate the molecular underlying mechanisms.
Conclusions. Obesity is characterized by features of premature $CD4^+$ T cell senescence in young individuals and enhanced immunosenescent remodeling in the elderly, indicating that obesity may induce immune senescence beyond chronological age.

INNATE IMMUNE CELL FUNCTIONS IN HEALTH AND DISEASE

Unveiling innate lymphoid cell states in colorectal liver metastases

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Liver metastases develop in up to 50% of colorectal cancer patients, and the prognosis of unresectable disease remains highly unfavourable, with 5-year survival below 2%. This poor outcome is largely driven by the liver's intrinsically tolerogenic microenvironment, which constrains anti-tumour immunity and promotes immune evasion. Within the hepatic niche, innate cells are central regulators of local immune responses and display marked phenotypic plasticity under pathological conditions, making them attractive targets for immunotherapy. Among these, NK cells and type 1 innate lymphoid cells (ILC1s) are particularly promising, as emerging evidence suggests complementary roles in controlling colorectal liver metastases (CRLM). However, how distinct NK and ILC1 subsets are shaped by the metastatic niche and interact with other immune cells remains unclear.
To address this, we employ two complementary syngeneic mouse models of CRLM: MC38 and AKTPF, both based on splenic injection of tumour cells to establish hepatic metastases. Notably, the AKTPF model closely recapitulates key molecular and histopathological features of human CRLM. Single-cell RNA sequencing and flow cytometry analyses reveal that ILC1s form a relatively homogeneous cluster across lesions, whereas NK cells segregate into three distinct subsets corresponding to progressive maturation states. In advanced metastases, ILC1s are significantly underrepresented, while NK cells display pronounced phenotypic plasticity and accumulate in partially activated states, potentially maintaining cytotoxic readiness while limiting terminal maturation. Together, these findings indicate that the metastatic niche selectively reshapes innate lymphoid compartments, promoting NK cell heterogeneity while constraining ILC1 diversity.
To further define NK/ILC1 cell states and their interactions within the CRLM microenvironment, we are integrating spatial transcriptomics across murine and human CRLM samples with intravital imaging. This multilayered approach will enable comprehensive characterisation of cell phenotype, spatial organisation and dynamic behaviour, with the aim of identifying innate immune mechanisms that could be exploited to overcome hepatic immune tolerance in CRLM.

Il22ra2-high cDC2 associate with TH17 remodeling in AOM/DSS colonic tumorigenesis

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Colorectal cancer (CRC) arises within a chronically inflamed and microbiota-rich environment, where epithelial repair programs and immune surveillance are tightly interwoven. Among cytokines orchestrating this crosstalk, IL-22 promotes epithelial regeneration and barrier integrity but, through sustained STAT3 activation in chronic settings, can also fuel tumor growth. At mucosal barriers, $CD4^+$ T helper 17 (T_{H17}) cells represent a major source of IL-22. Within this environment, dendritic cells (DCs) orchestrate tissue homeostasis by integrating microbial, metabolic, and cytokine-derived cues to regulate T cell-mediated immunity. Conventional type 2 DCs (cDC2) are emerging as key regulators of adaptive immunity at barrier sites, but their contribution to the immunological architecture of CRC remains only partially understood.
Using an AOM/DSS-driven model of colonic tumorigenesis combined with single-cell transcriptomics, we identify a specialized cDC2 subset characterized by high expression of IL-22 binding protein (IL-22BP, also known as *Il22ra2*), which emerges as top differentially expressed gene. IL-22BP is a soluble decoy receptor that specifically binds IL-22 and prevents its engagement of membrane-bound IL-22R1; in the intestine, IL-22BP expression is known to be downregulated during acute tissue damage. In addition to *Il22ra2* expression, this cDC2 subpopulation displays an inflammatory and tissue-adapted transcriptional profile and co-expresses high levels of *Il23a*, positioning it as a potential source of IL-23 in colonic cancer, a cytokine required for the expansion and maintenance of TH17 cells. In parallel, we observe by spectral flow cytometry a marked remodeling of both $CD4^+$ and $CD8^+$ T cell compartments, with a pronounced accumulation of $CD4^+$ TH17 cells and $ROR\gamma T^+$ T regulatory ($ROR\gamma T^+$ T T_{reg}) cells.
Altogether, our data uncover a previously unrecognized link between IL-22BP$^+$, *Il23a*$^+$ cDC2 and the IL-22 / IL-23 / T_{H17} axis in the colonic tumor microenvironment, pointing to a specialized function of this subset in T cell immunity that warrants further investigation in CRC.

An IL-18-responsive unconventional αβ T cell population with innate-like and antitumoral effector functions

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T cells are central mediators of anti-tumor immunity, and T cell-based therapies have transformed cancer treatment. While most studies have focused on conventional T cells, increasing evidence highlights a relevant contribution of unconventional T cells (UTCs), including invariant natural killer T (iNKT) and mucosal-associated invariant T (MAIT) cells, to tumor immunity.

We previously identified a subset of UTCs, double-negative αβ T cells (DNTαβ), as mediators of immune resistance in murine sarcoma. DNTαβ expressed a TCRαβ, lack CD4 and CD8, and did not bind CD1d or MR1-loaded tetramers, distinguishing them from iNKT and MAIT cells. However, their biology and function in homeostasis and cancer remain poorly defined.

In the current study DNTαβ were detected across multiple tissues of healthy mice at different ages, with the spleen representing their main reservoir. They expressed a polyclonal TCRαβ repertoire and developed independently of Cd1d, Mr1, β2m, and MHCII. Their transcriptomic profiling revealed a unique gene signature enriched for activation-related and innate-like markers, including high expression of IL-1 family receptors such as IL18R1. Interestingly, IL-18R1^{+}DNTαβ represented a mature subset with potentially high functional responsiveness. Consistently, DNTαβ responded robustly to IL-18 in combination with IL-12, exhibiting enhanced proliferation and increased IFN-γ and granzyme B production compared to conventional T cells. IL18R1+ DNTαβ infiltrated multiple murine sarcoma models, and injection of IL-2/IL-12/IL-18 activated DNTαβ limited tumor growth.

Mechanistically, activated DNTαβ promoted macrophage polarization toward an M1-like anti-tumor phenotype through an IFN-γ-dependent mechanism and directly mediated tumor cell cytotoxicity in vitro. Importantly, phenotypically and functionally analogous DNTαβ were identified in human blood and tumors. In a TCGA sarcoma cohort, a DNTαβ-associated gene signature correlated with type 1 immune response, IL-18/IL18R1 expression, and favorable prognosis.

Together, our findings position DNTαβ as a distinct UTC subset with potent anti-tumor functions with significant therapeutic potential in cancer.

WORKSHOP 1 – IMMUNOMEDIATED DISEASES

Chronic psoriasiform inflammation drives the expansion of CD8⁺ T cells recognizing subdominant and cryptic epitopes of cutaneous self-antigens

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Background: Psoriasis is a chronic-relapsing inflammatory disease characterized by polygenic susceptibility and an incompletely defined autoimmune component. Although primarily cutaneous, up to 30% of patients develop psoriatic arthritis years after skin onset. The strong association with HLA-C*06:02 links disease pathogenesis to HLA class I-restricted CD8⁺ T-cell responses. CD8⁺ T-cells recognizing cutaneous self-antigens, including ADAMTSL5 and LL37, have been detected in patients and may represent the "pathogenic bridge" between skin and joint manifestations. Here we aimed to investigate whether recurrent psoriasiform inflammation can drive expansion of CD8⁺ T-cell responses against self-antigens.

Experimental strategy: We used K5-mOVA C57BL/6 transgenic mice expressing ovalbumin in keratinocytes and induced chronic psoriasis-like inflammation through two cycles of imiquimod treatment. Antigen-specific CD8⁺ T-cells were identified ex-vivo and after in vitro expansion upon stimulation with H-2Kb-restricted OVA-derived peptides (SIINFEKL, KVVRFDK, CFDVFKEL), representing immunodominant, subdominant, and cryptic epitopes. As a readout of CD8⁺ T-cell activation, we evalueted Activation-Induced Markers (AIM: CD69, 4-1BB) by flow cytometry and IFN-γ production by ELISpot. To investigate the role of immunoproteasome-dependent cross-presentation, mice were topically treated with the inhibitor ONX-0914 before imiquimod administration.

Results and Conclusions: Recurrent psoriasis-like inflammation induced expansion of OVA-specific CD8⁺ T-cells recognizing subdominant and cryptic epitopes. Ex-vivo analyses revealed self-reactive CD8⁺ T-cells expressing CD69 and 4-1BB within the central memory compartment, mainly upon stimulation with the peptide corresponding to the cryptic epitope. After in vitro amplification, autoreactive responses were detected within the effector memory compartment by AIM and showed significantly increased IFN-γ production by ELISpot. Control mice showed no peptide-specific activation, and no activation was observed with an unrelated H-2Kb-binding viral peptide. Immunoproteasome inhibition reduced IFN-γ⁺ CD8⁺ T-cells, supporting a role for immunoproteasome-dependent cross-presentation in amplifying self-reactive responses.
These findings indicate that severe recurrent skin inflammation may promote tolerance breakdown and expansion of autoreactive CD8⁺ memory T-cells in psoriasis.

The HNF4α-IMP3-IL23R axis drives gut inflammation and anti-TNF resistance in inflammatory bowel disease

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Inflammatory bowel diseases (IBD) are driven by persistent intestinal inflammation sustained by dysregulated immune responses. Although targeted biologics have improved outcomes, many patients fail to achieve durable remission, indicating additional regulatory layers remain unexplored. Post-transcriptional control of immune transcripts is emerging as a key modulator in chronic inflammation. Here, we investigated the role of the RNA-binding protein IMP3 in IBD pathogenesis and its potential contribution to treatment-resistant immune responses.
IMP3 expression was analyzed in intestinal biopsies from IBD patients and healthy controls by qPCR, western blotting, and public transcriptomic datasets. Flow cytometry of lamina propria mononuclear cells (LPMCs) identified cell-specific expression. Functional studies using siRNA-mediated silencing and RNA immunoprecipitation (RIP) assessed IMP3 targets and downstream signaling. Transcriptional regulation of IMP3 was explored through ChIP-seq databases and transcription factor inhibition
IMP3 was markedly upregulated in IBD tissues, particularly in Th17 cells. Silencing IMP3 in IBD-derived LPMCs reduced IL23R expression — a key factor involved in T cell survival and associated with anti-TNF resistance — and downstream STAT3 activation, while increasing apoptotic and regulatory T-cell markers. RIP confirmed IMP3 binding to IL23R mRNA. Elevated IMP3 levels correlated with anti-TNF resistance and IL23R expression. HNF4α, a transcription factor linked to IBD susceptibility, emerged as a negative regulator of IMP3; its inhibition increased IMP3 levels in healthy LPMCs. In resistant patients, HNF4α was decreased and inversely correlated with IMP3.
These findings identify a novel HNF4α–IMP3–IL23R regulatory axis sustaining gut inflammation and contributing to anti-TNF resistance, suggesting IMP3 as a potential therapeutic target in refractory IBD.

Identifying mTOR activation as a druggable pathway in Lymphocytic Variant Hypereosinophilic Syndrome (L-HES)

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Lymphocytic variant hypereosinophilic syndrome (L-HES) is a rare, chronic disorder in which aberrant T-cells (often with a $CD3^{-}CD4^{+}$ phenotype) produce excessive cytokines, notably IL-5, causing severe eosinophilia and resultant tissue damage. It is characterized by prominent skin manifestations, and a good response to steroids and IL-5-targeting drugs, though it carries a risk of transformation into T-cell lymphoma.

Given the rarity of this condition, published data are typically case-reports or small case series, predominantly focusing on the immunophenotype of atypical T cells.

In this work we performed a multi-modal profiling of aberrant T cells from two patients with L-HES.

$CD3^{-}CD4^{+}$ T cells from both patients displayed heightened expression of CRTH2, a typical Th2 cell marker, together with increased IL-4, IL-5 and IL-13 secretion upon in vitro stimulation, compared to the normal $CD3^{+}CD4^{+}$ counterpart. Genomic profiling of sorted atypical T cells is ongoing, to identify molecular alterations occurring in these cells compared to the $CD3^{+}CD4^{+}$ counterpart. Preliminary epigenomic findings demonstrate a complex reshaping of the pattern of whole genome methylation, that goes far beyond the demethylation of genes involved in Th2 polarization.

By scRNAseq we identified a cluster of Th2 cells which included also all atypical T cells, identified by low surface CD3 expression via Abseq. Additionally, sc-TCR sequencing demonstrated that these cells are clonal. Regarding the transcriptional signature, $CD3^{-}CD4^{+}$ Th2 cells exhibited increased activation of the mTOR pathway compared to the $CD3^{+}CD4^{+}$ Th2 counterpart. This finding was functionally confirmed by assessing S6 ribosomal protein phosphorylation, that was consistently increased in $CD3^{-}CD4^{+}$ T cells of both patients compared to the normal $CD3^{+}CD4^{+}$ counterparts.

In conclusion, we provide evidence of complex deregulation of epigenomic and transcriptomic layers occurring in $CD3^{-}CD4^{+}$ T cells. We additionally propose mTOR as a druggable pathway to selectively target this atypical T cell population causing L-HES.

The IL-23/IL-17F axis drives pathogenesis in young patients with cystic fibrosis and allergic bronchopulmonary aspergillosis

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Despite being a widespread global disease, allergic bronchopulmonary aspergillosis (ABPA) still lacks a clear understanding of pathogenesis, which limits the diagnosis and hinders the development of therapeutic strategies. Cystic fibrosis (CF) patients can develop ABPA very early in life, which further accelerates their lung function decline.

Our study is focused on understanding the role of the Th17 immune response in the pathogenesis of ABPA in CF patients, with the aim of finding potential new therapeutic targets.

We have built a cohort of young CF patients with and without ABPA diagnoses and monitored them throughout two years with periodic sampling of blood and sputum.

We have cultured patient-derived peripheral blood mononuclear cells (PBMCs) with *Aspergillus fumigatus* (a.f.) and demonstrated how, exclusively in conditions of ABPA, patients develop a very clear cytokine signature induced by the chronic IL-23 production in response to the fungus. IL-23 induces a clear expansion of IL-17F and IL-22-producing cells, and this specific cytokine signature is steadily increased in ABPA patients' serum. We have also demonstrated that IL-17F is capable of impairing the bronchial epithelium integrity and increasing mucus production, an effect that is amplified with the presence of a.f. Furthermore, ABPA patient-derived B and T cells co-culture, after stimulation with a.f., was able to induce goblet cell metaplasia in CF patient-derived ALI bronchial epithelial layers while also increasing their expression of IL-17F receptor, IL-17RC. Finally, we have used single cell sequencing to study PBMCs in ABPA-CF compared CF revealing marked differences in both innate and adaptive immunity.

In conclusion, ABPA-CF patients' immunological landscape is characterized by a peculiar Th17 response led by the IL-23/IL-17F axis, which is supported by a.f. specific B and T cell cross-interaction. This cytokine signature promotes ABPA pathogenesis and may represent a new target for developing therapeutic strategies.

Antigenic imprinting drives durable memory T cell programs independent of chronological age

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Introduction: The determinants that sustain for decades durable antigen-specific T cell responses across the lifespan remain only partially defined, and indeed the molecular basis longevity of immunological memory itself is still not fully understood. Single-cell multi-omics now provides the resolution needed to dissect the functional, metabolic, and clonal heterogeneity that underpins life-long vaccine-induced immunity.
Objectives: To identify transcriptional, metabolic, and clonal features of SARS-CoV-2- and smallpox-specific T cells in middle-aged individuals ("boomers") and centenarians, and to determine how antigenic history versus chronological age shapes long-term immune memory.
Methods: Peripheral blood mononuclear cells were stimulated with SARS-CoV-2 spike or vaccinia peptide pools, and antigen-specific $CD4^+$ and $CD8^+$ T cells were FACS-sorted. Cells underwent VASA-seq single-cell total RNA sequencing integrated with TCR reconstruction, gene-set enrichment analysis, regulatory network inference (SCENIC), and metabolic modeling (COMPASS). Flow cytometry and single-cell metabolic regulome profiling (scMEP) complemented transcriptomic analyses. Differential expression, clonotype diversity, and functional signatures were assessed using standard bioinformatic pipelines.
Results: Antigen-specific T cells were present at comparable frequencies across age groups but differed in differentiation and functional states. SARS-CoV-2-specific T cells displayed dominant Th1 polarization and activation-associated metabolic programs, whereas smallpox-specific cells exhibited long-lived memory characteristics, enhanced clonal expansion, and metabolic signatures enriched for fatty acid oxidation and TCA cycle activity. Single-cell analyses revealed distinct transcriptional and clonal architectures influenced primarily by antigenic exposure rather than age.
Conclusion: Antigenic history imprints durable transcriptional, metabolic, and clonal programs on human memory T cells, supporting immune resilience even in extreme aging. These findings provide mechanistic insight into vaccine-induced long-term immunity and highlight the importance of functional memory quality over chronological age in shaping adaptive immune responses.

WORKSHOP 2 – INNATE IMMUNE CELLS IN HEALTH & DISEASE

Stimulation of type I interferon response activates innate immunity in mouse models of Colorectal Cancer Liver Metastasis

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Colorectal cancer liver metastasis (CRLM) is the leading cause of death in colorectal cancer patients, and its immune landscape is profoundly shaped by tumor-intrinsic pathways. Among the key regulators of liver immunity, type 1 innate lymphoid cells (ILC1), natural killer (NK) cells and macrophages fine tune their tissue residency properties, and effector and immunoregulatory function during metastasis development. However, it remains unclear how mismatch repair-deficient (dMMR) versus proficient (pMMR) tumors and their differential activation of type I interferon (IFN-I) programs, shape the recruitment and functional polarization of these immune populations.
Using two orthotopic liver metastasis models, MC38 (dMMR, immunogenic) and SL4 (pMMR, poorly immunogenic), we dissected mechanisms controlling NK/ILC1 recruitment, differentiation, and interaction with metastasis-associated macrophages (MAMs), and assessed whether STING activation could restore immune responsiveness in poorly immunogenic lesions.
Multiparametric flow cytometry, transcriptional profiling, conditional CXCR3 deletion in $NKp46^{+-}$ cells, macrophage-lymphocyte co-cultures, and in vivo treatments revealed striking differences between the two models.
MC38 metastases displayed abundant, mature $CD49a^+$ tissue-resident NK cells with enhanced cytotoxic function, whereas SL4 lesions showed limited NK recruitment and impaired effector maturation. Moreover, MC38 metastases were enriched in CXCL9-producing, IFN-I-programmed MAMs, that enhanced NK degranulation and IFN-γ production. CXCR3 deficiency reduced $CD49a^+$NK infiltration and acquisition of residency features, and increased metastatic burden, identifying CXCR3 as a key determinant of NK positioning into supportive metastatic niche.
Pharmacological STING activation induced IFN-I programs and upregulated PD-L1 expression by SL4 metastasis-infiltrating immune cells. Combining STING activation with PD-L1 blockade significantly

reduced SL4 metastatic burden and increased the frequency of proliferating CD8⁺ and NK cells. Antibody based *in vivo* depletion experiment demonstrated a prevalent protective role of NK cells in STING/αPD-L1 therapy.
These findings identify IFN-I programs as central regulators of macrophage activation and NK cell metastasis recruitment and support STING-based combinations to overcome immune resistance in pMMR CRLM.

Expansion of granulocyte macrophage colony stimulating factor–producing HLA-DR⁺ CD45RA⁺ Group 3 innate lymphoid cells in human colorectal cancer

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Growing evidence indicates that the antitumor functions of group 3 innate lymphoid cells (ILC3s) are linked to their ability to promote lymphoid neogenesis within tissues. While this activity has classically been attributed to lymphotoxin signaling, ILC3s also produce high levels of GM-CSF, a cytokine that drives immune cell mobilization and supports the maturation and organization of lymphoid structures. However, ILC3-derived GM-CSF expression in colorectal cancer (CRC) remains largely unexplored. Using in silico and ex vivo analyses, we observed increased GM-CSF expression in CRC compared with normal tissue and identified ILC3s as a major source of this cytokine among immune cells in both human samples and murine models. GM-CSF–producing ILC3s were enriched within lymphoid structures in CRC tissue and were predominantly found within an HLA-DR⁺ CD45RA⁺ subset, consistent with a less mature phenotype and suggestive of derivation from circulating ILC3s. Notably, transcriptomic analyses of peripheral blood from healthy donors identified an HLA-DR–expressing ILCp population displaying an ILC3-biased transcriptional program, characterized by elevated expression of GM-CSF-associated transcriptional regulators and lymphoid neogenesis–related genes. This subset was expanded in the peripheral blood of CRC patients, showing a shift in surface and transcriptional markers (downregulation of CD62L and upregulation of NKp46 and ROR⁺t) and markedly enhanced GM-CSF production, reflecting progressive ILC3-lineage commitment. Together, these findings identify an HLA-DR⁺ CD45RA⁺ ILC3 subset that accumulates in CRC patients and produces elevated GM-CSF both in tissue and in blood, potentially enhancing the recruitment and mobilization of immune cells and contributing to antitumoral immune responses.

Role of trained monocytes/T cell cross-talk in the control of immunological self-tolerance

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Immunological memory is also part of the innate immune system and can result in long-term functional reprogramming of innate immune cells with responsiveness to heterologous triggers ("trained-immunity"); however, if dysregulated, trained immunity can become maladaptive, perpetuating chronic inflammatory activation. Research over the past decade has highlighted benefits of trained immunity in immune-mediated disorders; however, the interplay between acquired and innate immune systems during trained processes, and the mechanisms by which trained immunity controls immunological self-tolerance, remain unknown.
Here, we evaluated the ability of human "trained"-monocytes to modulate autologous Tcell activation, proliferation, and differentiation. Monocytes were differentiated in vitro into macrophages upon Bacillus Calmette–Guérin (BCG) vaccine or β-glucan treatment (primary-stimulation), with or without LPS (secondary-stimulation). Cell culture supernatant and differentiated macrophages were used in co-culture with autologous Tcells. Tcells exposed to macrophage-conditioned medium, as well as extracellular-vesicles(EVs) isolated from macrophage-conditioned supernatants (without secondary-stimulation), showed increased differentiation toward a regulatory phenotype with higher FoxP3 expression, IL-10 production, and a metabolic shift toward glycolysis. Under these conditions, T cells showed reduced activation and proliferation,

with decreased expression of CD25, CD69, and Ki67. We corroborated the relevance of these findings in vivo, by employing the Experimental-Autoimmune-Encephalomyelitis (EAE) mouse model of multiple sclerosis. Fifteen days prior to EAE induction, mice were pre-conditioned with PBS, incomplete Freund's adjuvant (IFA) or complete Freund's adjuvant (CFA), which was used here as a strong innate immune stimulus analogous to BCG-vaccine exposure. Compared with the control groups, CFA-pretreated mice exhibited improved EAE clinical outcomes, including reduced disease scores and body weight loss. These effects were associated with reduced immune infiltration within the central nervous system (CNS) and increased frequency of Treg cells in the periphery.
Taken together these findings reveal a previously unrecognised feedback loop between macrophages and Tcells, where *trained*-macrophages can induce regulatory Tcells, supporting a tolerogenic microenvironment.

Aryl hydrocarbon Receptor shapes transcriptional and metabolic programs regulating conventional dendritic cell type 1 functional state

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Conventional dendritic cells type 1 (cDC1) are essential for cross-presentation and activation of cytotoxic $CD8^+$ T cells in anti-tumor immunity. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in immune regulation; however, its role in cDC1 differentiation and functional programming remains incompletely defined. Public transcriptomic data revealed that *Ahr* expression increases during DC differentiation, with higher levels in cDC1 compared with cDC2. Correlation analysis showed a positive association between *Ahr* and *Batf3*, a key regulator of cDC1 lineage commitment. Motif enrichment analysis of AhR ChIP-seq data generated in total DCs revealed enrichment of IRF8, BATF, and NFIL3 binding motifs, suggesting integration of AhR within transcriptional circuits governing cDC1 differentiation. In an inflammatory model, LPS-stimulated $Ahr^{fl/fl}Vav^{iCre}$ cDC1s showed reduced expression of cDC1-defining transcription factors (e.g., *Batf3, Irf8*) and increased proinflammatory cytokine expression alongside elevated cDC2-associated gene expression, indicating a transcriptional drift toward a cDC2-like program. Consistently, splenocytes from *Ahr*KO mice displayed increased cDC2 and pDC percentages but reduced cDC1s. To assess the functional impact of AhR loss in the tumor microenvironment, we used a murine fibrosarcoma model in which cDC1-specific *Ahr* deletion ($Ahr^{fl/fl}Xcr1^{Cre+}$) impaired tumor growth. Single-cell RNA-seq revealed that *Ahr*-deficient cDC1s adopted a more immunostimulatory state, characterized by enriched antigen presentation and activation signatures, increased cDC1–$CD8^+$ T cell communication. This inflammatory phenotype included increased cDC2-lineage gene expression and downregulation of fatty-acid biosynthesis genes (e.g., *Scd2, Elovl6*), paralleling the transcriptomic profile of cDC2s. To assess clinical relevance, we analyzed 259 TCGA sarcoma samples. High cDC1 signatures correlated with improved survival (p = 0.008), but AhR activation attenuated this protective effect (HR = 1.382). A human scRNA-seq atlas of 51 sarcoma samples confirmed AhR activation in cDC1s. Together, these findings identify AhR as a regulator coupling transcriptional and metabolic programs to modulate cDC1 immunogenicity and anti-tumor immunity.

Transcriptional profiling of Natural Killer cell activation driving immunotherapy response in triple-negative breast cancer patients

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Immunotherapy in combination with neoadjuvant chemotherapy is rapidly challenging the therapeutic perspective for Triple-Negative Breast Cancer (TNBC) patients. The use of immune checkpoint inhibitors enhanced the complete response rate achieved by chemotherapy alone. We previously demonstrated that enhanced Natural Killer cell (NK) tumor infiltration at diagnosis, assessed by spatial transcriptomics and immunohistochemistry, was associated with augmented chemotherapy efficacy. To dissect the specific role of NKs in predicting immunotherapy outcome in TNBC patients, we applied single-cell RNA sequencing (scRNA-seq) and multiparameter flow cytometry to profile circulating NKs. We compared patients who showed either pathological complete or partial response to the neoadjuvant schedule with pembrolizumab (KEYNOTE522 schedule). Results indicated that NKs in partial responders were significantly impaired and showed an immune-tolerant profile after therapy completion. These results confirmed the relevance

of activated NKs in defining immunotherapy response. However, the exact molecular profiling of NK effector functions toward TNBC cells is still lacking. To fill this gap, we built an NK-specific 300-gene signature. A predictive bioinformatic approach was applied, and, among predicted transcriptional regulators, we identified the zinc finger transcription factor VEZF1. ChIP-sequencing indicated that VEZF1 direct targets were involved in vesicle trafficking and lytic granule exocytosis. Mechanistically, we characterized for the first time VEZF1 transcriptional cooperation with the MYC-associated factor MAZ. The silencing of either VEZF1 or MAZ by CRISPR interference (CRISPRi) drastically impaired NK degranulation and cytotoxicity toward TNBC. Their governed transcriptional program was validated in an independent scRNA-seq dataset and correlated with immunotherapy response.
Collectively, our data suggest that NK activation is crucial in achieving a complete immunotherapy response in TNBC patients. In this context, we identified a novel regulatory transcriptional axis composed of VEZF1 and MAZ. The activity of these factors is relevant in sustaining NK anti-tumor response, thereby opening novel clinical applications for NK-based cell therapies.

WORKSHOP 3 – TUMOR IMMUNOLOGY

Atypical chemotactic receptor CCRL2 orchestrates the recruitment of protumor SiglecF^{+} neutrophils in lung cancer

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CCRL2 is an atypical seven-transmembrane receptor that binds the chemoattractant chemerin and regulates leukocyte trafficking. Although it lacks conventional G protein–coupled signalling, CCRL2 has emerged as a critical modulator of inflammatory responses in diverse pathological contexts, including arthritis and autoimmune encephalomyelitis. We previously demonstrated that genetic ablation of *Ccrl2* accelerates tumor progression in the Kras$^{G12D/+}$; p53^{LoxP} (TK) lung cancer model by impairing the recruitment of antitumor NK cells.
Here, we provide evidence that CCRL2 expression was selectively induced in tumour-infiltrating neutrophils, while remaining undetectable in circulating or tissue-resident populations, indicating that its expression is driven by tumor-specific environmental signals. Accordingly, exposure of bone marrow–derived neutrophils to lung tumour cell–conditioned supernatants for 24-72 hours resulted in robust upregulation of CCRL2 at both transcriptional and protein levels. Importantly, Ccrl2 deficient mice exhibited increased accumulation of protumor SiglecF^{high} neutrophils within the lung tumor microenvironment. As expected, SiglecF^{high} neutrophils displayed significantly higher ROS activity compared to SiglecF^{low} cells in both TK Ccrl2 WT and KO mice, indicating preserved intrinsic effector function. However, Ccrl2 deficient SiglecF^{high} neutrophils showed enhanced migratory responses to CXCR2 and CXCR4 ligands, suggesting that CCRL2 may limit the accumulation of tumor-associated neutrophils by modulating their recruitment or retention.
Collectively, these findings identify CCRL2 as a tumor-induced marker of neutrophil reprogramming and support a model in which CCRL2 acts as a regulatory checkpoint that restrains the expansion of protumor SiglecF^{high} neutrophils in lung cancer and may limit tumor progression.

xCT drives metastatic progression and immune–tumor interactions in breast cancer

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Breast cancer remains a leading cause of cancer-related mortality, primarily due to metastatic dissemination and therapy resistance. Tumor cells adapt to hostile microenvironments through metabolic and redox remodeling, a process in which the cystine/glutamate antiporter xCT, a key regulator of intracellular glutathione synthesis, plays a central role. xCT is frequently upregulated in aggressive breast cancers and correlates with poor prognosis, suggesting its involvement in metastatic progression.
Here, we investigated the contribution of xCT to breast cancer progression using genetically modified, immunocompetent and immunodeficient models, and advanced organ-on-a-chip platforms. While systemic xCT ablation in mice did not affect immune system development and primary tumor growth, tumor cell–specific depletion profoundly impaired invasion and lung metastatic dissemination. Notably, in immunodeficient mice, xCT-deficient tumor cells regained lung colonization ability, revealing a critical role for the immune system in the antimetastatic effects of xCT loss. Accordingly, xCT depletion was associated with altered immune cell recruitment within pre-metastatic and metastatic niches, supporting a model in which xCT promotes metastatic seeding through both tumor-intrinsic and immune-mediated mechanisms. These observations were confirmed in a human organ-on-a-chip model, where xCT silencing markedly limited tumor cell dissemination.
Mechanistically, xCT deficiency induced redox imbalance and metabolic reprogramming, leading to suppression of kinase signaling pathways critical for metastatic progression. Furthermore, tumor-derived extracellular vesicles mediated non–cell-autonomous xCT-dependent effects. Vesicles from xCT-expressing cells enhanced invasion, lung colonization, and immune cell recruitment, whereas those from xCT-deficient cells did not.
Building on these findings, we developed a structure-guided protein vaccine targeting the extracellular loops of xCT. Preliminary in vivo data demonstrate significant inhibition of metastatic dissemination, with ongoing optimization to enhance immunogenicity and therapeutic efficacy.
Overall, our work identifies xCT as a central regulator of metastatic competence and tumor–host communication, highlighting its potential as a safe and innovative therapeutic target in breast cancer.

Neutrophil dynamics and NETosis in the pathogenesis of multiple myeloma

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Background: Multiple Myeloma (MM) is the second most common hematological cancer, characterized by the clonal growth of plasma cells and the production of monoclonal paraproteins. This progression often leads to serious clinical complications, including osteolytic lesions, hypercalcemia, and kidney failure. A key feature of MM is immune dysfunction, demonstrated by a significant reduction in polyclonal immunoglobulins. Emerging research highlights the complex role of neutrophils (PMNs), which influence both innate and adaptive immune responses. Notably, studies indicate that the phagocytic ability of PMNs is severely decreased during induction therapy and remains impaired after autologous stem cell transplantation, pointing to a persistent immune deficiency. Therefore, our goal is to examine the functional role of PMNs in MM.
Methods: We measured various neutrophil-related mediators and neutrophil extracellular trap (NET) biomarkers in the sera of MM patients using ELISA kits. Key inflammatory markers, including MMP-9, MPO, neutrophil elastase (NE), CXCL8/IL-8, GM-CSF, IL-32, mast cell tryptase (MCT), and Arginase 1, were assessed. Additionally, NET formation was specifically identified by detecting MPO-DNA complexes and Citrullinated Histone H3 (CitH3) using high-sensitivity monoclonal antibodies.
Results: MM patients exhibited significantly higher serum levels of neutrophil-related mediators than healthy controls. Specifically, CitH3, MMP-9, and MPO levels increased steadily over time. While IL-32 and Arginase 1 were elevated, MCT levels were notably decreased. Correlation analyses indicated a complex inflammatory network in which NET biomarkers were positively correlated with key cytokines. Interestingly, IL-32 consistently exhibited a negative correlation with multiple neutrophil activation markers.
Conclusions: These findings demonstrate that MM patients experience ongoing neutrophil activation and increased NETosis, which intensifies as the disease progresses. The contrast between elevated Arginase 1 and decreased MCT suggests a functional reprogramming of myeloid cells. This self-sustaining inflammatory network likely affects the tumor microenvironment, offering new insights into the immune interactions that support MM development.

IgE-mediated peanut allergy reprograms the tumor immune microenvironment and limits colorectal cancer progression

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Background: Allergo-oncology proposes that allergic inflammation may shape tumor immunosurveillance. IgE-driven responses are characterized by FcεRI-mediated mast cell activation systemic immune priming, and enhanced antigen presentation, suggesting the capacity to reshape the tumor immune microenvironment (TIME). However, the impact of food allergy on colorectal cancer (CRC) progression remains undefined.
Purpose: To determine whether IgE-mediated peanut sensitization influences CRC development and to define its immunological imprint on the TIME.
Methods: Peanut allergy was induced by weekly oral sensitization with peanut extract (CPE) plus cholera toxin (CTX) for four consecutive weeks. Colorectal tumors were subsequently induced using the azoxymethane/dextran sodium sulfate (AOM/DSS) inflammation-driven CRC model. Tumor burden was evaluated and immune composition of tumors and adjacent colon were analyzed by multiparametric flow cytometry.
Results: Sensitization was confirmed by increased peanut-specific IgE levels and FcεRI-dependent mast cell activation. Sensitized mice exhibited a significant reduction in tumor burden compared with controls, indicating that IgE-mediated systemic sensitization is associated with attenuated CRC progression. Tumors from sensitized mice showed decreased frequencies of $CD4^+$ T cells alongside a relative enrichment of $CD8^+$ T cells. Although the overall $CD4^+$ compartment was reduced, Th1 cells were significantly expanded and Th17 cells showed an increasing trend, whereas Th2 and $FoxP3^+$ regulatory T cells were diminished. This profile suggests a shift toward a type 1–oriented, pro-inflammatory, and potentially cytotoxic tumor microenvironment. Additionally, the myeloid compartment was reshaped, with enhanced neutrophil infiltration and a marked expansion of dendritic cells, consistent with improved antigen-presenting capacity within the TIME.
Conclusions: IgE-mediated peanut sensitization is associated with reduced CRC progression and drives functional reprogramming of the TIME toward a Th1-dominant, less immunoregulatory state. Ongoing studies in mast cell–deficient mice will clarify whether mast cell activation represents the upstream orchestrator of this antitumor immune remodeling.

Tumor progression reprograms cardiac innate immunity and drives neutrophil-mediated myocardial dysfunction

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Introduction: Cardio-oncology has traditionally focused on therapy-induced cardiotoxicity. However, emerging evidence indicates that cancer and cardiovascular disease are biologically intertwined beyond treatment effects. Reverse cardio-oncology has demonstrated that cardiac injury can accelerate tumor progression by innate immune dysregulation, gut microbiota alterations, and the release of circulating pro-tumorigenic factors, whether tumor growth itself actively reprograms the myocardium remains unknown. To dissect the tumor-heart axis, we investigated how progressive cancer growth reshapes cardiac innate immunity and affects myocardial function in vivo.
Methods: To address this, we employed biologically distinct transplantable tumor models (FS6 and MN/MCA1 sarcoma models, MC38 CRC model, and LLC lung cancer model). Tumor-bearing mice were analyzed at 20- and 30-days post-implantation. Cardiac immune composition was characterized by flow cytometry, inflammatory mediators were quantified, and systolic function was assessed by echocardiography. To dissect the contribution of neutrophils, parallel experiments were performed in Csf3r-deficient mice.
Results: Across all tumor models, progressive tumor growth induced robust myocardial immune infiltration accompanied by a profound shift from lymphoid to myeloid dominance, a process that was particularly pronounced in the FS6 model. The myocardium of tumor-bearing mice exhibited marked accumulation of monocytes, macrophages, and neutrophils, associated with increased TNF and IL-6 expression. Importantly, in FS6 and MC38 model, these inflammatory changes correlated with significant systolic dysfunction. Strikingly, genetic neutrophil deficiency prevented tumor-associated cardiac functional impairment in FS6 model, identifying neutrophils as key effectors linking tumor progression to myocardial damage.
Conclusions: Our findings redefine the tumor-heart axis by demonstrating that cancer progression alone is sufficient to reprogram cardiac innate immunity and drive inflammatory cardiomyopathy. This work expands the paradigm of cardio-oncology beyond therapy-related toxicity and identifies neutrophil-dependent inflammation as a previously unrecognized mechanism of cancer-associated cardiac dysfunction. Targeting systemic innate immune remodeling may represent a novel strategy to protect the heart in patients with advanced malignancies.

WORKSHOP 4 – HOST-PATHOGEN INTERACTION

Viral infections accelerate hematopoietic stem cell aging

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During aging, hematopoietic stem cells (HSCs), responsible for life-long blood production and immune cells through self-renewal and differentiation, exhibit impaired regenerative capacity and skewed lineage output toward myeloid cells, contributing to immune-senescence and hematological dysfunction.
In response to pathogens, such as viral infections, HSCs sustain emergency hematopoiesis by increasing the production of immune cells to fight the insult. Pioneering studies have shown inflammation alone, or repeated inflammatory stimuli such as systemic LPS administration, can accelerate aging-associated features in HSCs, including myeloid skewing, and reduced clonal diversity. However, these models fail to capture key properties of bona fide viral infections, such as tissue tropism, spatial confinement, replication dynamics, and persistence. Whether viral infections can induce premature aging of the hematopoietic compartment remains largely unexplored. Here, we study the impact of viral infections on HSC biology. By using mouse models of acute/chronic and local/systemic infections, we investigate both early and persistent remodeling of HSCs and progenitor hierarchy. Preliminary data reveal that both acute and chronic LCMV infection induce rapid reorganization of hematopoiesis, acquiring hallmarks of aged HSCs such as myeloid skewing and persistent aging-like phenotypic changes in HSCs. Importantly, an acute local lung viral infection (Sars CoV-2) is sufficient to induce mobilization and an aged phenotype in HSCs, even after infection resolution, indicating systemic inflammation is not required to induce long-lasting HSCs remodeling. Therefore, our findings place viral infections as potential drivers of premature HSC aging. Ongoing work aims to determine the functional consequences of these changes, including their impact on clonal composition and the emergence of clonal hematopoiesis.
Given that aging-associated shifts in hematopoiesis are linked to worse immunological responses, and the aging of the immune system accelerates systemic aging, understanding how viral exposure reshapes this compartment may open new avenues for rejuvenating hematopoiesis and mitigating immune-senescence, thereby promoting healthy aging.

Bacterial lipopolysaccharide acylation patterns dictate hematopoietic stem cell fate and systemic immune responsiveness

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Bacterial infections remain a global health challenge, persisting despite advancements in vaccines and antibiotics due to sophisticated mechanisms of immune evasion. Lipopolysaccharide (LPS), a primary constituent of the Gram-negative outer membrane, undergoes acylation remodeling to modulate host recognition. While hexacylated LPS serves as a canonical pro-inflammatory ligand, LPS hypoacylation is exploited by both pathogens and commensal microbiota to enforce immune tolerance. Although hematopoietic stem and progenitor cells (HSPCs) are central determinants of infection outcomes—regulating the timing and direction of the immune response—the impact of LPS structural variation on HSPC reprogramming remains poorly understood.
In this study, utilizing a genetically engineered *Escherichia coli* strain developed in our laboratory, we characterize the in vivo impact of LPS hypoacylation on the hematopoietic landscape. Following intraperitoneal injection into C57BL/6J mice, hexacylated *E. coli* triggered robust systemic inflammation and emergency hematopoiesis. The hypoacylated strain bypassed the pro-inflammatory cytokine storm typical of septic infections caused by hexacylated bacteria, favoring instead the selective expansion of immunosuppressive progenitors in the bone marrow. These cells subsequently mobilized into the circulation and accumulated at the infection site, a shift accompanied by the enrichment of myeloid-derived suppressor cells (MDSCs) with potent immunoregulatory capacity. Further investigation using knockout mice deficient in receptors and signalling molecules involved in LPS sensing revealed that these effects are dependent on Toll-like receptor 2 (TLR4) and MyD88 signalling. Together, these findings identify the LPS acylation state as a key determinant of early hematopoietic fate. Sensing of hypoacylated LPS via TLR2–MyD88 diverts the hematopoietic output by suppressing inflammatory emergency responses and favoring the generation of immunosuppressive progenitors. This reveals a fundamental mechanism by which pathogens and the symbiotic microbiota shape host immunity.

Human TR3-56 cells in Multiple Sclerosis: a new pathogenic cell link between Epstein-Barr Virus infection and disease pathogenesis.

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Multiple Sclerosis (MS) is an autoimmune, neurodegenerative disorder driven by CNS-infiltrating T lymphocytes. Evidence suggests that Epstein-Barr virus (EBV) infection and impaired T-cell responses contribute to MS pathogenesis. We investigated a poorly explored subset, TR3-56 cells (CD3+CD56+), previously linked to altered functions in other autoimmune contexts.
We enrolled 46 newly diagnosed treatment-naïve Relapsing Remitting (RR)-MS subjects and 55 matched healthy controls. Ex vivo, we analyzed the frequency, exhaustion phenotype and cytotoxic function of TR3-56 cells. TR3-56 functional response to different viral stimuli, including EBV, was evaluated in both studied groups. Finally, we conducted a longitudinal analysis of 12 RR-MS subjects undergoing Ocrelizumab therapy, at 6 and 12 months post-treatment, to evaluate TR3-56 cell frequency and functional response to EBV.
Our findings revealed a significant reduction in the frequency of TR3-56 cells in the blood of RR-MS individuals, which inversely correlated with disease disability. We observed elevated levels of exhaustion markers on ex vivo TR3-56 cells from RR-MS patients compared to those from healthy controls. Analysis of TR3-56 cell functionality, including degranulation and cytokine production, unveiled a significant reduction in CD107a/LAMP-1 expression and IFN-γ production in the RR-MS group. In addition, TR3-56 cells from MS subjects exhibited a defective proliferative and cytotoxic response to EBV peptides. Following Ocrelizumab therapy, TR3-56 cell frequency significantly increased at 12 months. Notably, EBV-specific responsiveness was restored as early as 6 months post-treatment. Our findings suggest that the reduced frequency and impaired functionality of TR3-56 cells in RR-MS individuals are attributable to a specific defect within the subset of clones responsive to EBV antigens. Ocrelizumab therapy led to a significant restoration of TR3-56 cells, with EBV-specific responsiveness recovered as early as 6 months post-treatment. These results support a potential mechanistic link between EBV infection, B cell dysregulation, and TR3-56 cell impairment in MS pathogenesis.

Vδ2-extracellular vesicles contain multiple antiviral and immune-regulatory miRNAs

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Vδ2 T cells are promising candidates for immunotherapy due to their unique ability to mediate direct and bystander effector functions. We recently demonstrated that extracellular vesicles released by Vδ2 T cells (Vδ2-EVs) are able to induce several immunomodulatory effects. Specifically, Vδ2-EVs efficiently inhibit viral replication and activate antigen-presenting cells, thereby enhancing virus-specific T-cell responses.
To investigate how the molecular cargo of Vδ2-EVs mediates the observed immunomodulatory activities, miRNA sequencing was performed. Among the most abundant miRNAs carried by Vδ2-EVs, several converge on shared regulatory pathways that shape the amplitude, quality, and persistence of innate and adaptive immune responses (e.g., miR-106a-5p, miR-20b-5p, miR-155-5p, miR-23a-3p), and include miRNAs with documented direct antiviral activity (e.g., miR-342-3p, miR-17-5p, miR-16-5p). Gene Ontology (GO) enrichment analysis revealed significant overrepresentation of pathways involved in immune responses, and KEGG pathway analysis demonstrated marked enrichment of pathways associated with viral replication (e.g., HTLV-1, HBV, CMV, and HPV) as well as immune signaling.
To validate the bioinformatic predictions, quantitative analysis of miRNA target genes was performed in EV-targeted cells. We selected five predicted target genes of miRNA specifically enriched in Vδ2-EVs based on their roles in viral replication and immune responses. Specifically, we quantified these target genes in CMV-infected MRC5 cells or monocytes treated with Vδ2-EVs using qRT-PCR. After Vδ2-EVs stimulation, the expression of the target genes with immunomodulating properties (SOCS1, SOCS5 and TGFBR2) was significantly reduced in both MRC5 and monocytes. Furthermore, Vδ2-EVs treatment induced a reduction of the expression of target genes involved in viral replication (LDLR and PIKFYVE) only in CMV infected MRC5 cells, and not in monocytes, confirming the specificity of their activity. These results indicate that EVs released by Vδ2 T cells can promote antiviral immunity by downregulating key genes that inhibit interferon-mediated responses, facilitate viral replication, and restrain antigen-specific T-cell proliferation.

Pentraxin 3 KO mice exhibit increased susceptibility to Mycobacterium tuberculosis infection compared with Wild-Type controls

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To investigate the in vivo role of Pentraxin 3 (PTX3) during Mycobacterium tuberculosis (Mtb) infection, we monitored PTX3 KO and wild type (WT) mice that had been intranasally infected and with 2000 CFU of a chemiluminescent Mtb H37rv strain.
Survival was assessed three times per week over a 10-week period. PTX3 KO mice showed markedly reduced survival and all succumbed by week 10, at which time the WT mice survived.
In parallel, we found a pronounced increase in lung CFU in PTX3 KO mice, detectable as early as week 4 and consistently higher at all time points examined. The difference between PTX3 KO and WT lungs reached statistical significance at week 8, reinforcing the observation that bacterial replication in the lungs is insufficiently controlled in the absence of PTX3.
In the liver, CFU levels showed a trend toward higher bacterial burdens in PTX3 KO mice across all time points, although these differences did not reach statistical significance. Conversely, in the spleen, bacterial counts were higher in PTX3 KO mice, becoming statistically significant in week 4.
Taking together, these data demonstrate that the lungs of PTX3 KO mice are colonized earlier and more extensively by Mtb compared with WT lungs and suggest a critical role for PTX3 in the early containment of pulmonary Mtb infection.

WORKSHOP 5 – HOST-PATHOGEN INTERACTION

Harnessing Runx1 enhancer-reporter models to elucidate the specification of Haematopoietic Stem Cells in the mouse embryo

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Hematopoietic stem and progenitor cells (HSPCs) represent the lifelong source of cells supporting a functional immune system in mouse and humans. Because of this unique potential, great interest has been directed towards the understanding of the molecular mechanisms driving HSPC generation for both research and therapeutical purposes.
During embryogenesis, both hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) originate from hemogenic endothelium (HE) via a tightly regulated process termed endothelial-to-hematopoietic transition (EHT) that is critically dependent on the transcription factor Runx1. However, the regulatory events driving the specification of an HSC versus HPC fate are still poorly characterized. Elucidating signals and markers associated with these alternative fates is critical to guide the directed differentiation of HSC from pluripotent stem cells *in vitro*, and their potential application in cell-based therapies for blood-related disorders.
To dissect the specification of HSCs, we made use of two *Runx1* enhancer-reporter mouse models marking complementary subsets of *Runx1* expressing emerging blood cells in the dorsal aorta of the midgestation embryo, enabling their isolation, functional characterization and multiomic profiling. We found that differential activity of the *Runx1* +110 enhancer-reporter among the earliest blood precursors that emerge from HE, segregates them with the potential to develop into HSCs from those without this potential. In addition, we identified a specific gene signature associated with long-term repopulation potential *in vivo*. Our data suggest a model in which both HSCs and HPCs differentiate from a nascent, EHT-derived pool of cells, but while most differentiate into HPCs and contribute to fetal hematopoiesis, a small fraction up-regulate self-renewal genes and mature into the transplantable HSC lineage.

Tyrosine halogenation converts α-defensins into potent immunomodulators

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Neutrophils deploy potent cytotoxic mechanisms, combining reactive oxygen species (ROS), hypohalous acids, proteases, and antimicrobial peptides to eliminate invading microbes. These effectors are released simultaneously into confined spaces like phagolysosomes and inflammatory lesions — yet how these mechanisms cooperate, remains unclear. Here we demonstrate a previously unrecognized synergistic interaction between oxidative and non-oxidative neutrophil effector systems. Upon activation, myeloperoxidase (MPO)-derived hypohalous acids selectively chlorinate and iodinate α-defensin 1–3 (HNP1–3) at conserved tyrosine residues. This modification occurs in activated human and rat neutrophils and is abundant in neutrophil-rich pathological settings, including cystic fibrosis airways, bacterial pneumonias, and Staphylococcus aureus abscesses. Biophysical and structural analyses show that halogenation increases the hydrophobicity of HNP1 without disrupting its structure. Functionally, this modification transforms defensins into potent immunomodulators that amplify proinflammatory signaling, as demonstrated by single-cell and in vivo analyses. Our findings uncover a novel oxidative post-translational modification by which ROS reprogram defensins to orchestrate immune responses, revealing an oxidative–non-oxidative synergy that adds new complexity to neutrophil-mediated host defense.

NFAT signaling modulation promotes endogenous neural stem cell expansion and brain regeneration

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Background: Adult neural stem cells (NSCs) sustain brain regeneration throughout life, but neurogenesis declines with age and cannot compensate for neuronal loss in neurodegenerative diseases. Expanding endogenous NSCs represents a promising regenerative strategy. Calcium (Ca^{2+}) and Calcineurin (CaN) regulate Nuclear Factor of Activated T cells (NFAT) transcription factors controlling activation of immune cells and proliferation. We demonstrate that NFATs regulate inflammatory responses and terminal differentiation of dendritic cells. Considering the conserved role of Ca^{2+} signaling in stem cell biology, we hypothesized that CaN–NFATs signaling regulate NSC differentiation and that its selective inhibition promotes NSC expansion and enhances regenerative potential.

Methods: To inhibit CaN–NFAT signaling, we used Nestin-CreERT2-VIVIT mice, in which the VIVIT peptide, a specific inhibitor of CaN–NFAT interaction, is inducibly expressed in Nestin$^+$ NSCs. Primary NSCs isolated from the subventricular zone were analyzed by single-cell RNA sequencing. In vivo NSC expansion, migration, and differentiation were evaluated by immunofluorescence. Regenerative capacity was assessed in the MPTP model of Parkinson's disease.

Results: scRNAseq analysis showed that CaN–NFAT inhibition arrests NSCs differentiation, leading to their accumulation in stem and progenitor states. In vivo, NFAT inhibition significantly expanded endogenous NSCs and promoted their migration into various brain regions. VIVIT$^+$ NSCs expressed secreted factors predicted to remodel the stem cell niche, supporting a paracrine mechanism that enhances neurogenesis. In the MPTP model, NSC expansion promoted dopaminergic regeneration, demonstrated by restoration of tyrosine hydroxylase–positive neurons and improved tissue regeneration.

Conclusion: These findings identify CaN–NFAT signaling as a central regulator of adult NSC fate. Its inhibition promotes endogenous NSC expansion and enhances regenerative responses after injury. Targeting CaN–NFAT signaling represents a promising therapeutic strategy to stimulate endogenous brain repair and counteract neurodegeneration. This approach may enable innovative regenerative therapies for currently incurable neurological disorders.

Inflammasome inhibitor MCC950 prevents psoriasis-like skin manifestations and associated systemic inflammation

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Background: Psoriasis is a chronic, systemic inflammatory disease associated with cardiovascular and metabolic comorbidities. It is driven by a sustained cutaneous immune response that develops around dermal DC:T cell clusters. Early-phase pathology likely involves keratinocyte-innate immune interplay; however, the mechanisms driving subsequent adaptive immune responses remain largely unknown. The NLRP3 inflammasome may serve as a critical sensor in this process, translating cellular stress into inflammatory signaling via IL-1β and IL-18.
Aim: We aimed to investigate the effect of local NLRP3 inflammasome inhibition on the cutaneous and systemic psoriasis-like manifestations using both acute and long-term recurrent models of imiquimod-induced psoriasiform inflammation and MCC950 as inflammasome inhibitor.
Results: In the acute model, classical psoriasis features were observed, including acanthosis and immune cell infiltration with accumulation of myeloid cell and $CD3^+$ T cells, mainly $CD8^+$, together with upregulation of psoriasis marker genes *Il17*, Tnf, and *S100a8/a9*. Systemically, we observed elevated serum IL-6 and splenic immune remodeling marked by myeloid expansion and reduction of T cells consistent with their recruitment from the spleen to the inflamed skin. Topical MCC950 pre-treatment significantly reduced psoriasis-like clinical signs and immune cell infiltration, prevented pro-inflammatory gene upregulation, and restored systemic myeloid and T-cell balance.
In the long-term recurrent model, two months after the last flare, persistent cutaneous thickening, $CD8^+$ T-cell accumulation, and sustained expression of inflammasome-related genes *Nlrp3* and *Aim2* were still detectable. Early cutaneous administration of MCC950 effectively prevented long-term maintenance of this pro-inflammatory state-
Altogether, these findings highlight early upstream inflammasome inhibition as a potential strategy to prevent both cutaneous and systemic manifestations of psoriasiform inflammation and tissue adaptation in chronic inflammatory skin diseases.

Lysosomal acid lipase deficiency impairs metabolic reprogramming supporting antigen-presenting capacity of dendritic cells

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Lysosomal acid lipase deficiency (LAL-D), a rare autosomal recessive disorder, impairs the hydrolysis of cholesteryl esters and triglycerides within lysosomes and results in multisystem lipid accumulation. While the hepatic and metabolic consequences of the late-onset form of the disease (Cholesteryl Ester Storage Disease, CESD) are well characterized, the extra-hepatic effects have been less explored. Emerging evidence suggests that LAL-D affects immune homeostasis, with alterations in immune cell precursors, aberrant macrophage polarization, and reduced peripheral T cells with impaired function. On this line, aim of the study was to deepen the knowledge of the impact of LAL-D on innate immunity, focusing on dendritic cells (DCs).
Human monocyte-derived DCs were generated from blood samples of CESD patients and matched controls by GM-CSF and IL-4, and stimulated with lipopolysaccharide (LPS). Murine DCs were obtained from bone marrow monocytes of wild-type, treated or not with LAL inhibitor, and LAL-knockout (LAL-KO) mice by GM-CSF, and stimulated with LPS or with a peptide antigen (EαGFP). DCs phenotype was assessed by gene expression, metabolic profiling and functional assays.
When exposed to LPS, DCs from CESD patients exhibited significantly reduced CD80 and CD86 expression compared to control ones, suggesting an impaired maturation and defective antigen-presentation capacity. After LPS stimulation, murine LAL-inhibited and LAL-KO DCs versus control DCs showed an aberrant antigen-presentation capacity of both lipid and protein antigens, associated with an impaired ability to prime T cells. To assess whether this functional alteration is coupled to an impaired energetic metabolism, DCs underwent

a metabolic real-time analysis. Compared to WT ones, LAL-KO DCs showed an attenuated shift toward glycolysis while maintaining oxidative phosphorylation upon LPS stimulation, indicating a defective metabolic reprogramming required for full DC activation and immunogenicity.
In conclusion, genetic LAL-D impairs the metabolic reprogramming of DCs supporting antigen presentation, likely contributing to defective activation of T cells.

WORKSHOP 6 – TUMOR IMMUNOTHERAPY

hMENA-regulated secretory autophagy in cancer-associated fibroblasts shapes an immunosuppressive tumor microenvironment and promotes immunotherapy resistance in NSCLC

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Unconventional mechanisms of paracrine communication within the tumor microenvironment (TME), including secretory autophagy, are emerging as important regulators of tumor progression and therapeutic response. Understanding these mechanisms, particularly the interactions among cancer-associated fibroblasts (CAFs), tumor cells, and tumor-associated macrophages (TAMs), may reveal new therapeutic opportunities. The actin cytoskeleton regulatory protein hMENA and its isoform hMENAΔv6 are expressed in mesenchymal tumor cells and CAFs, where they promote CAF-tumor cell crosstalk through the secretion of pro-tumoral factors. Although hMENA has recently been implicated in autophagosome formation and trafficking in tumor cells, its role in secretory autophagy remains largely unexplored.
Here, we investigated the contribution of hMENA/hMENAΔv6 to CAF-mediated secretory autophagy and its potential involvement in resistance to immune checkpoint therapy (ICT) in non-small cell lung cancer (NSCLC). Depletion of hMENA/hMENAΔv6 in CAFs isolated from resected NSCLC tumors results in YAP accumulation, defective autophagosome trafficking, and reduced secretion of cytokines, including TGF-β1. Conversely, the secretome of hMENA/hMENAΔv6-overexpressing CAFs enhanced NSCLC cell proliferation and promoted metabolic reprogramming toward aerobic glycolysis. Clinically, a secretory autophagy-related gene signature including ENAH (hMENA gene) was enriched in hMENAΔv6high patients from the TCGA cohort and was associated with significantly poorer survival.
To further explore CAF heterogeneity, we constructed a single-cell atlas of CAFs by integrating 14 publicly available datasets. This analysis identified a subset of TGFβ/hMENA-enriched myofibroblastic CAFs characterized by elevated expression of secretory autophagy genes. Using a machine-learning approach, we derived a transcriptional signature from this CAF

subset that, together with secretory autophagy genes, stratified responders and non-responders to ICT in the Stand Up To Cancer RNA-seq dataset.
Given the emerging importance of CAF-TAM cross-talk, we also generated a single-cell atlas of macrophages to investigate interactions between TAMs and this CAF subset, aiming to elucidate their contribution to an immunosuppressive TME and reduced responsiveness to ICT.

Activin A blockade remodels the bone marrow niche and enhances chemotherapy response in paediatric B-cell Acute Lymphoblastic Leukemia

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Despite major advances in paediatric B-cell acute lymphoblastic leukaemia (B-ALL) therapy, outcomes for relapsed/refractory (r/r) patients remain poor. Increasing evidence implicates the protective bone marrow (BM) microenvironment in treatment resistance.
Aim of the work was to investigate the anti-leukemic potential of targeting Activin A, a microenvironment-derived factor, able to promote B-ALL migration and invasiveness.
Using multiple human B-ALL xenograft mouse models, we demonstrate that sequestration of Activin A with the chimeric antibody RAP-011 (ActRIIA-mIgGFc), the murine ortholog of Sotatercept, significantly delays disease progression. RAP-011 improved survival and reduced leukemic burden in both BM and extramedullary sites. The anti-leukemic effect was attributable both to a direct impact on leukemic cell migration and to broader modulation of the BM microenvironment, as demonstrated by single-cell RNA sequencing (scRNAseq). Specifically, Activin A sequestration attenuated the leukemia-induced TGF-β signalling and Cancer-Associated Fibroblast (CAF)-like transcriptional program within the mesenchymal stromal cell (MSC) compartment, identifying Activin A as a crucial regulator of leukemia-induced stromal reprograming. ScRNA-seq further demonstrated that Activin A blocking modulates pathways related to extracellular matrix remodeling and deposition. Beyond MSCs, RAP-011, also modulated the transcriptional programs associated with neutrophil activation, migration, and phagocytic function. Crucially, Activin A blockade synergized with dexamethasone-based chemotherapy, enhancing its pro-apoptotic effects and further reducing leukemic burden, beyond monotherapy. Notably, a synergistic effect between RAP-011 and dexamethasone was observed both when RAP-011 was administered at day 0 concomitantly with B-ALL cell infusion and when the combination treatment was initiated in mice with established leukemia, according to a treatment schedule more closely reflecting a potential clinical translation.
These findings support Activin A sequestration as a strategy to enhance treatment efficacy in B-ALL, offering a potential pathway to improve outcomes and enable chemotherapy de-escalation in high-risk patients.

Epigenetic activation of the IL-33/ST2 axis potentiates anti-tumor immunity and restores sensitivity to PD-1 blockade in melanoma

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IL-33 is an epithelial-derived alarmin with multifaceted functions in cancer. In melanoma, it facilitates anti-tumor immunity by activating multiple immune effector populations within the tumor microenvironment (TME). This study explores the combination of IL-33 with the hypomethylating agent decitabine (DAC) as a strategy to enhance immune recognition and therapeutic response in melanoma. In melanoma-bearing mice, combined DAC and IL-33 treatment suppressed tumor progression, extended overall survival and augmented immune cell infiltration, particularly $CD8^+$ T cells and eosinophils, within the TME. Moreover, the combined treatment induced upregulation of PD-1 on tumor-infiltrating lymphocytes and enhanced responsiveness to PD-1 blockade in vivo. In a vascularized human melanoma organoid model, DAC/IL-33 promoted tumor recruitment of PBMCs, particularly of PD-1+$CD8^+$ T, consistent with in vivo mouse studies. Furthermore, using a dual-TME competitive microfluidic chemotaxis assay, the DAC/IL-33-conditioned melanoma microenvironment selectively recruited $CD8^+$ T cells from wild-type but not IL-33 receptor–deficient (ST2-/-) mice, demonstrating the essential contribution of the IL-33/ST2 signaling axis for tumor-immune crosstalk. Consistently, DAC failed to

promote immune infiltration or reduce tumor burden in ST2-/- melanoma-bearing mice. Mechanistically, DNA methylation profiling showed that DAC enhances IL-33 transcription in both murine and human melanoma cells by demethylating a regulatory region enriched for transcription factor binding motifs within the IL33 locus. Collectively, these findings reveal a synergistic interaction between DAC and exogenous and endogenous IL-33/ST2 signaling in melanoma, mediated through epigenetic reprogramming and enhanced recruitment of immune effector cells that reshape the TME and sensitize to PD-1 blockade.

Reprogramming melanoma treatment by unlocking CSPG4-targeted combination approaches

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Malignant melanoma (MM) is the sixth most common cancer worldwide. Despite advances with BRAF/MEK-targeted therapies and immune checkpoint inhibitors, clinical efficacy is often limited due to resistance, highlighting the need for innovative strategies. Chondroitin sulfate proteoglycan (CSPG)4 is an attractive immunotherapeutic target, with limited expression in healthy tissues and high expression in MM, where it plays a key oncogenic role. To overcome immune-tolerance against this non-mutated self-antigen, we developed a chimeric DNA vaccine (HuDo-CSPG4), combining human and dog CSPG4 sequences. HuDo-CSPG4 potential was demonstrated in preclinical mouse MM models. In canine MM patients treated in the adjuvant setting with a monthly two-year vaccination schedule (P1), HuDo-CSPG4 elicited robust immune responses and improved survival. To facilitate clinical translation, we have refined the vaccination protocol in the veterinary setting: a condensed six-vaccination schedule (P2) elicited sustained immune responses, maintaining clinical benefit, while reducing repeated anesthesia. Moreover, shifting the vaccine backbone to the pVax vector, associated with fewer allergic reactions, to improve its safety profile, seems to not compromise its therapeutic potential. We have then investigated the rational of combining anti-CSPG4 vaccination with the BRAF inhibitor, Vemurafenib. In silico analyses revealed high CSPG4 expression in BRAF-mutated MM, both in primary tumors and metastases. CSPG4 silencing enhanced sensitivity to Vemurafenib, reducing proliferation, migration, clonogenicity, and sphere viability. Kinome profiling was performed on CSPG4-expressing or CSPG4-silenced cells, treated with Vemurafenib, and comprehensive analysis is currently underway to elucidate the role of CSPG4 in drug response and uncover novel resistance-associated signaling pathways. Also, vaccine-induced antibodies potentiated Vemurafenib efficacy in vitro. Finally, preliminary data in a murine model suggest enhanced tumor growth inhibition when HuDo-CSPG4 is combined with PD-L1 blockade. Mechanistic studies are needed. Overall, these preliminary results support the translational potential of HuDo-CSPG4, providing a rationale for its combination with standard-of-care treatments to enhance therapeutic outcomes.

Inducing a metastable tumour cell state triggers viral mimicry and enhances anti-tumour immunity

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Immune checkpoint blockade (ICB) has transformed cancer therapy, yet its efficacy remains largely restricted to tumours with pre-existing T cell infiltration. Immune-cold malignancies are refractory to ICB due to low tumour immunogenicity and active immune suppression. Tumour-intrinsic calcium signalling is frequently rewired to sustain plasticity, survival and immune evasion. In particular, the calcineurin (CN)–nuclear factor of activated T cells (NFAT) axis acts as a transcriptional hub promoting tumour progression. We hypothesized that selective disruption of CN–NFAT signalling in tumour cells could enhance anti-tumour immunity and improve therapeutic outcome.

To test this, we used a CN–NFAT inhibitory peptide that preserves CN catalytic activity while selectively blocking NFAT activation. Tumour cell–intrinsic CN–NFAT disruption increased the frequency of intratumoral progenitor exhausted $CD8^+$ T cells across multiple tumour models. This effect was abrogated by CXCR3 blockade, indicating chemokine-dependent recruitment. Importantly, these $CD8^+$ T cells remained responsive to ICB, resulting in reduced tumour growth. Lipid nanoparticle-mediated delivery of inhibitory peptide messenger RNA similarly overcame resistance to ICB.

Bulk RNA sequencing revealed induction of an interferon-like transcriptional program. In vitro analyses confirmed activation of the stimulator of interferon genes pathway and upregulation of CXCL9, CXCL10 and CXCL11. Single-cell RNA sequencing demonstrated that CN–NFAT disruption alters tumour cell-state transitions, leading to the accumulation of a metastable stem-like tumour cell population. These "trapped" metastable cells resulted in stress-associated activation of interferon-inducing pathways, including retinoic acid–inducible gene I (RIG-I)

and melanoma differentiation–associated gene 5 (MDA5), consistent with a viral mimicry phenotype.
In conclusion, cell-intrinsic activation of CN–NFAT pathway enables tumour cell-state transitions. Therefore, its inactivation drives the persistence of metastable populations that activate endogenous viral mimicry programs, converting immune-cold tumours into T cell–inflamed lesions and sensitizing them to ICB.

WORKSHOP 7 – MUCOSAL IMMUNOLOGY AND THE MICROBIOTA

Immunomodulatory effects and safety of fecal microbiota transplantation in ALS: results from the FETR-ALS clinical trial

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Background: The FETR-ALS clinical trial investigated fecal microbiota transplantation (FMT) as an immunomodulatory strategy in amyotrophic lateral sclerosis (ALS), a condition increasingly linked to gut–immune dysfunction. Regulatory T cells (Tregs), associated with slower disease progression, represent a key therapeutic target.
Methods: In this multicenter, randomized, double-blind, controlled trial, 42 ALS patients were randomized 2:1 to receive FMT (n=28) or sham procedure (SP, n=14). FMT was administered twice via nasojejunal infusion (baseline and 6 months) without antibiotic preconditioning. Longitudinal biospecimens (blood, stool, saliva, biopsies) were collected. Immune profiling was performed by polychromatic flow cytometry, cytokine quantification by multiplex Luminex assays, microbiota analysis by 16S rRNA sequencing, and serum free fatty acids (FFAs) by targeted GC–MS. The primary endpoint was change in circulating Tregs at 6 months. Secondary outcomes included ALS Functional Rating Scale-Revised (ALS-FRS-R), safety, and multi-omics analyses.
Results: At 6 months, 66.7% of FMT-treated versus 30.0% of SP participants showed increased Tregs (OR 4.67; p=0.07), with a ≥20% increase in 44% versus 20%, respectively. Mean Treg change differed significantly (+2.36 percentage points; p=0.04). ALS-FRS-R decline was slower with FMT (−0.31 points/month difference), with improved quality of life and BMI stability. No significant differences were observed in neurofilaments, respiratory function, or

cytokine profiles across matrices. Serum FFA analysis showed trends toward increased hexadecenoic and tetradecanoic acids and decreased butyric acid in the FMT group. Microbiota analysis revealed modest, heterogeneous compositional changes, without consistent donor engraftment. FMT was safe and well tolerated, with no treatment-related deaths and a comparable or lower rate of severe adverse events versus controls.
Conclusions: FMT demonstrated biological activity in ALS, promoting Treg expansion and modest clinical stabilization, supporting a gut–immune mechanism. These findings highlight microbiota-targeted interventions as promising immunomodulatory strategies requiring confirmation in larger trials with integrated longitudinal immune multi-omics.

A macrophage immunoreceptor-microbiota axis controls intestinal inflammation

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) driven by dysregulated interactions between the intestinal microbiota, epithelial barrier and immune cells. Intestinal macrophages act as key sentinels of microbial perturbations, and their activation state critically shapes inflammatory outcomes in the gut. However, the contribution of macrophage immunoreceptors to host–microbiome dynamics remains poorly defined. Here, we identify the myeloid receptor CD300e as a critical regulator of gut microbial ecology and colitis susceptibility. Analysis of intestinal biopsies from UC patients revealed elevated CD300e expression within the inflamed mucosa, and independent plasma proteomic datasets identified increased circulating CD300e levels, supporting a potential role in human disease. Taking advantage of CD300e knockout (KO) mice, we demonstrate that receptor deficiency stably reshapes gut microbial composition, enriching anti-inflammatory, butyrogenic taxa while depleting of colitis-associated bacteria. Functionally, CD300e deficiency confers marked protection against DSS-induced colitis, characterized by reduced disease activity, preserved epithelial integrity, and decreased immune infiltration and pro-inflammatory cytokine production in the colonic lamina propria. Fecal microbiota transplantation from CD300e KO donors into wild-type (WT) recipients significantly ameliorates colitis, demonstrating a microbiota-driven protective effect. Consistently, microbiota depletion with broad-spectrum antibiotic cocktail attenuates this protection, underscoring the contribution of microbial ecology. Notably, host CD300e expression continues to influence disease severity even in pseudo-germ-free mice, revealing a bidirectional host-microbiota interplay. Mechanistically, CD300e-deficient macrophages enhance epithelial barrier function in a macrophage and organoid-derived epithelial monolayer co-culture system, promoting tight junction integrity. Together, our findings uncover a macrophage immunoreceptor-microbiota axis that governs intestinal ecosystem stability and positions CD300e as a potential therapeutic target in UC.

Multiple sclerosis microbiota: driving trained innate and innate-like immunity

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, primarily driven by adaptive immune responses involving autoreactive T and B cells, which are targeted by highly effective immunomodulatory therapies. However, innate immune cells also play a crucial role in driving inflammation by producing pro-inflammatory cytokines, which contribute to neuroinflammation and neurotoxicity. Recent research has introduced the concept of trained immunity, where innate immune cells become hypersensitive to repeated stimuli, potentially worsening the chronic progression of MS and reducing the effectiveness of immunomodulatory treatments. Dysbiosis, or alterations in the gut microbiota, has been observed in MS patients, and understanding how these microbial changes influence trained innate immunity and adaptive immune cell responses could provide new avenues for improving MS treatment strategies.
This study investigated the immunostimulatory effects of bacterial and fungal extracts from faeces of persons with MS (pwMS) on peripheral blood mononuclear cells, focusing on induction of trained immunity in purified monocytes and differential activation of mucosal-associated invariant T (MAIT) cells, particularly cytokine profiles.
We find that fungi from MS patients' faeces, especially *Candida albicans*, potently induce trained immunity in pwMS monocytes, characterized by increased IL-6 and TNF-α production upon secondary LPS challenge, due to activation of CDK/MAPK signalling pathways. Fungal stimuli further drive monocyte differentiation toward pro-inflammatory non-classical phenotypes. Furthermore, *Escherichia coli* and *Candida albicans* activate MAIT cells through different mechanisms: bacteria act through direct activation mediated by the TCR Vα7.2, while fungi act via indirect activation. These divergent activation routes are mirrored by their

cytokine outputs, with *E. coli* inducing IFN-γ and TNF-α production, while *C. albicans* preferentially drives GM-CSF and IL-17 secretion.
These data suggest that microbial extracts from MS patients can stimulate key immune cell subsets, highlighting the potential role of gut microbiota in modulating immune responses in MS.

Characterising a γδ T cell – gut epithelial cell axis predisposing to Crohn's Disease

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Intestinal TCRγδ$^+$ intraepithelial lymphocytes (IELs) rely on organ-specific butyrophilin-like (BTNL) molecules expressed by mature epithelial cells. In mice, Btnl1 selects Vg7$^+$ cells, while in humans, BTNL3 selects Vg4$^+$ IEL. Variant *BTNL* alleles are associated with Crohn's disease (CD) severity and post-COVID childhood MIS-C. We hypothesise that idiopathic cases of such diseases reflect underlying disruption of the BTNL-γδ$^+$ IEL axis, impairing barrier integrity. To test this, we define the impact of acute and constitutive BTNL deletion on healthy gut tissues, including γδ IELs, and assess whether such signatures are exacerbated in inflammation, for example in human CD. Immediate impacts on γδ IELs are examined after inducible deletion of mouse Btnl1, using transcriptomics and flow cytometry. Long-term impacts on the epithelium are examined by s.c.RNA-seq and histological analysis of healthy mouse guts after constitutive Btnl1 deletion. The results are compared with s.c.RNA-seq phenotypes of healthy gut from donors with or without variant alleles of BTNL3+8 and with public CD datasets.
Constitutive mouse and human BTNL deletion leads to reduced numbers of Vγ7$^+$/Vγ4$^+$ cells, and residual cells display atypical traits phenocopying immature cells in mice (CD122^{lo}, Thy1$^+$, TIGIT$^-$, Lag3$^-$, CD8αα$^-$, CD5$^+$, CD24$^+$). Increase CD5 expression on human Vγ4$^+$ cells mirrors the Vγ4$^+$profile in inflamed IBD. Acute Btnl1 loss in mice do not deplete Vγ7$^+$ cells but alters their phenotype and transcriptomes, with acquisition of signatures associated with γδ IEL dysfunction in vivo and paralleling impaired immunosurveillance by dysregulated skin gd T cells (downregulated *Tnfrsf9*, *Xcl1*, *Ptpn6*, and decreased CD69 and TIGIT). Concurrent transcriptomic changes in epithelial, stromal and other immune compartments will be also presented. These results can establish whether BTNL-γδ$^+$ IEL axis disruption alone induces early biomarkers of gut inflammation, consistent with BTNL-deficiency pre-disposing toward penetrating CD, possibly offering actionable targets to new therapies.

Pro-inflammatory intestinal Th17-cells are tissue-resident, accumulate in the epithelia in Crohn's Disease and predict unresponsiveness to Vedolizumab

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We recently identified a population of pro-inflammatory intestinal CCR5+Th17-cells (pro-inflammatory Th17, pTh17) associated with intestinal inflammation in Crohn's Disease (CD) and activated by CD-associated adherent-invasive Escherichia coli.
Here we report that intestinal pTh17-cells in CD expressed genes characteristic of tissue-resident memory T-cells (TRM). They expressed high levels of CD69 and CD103 on the cell surface and accumulated in the intra-epithelial lymphocyte (IEL) compartment in CD. Consistently, single T-cell RNA sequencing in CD unveiled that intraepithelial Th17-cells displayed a gene signature of activated pTh17-cells.
In peripheral blood pTh17-cells with gut-homing potential were hardly detectable. Conversely, conventional CCR5-Th17-cells ("cTh17") were present and expressed increased levels of a4b7-integrin in the blood of IBD patients. Consistently, intestinal cTh17-cells were reduced after treatment with vedolizumab, which blocks a4b7-integrin mediated gut-homing of lymphocytes. Importantly, unresponsiveness to vedolizumab in CD was associated with increased frequencies of pre-existing intestinal pTh17-cells with high sensitivity and specificity.
We conclude that intestinal pTh17-cells are tissue-resident cells, which could be generated from circulating cTh17 precursor cells in the gut. Moreover, our findings are consistent with a key pathogenic role of intraepithelial Th17-cells in CD and suggest that monitoring intestinal pTh17-cells may predict unresponsiveness to vedolizumab.

WORKSHOP 8 – REGULATORY CELLS

Regulatory dendritic cells program metabolic immune suppression in the tumor microenvironment through IL4i1

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In the tumor microenvironment, amino acid metabolism, particularly tryptophan metabolism, is strongly associated with immune tolerance and unfavorable outcomes. Enzymes involved in tryptophan degradation, IL4i1, have recently been identified as an element shaping the tumor microenvironment (TME). IL4i1 sustains tumor hallmarks, including immune suppression and resistance to ferroptosis. However, the mechanisms through which IL4i1 shapes tumor immunity remain incompletely understood. Through single-cell RNA sequencing analysis, we demonstrated that in the TME of a transplantable fibrosarcoma model, IL4i1 is selectively expressed in regulatory dendritic cells (mregDCs). IL4i1^{+} mregDCs are characterized by the expression of mature dendritic cell markers including CCR7 and Zbtb46. Pseudotime trajectory analysis revealed that mregDCs arise along a differentiation trajectory originating from cDC2 cells within the tumor microenvironment. Moreover, the microenvironment is enriched with metabolites derived from tryptophan conversion to Indole-3-pyruvate (I3P) and 3-indole-acetaldehyde (3-Iald) by IL4i1. Il4i1$^{-/-}$ mice inoculated with fibrosarcoma cells display reduced tumor growth associated with enhanced CD8 T cell–mediated responses. Single-cell transcriptomic analysis of tumors from Il4i1$^{-/-}$ mice revealed that mregDCs remain present in the tumor microenvironment; however, the immune landscape shifts toward a more inflammatory state. Il4i1$^{-/-}$ tumors display increased inflammatory signatures and enhanced effector T cell responses. Consistently, conditional deletion of IL4i1 in mregDCs results in decreased tumor growth and reduced levels of IL4i1-derived metabolites in the TME. Additionally, IL4i1 metabolites contribute to suppressing CD8 T cell responses and promoting resistance to ferroptosis in tumor cells. The identification of AhR-expressing target cells responding to IL4i1-derived metabolites expands our understanding of how IL4i1 in myeloid cell subsets shapes a metabolic network affecting the TME and anti-tumor immunity. Moreover, IL4i1 emerges as a negative prognostic factor in sarcoma subtypes in the TCGA-SARC dataset. Our findings identify IL4i1 as a regulator of immune suppression and highlight IL4i1-driven metabolic signaling as a potential therapeutic target.

RBPJ as the fulcrum of a novel, HIF-independent hypoxia response in regulatory T cells

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Most environments in which regulatory T cells (T_{reg}) are hyperactive, including the tumor microenvironment (TME), are hypoxic. However, the role of oxygen restriction for T_{reg} identity and function has been insufficiently characterized and remains controversial. In this work, we investigated the impact of hypoxia on T_{reg} infiltrating the TME and discovered a direct correlation between hypoxia and localized infiltration of highly suppressive T_{reg}. We leveraged time-course RNA-seq of hypoxia-exposed human primary T_{reg} and extensive epigenomic analyses to elucidate the pathways that transduce oxygen tension in Treg and identified RBPJ as a potential mediator of this response. Perturbation of RBPJ in hypoxic conditions specifically reduced the transcription of hypoxia-responsive genes and the expression of suppressive surface markers in vitro as well as in human colorectal TMEs cultivated as tumor explants. Furthermore, RBPJ inhibition markedly abrogated the ability of hypoxia-cultured primary T_{reg} to suppress the proliferation of conventional CD4+ T cells. Chromatin immunoprecipitation of RBPJ revealed it is bound in both oxygen conditions at enhancers of genes upregulated in hypoxia, prompting us to perform immunoprecipitation of RBPJ followed by mass spectrometry (MS) on primary T_{reg} exposed to hypoxia, uncovering differential interactors at RBPJ-bound loci. We identified STAT5A as a hypoxia-exclusive interactor of RBPJ. We propose that a STAT5A-RBPJ transcriptional regulation complex forms at hypoxia-responsive enhancers in T_{reg}, driving the expression of tumor-infiltrating (TI-) T_{reg}-specific transcripts. We hypothesize that converging hypoxia and STAT5 signaling at these loci could activate immune regulatory programs in damaged tissue niches displaying both ongoing immune activation and either disrupted perfusion, high competition for oxygen or both, thus providing an evolutionary rationale for emergence of the hypersuppressive TI-T_{reg} transcriptome.

CD71-dependent iron metabolism in RORγt⁺ Tregs shapes intestinal homeostasis and tumor immunity

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Background: Cancer is a systemic disease shaped by interactions between gastrointestinal tract, microbiota, and immune system. Iron is essential for tumor, microbes, and immune cells. Regulatory T cells (Tregs) acquire iron via transferrin receptor CD71 to sustain expansion. In the gut, specific bacteria induce RORγt+ Tregs via antigen and metabolite signaling, promoting iron uptake and intestinal homeostasis. Since CD71 deficiency impairs colonic Treg development, we hypothesize that iron availability sustains Treg-mediated gut homeostasis but may promote immune suppression in tumor microenvironment.
Results: we observed that CD71 is more expressed in colonic Tregs than other tissues of healthy mice. To enable a functional analysis, we identified CCR6 and Neuropilin-1 (NRP1) as surface markers distinguishing microbiota-induced RORγt and thymic-derived Helios colonic Treg subsets. Transferrin uptake was higher in pre-activated CCR6+ than in NRP1+ Tregs, indicating enhanced iron uptake by microbiota-induced colonic Tregs.
A similar pattern was observed in human mucosal Tregs, with RORγt+ Tregs showing highest CD71 levels, supporting a conserved CD71 expression profile in gut-associated Tregs.
To assess the functional relevance of CD71+ Tregs in gut homeostasis, we conditionally deleted CD71 in Tregs. Deletion caused weight loss, colonic Treg reduction, and specific loss of microbiota-induced RORγt+ subsets, highlighting the role of CD71+RORγt+Tregs in gut homeostasis.
Given migratory capacity of colon-derived immunocytes, we hypothesized that colonic Tregs could access tumors. Following orthotopic injection of EO771 breast cancer cells, RORγt+ Tregs accumulated in tumor and expressed higher CD71 levels than Helios+ subset, suggesting a potential role in modulating local immunity.
Treg-specific CD71 deletion resulted in reduced tumor growth, decreased RORγt+ Tregs in tumors and colon and increased CD8 activation, supporting the role of CD71+RORγt+ Tregs during tumor progression
Conclusions: RORγt+CD71+Tregs maintain gut homeostasis and accumulate in breast cancer modulating anti-tumor immunity, linking mucosal and distal tumor immune responses.

A previously unrecognized regulatory T Cell subset in the breast cancer tumor microenvironment

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Introduction: Breast cancer (BC) is the most common malignancy among women worldwide. CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Tregs) represent a specialized subset of T lymphocytes that maintain immune tolerance and suppress anti-tumor immune responses. Their accumulation within the tumor microenvironment (TME) has been associated with poor clinical outcomes in several malignancies, including BC. However, the biological and functional characteristics of intratumoral Tregs remained still undefined. We have previously demonstrated that, among FOXP3⁺ Tregs, those expressing the FOXP3 exon 2–containing splice variant (FOXP3E2) exhibit the strongest suppressive activity.
Materials and Methods: Multiparametric flow cytometry (FACS) was performed on freshly isolated Tregs from either peripheral blood mononuclear cells (PBMCs) and breast tumor biopsies of newly diagnosed hormone receptor–positive (HR⁺) BC patients. We evaluated the expression of markers associated with proliferation, migration, and immunosuppressive function. These findings were correlated with the capacity of Tregs to suppress conventional T-cell (Tconv) proliferation in vitro and with patients' clinicopathological parameters. Additionally, RNA-sequencing data from approximately 1,000 subjects (990 breast tumor tissues and 112 tumor-adjacent normal tissues) were analyzed using the TCGA Splicing Variant Database (TSVdb).
Results and Discussion: We found that FOXP3E2⁺ tumor-infiltrating Tregs were enriched in the TME and peripheral blood of BC patients with poor prognosis and displayed a distinctive immunophenotypic profile. Comprehensive analysis across all TCGA breast cancer subtypes validated FOXP3E2 expression as an independent prognostic factor, identifying tu-

mors characterized by a highly immunosuppressive microenvironment and defective mismatch repair pathways. Functional in vitro assays using Tregs derived from BC patients confirmed the increased immunosuppressive capacity of FOXP3E2^{+} Tregs using BC-subject-derived Tregs.
Conclusion: Our findings suggest that FOXP3E2^{+} Tregs may serve as an independent prognostic biomarker in breast cancer and represent a promising target for the development of highly selective, depletion-based immunotherapeutic strategies.

Immune checkpoint expression in γδ T cells reflects structured regulatory circuits rather than a linear exhaustion trajectory

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Immune checkpoint receptors (ICRs) are commonly interpreted as hallmarks of T-cell exhaustion, yet their expression may also reflect physiological activation and differentiation programs. While this paradigm has been extensively studied in CD8 αβ T cells, the regulatory framework of ICR expression in human γδ T cells remains poorly defined.
Here, we investigated the dynamics of PD-1, TIM-3, LAG-3, TIGIT, and CTLA-4 expression across γδ and αβ T-cell subsets following both polyclonal activation and pharmacologic Vδ2 expansion with zoledronic acid (ZA), and in tumor-infiltrating lymphocytes (TILs), analyzed by single-cell transcriptomics before and after immune checkpoint blockade (ICB) therapy.
Polyclonal stimulation induced broad, time-dependent upregulation of multiple ICRs across lineages. In contrast, ZA-driven Vδ2 expansion generated a selective hierarchy characterized by sustained TIM-3 expression, transient PD-1 induction and limited modulation of TIGIT and CTLA-4. Differentiation analyses revealed structured, subset-specific patterns: TIM-3, LAG-3, and CTLA-4 were enriched in naive γδ T cells, TIGIT preferentially marked effector memory populations, and PD-1 displayed an intermediate distribution.
Integration with a single-cell atlas of tumor-infiltrating γδ and CD8 T cells confirmed lineage-specific checkpoint programs. Whereas CD8 T cells exhibited progressive ICRs upregulation along differentiation trajectories, consistent with exhaustion-associated states, γδ T cells displayed heterogeneous, state-dependent expression profiles. In basal cell carcinoma and melanoma samples, ICB therapy increased T-cell numbers but did not uniformly reverse inhibitory signaling; instead, compensatory upregulation of alternative checkpoints, particularly TIGIT and LAG-3, was observed.
Collectively, these findings demonstrate that ICR expression in γδ T cells reflects activation-coupled regulatory circuits rather than a canonical exhaustion program. Recognition of these lineage-specific checkpoint hierarchies, with TIM-3 emerging as a central node, may inform combinatorial immunotherapeutic strategies incorporating γδ T cells.

POSTER SESSIONS

POSTER SESSION 1

ALLERGY

P01

Integration of Basophil Activation test and CAP-inhibition into the classical diagnostic approach to develop a diagnostic algorithm for the choice of venom immunotherapy

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Hymenoptera venom allergy is a significant cause of anaphylaxis. Accurate identification of the culprit Hymenoptera is crucial for effective venom immunotherapy (VIT); however, diagnosis remains challenging. The Basophil Activation Test (BAT) and CAP-inhibition test may complement conventional IgE-based and skin tests.
This study aimed to develop a diagnostic algorithm combining classical tests with BAT and CAP-inhibition to guide VIT selection.
Ten patients with a history of Hymenoptera venom reactions underwent intradermal skin test (IDR), specific IgE (sIgE) measurement, and BAT; five also underwent CAP-inhibition assays. BAT was performed on whole blood stimulated with venom extracts from Apis mellifera, Vespula, Vespa crabro, and Polistes at 1 µg/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml. CAP-inhibition was conducted on serum samples using a ≥70% inhibition cut-off for positivity.
As an in vivo test, IDR directly evaluates patients but carries a risk of adverse reactions and showed 80% positivity to both vespid and apid venoms. sIgE indicates sensitization but does not correlate with clinical allergy or distinguish true polysensitization from cross-reactivity; 70% of patients tested positive to both venom groups. BAT, an ex vivo functional assay, differentiated apid from vespid allergy in 70% of cases, predominantly showing exclusive responses, although it was less effective in discriminating individual vespid species. CAP-inhibition helped distinguishing cross-reactivity from true polysensitization within vespids. Homologous inhibition with Polistes and Vespula was assessable in 100% of tested patients, whereas Vespa crabro was evaluable in only 20% without heterologous inhibition. Cross-reactivity was observed between Polistes and Vespula (40%) and between these species and Vespa crabro (60%). No single test is sufficient for VIT selection. An integrated diagnostic approach combining clinical history, conventional tests, BAT and CAP-inhibition can improve diagnostic accuracy. BAT helps distinguish apid from vespid allergy when routine tests are inconclusive, while CAP-inhibition helps identify the primary sensitizing vespid species.

P02

Modulation of airway inflammation by recombinant Der p 38 mutant immunotherapy in a murine model of allergic asthma

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Asthma is a chronic allergic airway disease frequently triggered by exposure to house dust mites (HDM). Der p 38, a major allergen derived from Dermatophagoides pteronyssinus, has been implicated in the development of mixed granulocytic asthma. However, the therapeutic potential of allergen immunotherapy (AIT) targeting Der p 38 remains poorly understood. In this study, we generated a recombinant mutant form of Der p 38 (D141A) using molecular biology techniques. Notably, D141A retained its ability to bind toll-like receptor 4 (TLR4) but lacked the capacity to induce basophil activation. In a murine model, D141A elicited significantly attenuated asthmatic responses compared to wild-type (WT) Der p 38. Furthermore, co-administration of WT with D141A effectively reduced key features of mixed granulocytic asthma. Importantly, AIT using WT, D141A, or their combination significantly suppressed Der p 38-induced airway inflammation. These findings suggest that the Der p 38 mutant protein, D141A, represents a promising therapeutic candidate for AIT, with potential applicability across diverse asthma endotypes and severities associated with HDM exposure.

P03

SFPQ/S100A8 complex induces filaggrin deficiency in atopic dermatitis

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Although atopic dermatitis (AD) is associated with a decrease of filaggrin levels, the underlying mechanisms are not clearly understood. Here, we aimed to investigate the pathogenic roles of the splicing factor proline- and glutamine-rich (SFPQ)/S100 calcium-binding protein A8 (S100A8) complex, a critical regulator inducing filaggrin deficiency in AD. The relocation of SFPQ from the nucleus to the cytoplasm, elevated S100A8 levels, and SFPQ/S100A8 complex formation were proportional to filaggrin deficiency and AD severity in the keratinocytes of AD patients and AD-like mice. This study demonstrates that the formation of SFPQ/S100A8 complex is a critical process in AD progression, which provides key insights into the pathogenic mechanisms of AD. The elucidation of the SFPQ/S100A8 complex axis in AD pathogenesis can be leveraged to develop therapeutic drugs for AD treatment.

AUTOIMMUNITY

P04

Immunomodulatory effects of dental pulp stem cell-derived secretome on immune checkpoint expression and cytokine production by lymphocytes and monocytes from rheumatoid arthritis patients

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Background/objective: Dental pulp stem cells (DPSCs) are mesenchymal stem cells exerting immunomodulatory activities by cell-cell contact and soluble factors. We aimed to investigate the effects of the secretome of DPSCs on lymphocytes and monocytes from rheumatoid arthritis (RA) patients unresponsive to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).
Methods: Supernatants were collected from DPSCs after 4 days of culture, and were used as DPSC secretome. Peripheral blood lymphocytes and monocytes were isolated from 8 RA patients unresponsive to csDMARDs. Lymphocytes activated using anti-CD3/CD28 beads, and monocytes activated using LPS were cultured in RPMI + 10% FBS with and without 60% DPSC supernatants. After 48 hours, the expression of immune checkpoints and adhesion molecules on lymphocytes and monocytes was analyzed by flow-cytometry. Moreover, 34 cytokines and 19 soluble immune checkpoints were quantified in supernatants.
Results: Activated T lymphocytes showed increased PD-1, 4-1BB, and LAG3 expression after treatment with DPSC supernatants (+1.7 fold, +1.7 fold, +7.8% respectively), while CTLA-4 and TIGIT expression did not change. Activated monocytes showed 2.3 fold increased CD14 expression after treatment, while TIM-3, CD11b, CD62L, and HLA-DR expression were not affected. Lower levels of soluble CD40, IDO, TIM-3, PD-L1 (fold changes: -1.4, -1.4, -1.4, -2.0 respectively), and higher levels of IL-2, IL-12p70, IL-17A, IL-23, IL-31 (fold changes: +1.8, +9.7, +2.6, +1.9, +2.1 respectively) were detected in supernatants of activated T lymphocytes after treatment. Lower levels of soluble OX40, 4-1BB, IDO, TIM-3, PD-L1, IL-12p70, IL-13, GM-CSF, TNFα (fold changes: -5.0, -2.5, -2.5, -2.5, -5.0, -2.0, -2.0, -2.0, -5.0 respectively), and 3.6 fold higher levels IL-10 were detected in supernatants of activated monocytes after treatment. All the reported changes were statistically significant ($p<0.05$).
Conclusions: DPSC-derived secretome modulated immune checkpoint expression and cytokine secretion in immune cells from RA patients, indicating a complex immunoregulatory profile that warrants further functional investigation.

P05

Predictive biomarkers discovery to anticipate Ocrelizumab effectiveness in relapsing-remitting Multiple Sclerosis patients

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Multiple Sclerosis (MS) is a chronic autoimmune neurodegenerative disorder of the Central Nervous System which affects more than 2.8 million people worldwide. MS remains incurable and patients can rely solely on disease-modifying therapies (DMTs) to contain inflammation and progression. Therefore, early and accurate selection of the most effective therapeutic strategy is therefore critical to maximize long-term clinical benefit while minimizing treatment-related risks.
Ocrelizumab (OCR) was recently introduced in the DMTs' catalogue as the first humanized anti-CD20

monoclonal antibody approved for the treatment of the most common MS form, namely Relapsing-Remitting (RR), and demonstrated its significant efficacy in slowing disease's progression. However, accumulating evidence reveals that a non-negligible subset of RR-MS patients exhibits suboptimal response to OCR, marked by premature B-cell repopulation within the initial dosing interval.
The underlying biological mechanism of such heterogeneity remains unclear and standardized dosing regimens may fail to fully exploit OCR's therapeutic potential across diverse patient profiles.
To address this unmet need, we sought to identify biomarkers capable of predicting OCR responsiveness prior to treatment initiation. This aim was pursued by applying an integrated analytical workflow combining statistical, machine-learning and stability-oriented features selection strategy to a set of previously underexplored clinical and individual parameters. This approach uncovered multiple candidate markers which, when considered collectively, may anticipate treatment outcomes with improved precision.
These findings provide a foundation for advancing personalized therapeutic strategies in RR-MS, offering a pathway to optimize OCR-based interventions and contributing to a deeper understanding of the complex and still incompletely defined mechanisms underlying MS pathogenesis and treatment variability.

P06
Lectin pathway functional deficit and early antiphospholipid titers as predictors of preterm delivery in Obstetric Antiphospholipid Syndrome

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Antiphospholipid syndrome (APS) is an autoimmune disease defined by thrombotic events and/or pregnancy morbidity in the presence of persistent antiphospholipid autoantibodies (aPL). Obstetric APS (OAPS) includes severe complications such as preterm delivery (PTD). Although complement activation is increasingly recognized as a key contributor to APS, obstetric manifestations cannot be fully explained by classical pathway activation alone.
In order to identify early predictive biomarkers, it was evaluated complement activation in aPL-positive and aPL-negative pregnancies, focusing on women who developed PTD. Patients were longitudinally monitored throughout pregnancy and postpartum. Preliminary findings showed higher titers of anti-β2GPI, and anti-phosphatidylserine/prothrombin antibodies in aPL-positive women who subsequently experienced PTD.
Functional analyses demonstrated preserved classical (CP) and alternative pathway activity across all time points. In contrast, lectin pathway (LP) activity was significantly reduced in aPL-positive women. Among aPL-positive PTD patients, LP activity was frequently below 10%, suggesting selective consumption of this pathway. LP activity positively correlated with gestational age at delivery, supporting its clinical relevance in pregnancy outcome.
Since mannose-binding lectin (MBL) is the key recognition molecule initiating LP cascade and MBL deficiency is present in approximately 10% of the Caucasian population, circulating MBL levels were measured at all gestational timepoints. MBL levels were lower at all timepoints in aPL-pos than aPL-neg PTD patients. 40% of our aPL-positive patients showed MBL deficiency. To determine whether the entire lectin pathway was consumed, MASP-2 levels were also assessed; MASP-2 displayed a physiological trend, suggesting that the observed LP impairment cannot be attributed to global pathway consumption and warrants further investigation.
Overall, these findings indicate that, not only CP but also LP could have an important role in pathogenesis of OAPS. The monitoring of LP factor levels, together with aPL titers, could represent relevant markers for identifying women at risk of PTD.

P07
Platelets' phenotype and mitochondrial function in Systemic Sclerosis and in the very early disease

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Introduction: Systemic Sclerosis (SSc) is an autoimmune disease characterized by vasculopathy, inflammation and fibrosis. Patients with very early diagnosis of SSc (VEDOSS) show vasculopathy and autoimmunity without skin and organ involvement typical of SSc. It is known that platelets are involved in thrombo-inflammation, and they can contribute to damage in several autoimmune diseases. Recent studies suggest that platelet activation may be relevant for SSc pathogenesis. This preliminary study investigated platelets and mitochondrial phenotype in patients with VEDOSS and SSc.

Methods: This single-center study enrolled consecutive adult patients diagnosed with SSc according to the ACR/EULAR 2013 and VEDOSS criteria, not in antiplatelet therapy. Age- and sex- matched healthy controls (HC) were selected. Platelet activation was assessed by staining blood with CD62P-PE and PAC1-FITC, with/without agonists (ADP, convulxin, TRAP-6). Mitochondrial function was studied with MitoTracker Green FM for mitochondrial mass and MitoTracker CMXRos for membrane potential on washed platelets.
Results: The study included 15 SSc patients [median age 58,9 (11,5)], 10 VEDOSS [age 51,5 (11,1)] and 12 HC [age 52,3 (10,6)], all females. Activated platelets (PAC-1+ CD62P+) were significantly higher in SSc than in HC after ADP [49,6 (12,9) vs 37,1 (10,2), $p<0.05$]. Conversely, platelets from VEDOSS patients showed higher reactivity to convulxin than SSc [89,9 (4,3)% vs 80,8 (8,7)%, $p<0.05$]. Platelets from VEDOSS exhibited significant mitochondrial dysfunction irrespective of stimulation compared with SSc and HC ($p<0.01$).
Conclusion: Overall, these findings indicate distinct platelet activation and mitochondrial patterns in VEDOSS and SSc, suggesting a different role depending on stage of disease. Of note, VEDOSS platelets seam to reflect the early endothelial dysfunction, while SSc platelets exhibit a chronic activation than could be linked to secretion and interaction with immune cells. Further studies on larger cohorts are needed to clarify the pathogenetic role of platelets and their potential as therapeutic targets in SSc.

P08
Chronically activated, yet not exhausted, AQP4-specific CD4⁺ T cells are associated with astrocytic injury in NMOSD

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Background: Neuromyelitis optica spectrum disorder (NMOSD) is a rare autoimmune inflammatory disease of the central nervous system characterized by pathogenic IgG1 autoantibodies against aquaporin-4 (AQP4) expressed on astrocytes. Antibody binding triggers complement-mediated astrocytic injury, resulting in severe neurological deficits such as blindness and paralysis. Although NMOSD is primarily antibody-mediated, increasing evidence suggests that CD4⁺ T helper cells contribute to disease initiation and progression.
Materials and Methods: AQP4-seropositive NMOSD patients and seronegative controls with other neuroimmunological diseases (ONID) were enrolled. Serostatus was determined using fixed and live cell-based assays. Peripheral blood mononuclear cells were stimulated for 18 hours with peptides derived from AQP4, MOG, CMV, EBV, and SARS-CoV-2 to assess antigen-specific responses. Antigen-specific CD4⁺ T cells (CD69+CD154+) were identified using the activation-induced marker (AIM) assay. Serum glial fibrillary acidic protein (GFAP) was measured as a biomarker of astrocytic injury.
Results: Total AIM CD4+ T cells were not significantly increased in NMOSD compared with ONID controls. However, NMOSD patients showed a higher proportion of CD3low AQP4-, CMV-, and SARS-CoV-2-specific CD4+ T cells, consistent with TCR/CD3 internalization following antigen recognition. AQP4-specific CD4+ T cells displayed reduced PD-1 and CD127 expression and a predominant central memory phenotype, suggesting chronic activation with preserved functional competence. Serum GFAP levels were modestly elevated in AQP4-seropositive patients and correlated with activated (HLA-DR+) and central memory AQP4-specific CD4+ T cells, whereas no correlation was observed in ONID controls.
Conclusion: Chronically activated, yet not exhausted, AQP4-specific CD4+ T cells are associated with astrocytic injury in NMOSD. A trend toward increased CMV-specific responses suggests CMV exposure may act as a potential trigger in susceptible individuals. Integrating cellular immune profiling with serum biomarkers may improve mechanistic understanding and patient stratification.

P09
Vaccination-induced trained immunity in myeloid cells confers protection in experimental autoimmune encephalomyelitis by promoting a suppressive Ly6Chigh phenotype

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Trained immunity is the process by which innate immune cells acquire a form of immunological memory following exposure to specific stimuli. While it can both enhance and suppress immune responses, its role in neuroinflammation and autoimmunity remains poorly understood.
In this study, we investigated the impact of trained immunity on autoimmune disease using an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. Trained immunity was induced via vaccination with *Mycobacterium tuberculosis* in complete Freund's adjuvant (CFA), followed by immunization against myelin oligodendrocyte glycoprotein. We assessed clinical disease progression and characterized innate immune cell populations in lymphoid tissues by flow cytometry.

CFA-vaccinated EAE mice developed a significantly milder clinical disease, confirmed by spinal cord histopathology. Flow cytometry revealed an expansion of a splenic CD45$^+$ CD11b$^+$ Ly6G$^-$ Ly6C^{int}/Ly6C^{high} myeloid population. Notably, only CFA Ly-6C^{high} cells completely suppressed autoreactive CD4$^+$ T cell proliferation and induced Foxp3$^+$ Treg differentiation, while Ly6C^{int} cells lacked this capacity — demonstrating a specific and essential role of *Mycobacterium tuberculosis* in shaping the suppressive phenotype.
CFA Ly6C^{high} cells promoted a shift from a Th1/Th17 toward a Th2/Treg immune response, providing a mechanistic basis for the observed clinical protection. Preliminary bulk RNA-seq analysis further suggests that these cells acquire a transcriptional program consistent with trained immunity, with emerging evidence of metabolic reprogramming and epigenetic regulation as key drivers of their immunosuppressive function.

P10
Target therapy in Multiple Sclerosis: longitudinal analysis of lymphocytes populations

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Multiple sclerosis is a chronic autoimmune disease of the central nervous system characterized by inflammation, demyelination, and neurodegeneration. Although traditionally considered mainly driven by autoreactive T lymphocytes, increasing evidence highlights the central role of B cells. These cells contribute to pathogenesis not only through antibody production but also via interactions with T lymphocytes. The high efficacy of anti-CD20 therapies further supports their key role; however, variability in B-cell repopulation and clinical outcomes suggests that fixed dosing regimens may not be optimal for all patients, supporting the need for more personalized treatment strategies.The aim of this study was to evaluate the long-term impact of anti-CD20 therapy on immune profiles and clinical outcomes in MS, focusing on the relationship between B-cell repopulation kinetics and the risk of progression independent of relapse activity (PIRA), as well as changes in T-cell populations.
Peripheral blood samples from patients with MS (n=61) were analyzed before and after treatment with anti-CD20 therapies (ocrelizumab or ofatumumab) using flow cytometry. Based on B-cell frequency and absolute counts measured within six months after infusion, patients were classified as responders or non-responders.
After therapy, the B-cell compartment showed enrichment in transitional naive B cells and plasmablasts, with a marked reduction in memory B cells. Non-responders had a higher risk of PIRA and a higher baseline percentage of B lymphocytes, with a discriminative cut-off of 12.99%.
Anti-CD20 therapy was also associated with reduced effector memory T cells, follicular helper T cells and γδ central memory T cells, together with an increase in naive and central memory CD4 T cells, suggesting a shift toward a less inflammatory immune profile.
These findings suggest that anti-CD20 dosing could potentially be personalized according to baseline CD19 B-cell counts. Immunological monitoring may help identify biomarkers predictive of treatment response and support personalized therapeutic strategies in MS.

P11
HLA class I immunopeptidomics of the rheumatoid arthritis synovial compartment reveals candidate CD8$^+$ T-cell targets

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease resulting from a response driven by self-reactive CD4$^+$ T cells that recognize autoantigenic peptides presented by antigen-presenting cells (APCs). Recent evidence suggests that CD8$^+$ T cells are also important players in this process. This study aims to define the immunopeptidome of human leukocyte antigen (HLA) class I molecules (HLA-ABC) from RA synovial tissue (ST)- APCs and synovial fluid (SF)-pulsed monocyte-derived dendritic cells (DCs), and to identify peptides recognized by CD8$^+$ T cells from RA patients. Methods: HLA-ABC/peptide complexes were obtained from DCs generated from healthy subjects (HS), which were pulsed with a pool of RA SF (SF-DCs) or left unpulsed (UP-DCs), or obtained directly from RA ST. Isolated peptides were sequenced by mass spectrometry. A theoretical affinity algorithm was used to assign peptides to given HLA class I allotypes. The autoantigenicity of selected peptides was estimated by their ability to specifically activate CD8$^+$ T cells from RA patients compared with HS, as measured by the induction of

intracellular IFN-γ expression and surface exposure of CD107a by flow cytometry.
Results: After discarding contaminant sequences, between 107 to 663 peptides were obtained from DC samples, while over 3,500 class I peptides were identified from each ST sample. A stepwise prioritization strategy was applied to reduce the number of peptides up to ten for further functional assays. The frequencies of $CD8^+$ T cells co-stained for IFN-γ and CD107a were significantly higher in RA patients than in HS in response to peptides derived from the proteins MIF, ETS1, USF1, VIM, and AHR.
Discussion: In the present work, we employed an immunopeptidomic strategy to identify a series of novel autoantigenic $CD8^+$ T-cell epitopes for RA, derived from synovial, mostly immune-related proteins, which may be useful for future clinical applications.
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P12
Celiac disease within a suspected cohort: evaluation of prevalence, sex distribution, and the clinical necessity of emerging immunological markers

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Background: The diagnosis of celiac disease (CD) relies on clinical, histological, and immunological findings. Serological markers are critical for identifying the disease, but IgA-class tests fail in selective IgA deficiency (SIgAD), and early self-initiation of a gluten-free diet (GFD) leads to false negatives. This study evaluates anti-tissue transglutaminase (tTG-IgA) positivity, characterizes high antibody titers, and discusses integrating IgG-class anti-endomysium antibodies (EMA-IgG) and gluten-specific interferon-gamma release assays (IGRAs) into diagnostic algorithms.
Materials and Methods: A retrospective, single-center audit was conducted on 1,697 unique patients on a gluten-containing diet. Immunological profiling included quantitative tTG-IgA (multiples of the upper limit of normal, xULN), total serum IgA, and EMA-IgA screening. For total IgA deficiency, an automated reflex loop triggered tTG-IgG and EMA-IgG testing. A literature review positioned gluten-specific IGRAs—measuring $CD4^+$ T-cell immune memory—against diagnostic impasses caused by premature gluten restriction.
Results: Among 1,697 patients, 135 tested positive for tTG-IgA, yielding a targeted prevalence of 7.96% (95% CI: 6.8–9.3%). Of these, 67.4% (91/135) exhibited high titers >= 10xULN, fulfilling criteria for a biopsy-free pathway when secured by EMA-IgA positivity. Total IgA screening revealed a SIgAD rate of 1.22% (16/1,308; 95% CI: 0.75–1.98%). Implementing tTG-IgG and EMA-IgG in these deficient patients successfully rescued the CD diagnosis.
Discussion & Conclusion: A targeted seropositivity rate of ~8% underscores the efficiency of risk-based screening. While titers >= 10xULN support biopsy-free strategies, 28.9% of requests lacked total IgA. To maximize safety, embedding EMA-IgG within an automated reflex testing panel is imperative when low total IgA is flagged. Finally, for patients presenting after early gluten eviction, gluten-specific IGRA represents the future of exploration, establishing the diagnosis while eliminating the burden of prolonged oral challenges and invasive endoscopies.
Keywords: Celiac disease, tTG-IgA, EMA-IgG, Selective IgA deficiency, Gluten-specific IGRA, xULN.

P13
Differential Impact of single macronutrient-enriched diets on self-immunetolerance and systemic neuroinflammation

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Emerging evidence highlights a fundamental connection between host metabolism and immune tolerance, driven by nutrient-dependent modulation of intracellular energy sensors like the mTOR pathway. This nutrient availability critically dictates $CD4^+$ regulatory T (Treg) cell expansion and suppressive capacity. Consequently, caloric excess induces "metabolic pressure," an environmental determinant that compromises Treg homeostasis and promotes the loss of self-tolerance. To elucidate these dynamics, we evaluated the *in vivo* effects of single macronutrient-enriched regimens (high-fat, high-carbohydrate, or high-protein diets) on the murine immune system under homeostatic conditions and during experimental autoimmune encephalomyelitis (EAE). During a 6-week pre-EAE diet conditioning phase, chronic consumption of lipids or carbohydrates—but not proteins—induced a systemic inflammatory response in healthy mice, characterized by elevated circulating proinflammatory cytokines and reduced peripheral Treg presence compared to standard controls. Within the EAE model, high-fat and high-carbohydrate diets exacerbated disease pathogenesis and significantly augmented central nervous system immune infiltration. Peripherally, these metabolic pressures exacerbated the MOG-specific immune response, evidenced by increased

MOG-reactive T cell representation and enhanced proinflammatory cytokine production. Extracellular flux analysis (Seahorse assay) revealed that lipid and carbohydrate overload altered cellular metabolic fitness, driving an enhanced glycolytic rate not observed with protein overload. Crucially, lipid and carbohydrate diet-driven conditioning reduced the percentage of peripheral Treg cells and impaired their suppressive function, whereas protein excess preserved both. These findings demonstrate that metabolic pressure from dietary fats and carbohydrates uniquely undermines Treg-mediated suppression, driving systemic inflammation and autoimmune susceptibility.

BIG DATA, OMICS AND ARTIFICIAL INTELLIGENCE

P14
SPHERE Spatial Profiling of Heterogeneity in the microEnvironment

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The composition and spatial organization of the Tumor Immune Microenvironment (TIME) play a key role in shaping anti-tumor immune responses and influencing clinical outcomes, including response to immune checkpoint blockade (ICB). Interactions between immune, stromal, and tumor cells within the TIME affect tumor progression and therapeutic efficacy. However, understanding how specific cellular niches are organized and interact within the tissue microenvironment remains a major challenge in cancer immunology. Here we present SPHERE (*Spatial Profiling of Heterogeneity in the microEnvironment*), a computational framework designed to characterize phenotype-centered spatial niches and their surrounding microenvironment. Starting from a phenotype of interest, inferred from spatial deconvolution methods, or from a gene signature, SPHERE defines customizable radius-based neighbourhoods around a cell type of interest to characterize composition and spatial organization of the surrounding cellular components. This strategy enables systematic investigation of how immune-stroma- or tumor-associated features are embedded within the tissue architecture.
We applied SPHERE in a pilot study involving eleven treatment-naïve non-small cell lung cancer patients who received immune checkpoint blockade therapy at recurrence. Spatial transcriptomics profiles were generated using the 10x Genomics Visium platform, enabling identification of niches and characterization of their local cellular environments. SPHERE analysis reveals differences in microenvironmental niches between patients responding and non-responding to immunotherapy. Neighbourhoods surrounding selected cell types show distinct configurations and interaction patterns. SPHERE uncovers spatial immune/stromal patterns associated with therapeutic outcomes and may distinguish microenvironments associated with poor or good responders.
Overall, these findings highlight the importance of spatially resolved analyses to dissect the tumor immune microenvironment. We envisage, SPHERE may provide a flexible framework applicable to spatial transcriptomics platforms including 10x Visium and Visium HD, and adaptable to diverse tissue types and pathological contexts, enabling systematic exploration of spatial niches and their surrounding cellular ecosystems.

P15
Characterization of B-cell subsets and BCR repertoire in NSCLC patients with Tertiary Lymphoid Structures in the primary tumor

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Tertiary Lymphoid Structures (TLS) detection in solid tumors has brought back to stage B cell and their possible contribution in mounting local immune response against tumors. Although the presence of TLS has been associated with favorable prognosis and improved response to immunotherapy in several cancers, the functional role of B cells within the tumor microenvironment remains controversial. In non-small cell lung cancer (NSCLC), we have previously shown that intratumoral TLS correlate with improved clinical outcome and that TLS-related gene signatures predict response to immune checkpoint therapy (ICT), suggesting a key role for the TLS-B cell axis in antitumor immunity. To better characterize tumor-associated B cells, we per-

formed whole-transcriptome single-cell RNA sequencing (scRNA-seq) combined with single-cell B-cell receptor sequencing (scBCR-seq) on sorted B cells from paired tumor tissue, adjacent non-tumoral lung tissue, and peripheral blood of treatment-naïve NSCLC patients. Unsupervised clustering identified six distinct B-cell subpopulations, spanning differentiation states from naïve and pre-GC B cells to GC B cells and plasma cells. Tumor-infiltrating B cells displayed transcriptional profiles distinct from lung tissue-resident memory B cells and showed features of activated populations, including GC-like switched memory B cells and antigen-experienced IgM$^+$ B cells expressing TCL1A, FCRL5, ITGB1, and ITGAX. These markers have been associated with autoreactive and age-associated B cells, although they do not define a discrete subset in this context. Tumoral B cells presented a restricted BCR repertoire, characterized by switched BCRs and increased CDR3 length, strongly suggesting a germinal center origin. Further analyses in larger patient cohorts are ongoing to define the relationship between TLS-associated B-cell subsets, BCR features, and response to ICT.

CELL SIGNALING

P16
Differential activation of Toll-like Receptor 8 signaling pathways in human neutrophils and monocytes

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Toll-like Receptor 8 (TLR8) is an endosomal pattern-recognition receptor that senses GU- and AU-rich single-stranded RNA, enabling detection of viral and bacterial pathogens as well as endogenous RNA from damaged cells. Stimulation with the TLR8 agonist R848 induces markedly distinct transcriptional responses in neutrophils and autologous monocytes. In particular, TLR8-activated neutrophils fail to induce interferon-stimulated genes. This study aimed to define the molecular basis of this impaired type I interferon response.
Gene expression analyses showed that, unlike monocytes, R848-stimulated neutrophils fail to induce IFNβ. Interferon Regulatory Factor 5 (IRF5), a key transcription factor driving IFNβ expression downstream of TLR8, was strongly expressed at both mRNA and protein levels in freshly isolated CD14$^+$ monocytes, whereas its expression was markedly reduced in neutrophils. Consistent with this difference, IRF5 activation was observed exclusively in R848-stimulated monocytes. To define IRF5 target genes on a genome-wide scale, we performed IRF5 Chromatin Immunoprecipitation sequencing analyses. Following R848 stimulation, monocytes showed strong and widespread recruitment of IRF5, including at the IFNβ promoter and multiple inflammation-related loci, whereas neutrophils displayed no appreciable IRF5 binding. Despite ongoing debate, studies using kinase inhibitors have shown that TLR8-induced IFNβ expression in monocytes is mediated by the TAK1/IKKβ pathway, not by TBK1. Notably, TAK1 and IKKβ were both expressed and activated in neutrophils upon R848 stimulation, indicating that the absence of type I interferon production in neutrophils is mainly attributable to low IRF5 expression rather than defects in upstream signaling pathways.
These findings highlight cell-type specific regulation of TLR8 signaling and provide insight into mechanisms shaping innate immune responses.

CYTOKINES AND OTHER SOLUBLE MEDIATORS

P17
Investigating whether human mature polymorphonuclear myeloid-derived suppressor cells produce interleukin-10

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Neutrophils are the most abundant circulating leukocyte population and represent the first line of defense against noxious insults. They can exert a wide array of functions like degranulation, production of oxygen radicals, and phagocytosis. Additionally, once activated, they may also perform de novo cytokine synthesis. In this context, conflicting results have been published regarding neutrophils' ability to release interleukin-10. Nevertheless, previous data from our group demonstrated that the IL-10 locus displays a closed chromatin conformation in neutrophils from healthy donors (HDs). In addition, both SAA-1 and lipopolysaccharide failed to induce neutrophil-mediated IL-10 release, as quantified by enzyme-linked immunosorbent assay (ELISA). On the other hand, we have recently observed that the IL-10 transcript is included in the gene signature of mature polymorphonuclear myeloid-derived suppressor cells (mPMN-MDSC) isolated from cancer patients and granulocyte-colony stimulating factor (G-CSF)-treated donors (GDs) generated in our laboratory. To test whether this increase in IL-10 transcript expression could be coupled to a potential protein production, we performed a cytokine

secretion assay (CSA) on mPMN-MDSCs from GDs. Our preliminary data obtained with this approach showed that both low-density and normal-density neutrophils from GDs harbored a small subset of cells releasing IL-10 in response to R848 stimulation. As expected, using the same experimental setting, we also confirmed that neutrophils from HDs did not secrete IL-10. Although interesting, our results remain preliminary, as additional experiments are required to confirm the reliability of our CSA data using a quantitative assay. Moreover, we need to better characterize the epigenetic regulation of IL-10 transcript expression, as it must be associated with chromatin opening processes, possibly triggered by G-CSF. In conclusion, our data suggest a novel, yet unexplored mechanism that may contribute to neutrophil-mediated immunomodulation in humans and warrant further investigation to provide valuable insights into mPMN-MDSC features.

P18

IL-1R8 directs hematopoietic fate decisions by counterbalancing IL-1-driven emergency myelopoiesis

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Emergency hematopoiesis represents a rapid systemic adaptation to conditions requiring an expanded hematopoietic pool and accelerated leukocyte production. Within this context, Interleukin-1 (IL-1) serves as a pivotal mediator; while essential for homeostasis, its chronic induction during inflammatory stress drives persistent myeloid skewing and a concomitant exhaustion of the hematopoietic stem cell and progenitor (HSPC) reservoir. IL-1R8 is an atypical IL-1 receptor (ILR) family member that curtails ILR- and Toll-like receptor (TLR)-mediated pathways. In this study, we demonstrated that IL-1R8 is highly expressed in both mouse and human HSPCs. IL-1R8-deficiency impaired self-renewal, compromised long-term engraftment and promoted age-associated exhaustion of HSPCs, leading to sustained myeloid bias under inflammation. Notably, these defects were rescued in *Il1r8*$^{-/-}$/*Il1r1*$^{-/-}$ double knock-out mice, confirming that the impairments observed in the hematopoietic niche were driven by dysregulated IL-1 signaling. Transcriptomic profiling further revealed that IL-1R8 deficiency upregulated pathways associated with myelopoiesis, immune and cell cycle activation, and hematologic malignancies in Lineage-, Sca-1^{+} and c-Kit^{+} cells (LSKs) and HSCs upon stimulation with IL-1β. Consistent with these findings, reduced IL-1R8 expression in human CD34^{+} HSCs correlated with unfavorable outcomes in myelodysplastic syndrome with low blasts (MDS-LB), positioning IL-1R8 as a gatekeeper against preleukemic transformation. Collectively, IL-1R8 emerges as a regulator of the balance between stemness and myeloid differentiation, with profound implications for bone marrow failure and neoplastic progression.

DENDRITIC CELLS

P19

Immunostimulant-Induced Maturation of Monocyte-Derived Dendritic Cells in Respiratory Infection Prophylaxis

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A well-known family of drugs, represented by the bacterial lysates, has been shown to have a significant effect on the number of infectious episodes, the day of fever and the use of antibiotics. The capacity of bacterial lysate to induce the maturation of human monocyte-derived dendritic cells, has been indicated as the first and fundamental effect focused on the activation of an efficient immune response. Other drugs, used in clinics in the prophylaxis of Recurrent Respiratory Tract Infections (RRTIs), such as synthetic molecules, for example Pidotimod, have been described to induce the maturation of dendritic cells as well. However, experimental results seem to be poorly convincing. We evaluated the capacity of Pidotimod inducing the maturation of dendritic cells, using a well-established bacterial lysate, Lantigen B, as positive controls. This maturation was detected in triplicate using monocyte-derived dendritic dell maturation assay, measured as increase of the expression of the CD83 and CD86 co-stimulatory molecules and the secretion of IL1b, IL12 and IL23, three cytokines suitable to positively influence the activity of the immune system cells. At least in our experimental

conditions, 10, 1 and 0.1 micrograms of Pidotimod had no effects on dendritic cells, while Lantigen B was able to induce a complete maturation of dendritic cells in vitro. Of note, the same different doses of Pidotimod, added to Lantigen B, did not modify or better improve the capacity of the bacterial lysate of inducing dendritic cell maturation. These findings question the purported immunostimulatory effects of Pidotimod and its claimed role in decreasing the incidence and severity of airway infections by acting directly on the immune system cells.

P20
Broncho-Vaxom® modulates dendritic cell metabolism and immune activity via Aryl Hydrocarbon Receptor

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The OM-85 bacterial lysate (commercially known as Broncho-Vaxom®) is widely used for the prevention of recurrent respiratory tract infections and has been traditionally recognized for its ability to broadly stimulate the immune system. Our study moves beyond this conventional view, investigating the molecular mechanisms by which OM-85 influences the metabolism and the regulatory function of dendritic cells (DCs), focusing on the Tryptophan (Trp) catabolism and the Aryl Hydrocarbon Receptor (AhR) pathways.
We found that OM-85 was able to directly activate AhR signaling in a dose dependent manner in DCs, confirmed by a positive luciferase reporter assay. Consistent with this, within OM-85 itself we quantified several immunomodulatory metabolites, including those from the kynurenine, indole, and Phe-Tyr pathways, as potential AhR ligands.
Treatment of bone marrow-derived DCs (BMDCs) and sorted conventional (cDCs) or plasmacytoid (pDCs) subsets with OM-85 induced Indolamine 2,3-dioxygenase 1 (IDO1) expression and subsequent Kynurenine (KYN) release. Crucially, this metabolic response was strictly AhR-dependent and limited to a specific cDC subset, but not in pDCs. Moreover, in the cDC1/OT1 (OVA-specific $CD8^+$) model, OM-85 enhanced T cell proliferation and IFNg production, an effect significantly increased in the absence of AhR. Conversely, in the cDC2/OTII (OVA-specific $CD4^+$) model, IFNg production, associated with AhR-deficiency, was suppressed by OM-85 treatment. Translational relevance of these results is supported by preliminary human data, which reveals a clear dose-response induction of IDO1 following OM-85 stimulation in human peripheral blood mononuclear cells (PBMCs).
In conclusion, OM-85 modulates Trp metabolism and immune function in an AhR-dependent manner in specific DC subsets. The translational validation provided by the dose-dependent IDO1 induction in human PBMCs supports that OM-85's immunomodulatory properties rely on complex metabolic signaling to orchestrate distinct innate and adaptive immune responses, highlighting its newly investigated immunoregulatory role.

P21
Extracellular vesicle subpopulations from human amniotic fluid differentially induce IDO1-dependent tolerogenicprogramming in dendritic cells

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Extracellular vesicles (EVs) present in human amniotic fluid are increasingly recognized as important mediators of immune regulation and represent promising candidates for cell-free immunotherapies. However, the mechanisms through which amniotic fluid–derived EVs modulate dendritic cell (DC) responses remain poorly defined. Here, we investigated the immunomodulatory activity of EVs isolated from human amniotic fluid on conventional dendritic cells (cDCs) and examined whether distinct EV subpopulations differentially regulate DC function.
EVs were isolated from human amniotic fluid and fractionated by differential ultracentrifugation into two major vesicle populations, referred to as p10 and p100 fractions. Both EV subsets were characterized and evaluated for their capacity to influence dendritic cell activation and inflammatory responses.
Exposure of cDCs to amniotic fluid–derived EVs resulted in the induction of indoleamine 2,3-dioxygenase 1 (IDO1), a key regulator of tolerogenic dendritic cell programs. Notably, the two EV fractions displayed distinct immunomodulatory activities. The p10 fraction induced a markedly stronger upregulation of IDO1 expression in cDCs compared with the p100 fraction. In parallel, p10-conditioned cDCs exhibited a pronounced reduction in IL-6 production following LPS stimulation, indicating attenuation of pro-inflammatory signaling pathways. Consistent with these changes, dendritic cells exposed to amniotic fluid–derived EVs displayed a reduced capacity to support antigen-driven proliferation of OT-II $CD4^+$ T cells, supporting the acquisition of a regulatory DC phenotype.
Collectively, these findings demonstrate that EVs present in human amniotic fluid actively shape

dendritic cell functional programming. Moreover, the differential activity of EV subpopulations highlights the importance of EV heterogeneity in immune regulation and identifies specific EV fractions as potential tools for the development of EV-based immunomodulatory strategies.

P22
Generation of a neoantigen-rich NSCLC model to study dendritic cell-based vaccination

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Despite advances in therapeutic strategies, non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality worldwide. High tumor mutational burden (TMB) has emerged as a favorable prognostic factor, as it increases neoantigen generation and promotes $CD8^+$ T cell-mediated anti-tumor immune responses.
To investigate tumor-immune interactions in NSCLC, we developed a preclinical model based on the KP (KrasG12D/WT; Tp53fl/fl) strain. A stable and slowly growing tumor cell line was isolated from advanced lung lesions and subsequently engineered to knock out Mlh1, a key component of the DNA mismatch repair pathway, thereby increasing TMB and neoantigen generation.
In vivo validation demonstrated that orthotopic implantation of Mlh1-deficient cells resulted in significantly slower tumor growth compared to the wild-type counterpart. This phenotype was associated with increased infiltration of $CD8^+$ T cells, consistent with enhanced immune recognition driven by the elevated neoantigen load.
Given the central role of type 1 conventional dendritic cells (cDC1) in cross-presentation and $CD8^+$ T-cell priming, we next explored a cDC1-based vaccination strategy. cDC1 were incubated with live Mlh1-KO tumor cells and administered as a prophylactic vaccine prior to tumor challenge. Unexpectedly, vaccinated mice developed tumors that grew faster than those in control animals. Future work will focus on defining how the nature of tumor-derived material influences cDC1 antigen acquisition and cross-presentation in vivo by comparing UV-irradiated, live, and CTL-killed tumor cells.
Overall, we established a stable and immunogenic NSCLC model characterized by increased neoepitopes expression and enhanced T cell infiltration. This system provides a valuable platform to study cDC1-mediated antigen presentation, tumor-immune crosstalk, and dendritic cell-based vaccination strategies in lung cancer.

EPIGENETICS

P23
EZH2 inhibition shapes histotype-specific immune recruitment in 3D malignant pleural mesothelioma models and supports rational combination strategies

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Malignant pleural mesothelioma (MPM) is an aggressive, treatment-refractory malignancy characterized by low immunogenicity and a highly immunosuppressive tumor microenvironment (TME), limiting immune checkpoint blockade efficacy. Strategies targeting myeloid cells or modulating epigenetic programs may enhance immunotherapy. Tazemetostat, a selective EZH2 inhibitor, shows anti-proliferative activity in MPM, but its impact on the TME remains poorly defined. We investigated how EZH2 inhibition reshapes immune pathways across MPM histotypes and identified putative macrophage-related targets for combination therapies.
Using optimized 3D multicellular spheroids (MCS) representing epithelioid, biphasic, and sarcomatoid histotypes, we found that Tazemetostat significantly reduced MCS volume across all models, confirming EZH2 as a conserved therapeutic target. Notably, EZH2 inhibition induced inflammatory gene expression in a histotype-specific manner: sarcomatoid MCS strongly upregulated T-cell chemoattractants; biphasic models induced both monocyte and T-cell attractants; whereas epithelioid models showed a weaker response, primarily inducing IL-8.
Functional migration assays confirmed these shifts: epithelioid and biphasic MCS preferentially recruited monocytes, while T-cell infiltration was enhanced predominantly in non-epithelioid models. In co-culture, monocytes counteracted tazemetostat efficacy across all histotypes, whereas anti-tumor effects were maintained with T-cells. Mechanistically, tazemetostat upregulated PTGS2 (COX2) in both tumor and infiltrating monocytes, identifying the PGE2 pathway as a driver of myeloid-mediated resistance.
Overall, EZH2 inhibition reshapes the MPM TME histotype-dependently but triggers compensatory myeloid immunosuppression. Targeting the PGE2-EP2/4 axis may overcome this resistance, supporting rational, histotype-tailored immuno-epigenetic combination strategies.

HOST-PATHOGEN INTERACTION

P24
Organoid cultures as a three-dimensional platform to study fungal infectious diseases

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A major advancement in modeling human diseases has been made through the organoid technology. Organoids are three-dimensional structures composed of tissue specific cells types and that mimic the organ of origin morphology and some functions. These mini-organ cultures can be set up starting from adult tissue-specific stem cells isolated from a biopsy. The so-obtained organoid culture is able to recapitulate patient-specific disease signature features, making it a unique research and translational tool. Given the epithelial nature of organoid cultures, they represent a strategic technique to model fungal infections. We have recently developed organoid cultures from different patient-derived biopsies and mouse organs, such as gut, liver and lung organoids. These organoid cultures have been employed as a 3D platform to test three different Candida albincans and Aspergillus fumigatus infection approaches: microinjection into the organoid lumen, organoid-derived monolayers and infection during splitting. Infection outcome has been monitored through organoid or monolayer and fungi vitality assays and key cytokine production has been quantified to assess epithelia immunological reactivity. Thus, we have compared the three different infection methods. These fungi-infected organoid cultures not only provide an infectious disease model, but also offer the opportunity to study patient-specific susceptibility to fungal infection and a better understanding of host-pathogen interactions.

P25
Microbial adhesion promotes Piezo1 activation to initiate innate immunity

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How mammals mount an effective immune response against infectious agents remains unresolved. Here, we identify microbial adhesion to myeloid cells as a critical initiating event that precedes Pattern Recognition receptor (PRR) engagement. Using a skin infection model with pathogenic bacteria and fungi injected intradermally, we demonstrate that neutrophil recruitment occurs in two sequential phases. The early phase is PRR-independent and instead driven by microbial adhesion, which engages the mechanosensitive ion channel Piezo1 to promote leukotriene (LT)B4 production. Together with interleukin (IL)-1α, LTB4 induces CXCL1 release, triggering neutrophil infiltration via the same circuit at play during sterile inflammation. In contrast, the late phase is Toll like Receptor (TLR)- and CXCL2-dependent, marking a transition to the canonical, pathogen-driven response. Our findings uncover microbial adhesion as a previously unrecognized danger signal that activates innate immunity via mechanotransduction, revealing a novel paradigm of how immune responses to infection are initiated.

P26
Optimization of peptide-based enzyme-linked immunosorbent assay for the Mpox B-cell humoral response: an investigation of the A42R core protein model

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Mpox is a zoonotic disease caused by the monkeypox virus (MPXV) which recently showed unprecedent global spread and enhanced human-to-human transmission. The assessment of the specific humoral response is hindered by the complexity of the viral particles and by the cross-reactivity among members of the Orthpoxvirus genus. MPXV A42R-core-protein shares substantial amino acid sequence identity with eukaryotic profilins and plays a key role in viral particle organization. It was recently identified as a target for antiviral compounds to inhibit MPXV replication. Limited data are known about the A42R immune response. We assessed humoral responses in acute (n. 21 patients) and convalescent (n. 20 patients) MPXV infection using a homemade ELISA with twelve A42R-based 20-mer (10-amino acid overlapped) peptides. Serum samples from 27 healthy, unexposed, unvaccinated donors served as controls. Statistical analysis was

performed using ROC analysis and the Mann-Whitney non-parametric test. ROC significant values for five out of twelve peptides for IgM and IgG response were obtained. Acute patients exhibited a significant IgM response compared to convalescent (pep1: p=0.0002, pep2: p=0.0008, pep3: p=0.002, pep5: p=0.001, pep6: p=0.002) and control groups (all peptides p<0.0001). IgG levels were lower than IgM levels. Moreover, individuals in the acute group generally exhibited higher IgG levels than those in the convalescent group (pep3: p=0.04, pep5: p=0.007, and pep6: p=0.001), as well as compared to the control group (pep1: p=0.0003; pep2, 3, 5, and 6: p<0.0001). We highlighted the peptide positions in the 3D structure of the MPXV A42R profilin-like protein (PDB ID 4QWO), identifying a potential antibody binding region in the dimer. Although verified in a small group, these data show promise for serological studies on MPXV infection and could help identify new immunogenic proteins and therapeutic targets relevant to patient care.

IMMUNODEFICIENCIES

P27
Characterization of progenies from circulating inflammatory precursors in Common Variable ImmunoDeficency (CVID) patients

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Common Variable Immunodeficiency Disorders (CVID) represent the most prevalent category of clinically significant primary immunodeficiency disorders, estimated to affect up to 1 in 25.000 individuals. CVID includes a heterogeneous spectrum of disorders characterized by the disruption of B cell differentiation and a switch of CD21, resulting in impaired immunoglobulin production, in particular IgG, IgA and sometimes IgM associated to typical adverse complications as cancer and infections. Sporadic invasive Cytomegalovirus (CMV) infections have been reported. Recently, failure to control CMV replication has been shown in a subset of CVID patients associated with splenomegaly, circulating $CD8^+$ T-cell activation and expansion, and decreased NK cell activation and numbers. CMV has been associated with both T-cell and NK-cell control, and to inflammatory precursor exit from the bone marrow. Two distinct peripheral blood precursor populations, $CD34^+DNAM\text{-}1^{bright}CXCR4^+$ and $Lin^-CD56^-CD16^+Perf^-CD94^-CXCR4^+$, are released from the bone marrow and generate in vitro highly functional NK-cells during CMV reactivation in Bone Marrow (BM) transplant patients. To characterize in CVID patients the defect in CMV control, we decided to characterize by FACS and functional assay the peripheral NK cells and the circulating inflammatory progenitors

P28
Autosomal dominant Hyper-IgE Syndrome patients retain IL10-producing preTh17-cells that are activated by opportunistic pathogens and support IgE production

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Background: Autosomal Dominant-Hyper-IgE Syndrome (AD-HIES) is caused by dominant-negative (DN) STAT3 mutations and characterized by high IgE levels, a lack of Th17-cells and recurrent infections with extracellular pathogens. We previously identified an enigmatic population of IL-10 producing CCR6+B-helper T-cells and investigated here their relationship to Th17-cells and STAT3 signalling requirements.
Methods: Human blood lymphocytes were analysed by multiparametric flow cytometry in healthy donors and AD-HIES patients. Analysis was performed by conventional gating or with bioinformatic tools. FACS-purified T-cell subsets were activated in vitro and Th17 differentiation assessed. T-cell antigen specificities were assessed by activation with heat-killed pathogens or antigenic peptide pools. B helper capacities were determined according to antibody secretion in B-T co-cultures by ELISA.
Results: CCR6+Th-cells that lacked subset-defining differentiation markers ("CCR6SP") were mostly non-polarised central memory T-cells (TCM) that produced IL-10 and expressed RORγt. They were pre-committed to a Th17 fate, since TCR stimulation in the absence of polarising cytokines induced efficient Th17 differentiation. The latter was promoted by an autocrine loop of STAT3-activating cytokines. CCR6+Th-cells were reduced in patients with DN-STAT3 mutations but contained activated CCR6SPT-cells that produced IL-10 and responded vigorously to AD-HIES-associated pathogens. These

residual CCR6+Th-cells provided B-cell help for IgG and IgE production.
Conclusions: Th17 differentiation in AD-HIES patients was not completely impaired but arrested at an intermediate stage of IL-10 producing "pre-Th17"-cells. Surprisingly, DN-STAT3 mutations did not inhibit IL-10 production by CD4+T-cells. Pre-Th17-cells were activated by AD-HIES-associated pathogens and possessed B-helper functions, suggesting that they are not protective but may promote aberrant IgE production.
Allergy 2026, in press

P29
Uncovering Natural Killer cell dysregulation in a novel RAB27A variant of Griscelli syndrome type 2

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Griscelli syndrome type 2 (GS2) is a rare autosomal recessive immunodeficiency caused by mutations in the *RAB27A* gene, which encodes a protein involved in vesicular trafficking. Rab27a defects affect anti-viral responses of Natural Killer (NK) cells, crucial effectors of innate immunity, by impairing cytolytic granule secretion.
Here, we describe a 10-year-old female carrying a novel homozygous *RAB27A* variant, p.(Gln71His), presenting with mosaic albinism, hypopigmentation, complications following Epstein-Barr virus (EBV) infection, and early-onset hemophagocytic lymphohistiocytosis. To define the impact of this mutation on NK-cell biology, peripheral blood mononuclear cells (PBMCs) from the patient were analysed in comparison with healthy donors (HDs). Flow cytometric evaluation revealed normal expression of major activating receptors (i.e., NKp30, NKp44, NKp46, NKG2D, and CD16), inhibitory KIR receptors, CD57 maturation marker, and perforin. Functional assessment showed that, after IL-2 stimulation, patient NK cells retained efficient degranulation against HLA-I-deficient K562 human erythroleukemia target cells, unexpectedly preserved compared with what is generally reported in *RAB27A* deficiency. In contrast, degranulation against the 721.221 B-lymphoblastoid cell line was reduced. Furthermore, redirected killing assays using murine mastocytoma P815 cells demonstrated impaired signalling downstream of key activating receptors, including NKp30, NKp46, NKG2D, and CD16.
Despite a normal phenotypic profile and partially preserved cytotoxic capacity, these findings indicate a defect in receptor-mediated NK-lymphocyte activation that may compromise antiviral responses. These results highlight the need for further studies to better define NK cell dysfunction in the context of *RAB27A* deficiency.

P30
Defective myeloid maturation in MIRAGE syndrome: insights from deep immunophenotyping and transcriptomic profiling

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Background and aims: Germline gain-of-function variants in SAMD9 cause MIRAGE syndrome, a multisystem disorder associated with bone marrow failure, myelodysplasia, and infectious susceptibility. We investigated deep immunophenotypic and transcriptomic features of myeloid cells in a child with MIRAGE syndrome.
Methods: Peripheral blood neutrophils and PBMCs were isolated via Ficoll density-gradient centrifugation and analyzed by multiparametric flow cytometry and bulk RNA sequencing (RNAseq).
Results: A 15-month-old boy presented with severe respiratory viral infections, pancytopenia, oral candidiasis, chronic diarrhea, growth failure, and neurodevelopmental delay. Whole-genome sequencing identified a novel de novo c.1938A>C (p.Glu646Asp) SAMD9 variant, while bone marrow evaluation showed myelodysplasia with chromosome 7 monosomy.
Peripheral blood immunophenotyping showed a marked reduction of B, NK and dendritic cells. Deep myeloid profiling showed a noticeable decrease in mature $CD10^{-}$ neutrophils alongside an expansion of immature CD10- neutrophils (87*106 cells/L vs 3-22 in healthy donors) consistent with circulating band cells and metamyelocytes. Monocyte subsets were also reduced, with absence of non-classical/slan+ monocytes, supporting a defect in peripheral monocyte maturation associated to neutrophil immaturity.
Differential gene expression analysis of neutrophils isolated from patient showed an enrichment of signatures related to emergency granulopoiesis, myeloid immaturity, acute inflammation and immunoregulatory pathways, with downregulation of interferon-related genes. Transcriptomic analysis

of patient PBMC confirmed a gene expression signature of neutrophil immaturity, consistent with the presence of immature low-density neutrophils within the PBMC fraction and showed a downregulation of NK- and monocyte-related pathways.
Conclusions: These findings identify defective peripheral myeloid maturation, affecting both neutrophil and monocyte compartments, as a prominent feature of MIRAGE syndrome. This defect likely contributes to infectious susceptibility and supports early consideration of curative treatment strategies, including hematopoietic stem cell transplantation.

P31
Integrated neutrophil profiling reveals impaired survival and type I Interferon signature in Dominant-Negative STAT3 Hyper-IgE Syndrome
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Background and Aims: Dominant-negative (DN) STAT3 variants cause Hyper-IgE Syndrome Type 1 (HIES1). Dysregulated STAT3 activity may drive cell-intrinsic transcriptional and functional programs across innate immune subsets, shaping phenotypic manifestations. Neutropenia, impaired neutrophil chemotaxis, and altered chemoattractant receptor expression have been variably reported in HIES1 and proposed to contribute to infectious susceptibility.
Methods: Five DN-STAT3 patients (M/F:2/3; age 7-52 years, median 15 years) and healthy donors (HDs) underwent deep myeloid immunophenotyping with functional assays by flow-cytometry and single-cell RNA sequencing (scRNAseq). Additionally, isolated neutrophils were stimulated and analyzed by RT-qPCR.
Results: Intermittent mild-to-moderate neutropenia was observed in 4/5 patients. In DN-STAT3 patients, reduced neutrophil counts showed a predominantly mature phenotype with nearly absent low-density neutrophils. Monocyte and dendritic cell subsets were similarly represented in PBMCs from HDs and DN-STAT3 patients. None of them displayed eosinophilia. Compared with HDs, neutrophils from DN-STAT3 patients displayed significantly impaired viability after G-CSF stimulation, with no differences were detected after TLR8 stimulation or at baseline. Gene expression analysis in stimulated PBMCs and neutrophils from DN-STAT3 patients showed significantly reduced SOCS3 expression after IL-10 stimulation in PBMCs and G-CSF stimulation in neutrophils. Moreover, neutrophil scRNAseq revealed enrichment of a transcriptional subset significantly expressing interferon-stimulated genes (IFIT1-3, ISG15, OAS1-3, RSAD2), consistent with a type I interferon signature.
Conclusions: Our data support a specific role of STAT3 in regulating neutrophil survival and response to cytokines and TLR ligands. Moreover, we identified an interferon-driven transcriptional reprogramming of neutrophils in HIES1, suggesting that this pathway may contribute to disease pathophysiology and become a potential actionable therapeutic target.

IMMUNOTHERAPY

P32
LNP co-Loaded with TNF-α siRNA and a synthetic corticosteroid to sepsis-induced lung inflammation
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Sepsis, a life-threatening organ dysfunction from dysregulated immune response to infection, carries high global mortality. Acute lung injury (ALI) is often the principal cause of sepsis. TNF-α, rapidly released by alveolar macrophages (AM), acts as master upstream regulator of pulmonary hyperinflammation by recruiting immune cells, amplifying cytokines (IL-6, IL-1β), and promoting vascular leakage, key ALI hallmarks. Systemic anti-inflammatories like corticosteroids have limited efficacy and off-target effects. To address this, we developed nebulized lipid nanoparticles (LNPs) co-delivering TNF-α siRNA and dexamethasone palmitate (DXP) directly to lungs, enabling precision and persistent suppression of hyperinflammation while minimizing systemic exposure.
Building on prior work showing TNF-α siRNA LNPs reduce lung inflammation without toxicity (Wang et al., 2025), we engineered next-generation LNPs via microfluidics to co-load TNF-α siRNA and DXP while maintaining optimal pulmonary delivery properties: negatively charged, monodisperse particles (90 nm) and stable for 14 days. Interestingly, siRNA

encapsulation efficiency decreased with increasing DXP content, indicating a formulation arrangement. Preliminary *in vitro* studies in LPS-activated RAW264.7 cells have revealed that our co-loaded LNPs has no cytotoxic effect. Moreover, incorporating DXP into TNF-α siRNA-loaded LNPs significantly enhanced anti-inflammatory efficacy, characterized by the suppression of TNF-α and IL-6. These findings indicate an immunomodulation strategy in which TNF-α gene silencing and DXP-mediated anti-inflammatory signaling jointly regulates the inflammatory cascade.
Based on those results we are currently evaluating the dual therapeutic effects on isolated AM from mouse bronchoalveolar lavages, before moving to a mouse LPS-induced ALI model and assessing drug distribution, immune cells targeting and therapeutic efficacy. Based on our latest results, we believe that our approach represents a significant advancement toward precision nanomedicine for sepsis-induced lung injury, with life-changing potential for critical care.
Wang et al., (2025) Pulmonary delivery of siRNA anti-TNFα-loaded lipid nanoparticles for rapid recovery in murine acute lung injury, Adv Health Mater (DOI: 10.1002/adhm.202500695)

P33
Clinically oriented nanovectors for advancing cancer immunotherapies

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Genetic modification of immune cells has opened the door to a new era of immunotherapies, representing a highly innovative and promising therapeutic strategy for fighting cancer.
In this framework, employment of nanotechnology-based vectors, non-viral nanovectors (NVs) as safe and clinically translatable alternatives with respect to viral vectors, could pave the way to expand delivery of nucleic acids to immune cells to be genetically modified. To reach this challenging milestone, we are deeply investigating several non-toxic modular NVs for gene delivery. These nanostructured delivery systems are formulated by employing an appropriate combination of FDA-approved synthetic, bio-inspired/natural polymers with the objective to achieve accurate nanostructuration and efficient payloading. Results showed efficient nucleic acid loading, sustained/controlled release over time/space, and a reliable transfection efficacy in human immune cells. In addition, these NVs showed a good penetration index into 3D complex structures, with valuable implications for their *in vivo* application. Additionally, by using nanostructuration strategies based on biomimetic coating, it was possible to confer a precise biomimetic asset to the NVs for specific cell targeting. Reaching these outcomes required a continuous assessment and then biocompatibility optimization, since this point appeared to represent a key point for the ability to affect cell functionality and NV efficacy strongly. Our evidences indicate that primary immune cells are sensitive to engineering with NVs. Therefore, providing a safe profile is a challenging frontier to obtain effective nanotechnology-based solutions for engineering immune cells, increasing efficacy, and limiting adverse effects, a key point in improving immunotherapies using NVs.
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P34
Targeting the Discoidin domain receptor 2 /type I collagen axis to enhance immunotherapy in serous ovarian carcinoma

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Introduction: Serous ovarian carcinoma (SOC) is a highly lethal gynaecological malignancy characterised by frequent relapse, chemoresistance, and limited responsiveness to immunotherapy. A key contributor to immune evasion is the dense collagen-rich stromal matrix, which forms a physical barrier that restricts tumour-reactive T-cell infiltration. Discoidin domain receptor-2 (DDR2), a tyrosine kinase activated by type I collagen (Col1), is implicated in tumour progression and reduced immunotherapy effectiveness across solid malignancies. Nonetheless, the involvement of DDR2 in modulating immunotherapeutic responses in SOC has not yet been defined.
Methods: OVCA433 and OVCAR3 cell lines were used as in vitro models of low-grade and high-grade SOC, respectively. Tumour-specific T-cell lines (TOVC) were generated by stimulating peripheral blood mononuclear cells from healthy donors with

irradiated tumoral cells in the presence of IL-2 and IL-7. After two restimulation, the effects of disrupting the DDR2/Col1 axis were assessed using the selective allosteric DDR2 inhibitor WRG-28, alone or combined with bispecific antibodies (BsAbs) targeting CD28 and mucin 1 (MUC1). T-cell activation markers were analysed by flow cytometry, cytokine production by ELISA, and tumour apoptosis by fluorescence microscopy measuring caspase-3. Confocal microscopy evaluated T-cell recruitment and cytotoxic activity through granzyme B release.
Results and Discussion: Pharmacological inhibition of the DDR2/Col1 interaction with WRG-28 significantly enhanced SOC-specific T-cell responses, as demonstrated by increased expression of activation markers and elevated production of IFN-γ and GM-CSF. Moreover, WRG-28 treatment improved the recruitment and cytotoxic function of tumour-specific TOVC cells, leading to augmented GZMB secretion and increased Casp3 activation in tumour cells. Notably, These effects were further amplified when WRG-28 was combined with CD28xMUC1 BsAbs.
Conclusion: The DDR2/Col1 axis is a key mediator of immune escape in SOC. Targeting DDR2 may help disrupt stromal barriers, enhance T-cell infiltration, and improve the efficacy of bispecific antibody-based immunotherapy.
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P35
Rafoxanide potentiates anti-PD-1 therapy by activating a VDAC1-dependent mtDNA-type I interferon axis in colorectal cancer

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Resistance to immune checkpoint inhibitors (ICIs) and in particular to anti-programmed cell death-1 (PD-1) protein remains a major obstacle in the treatment of colorectal cancer (CRC), underscoring the need for combination strategies that enhance therapeutic efficacy. In this context, approaches that stimulate antitumor immunity, particularly through the induction of immunogenic cell death (ICD), represent a promising strategy. Rafoxanide, an anthelmintic drug showed to exert potent anti-neoplastic activities in multiple cancer types, has previously been identified as a bona fide ICD inducer in CRC models. In this study, we evaluated whether rafoxanide could synergize with anti-PD-1 therapy thereby improving its efficacy. We demonstrate that combined anti-PD-1 and rafoxanide treatment significantly reduced tumor growth in a syngeneic CRC graft model compared with anti-PD-1 monotherapy. This enhanced antitumor effect was associated with increased functional activity of intratumoral $CD8^+$ T cells, characterized by elevated IFN-γ production. Proteomic analysis of tumor tissues revealed a marked upregulation of type I interferon (IFN)-related proteins following combination therapy, suggesting a central role for this pathway in mediating therapeutic synergy. Of note, blockade of the interferon-α/β receptor (IFNAR) significantly impaired the antitumor efficacy of combined rafoxanide and anti-PD-1 therapy in vivo, confirming the dependence of therapeutic benefit on type I IFN signaling. In vitro evidence on CRC cells suggested that rafoxanide triggers the accumulation of mitochondrial DNA (mtDNA) in the cytosol by oligomerizing the voltage-dependent anion channel 1 (VDAC1), an outer mitochondrial membrane channel that regulates metabolite transport and mitochondrial permeability, thereby activating type I IFN signaling.
In conclusion, our data suggest that rafoxanide may enhance anti-PD-1 efficacy by activating a VDAC1-dependent mtDNA–type I IFN axis in CRC cells. This mechanism may contribute to improved anti-tumor immune response and lower resistance to immune checkpoint blockade.

P36
Oral plant-derived ENO3PEP vaccination reprograms the tumor microenvironment and delays PDAC progression

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, characterized by an immunosuppressive tumor microenvironment (TME) and resistance to standard therapies. Alpha-enolase (ENO1) is a tumor-associated antigen overexpressed in PDAC, and its immunogenic peptides (ENO3PEP) represent a promising vaccination target. In this study, we evaluated the therapeutic potential of ENO3PEP vaccination, delivered either intramuscularly or as plant-derived oral formulations, in an orthotopic PDAC model to investigate immune cells infiltration and microbiota composition.
Methods: Syngeneic PDAC cells were orthotopically injected into the pancreas of C57/BL6 mice. Twelve days after tumor implantation, mice received dried leaves of Nicotiana benthamiana plants expressing ENO3PEP (ENO3PEP-Nb) or control leaves (LBA) (2.5 µg dried leaves/mouse, administered twice a week), or intramuscular ENO3PEP DNA vaccination (50 µg DNA/ mouse, administered twice, 10 days apart). Thirty days after tumor implantation mice were sacrificed and tumor weight, metastases, cellular and humoral immune response and gut microbiota composition were analyzed.

Results: Both DNA- and protein-based ENO3PEP vaccination strategies significantly reduced tumor weight and lung and liver metastases compared with controls, showing association with distinct TME and gut microbiota profiles. Notably, ENO3PEP-Nb, compared with LBA controls, showed: i) increased IgG2b and IgA levels, ii) enhanced IFNγ secreting T cells, iii) increased $CD8^+$ T cell infiltration, iv) macrophages with elevated MHC class II expression, v) reduced stromal collagen deposition, and vi) different gut microbiota profile, with enrichment of Lactococcus, Verrucomicrobiota and Bacteroidota taxa.
Conclusions: ENO3PEP-Nb and ENO3PEP-DNA delay PDAC progression through distinct antitumor mechanisms. Notably, the plant-based oral vaccination reshapes the TME by enhancing macrophage activity and enriching bacterial taxa associated with improved antitumor immunity. This non-invasive strategy represents a promising therapeutic alternative for the management of this aggressive malignancy.

P37
ENO3PEP vaccination potentiates chemotherapy by reprogramming the immunosuppressive microenvironment in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDA) remains one of the most lethal malignancies, owing to late diagnosis, aggressive progression, and profound resistance to conventional therapies. Standard first-line regimens, including gemcitabine plus nab-paclitaxel (GemNab), confer only modest survival benefit, highlighting the urgent need for innovative combinatorial strategies. Given the highly immunosuppressive tumor microenvironment (TME) of PDA, immunotherapeutic approaches capable of restoring effective anti-tumor immunity are particularly attractive. Alpha-enolase (ENO1), a glycolytic enzyme overexpressed in PDA cells, represents a validated PDA-associated antigen. Here, we evaluated whether an ENO1-based DNA vaccine (ENO3PEP) could enhance the therapeutic efficacy of GemNab chemotherapy.
Immunodominant ENO1-derived peptides were selected based on proliferative and cytokine responses in peripheral blood mononuclear cells from healthy donors and PDA patients. Selected peptides were cloned into the pVax vector to generate the ENO3PEP vaccine. Therapeutic efficacy was assessed in KPC mice, which spontaneously develop PDA. Mice were vaccinated starting at 8 weeks of age with four biweekly boosts. GemNab was administered at weeks 9 and 11, alone or in combination with ENO3PEP. Mice were euthanized at 18 weeks.
ENO3PEP vaccination significantly reduced primary tumor burden and lung and liver metastases, synergizing with GemNab to further enhance disease control. Vaccinated mice developed robust humoral immunity, with increased anti-ENO1 IgG and IgG2c titers, indicative of Th1 polarization. Enhanced ENO1-specific T-cell responses were confirmed by increased IFNγ secretion upon antigen stimulation. TME remodeling was evident, with reduced collagen deposition and increased infiltration of activated cytotoxic immune subsets in tumors and draining lymph nodes, as assessed by histology, immunohistochemistry, imaging mass cytometry and multiparametric flow cytometry.
Collectively, ENO3PEP potentiates the efficacy of chemotherapy by eliciting coordinated humoral and cellular anti-tumor immunity and by reprogramming the desmoplastic and immunosuppressive TME. These findings provide a strong preclinical rationale for integrating ENO1-targeted vaccination into standard chemotherapy regimens for PDA.

P38
Evaluation of the immunomodulatory role of novel A2A adenosine receptor antagonists and anti-PD1 in murine model of breast carcinoma

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Adenosine is a purine nucleoside involved in several physiological and pathological processes through interaction with G protein-coupled membrane receptors A1, A2A, A2B, and A3. In tumors, adenosine exerts a strong immunosuppressive effect by recruiting regulatory T cells and suppressing effector T-cell activity; moreover, it promotes tumor growth primarily through the A2A receptor (A2A-R).
This study aimed to evaluate novel selective A2A-R antagonists as a potential antitumor immunotherapy in a murine BALB/c model of breast carcinoma. Two newly synthesized compounds, previously shown to be non-toxic in healthy animals, were tested alongside ZM241385, a well-characterized A2A-R antagonist widely described in the literature and used in neurodegenerative research.
Two experimental sessions were conducted, each including 30 mice inoculated with 4T1 tumor cells to induce breast cancer. A2A-R antagonists were administered either alone or in combination with the monoclonal antibody anti-PD-1 (αPD-1), at 10 mg/kg every other day for 15 days. Following sacrifice, histological analyses were performed on lung and tumor tissues. Additionally, cells isolated from

spleen, peri-tumoral lymph nodes, and remaining tumor tissue were analyzed by flow cytometry to characterize immune populations.
Results confirmed successful establishment of the experimental model and showed that all treatments were well tolerated and non-toxic. However, no significant reduction in tumor growth was observed compared with controls. Flow cytometry revealed that αPD-1, both alone and combined with A2A-R antagonists, enhanced immune activation within the lymph node lymphocytic compartment. This was evidenced by increased expression of immune checkpoint activation markers, expansion of effector and memory T-cell populations, and elevated proliferation markers.
Despite these immunological changes, no effective antitumor response was observed within the tumor microenvironment, which displayed insufficient functional T-cell activation and increased neutrophil infiltration. Overall, these treatments demonstrated immunomodulatory potential and may enhance combination immunotherapy effects, but under the tested conditions they were insufficient to reduce tumor mass.

P39
Dissecting a dual tumor-suppressive role of microRNA203a in melanoma

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Cutaneous melanoma is the most aggressive form of skin cancer, characterized by high metastatic potential and poor prognosis in advanced stages. Despite recent advances produced by immune checkpoint inhibitors and targeted therapies, therapeutic benefit remains limited to a small fraction of patients, highlighting the urgent need for innovative strategies to restore effective antitumor immunity.
Aberrant microRNA (miRNA) expression is a hallmark of cancer, and downregulation of miR203a has been closely linked to melanoma progression and poor prognosis. Based on these observations, this project investigates miR203a as a novel dual-action anticancer strategy capable of simultaneously suppressing tumor growth and enhancing immune responses.
Indeed, restoration of miR203a expression through the administration of a synthetic mimic of mature miR203a markedly impaired melanoma cell proliferation and survival. This effect is likely mediated through post-transcriptional mechanisms, as a scrambled miRNA sequence failed to reproduce these effects.
Beyond this canonical role, our group recently reported that miR203a can act as an unconventional Toll-like receptor (TLR) 7/8 ligand, promoting a potent pDC/monocyte/NK cell crosstalk. Thus, the treatment with miR203a can induce the TLR7/8-dependent activation of NK cells, key effectors in antitumor immunity, whose cytotoxic activity is frequently compromised during melanoma immunoediting.
Importantly, intravenous delivery of miR203a markedly reduced melanoma growth in a lung metastatic model, and the treatment was associated with increased abundance and activation of NK cells in the spleen and lungs of treated mice.
Together, these findings highlight miR203a as a dual antitumor agent, directly inhibiting melanoma growth while enhancing NK cell-mediated immunity. In this view, miR203a administration may represent a valuable support, or even a de-escalation strategy, for present melanoma treatments.

P40
Circulating dendritic cell subsets as potential biomarkers of response to immune checkpoint inhibitors

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Tumor immunotherapy encompasses a range of strategies designed to harness the host immune system to eradicate established cancers, including immune checkpoint inhibitors (ICIs). ICIs have demonstrated remarkable potential to improve prognosis; however, clinical benefit is limited to subsets of patients that remain difficult to identify in advance. In this context, comprehensive immune profiling is emerging as a promising approach to better characterize patients' immune status, which may influence both therapeutic response and the development of immune-related toxicities.
As part of a broader research program aimed at identifying immune profiles predictive of response to ICIs and associated toxicities, we analyzed circulating dendritic cell (DC) subsets in peripheral blood samples from 67 cancer patients collected before treatment initiation (T0) and after the first ICI cycle (T1). Multiparametric flow cytometry was used to quantify plasmacytoid DCs (pDCs) and conventional DC subsets.
At baseline, pDC levels were significantly reduced in cancer patients compared with healthy controls and were further decreased in patients who experienced disease progression or death within six months. No significant association was observed between baseline pDC levels and the development of immune-related adverse events. In addition, a reduction in conventional DC1 (cDC1) frequency at T1 was observed in patients who achieved an objective response or maintained stable disease.
Although preliminary, these findings identify pDCs as a sensitive immune population associated with clinical outcome and prognosis in patients receiving ICIs. Upon validation in larger cohorts, this

parameter may contribute to the definition of integrated immune profiles to improve patient stratification and optimize the clinical management of immunotherapy.

P41

Rewiring granulopoiesis to promote antitumoral neutrophils in colorectal cancer

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide. Although immunotherapies, particularly immune checkpoint inhibitors (ICIs), have significantly improved outcomes in several cancer types, but almost 95% of CRC cases remain unresponsive to these treatments. This lack of efficacy is largely attributed to poor T cell infiltration and low tumor antigenicity. Based on this, our hypothesis is to harnessing and stimulate the innate immune system, particularly neutrophils, to mount an effective anti-tumor response. Neutrophils are known to exhibit functional plasticity, adopting either a pro-tumoral or anti-tumoral role depending on the characteristics of the tumor microenvironment (TME). Published results and preliminary data obtained in our lab indicate that neutrophil progenitors can be trained in order to promote their differentiation toward an antitumoral phenotype. Treating mice with beta-glucan, an immunomodulatory polysaccharide modulates neutrophil activity within the TME enhancing tumor killing.
We have obtained similar results using mice with genetic deletion of ACKR2, an atypical chemokine receptor that regulate the differentiation and release of antitumoral neutrophil from the bone marrow in different tumor models.
Our aim is to increase the antitumor efficacy of neutrophils by combining beta-glucan treatment or ACKR2 inhibition with immunotherapeutic agents already known to selectively unleash the antitumoral potential of neutrophils. In particular we will focus our attention on a chimeric antibody developed by our collaborators at Helsinki University that will specifically activate the antibody dependent cytotoxicity of neutrophils against colon cancer. For this challenge, we will use in vivo and in vitro models to evaluate the effectiveness of these combination strategies, ultimately aiming to identify a non-invasive and effective therapeutic option for CRC patients.

P42

Targeting leukemia-associated macrophages within the B-Cell Acute Lymphoblastic Leukemia bone marrow niche using Chimeric Antigen Receptor-redirected Cytokine Induced Killer Cells

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B-Cell Acute Lymphoblastic Leukemia (B-ALL) is the most common childhood cancer. Despite high survival rates, outcomes remain poor in relapsed or treatment-resistant patients. Recent evidence shows that the bone marrow (BM) niche supports leukemic cell survival, proliferation and chemoresistance, with macrophages emerging as key drivers of microenvironment remodeling in solid and hematological tumors. This study investigates the interaction between B-ALL cells and macrophages and explores a new strategy to selectively target pro-leukemic M2-macrophages by engineering Cytokine Induced Killer (CIK) cells with an anti-FRβ Chimeric Antigen Receptor (CAR).
We demonstrated that, while patient-derived B-ALL blasts undergo spontaneous apoptosis when cultured alone, M2-macrophages maintain leukemic cell viability at 92.2%±3.9, whereas this protection is absent with M1-macrophages. Moreover, M1-macrophages significantly impaired B-ALL cell lines proliferation, consistent with an anti-leukemic role, while M2-macrophages failed to restrain leukemic cell growth. Importantly, M2-macrophages also protected leukemic cells from chemotherapy, restoring cell viability to levels comparable to those of untreated cells, whereas M1-macrophages failed to confer any protection. Further analysis revealed that co-culturing B-ALL cells with monocytes induces the upregulation of the M2 markers FRβ, CD163 and CD206. Together with our preliminary evidence of widespread FRβ expression in B-ALL BM biopsies and its limited expression in healthy tissues, FRβ emerged as a promising targetable antigen. Thus, we engineered CIK cells with an anti-FRβ CAR to target FRβ-expressing M2-macrophages. FRβ-CARCIK cells efficiently lysed FRβ+ THP-1 cells (lysis: 45.3%±21.9) after 4 hours at an effector-to-target (E:T) ratio of 1:1. Their killing efficiency was even more pronounced in long-term assays (98.5% lysis after 7 days, E:T 1:10). FRβ-CARCIK cells efficiently lysed also primary M2-macrophages (IncuCyte live-cell imaging, E:T 1:1).
Our findings underscore the importance of M2-macrophages in promoting B-ALL survival and chemoresistance. Their targeting with FRβ-CARCIK cells may be a promising strategy to be combined with current therapies in B-ALL.

P43

Phenotypic and functional characterization of NK cell subsets in the bone marrow of neuroblastoma patients: implications for NK cell-mediated immunotherapy

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Introduction: Neuroblastoma (NB) is the most common extracranial solid tumor in children, with about 50% of patients classified as high-risk (HR), of whom 60–70% relapse or develop therapy resistance despite intensive multimodal treatment. Mortality in relapsed/refractory HR patients can reach 90%, often due to uncontrolled bone marrow (BM) disease. NK cells play a central role in innate anti-tumor immunity and antibody-dependent cellular cytotoxicity, particularly during the standard immunotherapy with anti-GD2 mAbs. However, NK populations in BM, the most common site of relapse, are still poorly characterized.
Objectives: This study aimed to characterize BM-NK cell populations from NB patients, and to evaluate their potential responsiveness to tetrameric engineered immunotherapeutics.
Methods: BM and paired peripheral blood (PB) samples were collected from 46 NB patients at diagnosis and during follow-up. Samples were analyzed by multiparametric flow cytometry combined with high-dimensional clustering to define NK-cell subsets. Correlations indexes with clinical parameters were calculated. As proof of concept we compared anti-CD20 engineered tetrameric molecules with anti-CD20 IgG1 mAbs for their ability to activate BM-NK cells from NB patients.
Results: We identified a distinct CD56^{int} NK subset (CD69^{high}, CXCR6^{+}, NKp46^{high}, CD16$^{low/neg}$, KIR^{low}, TIGIT^{high}, NKG2A^{high}, unconventional chemochine receptor profile) in BM, rarely detectable in PB. Its frequency varied across patients (5–54%) and negatively correlated with BM infiltration. Maximum frequency of CD56int NKs was observed post-aHSCT, during the immunotherapeutic window. Importantly, CD56int NKs responded poorly to IgG mAbs, according to their low/neg CD16 expression but were effectively expanded ($p<0.05$) by anti-CD20 engineered molecules, which also triggered superior NK cell degranulation ($p<0.001$).
Conclusion: We identified a peculiar BM-NK cell population in NB patients that is highly responsive to engineered tetrameric molecules. Our findings show that these agents effectively harness this subset offering a promising immunotherapeutic strategy in patients with relapse/refractory HR-NB.

P44

Halting autoimmune disease progression by the use of regulatory T-Cell derived extracellular vescicles

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the immune-mediated destruction of pancreatic β-cells. While regulatory T (Treg) cell-based adoptive therapies hold promise for restoring immune tolerance, these "living drugs" face limitations, including *in vivo* phenotypic instability. Here, we investigated whether extracellular vesicles derived from expanded Treg cells (expTreg-EVs) can recapitulate the immunomodulatory properties of their parental cells, offering a more stable, cell-free alternative.
Treg cells were isolated from the peripheral blood of healthy donors (HD), and expanded *in vitro* for 14 days upon which expTreg-EVs were isolated via size-exclusion chromatography. Notably, *in vivo* administration of expTreg-EVs from healthy donors significantly delayed hyperglycemia onset and improved progression-free survival in non-obese diabetic (NOD) mice. To identify expTreg-EV mechanism of action, *in vitro* assays was performed and revealed that the expTreg-EVs significantly downregulated the metabolic and proliferation marker CD71 and effectively suppressed the proliferation of both CD4^{+} and CD8^{+} T cells. By a proteomics approach, expTreg-EVs were shown to contain the EV marker CD81 and the mediator ANXA2, known to foster and anti-inflammatory environment; furthermore, an RT-qPCR microRNA profiling demonstrated that those EVs were also enriched in miR-21-5p, which is recognized to fine-tune the TCR-dependent activation of T cells.
Our findings identify allogeneic expTreg-EVs as a highly promising, cell-free immunoregulatory platform capable of halting autoimmune diabetes progression, potentially exploiting both miRNA transfer and iron-deprivation mechanisms to suppress autoreactive T cells.

MAST CELLS AND GRANULOCYTES

P45
Effect of anti-IL-5 monoclonal antibody therapy on eosinophil and neutrophil extracellular traps in patients with chronic rhinosinusitis with nasal polyps

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Chronic rhinosinusitis with nasal polyps (CRSwNP) is a predominantly chronic inflammatory disease of the nose/paranasal sinuses driven by type 2 inflammation. However, increasing evidence support the coexistence of eosinophilic and neutrophilic activation within the same inflammatory microenvironment. Extracellular traps formation by eosinophils (EETs) and neutrophils (NETs) represents a potent effector mechanism capable of amplifying tissue inflammation, by releasing DNA scaffolds, granular proteases, and damage-associated molecular patterns. To date, the biological impact of IL-5-targeted therapy (mepolizumab) on EETosis and NETosis in type 2 inflammation is poorly evaluated. Therefore, the aim of our study was to investigate the effect of mepolizumab on EETs formation (EETosis) and NETs formation (NETosis) in CRSwNP patients, and their potential role as biomarkers of treatment response.
In this longitudinal study, 22 patients (9 female and 13 male) with severe CRSwNP (mean age 60.04 ± 10.98 years) were treated with mepolizumab at 100 mg every 4 weeks. At baseline (T0) and after 6 (T1) and 12 (T2) months of treatment, spontaneous EETosis and NETosis from peripheral blood granulocytes was evaluated *in-vitro* by indirect immunofluorescence staining, using anti-Major Basic Protein (MBP) and anti-Myeloperoxidase (MPO), respectively. DAPI was used to visualise the nuclei. EETs and NETs were expressed as percentage of the total area of cells in the microscope field. At T1 and T2, mepolizumab was able to induce a significant reduction of absolute eosinophil count in the peripheral blood ($p<0.0001$ and $p<0.01$, respectively) as well as EETosis ($p<0.01$ and $p<0.01$, respectively). In parallel, NETosis significantly reduced at T2 ($p<0.05$), despite unchanged absolute neutrophil counts, supporting an indirect effect of eosinophil-targeted therapy on neutrophil effector function. Our findings strongly suggest that by inhibiting IL-5 axis in CRSwNP it is possible to reduce EETosis and NETosis beyond simple eosinophil depletion, providing new insight into granulocyte interplay in type 2 inflammation.

P46
Analysis of transcriptional profile of circulating neutrophils from young and elderly donors

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The innate and adaptive immunity are influenced by genetic and demographic factor such as sex and age. Females show stronger immune response against infection but higher susceptibility to autoimmune diseases, while males are more susceptible to severe infections. In innate immunity, neutrophils constitute the first line of defense against pathogens and display sex-based functional differences. To investigate these differences, a transcriptomic analysis of freshly isolated neutrophils from healthy young and elderly donors was performed.
39 young and elderly healthy subjects were enrolled from Borgo Roma and Borgo Trento hospital, Verona. Neutrophils were isolated using EasySep and processed. Libraries were prepared using the Smart-seq2 protocol and aligned to GRCh38 using STAR. Gene counts were obtained with HTSeq-count and differential expression analysis was performed with DESeq2 Gene-ontology (GO) and Gene Set Enrichment Analysis (GSEA) were performed using the ClusterProfiler package, applying Benjamini–Hochberg correction.
The donors were analyzed in an age-disaggregated manner and a PCA of young and elderly donors showed that sex affect the transcriptional profile. Differential expression analysis identified interferon-dependent genes that were significantly up-regulated in females of both age groups. GO and GSEA, using reactome interferon signaling, showed an enrichment of biological process associated with the interferon response in both young and elderly females. Motif enrichment of androgen receptor in the promoters of these interferon-dependent genes suggests that testosterone may modulate their expression. Moreover, sex difference is present when performed a GSVA on maturity genes and a positive correlation with the GSVA of hallmark of interferon alpha response is observed.
Sex differences in immunity are increasingly recognized. Statistical analysis of transcriptome can help to identify sex-based difference in the neutrophils, and the next goal of this study will expand the transcriptomic analysis to monocytes.

METABOLISM

P47

Effects of intermittent hypoxic training on the health and mitochondrial metabolism of non-professional athletes

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Introduction: Intermittent hypoxic training (IHT) is increasingly adopted by athletes to mimic altitude adaptation and enhance performance. Hypoxia is a key regulator of mitochondrial function and innate immune responses. Through hormetic mechanisms, controlled hypoxia may induce adaptive bioenergetic remodeling, whereas excessive exposure can activate inflammatory pathways. Monocytes, central mediators of innate immunity, undergo bioenergetic and phenotypic reprogramming under hypoxic stress, potentially shifting their inflammatory profile. Despite the widespread use of hypoxic masks, data on their immunometabolic safety remain limited. This study aimed to evaluate the immunometabolic effects of a mask-based IHT protocol in non-professional athletes.
Methods: Ten non-professional athletes underwent three 50-minute spinning sessions (once weekly for three consecutive weeks) following an IHT protocol using a training mask with progressive airflow resistance. Assessments were performed at baseline (T0), immediately after the first session (T1), after the third session (T2), and one month post-intervention (T3). Vital parameters were recorded at each time point. Venous blood samples were analyzed for 52 hematochemical parameters. Monocyte subsets (classical, intermediate, non-classical) were quantified by flow cytometry. Mitochondrial morphology and function were assessed, including respiratory activity and ROS production. Circulating cell-free mtDNA and pro-inflammatory cytokines (IL-6, IL-8, TNF-α) were measured in plasma.
Results: Blood pressure remained stable throughout the study. Heart and respiratory rates increased at T1 and T2, returning toward baseline at T3. A transient increase in ROS production and circulating mtDNA was observed after exercise sessions. Monocyte subset distribution and cytokine levels did not show significant persistent changes. All hematochemical parameters, including oxygen-related indices, remained within physiological ranges.
Conclusions: In this pilot study, mask-based IHT induced transient redox and mitochondrial stress signals without evidence of sustained inflammatory activation or systemic immune dysregulation. Although limited by sample size, these findings suggest short-term immunological safety in healthy subjects and support further investigation of hypoxia-driven immunometabolic adaptations.

P48

Immunomodulatory impact of systemic Vitamin D deficiency: breakdown of immune tolerance in a Saharan female cohort

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Background and Aims: Vitamin D is a crucial immunomodulatory hormone maintaining peripheral tolerance. Via the Vitamin D Receptor (VDR), it suppresses dendritic cell maturation, curtails pro-inflammatory Th1/Th17 pathways, and promotes functional FOXP3+ regulatory T cell (Tregs) differentiation. In the Saharan region of Ouargla, an ecological paradox exists where intense sunlight does not match clinical status. This study evaluated systemic vitamin D deficiency in a local female cohort and its implications for immune tolerance breakdown.
Methods: A cross-sectional study was conducted on a female cohort in Ouargla. Serum 25-hydroxyvitamin D [25(OH)D] was quantified using a fully automated Chemiluminescence Immunoassay (CLIA) platform, selected for its superior sensitivity, wide dynamic range, and high reproducibility over conventional assays. Thresholds followed international consensus: sufficiency (≥30 ng/mL), insufficiency (20-29 ng/mL), and deficiency (<20 ng/mL).
Results: High-precision CLIA data revealed a critically high prevalence of systemic hypovitaminosis D. An overwhelming majority displayed serum 25(OH) D levels severely below the 30 ng/mL sufficiency threshold, with many showing profound deficiency (<12 ng/mL), confirming the Saharan paradox. Immunologically, this depletion represents a loss of the physiological brake on autoreactive T-cells, implying impaired Treg suppression and compromised epithelial barriers.
Conclusions: Systemic hypovitaminosis D is an established reality in Ouargla, robustly validated by CLIA performance. This deficit is a potential driver for peripheral tolerance breakdown, suggesting heightened auto-immune susceptibility in arid regions. Multidisciplinary investigations targeting local immunogenetics (VDR, CYP27B1 polymorphisms) and cultural habits are mandatory to decipher this vulnerability.

MONOCYTES AND MACROPHAGES

P50

Spatial and molecular profiling of multinucleated giant macrophages in pancreatic ductal adenocarcinoma

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Macrophages constitute a dominant and heterogeneous immune population within the microenvironment of pancreatic ductal adenocarcinoma (PDAC), but how specific macrophage states contribute to tumor behavior remains poorly understood. In an institutional series of 145 PDAC specimens, we identified a distinct subset of multinucleated giant cells (MGCs) of macrophage origin, an entity well described in chronic inflammation but rarely characterized in cancer. $CD68^+$ MGCs were found in about 28% of cases, enriched in squamous, non-glandular regions, and more frequent after neoadjuvant chemotherapy. Spatial and high-dimensional profiling, including NanoString GeoMx® Digital Spatial Profiling, Hyperion Imaging, and AI-guided histopathology, defined the morphological, phenotypic, and transcriptional features of these cells. PDAC-associated MGCs lacked canonical polarization markers (HLA-DR, CD163) and instead displayed a unique transcriptional program involving POLR2K, TUBA8, COX5B, and VDAC1, genes linked to oxidative stress, DNA repair, and MYC signaling. Gene set enrichment and spatial analyses indicated that MGC-rich regions coincide with hypoxic and extracellular matrix–remodeling niches. Experimental hypoxia promoted MGC formation *in vitro*.

Morphometric assessment revealed abnormal nuclear architecture and increased $53BP1^+/Ki67^+$ nuclei in MGCs, suggesting proliferative activity despite DNA damage. A macrophage MGC gene signature was enriched in the squamous subtype of PDAC and correlated with shorter overall survival in TCGA datasets ($p = 0.018$).

Collectively, these data identify multinucleated macrophages as a previously unrecognized immune cell state driven by microenvironmental stress and hypoxia. Their distinctive transcriptional and spatial profiles associate with aggressive tumor phenotypes, highlighting potential diagnostic and prognostic relevance in pancreatic cancer.

P51

Sodium butyrate abolishes atherogenic-LDL-induced trained immunity in THP-1 macrophages: a transcriptomic and pharmacological dissection

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Background: Trained immunity protects against infection but, in chronic sterile inflammation such as atherosclerosis, promotes a maladaptive intolerant response in monocyte-lineage cells. The chromatin-remodeling and non-coding RNA (ncRNA) arms of this response remain incompletely characterized, and it is unclear which natural histone-modifying compounds dismantle low-density lipoprotein (LDL)-induced training rather than only suppress cytokine output.

Methods: A panel of 13 cytoplasmic hybrids (cybrids) derived from human monocytic THP-1 cells, each carrying an atherosclerosis-associated mitochondrial DNA (mtDNA) variant, plus parental THP-1, was profiled using LPS-LPS and LDL-LPS two-hit protocols with atherogenic LDL isolated from patients with atherosclerosis (LPS 1 μg/ml; LDL 100 μg/ml protein; n = 3 biological replicates per condition). IL-1β, IL-6, IL-8, MCP-1 and TNF were quantified by ELISA. RNA sequencing (RNA-seq) was used to identify transcripts co-expressed with intolerant cytokine output (Spearman $\rho \geq 0.9$, false discovery rate < 0.05). Sodium butyrate (1.5 mM), resveratrol (50 μM), curcumin (10 μM) and epigallocatechin-3-gallate (EGCG, 100 μM) were then tested in parental THP-1 under the same LDL-LPS protocol; intolerance was defined as higher IL-1β secretion under LDL+LPS than under LPS alone.

Results: Of the four compounds, only sodium butyrate abolished the LDL-LPS versus LPS-only differential in IL-1β secretion ($p > 0.05$); resveratrol, curcumin and EGCG reduced absolute cytokine secretion but preserved the trained-immunity differential. Across the cybrid panel, intolerance segregated by cytokine and by cybrid: IL-8 was intolerant in 8/14 cybrid lines, MCP-1 in 6/14, IL-6 in 3/14, IL-1β in 2/14; TNF showed uniform tolerance. Twenty-three epigenetic regulators co-expressed with the intolerant signature included DNA-methylation writers and erasers, SWI/SNF and ISWI chromatin remodelers, and miRNA/long ncRNA processing machinery.

Conclusions: Sodium butyrate dismantled, rather than merely suppressed, atherogenic-LDL-induced trained immunity in THP-1 macrophages, unlike the three other natural compounds tested.

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P52

Targeting the HO-1–Ferroptosis Axis to Reprogram Immunosuppressive Macrophages and Enhance T-Cell Activation

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Tumor-associated macrophages (TAMs) are central mediators of immune suppression within tumor microenvironment, facilitating tumor progression, immune escape, and unresponsiveness to immunotherapy. Ferroptosis, an iron-dependent form of regulated cell death executed by lipid peroxidation (LPO), has recently emerged as an important regulator of cancer biology; however, its impact on macrophage immune suppressive activity remains poorly understood. Heme oxygenase-1, a key regulator of heme degradation and iron metabolism, is closely linked to pro-tumoral macrophage polarization and oxidative stress responses. We previously identified HO-1 modulation via Zinc protoporphyrin IX (ZnPPIX) as an effective strategy to relieve macrophage-driven immune suppression.
Here, we investigated macrophage susceptibility to ferroptosis and evaluated how HO-1 modulation influences ferroptosis progression and immune suppressive function. Differentiated macrophages displayed moderate susceptibility to RSL3-induced ferroptosis, with an LC50 of 500 nM after 24 h treatment. Kinetic analysis of LPO using BODIPY 581/591 C11 identified 1 h treatment as the optimal window for functional studies. Transcriptomic profiling of ZnPPIX-treated macrophages revealed an enrichment of ferroptosis-related pathways and a significant up-upregulation of ferroptosis-protective genes, supporting a role for HO-1 in maintening redox homeostasis. Accordingly, ZnPPIX pretreatment significantly attenuated RSL3-induced LPO and rescued macrophages' viability in different experimental settings, indicating that HO-1 actively modulates ferroptotic stress responses. Notably, in co-culture assays with activated T cells ferroptosis induction significantly impaired the suppressive activity of macrophages, mirroring the reprogramming effect of ZnPPIX mono-treatment. Combined ZnPPIX/RSL3 treatment similarly enhanced lymphocyte proliferation and activation.
Overall, our findings suggest that ferroptosis and HO-1 pathways are interconnected nodes regulating of macrophage immune suppressive function. Targeting this axis represents a promising therapeutic strategy to successfully reprogram TAMs and improve anti-tumor immune responses.

NK CELLS AND ILC

P53

μ-opioid receptor expression and degranulation in NK cells in neuropathic pain: a possible role for bergamot polyphenol fraction

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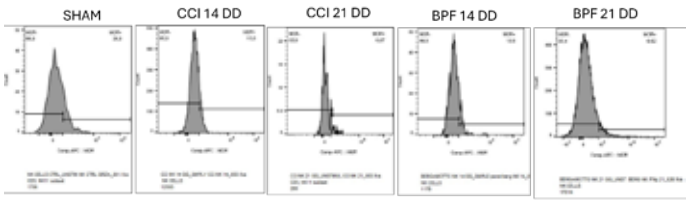
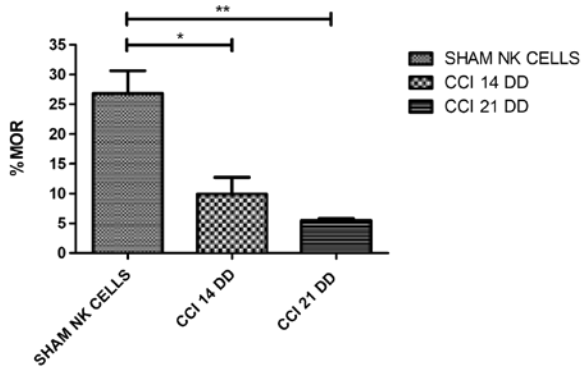
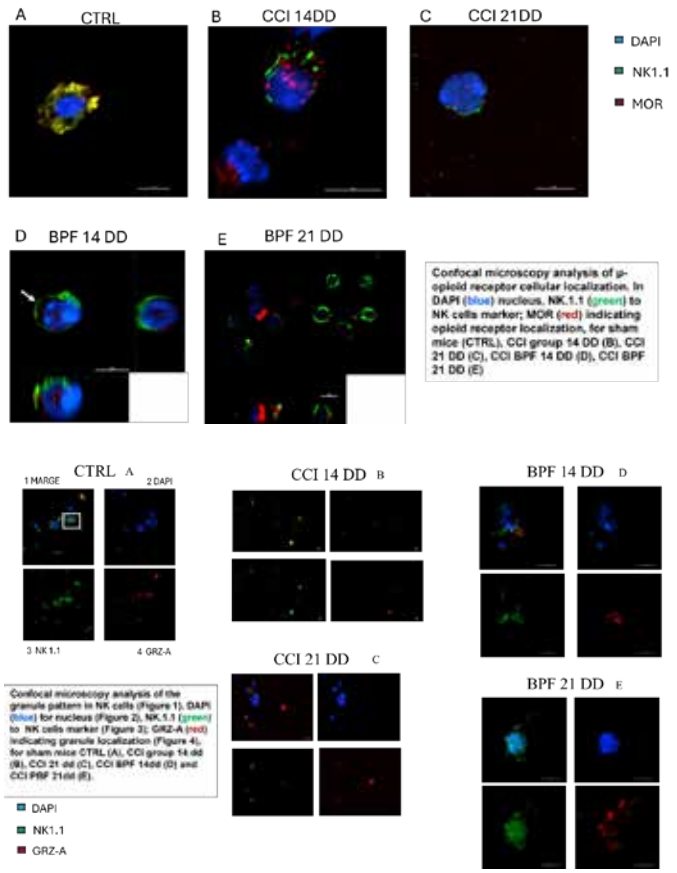
Introduction and Aims: Pain is a major global health challenge, often lacking objective diagnostic tools. Natural killer (NK) cells are cytotoxic effectos of the innate immune system. Recent evidence suggests that μ-opioid receptor (MOR) expression on B cells and NK cells may serve as a pain biomarker. In this study, we used a murine chronic constriction injury (CCI) model to investigate MOR expression on NK cells and to evaluate the effects of a bergamot-derived polyphenolic fraction (BPF) in comparison with CCI. We also assessed NK cell degranulation, IFN-γ production, and the role of the inhibitory Ly49C/I receptor in NK cell function.
Methods: Male C57BL/6 (B6) mice underwent chronic constriction injury (CCI) of the right sciatic nerve according to the Bennett model. Mice were sacrificed 14 or 21 days after CCI, with or without BPF treatment (25 mg/kg). NK cell degranulation was assessed by CD107a assay and cells were stained with following antibodies: LIVE/DEAD-APC-Cy7, NK1.1-BV605, CD3-BV886, Ly49A-BV421, Ly49C/I-BB700, CD107-PE, and MOR-APC, using LSR Fortessa flow cytometer. Granzyme-A (GRZ-A) production and MOR localization in NK cells were analyzed by confocal microscopy.
Results: Flow cytometry analysis revealed alterations in the expression of MOR on NK cells compared to sham controls.CCI mice exhibited reduced NK cell degranulation, which was modulated in the BPF-treated. The CD107a surface assay and IFN-γ production post-injury showed an NK cell exhaustion phenotype. Confocal microscopy demonstrated predominantly intracellular MOR localization in NK cells from CCI group, in contrast to the mainly surface-associated expression observed in sham controls. In BPF-treated CCI mice, MOR showed a partial internalization at 14 days. Moreover, in the same group, GRZ-A tended to remain predominantly intracellular at 14 days.



Flow cytometry analysis of MOR expression on total NK cells from SHAM (CTRL) and on CCI 21days, CCI 21 days, BPF 14 days and BPF 21 days. Data are representative of one independent experiment.



Flow cytometric analysis to measure MOR expression on total NK cells at 14 and 21 days after CCI. Results are expressed as mean ± SEM, for 10 mice per treatment. Events were gated on live singlet lymphocytes, CD3−NK1.1+ *p<0.050, **p <0.0050 vs sham.



Conclusions: MOR expression on NK cells may serve as a biomarker for neuropathic pain, warranting further investigation into the modulatory effects of BPF and pharmacological agents.

P54
HLA-E/NKG2A reprograms Natural Killer cells toward a granzyme K⁺ poorly cytotoxic phenotype in multiple myeloma

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Natural Killer (NK) cells are critical for immune surveillance in Multiple Myeloma (MM), yet malignant plasma cells employ multiple strategies to evade NK-mediated recognition. The non-classical HLA-E molecule emerges as a key determinant of NK cell resistance, with expression on MM plasma cells progressively increasing during disease evolution. While HLA-E is typically recognized for its inhibitory signaling, whether it can also reprogram NK cells toward a dysfunctional phenotype remains unclear. Stimulation of NK cells with recombinant HLA-E or antibody-mediated NKG2A triggering promoted the expansion of GZMK⁺ cells with lower CD16 and GZMB, intermediate CD56, and reduced CD57, indicating a shift toward a less mature and poorly cytotoxic phenotype. These in vitro observations were reflected in patients, as single-cell RNA sequencing identified a distinct NK cell cluster in MM bone marrow with low expression of cytotoxic genes (FCGR3A, PRF1, GZMB) and high GZMK. Moreover, cytometric analysis of paired peripheral blood and bone marrow samples revealed a significant expansion of GZMK⁺ NK cells, detectable in the blood and also in pre-malignant lesions (MGUS/SMM). Overall, our findings reveal a previously underappreciated effect of NKG2A on NK cell biology, driving the accumulation of GZMK⁺ NK cells with poor cytotoxic activity in MM. These results also highlight this pathway as a potential target to restore effective NK-mediated immunity, even at pre-malignant stages.

P55
Antibody response to Recombinant Zoster Vaccine and selective perturbation of NK cell compartment in Primary Antibody-Deficient patients

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Although the adjuvanted glycoprotein E (gE)-based Recombinant Zoster Vaccine (RZV, Shingrix, GSK) has been licensed for aged and immunocompromised subjects, its efficacy in Primary Antibody Deficient (PAD) patients is completely unknown. Natural Killer (NK) cells contribute to the protective effects of vaccinal antibodies (Abs) thanks to the IgG receptor, FcγRIIIA/CD16, whose aggregation leads to the killing of infected cells and IFNγ release, which potentiates adaptive responses. Recently, a memory NK cell population, specialized towards Ab-dependent functions, is emerging as regulator of vaccine-induced T and B cell responses.
Here we assessed the humoral response and the modulation of NK cell compartment in RZV-receiving Common Variable Immunodeficient (CVID) and unclassified PAD (unPAD) patients. While the vast majority of unPAD patients exhibited significant Ab production, half of the CVID cohort was unable to produce anti-gE IgG. Compared to responders, non-responder CVID displayed a more severe immune-dysregulated phenotype. Patient-derived vaccine-induced Abs were effective in activating gE-specific, CD16-dependent degranulation and IFNγ production, with a magnitude that correlated with Ab levels.
Interestingly, the conventional NK pool is selectively exhausted in CVID patients, more markedly in non-responders, in a maturation-dependent fashion, as evidenced by an increased frequency of TIGIT+ NK cells, mainly in the less mature CD57- NK subset. At variance, an expansion of memory NK compartment, that preserve normal phenotypic and functional hallmarks, including amplified Ab-dependent functions and reduced susceptibility to exhaustion, was selectively observed in CVID patients.

P56
Type 1 innate lymphocytes are critical mediators of protection in colorectal cancer

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Colorectal cancer (CRC) remains one of the major causes of cancer-related mortality worldwide. Natural Killer (NK) cells and ILC1-like NK cells constitute the predominant population of innate lymphocytes infiltrating CRC. NK cell activity has been linked to a favourable clinical outcome in CRC patients; nevertheless, accumulating evidence has also pointed to their potential involvement in dampening T-cell responses and reducing the effectiveness of immune checkpoint blockade therapies. In this work, we integrated genetic mouse models with single-cell RNA sequencing and flow cytometric analyses to dissect the molecular pathways regulating type 1 innate lymphocyte functions throughout CRC progression. Using a conditional mouse model selectively lacking *Stat4* in NKp46-expressing innate lymphocytes, we found that STAT4 deficiency resulted not only in aggravated chronic inflammation but also in enhanced tumor growth and profound reshaping of the tumor microenvironment. Collectively, our findings reveal distinct STAT4-dependent transcriptional signatures in tumor-infiltrating NK cells and ILC1, highlighting the protective role of STAT4-driven innate lymphocyte responses in colorectal cancer.

P57

Unraveling the role of Bone Morphogenetic Protein 4 in immunomodulation of Natural Killer lymphocytes

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Bone Morphogenetic Protein 4 (BMP4) is a member of the Transforming Growth Factor β (TGFβ) superfamily. It has been implicated in tumorigenesis, angiogenesis, and immune regulation. While its effect on T lymphocytes and macrophages have been described, its impact on Natural Killer (NK) lymphocytes remains unexplored. NK cells are essential effectors of innate immunity, tasked with eliminating tumor cells. However, within the tumor microenvironment, they can be polarized towards a decidual-like (dNK-like) state characterized by compromised cytotoxic activity, due to the presence of immunomodulatory factors such as TGFβ.
Here, we investigated whether BMP4 induces phenotypical and functional alterations of NK cells, and whether it may play a role in disabling NK cells in the tumor microenvironment. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and cultured with recombinant BMP4 for 72 hours in the presence of the activating cytokine IL-2. Flow cytometric analyses revealed that BMP4 induces a downregulation of activating receptors NKp30, NKG2D and DNAM-1, an upregulation of dNK-like markers CD9 and CD49a, as well as a reduction in the levels of lytic enzymes perforin and granzyme B. Functionally, degranulation against the HLA-I– human erythroleukemia K562 cell line was compromised, demonstrating an impairment of cytotoxic activity. These results were further corroborated by experiments using the conditioned media from primary human uveal melanoma cell lines (92.1, Mel270, Mel285), known to produce high amounts of recombinant BMP4, which recapitulated the observed phenotypic and functional alterations.
Altogether, our results sustain the hypothesis that BMP4 actively promotes a dNK-like phenotype and compromises NK lymphocyte cytotoxic functions, suggesting a previously unrecognized role for BMP4 in tumor immune evasion, particularly in the context of uveal melanoma. Targeting BMP4 may represent a novel strategy to restore NK responses within the tumor microenvironment.

P58

Efficient CRISPR–Cas9 RNP genome editing in primary murine ILC2

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Group 2 innate lymphoid cells (ILC2s) are central regulators of tissue integrity and type 2 immunity, contributing to epithelial barrier preservation, tissue remodeling, and inflammatory orchestration. Beyond their established roles in allergy and host defense, ILC2s are increasingly recognized as active participants in the tumor microenvironment, where they can influence immune regulation and disease progression. A deeper understanding of the molecular circuits controlling ILC2 activation, inhibitory signaling, and effector functions is essential to clarify their contribution to protective immunity, chronic inflammation, and cancer, and to evaluate their potential as therapeutic targets. Here, we developed and optimized a non-viral genome editing approach based on CRISPR-Cas9 ribonucleoprotein (cRNP) delivery for efficient gene modification in mature primary murine ILCs. ILCs were isolated from mouse tissues and enriched either by magnetic bead-based separation or fluorescence-activated cell sorting (FACS). Purified cells were cultured short-term in cytokine-supplemented conditions to maintain viability and responsiveness prior to electroporation with pre-assembled Cas9-sgRNA complexes. This strategy enabled efficient gene disruption while preserving high cell viability and functional competence after editing. Overall, our optimized protocol provides a robust, reproducible, and scalable platform for functional genetic interrogation of primary ILC2s. This approach opens new opportunities for dissecting ILC2 biology and supports the development of innovative ILC2-directed immunomodulatory strategies in inflammatory disorders and cancer.

P59

Perturbation of NK cell functions by targeting STATs

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Natural killer (NK) cells are essential components of host defence, eliminating infected or transformed cells and secreting cytokines that coordinate type 1 immune responses. NK cell effector activities are stringently controlled by dedicated transcription factor networks, particularly those operating downstream of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling cascade. This evolutionarily conserved pathway is pivotal for NK cell differentiation, survival, and functional maturation, and is commonly dysregulated across a broad range of malignancies. Among STAT family members, STAT5, STAT4, and STAT1 are well recognized for their roles in sustaining NK cell viability, cytotoxic effector functions, and adaptive-like properties. In contrast, the contribution of STAT3 to human NK cell biology remains insufficiently characterized, as comprehensive genome-wide studies defining STAT3 target genes and regulatory circuits are still limited. Moreover, murine models have produced conflicting results, depending on whether Stat3 ablation was implemented throughout the hematopoietic system or selectively confined to NK cells. To elucidate the role of STAT3 in NK cell regulation, we optimized a CRISPR-Cas9-based genome-editing approach in primary mouse and human NK cells. Human NK cells were isolated from peripheral blood mononuclear cells, whereas murine NK cells were derived from splenic tissue. Cells were purified using magnetic bead-based separation and maintained for three days in a cytokine-enriched culture medium. NK cells were then electroporated with Cas9-sgRNA ribonucleoprotein complexes under optimized parameters. Editing efficiency was assessed 48 hours post-electroporation by flow cytometry. In parallel, we examined the phenotype of patients carrying autosomal dominant negative STAT3 mutations. This strategy enabled us to delineate a role for STAT3 not only in regulating NK cell differentiation but also in regulation of NK cell cytotoxic function.

POSTER SESSION 2

MUCOSAL IMMUNOLOGY AND THE MICROBIOTA

P60

Iron-sequestration prevents dysbiosis and intestinal inflammation

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In healthy conditions, intestinal immune cells are educated to tolerance, but a combination of genetic and environmental condition may trigger chronic inflammation favoring cancer onset. Several studies indicate that high intake of iron-rich food is associated with CRC risks. At cellular level, the upregulation of iron-metabolism related proteins is essential for tumor progression, in the lumen heme-rich environment is associated with intestinal dysbiosis.
We created a murine model (Winnie-APC$^{Min/+}$) which combines the genetic predisposition to develop CRC (APC$^{Min/+}$) with a model of mild, spontaneous, and progressive colonic inflammation caused by a mutation in the Muc2 gene (Winnie).
Furthermore, we demonstrated that Winnie-APC$^{Min/+}$ are characterized by an intestinal dysbiosis. Winnie dysbiotic microbiota is sufficient to promote CRC into a genetically predisposed recipient APC$^{Min/+}$.
Several studies are exploring the possibility of using favorable gut microbiome to improve therapy efficacy in patients with tumor. With the present proposal we aim to explore the link between elevated iron intake, dysbiosis and CRC development, and, in parallel, iron chelation as strategy to prevent CRC and correct dysbiosis.
Our hypothesis is that by modulating iron availability we will be able to reduce intestinal inflammation, dysbiosis and tumor progression. This approach may represent an innovative adjuvant strategy for genetically predisposed individuals.
We treated 4-week-old WT and Winnie with the iron chelator or iron supplements for 8 weeks. 16S sequencing was used to define the intestinal microbial response to luminal iron availability. The intestinal tract was collected and analyzed by histological and immune infiltrate.
Intestinal iron-chelation is able to interfere with iron-abundance mediated dysbiosis and consequently reduce inflammation. Next, we wil repeat this experimental setting on Winnie-APC$^{Min/+}$ mice to evaluate the effect of the iron-axis in tumor development.

P61
Characterization of nasal virus-specific T cell responses in immunocompromised patients

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Respiratory viral infections pose a major clinical challenge in immunocompromised individuals, who are at increased risk of severe disease. The nasal mucosa represents a critical frontline barrier of immune defence, where robust local immune responses play a crucial role in preventing the spread of viruses to the lower respiratory tract. While nasal immune responses have been characterized in healthy subjects, they remain poorly understood in immunocompromised patients.

To address this, we enrolled 17 patients with solid tumors under active anti-cancer treatments (8 chemotherapy, 5 chemo+immunotherapy, 2 targeted therapy alone, 2 immunotherapy alone) and 15 healthy individuals. We collected paired blood and nasal samples. Nasal lymphocytes were obtained through nasal swabs performed at the level of the middle-superior turbinate, quantified by flow cytometry, and stimulated with peptide pools from SARS-CoV-2, Influenza A, Rhinovirus and Respiratory Syncytial Virus (RSV) in an IFN-γ ELISpot assay. Virus-specific T cell responses were also evaluated in paired blood samples.

Both cohorts displayed broad nasal T cell responses to multiple viral antigens, comparable in specificity to circulating T cells. Remarkably, virus-specific T cell responses in the nasal mucosa were often of higher magnitude than those detected in peripheral blood mononuclear cells, with Influenza A-specific responses being the most frequently observed. Preliminary phenotypic analyses revealed nasal virus-specific T cells enriched for tissue-residency markers (CD69+, CD103+) compared to the circulating counterparts, forming a local memory compartment preserved in cancer patients, similarly to healthy controls. Longitudinal analyses to assess the kinetics and persistence of these responses are ongoing.

These initial findings provide new insights into the nasal memory T cell responses in immunocompromised cancer patients. Importantly, they suggest that peripheral blood monitoring significantly underestimates mucosal antiviral immunity, underscoring the need for tissue-informed immunomonitoring strategies and novel mucosal vaccines targeting local immune responses.

P62
Disruption of the microbiota–immune axis by mixed nano- and microplastic wxposure in C57BL/6J mice

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Background: environmental plastics gradually degrade into microplastics (MPLs) and nanoplastics (NPLs) through biological, chemical, and physical processes. Smaller particles can enter the bloodstream via the respiratory tract and potentially affect the gut–lung immune axis, whereas larger particles interact mainly with the intestinal environment, potentially impairing immune homeostasis. To date, data on the accumulation of N/MPLs in mammalian tissues are limited, yet such information is essential for evaluating potential human health risks.

Aim: to investigate the effects of N/MPL exposure on the microbiota–immunity axis in vivo using a C57BL/6J wild-type mouse model.

Methods: in C57BL/6J wild-type mice were administered polyethylene (PE) and polyethylene terephthalate (PET) particles via oral gavage. The V3–V4 hypervariable region of the 16S rRNA gene was sequenced from lung and colon samples to assess microbiota composition. In addition, inflammatory cytokines were quantified in plasma by multiplex technology. Finally, T cell populations were characterized in the spleen by cytofluorimetry and the colon metabolism gene expression profiles were analysed by using Nanostring approach.

Results: we documented some differences at genus level after microplastic treatment respect to control in the colon microbiota. In addition, we observed an increase in plasma of IL-1β, a renowned pro-inflammatory cytokine, in PE treatment compared to control and PET treatment.

Conclusions: we documented some significant changes in the microbiota-immunity axis after the treatment with N/MPLs. Finally, the assessment of MP and NP exposure in mice models offers a valuable tool to assess health risk of plastic exposure to animals and parallel to humans.

P63

Atypical chemokine receptor CCRL2 as a functional gatekeeper in intestinal inflammation and epithelial barrier function

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Inflammatory bowel disease (IBD) is a chronic immune-mediated disorder characterized by excessive leukocyte infiltration and disruption of the intestinal epithelial barrier. Chemokine receptors are key regulators of immune cell trafficking during intestinal inflammation. CCRL2 is an atypical seven-transmembrane receptor for the non-chemokine ligand chemerin that does not signal through classical G protein nor β-arrestin–dependent pathways and is expressed by both leukocytes and intestinal epithelial cells. CCRL2 expression by epithelial cells was investigated by RNA-scope and scRNAseq in gut tissue and the role of inflammatory infiltrate by multiparametric cytofluorimetry.
In addition, to investigate the role of CCRL2 in intestinal inflammation, wild-type (WT) and CCRL2-deficient (KO) mice were subjected to dextran sodium sulfate (DSS)-induced colitis. CCRL2 KO mice developed a markedly exacerbated disease phenotype, characterized by greater weight loss, increased intestinal bleeding, colon shortening, and more severe histopathological damage compared with WT controls.
Mechanistic insights are currently investigated using colonic organoids derived from WT and CCRL2 KO mice and spatial proteomics. Overall, these findings identify CCRL2 as a protective regulator of intestinal homeostasis, acting at both immune and epithelial levels to restrain inflammation and preserve epithelial barrier function.

OTHER TOPICS

P64

The Pala Single Cell Sorter Enables High-Throughput iPSC Engineering and Preserves Colony Stemness and Viability

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Stable cell line development is a critical process for developing and manufacturing biologics, generating disease relevant cellular models, and creating the next generation of cell & gene therapies. The utility of new cell models (patient derived iPSCs), the development of new genome editing technologies (CRISPR) and the deployment of automation and AI for high-throughput screening are creating a greater demand for scaling cell line development capabilities and workflows. R&D Systems has developed the Pala single cell sorter and dispenser that incorporates microfluidics, flow cytometry, and liquid dispensing in a portable, easy to use, and gentle device for generating high rates of single cell derived clones for enhancing cell line engineering workflows.
First, Pala is compared to traditional limiting dilution (LD) to dispense induced Pluripotent Stem Cells (iPSCs) for single cell cloning and colony outgrowth. Single dissociated iPSCs were dispensed in treated 96-well plates or hand pipetted at 0.4 cells/well using LD. Colony health and stemness are compared between each method using clone area and TRA-1-60 immunofluorescence staining. Pala, on average, generated ~3-fold greater numbers of single cells deposited, and colonies generated than LD. Additionally, cell health, as determined by clone area, and stemness, as determined by TRA-1-60 immunofluorescence, showed equivalence between the two methods demonstrating that Pala derived clones are as healthy as clones derived with LD. Finally, beta-2-microglobulin, a component of MHC class II molecules and a target for immune rejection of cell therapy products, was knocked out using CRISPR and edited iPSCs were dispensed using Pala. Single cell derived colonies were verified and characterized showing high outgrowth rates and expression of stemness markers Nanog, Oct4, and SSEA4 to demonstrate undifferentiated state. This work demonstrates a high-throughput workflow for cell line engineering and cell therapy manufacturing using gentle, fluorescent, microfluidic single cell sorting with high efficiency and quality.

P65

Mapping brain activation in response to peripheral immune challenge

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The brain and the immune system entertain a continuous, bidirectional dialogue that begins in the early development and persists throughout life. When the immune system responds to a peripheral challenge, it generates signals that do not remain confined to the site of activation, but instead reach the brain. This project aims to understand how the brain encodes and integrates the signals generated by a peripheral immune challenge.
To address this, we used TRAP2 mice, a genetic model that allows permanent labelling of neurons

based on their activity at a defined time window. Peripheral inflammation was induced by topical application of imiquimod, and neuronal tagging was performed either at the inflammatory peak or during recovery. After full recovery, animals received a second imiquimod challenge. By comparing which neurons were tagged during the first episode with those active during the second, we can directly assess whether repeated inflammation engages the same brain circuits.
Labelled neurons were quantified across the brain using a standardized atlas registration pipeline. Preliminary results show robust neuronal activation in cortical and subcortical regions following peripheral immune challenge.
Finally, while this study focuses on skin inflammation, the same approach can be extended to other peripheral organs, with the broader goal of building a comparative map of how the brain responds to immune challenges arising from different tissues

P66

Interaction of different Lipid Nanoparticles formulations with immune cells

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Introduction: Lipid nanoparticles (LNPs) represent an advanced platform for nucleic acid delivery. Compared to lamellar liposomes, non-lamellar LNPs (featuring cubic, hexagonal, or sponge phases) offer a larger surface area, higher stability and enhanced membrane interactions. However, the mechanisms of their interaction with the immune system remain to be fully elucidated.
Aim: This study compares three LNP formulations (A: Glycerol Monooleate (GMO)/cationic lipid (DOEPC)-PolyA, B1: GMO/ ionizable lipid (DODMA)-PolyA, and B2: GMO/DODMA) to evaluate their toxicity and binding affinity across different subsets of Peripheral Blood Mononuclear Cells (PBMCs).
Materials and Methods: PBMCs were isolated from healthy donors using density gradient centrifugation and incubated with formulations A, B1, and B2 at various concentrations (50 μM, 100 μM, 200 μM) for 2h, 6h, and 24h. All formulations were fluorescently labelled with the lipophilic probe beta-BODIPY to enable intracellular tracking. Cellular interactions and viability were analyzed by flow cytometry, while intracellular localization was investigated using confocal microscopy.
Results: Cell viability remained high at 2h and 6h for all tested concentrations. However, formulation A showed higher toxicity and faster interaction ability compared to B1 and B2. Preliminary data on confocal microscopy confirmed cytoplasmic localization for all studied LNPs.
Conclusions: These results suggest that lipid composition influences the interaction of LNPs with immune cells. In particular, the cationic formulation (A) showed faster interaction but also higher toxicity, whereas the ionizable formulations (B1 and B2) appeared better tolerated but showed slower interaction kinetics. In addition, all formulations were internalized into the cytoplasm. These results provide preliminary insights for further investigations, including the analysis of immune cells activation and the evaluation of cargo delivery efficiency and release.

P67

CCRL2 role in the regulation of colon epithelial homeostasis

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CCRL2 is a seven-transmembrane receptor structurally related to atypical chemokine receptors (ACKRs) and lacks classical G-protein signaling activity. It binds the non-chemokine ligand chemerin without inducing ligand scavenging or receptor internalization. CCRL2 expression is detected in multiple leukocyte populations as well as barrier cells, including vascular and lymphatic endothelial cells and intestinal epithelial cells. Although CCRL2 has been implicated in the regulation of leukocyte recruitment and immune responses in inflammatory diseases and cancer, its role in intestinal epithelial homeostasis under physiological conditions remains poorly understood.
The contribution of CCRL2 to colon epithelial homeostasis under basal conditions was investigated using wild-type (WT), CCRL2 knockout (KO), and epithelial-specific VillinCre;CCRL2 fl/fl conditional KO mice. Histological and immunofluorescence analyses of FFPE colon sections were performed to evaluate epithelial proliferation, apoptosis, mucus production, and epithelial barrier integrity.
CCRL2 deficiency resulted in reduced epithelial proliferation without significant changes in apoptosis, indicating a selective effect on epithelial turnover

under basal conditions. Moreover, CCRL2 KO mice displayed a significant reduction in MUC2-positive goblet cells, suggesting impaired mucus production and altered epithelial barrier properties. Consistently, increased bacterial translocation was observed both within crypt structures and intraepithelial compartments. Similar alterations were detected in VillinCre;CCRL2 fl/fl mice, demonstrating that epithelial-specific deletion of CCRL2 compromises barrier integrity during homeostasis.
Collectively, these findings identify epithelial CCRL2 as an essential modulator of crypt homeostasis and mucosal barrier function in the steady-state intestine.

P68
Optimizing ADAR1-silencing as a novel immunotherapeutic strategy to boost NK cell responses in Cervical Cancer
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ADAR1 catalyzes the adenosine-to-inosine editing of double-stranded RNA, thereby preventing aberrant activation of innate immune sensing pathways. In cervical cancer (CC), ADAR1 is overexpressed in patient lesions, which correlates with reduced $CD7^+$ cell infiltration and functional alterations of NK cells, supporting its role in promoting an immunosuppressive tumor microenvironment. Our previous *in vitro* studies demonstrated that ADAR1 inhibition reduces tumor cell proliferation and enhances NK cell activation, providing a strong rationale for in vivo validation. To assess the therapeutic potential of ADAR1 targeting *in vivo*, we established subcutaneous xenograft in $RAG^{2-/-}$ mice using the SiHa CC cell line, which had previously been engineered by virus-like particles to express a doxycycline-inducible shRNA against ADAR1. This approach significantly impaired tumor growth and promoted phenotypic maturation of tumor-infiltrating NK cells, highlighting a critical role for ADAR1 in restraining innate immune surveillance. To improve the accuracy of our clinically therapeutic targeting approach, we are developing new delivery systems for ADAR1-specific siRNA. The initial *in vivo* delivery studies were performed using in vivo-jetPEI. However, to improve delivery efficiency, biocompatibility and translational potential, we are currently developing lipoplex-based formulations that encapsulate ADAR1-specific siRNAs. Compared with conventional transfection reagents, lipoplexes offer enhanced biocompatibility, systemic delivery potential, and passive tumor accumulation. Notably, *in vitro* pre-incubation of lipoplexes with human plasma markedly reduced silencing efficacy, likely due to the formation of a protein corona affecting intracellular trafficking rather than cellular uptake. Analysis by confocal microscopy revealed that both standard and plasma-coated lipoplexes are efficiently internalized, suggesting that impaired endosomal escape or cytoplasmic release may limit the effectiveness of RNA interference. Ongoing studies aim to dissect these intracellular mechanisms to optimize lipoplex delivery systems and maximize the translational potential of ADAR1-targeted therapies in cervical cancer.

T AND B LYMPHOCYTES

P69
Sex-bias immune aging and its interplay with X-inactivation escape genes at single-cell level
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The world is aging, and improving the health of the elderly is crucial. Elderly individuals are particularly susceptible to immune system failure, leading to increased vulnerability to infectious diseases. Immune responses exhibit sex-specific patterns due to a combination of hormonal and genetic factors, including the number of X chromosomes and the expression of genes escaping X-inactivation in females. However, which genes escape X-inactivation throughout life and their impact on immune cell functions remain unclear.
Using single-cell multiomics methodologies, we assess whether age-related changes in cellular composition, chromatin accessibility, and transcription are sex-specific in mice. Our findings reveal a similar aging-specific remodeling of the T-cell compartment in both sexes and a pronounced sex influence on the B-cell transcriptome. Moreover, we describe a cell-type-specific landscape of X-inactivation, with escape genes contributing to female-biased expression. Notably, a subset of aged T-cells, which play a key role in aging and cancer progression, demonstrates increased transcriptional activity from the inactive X chromosome, accompanied by heightened chromatin accessibility. Our work sheds new light on the intricate interplay between sex and age, highlighting cell-type-specific escape dynamics in shaping sex-specific immunological trajectories and advantages.

P70
Identification of Novel T-cell Targets for a SARS-CoV-2 and Pan-coronavirus Vaccination

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Background: SARS-CoV-2 vaccine efforts have reduced infection rates and fatalities associated with COVID-19. However, the high mutation rate of vaccination target, Spike, has led to the evolution of variants of concern able to evade vaccine induced immunity. Preparation for emerging human coronaviridae (HCoV) is also imperative. Therefore, this work investigates T-cell targets for pan-coronavirus vaccination, for SARS-CoV-2 and emerging HCoV protection. Specifically, we investigate non-structural proteins (NSPs), with indispensable roles early in the virus life cycle and high conservation across HCoVs.
Methods: To investigate cellular NSP immunity, we first assessed the responses of SARS-CoV-2 convalescent donors following in vitro expansion with 47 highly conserved peptides, derived from SARS-CoV-2 NSP proteins. Subsequently, NSP-specific T-cell clones were generated and characterised to understand in greater detail individual NSP-specific T-cell responses.
Results: Screening uncovered novel NSP T-cell epitopes; both CD4+ and CD8+ T-cell responses were identified. Isolated NSP-specific T-cell clones were of high avidity and restricted through several common HLA alleles, suggesting that responses to these epitopes following vaccination are likely to be widespread. Furthermore, NSP-specific T-cell clones cross-recognised corresponding HCoV peptide, highlighting the potential for generating pan-coronavirus protection. Finally, T-cell clones recognised naturally processed antigen. Interestingly however, CD4+ T-cell recognition was only achieved when protein was presented by an epithelial cell line, rather than a routinely used lymphoblastoid cell line (LCL). This not only implicates epithelial cells as APCs during SARS-CoV-2 infection but also indicates differences in antigen processing pathways between the two cell types.
Conclusion: NSP-specifc T-cell responses identified are restricted by common HLA alleles and cross-recognise peptide from circulating HCoVs. T-cell clones recognise processed antigen however future work will involve SARS-CoV-2 live infection assays to prove that expansion of NSP-specific T-cells can induce HCoV protection.
T-cells can induce HCoV protection.

P71
Regulation of cytotoxic functions of CD4⁺ effector and regulatory T cells by Natural killer cell receptors

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Cytotoxicity is classically attributed to CD8⁺T cells and Natural killer (NK) cells; however, CD4⁺T cells can also acquire cytotoxic features. Thus, CD4⁺T cells, may express cytotoxic molecules like Granzymes (Gzm) and Perforin. These cytotoxic CD4⁺T cells express the transcription factor Eomesodermin (EOMES) and comprise cytotoxic effector T lymphocytes (CD4⁺CTLs), pro-inflammatory Th1-cells and anti-inflammatory type 1 regulatory-like T-cells (EOMES+Tr1-like). Within this spectrum, Th1-cells and Tr1-like cells express predominantly GzmK, and CD4⁺CTLs GzmB. These cells could mediate the elimination of tumor cells and/or antigen-presenting cells; however, the molecular mechanisms governing their cytotoxic functions remain poorly defined. NK receptors (NKRs), known to regulate cytotoxicity in NK and CD8⁺T cells, may also modulate cytotoxic CD4⁺T cell responses, but their expression and roles remain underexplored among CD4⁺T cells.
To investigate the expression of activating and inhibitory NKRs on cytotoxic CD4⁺T cell subsets, multiparametric FACS analysis performed on peripheral blood of healthy donors. Cytotoxic CD4⁺T cell subsets displayed characteristic NKR expression profiles. The NKR profile of Tr1-like cells was characterized by the co-expression of inhibitory receptors such as NKG2A, KLRG1 and CD85K with activating receptors including NKG2C, D and NKp80. Notably, Tr1-like cells that expressed NKG7 expressed also higher levels of cytotoxic NKRs and degranulated upon TCR stimulation. Bioinformatic analysis predicted that they could differentiate further to effector CD4⁺CTLs. Consistent with a progressive acquisition of cytotoxic effector functions, CD4⁺CTLs exhibited higher expression of activatory NKRs.
Together, these data support a model in which CD4⁺ cytotoxic T cells exist across a dynamic range of differentiation at the transcriptional and functional level that is associated with distinct NK receptor patterns. We plan to evaluate if the NKR patterns determines the target specificity of pro- and anti-inflammatory CD4⁺T cell subsets.

P72

Ectopic neurogranin expression in CD8⁺ T cells drives intrahepatic dysfunction

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Hepatitis B virus (HBV) establishes chronic infection in over 250 million people worldwide and remains a leading cause of cirrhosis and hepatocellular carcinoma. In chronic hepatitis B (CHB), virus-specific $CD8^+$ T cells acquire a dysfunctional state that is mechanistically and phenotypically distinct from exhaustion observed in extrahepatic chronic infections and cancer. However, the molecular programs enforcing this liver-imprinted dysfunction remain poorly defined.

Using a relevant HBV preclinical model of intrahepatic priming, we performed integrated transcriptomic analyses to identify regulators of hepatocyte-induced $CD8^+$ T cell dysfunction. Across orthogonal approaches, including single-cell RNA sequencing, spatial transcriptomics, high-dimensional flow cytometry, and confocal imaging, we consistently identified Neurogranin (Nrgn) as selectively upregulated in hepatocyte-primed HBV-specific $CD8^+$ T cells. Importantly, this signature was conserved in $CD8^+$ T cells from patients with chronic HBV infection.

Nrgn is a neuron-associated calmodulin-binding protein that regulates calmodulin availability during Ca^{2+} transients, implicating calcium sensing in the enforcement of liver-imprinted T cell dysfunction. Consistent with this model, we modulate Neurogranin expression in dysfunctional $CD8^+$ T cells using antisense oligonucleotides (ASOs), revealing a functional contribution of Ca^{2+}, calmodulin signalling to the maintenance of the liver-imprinted dysfunctional state.

Collectively, our findings establish Neurogranin as a conserved marker and functional regulator of hepatocyte-primed $CD8^+$ T cell dysfunction and nominate calcium-TCR signalling circuitry as a targetable vulnerability to enhance immune restoration strategies in chronic HBV.

P73

Omalizumab and dupilumab differentially modulate follicular helper T and regulatory B cells in severe allergic asthma

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Severe allergic asthma (SAA) is a type 2-high asthma endotype characterized by IgE-mediated inflammation and dysregulation of adaptive immune cell subsets, including follicular helper T (Tfh) and regulatory B (Bregs) cells. Although omalizumab and dupilumab are approved biologic therapies for SAA, their impact on these immune subsets remains poorly investigated. Aim: To investigate the effects of omalizumab and dupilumab on Tfh and Breg phenotypes and their correlation with lung function test in SAA patients. Methods: Peripheral blood mononuclear cells from 8 SAA patients treated with omalizumab, 5 SAA patients treated with dupilumab, and 8 healthy controls (HCs) were analysed by flow cytometry. Tfh and Breg cell changes were evaluated at baseline (T0) and after six months of treatment (T6). Clinical and functional parameters were also collected. Results: At T0, mature B cells ($CD19^+$ $CD10^-$) positively correlated with ACT scores and FVC, while immature and naïve B cells inversely correlated with these clinical parameters. FEV1 was inversely associated with serum IgE levels. At T6, Breg cell frequencies positively correlated with ACT, FEV1 and FVC percentages. Omalizumab increased mature B cells and Breg subsets ($CD19^+CD24^+CD27^+$ and $CD19^+CD24^+CD38^+$), alongside reduced plasma cells, transitional, and memory B cells. Conversely, dupilumab was associated with lower circulating Tfh cells percentages. Conclusion: Omalizumab and dupilumab exert differential modulation of immune cells in SAA, particularly targeting B-cell and T-cell compartments, respectively. The association between Breg cells and clinical-functional parameters suggests their potential role as a biomarker of therapeutic response.

P74

Metabolic and immune signatures associate with rejection and tolerance in lung transplantation

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Introduction: Lung transplantation (LT) is a surgical procedure designed to replace one or both diseased lungs with healthy donor organs in patients with end-stage pulmonary diseases.

In the years following transplantation, chronic rejection, clinically defined as chronic lung allograft dysfunction (CLAD), remains the leading cause of graft failure, with a 5-year survival rate of 55%.

A deeper knowledge of the immunological scenarios associated with graft tolerance or rejection, could make possible to selectively target alloreactive T cells.

Methods: We collected transbronchial biopsies from transplanted patients and performed gene

expression analysis by RT-PCR, focusing on immunometabolic pathways, including MYC and PD-1 related pathways. In parallel, we collected peripheral blood samples from patients. We quantified circulating cell-free mtDNA in plasma and we isolated CD8+ T cells from the cellular fraction and assessed their metabolic profile at baseline and after stimulation with antiCD3/antiCD28 antibodies, to mimic T cell activation.
Results: We observed a strong negative correlation between PD-1 expression and genes associated with T cell activation, while we did not find any significant association with the transplant clinical outcome.
$CD8^+$ T cells from patients without rejection showed higher basal PD-1 expression with only a modest increase after stimulation, consistent with a partially exhausted or regulated T cell state. In contrast, rejecting patients displayed higher baseline MYC levels, reflecting active effector and metabolic responses. Subjects experiencing rejection also showed higher level of cell free mtDNA in plasma.
Conclusions: Gene expression analysis in transbronchial biopsies suggests that these factors might be part of the same regulatory network involved in metabolic pathways after transplantation. Elevated circulating cell-free mtDNA in rejecting patients may reflect tissue damage and systemic inflammation, while findings in peripheral $CD8^+$ T cells indicate that targeting metabolic pathways associated with effector function, while supporting exhaustion-related programs, could help modulate alloreactive responses and enhance graft tolerance.

P75
Decoding the short- and long-lived components of the antibody response to SARS-CoV-2 nucleoprotein at the individual level

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The antibody response to the SARS-CoV-2 Nucleoprotein (NP) after infection shows substantial inter-individual variability in durability, with antibody levels becoming undetectable within six months in a significant proportion of individuals. The immunological basis of this variability remains incompletely understood.
We developed a mathematical model of antibody decay — the 2-ASC (two–antibody-secreting cell) model — grounded in established immunological principles of humoral immunity. The model assumes that both the plasmablasts-derived and the plasma cells-derived components of the response decay exponentially, with two distinct half-lives, conserved across individuals. Inter-individual variability in antibody kinetics is thus attributed exclusively to differences in the relative size of these two components, rather than to heterogeneity in plasma cell longevity.
We applied this framework to longitudinal anti-NP IgG data from 40 unvaccinated individuals with PCR-confirmed SARS-CoV-2 infection, from the first epidemic wave. The half-lives of antibodies and plasma cells were estimated by fitting the model to longitudinal data using a grid-search optimization approach combined with bootstrap resampling to quantify uncertainty. Model-based decomposition of individual antibody trajectories revealed that a plasma cell–derived component was detectable in the vast majority of individuals (39/40).
These findings indicate that seroreversion of anti-NP responses does not generally reflect a failure to generate long-lived antibody-secreting cells, but rather arises from quantitative features of the initial immune response and technical detection limits. This work illustrates how mathematical modeling of antibody responses can disentangle sources of variability, offering a principled framework for interpreting the durability of the serological response at the individual level.

P76
B-1 innate-like human B cells: developmental origins and tissue distribution

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In the mouse, two successive waves of hematopoiesis have been described. The pro-definitive wave generates B-1a B cells expressing the marker CD5.
In humans, the pro-definitive wave of development generates T cells, B cells, and innate lymphoid cells in second trimester of pregnancy. After birth, the adaptive immune system gradually matures and takes over the protective role.
There are no reliable markers enabling the identification of innate-like B cells in humans. Recent work showed that the expression of CD5 and CD43, in combination with the transcription factor TCF1, identifies murine B-1a B cells. These markers were also detected in B cells of pleural exudates from patients with bacterial infections suggesting they may be used to discriminate the B-1a population in humans.
We stained different samples from peripheral blood and body fluids. Our data indicate that in human peripheral blood (PB) a minor fraction of the CD5+ B cells express CD43 and TCF1. TCF1, however, is also expressed by two defined fractions of CD5- memory B cells. Our findings suggest that TCF1 expression reflects a functional state within the B-cell compartment and is not restricted to CD5+ B cells.
Cord blood (CB) is enriched in cells derived from ear-

ly developmental hematopoiesis. Our preliminary analyses show that CD5+ B cells are more abundant in CB and a few of them also express TCF1 and CD43. Most CD5+ B cells lack both CD43 and TCF1.
The spleen plays a critical role in the generation and maintenance of innate-like B cells. We are investigating the TCF1$^+$CD5$^+$CD43$^+$ B-cell population in human spleen to determine whether this organ represents a key physiological niche for these cells.
Together, these analyses aim to clarify the developmental origin, tissue distribution, and phenotypic definition of human innate-like B cells.

P77
Phenotypical and functional characterization of cytokine-producing Regulatory T cells in colorectal cancer

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Regulatory T cells (Tregs) are key players in determining the outcomes of immune responses. In cancer context, Tregs accumulate at the tumor site, and this condition is often associated with tumor growth promotion and neoplastic progression, as it may contribute to the inhibition of the antitumor immune response. In colorectal cancer (CRC), Treg presence in tumor milieu positively correlates with enhanced tumor control. However, the prognostic value of Tregs remains complex and controversial, depending on the molecular tumor microenvironment, cell interactions, and presence of different cell subtypes.
Recently, two Treg populations with differential IL-10 expression has been described to constitute two functionally distinct subsets in CRC, whose abundances correlate with opposite prognoses. In contrast to IL-10$^-$ cells, which exhibited pro-tumoral activity, IL-10$^+$ Treg contributed to restrain tumor growth by suppressing IL-17 production by CD4+ T cells. In CRC patients, this Treg subsets presented specific enrichment for gene modules that included a differential expression of Helios, IL2RA and STAT4.
In our CRC patient cohort, a preliminary flow cytometry analysis revealed a higher frequency of IL10 and IL17-producing Tregs in tumor and normal adjacent tissue (NAT) compared to peripheral blood, with IL-10$^+$ Tregs specifically enriched in NAT, consistent with the literature. These populations tend to be more abundant within the Treg subset expressing low Helios levels, particularly in tumor. In this microenvironment, IL-10$^+$ cells tend to exhibit higher CD25 expression, whereas IL-17$^+$ cells show increased CD71 expression, suggesting differential phenotypical, functional and metabolic features of the two populations. Finally, we observed a trend toward a positive correlation between IL-10$^+$ Treg frequency and the percentage of CD8$^+$ cells, confirming a beneficial role of this Treg population in cancer.
Furthering our understanding of the biology of these distinct subsets could help selectively target and, perhaps, harness specific characteristics for therapeutic benefit in various diseases.

P78
Immuno-antigenic deciphering of Pre-eclampsia

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Preeclampsia (PE) is a hypertensive disorder of pregnancy, affecting 2-8% of pregnancies worldwide, and remains a leading cause of maternal and foetal morbidity and mortality. It is characterized by new-onset hypertension after the 20th gestational week, often accompanied by proteinuria and multi-organ dysfunction. The pathogenesis of PE is multifactorial, involving placental dysfunction, immune dysregulation, and cardiovascular maladaptation. Currently, the only available treatment is delivery of the impaired placenta, which may lead to adverse postnatal outcomes for the offspring. Additionally, evidence suggests that offspring of PE mothers have greater propensity for cardiovascular disease in adulthood.
Research evidence suggests a possible role for the immune system in PE progression. Our own work has shown that pro-inflammatory T cells, kept in check by anti-inflammatory regulatory T cells, have fundamental roles in generating and/or progressing pathology in pregnancy (Aluvihare et al Nat Immunol 2004), and in cardiovascular disease (Kallikourdis et al Nat Comms 2017, Martini et al Circ Res 2020, Martini et al Circ Res 2025). We have also developed methods to decipher the antigens involved in such noxious adaptive immune responses (Cremonesi et al Circ 2023, Martini et al Circ Res 2025). In this ongoing project, we have applied these to PE, with promising findings for diagnosis and therapy.

TRANSLATIONAL IMMUNOLOGY

P79

BRD4 as a key modulator of IL-13RA2^{+} inflammatory fibroblasts in inflammatory bowel disease

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Aims/Purpose: Inflammatory-associated fibroblasts (IAFs), a subset of fibroblasts characterized by the expression of IL13RA2, are expanded in IBD patients and associated with PGE2-driven epithelial cell–damaging pathways. The intracellular pathways underlying the differentiation are unknown. BRD4 is a transcriptional regulator over-expressed in inflamed gut of inflammatory bowel diseases (IBD) patients and supposed to play a role in fibrogenesis in other settings. This study aims to investigate whether BRD4 regulates the differentiation of IAFs in IBD.
Methods: IAFs from IBD patients were characterized using publicly available single-cell RNA sequencing (scRNA-seq) datasets. Flow cytometry was used to assess BRD4 and IL13RA2 expression in fibroblasts isolated from mucosal samples of IBD patients. The role of BDR4 in the IAFs induction was also evaluated using BRD4-targeting antisense oligonucleotide and PROTAC.
Results: IBD IAFs expressed elevated levels of BRD4 and BRD4-related effector cytokines and chemokines. Patient-derived primary fibroblasts displayed elevated BRD4 levels but lacked IL13RA2 expression, indicating that fibroblasts lose their inflammatory phenotype under in vitro culture conditions. This phenotype could be restored upon stimulation with IL-13, IL-4 and TNF-α. Selective downregulation of BRD4 significantly reduced the percentage of cytokines-induced IAFs in vitro.
Conclusion: These findings suggest that BRD4 plays a key regulatory role in the differentiation/and or maintenance of IAFs in IBD.

P80

Invasive fungal infections and targeted therapies in chronic lymphocytic leukemia

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Humans are generally resistant to fungal infections; however, Aspergillus fumigatus remains a major cause of morbidity and mortality in immunocompromised patients. In chronic lymphocytic leukemia (CLL), targeted therapies, including kinase inhibitors and BCL-2 antagonists, have improved disease outcomes. However, these agents may interfere with immune pathways essential for antifungal defense, contributing to the increased incidence of invasive fungal infections, particularly invasive pulmonary aspergillosis. LC3-associated phagocytosis (LAP) is a critical non-canonical autophagy pathway in antifungal immunity, but its role in CLL patients receiving targeted therapies remains unclear. In this study, we demonstrate for the first time that venetoclax, unlike ibrutinib, induces LAP, enhancing clearance of Aspergillus and improving lung pathology. Based on these findings, we are investigating whether venetoclax has direct antifungal activity, characterized by impaired fungal growth, inhibition of germination, and fungal cell damage. In leukemic mouse models, treatment with venetoclax, alone or with ibrutinib, leads to leukemia remission and effective control of Aspergillus infection, as evidenced by reduced fungal burden and amelioration of pulmonary inflammation. Preliminary data from human CLL samples support the translational relevance of our findings, showing significant changes in patients treated with venetoclax plus ibrutinib. Overall, our results identify venetoclax as a key modulator of LAP-mediated antifungal immunity in CLL. Enhancing LAP may represent a novel therapeutic strategy to prevent or limit invasive aspergillosis, a life-threatening complication with high mortality in hematological malignancies. We are also testing other kinase inhibitors to explore their potential in modulating LAP-mediated antifungal responses. These findings contribute to

a deeper understanding of how targeted therapies modulate host immune mechanisms, such as LAP, in the context of fungal pathogen interactions, with potential implications for antifungal host defense strategies.

P81

Differential modulation of regulatory T cells by interleukin-33 in acute rejection and chronic lung allograft dysfunction

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Background: Interleukin-33 (IL-33) is a pleiotropic cytokine with a pivotal role in regulatory T cell (Treg) modulation. Although its role in lung transplantation (LTX) remains unclear, current evidence implicates IL-33 in acute rejection (AR) and chronic lung allograft dysfunction (CLAD).
Aim: To investigate the effect of IL-33 on Treg subsets and proliferative capacity in AR and CLAD patients.
Methods: Peripheral blood mononuclear cells were obtained from 6 AR patients, 9 CLAD patients with bronchiolitis obliterans syndrome (BOS), and 9 healthy controls (HC). Purified Treg cells were stimulated in vitro with IL-33 for 24 hours. Treg subsets were characterized by multicolour flow cytometry and proliferation was assessed by live-cell imaging.
Results: IL-33 increased Treg frequencies in HC and AR patients, whereas a significant reduction was observed in CLAD patients ($p<0.05$). In AR, IL-33 enhanced terminally differentiated Tregs, while CLAD patients showed an increasing trend in naïve CD45RA+CCR7+ Tregs. Across all groups, IL-33 significantly increased effector memory CD45RA-CCR7- Tregs. IL-33 stimulation reduced central memory CD45RA-CCR7+ Tregs in HC and CLAD ($p<0.05$), while a decreasing trend was detected in AR. PD-1 and CTLA-4 expression on Tregs was significantly downregulated in HC and AR after stimulation. Moreover, IL-33 induced a significant decrease in PD-1 expression ($p<0.01$) in CLAD patients. Following IL-33 stimulation, proliferation assays revealed a significant reduction in Treg proliferative capacity in CLAD compared to HC and AR groups.
Conclusions: IL-33 differentially modulates Treg subsets and functionality in AR and CLAD patients, acting as a contextual amplifier that reinforces effective immunoregulation in AR and reveals regulatory dysfunction in CLAD

P82

Harnessing tetraspanins: a first-incClass anti-MS4A4A antibody stimulates NK degranulation against M2 macrophages

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MS4A4A is a member of tetraspanin superfamily A (MS4A), which also comprises CD20 (MS4A1), a pan-B cell marker and a pivotal target for antitumor immunotherapy. Anti-CD20 antibodies can bind both the first and the second extracellular loop (Ofatumumab) or the second alone (Rituximab), because of the “eight-shape” resulting from a disulfide bridge. Conversely to CD20, MS4A4A expression pattern is restricted to innate immune cells, mainly to anti-inflammatory and pro-tumor M2 macrophages. Indeed, MS4A4A acts as a biomarker in several diseases, including autoimmune disorders and cancer. Thus, MS4A4A represents a promising target for diseases with high rate of M2-like macrophages. The present study aimed at generating an anti-MS4A4A antibody to evaluate its specificity and effector functions. Therefore, FACS analysis were carried out on both CHO-4A and human M2 macrophages from buffy coat, revealing specific binding to MS4A4A. Moreover, anti-MS4A4A antibody recognized CD20 on Raji cells. The cytotoxic assay highlighted autologous NK degranulation triggered by anti-MS4A4A antibody upon co-culture with M2 macrophages. Computational analysis by BLASTp and Abysis unmasked requirements for a feasible homology of the second extracellular loop conformation compared to CD20 and showed that the complementarity-determining regions (CDRs) of MIMAbs anti-MS4A4A antibody resembled Ofatumumab but not Rituximab CDRs. Accordingly, the first CD20 epitope partially covered the corresponding MS4A4A aligned sequence. Taken together, these results unravel the potentiality of MS4A4A as a novel therapeutic target in cancer immunotherapy and sustain the eligibility of MImAbs antibody for pre-clinical studies. Further studies will evaluate killing rate of both M2 and tumor-associated macrophages upon anti-MS4A4A antibody.

P83

Plasma IL-18 associates with impaired monocyte antibacterial function in non–small cell lung cancer

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Patients with non–small cell lung cancer (NSCLC) exhibit increased susceptibility to bacterial infections despite evidence of systemic inflammatory activation. We recently demonstrated a dissociation between exaggerated inflammatory responses and impaired phagocytic competence in circulating monocytes. Here, we investigated whether systemic IL-18, a key inflammasome-related cytokine, is associated with this dysfunctional antibacterial phenotype.

Plasma IL-18 levels were measured in NSCLC patients and healthy donors. Monocyte phagocytic capacity was assessed using pH-sensitive bacterial particle uptake assays following stimulation with heat-killed bacteria (Staphylococcus aureus, and Escherichia coli) and lipopolysaccharide (LPS). Surface expression of Toll-like receptors (TLR) was evaluated by flow cytometry.

NSCLC patients displayed significantly elevated circulating IL-18 levels compared with healthy donors. Higher plasma IL-18 concentrations were inversely correlated with LPS-induced monocyte phagocytic capacity ($\rho = -0.68$, $p < 0.05$). Similar inverse trends were observed for Staphylococcus aureus and Escherichia coli-induced phagocytosis, although these did not reach statistical significance. Elevated IL-18 levels were associated with a non-significant trend toward higher TLR2 and TLR4 expression on circulating monocytes.

These findings suggest that systemic IL-18 elevation in NSCLC is associated with impaired antibacterial effector function, potentially contributing to the functional uncoupling between inflammatory activation and microbial clearance. Rather than reflecting simple immune suppression, this pattern supports a model of chronic inflammasome-driven signaling linked to innate immune dysfunction.

Ongoing mechanistic studies aim to determine whether IL-18 directly modulates monocyte antibacterial competence or represents a biomarker of a broader inflammatory reprogramming state in NSCLC.

P84

Endothelial hyper-responsiveness to physiological estradiol fluctuations in women with hereditary angioedema

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Hereditary angioedema due to C1-inhibitor deficiency is a bradykinin-driven disorder of vascular permeability characterized by recurrent episodes of tissue edema In women, disease expression fluctuates during the menstrual cycle, suggesting hormone-dependent modulation of vascular responsiveness. Whether endogenous hormonal variations directly influence endothelial activation remains incompletely understood.

In a multicentre prospective study within the Italian ITACA network, fertile women with C1INH-HAE and age-matched healthy controls were evaluated during defined ovulatory and luteal phases. Plasma levels of luteinizing hormone, follicle-stimulating hormone, estradiol, progesterone, and vitamin D were measured together with endothelial activation mediators, including vascular endothelial growth factors (VEGF-A, VEGF-C, VEGF-D), angiopoietins (ANGPT1, ANGPT2), and secretory phospholipase A2. Complement components and patient-reported outcomes assessing disease activity, headache-related disability, and sleep quality were also recorded.

Hormonal concentrations did not differ between patients and controls across menstrual phases. In contrast, endothelial mediators were significantly increased in C1INH-HAE women. Phase-dependent modulation emerged exclusively in patients, with higher VEGF-C levels during ovulation. Estradiol levels directly correlated with VEGF-A concentrations only in C1INH-HAE, indicating disease-specific hormone–endothelial coupling. Clinically, disease activity was overall mild; however, ovulation was associated with increased headache-related disability, supporting a functional vascular susceptibility state.

These findings indicate that C1INH-HAE is characterized by an intrinsic endothelial hyper-responsiveness to physiological estrogen fluctuations rather than by systemic hormonal excess. Cyclical estradiol exposure may therefore amplify vasoactive signaling and precondition the vasculature to bradykinin-mediated permeability.

This hormone-dependent endothelial priming provides a mechanistic framework linking reproductive biology and vascular inflammation and identifies the endothelium as a potential immunomodulatory target in women with hereditary angioedema.

P85
High-dose intravenous immunoglobulin restores immune homeostasis in Long COVID: insights from longitudinal immunomonitoring

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Long COVID (post-acute sequelae of SARS-CoV-2 infection) is characterized by persistent multisystem symptoms and immune dysregulation, with limited therapeutic options. We report a clinically and immunologically detailed case of a previously healthy 39-year-old man with severe, treatment-refractory Long COVID, successfully treated with high-dose intravenous immunoglobulin (IVIg).
The patient presented with debilitating fatigue, cognitive impairment, and dysautonomia, associated with elevated autoantibodies against G protein–coupled receptors (GPCRs). Longitudinal immunomonitoring revealed profound T-cell dysregulation, including CD4/CD8 inversion, expansion of effector $CD8^+$ T cells, and spontaneous IFN-γ and TNF-α production. Notably, a population of $CD8^+$ T cell–$CD14^+$ monocyte complexes was identified, associated with persistent inflammatory activation. High-dose IVIg administration led to rapid and sustained clinical improvement, with normalization of fatigue, recovery of cognitive performance, and restoration of quality of life over one year. Immunologically, IVIg was associated with a progressive reduction in spontaneous cytokine production, disruption of pathogenic T cell–monocyte complexes, decreased endothelial activation markers, and a decline in circulating GPCR autoantibodies. These effects were dose-dependent and temporally correlated with clinical response. Mechanistically, our findings support a model in which IVIg modulates post-viral immune dysregulation by targeting both cellular and humoral pathways, including Fc receptor–mediated inhibition of immune synapse formation and neutralization of pathogenic autoantibodies. Although limited by its single-case design, this study identifies $CD8^+$ T cell–monocyte interactions and GPCR autoantibodies as potential biomarkers and therapeutic targets in Long COVID. These results provide a rationale for biomarker-guided immunomodulatory strategies and support further evaluation of high-dose IVIg in controlled clinical trials.

P86
High-parameter spectral phenotyping of platelets in familial chylomicronemia syndrome

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Background: Familial chylomicronemia syndrome (FCS) is a rare monogenic disorder of lipid metabolism leading to severe hypertriglyceridemia and to increased risk of pancreatitis. Thrombocytopenia has been observed in FCS patients. However, the relationship between triglyceride (TG)-rich lipoproteins, platelet function and the inflammatory complications of FCS are not fully understood.
Objective: To perform a multi-parametric analysis of platelet phenotype and functionality in patients with genetically confirmed severe chylomicronemia syndromes.
Methods: Eight FCS with a median [interquartile range] TG concentration of 1389 [936-2704] mg/dL and a platelet count of 160 [120.5-168.8]x103/µl, were compared to an age- and sex-matched cohort (n=16) of *Multifactorial Chylomicronemia Syndrome* (MCS) patients with a TG concentration of 269 [174-530] mg/dL and a normal platelet count (276 [214.5-295.8]x103/µl). Platelet responsiveness and phosphatidylserine exposure were measured by conventional flow cytometry. The platelet phenotype was characterized by spectral flow cytometry with a newly developed 16-color panel.
Results: FCS displayed a significantly larger fraction of circulating phosphatidylserine-positive and P-selectin negative platelets, i.e. apoptotic platelets (FCS: 14.8 [6.8-29.3]% vs MCS:0.4 [0.1-3.2]%). The percentage of apoptotic platelets was inversely related to the platelet count (p=.014), independently of age, TG and mutation burden. Upon stimulation, FCS platelets displayed an impaired response to all tested agonists. Multiparametric spectral phenotyping detected significantly higher platelet expression of CD44, CD36, CD42b, CD31 in FCS compared to MCS. The 'don't eat me signal" CD31 was the most distinctive feature of apoptotic platelets in FCS. The platelet hypo-responsiveness to agonists and the frequency of apoptotic platelets associated with a higher incidence of splenomegaly and pancreatitis.
Conclusions: In FCS the reduced platelet count is associated to an increase of apoptotic death, reduced responsiveness and a phenotypical shift of the circulating platelet pool. Ongoing mechanistic studies may provide insight on the relationship between TG-rich lipoproteins, platelets and the inflammatory complications of hypertriglyceridemia.

P87

Metabolic reprograming by oxidized lipids drives platelet dysfunction in metabolic dysfunction-associated steatotic liver disease

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Background: The innate immune system uses oxidized phospholipids (oxPLs) as a generic indicator of threat to the host. oxPLs accumulate in liver and serum of humans and mice with metabolic dysfunction-associated steatotic liver disease (MASLD) and neutralization of oxPLs prevents disease progression. MASLD is associated with an increased risk of cardiovascular disease and platelets have been implicated in MASLD pathogenesis; however, what triggers platelet activation and docking to the liver remain overlooked.

Aim and methods: Here we conducted a cross-sectional study to investigate if oxPLs contribute to platelet function in MASLD. The causal relationship between oxPLs and the platelet phenotype was investigated by comparing AMLN-fed Ldlr-/- mice, which develop MASH in 30 weeks, to AMLN-fed E06-scFv Ldlr-/-, that express a natural antibody (E06) that neutralizes oxPLs.

Results: At all stages of MASLD, circulating platelets displayed upregulation of both glycolytic and mitochondrial metabolism and a phenotypical and functional shift. Since the earliest stage of MASLD (simple steatosis), cellular and soluble markers of platelet secretion were detectable in blood. In advanced MASLD (MASH and cirrhosis), platelets were hypo-responsive to G-protein-coupled receptor stimulation and hyper-responsive to immune-like stimulation, which promoted a pro-inflammatory and procoagulant phenotype. In vivo, platelet functional reprogramming correlated with increased platelet mitochondrial activity and with plasma oxLDL levels. Ex vivo, oxPLs recapitulated the increased mitochondrial activity and functional reprogramming observed in MASH platelets. In a mouse model of MASLD, neutralization of oxPLs mitigated diet-induced platelet pre-activation and platelet binding to liver macrophages.

Conclusion: In conclusion, in MASLD, platelets are in a hypermetabolic and hyperactive state. Ex vivo and in vivo experiments identify oxPLs as key molecular links between systemic metabolic dysfunction and platelet metabolic and functional reprogramming. These findings provide a framework for developing early non-invasive biomarkers and new pharmacological strategies to prevent hepatic and extra-hepatic complications associated with MASLD.

P88

LINE1 RNA as a novel therapeutic target for T cell dysfunction in cancer and ageing

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T cell dysfunction, defined by defective effector responses, limited proliferative capacity, and persistent expression of inhibitory receptors, represents a major barrier to effective immunity in cancer and during aging. Although immunotherapeutic strategies have improved clinical outcomes, the molecular mechanisms driving dysfunctional T cell states remain incompletely understood, hindering the development of durable interventions. Here, we identify a previously unappreciated role for LINE1-containing transcripts in regulating T cell fate. LINE1 is the most abundant transposable element in the human genome, accounting for approximately 18% of genomic content, yet its functional relevance in immune cells has remained largely unexplored.

We demonstrate that specific LINE1-derived splicing variants are retained at chromatin in quiescent T cells, where they repress transcription of key effector genes. Upon T cell activation, LINE1 transcripts expression is normally downregulated through a PTBP1-dependent post-transcriptional mechanism. This regulatory pathway is defective in dysfunctional T cells, resulting in aberrant accumulation of LINE1 transcripts. Notably, LINE1 transcripts progressively accumulate over the human lifespan, from low levels in neonatal T cells to markedly elevated levels in elderly individuals, contributing to immune senescence. A comparable increase is observed in TILs, where LINE1 accumulation correlates with impaired anti-tumor activity.

Mechanistically, we show that LINE1 transcripts negatively impact global protein synthesis and cell cycle progression, two tightly regulated processes essential for effective T cell responses. Importantly, pharmacological targeting of LINE1 transcripts using antisense oligonucleotides restores T cell functionality in both aged and tumor-associated contexts. LINE1 inhibition rescues protein synthesis, enhances effector cytokine production, reduces inhibitory receptor expression, and reinforce cytotoxic capacity.

Collectively, our findings uncover LINE1 RNA as a novel nuclear immune checkpoint that constrains T cell function through control of translational and

proliferative programs. The reversibility of this pathway highlights LINE1 targeting as a promising immunotherapeutic strategy to restore immune competence in cancer and aging.

TUMOR IMMUNOLOGY

P89

Exploiting the ferroptotic vulnerability of malignant pleural mesothelioma to prime the immune microenvironment

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Malignant pleural mesothelioma (MPM) is a highly aggressive, therapy-resistant malignancy. While immune checkpoint blockade (ICB) offers benefits for non-epithelioid subtypes, many patients remain unresponsive due to low tumor immunogenicity and an immunosuppressive microenvironment. Ferroptosis, an iron-dependent form of immunogenic cell death (ICD), represents a promising therapeutic avenue to reshape the tumor microenvironment (TME); however, the relative susceptibility of different MPM histotypes remains poorly defined. We investigated ferroptosis vulnerability in human epithelioid (REN, BR95, NCI-H2452), biphasic (MSTO-211H), and sarcomatoid (NCI-H2052) MPM cell lines. Treatment with the GPX4 inhibitor RSL3 revealed heterogeneous sensitivity: sarcomatoid cells—typically the most chemoresistant—exhibited the highest vulnerability, while epithelioid lines were markedly resistant. Cell death was confirmed by lipid-ROS accumulation and rescued by Ferrostatin-1. Mechanistically, sarcomatoid sensitivity correlated with higher basal labile iron pool (LIP) levels. Conversely, epithelioid resistance was associated with increased antioxidant defenses, specifically Ferroptosis Suppressor Protein-1 (FSP1) and aldo-keto reductases (AKR1C1–3). In multicellular spheroids containing tumor cells and monocytes (MCS-Mo), the GPX4-degrader N6F11 selectively induced ferroptosis in MPM cells while sparing primary monocytes and T lymphocytes.
In conclusion, our findings demonstrate that GPX4 targeting is a potent strategy for hitting aggressive MPM subtypes, while blocking FSP1 and AKR1C1-3 could broaden the efficacy of these pro-ferroptotic strategies across all MPM histotypes. Furthermore, as a form of ICD, ferroptosis can reshape the TME, turning "cold" MPM into "hot" tumors more susceptible to immune-mediated clearance.

P90

Innate immune alterations in bladder cancer: evidence of NK cell dysfunction and monocyte reprogramming

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Background: Bladder cancer (BC), particularly the muscle-invasive form (MIBC), is characterized by poor clinical outcome and limited response to immunotherapy, largely due to immune dysfunction within the tumor microenvironment (TME). Innate immune cells play a pivotal role in regulating tumor immunity, yet their contribution to immune escape in BC remains incompletely defined. This study investigates phenotypic and functional alterations of circulating NK cells and monocyte subsets in BC patients.
Methods: Peripheral blood mononuclear cells were isolated via Ficoll-Hystopaque density gradient centrifugation from BC patients (n = 17) and healthy donors (n = 12). Flow cytometry was used to characterize monocyte and NK cell subsets, focusing on markers associated with activation, inhibition, and immunomodulatory functions. NK cell activity was assessed by degranulation (CD107a) and cytotoxicity assays against K562 target cells.
Results: BC patients exhibited a redistribution of circulating monocytes toward the intermediate $CD14^{+}CD16^{+}$ subset, consistent with enhanced immune regulatory potential. NK cells displayed marked phenotypic remodeling and preserved degranulation capacity compared to controls, suggesting chronic or dysregulated activation rather than effective cytotoxic function.
Conclusions: Our data reveal profound innate immune remodeling in BC, characterized by monocyte plasticity and NK cell functional imbalance. The coexistence of preserved NK degranulation with dysfunctional immune features may contribute to tumor immune evasion. These alterations highlight innate immune cells as relevant biomarkers and potential targets for improving immunotherapeutic responses in BC.

P91
Lipid-loaded macrophages promote tumor fibrosis and immunotherapy resistance in melanoma

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Immune checkpoint inhibitor (ICI) resistance is profoundly shaped by the tumor microenvironment, which suppresses T-cell-mediated anti-tumor immunity through multiple mechanisms. Among the major cellular mediators of this process are tumor-associated macrophages (TAMs), the predominant immune infiltrate in many solid tumors, which actively sustain tumor progression and therapeutic resistance. Emerging evidence indicates that metabolic rewiring critically influences macrophage phenotype and function. In this context, lipid-laden macrophages (LLMs) have been identified across several malignancies, including prostate cancer, ovarian cancer, and melanoma; however, the mechanisms driving lipid accumulation and the functional consequences of this phenotype remain poorly understood.

To investigate the biology of LLMs in melanoma, we combined high-throughput approaches including single-cell RNA sequencing, bulk transcriptomics, and multiparametric flow cytometry. Our analyses confirmed that lipid accumulation is a defining feature of tumor-infiltrating macrophages and positively correlates with tumor burden. Furthermore, lipid-loaded TAMs displayed increased expression of immunosuppressive markers, including PD-L1 and Arginase-1, supporting their contribution to immune evasion and resistance to ICIs.

Importantly, transcriptomic profiling revealed that LLMs acquire a robust pro-fibrotic transcriptional program characterized by enhanced expression of collagen and extracellular matrix (ECM)-related genes. Mechanistically, we identified activation of the transcriptional co-regulator YAP1 in lipid-loaded macrophages. Both genetic ablation and pharmacological inhibition of YAP1 significantly reduced collagen production and suppressed ECM-associated gene and protein expression in macrophages. Consistently, in vivo studies confirmed the presence of collagen-producing LLMs within the tumor microenvironment, indicating their direct contribution to tumor-associated fibrosis. Moreover, melanoma patients resistant to ICIs exhibited an enrichment of pro-fibrotic macrophage signatures.

Overall, our findings uncover a mechanistic link between lipid metabolism, macrophage reprogramming, fibrosis, and immunotherapy resistance in melanoma. These results identify YAP1-dependent pro-fibrotic activity in lipid-loaded macrophages as a potential therapeutic target to enhance the efficacy of cancer immunotherapy.

P92
Release of tumor antigens drives systemic immune evasion through hepatocyte cross-presentation

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Cancer immunotherapy has shown promising results, yet its efficacy remains limited in patients with advanced gastrointestinal tumors (GIT). Here, we identify a liver-imposed mechanism of immune escape whereby trafficking of tumor antigens (TAs) from GITs induces systemic T cell dysfunction.

Using mouse models bearing GITs engineered to express fluorescent TAs secreted as soluble proteins, we found that circulating TAs were uptaken and cross-presented by hepatocytes. Hepatocyte-mediated presentation of TAs reprogrammed tumor-specific $CD8^+$ T cells into a dysfunctional phenotype, enabling immune escape of TA-expressing tumor clones and promoting tumor progression.

Immunopeptidomic analysis of human colorectal cancer (CRC) samples revealed the presence of TAs in histologically normal liver tissue, supporting direct TA transfer and presentation in the livers of cancer patients. Consistently, transcriptional signatures of hepatocyte-educated T cells in patients with GITs correlated with poor survival.

In mouse models, interfering with hepatocyte-mediated TA cross-presentation reduced immune suppression and T cell dysfunction. Therapeutically, treatment with an agonistic monoclonal antibody targeting 4-1BB restored T cell function, eliminated TA-expressing tumor cells, and suppressed tumor growth in a CRC mouse model.

Collectively, our findings uncover a novel liver-mediated immunosuppressive pathway that limits antitumoral immune efficacy in GITs and identify 4-1BB agonism as a strategy to overcome tumor-induced T cell dysfunction.

P93

Immunological characterization of the tumor microenvironment in Non-Muscle Invasive Bladder Cancer: preliminary analysis of T lymphocyte populations and immune checkpoints

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High-risk non-muscle-invasive bladder cancer (NMIBC) exhibits high recurrence rates despite transurethral resection of the bladder (TURB) and intravesical immunotherapy with Bacillus Calmette-Guérin (BCG). The tumor microenvironment (TME) modulates the immune response through the exhaustion of effector T cells and the accumulation of immunosuppressive populations, such as regulatory T cells (Tregs), which may represent potential prognostic biomarkers of recurrence.
Peripheral blood (PB), healthy tissue (HT), and tumor tissue (TT) samples were collected at the time of TURB from 12 patients with high-risk, BCG-naïve NMIBC. Immunophenotypic characterization was performed by multiparametric flow cytometry using a 22-parameter panel (BD FACSymphony™ A5SE) encompassing: markers of naïve/memory differentiation (CD45RA, CCR7, CD28), regulatory phenotype (CD127, CD25, FoxP3, CD39), tissue residency (CD103), senescence (CD57), homing and trafficking (CXCR4, CXCR7), cytotoxic function (Granzyme B, EOMES), and exhaustion (PD-1, TIM-3, LAG-3, TIGIT).
The analysis of $CD8^+$ Tregs was challenging given that this is a physiologically rare population whose detectability was frequently limited by the low cellular yield of tissue samples; nonetheless, expression of $PD\text{-}1^+$, $TIM\text{-}3^+$, and $CD39^+$ was detected in analyzable tissues. Within TT, $CD4^+$ Tregs exhibited marked upregulation of $CD39^+$, $TIM\text{-}3^+$, and PD-1+, along with an expansion of the $CD28^+CD39^+$ fraction, indicative of a progressive loss of the naïve phenotype and acquisition of an activated-suppressive profile. Conventional $CD4^+$ and $CD8^+$ T lymphocytes displayed altered expression of $PD\text{-}1^+$, $TIGIT^+$, $LAG\text{-}3^+$, and $TIM\text{-}3^+$ in TT, consistent with functional exhaustion and TME-induced dysfunction. Conventional $CD8^+$ T lymphocytes showed a reduction in both the $CD57^+GranB^+$ and $CD57^-GranB^+$ subsets, suggesting an impaired effector cytotoxic profile.
These preliminary findings delineate a TME characterized by a suppressive phenotype of regulatory populations and signs of functional exhaustion of conventional T lymphocytes.

P94

Unveiling the role of lipid-loaded macrophages in cancer

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High-grade serous ovarian cancer (HGSOC) is the most common and aggressive subtype of ovarian cancer and it is characterized by high lethality and resistance to current therapies. Tumor-associated macrophages (TAMs) are key players in the tumor microenvironment (TME), sustaining tumor growth and promoting an immune suppressive TME. Within the TME of HGSOC patients, we identified lipid-loaded macrophages (LLMs) as a distinct subset of TAMs, characterized by intracellular lipid accumulation. The presence of LLMs positively correlates with tumor progression and poor prognosis. Based on these observations, we aimed to investigate the contribution of LLMs in shaping the TME in HGSOC. To this aim, we profiled the TME using an orthotopic murine model of ovarian cancer, in which UPK10 cells were directly injected into the ovarian bursa. Flow cytometry analysis revealed the presence of LLMs displaying a pro-tumorigenic phenotype. Building on these findings, we generated LLMs in vitro that displayed pro-tumoral features, including increased expression of immunosuppressive markers as their in vivo counterparts. Notably, we also detected LLMs in other tumor types, including prostate and breast cancer, highlighting a broader, cross-tumor role for this macrophage population in promoting an immunosuppressive TME. To further characterize this macrophage subset and define its transcriptional signature, LLMs and non-LLMs were sorted from murine models of prostate, breast, and ovarian tumors. Through comparative bulk transcriptomic analysis we identified differentially expressed genes shared across the three tumor models. Using top ranked genes (fold change ≤ -0.25 or ≥ 0.25, p-value ≤ 0.05), we generated a CRISPR-library to identify regulators of lipid accumulation and pro-tumoral activity in LLMs. Overall, our findings highlight LLMs as a tumor supporting subpopulation of TAMs in HGSOC. Identifying important regulators of lipid accumulation may uncover new therapeutic targets to enhance therapy responses and improve patient outcomes.

P95
Investigating B cells in the immune landscape of high-grade serous ovarian cancer

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High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy, with relapse occurring in ~75% of patients despite initial response to platinum-based chemotherapy. In the absence of reliable predictive biomarkers, recurrence remains difficult to manage clinically. Growing evidence indicates that immune cells within the tumor microenvironment influence tumor progression and therapeutic response. Among them, B-cells can act not only as antibody-secreting cells (ASCs) but also as antigen-presenting cells (APCs) for T-cells, which are known contributors to HGSOC progression. However, the prognostic significance of B-cells in this context remains unclear.

We enrolled 31 patients and analyzed B-cells from peritoneal biopsies by combining flow cytometry, immunohistochemistry, and immunofluorescence. Tumor tissues compared to healthy controls displayed enrichment of total $CD19^+$ B-cells and ASCs, along with a reduction in potentially active $CD19^+CD21^+$ B-cells, suggesting terminal differentiation and local exhaustion of B-cells. Longitudinal analysis of matched samples collected before and after neoadjuvant chemotherapy at interval debulking surgery (IDS) revealed partial recovery of $CD19^+CD21^+$ B-cells, indicating possible functional reactivation.

Stratification by clinical outcome showed that relapsing patients exhibited lower $CD19^+$ B-cells infiltration, fewer ASCs, and a higher proportion of $CD27^-IgD^-$ double-n5egative B-cells, suggesting that early B-cell profiles may correlate with therapeutic response.

Given the interplay between B-cells and T-cells within tumors, we examined their spatial relationship through immunohistochemistry, observing colocalization within lymphoid aggregates both before and after treatment. Although B-cell abundance at IDS did not consistently differ between responders and relapsing patients, baseline samples from responders presented more numerous and better-organized lymphoid aggregates, indicating a more favorable immune microenvironment for an effective anti-tumor activity.

Overall, our findings suggest that B-cells may serve as early indicators of treatment response in HGSOC, particularly within lymphoid structures where they can function as both APCs and ASCs, supporting further investigation into B cell role in this tumor setting.

P96
Single-cell RNA profiling of mature polymorphonuclear-myeloid-derived suppressor cells and tumor-associated neutrophils from cancer patients

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Human polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs) consist of circulating low-density neutrophils (LDNs) characterized by the ability to inhibit T-cell responses. In previous studies, we demonstrated that the mature fraction of PMN-MDSCs (i.e. mPMN-MDSCs) exerts more potent immunosuppressive functions than its immature counterpart. More recently, we defined a specific gene signature of mPMN-MDSCs from cancer patients and G-CSF-treated donors (GDs) by bulk RNA sequencing (RNA-seq) experiments. In this study, by performing single-cell RNA-seq (scRNA-seq) experiments of circulating mPMN-MDSCs from non-small cell lung cancer (NSCLC) patients, we identified a major scRNA-seq cell cluster (arbitrarily named NSCLC c6) specifically displaying immunosuppressive and protumor transcriptomic features. Then, by analyzing publicly available scRNA-seq datasets from human tumor-associated neutrophils (TANs), we uncovered three TAN clusters (arbitrarily named TAN c6-c8) that were found to share with NSCLC c6 several common genes and transcription factor (TF) regulons associated with response to hypoxia, positive regulation of angiogenesis, and metabolic reprogramming. Furthermore, by performing scRNA-seq experiments of GD mPMN-MDSCs, we uncovered four scRNA-seq cell clusters (arbitrarily named GD c4-c7) that were enriched for the same genes and pathways characterizing NSCLC c6 and TAN c6-c8 cells. Altogether, these data uncover that human circulating mPMN-MDSCs and TANs from different cancer types share scRNA-seq cell clusters with transcriptomic similarities, supporting the notion that they might be strictly related.

P97

Implementation of Patient-derived Tumor Explant 3D-model to Assess the Efficacy of Novel Therapies

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Cancer research is advancing rapidly, yielding numerous promising therapeutic candidates. However, the capacity of clinical trials and resource constraints represent a major bottleneck for the evaluation of these emerging therapies. To address this challenge, we have implemented a sophisticated ex vivo approach—patient-derived tumor explants (PDTEs). This platform allows the culture of human tumor tissues while preserving the native architecture and complex cellular ecosystem of the tumor microenvironment for several days. Our findings on established PDTE cultures from tumors demonstrate that this platform enables monitoring of key immune populations, including T cells, macrophages, and dendritic cells allowing for comprehensive assessment of their phenotypes and states in response to drug treatments.

T regulatory cells (Treg), endowed with functional and transcriptional plasticity, are major tiebreakers in cancers. Consequently, selective targeting of tumor-infiltrating Tregs is a pivotal strategy in novel treatments, minimizing the risk of autoimmunity that arises from systemic Treg depletion. Ikaros zinc-finger transcription factor family members HELIOS (Ikzf2) and EOS (Ikzf4) have been identified as preferentially expressed in tumor infiltrating Treg, and their *in-vitro* and *in-vivo* perturbation has proven to lead to Treg instability and consequent acquisition of effector functions along with enhanced antitumor immunity, making them perfect targets for immunotherapy.

We leveraged PDTEs from colorectal and breast cancer to assess the effects of a new small molecule that selectively degrades HELIOS and EOS. Upon treatment we confirmed a loss of stability occurring in tumor infiltrating Treg highlighted by an increase of the activity for IFN response signatures at single-cell transcriptional level. This was accompanied by enhanced activation of effector $CD4^+$ and $CD8^+$ T cells. Collectively, our findings highlight the utility of PDTEs as a physiologically relevant platform for functional drug testing and underscore the therapeutic potential of HELIOS and EOS degradation in reprogramming Treg function to boost anti-tumor immunity.

P98

Zinc finger E-box Binding homebox 1 restrains stress-induced antigen presentation and shapes immune escape in Acute Myeloid Leukemia

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Introduction: Acute myeloid leukemia (AML) is a hematologic malignancy characterized by the expansion of immature myeloid blasts and disruption of bone marrow homeostasis. The disease displays marked cellular and immunologic plasticity, allowing leukemic cells to adapt to therapeutic and inflammatory pressures. ZEB1, a chromatin remodeling transcription factor known to regulate cellular plasticity, has been shown to shape the immunological niches of AML by suppressing $CD8^+$ T cell activity while fostering Th17 cell expansion. We examined whether ZEB1 also controls major histocompatibility complex (MHC) antigen presentation pathways to foster immune tolerance and leukemic escape.

Methods: Single-cell RNA sequencing using the 10x Genomics platform profiled 58,856 cells from 14 AML patient samples. Blasts were stratified into ZEB1-high and ZEB1-low subsets, followed by differential expression and Hallmark gene set enrichment analyses. In a murine cell line (C1498 NPMc+), single-cell subcloning correlated ZEB1 levels with antigen presentation machinery. Functional experiments in C1498 NPMc+ and human K562 cells employed lentiviral ZEB1 silencing. Antigen processing components were quantified by RT-PCR and ChIP-qPCR at baseline and after cytarabine (Ara-C) 0,5 µg/ml–induced stress. In vivo studies assessed T cell-mediated control, immune escape, and memory.

Results: scRNA-seq analysis revealed that antigen processing and presentation pathways (MHC I-II) were significantly downregulated in ZEB1-high blasts compared to ZEB1-low. Subcloning confirmed correlations between ZEB1 and key MHC-I genes. ZEB1 depletion reduced ribosomal transcripts including RPL28 and RPL38, and impaired antigen supply regulators such as TAP1, CUL3, and B2M. After cytarabine, TAP1 and B2M increased exclusively in ZEB1-deficient cells, indicating that ZEB1 restrains inflammation-induced presentation. ChIP-seq identified direct ZEB1 binding at HLA-A and RPS28, supporting context-dependent remodeling. In vivo, ZEB1 loss initially enhanced T cell control but ultimately permitted aggressive immune escape under sustained stress.

Discussion: Overall, ZEB1 links leukemic plasticity with immune regulation, and its targeting may potentiate antileukemic immunity.

P99

FcγRIIIA (CD16)$^+$CD8$^+$ T cells emerging following mRNA vaccination for COVID-19 accumulate in human colorectal cancer

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Emerging reports revealed the potential of mRNA-based COVID-19 vaccines to stimulate anticancer immunity and promote tumor regression. It was shown that two doses of mRNA COVID-19 vaccine can enhance the infiltration of cytolytic CD8+ T cells expressing TIGIT. We have recently observed that mRNA COVID-19 vaccination expands a subset of CD8$^+$ T cells bearing CD16 and endowed with both innate cytotoxic potential and antibody-dependent cell cytotoxicity (ADCC). Here, we report that this CD16$^+$CD8$^+$ T cell subset expresses TIGIT, high levels of activating receptors, and shows superior killing properties against cancer cells. Notably, CD16$^+$CD8$^+$ T cells accumulate in colorectal cancer (CRC) tissues of patients who had previously received mRNA COVID-19 vaccination. They responded to SARS-CoV-2 peptides and displayed a marked expression of ICOS, a costimulatory molecule involved in the generation of CD8+ tissue-resident populations. Interestingly, evaluation of CD16 expression on CD8$^+$ T cells within CRC tissue reveals an increased frequency of CD16$^+$ CD8$^+$ T cells, which localize in close proximity to tumor nests. Taken together, these observations raise the hypothesis that mRNA COVID-19 vaccination might enhance antitumor immunity by promoting the expansion of CD16$^+$CD8$^+$ T cells capable of infiltrating human tumors and potentially mediating cytotoxic responses against IgG-coated cancer cells.

P100

Linking histone lactylation to the epigenetic reprogramming of CD4+ T regulatory lymphocytes in the tumor ecosystem

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Metabolic restrictions and suppressive cells in the tumor microenvironment (TME) limit immunotherapy effectiveness. FOXP3$^+$ regulatory T cells (Treg) are key mediators of immune suppression and adapt metabolically to the hypoxic, lactic acid-rich and nutrient-deprived TME. Recent evidences from murine models indicates that tumor-infiltrating Tregs (TI-Tregs) preferentially utilize lactic acid to foster their suppressive activity in the TME. Given that the epigenome links extracellular cues to cellular behavior, we are exploring if lactate-derived histone lactylation affects the epigenetic landscape of TI-Tregs to promote their adaptation to the TME. Using cutting-edge single-cell omics technologies, we identified increased expression of lactate metabolism-related and immunosuppressive genes in Tregs from colorectal cancer (CRC) compared to Tregs from normal colon tissue. Additionally, Tregs located in close spatial proximity to highly glycolytic, hypoxic and lactic-acid rich tumor niches exhibited elevated expression of immune-checkpoint molecules and maintained a strong immunosuppressive activity. Afterwards, we quantified global histone and non-histone lactylation in human primary TI-Tregs with a flow-cytometry based approach and detected elevated signals across tumor types. Relatedly, lactylation in Tregs was induced and modulated by key TME-like cues, like hypoxia and lactic acid and was profoundly enhanced when Tregs were exposed to both stimuli concurrently. Considering our interest in decoding the epigenetic relevance of lactylation, we explored its subcellular distribution and observed a predominant nuclear localization. Most remarkably, integrated ChIP-seq and bulk-RNA-seq analysis, revealed enriched lactylation at the transcription start site of hypoxia and lactic acid-responsive genes, linking lactylation deposition to TME-driven transcriptional programs for the first time in Tregs. Next, we leveraged post-translational modification mass-spectrometry of human primary Tregs and singled-out three lactylated histone lysines as candidate epigenetic regulatory hubs responsive to hypoxia and lactic acid. Profiling newly discovered lactylated histone lysines in TI-Tregs may uncover therapeutic targets to limit their suppressive activity via metabolic and epigenetic pathways.

P101
HSP70-Driven Cytoskeletal Remodeling and Metalloproteinase Activation Enhance Cancer Cell Invasiveness

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Introduction: Breast cancer is one of the leading causes of cancer-related mortality among women worldwide, particularly in developing countries. Heat shock proteins (HSPs) and heat shock factor 1 (HSF1) are central components of the heat shock response (HSR), which has been implicated in breast cancer development. Previous studies indicate that extracellular HSP70 exhibits strong motogenic activity in fibroblasts, vascular, and epithelial cells, and can promote angiogenesis and extracellular matrix remodeling. These findings suggest a potential role for HSP70 in tumor progression and dissemination. Therefore, this study investigated the effect of HSP70 on the migration and invasion capacity of breast cancer cells in vitro.
Materials and Methods: Cellular motility and invasion were studied using the human breast cancer cell line MDA-MB-231, which reflects characteristics of triple-negative breast cancer in vivo. Stimulation with recombinant human HSP70 was examined in several cancer cell lines (PANC-1, MDA-MB-231, and JAR) using migration and invasion assays. Gelatin zymography was used to evaluate the activity of matrix metalloproteinases MMP-2 and MMP-9 during invasion. Cytoskeletal rearrangements induced by rh-HSP70 stimulation in MDA-MB-231 cells were analyzed using double immunofluorescence staining of actin and tubulin.
Results: Extracellular HSP70 significantly increased migration and invasion in multiple human cancer cell lines. During migration, MDA-MB-231 cells exhibited pronounced cytoskeletal reorganization dependent on HSP70 stimulation. Additionally, HSP70 promotes activation of MMP-2/MMP-9, an enzymes associated with cell migration, invasion, and metastasis.
Conclusion: These findings indicate that HSP70 may facilitate cancer cell invasion and extravasation, key steps in metastatic dissemination. Targeting HSP70-mediated pathways, and MMP-2/MMP-9 activity, could represent a potential therapeutic strategy for preventing the formation of a tumor-permissive microenvironment and limiting metastasis.
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P103
Inflammatory hyperactivation Is uncoupled from antibacterial effector function in non–small cell lung cancer

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Patients with non–small cell lung cancer are highly susceptible to pulmonary infections, a phenomenon traditionally attributed to cancer-associated immunosuppression. However, whether innate immune dysfunction reflects reduced immune activation or a qualitative functional rewiring remains unclear. We hypothesized that antibacterial immunity in non–small cell lung cancer (NSCLC) is characterized by a dissociation between inflammatory sensing and effector function.
Peripheral blood monocytes from treatment-naïve patients and healthy donors were stimulated with heat-killed bacteria or lipopolysaccharide. Cytokine production, Toll-like receptor (TLR) expression, and phagocytic activity were assessed using functional and flow cytometric approaches. Human lung macrophages (HLMs) isolated from surgical specimens of NSCLC patients were analysed to determine whether systemic alterations were mirrored at the tissue level.
Contrary to the concept of global immune suppression, monocytes from patients displayed exaggerated inflammatory responses following bacterial stimulation, producing higher levels of TNF-α, IL-6, IL-1β and CXCL8, together with reduced IL-10 induction. This hyperinflammatory phenotype was associated with an expanded fraction of TLR2 and TLR4-positive monocytes. Strikingly, despite enhanced inflammatory activation, patient monocytes exhibited impaired phagocytosis and reduced HLA-DR expression, indicating defective antibacterial competence.
HLMs of NSCLC patients reproduced this functional pattern, showing preserved inflammatory responsiveness but incomplete or stimulus-dependent phagocytic activation. Notably, the hierarchy of stimulus potency was preserved across circulating and tissue-resident compartments, with identical stimulus ranking in monocytes and lung macrophages, supporting a shared hierarchy of innate immune activation.
These findings challenge the paradigm of simple immune suppression in lung cancer and instead identify a state of inflammatory hyperactivation with impaired antibacterial function. This function-

al decoupling of innate immunity provides a mechanistic explanation for infection susceptibility in NSCLC and suggests that restoring immune balance, rather than boosting inflammation, may represent a more effective therapeutic strategy, with potential implications not only for antibacterial host defence but also for anti-tumor immune competence.

P104

Role of cancer-associated fibroblasts in pancreatic adenocarcinoma progression through the modulation of neutrophil pro-tumoral activity

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy projected to become the second leading cause of cancer-related mortality within the next decade. Among solid tumors, PDAC is characterized by delayed diagnosis due to nonspecific symptomatology and the absence of reliable early diagnostic biomarkers. The pro-tumorigenic microenvironment in PDAC is sustained by a highly fibrotic stroma and extensive infiltration by immunosuppressive cell populations, including tumor-associated macrophages, regulatory T cells, and myeloid-derived suppressor cells. The multifaceted and often opposing roles of neutrophils in cancer have been highlighted; however, clinical evidence supports the notion that neutrophils promote cancer progression through the release of myeloperoxidase (MPO), neutrophil elastase (NE), and matrix metalloproteinase-9 (MMP-9), which degrade the extracellular matrix and activate downstream signaling pathways — including NF-κB — thereby stimulating angiogenesis and facilitating tumor advancement. Defining which components of the tumor microenvironment contribute to tumor progression remains a critical unresolved question.
Using spectral cytofluorimetry, we characterized the phenotype of circulating and tumor-infiltrating neutrophils in 25 treatment-naïve patients with pancreatic cancer at initial diagnosis. Peripheral blood-derived neutrophils were further assessed for their functional properties through immunofluorescence assays and monitored in a time-course experimental setting. Our findings demonstrate that pancreatic tumor cells drive the establishment of a microenvironment promoting neutrophil activation, whereas cancer-associated fibroblasts sustain this activated state over time. Secretome analysis revealed distinct molecular mediators produced by fibroblasts that maintain neutrophil functional competence, as well as molecules associated with angiogenesis and metastatic dissemination, distinguishing fibroblast-derived signals from those originating from tumor cells. In conclusion, our data delineate distinct and complementary contributions of tumor cells and cancer-associated fibroblasts in maintaining neutrophil activation within the tumor microenvironment of pancreatic cancer patients. Collectively, these results may inform the design of novel immunotherapeutic approaches targeting neutrophil activation pathways, with the potential to interfere with tumor-promoting processes within the pancreatic cancer microenvironment.

P105

Eosinophil Trogocytosis of Tumor-Derived PD-1 via the PD-L1/CD11b/CD18: A Potential Mechanism of Resistance to PD-1 Immunotherapy

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Trogocytosis, despite being a relatively rare event, has remained largely understudied and underestimated among the mechanisms of cancer immune evasion. Nevertheless, accumulating evidence indicates that it can profoundly influence immune cell function and tumor–immune interactions. We recently showed that IL-33–activated eosinophils acquire tumor-derived membrane fragments through the CD11b/CD18 integrin complex early after contact. Here, we examined whether eosinophils can acquire immune checkpoints from tumor cells, specifically PD-1, as a process with potential implications for immunotherapy resistance. Eosinophils stimulated with IL-5 (EO-5) or IL-33 (EO-33) do not intrinsically express PD-1. However, following co-culture with PD-1–expressing EG.7-OVA lymphoma or RBL-5 leukemia cells, EO-33, but not EO-5, acquired PD-1 through trogocytosis. This transfer required CD11b/CD18 signaling, which mediates eosinophil–tumor cell contact. Consistently, RNA-seq analysis revealed enrichment of pathways related to cell adhesion, immune synapse organization, and membrane dynamics in EO-33 relative to EO-5. Eosinophils displayed low PD-L2 but detectable PD-L1 expression, which was further upregulated by IL-33 and co-localized with tumor-derived PD-1 during co-culture. Blocking PD-1/PD-L1 interaction with anti–PD-L1 antibody abolished PD-1 acquisition by EO-33, demonstrating a receptor–ligand-dependent mechanism. FRET analysis supported the physical association of CD11b/CD18 with the PD-1/PD-L1 axis, suggesting that this complex stabilizes the immunological synapse during trogocytosis.

Sequential protein docking further confirmed the structural feasibility of such a supercomplex. The uptake of tumor-derived PD-1 through trogocytosis could modulate eosinophil signaling, reshape the tumor immune microenvironment, and potentially contribute to reduced responsiveness to PD-1–targeted immunotherapies.

P106

5-ALA-dye assisted surgery reveals inhibitory, layer-specific, immune cells transcriptional profile and an enrichment of tumor Granzyme K+ T and NK cells

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Introduction and Aim: Glioblastoma (GBM) tumor microenvironment (TME) is characterized by immune infiltrate and by different metabolic areas, so we hypothesize that different immune cells could play a specific role in anti-tumor response and glioblastoma development, according to their localization in the TME.

Methods and Results: sc-RNAseq, including TCR-seq and Cite-seq, was performed on peripheral blood (PB) and on different GBM layers (intermediate and marginal) identified with 5-ALA-dye assisted surgery, from 3 patients. Bioinformatic analysis revealed substantial differences in activation, effector function, inhibitory signalling and metabolic pathways among PB and tumor layers. GBM naïve $CD4^+$ T cells retain a quiescent phenotype, whereas infiltrating Th effector/memory and Th1/Th17 cells display features of chronic activation with upregulated inhibitory checkpoints, non-classical cytotoxic programs, and a tissue-resident memory (Trm) signature. Tumor is enriched of Treg cells, expressing broad inhibitory receptors and immunoregulatory cytokines and of $CD8^+$GZMK$^+$ T cells, expressing low GZMB (granzyme B) and GNLY (granulysin). Clonally expanded GBM $CD8^+$GZMK$^+$ T cells, identified by single-cell TCR sequencing, exhibit in the intermediate region a stress- and biosynthesis-driven transcriptional program, whereas in the marginal area display migratory and immune-regulatory features. Tumor was poorly infiltrated by NK cells, predominantly CD56bright, enriched of GZMK$^+$ cells, with markers of exhaustion and a Trm phenotype. Several T and NK cell subsets from marginal layer are characterized by enriched expression of CXCR4 and interactome analysis suggests gained interaction with CXCL12+ macrophages.

Conclusions: These data support the concept that glioblastoma represents an immunologically active but metabolically constrained tumor, where immune cells are present, clonally engaged, and functionally competent yet restrained by chronic inhibitory signaling and metabolic stress. This suggests that therapeutic strategies combining immune checkpoint blockade with metabolic modulation may be required to restore effective anti-tumor immunity. Moreover, layers' heterogeneity further predicts that treatment efficacy may vary across tumor regions.

P107

Chemerin orchestrates spatially defined CAF-macrophage niches in lung tumors

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Cytokines and chemokines guide the recruitment, positioning and functional polarization of leukocytes that infiltrate tumors, thereby shaping disease progression and therapy response.
The CCRL2/Chemerin/ChemerinR1 axis represents an alternative chemotactic pathway where CCRL2-expressing barrier cells present the chemoattractant Chemerin to recruit ChemerinR1+ immune cells. While we previously identified this axis as critical for NK cell lung homing and immune surveillance, the presence of ChemerinR1+ myeloid cell within the tumoral microenvironment is linked with an aggressive disease, but its role is still unclear.
Using single-cell and spatial transcriptomics in human and mouse models, we mapped the cellular composition and genomic regulation of Chemerin/ChemerinR1+ lung cancer niches. Conserved between human and mouse, this axis showed a strong segregation: *RARRES2* was expressed only by cancer associated fibroblasts (CAFs), and its levels were increased in immune checkpoint blockade treated patients. Meanwhile, *CMKLR1* marked only monocyte-derived tumor associated macrophages (TAM), with an M2 phenotype and a Lipid Associated Macrophages (LAM) transcriptional program. Topologically, these *RARRES2+/CMKLR1+* CAFs-TAMs niches localize into metabolically dysregulated regions that promote the LAM transition, T cell exclusion, and an immunosuppressive microenvironment. Finally, human–mouse *CMKLR1+* TAMs shared a common core gene signature, suggesting a conserved macrophage state in lung tumors.
Our results suggest that dysregulated Chemerin/ChemerinR1 crosstalk defines a stromal–myeloid circuit that is likely therapeutically actionable in lung tumors and potentially extendable to other

solid tumors. Modulating CMKLR1 signaling to reprogram macrophage states and influencing myeloid positioning could provide a practical route to reshape the tumor microenvironment and improve therapeutic targeting strategies.

P108
HPV-specific T cell responses and tumor microenvironment characterization in head and neck squamous cell carcinoma

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Background: HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) represents a distinct clinical entity with superior prognosis compared to HPV-negative tumors, partly attributed to robust anti-viral immune responses. A detailed characterization of HPV16-specific $CD4^+$ and $CD8^+$ T cells in patients with head and neck cancer may inform the design of immunomonitoring strategies and immune-based interventions.
Methods: We performed deep immunophenotyping of 13 OPSCC patients (blood, n=13; lymph nodes, n=11; tumor tissue, n=9; non-tumor mucosa, n=3). HPV-specific $CD4^+$ and $CD8^+$ T cells were identified using activation-induced marker (AIM) assays following overnight stimulation with overlapping peptide pools covering HPV16 E6, E7, L1, and L2 proteins. Phenotypic characterization employed multi-parameter flow cytometry to assess differentiation status, activation markers, tissue residency, and exhaustion. The broader immune landscape included Tregs, B cell subsets, and germinal center signatures.
Results: HPV-specific $CD8^+$ T cells were detected in all compartments, with higher frequencies in tumor tissue (mean ~0.15%) and non-tumor mucosa than in lymph nodes and in peripheral blood. Tumor-infiltrating HPV-specific $CD8^+$ T cells displayed predominantly effector memory and terminally differentiated effector memory phenotypes (T_{EM} and T_{EMRA}) and showed increased expression of CD25, HLA-DR, and PD-1 compared with peripheral blood and lymph nodes, indicating highly activated and partially exhausted populations. Oncogenic proteins E6 and E7 elicited stronger $CD8^+$ T cell responses than capsid proteins L1 and L2, especially in the tumor. HPV-specific $CD4^+$ T cells showed similar enrichment in tumor and lymph nodes, with central and effector memory phenotypes and increased activation marker expression. E7-specific $CD4^+$ responses were prominent. The broader immune landscape showed distinct Treg and B cell subset distributions.
Conclusions: Deep immunophenotyping reveals robust, compartmentalized HPV16-specific $CD8^+$ and $CD4^+$ T cell responses in OPSCC, supporting multi-compartment immune monitoring and HPV-specific therapeutic strategies.
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P109
Sex differences in allergic asthma–driven airway inflammation and lung cancer susceptibility

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Chronic inflammatory lung diseases, such as those associated with allergic asthma represent a risk factor for lung cancer. Allergic asthma drives eosinophilic airway inflammation, excessive mucus production, and increased airway hyperresponsiveness. These changes prompt lung epithelial cells to release alarmins, some tied to carcinogenesis and tumor immunity, alongside activation of oxidative stress–response and tissue-repair genes. Furthermore, sex differences may influence susceptibility and severity of asthma and associated inflammation, making sex a possible biological variable for lung cancer progression. To gain knowledge on the mechanisms linking chronic pulmonary inflammation and carcinogenesis, we investigated the immunological, inflammatory, oxidative and metabolic events in a mouse model of allergic asthma to house dust mite (HDM) and urethane-induced carcinogenesis in relation to sex. After two week-immunization with intranasal HDM, females exhibited higher serum IgE levels and pulmonary eosinophils than males, indicating greater allergic sensitization to HDM. After 10-week treatment with urethane (tumor initiation stage), chronic exposure to HDM induced in females more elevated pro-inflammatory cytokines, chemokines, pro-angiogenic factors and metalloproteinases in both lung tissue and BALF than males under the same regimen. After a 15-week urethane-resting period (tumor promotion stage), lungs from females receiving HDM plus urethane displayed a higher inflammatory burden and increased levels of many tumor markers than males. NMR spectroscopy detected gender-specific metabolic changes across all time points, particularly in lung lipid profiles, which was more disrupted in females, especially in response to the allergen. Conversely, EPR analysis

indicated higher reactive oxidizing species in the blood of males with respect to females in response to urethane and to urethane plus HDM. Our results indicate sex-specific immunological, inflammatory and biochemical responses to both allergen and carcinogen, which may affect neoplastic transformation.

P110

Ex-vivo induced lung tumor organoids co-culture system to study the crosstalk between alveolar macrophages and lung cancer cells at early stages of tumorigenesis

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Non-small-cell lung cancer (NSCLC) is one of the major causes of cancer-related death worldwide, representing an urgent unmet clinical need. Since the introduction of immune-checkpoint-blockade (ICB) therapies, increasing effort was spent to study the immunological landscape in NSCLC patients, in order to develop better therapies and increase the efficacy of those that are available.
Accumulating evidence shows the critical role of alveolar macrophages (AM) as crucial players in the evolution of cancer in the lungs, playing dynamic roles and adapting to local niches as the disease progresses. However, the interplay between AM and early stages of tumorigenesis is still a major gap, due to the lack of appropriate experimental models to capture this phase.
In this work, we propose 3D organoid cultures from the $Kras^{G12D/+}/p53^{flox/flox}$ (KP) inducible NSCLC model to recapitulate transformation *ex-vivo* and reconstitute the system with AM in a highly controlled fashion.
We have established the procedure to trigger transformation of normal lung organoids (from KP not-induced tissues) into tumor organoids *ex-vivo* by Cre delivery and to monitor the underlying morphological and molecular changes. Owing to the full control over the timing of each step, we are now testing the impact of AM on transforming KP organoids, monitoring the co-culture in terms of phenotypic and transcriptional changes occurring in both tumor and immune component. Moreover, besides timing, this *in-vitro* model allows us to widely manipulate organoids (changing cell composition or proportion of initially induced cells) and macrophages (pre-conditioning them *ex-vivo* or extracting them from tumor-bearing animals at different stages), to test multiple scenarios of crosstalk.

P111

γδ T cells mediate antitumor immunity and tertiary lymphoid structure organization in colorectal cancer

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γδ T cells represent a distinct subset of T lymphocytes that bridge innate and adaptive immunity and are increasingly recognized as important players within the tumor microenvironment. In colorectal cancer (CRC), γδ T cells may contribute to antitumor immunity through direct cytotoxic activity, cytokine production, and by shaping local immune organization. Their potential involvement in the formation of tertiary lymphoid structures (TLS), which are associated with enhanced immune responses in tumors, remains to be fully elucidated.
To explore the functional landscape of γδ T cells in CRC, we analyzed single-cell RNA sequencing datasets and performed pseudobulk transcriptomic analyses of γδ T cell populations within tumor and normal tissues. In addition, the chemokine CXCL13—known to play a key role in lymphocyte recruitment and TLS formation—was further evaluated at the protein level, either by assessing CXCL13 levels in the plasma of patients with colorectal cancer, and its expression by γδ T cells.
Transcriptomic analysis revealed increased expression of CXCL13 within the tumor compartment compared with normal tissue. Notably, this enrichment appeared particularly pronounced in tumors characterized by mismatch repair deficiency (MMRd), a molecular subtype associated with high immunogenicity and strong immune infiltration. These findings support a potential role for γδ T cells not only in direct antitumor activity but also in promoting immune cell recruitment and contributing to the organization of TLS within the tumor microenvironment.
Overall, our data highlight the multifaceted functions of γδ T cells in CRC, encompassing cytotoxic activity, cytokine production, and possible involvement in TLS formation. These observations suggest that γδ T cells may play a broader role in orchestrating antitumor immunity in CRC, particularly in highly immunogenic tumor subtypes.

P112

Transforming Growth Factor β drives Natural Killer Cell reprogramming and impairs antitumor immunity in primary Uveal Melanoma

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Natural Killer (NK) lymphocytes are key mediators of tumor immune surveillance. In several solid cancers, tumor-derived Transforming Growth Factor β (TGFβ) promotes NK cell reprogramming toward a poorly cytotoxic, pro-tumoral, tissue-adaptive phenotype. Uveal melanoma (UM) is an aggressive intraocular malignancy, characterized by early metastatic dissemination and limited response to immunotherapy. Consistently, analysis of primary UM patient specimens revealed scarce NK cell infiltration, suggesting an immunologically non-permissive microenvironment. Here, we investigated whether TGFβ-driven NK lymphocyte dysfunction contributes to immune escape in UM and represents a therapeutically actionable pathway.
Analysis of The Cancer Genome Atlas dataset demonstrated elevated TGFβ expression in primary UM, correlating with reduced overall survival. UM cell lines (92.1, Mel285, Mel270) recapitulated this phenotype, exhibiting high TGFβ expression and secretion. Exposure of peripheral blood NK cells from healthy donors to UM-conditioned medium induced profound phenotypic and functional reprogramming, characterized by downregulation of activating receptors (NKp30, NKG2D, DNAM-1) and acquisition of tissue-adaptive markers (CD9, CD49a). This polarization was associated with reduced granzyme B and perforin expression, impaired degranulation, and diminished invasive capacity in three-dimensional UM spheroids. In addition, in an orthotopic zebrafish xenograft model, UM-primed NK cells failed to control tumor growth, demonstrating that tumor-driven reprogramming results into loss of antitumor activity in vivo. Notably, pharmacological inhibition of TGFβ signaling, using either receptor blockade or ligand neutralization, restored NK cell phenotype, infiltration, and cytotoxic function.
Collectively, our findings identify TGFβ as a central driver of NK cell inactivation in UM and uncover a mechanistic axis of tumor immune escape. Targeting TGFβ signaling may therefore represent a strategy to restore NK-mediated tumor control and enhance immunotherapeutic efficacy in uveal melanoma.

P113

Impact of radiotherapy on circulating immune cell subsets in oropharyngeal HNSCC

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Head and neck cancer (HNC) frequently progresses toward immune escape and metastatic dissemination. Approximately 90% of cases are head and neck squamous cell carcinomas (HNSCC). Besides surgery, radiotherapy (RT), often combined with chemotherapy, represents a cornerstone of treatment. However, these conventional approaches are not tumor-specific and may also affect healthy tissues. An effective antitumor immune response is crucial to prevent recurrence and metastasis, yet patients' systemic immunity can be significantly altered by (chemo)radiotherapy.
This study aimed to investigate the impact of RT on systemic immune status by evaluating changes in circulating immune cell phenotypes in patients with oropharyngeal HNSCC. Peripheral blood samples from 40 patients were collected before RT (T0) and one month after treatment completion (T1). Multicolor flow cytometry was performed to characterize major immune cell subsets, including naïve and memory T and B lymphocytes, natural killer (NK) cells, monocytes, and dendritic cell subsets.
RT induced a significant reduction in total leukocytes ($CD45^+$) and total T lymphocytes ($CD3^+$). Naïve and memory $CD4^+$ T cells, B cells, and NK cells significantly decreased at T1 compared to baseline. Monocyte and dendritic cell subsets did not show significant changes. Although total $CD8^+$ T cells were reduced after RT, no significant differences were observed within their naïve and memory compartments. Immune populations were further analyzed according to sex, age, and HPV status.
Overall, these findings demonstrate that RT significantly reshapes the systemic immune landscape in HNSCC patients, revealing a persistent immunosuppressive effect detectable one month after treatment. A better understanding of RT-induced immune modulation may contribute to the optimization of combined therapeutic strategies and improve clinical outcomes.

P114
The role of hMENA in shaping extracellular matrix properties and anti-tumour immune response in Non-Small Cell Lung Cancer

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Mechanical alterations in tumours, as increased stiffness and solid stress significantly influence anti-tumour immunity in cancer. The extracellular matrix (ECM), a key Tumour Immune MicroEnvironment (TIME) component, is emerging as a functional unit governing immune cell recruitment, spatial positioning, and functions, yet a deep understanding of the molecular programs connecting ECM remodelling, and immune regulation remain poorly understood.
Among stromal components, cancer-associated fibroblasts (CAFs), particularly the ECM-producing myofibroblastic subset (ecm-myCAFs), drive ECM deposition and assembly and regulate immune infiltration and functionality within the TIME. Previous work from our laboratory demonstrated that hMENA, an actin-cytoskeleton regulator, controls the fibronectin/β1-integrin axis in Non-Small Cell Lung Cancer (NSCLC) and is associated with tertiary lymphoid structures (critical sites for local anti-tumour immune activation) presence, spatial organization, and improved response to immune checkpoint blockade. Furthermore, hMENA contributes to transcriptional rewiring via cytoskeleton-nucleus coupling, influencing gene programs involved in immune modulation.
Here, we explored how hMENA expression in CAFs isolated from NSCLC patients controls ECM organization and impacts anti-tumour immunity. Integrative analysis of an in-house CAF atlas revealed that hMENA-enriched ECM-myCAFs exhibit upregulation of Lysyl Oxidase (LOX), a copper-dependent amine oxidase responsible for ECM crosslinking, and its regulatory network, linking hMENA signalling to ECM properties. Silencing hMENA in patient-derived CAFs reduced fibronectin and LOX expression and generated decellularized ECMs with reduced anisotropy and altered viscoelastic proprieties as assessed by Brillouin microscopy. These ECM changes reshaped immune cell states, decreasing immunosuppressive populations such as regulatory B cells and M2-like macrophages, while enriching memory B cells and pro-inflammatory myeloid phenotypes.
Collectively, our findings identify hMENA as key regulator connecting ECM programs to immune functions. We envisage that targeting the hMENA-LOX axis may offer a novel strategy to reprogram tumour stroma, foster immune-permissive TIME, and enhance immunotherapy responsiveness in NSCLC.

P115
Immunomodulation of γδ T cells in glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor, characterized by poor prognosis and limited therapeutic options, highlighting need for effective immunological strategies. Tumor progression is strongly influenced by the immune microenvironment; therefore, understanding composition and state of infiltrating lymphocytes is essential for clarifying mechanisms driving GBM development. This study investigated interaction between tumor cells and γδ T lymphocytes in patients with GBM.
Flow cytometry demonstrated variable proportion of tumor-infiltrating lymphocytes, with γδ T cells accounting 1.3–1.8% of the total infiltrate and predominantly with Vδ2 phenotype. Transcriptomic analyses revealed overexpression of *TRDC* in GBM, confirming enrichment of γδ T-cell signatures. Genes encoding butyrophilins 2 and 3 were upregulated, together with stress-induced ligands MICA/B, and ULBP1–6, recognized by both Vδ1 and Vδ2 subsets.
Accordingly, phenotypic characterization revealed distinct differentiation profiles. In GBM, tumor-infiltrating Vδ1 cells were enriched in the naive and effector memory subsets, whereas peripheral Vδ2 cells predominantly exhibited effector memory and TEMRA phenotypes. In meningioma, γδ T cells showed prevalent effector memory differentiation with reduced naive.
Moreover, *TRGC1* and *TRGC2* expression were correlated with *PDCD1* and *HAVCR2*, suggesting co-expression of these checkpoints by tumor-infiltrating γδ T cells. Flow cytometry analysis confirmed that

approximately 35% of GBM-infiltrating Vδ1 and Vδ2 cells, as well as peripheral Vδ2 cells, expressed PD1 and TIM3. About 30% of Vδ1 and Vδ2 cells expressed NKG2A in tissue and blood, whereas expression was lower in meningioma samples. Notably, 90% of tissue Vδ1 and 65% of Vδ2 cells expressed the activating receptor NKG2C. Finally, Luminex analysis revealed elevated IL-10 and IL-17A in GBM, with a cytokine milieu that may influence γδ T-cell function.
These preliminary findings may help improve our understanding of the tissue microenvironment composition in GBM and mechanisms underlying resistance to immunotherapy, potentially guiding the development of new therapeutic strategies.

VACCINES

P116
Discriminating maternal and infant immune responses in early life using quantitative antigen-specific Fc glycosylation profiling

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In the neonate, serum immunoglobulin G (IgG) antibodies include maternally transferred antibodies and antibodies produced by the infant following infection or vaccination. This coexistence complicates the interpretation of immune responses in early life, as conventional serological assays quantify antigen-specific antibody levels but cannot determine their origin. Consequently, residual maternal antibodies may confound the evaluation of vaccine-induced immune responses in infants, limiting the interpretation of vaccine immunogenicity when administered in the first weeks of life.
Recent studies have shown that antibodies transferred across the placenta and antibodies produced during early-life immune responses display distinct glycosylation patterns in their Fc fragment. These glycan motifs differences, such as fucosylation, galactosylation and sialylation, have been observed between maternally derived and infant-produced IgG. Studies in pediatric cohorts from high-income countries have characterized these differences using high-resolution techniques, such as mass spectrometry and liquid chromatography, revealing reproducible variations in Fc glycan composition. These observations suggest that Fc glycan signatures may serve as molecular markers capable of distinguishing maternally derived antibodies from antibodies produced by the infant.
The project evaluates multiple high-throughput immunoassays to measure Fc glycosylation in antigen-specific antibodies, including multiplex bead-based assays (Luminex), ELISA-based strategies and biolayer interferometry (BLI), using glycan-binding lectins to detect specific Fc glycan motifs.
Preliminary results obtained testing monoclonal antibodies digested with different glycosidases indicate that the ELISA approach shows limited sensitivity for Fc glycan discrimination. In contrast, multiplex platforms such as bead-based (Luminex), and electrochemioluminescence (Meso Scale Discovery) assays, appear more suitable for antigen-specific Fc glycosylation profiling in non-complex samples.
By integrating antigen specificity with Fc glycosylation profiling in a scalable platform compatible with limited sample volumes, this work aims to establish a glyco-serological framework to investigate antigen-specific antibody origin in early life and improve the interpretation of vaccine-induced immune responses in infants.

P117
Single-cell transcriptional profiling of antigen-specific T effector and regulatory cells reveals divergent immune signatures in hybrid versus vaccine-induced immunity

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Backgorund: Hybrid immunity, resulting from natural SARS-CoV-2 infection and vaccination, and vaccine-induced immunity alone may elicit distinct transcriptional programs in antigen-specific T and B cells. We leveraged a cohort collected during 2021, including infection-naïve vaccinated (N) and hybrid immunized (HI) subjects, as a model to dissect these differences at single-cell resolution.
Methods: PBMCs from 3 N and 3 HI subjects were collected one month after the second mRNA vaccine dose, stimulated overnight with Spike peptides, and sorted by activation markers. Antigen-specific B cells were captured using recombinant Spike to catch specific BCRs. All populations underwent scRNA-seq with paired TCR/BCR profiling. The pilot analysis was performed on one N and one HI subject.
Results: A discrete cluster (cluster 10) of antigen-specific T cells emerged from unsupervised analysis, populated by both N and HI cells, upregulating the fatty acid metabolism as a preferential energetic strategy. Comparing N and HI cells, HI antigen-spe-

cific T effectors upregulated TNF-α, IFN-γ, and type I IFN pathways. A subset of antigen-specific T cells from both groups co-populated the Treg cluster, enriched for activation and cytoskeletal remodeling pathways; HI-derived antigen-specific Tregs selectively upregulated IFN-γ and type I IFN-related and modulatory genes. Recovered Spike-specific B cells were a low number, but showed activation and cytoskeletal remodelling. BCR sequencing and TCR clonal expansion analysis are ongoing.

Conclusions: Hybrid immunity imprints a more inflammatory transcriptional program on antigen-specific T cells than vaccination alone. While both groups share fatty acid metabolic adaptation, HI subjects display a heightened type I and γ IFN signatures in effector and regulatory compartments, suggesting that prior infection qualitatively reshapes T cell inflammatory and modulative functional states in a trained comprehensive response. Further investigation is needed to answer questions about the activation/regulation balance in hybrid immunity, with potential implications for understanding its superior protective efficacy.

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