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# ABSTRACT BOOK

SIICA
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Immunologia Clinica e Allergologia

# NON-CONVENTIONAL CELL-TO-CELL COMMUNICATION AND IMMUNE REGULATION

## Amniotic fluid stem cell-derived extracellular vesicles educate type 2 conventional dendritic cells to rescue autoimmune disorders in a multiple sclerosis mouse model

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Dendritic cells (DCs) are essential orchestrators of immune responses and represent potential targets for immunomodulation in autoimmune diseases. Human amniotic fluid secretome is abundant in immunoregulatory factors, with extracellular vesicles (EVs) being a significant component. However, the impact of these EVs on dendritic cells subsets remain unexplored.
In this study, we investigated the interaction between highly purified dendritic cell subsets and EVs derived from amniotic fluid stem cell lines (HAFSC-EVs). Our results suggest that HAFSC-EVs are preferentially taken up by conventional dendritic cell type 2 (cDC2) through CD29 receptor-mediated internalization, resulting in a tolerogenic DC phenotype characterized by reduced expression and production of pro-inflammatory mediators. Furthermore, treatment of cDC2 cells with HAFSC-EVs in coculture systems resulted in a higher proportion of T cells expressing the regulatory T cell marker Foxp3 compared to vehicle-treated control cells. Moreover, transfer of HAFSC-EV-treated cDC2s into an EAE mouse model resulted in the suppression of autoimmune responses and clinical improvement.
These results suggest that HAFSC-EVs may serve as a promising tool for reprogramming inflammatory cDC2s towards a tolerogenic phenotype and for controlling autoimmune responses in the central nervous system, representing a potential platform for the study of the effects of EVs in DC subsets.

## Extracellular vesicles from γδ T cells exert adjuvant antiviral responses enhancing antigen presenting capabilities

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γδ T cells play a critical role in the immune response to viral infections, and may represent a good target to improve the immune response in immunocompromised hosts. We recently identified an antiviral signature of Vδ2 T cells in children given hematopoietic stem cell transplantation and demonstrated their ability to exert a direct and adjuvancy activity on virus-specific αβ T cell response. Furthermore, several evidences suggest that extracellular vesicles produced by Vδ2 T cells (Vδ2-EV) can exert deliver immunomodulatory signals.
The aim of this work was to evaluate the impact of Vδ2-EV on virus-specific T cells and to define the molecular mechanism.
Vδ2-EVs were isolated by ultracentrifugation from healthy Vδ2 T cell lines. Vδ2-EVs and their tropism were characterized by flow cytometry. The functional activity of Vδ2-EVs on viral-specific T cells as well as on dendritic cell (DC) maturation were tested on healthy donors and analysed by flow cytometry and Elispot.
Vδ2-EVs (150 nm size) were positive for CD63, CD9, Vδ2 and MHC- II markers and were preferentially internalized in monocytes, at lower extent in T cells. The pre-treatment of PBMC with Vδ2-EVs induce a significant increase of the frequency of CMV-specific IFN-γ producing T cells. Moreover, Vδ2-EVs were able to activate monocytes inducing MHC-II and CD86 expression, suggesting that Vδ2-EVs could promote APC functions. Accordingly, Vδ2-EVs improved the ability of iDC to trigger the CMV-specific T cell proliferation of autologous T cells. Finally, the analysis of the miRNA content in Vδ2-EVs compared to ctrl-EV showed the overexpression of several miRNA with important role in promoting antigen presenting function (eg. hsa−miR−106a−5p).
Altogether, these data demonstrate a role of signals delivered by Vδ2-EVs in improving the APC maturation and the virus specific T cells and provide a proof-of-concept for using the allogeneic Vδ2-EVs -based therapy in immunocompromised patients to boost antiviral response.

## JF SYMPOSIUM
## RISING STARS IN IMMUNOLOGY

### Microbial-derived metabolites promote NKT22 responses in colitis associated colorectal cancer patients

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Inflammatory bowel diseases (IBD) patients manifest an increased risk of developing colitis-associated colon cancer (CAC). Interleukin 22 is a cytokine involved in proliferation and survival of epithelial cells. However, it also correlates with the development of tumoral lesions. IL22 is secreted by different immune cell types, including tissue resident iNKT cells (iNKTs), exerting cytotoxic activity. In IBD patients, iNKTs perform both pro-inflammatory or tolerogenic functions in a microbiota-dependent fashion, opening questions on their functional role in CAC development.
Immune cell composition and iNKT phenotype of inflamed and cancerous tissue lesions of CAC, IBD and sporadic CRC patients were analysed in samples obtained from Policlinico Hospital, Milan, and IOV-IRCCS, Padua. High-dimensional single-cell flow cytometry, metagenomics, RNAseq, spatial immunophenotyping and transcriptomic were performed to evaluate the phenotype and function of human iNKT cells. Metabolomics was performed to identify microbial-derived molecules activating iNKTs. Mechanisms were dissected in CAC murine models, either iNKT proficient or deficient.
An increased NKT22 infiltration was observed in CAC tissues, together with an enrichment of *Odoribacter*, a Gram-negative bacteria implicated in tryptophan metabolism. Stimulation of iNKTs with *Odoribacter*-derived metabolites induced aryl hydrocarbon receptor (AhR)-dependent IL22 production by iNKTs. Metabolomic analyses revealed an increase in tryptophan metabolites in the *Odoribacter* supernatant, promoting NKT22 differentiation both in vitro and in vivo models. Moreover, the administration of *Odoribacter* supernatant or of the single purified metabolites were sufficient to increase tumorigenesis in CAC mouse models, but not in iNKT-deficient mice. Spatial proteomics and transcriptomic analyses of human CAC tissues confirm the microbiota-dependent functional skewing of iNKTs residing closely to cancer cells.
iNKTs in CAC patients are the major IL22 producing cells and are induced by the recognition of microbiota-derived metabolites implicated in tryptophan metabolism and AhR stimulation. NKT22-microbiota interaction in CAC patients might play a crucial role in tumor development.

### Microbial bile acid metabolites are key regulators of central nervous system autoimmunity

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Changes in the gut microbiota composition and gut mucosal immunity, i.e., high frequency of Th17 cells, are detected in both preclinical models (EAE) and relapsing-remitting multiple sclerosis (RRMS) patients and correlate with disease activity. The microbial metabolic profile is crucial for modulating immunity at the intestinal and systemic level. Secondary bile acid metabolites (BAM) produced by the commensal microbiota exert immunoregulatory functions by increasing FOXP3$^+$ Treg cell differentiation at the expense of Th17/Th1 cells. We analyzed the presence of secondary BAM and BAM-producing bacteria in RRMS patients and tested whether immunoregulatory BAM counteract brain autoimmunity by modulating gut immune homeostasis.
We performed WGS metagenomic analysis together with metabolomic analysis (UPLC-MS) to search for alterations in the relative abundance of BAM-producing bacteria and BAM concentrations in the intestine of RRMS patients and healthy controls and correlate with the immunological profile. To test the in vivo effect and mechanism of action of BAM we administered two immunomodulatory BAM, lithocholic acid (LCA) and deoxycholic acid (DCA), to EAE-immunized mice.
Our analysis revealed a reduced abundance of BAM-producing strains and decreased concentration of immunoregulatory DCA in RRMS patients which correlate with an increased percentage of Th17 cells in the peripheral blood. Oral administration of BAM significantly ameliorated clinical and neuropathological signs of EAE. BAM-treated EAE mice showed decreased Th17/Th1 cell frequency and increased percentages of FOXP3$^+$ Treg in the



gut but also in draining lymph nodes and brain tissue. Notably, we found that BAM modulate the functional profile of dendritic cells by reducing their capacity to trigger MOG-reactive Th17 cells.
Our data highlight the key role of immunoregulatory BAM in modulating autoimmunity in the central nervous system. Our findings could pave the way to new therapeutic approaches in RRMS based on restoration of a healthy BAM-producing gut microbiota and a functional immunoregulatory biliary network.

## AhR-expressing type 1 conventional dendritic cells play a protective role in the experimental model of sepsis

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Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. During sepsis, conventional dendritic cells (cDCs) - professional antigen presenting cells - limit the inflammatory response. We have shown that the activation of aryl hydrocarbon receptor (AhR) in cDCs is required for survival during lipopolysaccharide (LPS) induced sepsis in vivo. However, which specific cDCs subset - either type 1 (cDC1) or type 2 (cDC2) - exert this protective function is still unclear. To address this point, we used the experimental model of sterile infection resembling a sepsis-like condition by exposing mice to a sub-lethal dose of LPS. First, we used a bone marrow Zbtb46DTR chimera to deplete cDCs in vivo and thus to verify the cDCs involvement in sepsis. We next assessed AhR engagement in cDCs function in experimental sepsis, by generating a mouse model lacking AhR in cDCs. Results demonstrated that Zbtb46DTR chimeras treated with diphtheria toxin are unable to survive sub-lethal LPS challenge compared to WT mice reconstituted with WT bone marrow. We also found that the loss of AhR in cDCs abrogate such protective function, as Ahrf/f Zbtb46 $Cre^+$ mice were highly susceptible to infection. To assess the role of cDCs subset in sepsis, we used $Batf3^{-/-}$ mice (lacking $CD24^+$ cDC1) and Notch2fl/fl CD11c Cre mice (lacking $CD11b^+$ cDC2). We found that $Batf3^{-/-}$ mice are susceptible to sub-lethal LPS challenge, as opposed to Notch2 mice. Whole genome analysis showed that AhR deletion in cDC1 abrogated cDC1 tolerogenic signature. Consistently, we found that WT mice challenged with LPS survive only when receive AhR competent cDC1. Our data demonstrated that AhR-expressing cDC1 are required for protection during sepsis and thus may represent a potential therapeutic target in sepsis.

## Key role of microRNA-142-3p shuttling via extracellular vesicles in regulatory T cell function

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Human $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells are a subset of $CD4^+$ T cells that control immune self-tolerance by inhibiting autoreactive cell responses and thus playing a key role in the pathogenesis of autoimmune diseases. In multiple sclerosis (MS), the balance between Treg and conventional T cells (Tconv) is disturbed, but the mechanism underlying Treg dysfunction has not been elucidated. It is known that Treg cells secrete significant amounts of extracellular vesicles (EVs), lipid bilayer particles capable of transferring their molecular cargo (i.e. proteins and microRNAs) and

engaging in intercellular communication. However, the involvement of EVs in Treg cell inhibitory function in healthy conditions and the potential pathogenetic role of EVs in autoimmune diseases remain unclear.
Here we showed that human Treg-EVs exhibited a significant inhibitory effect on $CD4^+$ T cell proliferation and activation in vitro and were able to in vivo protect mice from development of experimental autoimmune encephalomyelitis (EAE), the mouse model of MS.
microRNA analysis of Treg-EVs revealed that miR-142-3p was significantly enriched in Treg-EVs compared to Tconv-EVs from healthy donors. Moreover, the shuttling of this miRNA was a crucial molecular event for the down-regulation, in EV-target cells, of mRNAs necessary to engage cell growth and division, including the cystine carrier SLC7A11, a key determinant of redox homeostasis in proliferating T cells
Furthermore, Treg cells from subjects with multiple sclerosis released EVs with lower amount of miR-142-3p and dramatically reduced suppressive capacity.
Taken together, these findings delineate a previously unrecognised mechanism for Treg cell suppressive activity and elucidate a physiological function of miR-142-3p shuttling by Treg cells via EVs, unveiling its pathogenetic implication in human autoimmunity.

## WORKSHOP 1 - TUMOR IMMUNOLOGY 1

### Mast cells may contribute to recalling B cells in the colorectal cancer milieu

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In colorectal cancer (CRC), mast cells (MCs) are crucial modulators of angiogenesis and tumor progression. They orchestrate the functions of both cancer and immune cells in the tumor environment, which impacts patient prognosis. An intimate interaction between MCs and B cells is settled in the intestine, both in physiology and inflammation, where MCs provide support for the effector functions of B cells. We are now exploring a connection between the activation of MCs in CRC and B cells recruitment/accumulation in the tumor micro/macro milieu. To this aim, we established subcutaneous MC38 CRC cells growth in wild-type, KitW-sh MC-lacking mice and MCs-reconstituted KitW-sh mice. In vitro co-cultures between MCs and the MC38 cell line were further established. In parallel, we also developed a method to isolate and analyze both MCs and B cells from human biopsies of CRC patients.
We observed B cell accumulation in CRC biopsies and co-colocalization with MCs in the tumor tissue. Moreover, increased B cell density in tumor lymph nodes (LNs) was found both in human patients and in the mouse model.
In mice, where we uncovered a CCL20 chemokine gradient in tumor draining LNs, we detected CCR6hi B cells accumulation. Similarly, an increase of CCL20 expression was assessed in cancer tissue when compared to normal colon. Interestingly, we proved that TNF-$\alpha$ released by activated-MCs was required for the CCL20 increased expression in cancer cells in vitro.
The accumulation of $CCR6^+$ B cells in the tumor micro/macro environment may rely on the crosstalk between CRC cells and MCs. A deeper understanding of MCs activation in CRC can help to predict the behavior of other immune cells in CRC and opens to the possibility that, inhibiting TNF-$\alpha$ derived from MCs, B cell immunosurveillance may be indirectly affected.

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## Decitabine and IL-33/ST2 axis cooperate against melanoma through remodeling the tumor microenvironment to overcome resistance to PD-1 blockade

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IL-33 plays a crucial role in cancer, particularly in melanoma, where it exhibits anti-tumor properties by stimulating immune cells. In this study, we explored the combination of IL-33 with decitabine (DAC), a DNA methylation inhibitor that promotes immune recognition by re-activating silenced genes, for melanoma treatment. In multicellular spheroids of mouse and human melanoma cells, DAC alone inhibited tumor cell aggregation, suggesting its direct effect on tumor cells. In vivo, DAC combined with IL-33 reduced tumor growth and prolonged the survival of mice transplanted with melanoma cells, outperforming single treatments. This combination therapy was the most efficient in promoting immune recruitment (i.e., T cells and eosinophils) at the tumor site and increased PD-1 expression, leading to a better response to PD-1 blockade. In a microfluidic competitive migration assay, DAC/IL-33 treatment generated the strongest chemotactic response, attracting immune cells, including $CD8^+$ T cells, from naïve wild type mice, but not from mice lacking the IL-33 receptor ST2 ($ST2^{-/-}$), indicating that IL-33 signaling was crucial for immune cell recruitment. In vivo, DAC failed to induce tumor immune infiltration and was ineffective in reducing tumor growth in $ST2^{-/-}$ mice. Moreover, DAC increased the expression of ST2 and IL-33 at the tumor site, suggesting it may enhance endogenous IL-33 production. Methylation studies indicated that DAC increased the expression of IL-33 in mouse and human melanoma cells through demethylation of a transcription factor-binding site located inside the IL33 gene. Our findings underscore a cooperative action between DAC and the IL-33/ST2 axis against melanoma, potentially through immune cell recruitment and epigenetic regulation of gene expression, thus remodeling the tumor immune microenvironment to overcome resistance to PD-1 blockade.

## In silico and in vitro evaluation of the role of bone marrow-derived $CD8^+$ tissue-resident memory cells in multiple myeloma disease

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Multiple myeloma (MM) is a complex blood cancer caused by the uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM). It progresses through a multi-step transformation from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma (SMM) before developing into MM.

Recent research highlights the role of $CD8^+$ T memory cells in MM progression, particularly the decline of $CD8^+$ T memory stem cells in MGUS, which may trigger disease advancement. This study focuses on $CD8^+$ tissue-resident memory T cells (TRM) and their significance in MM progression and immune defense mechanisms.

By analyzing single-cell RNA sequence data on over 600,000 cells from the BM of 183 patients across various disease stages, the study emphasizes the molecular and phenotypic evolution of $CD8^+$ TRM cells. Further analysis of 161,000 high-quality $CD8^+$ T cells and immunophenotyping by flow cytometry of a 35-patient independent cohort reveals a correlation between $CD8^+$ TRM cell prevalence and patient outcomes.

Pseudotime trajectory analysis identifies two distinct subsets of $CD8^+$ TRM cells in human BM, characterized by granzyme K (GZMK) and granzyme B (GZMB) expression. In SMM and MM, the $GZMK^+$ subset displays diminished cytotoxic capabilities and increased expression of exhaustion markers. In MM patients, the abundance of effector memory and terminally differentiated $CD8^+$ TRM cells are inversely correlated with patient progression-free survival, indicating its potential as a prognostic marker and a target for therapeutic intervention.

To further investigate $CD8^+$ TRM functionality, we expanded these cells from MM patients' BM. Upon stimulation, $CD8^+$ TRM cells developed a cytokine response upon recognition of autologous myeloma cells and this response was inhibited by an anti-CD3 monoclonal antibody. This confirms that MM-derived $CD8^+$ TRM cells retain antigen recognition and clonal expansion ability, offering new therapeutic chances for MM treatment.

## Granulocyte-macrophage colony-stimulating factor–producing RORγt⁺ Group 3 innate lymphoid cells accumulate in human colorectal cancer and correlate with tertiary lymphoid structure-associated cell types

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Accumulating evidence indicates that Group 3 innate lymphoid cells (ILC3s) play a key role in shaping gut tumor immunity. Here, we found an increased number of ILC3s that do not express NKp44 but acquire an NK-like phenotype maintaining high level of RORγt in human colorectal cancer (CRC). Functionally, this subset releases large amount of granulocyte-macrophage colony-stimulating factor (GM-CSF) and represents, among leukocyte subsets, the major cellular source of this cytokine in CRC. In a mouse model of colitis, it has been reported that GM-CSF promotes ILC3 mobilization and tissue reorganization. In line with these findings, tumor infiltrating ILC3s upregulate the homing receptor CCR7. Moreover, stimulation of patient-derived tumor fragment with recombinant GM-CSF results in the activation of T cell compartment that acquires a T follicular helper (Tfh) cell phenotype and gains the capability to produce CXCL13, a powerful chemokine able to attract B cells. Accordingly, the frequency of GM-CSF-producing ILC3s in CRC specimens correlates with those ones of both Tfh cells and B cells and analysis of ILC3 localization reveals their close proximity to T cells within tertiary lymphoid structures as well as in tumor stroma. Together, these results highlight the importance of GM-CSF as a critical cytokine by which ILC3s could shape tumor immune microenvironment in the context of CRC.

## Aryl hydrocarbon receptor-mediated immune suppression in soft tissue sarcomas: implications for conventional dendritic cell subset 1-mediated antitumor immunity

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Immune dysregulation is a hallmark of cancer, limiting the efficacy of immune checkpoint inhibitors (ICIs) in many solid tumors, including soft tissue sarcomas (STS). The Aryl Hydrocarbon Receptor (AhR), an environmental xenosensor, promotes tumor immune evasion by responding to ligands in the tumor microenvironment (TME). In contrast, conventional type 1 dendritic cells (cDC1) are essential for initiating antitumor immunity. In this study, we explored: (i) the prognostic value of cDC1 and AhR activation signatures (330 and 163 genes, respectively) in STS using Cox regression analysis of 259 TCGA-SARC tumor samples. High cDC1 expression (coef = -1.474, HR = 0.229, $p < 0.001$) correlated with better survival, while AhR alone had no significant impact (coef = -0.123, HR = 0.884, $p = 0.654$). However, their interaction (coef = 1.796, HR = 6.024, $p < 2e-16$) significantly attenuated cDC1's protective effect, suggesting AhR-mediated immune suppression. Functional enrichment of differentially expressed genes (DEGs) in cDC1/AhR high and low groups revealed enrichment in immune-related pathways, indicating potential immune exhaustion; (ii) AhR gene expression significantly correlates with its activation signature in STS tumor samples. Based on this correlation, we inferred possible AhR activation and protein expression, observing variable levels of AhR across different STS histological subtypes within the TME; (iii) Selective AhR deletion in TME-derived cDC1 improved tumor rejection, as Ahrf/f XCRI Cre mice injected with D42M1-ES2 cells exhibited significantly reduced tumor volume compared to Ahrf/f mice; (iv) In addition, single-cell analysis of CD45⁺ TME cells from these mice confirmed the restoration of immune function. Our findings suggest that AhR suppresses cDC1-mediated immunity in STS, revealing a potential target to enhance antitumor responses.

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# WORKSHOP 2
# IMMUNE RESPONSE TO PATHOGENS 1

## Modeling the dynamics of long-lasting antibody responses: insights into persistence and variability following infection and vaccination

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Long-lasting antibody responses are essential for protective immunity following infection or vaccination, yet the mechanisms underlying their persistence and the variability observed between individuals remain only partially understood.
Using an agent-based in silico model, we investigated the dynamics of antibody-secreting cells and their impact on the trajectory of the antibody response. Our model proposes that antibody-secreting cells probabilistically acquire an extended half-life, with long-term antibody levels being influenced by the initial pool of plasmablasts, their probability of differentiating into long-lived plasma cells, and the half-life of these long-lived cells.
Interestingly, our simulations reveal two distinct patterns in antibody titer trajectories: "sustainers," who maintain high antibody levels over time, and "decayers," whose antibody levels decline rapidly. The key factor driving this decline is the absence of long-lived plasma cells. Stochastic variations in immune repertoire and priming efficiency between individuals contribute to the observed divergence between these groups.
To refine and validate our model, we compared its predictions with clinical data on antibody responses to SARS-CoV-2 nucleoprotein and Spike following infection or vaccination. Our findings demonstrate a strong correlation between the model's predictions and empirical data, highlighting the robustness of our approach.
We present a generalized mathematical model that effectively captures the decline in antibody titers following adenoviral and mRNA COVID-19 vaccinations and SARS-CoV-2 infection.

## Neutrophils specific CCRL2 genetic variants contributes to COVID-19 severity

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Severe COVID-19 patients have a limited antiviral response due to local neutrophilia and exacerbated inflammation. The 3p21.31 locus is the most robust genomic region associated with COVID-19 severity. However, due to the complex linkage disequilibrium (LD) structure of the region and its elevated gene density, further analyses are needed to better resolve the association signals in this locus. This region contains a main chemokine receptor (CKR) cluster, which function is to orchestrate the immune response by regulating the trafficking of leukocytes to tissues. Given that the role of neutrophils in COVID-19 severity has been extensively described, but little is still known on the impact of the 3p21.31 CKR cluster on neutrophils, we decided to investigate this matter.
To this aim, we tested expression quantitative trait loci (eQTL) targeting the 3p21.31 CKR cluster linked to COVID-19 hospitalization in individuals of European ancestry from the COVID-19 HGI meta-analysis. Among these, CCRL2, a key regulator of neutrophil trafficking, was targeted by neutrophil-restricted eQTLs. We confirmed these eQTLs in an Italian COVID-19 hospitalized patients. In addition, our haplotype analysis revealed a link between an increased CCRL2 expression and COVID-19 severity and hospitalization.
By the exposure of neutrophils and monocytes to a TLR8 ligand, thus mimicking a viral infection, we revealed specific chromatin domains within the 3p21.31 locus exclusive to neutrophils. In addition, the identified variants mapped within these regions altered the binding motif of neutrophils-expressed transcription factors.
These results support the contribution of CCRL2 eQTL variants to the risk of severe COVID-19 by selectively affecting neutrophil trafficking, and therefore we propose CCRL2 as a novel susceptibility factor for COVID-19 severity and hospitalization.

**Altered miRNAome impacts on immune responses, inflammatory processes and tissue repair in children affected by long COVID**

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Epigenetic seems to be implicated in antiviral responses and interaction with the SARS-CoV-2. In long COVID affected patients during adulthood, distinct miRNAs are deregulated and appear to be involved in inflammatory responses and responsible for organ-specific complications. This study dissected the potential implications of epigenetics in paediatric long COVID, to better understand their role and potential clinical applications.
Children were evaluated at least 8 weeks after their initial infection and classified as "fully recovered" (no persisting symptoms and return to pre-COVID activity levels) or as having "long COVID" (persistent symptoms impacting daily life for at least eight weeks, with other diagnoses excluded). The miRNAomes analysis of peripheral blood immune cells from 48 patients with persistent symptoms and children who fully recovered revealed a combination of miRNAs (such as -26a-5p, -423-3p, -29a-3p, -424-5p) significantly deregulated in long-COVID group. Moreover, the analysis predicted the modulation of different pathways possibly involved in the long-lasting persistency of long-COVID. The deregulated miRNAs were predicted to be involved in the upstream regulation of AKT cascade. In vitro analysis confirmed that selective inhibitors of AKT were able to counteract immune cells dysregulation possibly responsible for cell activation and proliferation and antibody production.
The identification of specific miRNA signatures could help in diagnosing, prognosing, and monitoring paediatric long COVID, and miRNAs may serve as potential therapeutic targets. Moreover, targeting the gene(s) modulated by dysregulated miRNAs could represent an alternative strategy to counteract the long-lasting persistency of symptoms during childhood.

**Different lipopolysaccharide (LPS) acylation patterns modulate hematopoietic progenitor responses and influence infection susceptibility**

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Bacterial infections are a significant global health threat, despite advancements in vaccines and antibiotics, largely due to bacteria's ability to evade the host immune system. Lipopolysaccharide (LPS), a major component of the bacterial outer membrane, exhibits diverse acylation patterns, with hexa-acylated LPS being the most prevalent. Some bacteria, including those with pandemic potential such as *Yersinia pestis*, use LPS hypoacylation as a strategy to escape immune detection. Hematopoietic stem and progenitor cells (HSPCs) play a crucial role in determining infection outcomes, as they regulate the timing of the immune response and influence its direction by producing cells with either pro-inflammatory or immunosuppressive properties. Despite their importance, the effects of LPS acylation patterns on HSPC function remain poorly understood
To explore the impact of LPS hypoacylation on HSPCs, we engineered an *Escherichia coli* strain to express pentacylated LPS. Upon intraperitoneal injection of pentacylated or hexaylacted *E. coli* into C57BL/6J mice, we found that mice infected with pentacylated *E. coli* exhibited higher survival rates compared to those infected with hexa-acylated *E. coli*. At early time points, hexa-acylated *E. coli* infection triggered a greater expansion of circulating emergency pro-inflammatory multipotent progenitor, whereas at later time point, pentacylated *E. coli* promoted the expansion in the bone marrow, and expansion/recruitment in blood and infection sites of multipotent progenitor with potential immunosuppressive functions. In knockout mice with impaired LPS sensing, these effects are dependent on Toll-like receptor 4 (TLR4) and MyD88 signalling.
In summary, this study demonstrates that variations in LPS acylation patterns affect the hematopoietic system, with implications for infection outcomes. It highlights that the sensing of hypoacylated LPS through TLR4 and MyD88 signalling promotes the expansion and mobilization of hematopoietic progenitors with potential immunosuppressive functions, rather than progenitors associated with emergency hematopoiesis.

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### LINE1 modulate human T cell function by regulating protein synthesis during lifespan

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Neonatal T cells represent a distinct developmental stage, characterized by the absence of memory T lymphocytes and a predominance of naïve T cells. Upon stimulation, neonatal naïve T cells display an enhanced activation propensity, yet the molecular mechanisms underlying this rapid responsiveness remain largely uncharted. In our previous study, we identified a novel epigenetic mechanism governing T cell quiescence in adult naïve T cells, involving the expression of LINE1 elements - the most abundant class of retrotransposons - into previously unrecognized splicing variants. Notably, their down-regulation signals the transition to an activated state (Marasca et al, Nat Gen 2022).

In this study, we found that neonatal naïve T cell quiescence is characterized by increased energy production, early cell cycle entry, and enhanced protein synthesis. We investigated the dynamics of LINE1 expression and its role in regulating the activation readiness of neonatal naïve $CD4^+$ T cells. Our findings reveal that neonatal naïve T cells lack LINE1 expression due to tonic TCR activation driven by self-antigen presentation. This signaling cascade sustains basal mTORC1 activation, which in turn suppresses LINE1 splicing through PTBP1. Strikingly, we demonstrated that the absence of LINE1 primes neonatal T cells for the rapid execution of the activation program by modulating protein synthesis and cell cycle progression. Furthermore, our analysis of LINE1 expression across the human lifespan revealed a progressive increase from childhood to adulthood, peaking in elderly individuals. This accumulation of LINE1 transcripts contributes to immune senescence by reducing protein synthesis in aging T cells.

Our study uncovers a novel role for LINE1 in T cell development, highlighting their expression as a quantitative traits of human T cell activity across the lifespan, with implications for immune function in both health and disease.

## WORKSHOP 3
## ERRORS OF IMMUNITY

### The role of Interferon-gamma in autoimmune polyendocrinopathy syndrome 1

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a life-threatening, autosomal-recessive, autoimmune syndrome caused by autoimmune regulator (AIRE) deficiency. In APECED, self-reactive T cells escape thymic negative selection, infiltrate target organs, and drive autoimmune injury. Yet, the effector mechanisms governing T cell-mediated organ damage in APECED remain poorly understood. In a three-phased study we present evidence supporting the classification of APECED as a disease mediated by interferon-gamma (IFN-γ). APECED patients exhibited enhanced IFN-γ responses in blood and all examined autoimmunity-affected tissues. $Aire^{-/-}$ mice

exhibited selectively increased IFN-γ production by T cells, and enhanced IFN-γ, phosphorylated STAT1 (pSTAT1), and CXCL9 signals in multiple autoimmunity-affected organs. Ifng ablation or ruxolitinib-induced JAK/STAT blockade in Aire$^{-/-}$ mice normalized IFN-γ responses and averted T cell infiltration and damage in target organs. Ruxolitinib treatment of five APECED patients was well-tolerated, decreased T cell-derived IFN-γ, normalized IFN-γ and CXCL9 levels, and remitted alopecia, oral candidiasis, nail dystrophy, gastritis, enteritis, arthritis, Sjögren's-like syndrome, urticaria, and thyroiditis. AIRE-deficiency features excessive, multiorgan IFN-γ-mediated responses. JAK inhibition with ruxolitinib may be a promising strategy for the management of APECED.

**Dissecting the genetic heterogeneity in common variable immunodeficiency: focus on a missense mutation in the CD40L gene**

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A total of 44 patients with a clinical phenotype consistent with common variable immunodeficiency (CVID) were recruited to study immunophenotypic, genomic, and transcriptomic aspects of the disease. Flow cytometric immunophenotyping of main lymphocyte populations was performed in both patients and healthy controls.
Results showed a significantly higher frequency of CD8$^+$ T lymphocytes and a lower number of NK cells in patients compared to controls. Among NK cells, patients exhibited a higher percentage of CD56$^{bright}$ cells. CD4$^+$ T lymphocytes were reduced, though not significantly compared to controls. Total B lymphocytes were comparable between patients and controls, although the memory compartment was reduced in patients.
Whole exome sequencing (WES) identified putative genetic alterations in 50% of the patients. Among the others, we identified a novel mutation in the transmembrane domain of the CD40L gene. We demonstrated that this mutation, reduces but does not completely abolish CD40L expression on CD4$^+$ T cells membrane after polyclonal stimulation. Altogether, these findings demonstrate a novel mutation responsible for X-linked hyper-IgM syndrome. Literature review suggests that transmembrane domain mutations may lead to milder or atypical phenotypes, resembling CVID, as they alter protein expression on the cell surface. In this patient, blood counts were normal, with elevated CD8$^+$ T cells and a low CD4$^+$/CD8$^+$ ratio. NK and B cells were reduced, along with low IgG, borderline IgA, and slightly elevated IgM, which are typical of XHIGM. Memory B cells were significantly reduced, particularly switched memory B cells, while T lymphocyte subpopulations were mostly normal, except for reduced Tfh cells. Additionally, a decreased production of IFN-γ by CD4$^+$ T lymphocytes was observed.
Molecular investigations using single-cell RNA sequencing, including BCR and TCR sequencing, are ongoing on both resting and polyclonally stimulated peripheral blood mononuclear cells of this patient for a deep transcriptomic profile linked to this mutation.

**Lymphoproliferative disorders and inborn errors of immunity: a model for unraveling signaling pathways targetable by precision therapies**

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Introduction: Lymphoproliferative disorders (LPDs) can be the presenting clinical feature or a complication of inborn errors of immunity (IEI).
Benign and malignant LPDs may be driven by genetically determined mechanisms leading to alteration of specific signaling pathways. In IEI-related LPDs, flow-cytometric or molecular monoclonality/oligoclonality does not necessarily indicate malignancy: differential diagnosis is challenging and



cannot disregard an expert pathological review. Currently available investigations do not strongly correlate with disease severity or prognosis, so it's crucial to identify/validate further biomarkers and functional tests to assess if an early targeted therapy can reduce immune dysregulation, preventing lymphoproliferation and malignant evolution.

**Methods:** We are retro-prospectively collecting clinical, immunological, genetic and histological data from a cohort of IEI patients with LPDs, presenting with persistent lymphadenopathy lasting more than 3 months.

**Results:** We included 38 patients, with a median age of 5 and 12.5 years at clinical onset and IEI clinical diagnosis, respectively. LPDs were present at onset in 14 patients; a genetic diagnosis was made in 15 patients at a median age of 14.5 years. 23 patients received immunomodulatory therapies.An imbalance towards memory differentiation in $CD4^+$/$CD8^+$ subsets was observed in patients who later developed lymphoma. Histological analysis showed common patterns, including follicular hyperplasia, irregularly shaped germinal centers, paracortical expansion, Castleman-like, progressive transformation of germinal centers-like, and predominant $IgM^+$ over reduced/absent $IgG^+$ plasma-cells. In a 33-year-old female with a complex history of immune dysregulation, later genetically diagnosed as APDS2 (activated phosphoinositide-3-kinase-delta syndrome-2), histological revision revealed non-malignant interfollicular hyperplasia instead of the previous histologic diagnosis of Hodgkin lymphoma at 19 years of age.

**Conclusions:** LPDs are common onset features of IEI. Specific histological features may raise suspicion of underlying IEI, emphasizing the crucial role of pathologist expertise, that could drive together with clinician toward a correct diagnosis in order to choose the more appropriate treatment.

## Human IL12RB1-deficiency reveals essential roles of IL-12 and IL-23 in innate and adaptive immune responses

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Mendelian susceptibility to mycobacterial infections (MSMD) is a primary immunodeficiency characterized by increased frequency of infections from non-tubercular mycobacteria, due to genetic defects affecting the IL-12-IFN-γ axis. Autosomal recessive deficiency of IL-12RB1 is the most common cause of MSMD. IL-12Rb1 pairs with IL-12Rb2 or IL-23R to form complete IL-12 or IL-23 receptors, respectively. Thus, IL-12Rb1 deficiency impairs both sensitivity to IL-12 and IL-23. Here, we studied PBMNC from an IL12RB1 deficient patient to dissect how IL-12 and IL-23 control innate and adaptive immune cells' functionality. We performed single cell RNA sequencing on PBMC either resting or after TCR stimulation. IL12RB1-deficient $CD8^+$ T cells displayed a marked contraction of the most cytotoxic clusters expressing GZMB. On the contrary, GZMK-expressing $CD8^+$ T cells were not affected. $CD4^+$ T cells displayed an impaired expression of genes related to cytotoxicity as well. IL12RB1-deficient $CD4^+$ and $CD8^+$ T cells expressed lower levels of IFNG than cells from healthy individuals, as previously demonstrated. In addition, we demonstrated that IL12RB1-deficient $CD4^+$ and $CD8^+$ T cells capable to express IFNG have a distinct transcriptional signature compared to cells from donors. This finding suggests that although some cells can become IFNG producers even in absence of IL12RB1, their differentiation is not complete. Additionally, scRNAseq data demonstrated that IL12RB1-deficient T cells have an impaired capability to trigger oxidative phosphorylation upon TCR stimulation. We performed functional experiments that confirmed an impaired energy production. NK cells were significantly reduced in the circulation of the IL12RB1-deficient patient, but their cytotoxic profile was not affected, suggesting that IL-12 and/or IL-23 are fundamental for NK cells' expansion. B cells were also reduced in circulation and exhibited contraction of the memory cluster. Finally, monocytes demonstrated a significant downregulation

of gene signatures related to IFN signaling and antigen processing capability.

**Study of the pro-inflammatory role of cytokine nicotinamide phosphoribosyltrasferase in inflammatory bowel disease and its possible therapeutic use**

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Nicotinamide phosphoribosyltrasferase (NAMPT) is the crucial enzyme in the salvage pathway for NAD synthesis. This protein exists in two different forms: within or outside the cell. The intracellular form, iNAMPT, enables NMN synthesis, an important precursor of NAD. The extracellular form, called eNAMPT, functions as a cytokine, exerting paracrine and autocrine influences on various immune cells. Although the precise mechanism by which eNAMPT operates remains unknown, its role in many diseases is significant. Increased levels of eNAMPT have been linked to different inflammatory conditions such as inflammatory bowel disease (IBD).
In our work we have studied the role of eNAMPT on a chemically induced (i.r. 3 mg/mouse DNBS 5 days) in vivo colitis murine model. We have evaluated animal phenotype (weight and colon length) after recombinant eNAMPT administration. In addition, colons of treated animals were analysed through immunohistochemistry and PCR for the presence of fibrosis. We have demonstrated that eNAMPT exacerbates colitis symptoms. Therefore, we have deeply investigated eNAMPT proinflammatory activity on immune cells in colon (lamina propria), bone marrow and blood of treated and untreated animals through flowcytometry (FACS). We observed that eNAMPT controls myeloid cells recruitment, especially monocytes and macrophages $CD86^+$, in colon of DNBS induced mice and controls immune cells differentiation in healthy mice, promoting LSK cells and common myeloid precursors (CMP) differentiation in bone marrow.
In this work we have also investigated the efficacy of the recently developed eNAMPT neutralizing mAb ALT-100 in the treatment of DNBS induced colitis. ALT-100 demonstrated to be able to reduce myeloid cells recruitment in the colon and myeloid differentiation in the bone marrow.
In conclusion we can now affirm that eNAMPT induces myeloid cells differentiation and recruitment resulting in inflammation worsening and myeloid cells maturation in healthy mice and in IBD model, that can be reverted through eNAMPT antibody neutralization.

## WORKSHOP 4
## INNATE IMMUNITY

**STAT4 suppresses type I interferon transcriptome in inflammatory ILC2**

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Type 2 innate lymphoid cells (ILC2) are required for protection against helminthic infection and can promote inflammation in pathological conditions, such as allergy and asthma. Under specific stimuli, ILC2 change their phenotype and acquire the ability to produce the typical type 3 cytokine, IL-17; these cells are defined as inflammatory ILC2 (iILC2) and are enriched in the lung. Members of the signal transducer and activator of transcription (STATs) play a crucial role in the regulation of ILC2 functions, not only at steady state but also during activation. Herein, to evaluate the dynamics of the expression of STATs in ILC2, we first mined bulk RNA-seq data of murine ILC2 from distinct tissues. Using this dataset, we found that the expression of Stat4 in activated ILC2 reached the levels observed in prototypical type 1 innate lymphocytes, namely NK cells and ILC1. By using an in vivo model based on IL-25 administration, we validated that STAT4 was expressed by ILC2 at protein level, and selectively expressed in iILC2. This expression pattern is also conserved in iILC2 present in the peripheral blood of non-controlled asthma patients. By using a genetic approach, we observed that Stat4-deficient ILC2 showed impaired differentiation towards the iILC2 fate after IL-25 administration. At the transcriptional level, Stat4-deletion unleashed a type I IFN-regulatory circuit in iILC2, which is detrimental for their functions. Despite the well-known antagonistic function of STAT4 in type 2 cells, our data provide evidence for a positive feedback for iILC2 differentiation by limiting the action of type I IFNs.

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## Cyp1a1 deficiency endows type-2 dendritic cells with immunoregulatory functions

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**Introduction:** Classical dendritic cells (cDCs) encompass two main lineages: cDC1s and cDC2s, each marked by distinct surface proteins. cDC1s are pivotal for $CD8^+$ T cell immunity to pathogens and tumors, while cDC2s initiate $CD4^+$ T cell responses. cDCs adapt to environmental changes influenced by factors like diet and microbiota. The aryl hydrocarbon receptor (AhR) detects environmental signals, regulating immune responses by inducing cytochrome P4501 (CYP1) enzymes to metabolize AhR ligands, although their specific role in cDC subsets is not fully understood.

**Purpose:** To Investigate the functional role of Cyp1a1 in dendritic cell subsets and its impact on immune responses.

**Results:** We demonstrated that Cyp1a1 deficiency does not alter the DC developmental program. Moreover, we observed significant upregulation of Cyp1a1 mRNA in cDC2 compared to cDC1 upon LPS stimulation. Using a Luminex approach, we found that Cyp1a1 deficiency strongly suppressed the production of proinflammatory cytokines, such as IL-6, in activated cDC2s compared to WT controls. In a cDC2-OTII co-culture system, OTII cells proliferation was significantly reduced without Cyp1a1 in cDC2. To further assess the impact of Cyp1a1 in cDC2 in vivo, we employed a skin test assay in female mice sensitized with the H-2Db-restricted HY peptide. Notably, reactivity to HY peptide was significantly diminished when co-administered with $Cyp1a1^{-/-}$ cDC2. These findings indicate that Cyp1a1 upregulation may metabolize AhR ligands, boosting cDC2 inflammatory function.

**Conclusion:** Interfering with Cyp1a1-mediated degradation of AhR ligands could offer a novel strategy to control immune responses in autoimmune and inflammatory diseases.

## Uncovering two neutrophil-committed progenitors that immediately precede the promyelocytes during human neutropoiesis

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Technological advances have changed our view of haematopoiesis, switching from the classical hierarchical tree-like model to that envisioning a continuous differentiation of progenitors along discrete lineages. Several precursors of myeloid cells have been identified based on flow cytometry approaches, including the granulocyte-monocyte-dendritic cell (DC) progenitors (GMDPs), monocyte-DC progenitors (MDPs), common DC progenitors (CDPs) and common monocyte progenitors (cMoPs). Concerning neutropoiesis, some progress has been made, for instance in identifying proNeu1s and eNePs, but since these cells express the CD66b and CD15 lineage markers, they resemble the promyelocyte (PM). In this research area, however, our group has recently identified four $SSC^{lo}Lin^-CD66b^-CD45^{dim}CD34^+/CD34^{dim/-}CD64^{dim}CD115^-$ cells as the earliest precursors specifically committed to the neutrophil lineage present in human bone marrow (BM), which we called neutrophil-committed progenitors (NCPs, from NCP1s to NCP4s).

Now we report the discovery of two new $SSC^{hi}CD66b^-CD64^{dim}CD115^-$ NCPs, which have been fully characterized for their flow cytometric side-scattered light (SSC) profile, in addition to their phenotypic, transcriptomic, maturation and immunohistochemistry properties. These cells were found to precede PMs, but to follow NCP4s in the neutropoiesis cascade. Similarly to $SSC^{lo}CD45RA^+$ NCP2s/NCP3s and $SSC^{lo}CD45RA^-$ NCP1s/NCP4s, these cells exhibit phenotypic differences in CD45RA expression levels and, therefore, were named as $SSC^{hi}CD45RA^+$ NCP5s and $SSC^{hi}CD45RA^-$ NCP6s. Moreover, NCP5s resulted more immature than NCP6s, as determined by cell differentiation and proliferative potential, as well as by transcriptomic and phenotypical features. Finally, by examining whether NCPs and all other $CD66b^+$ neutrophil precursors are altered in representative hematological malignancies, we could observe that, in patients with chronic-phase chronic myeloid leukemia (CP-CML), but not with systemic mastocytosis (SM), there is an increased frequency of BM NCP4s, NCP6s, and all downstream CD45RA-negative neutrophil progenitors, suggesting their expansion in CML pathogenesis. Collectively, our data improves the understanding of human neutropoiesis and shed new

light on the pathogenesis of CML (Cellular&Molecular Immunology, 2025, in the press).

**Phenotype and function of suppressive monocytes during viral infections**

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Monocytes can play context-dependent roles during infection, by either enhancing or hindering immune responses. Our group has shown for the first time that inflammatory monocytes (IMs) can suppress B lymphocytes and dampen antibody responses during non-cytopathic lymphocytic choriomeningitis virus (LCMV) infection. This novel suppressive function appears to be context-dependent, as monocytes recruited in response to the cytopathic vesicular stomatitis virus (VSV) do not affect B cell responses.
To better characterize suppressive monocytes, we investigated their phenotype through a combination of transcriptomics, flow cytometry, and confocal microscopy. Interestingly, both bulk and single-cell (sc) RNA sequencing showed that a subset of monocytes recruited to draining lymph nodes (dLNs) during LCMV infection expresses the cytotoxic molecule granzyme A (GzmA) 3 days post-infection. Flow cytometry and confocal imaging confirmed that some LCMV-recruited monocytes express GzmA also at the protein level and localize close to LCMV-specific B cells. Preliminary data from functional studies suggest that GzmA and perforin (Prf-1) might indeed affect LCMV-specific B cell survival, thus impacting also antibody responses. Overall these findings suggest that monocyte-mediated release of GzmA and Prf-1 could suppress antigen-specific B lymphocytes, although further experimental corroboration of this hypothesis is ongoing. Deep characterization of the molecular profile of suppressive monocytes may provide insights into new mechanisms employed by viruses to evade immune clearance and facilitate persistence within infected hosts.

**Macrophage-driven NK cell plasticity associates with CXCR3 axis in mouse liver metastasis models**

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**Background:** NK cells are innate lymphoid cells (ILC) that limit tumor growth by cell cytotoxicity and by producing cytokine and chemokines. ILC1-like cells, marked by CD49a expression, have been linked to NK cell plasticity driven by TGF-β1. Nevertheless, the cells implicated in NK cell plasticity and their role in regulating liver metastasis derived from colorectal cancer (CRC) remain poorly understood.

**Aim:** To investigate the role of CD49a$^+$NK cells in CRC liver metastasis and their interaction with metastasis-associated macrophages (MAMs).

**Methods and Results:** We employed two liver metastasis mouse models based on the intrasplenic injection of CRC cell lines (MC38 and SL4). CD11bhighF4/80highMAMs infiltrated MC38-induced metastasis characterized by the expression of CD206, Arginase 1, TREM2, PD-L1 and MHC-II. MAMs expressed IL-15 and TGF-β1 transcripts and secreted CXCL9, CXCL10 and IL-15/IL15Rα proteins. CD49a$^+$NK cells displayed high anti-tumor function, and their accumulation well correlated with CXCL9$^+$MHCII$^+$ macrophage frequency in the metastasis. Moreover, co-culture experiments of NK cells with MAMs isolated from MC38-induced metastases resulted in NK cells acquiring ILC1-like characteristics. On the other hand, mice treated with anti-CSF1R mAb, which selectively impairs F4/80highMAM infiltration, displayed marked inhibition of CD49a$^+$NK cell development. To further investigate whether CD49a$^+$NK cell accumulation was influenced by the immune microenvironment we analyzed SL4-induced metastases, which were characterized by the increase of F4/80int macrophages and by F4/80high macrophages with reduced CXCL9 and TGF-β1 expression compared to MC38 metastatic infiltrate. This altered myeloid compartment was markedly associated to a reduced accumulation of CD49a$^+$NK cell, supporting the hypothesis that CXCL9-mediated interaction with macrophages plays a crucial role in shaping the NK cell phenotype in liver metastasis.



**Conclusion:** Altogether, these results suggest that CD11bhighF4/80highMHC-II$^{+}$CXCL9$^{+}$ macrophages arise in an immunogenic metastatic context and could contribute to generation of tissue niches critical for NK cell trans-differentiation into CD49a$^{+}$NK cells in CRC.

# WORKSHOP 5
# IMMUNOTHERAPY

## Interleukin-1 receptor 8 deficiency enhances the anti-tumour activity of doxorubucin and reduces cardiotoxicity

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Chemo-immunotherapy, combining anthracyclines like doxorubicin (Doxo) with immune checkpoint inhibitors (ICI), enhances immunogenic cell death and tumor-specific immune responses against cancer. It increases the risk of severe side effects, like inflammation and T cell-mediated cardiotoxicity. Interleukin-1 (IL-1) plays a key role in both cancer- and cardiac-related inflammation. IL-1 receptor 8 (IL-1R8), a negative regulator of IL-1 and TLR signaling, functions as an IC for NK and CD8$^{+}$ cells, enhancing anti-tumor immunity in murine models [1]. To explore IL-1R8 as a novel IC, we investigated the role and potential side effects of its deficiency in combination with chemotherapy.
We investigated the impact of IL-1R8 deficiency on tumor growth and cardiac toxicity after Doxo treatment. Wild-type (wt) and Il1r8$^{-/-}$ mice were subcutaneously transplanted with FS6 fibrosarcoma cells and treated with high or low (20-10 mg/kg) Doxo concentration. Tumor growth was monitored and immune infiltration was analyzed. Cardiac function, fibrosis, immune infiltration, and inflammation were examined in cardiac tissue.
The high-dose regimen completely eradicated the tumor in both genotypes, while low-dose Doxo significantly reduced tumor size in Il1r8$^{-/-}$ mice, accompanied by more mature CD8$^{+}$ cells, compared to wt mice. High-dose Doxo impaired cardiac function, with no genotype differences, indicating that IL-1R8 blockade does not impact on cardiotoxicity. In contrast with the hypothesis, Il1r8$^{-/-}$ mice exhibited reduced cardiac fibrosis, lower inflammation, and fewer CD4$^{+}$ cells in cardiac tissue after Doxo treatment, compared to wt counterpart. Co-culture experiments (CD4$^{+}$Th1 cells plus fibroblasts or mac-

rophages) revealed that Il1r8$^{-/-}$ Th1 cells had reduced pro-fibrotic and inflammatory activity compared to wt cells, in line with a previous study identifying MyD88 as a regulator of cardiac fibrosis through modulation of T cell activation [2].
Our results show that IL-1R8 targeting enhances the anti-tumor activity of Doxo, while reducing cardiotoxicity and fibrotic effects of CD4$^{+}$ cells.

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**Dissecting a dual tumor-suppressive role of microRNA203a in non-small cell lung cancer**

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Lung cancer is the most frequent cause of cancer-related death, with non-small cell lung cancer (NSCLC) attesting for the 85% of the novel diagnosis every year. Chemotherapy against NSCLC is widely inefficient, while immune checkpoint inhibition produced appreciable results just in subsets of patients.
Aberrant expression of microRNAs (miRNAs) represents a common hallmark of cancer and, accordingly, miR203a downregulation was recently associated with NSCLC progression and poor prognosis. This project aims to propose miR203a replenishment as a novel anticancer strategy, acting through a double combined effect to enhance immune responses and reduce tumor growth.
Based on our findings that extracellular miRNAs, including miR203a, can act as unconventional toll like receptor (TLR) 7/8 ligands, we show here that miR203a efficiently activates a potent pDC/monocyte/NK anticancer crosstalk. Specifically, full NK cell activation, including IFN-γ production and cytotoxicity, depends on the release of IFN-α, IL-12 and IL-18 by miR203a-activated pDCs and monocytes. Importantly, these effects are abolished by the TLR7/8 antagonist Enpatoran.
At the same time, miR203a treatment also exerts a direct anticancer function on NSCLC cell lines by hampering tumor viability, proliferation and clonogenic capability: the underlying mechanisms are currently being investigated.
Consistent with these *in vitro* results, *in vivo* miR203a administration results in the drastic reduction of NSCLC tumor burden and in the restoration of NK cytotoxic functions, which are usually hampered in this tumor microenvironment.
In conclusion, miR203a emerges as a novel multifaced therapeutic weapon against NSCLC, acting synergically on tumor and immune cells, possibly contributing to the future reduction of chemoresistance and side effects of current therapies.

**A private crosstalk established by tumor-targeted cytokine release rescues CAR-T activity and engages host T cells against glioblastoma**

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Chimeric antigen receptor T cells (CAR-Ts) have shown limited efficacy in solid tumors, due to poor penetration, constrained activity, and early exhaustion into the immunosuppressive tumor microenvironment (TME). While stimulatory cytokines can counteract immune suppression, their systemic administration entails risk of toxicities and counter-regulatory responses. Here we leveraged a population of tumor-associated TIE2 expressing macrophages (TEMs) to release IFN-α and/or orthogonal IL-2 (oIL2) at the tumor site. Targeted cytokine delivery rescued CAR-T functionality against the clinically relevant tumor antigen B7-H3 in an immunocompetent mouse model of glioblastoma (GBM), which was refractory to treatment with CAR-Ts alone. Immunophenotypic and transcriptomic analyses showed that TEM-base cytokine delivery alleviated terminal exhaustion of CAR-Ts, while inducing effector/memory states associated with enhanced anti-tumor activity. Furthermore, IFN-α delivery elicited potent endogenous T cell responses against multiple tumor-associated antigens, leading to delayed GBM growth and prolonged mice survival even with tumors expressing B7-H3 in only a fraction of cells. Our data suggest that the combination of TEM-based cytokine delivery and CAR-T treatment could achieve synergistic effects in GBM patients, this providing greater therapeutic benefits that monotherapies.

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## Stimulating resolution of inflammation unleashes CD8 T cell anti-cancer immunity

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Chronic non-resolving inflammation is a major hurdle to overcome for a successful antitumor response, as it induces an immunosuppressive tumor microenvironment (TME) that hampers anticancer immunity. Recent evidence suggests that inflammatory signals released in the TME alter the actions of key anticancer effectors, i.e., CD8 T cells, and promote their differentiation into a dysfunctional, exhausted phenotype (Tex). Thus, re-invigorating resolution (the ideal outcome of inflammation) with mediators including resolvins (RvD) could shape the plasticity of CD8 T cells and restore their antitumor functions.
To address this, we used head and neck cancer as an experimental system. Bioinformatic analysis of patient tumors, coupled with in vitro, in vivo, and omics approaches were used to dissect how restoring resolution reinvigorate T CD8.
In patients, Tex were significantly enriched in cytokine pathways, suggesting that inflammatory programs drive T CD8 antitumor immunity. Exposure to RvD5 reduced cancer cell proliferation by enhancing T CD8 cell activity in vitro. Mechanistically, RvD5 stimulated T CD8 to block cancer cells in G2/M phase and downregulated markers of exhaustion in both $CD8^+$ T (PD-1) and tumor cells (PD-L1). In vivo, RvD5 reduced cancer growth by decreasing the inhibitory PD-L1/PD-1 axis in the TME. Cytokine analysis, lipidomic, bulk and single-cell RNAseq revealed increased production and release of mediators involved in the antitumor response in the TME of RvD5 treated mice. Importantly, RvD5 increased the response rate of mouse tumors to anti-PD-1 treatment, suggesting that shifting cancer inflammation toward resolution is beneficial to enhance the efficacy of currently used immune checkpoint inhibitors.
Thus, RvD5 reduces tumor growth by modulating inflammation and delaying Tex in the TME. Therefore, stimulation of resolution shape T CD8 actions against cancer cells and could represent a new therapeutic strategy to potentiate antitumor immunity.

## Targeting chondroitin sulfate proteoglycan 4 with DNA vaccination to unlock new potential combination therapies for melanoma

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Malignant melanoma (MM) is the sixth most common cancer worldwide. Despite advances thanks to the introduction of BRAF/MEK-targeted therapies and immune checkpoint inhibitors, many patients still do not benefit. Combining standard therapies with cancer vaccines could be pivotal. Chondroitin sulphate proteoglycan (CSPG)4 is an ideal immunotherapeutic target, with limited expression in healthy tissues and high expression in MM, where it plays a key oncogenic role. However, as a non-mutated self-antigen, CSPG4 is poorly immunogenic. To overcome this, we developed a chimeric DNA vaccine (HuDo-CSPG4) combining human and dog CSPG4 portions. In preclinical mouse models, HuDo-CSPG4 immunization showed immunogenicity and antitumor potential. We tested this approach in a veterinary trial enrolling 80 client-owned dogs with surgically-resected, CSPG4-positive oral MM; 52 were included in the immunization arm, following a monthly (up 2 years) vaccination protocol (P1). This protocol includes the need for monthly anesthesia, potential T cell retention at the injection site and the risk of T cell exhaustion. Therefore a 6-vaccination protocol, followed by boosters every 6 months (up to 2 years, P2) was also tested. Anti-CSPG4 vaccination in canine MM patients was safe, immunogenic and with clinical benefit, significantly increasing survival of vaccinated dogs compared to controls. Induced immune responses correlated with patients' survival. Dogs receiving P2 showed similar clinical outcomes despite fewer vaccinations, proposing P2 as an optimal schedule. As a step forward for the human clinic, HuDo-CSPG4 vaccine in combination with standard-of-care therapies, such as anti-BRAF (Vemurafenib)/MEK inhibitors and anti-PD-L1, is under investigation. Preliminary studies suggest that CSPG4 could be related to vemurafenib-resistance and targeting CSPG4 could enhance drug sensitivity. Thanks to the predictive power of spontaneous canine tumors and the unique hybrid structure of the vaccine, these results may hold significant translational implications, providing a rationale for proposing HuDo-CSPG4 vaccination in combination therapies, to finally improve MM patients' survival.

## WORKSHOP 6
## VACCINES

### Characterization of DC–T Cell interaction dynamics in a vaccination model through in vivo enzymatic labeling

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Dendritic cell (DC)-T cell interactions are critical for activating adaptive immunity, shaping the intensity, duration, and nature of the immune response.
In this research, we investigate DC-naïve T cell interactions in vivo in a vaccination setting. Using an enzymatic labeling approach, we quantified interactions between DCs and antigen-specific $CD4^+$ and $CD8^+$ T cells at different time points upon vaccination. Our findings reveal a progressive increase in interacting DCs, peaking at 72 hours post-immunization. Notably, this kinetic was independent of migratory DCs accumulating into the lymph node but correlated with the clonal expansion of antigen-specific T cells. By combining enzymatic labeling with mitotic division tracking, we observed that DC-T cell interactions extend beyond initial antigen presentation and persist throughout T cell replication.
Furthermore, CITE-seq analysis identified distinct DC states engaged with T cells at different stages of the immune response, suggesting specialized functional roles for DCs during early priming versus the expansion phase. Ongoing research aims to elucidate the impact of these post-mitotic interactions on shaping and regulating T cell responses.

### Exploiting hyaluronan as a natural and effective immunological adjuvant for protein-based vaccines against HER2-expressing breast cancer

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Background: Protein-based vaccines represent a safe and practical platform for disease treatment and prevention yet necessitating an efficient adjuvant to effectively induce an immunological memory. HER2 overexpression occurs in 15-30% of breast cancer cases in women and correlates with adverse prognostic outcomes and frequent relapse. Indeed, anti-HER2 vaccines could serve therapeutically or preventatively to inhibit progression or target early disease stages. Recently, our group patented a method where protein antigens are conjugated to 200 kDa hyaluronan (HA) fragments, acting as toll-like receptor 2/4 agonists. Our final goal relies on the validation of HA-based protein vaccination approach in efficacy and safety against HER2-expressing BC.

Methods: Syngeneic HER2/neu-positive breast tumors were established in female Balb/c mice and transgenic spontaneous Balb/NeuT mice. Preventive vaccination in both models followed a three-dose schedule and Balb/c mice were challenged and rechallenged respectively 30 and 90 days after the first dose, with HER2 expressing TUBO cells; Balb/NeuT mice were monitored for tumor onset. Therapeutic vaccination, applied to Balb/c mice only, started 12 days after tumor challenge with a three-dose schedule.

Results: Efficacy was evaluated by the assessment of magnitude and duration of humoral and cellular specific immune responses all three settings. Remarkably, one-year post-vaccination, antigen-specific antibodies were still detectable and functional, as reported in inhibition of cell proliferation and induction of complement, antibody and phagocytosis-dependent cytotoxicity assays. HA-induced Th1/Th2 humoral and cellular responses were effective in both prophylactic (100% survival) and therapeutic (tumour regression in 2/12 mice) contexts, breaking tolerance against rHER2/neu and delaying spontaneous tumour growth in BALB-neuT mice.

Conclusions: HA proved efficient in generating robust and long-lasting humoral and cellular responses with very low antigen doses, suggesting potential advantages for poorly responding subjects. This innovative solution will provide new preventive solutions for hereditary neoplasms and disease relapse prevention, alongside effective therapies for existing neoplasms.



## Smallpox-specific T cells in vaccinated centenarians exhibit phenotypic and metabolic traits of long-term memory compared to SARS-CoV-2-specific T cells

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Introduction: The memory of adaptive immune system cells is critical for pathogen protection, with vaccines relying on the induction of long-term memory responses. The duration of immunity depends on the quality and quantity of these responses. Smallpox vaccination, received at the age 5-9 years, in Italy was discontinued in the late 1970s. Vaccinia-specific immunity persist for several decades. However, detailed data on the characteristics of such memory cells and their potential cross-reactivity against Mpox remain limited.

Objectives: This study aimed to identify and characterize smallpox-specific memory T cells in individuals vaccinated before 1940 or around the '70s, and compare their features with memory cells generated after SARS-CoV-2 vaccination.

Methods: We enrolled 25 individuals (defined: "boomers"; mean age 68 ± 8.5 years) and 16 individuals ("centenarians"; mean age 97 ± 5.5 years). Using activation-induced markers assay and 21-parameter flow cytometry, antigen-specific T cells were identified following stimulation with either SARS-CoV-2-Spike glycoprotein or vaccinia peptide pool. Single-cell transcriptome analysis was performed using VASA-seq.

Results: Transcriptome of smallpox-specific T cells was characterized by a gene signature of mainly resting memory with stem-like properties (LEF1, IGFR1, TCF7, CD27), with a metabolic hallmark gene signature of oxidative phosphorylation, while SARS-CoV-2-specific T cells express SLAMF1, LAG3, SELPG, CD74, PRF1, IRF, mainly relying on glycolysis. Smallpox-specific $CD4^+$ T cells are skewed towards central memory Th0-2 phenotype, with less naive and transitional memory Th1-17 $PD1^+$. This observation is more marked in centenarians. Centenarians display low percentage of SARS-CoV-2-specific $CD8^+$ T cells and still detectable smallpox-specific $CD8^+$ T cells with resting memory phenotype. TCR clonotype analysis revealed that centenarians were characterized by a lower quantity of small clonotypes in all clusters but MAIT cells when compared to boomers.

Conclusion: These findings highlight the durability of smallpox-specific T cells, even decades post-vaccination, and suggest unique immune properties relevant for cross-reactivity against Mpox.

## Characterization and monitoring of T cell-specific immune response in subjects at high risk of Monkeypox infection undergoing anti-smallpox vaccination

**Laura Maggi[1], Anna Vanni[1], Valentina Cosi Becchi[1], Giulia Lamacchia[1,2], Lorenzo Salvati[1,3], Alessio Mazzoni[1,2], Manuela Capone[1,4], Francesca Matani[1], Lucia Bartoli[1,5], Stefania Francalanci[1,2], Seble Tekle Kiros[2], Filippo Lagi[2], Alessandro Bartoloni[1,2], Lorenzo Cosmi[1,2], Francesco Liotta[1,2], Alessandro Sette[6], Francesco Annunziato[1,2]**

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Introduction: Currently, the MVA-BN vaccine, Modified Vaccinia Virus Ankara, is used for subjects at risk of infection by Monkeypox virus (MPXV). While some studies shown that the antibody response in individuals vaccinated against Variola virus is maintained over time, the duration and cross-reactivity of the antigen-specific T response induced by vaccination towards different strains of Orthopoxvirus has not yet been investigated.

Purpose: Characterization of the antigen-specific T cell response in terms of frequency and cross-reactivity between Vaccinia virus (VV) and MPXV, comparing: subjects vaccinated at young age against smallpox and now vaccinated with one dose of MVA-BN (ex-vax), and subjects vaccinated ex-novo with two doses of MVA-BN (naïve). 80 subjects were sampled before and after vaccination with MVA-BN, at different time points and analyzed by 25-color spectral flow cytometry, post ex-vivo stimulation with conserved peptides of the Orthopoxvirus family or specific for MPXV.

Results: Preliminary data on 8 ex-vax and 8 naïve subjects revealed the presence of Orthopox and MPX specific T cells in patients vaccinated during childhood at T0 (before the new vaccination) and their increment after only a single dose of vaccine. At later time point after MVA vaccination we observed an appreciable response to both MPX and Orthopox pool in both cohorts although with no difference in the magnitude of response.

Conclusions: These data suggest a persistence of immunological memory in subjects vaccinated with the previous Variola virus vaccine and the efficacy of a single dose of MVA-BN in reactivating it and in inducing a comparable response in naïve subjects.

Cross-reactivity between VV and MPXV is also present, suggesting a possible protection against other Orthopoxvirus strains. This experimental setting will be applied on the other enrolled subjects including also a sub-cohort receiving the same MVA-BN vaccine via intramuscular administration despite intradermal one, as the first analysed cohort.

**Machine learning approaches to detect individuals who were unaware of SARS-CoV2 infection: dissection of hybrid and vaccine-induced immunity**

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**Background:** The emergence of the SARS-CoV-2 Omicron variant and its subvariants has increased the cases of unreported infections. Identifying unaware infected individuals is crucial for estimating the true prevalence of infection and evaluating the breadth of hybrid immunity. In this study, we addressed this challenge by applying a machine learning approach.

**Methods:** A group of 116 healthy participants vaccinated against SARS-CoV-2, were enrolled in the IMMUNO_COV study, at Siena University Hospital. A blood sample was collected six months after the third vaccine dose. SARS-CoV-2 infection was self-reported by participants. Serological parameters used for the computational modelling were antibody responses specific for the wild type strain and Delta, Omicron BA.1 and Omicron BA.2 variants. Spike- and nucleocapsid-specific B cells were also assessed in each participant.

**Findings:** By applying dimensionality reduction and unsupervised clustering, participants were grouped into high and low responders, with a distribution of infected participants mainly within the high responder group. The implementation of a k-Nearest Neighbours model identified a subset of 14 participants who were unaware of a previous infection. Their immunological profile, characterized by strong Spike- and nucleocapsid-specific humoral and B cell responses, significantly differed from what observed in non-infected participants.

**Conclusions:** Machine learning approaches have been used to identify participants unaware of their infection and dissecting their hybrid immunity profile. Based on widely available serological data, this rapid and cost-effective method can be a tool for understanding the true prevalence of a pathogen infection, improving comprehension of immune responsiveness generated by both vaccination and hybrid immunity, and tailoring public health interventions.



# WORKSHOP 7
# TUMOR IMMUNOLOGY 2

## Interleukin 10 produced by tumor-reactive EOMESODERMIN⁺Tr1-like cells is critical for immunotherapy of melanoma

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Regulatory T cells that express FOXP3 block tumor destruction by CD8⁺T cells and could inhibit the efficiency of immunotherapy. We previously published that EOMESODERMIN-expressing type-1 regulatory T cells, which secrete the tolerogenic cytokine IL-10 (EOMES⁺Tr1-like cells), also play a key role in human tumors. Notably, depending on the context, IL-10 may have pro- or anti-tumor effects. Bioinformatic analysis suggested however that tumor infiltration of EOMES⁺Tr1-like cells is associated with poor patient's survival, and with responsiveness to anti-PD1 immunotherapy of melanoma patients.
In the B16-OVA mouse melanoma model, EOMES⁺Tr1-like cells and FOXP3⁺Tregs accumulated in tumors. Moreover, melanoma-bearing IL-10-reporter mice unveiled that tumor-infiltrating regulatory T cells produced high levels of IL-10. Adoptive transfer experiments with IL-10-deficient or sufficient T cell populations suggested a critical role of Tr1-derived IL-10 to inhibit cytotoxic anti-tumor T cell responses. Moreover, anti-PD-1L immunotherapy reduced T cell IL-10 production, and was ineffective in the absence of IL-10-producing Tr1-cells. Both EOMES⁺Tr1-like cells and FOXP3⁺Tregs responded to the model tumor neoantigen OVA. Single cell RNA sequencing of neoantigen-specific CD4⁺ T cells unveiled largely conserved gene signatures of these tumor-infiltrating regulatory T cell subsets in humans and mice.
In resected tumors from melanoma patients EOMES⁺Tr1-like cells and FOXP3⁺Treg were significantly increased and expressed Ki67, indicating recent activation. Moreover, circulating Tregs and Tr1-cells from melanoma patients, but not from healthy controls, produced IL-10 in response to melanoma-associated antigens (MelanA, GP100, Tyrosinase) and to the neo-antigen BRAFV600E. Finally, melanoma patients undergoing immunotherapy showed significant lower IL-10 production of Tr1-cells, but not of FOXP3⁺Tregs, in response to melanoma-associated antigens.
These findings suggest a key role for IL-10 produced by melanoma-reactive EOMES⁺Tr1-cells to inhibit anti-tumor CD8⁺T cell responses. Moreover, they suggest that anti-PD1 immunotherapy does not necessarily act directly on exhausted CD8⁺T cells, but may unleash cytotoxic anti-tumor responses indirectly, by blocking pro-tumorigenic IL-10 production of EOMES⁺Tr1-like cells.

## IL4I1 catalytic activity confers protection against ferroptosis in a murine fibrosarcoma cell line

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**Background:** Interleukin-4-induced gene 1 is an L-amino acid oxidase endowed with immunoregulatory properties. Secreted in the synaptic cleft by antigen-presenting cells, it acts via TCR signaling inhibition, modulation of naïve T cell differentiation and proliferation, overall facilitating cancer immune escape. Among amino acid catabolizing enzymes, IL4I1 is the most expressed in a variety of human tumors, and its expression correlates with decreased survival and a pejorative outcome.

**Purpose:** Despite numerous evidence of IL4I1's role in various tumor types, the mechanism by which this enzyme promotes an immunosuppressive tumor microenvironment is currently unclear. Therefore, our work aims at understanding, through an immunogenic tumor cell line that does not express IL4I1, how its overexpression can favor immune evasion mechanisms.

**Results:** To evaluate IL4I1 potential to shape the TME, we engineered an immunogenic fibrosarcoma cell line using a retroviral vector containing the IL4i1 enzyme-coding sequence. Enzyme expression was confirmed at both transcript and protein level in the fibrosarcoma cells. Additionally, we demonstrated that IL4I1 was secreted and catalytically active. Mass spectrometry analysis highlighted significant production of IALD, I3P, and PPA among the 33 metabolites of tryptophan metabolism. Of note, IL4I1 over-expression reverted the fibrosarcoma's behavior in vivo, leading to the establishment of a tumor mass. Moreover, transcriptomic and proteomic characterization revealed a downregulation of genes associated with ferroptosis in IL4I1-overexpressing cells. Remarkably, these cells displayed notable resistance when exposed to ferroptosis-inducing stimuli such as RSL3, which is lost in IL4I1 catalytic site mutants. When injected in vivo, these

mutants showed a complete reversion of IL4I1-induced phenotype.

**Conclusion:** Our findings highlight IL4I1 influence on the tumor microenvironment's immunogenicity, particularly its role in promoting resistance to ferroptosis. These insights may guide the development of targeted cancer therapies.

### Defining breadth and dynamics of tumor-specific T lymphocytes in oesophageal adenocarcinoma

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Locally advanced esophageal adenocarcinoma (EAC) is a highly aggressive tumor treated with neoadjuvant chemotherapy (nCT), followed by surgery. Less than 30% of patients achieve a pathological complete response to nCT, which correlates with increased 5-year overall survival. Our recent multidimensional analysis of pre-treatment tumor biopsies correlated pre-existing intra-tumor immunity with clinical response to nCT, suggesting that T cells specific for tumor antigens may participate in tumor control. Because EAC is highly mutated, we sought to investigate breadth and dynamics of T cell responses against tumor neoantigens (TNAs) in blood, tumor-infiltrating lymphocytes (TILs) and resected tumor-draining lymph nodes (LNs) in responders versus non-responders to nCT.

TNAs derived from missense mutations, and in frame insertions/deletions and gene fusions, are identified by computational analysis of tumor whole exome and RNA sequencing from pre-treatment tumor biopsies. Autologous antigen presenting cells (APCs) are generated from patient's B lymphocytes, immortalized and either pulsed with synthetic TNAs-related peptides or transduced with TNAs-encoding minigenes, either with small selected antigenic libraries or via HLA-Agnostic Neoantigen Screening (HAN-Solo) method to comprehensively assess patient T cell reactivities against the entire tumor mutanome.

Preliminary results with PBMCs and tumor-draining LNs from EAC patients show recognition of autologous TNAs by either $CD8^+$ or $CD4^+$ T cells, supporting the potential immunogenicity of one or more of tested TNAs. TCR sequencing identifies clonally related tumor-specific $CD8^+$ T cells in circulation and tumor draining LNs of the same patient. We are also pursuing HAN-Solo screening is ongoing with the first two patients to quantitatively measure differences in TNAs recognition by T cells comparing responders versus non-responders patients.

### Neutrophil-like monocytes increase in patients with colon cancer and induce dysfunctional TIGIT$^+$ natural killer cells

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous family of immune cells including granulocytic ($CD14^{neg}/CD15^+/HLA\text{-}DR^{neg}$) and monocytic subtypes ($CD14^+/CD15^{neg}/HLA\text{-}DR^{neg}$). In the present study, we found a population of monocytes expressing the granulocyte marker CD15 that significantly increased in both peripheral blood (PB) and tumoral tissues of patients with colorectal cancer (CRC). Further phenotypical analysis confirmed the granulocytic-like features of this monocyte subpopulation that is associated with an increase in granulocyte-monocyte precursors (GMPs) in the PB of these patients (pts). Mechanistically, this granulocyte-like monocyte population suppressed NK cell activity by inducing TIGIT and engaging NKp30. Accordingly, an increased frequency of TIGIT$^+$ NK cells with impaired functions was found in both the PB and tumoral tissue of CRC pts. Collectively, we provided new mechanistic explanations for tumor immune escape occurring in CRC by showing the increase in this new kind of MDSC, in both PB and CRC tissue, which is able to significantly impair the effector functions of NK cells, thereby representing a potential therapeutic target for cancer immunotherapy.



## Integrating immune cell profiling and microbiota analysis to understand multiple myeloma disease progression

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Multiple myeloma (MM) is an incurable hematologic cancer caused by the abnormal growth of plasma cells in the bone marrow (BM). It typically follows a precursor stage, monoclonal gammopathy of undetermined significance (MGUS), and an intermediate phase, smoldering MM (SMM). Although only 5-10% of MGUS cases progress to MM, predictive factors remain poorly understood. Immune microenvironment and microbiota changes may drive disease progression.

We analyzed the immune composition, in both BM and peripheral blood, of 106 patients, including MGUS, SMM, and MM. Cytokine and chemokine levels were assessed using a 48-plex Luminex plate on 72 samples from these patients and 4 healthy donors (HDs). Moreover, fecal microbiome profiling was conducted on samples from 10 MGUS, 15 SMM and 16 MM patients.

We identified an increase in circulating TEMRA CD8 T cells, particularly $CD57^+$ cells, accompanied by a decrease in naïve $TIGIT^+$ and $TIGIT^+$ $TIM3^+$ CD8 T cells. Within CD4 T cell subsets, a reduction in BM effector memory phenotype and an elevation in IL-17-producing BM CD4 T cells were observed throughout disease progression. Concurrently, non-classical monocytes HLA-$DR^+$ $CD11c^+$ and granulocytes in the BM diminished.

The evaluation of cytokines and chemokines levels within BM plasma indicated a reduction in GM-CSF, IFN-α2, IFN-γ, IL-1β, IL-2, IL-2Rα, IL-3, IL-10, IL-13, LIF, and MCP-1/CCL2 levels in MM patients compared to MGUS/SMM and HDs, suggesting a potential impairment in their T cell activation and functionality.

Finally, fecal microbiota analysis showed increased Proteobacteria, *Enterobacterales*, *Enterobacteriaceae*, and *Streptococcaceae* in MM patients, with a decline in beneficial phyla like *Actinobacteriota* and *Verrucomicrobiota*. MGUS exhibited a more balanced microbiota, enriched in *Lachnospirales*, *Oscillospirales*, *Bacteroidales*, *Lachnospiraceae*, and *Bifidobacteriaceae*, while SMM showed a gradual shift towards dysbiosis.

These findings emphasize the potential role of immune and microbiota profiling, as predictive biomarkers for monitoring MM progression and as targets for therapeutic interventions.

## WORKSHOP 8
## IMMUNE RESPONSE TO PATHOGENS 2

### A SARS-CoV-2 nucleocapsid protein-derived peptide modulates MHC-I expression and impairs antigen presentation

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CD8⁺ T cells are essential in vaccine-induced immunity and to control viral infections, including SARS-CoV-2, which continuously evolve to escape immune detection. While much attention has been given to spike-targeted immunity, emerging evidence suggests that the virus actively suppresses antigen presentation through ORF complex proteins (ORF3a-ORF7-ORF8) and proteins E and N. Here, we investigate how a SARS-CoV-2 nucleocapsid-derived peptide (N219-227, LALLLLDRL-"LAL") interferes with MHC-I expression and antigen presentation, shaping CD8⁺ T cell responses in ways that could potentially impact viral persistence, immune memory, and vulnerability to co-infections.
We first assessed peptide-MHC binding and impact on HLA expression in T2 and LCL cell lines. The LAL peptide significantly reduced MHC-I surface expression in T2 and LCL cells, causing intracellular retention and disrupting endoplasmic reticulum architecture. We next investigated the impact of the LAL peptide on the priming of naive CD8⁺ T cells specific for the melanoma-derived EV10 using an in vitro approach starting from PBMCs isolated from healthy SARS-CoV-2 unexposed and melanoma free donors. LAL exposure impaired the priming of EV10-specific naïve CD8⁺ T cells, reducing their frequencies.
We examined the effect of this peptide on memory antigen-specific responses, elicited by a pool of commonly recognized viral epitopes, as well as on general TCR ligation, by Intracellular cytokine staining and by evaluating proliferation as well as the expression of other functional markers. LAL decreased the production of TNF-α in memory CD8⁺ T cells in response to viral epitopes. Lastly, monocytes exhibited a reduction in CD86 expression and inflammatory cytokine production upon LAL exposure, suggesting interference with costimulatory signalling. From this peptide, ten fragments were generated from the C- and N-terminal portion, so that the inhibitory portion could be identified and associated with other pathogens.
In conclusion, we identified a N protein sequence that could impair heterologous immune responses likely representing a viral immune escape mechanism.

### P91
### Single-cell analysis reveals SARS-CoV-2 modulation of monocyte differentiation and immune response during Omicron BA.1 in vitro stimulation

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Single-cell technologies provide valuable insights into heterogeneous immune cell populations. To investigate how SARS-CoV-2 shapes host immunity, we employed a high-throughput single-cell approach on HLA-DR enriched human peripheral blood mononuclear cells stimulated in vitro with the Omicron BA.1 variant. This analysis yielded integrated data on protein and gene expression at a single-cell resolution.
RNA-seq data and pathway analysis of differentially regulated genes revealed intricate regulatory patterns in monocytes, natural killer cells, plasmacytoid and conventional dendritic cells. Among all cell types, monocytes were most strongly affected in terms of their differentiation status, expression of adhesion markers, cytokine and chemokine signaling and antigen processing and presentation.
We further investigated the effect of viral entry on the stimulated cells by aligning the Omicron BA.1 genome to single-cell reads and found that, although SARS-CoV-2 RNA was detected across all cell types, CD14⁺ and CD16⁺ monocytes were preferentially virus-positive. Genes upregulated in virus-positive monocytes were associated with cellular responses to stress and infection, inflammation and metabolism, while genes related to antiviral alpha-beta interferon signaling and antigen processing and presentation appeared to be downregulated.
This study gives new insights on intersection be-



tween SARS-CoV-2 dynamics and host innate immunity and further elucidate mechanisms that potentially contribute to the invasion of immune cells by SARS-CoV-2 leading to control and/or pathogenesis of the infection.

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## HLA-E-restricted CD8⁺ T cell response to conserved SARS-CoV-2 peptides is promising for improving vaccination and therapeutic strategies

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The SARS-CoV-2 pandemic has had significant effects on global health and the economy. While mRNA vaccines have effectively prevented disease, they do not ensure long-term immunity. In this study, we identified eight peptides from the complete genome SARS-CoV-2 (MT020880) that fit the HLA-E pocket and are recognized by CD8⁺ T cells to provide long-term immunity against SARS-CoV-2 infection.
The peptide-MHC binding affinity and the identified potential immunogenic epitopes were predicted by NetMHC version 4.0. After investigating the immunogenicity of synthesized epitopes, we analyzed HLA-E-restricted CD8⁺ T cells from peripheral blood mononuclear cells of 20 COVID-19-recovered individuals, 20 SARS-CoV-2 seropositive hospitalized patients, and 20 individuals who received three doses of the mRNA BNT162b2 vaccine, using flow cytometry to evaluate their phenotype and functional characteristics. Intracellular cytokine staining measured the expression of effector cytokines (IFN-γ, TNF-α, IL-2) following stimulation by our designed peptides pool. Blocking monoclonal antibodies (mAbs) confirmed cytokine production mediated by HLA-E-restricted TCR-peptide recognition.
A subset of CD8⁺ T cells recognized and reacted to the pool of HLA-E-restricted peptides by producing effector cytokines. The HLA-E-restricted SARS-CoV-2-specific CD8⁺ T cell response was more frequently observed in convalescent individuals than in hospitalized patients. These CD8⁺ T cells predominantly expressed TNF-α, with lower levels of IFN-γ and IL-2. Significant inhibition of TNF-α production was observed in the presence of blocking mAbs targeting TCR αβ and HLA-E molecules, confirming specific recognition by TCR αβ of the peptide presented by the HLA-E.
These peptides, which are highly conserved across major SARS-CoV-2 variants, suggest that HLA-E-restricted CD8⁺ T cells may contribute to controlling SARS-CoV-2 infection, reducing disease severity, and producing long-term immunity. Hence, understanding HLA-E-restricted CD8⁺ T cell-mediated immunity is crucial for improving therapeutic and vaccine strategies.

## The long pentraxin PTX3 is a pathogenic host factor in the *Staphylococcus aureus* infection of the bone

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Rationale: Osteomyelitis is a severe bone infection mainly caused by the Gram-positive bacterium *Staphylococcus aureus*. Immune and non-immune elements of the bone microenvironment cooperate to detect, contain, and eliminate *S. aureus*, yet this has evolved strategies to evade the host response, acquire antibiotic resistance, and chronically colonize the musculoskeletal tissue. The soluble pattern recognition molecule (PRM) PTX3 exerts protective roles against opportunistic pathogens and is increasingly recognized as a key player in bone pathophysiology. This study was therefore conceived to address the role of PTX3 in the pathogenesis of bacterial osteomyelitis.

Methods: A mouse model was developed that recapitulates the fracture/surgery-related infection of the lower limbs in humans. Local and systemic infection, antimicrobial reaction and bone remodeling were comparatively assessed in WT and Ptx3$^{-/-}$ mice using microbiology, flow cytometry, histochemistry and micro-CT techniques.

**Results:** Ptx3 expression was upregulated in non-hematopoietic bone cells, with most of the protein being synthesized by CD45$^-$Sca-1$^+$ osteoprogenitors and accumulating in the bone tissue during the acute phase of the infection. Notably, Ptx3$^{-/-}$ mice exhibited a lower bacterial load in the infected limbs and reduced systemic inflammation, with decreased serum levels of IL-6 and limited splenic infiltration of Ly6-G$^+$ neutrophils and Ly6-C$^{hi}$ monocytes. This phenotype was specific to *S. aureus*, as Ptx3$^{-/-}$ mice were not protected from *S. epidermidis* (common isolate in osteomyelitis), and independent of sex or bacterial dose. Furthermore, administration of a PTX3-targeting antibody dampened infection in the bones of SA-infected WT mice. Interestingly, the Ptx3$^{-/-}$ bone mounted more pronounced inflammatory reactions, with elevated levels of chemokines and cytokines with antimicrobial activity and increased expression of cell-associated and soluble PRMs in non-hematopoietic bone cells.

**Conclusion:** PTX3 plays a pathogenic role in the acute phase of *S. aureus*-dependent osteomyelitis and might be exploited as a novel target for prophylaxis and therapy of bone infections.

## Mechanoreceptors initiate innate immunity in response to microbial infections

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How mammals mount an effective immune response against infectious agents remains an unresolved fundamental issue in biology. During inflammation, the recruitment of circulating neutrophils into tissue is one of the early events and it has been linked with the rapid containment of infections. This process, traditionally associated with the expression of pattern recognition receptors (PRRs) and with pathogen recognition, appears to be more strongly influenced by tissue damage signals. Among them, tissue detection of mechanical stress has been recently linked to the activation of immune cells during inflammation. Building on these observations, we sought to identify the signals that trigger the beginning of the inflammatory process during microbial infections. To do so, we utilized a skin infection model wherein microbes (*Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) are injected intradermally to minimize tissue damage while introducing PAMPs in substantial amounts. To study the molecular mechanism leading to neutrophil recruitment, we characterized the immune infiltration through multi-parametric flow cytometry and multiplexed imaging and the molecular cascade through qPCR and ELISA, both in WT and mice KO for PRRs or interleukin receptors. Moreover, results in KO mice were validated with pharmacological and genetic approaches. Here, we discovered an unforeseen two-tier mechanism of neutrophil recruitment during infections, in which mechanoenzyme is key to initiating innate immunity. Mechanistically, neutrophil recruitment is initiated by a mechanosensory-dependent pathway, involving the activation of PIEZO1 channels. This leads to LTB4 production, which, along with IL-1α, induces the release of CXCL1, promoting neutrophil arrival to the site of infection. In contrast, at alter time points neutrophil recruitment is TLR- and CXCL2-dependent, highlighting a shift towards a pathogen-driven response to sustain inflammation.

Società Italiana di Immunologia Immunologia Clinica e Allergologia

# WORKSHOP 9
# METABOLISM

## Defective OPA-1 dependent mitochondrial fusion in Kupffer cells affects immunometabolic response and systemic metabolism

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**Background:** Kupffer cells (KCs) are hepatic resident macrophages essential for liver physiology that contribute to the immunoinflammatory response associated with the development of metabolic associated fatty liver disease (MAFLD). KCs respond to liver metabolites by reprogramming their metabolism via modulating mitochondrial plasticity and function. OPA-1 is a mitochondrial dynamin whose activity promotes mitochondrial fusion and modulation of oxidative phosphorylation an is differentially expressed in the liver of MAFLD patients compared to healthy liver.

**Aim:** Given the close interaction that KCs have with other cells in the hepatic niches, this work aims to investigate how the modulation of OPA1-driven mitochondrial fusion in KCs affects lipid metabolism and immune response in the liver and systemically.

**Methods:** KC selectively OPA1 deficient mice (Clec4F_Cre/Opa-1 Fl/Fl) were fed a Standard Diet or a High-Fat Diet for 20 weeks. The immune phenotype was assessed by cytofluorimetry while the metabolic profile was evaluated by in vivo indirect calorimetry and following plasma and tissue lipid profile analysis. Single cell RNA sequencing was also performed to profile the impact of OPA1 deficiency on KCs function and possible paracrine effects on hepatocytes.

**Results:** Clec4F_Cre/Opa-1 Fl/Fl mice present immunophenotype characterized by a higher proportion of pro-resolving KC2s than pro-inflammatory KC1s. Functionally, KCs also exhibit different phagocytic and proliferative capacity. Systemically, this translate into the preference of the utilization of carbohydrates.

**Conclusions:** These data suggest that OPA1 plays a key role in the function of Kupffer cells and that the lack of OPA1, by affecting cellular metabolic reprogramming, impacts their crosstalk with hepatocyte and potentially other resident liver cells, thus influencing the development of metabolic liver disorders.

## The GDP metabolism triggering RANBP1-dependent modulation, form the basis of a new differential genetic-metabolic regulation of the lymphocyte fate: a novel pathway for targeted CD4⁺ re-editing

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Recent studies have demonstrated that the protein RANBP1 (RAN binding protein 1) plays a regulatory role in nuclear-cytoplasmic transport by modulating the hydrolysis of RAN-bound GTP to GDP. This process generates a GDP/GTP gradient, which in turn allows the nuclear/cytoplasmic transport of micro-RNAs, proteins, and mRNAs (Dattilo V et al 2017, Audia S et al 2023). In addition, recent studies have indicated the potential of RANBP1 in the differentiation of Th17 cells, a type of T lymphocyte involved in immune response and autoimmune disease, by regulating nuclear trafficking and cytokine response (Brescia C et al 2023). Preliminary studies have shown that purine metabolism, particularly GDP, also by modulating RANBP1, is able to impact a dramatic immunophenotypic plasticity of CD4⁺ and Th17⁺. Buffy coats from healthy donors were used to isolate CD4⁺ cells, which were cultured or differentiated into Th17 lymphocytes in the presence of an appropriate pool of cytokines and co-stimulators. Lentiviral vectors and treatment with GDP 300nM and lactate 10 mM were then used to modulate RANBP1 expression. Expression studies including real-time PCR, FACS and RNA-Seq and functional metabolomics studies using Sea-Horse, HPLC, Raman spectroscopy and fluorometric assay were then used to assess metabolic/immunophetopic and genetic modulations. The results show that both RANBP1 and its metabolic product GDP are potent activators of lactate metabolism, resulting in differential reorganization of CD4 and Th17 lymphocytes. This process is characterized by a tendency of CD4 lymphocytes to adopt a Th17 phenotype. In contrast, Th17 lymphocytes adopt a mixed Th17/Treg phenotype. It was also observed that lactate produced after stimulation with GDP or modulation of RANBP1 can influence purine metabolism, forcing the guanosine pool. This has allowed for the first time to dissect a new immune-metabolic circuit, to elucidate its underlying

genetic background and to suggest new dynamics of targeted lymphocyte reprogramming.

**Impact of specific nutrient overload on the balance between peripheral immune tolerance and autoimmunity**

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Compelling experimental evidence underlines the strong link between metabolic status and immune tolerance through a direct control exerted by nutrients on intracellular nutrient-energy sensors in immune cells. In this context, nutrient availability has been shown to influence the expansion and function of $CD4^+$ regulatory T (Treg) cells, by modulating the activity of mammalian-target of rapamycin (mTOR) pathway. Moreover, excessive caloric intake leads to a state of "metabolic pressure", which may act as a dominant environmental factor able to alter immune tolerance impairing Treg homeostasis, potentially contributing to the breakdown of self-tolerance. Building on these findings, we investigated the impact of single nutrient-enriched diets (high fat, high carbohydrate or high protein diets), mimicking signals of metabolic pressure, on immune system of mice in healthy condition or during experimental autoimmune encephalomyelitis (EAE, the mouse model of MS). We found that an excess of lipids or carbohydrates in diet induced an increased inflammatory state in periphery compared to standard diet, as testified by higher level of circulating proinflammatory cytokines associated with impaired peripheral Treg cell activity. Also in the context of EAE, high-fat diet and high-carbohydrate diet exacerbated the disease progression, increasing immune cell infiltration within the central nervous system. Moreover, we observed an altered Treg suppressive function induced by lipid or carbohydrate but not by protein overload also in EAE mice, suggesting a selective and specific effect of the different nutrients on immune tolerance, systemic inflammation and, eventually, on susceptibility to autoimmune disorders.

**Aging with HIV: assessing premature immunosenescence as a strategy to monitor and prevent age-related co-morbidities**

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Unlike in the early days of the epidemic, people are now living with HIV, presenting new clinical challenges, particularly in terms of managing their aging process with HIV. Indeed, despite the success of ART in controlling viremia, residual immune alterations contribute to the development of a premature senescent phenotype in People Living with HIV (PLWH), resulting in a higher burden of age-related comorbidities - a trend that may be further exacerbated by life-long ART treatment.
Evaluation of premature senescence features at immune level was performed. We assessed activation and senescence markers on T cells and characterized memory and primary responses both after in vitro induction and ex vivo, analysing SARS-CoV-2-specific responses induced by vaccination. Metabolic properties of T cells were evaluated by means of fluorescent probes. All analyses were conducted on ART-treated PLWH, and healthy adults matched for age and sex.
Although ART preserves primary responses, our findings uncover a senescent T cell phenotype marked by a peculiar metabolic status. In particular, altered mitochondrial traits were observed in T cells from PLWH and linked with oxidative stress but dissociated from typical bioenergetic pathways. Instead, we found a strict correlation between cellular metabolic alterations and multiple aging pathways, including loss of naïve and accumulation of differentiated T cells, expression of senescent markers, SASP levels and increased CVD risk. Nevertheless, PLWH presented with preserved primary and recall T cell responses, indicating the effectiveness of ART in restoring adaptive immunity.

Collectively, our findings provide insights into the presence of alterations associated with a senescent profile in PLWH that clues to monitor different steps of aging also in the presence of effective therapy. These data further emphasize the importance of early ART initiation in mitigating the long-term consequences of HIV infection.

**Cardiovascular-immunometabolic profile in survivors of childhood acute lymphoblastic leukaemia (sALL)**

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**Background and Aims:** Acute lymphoblastic leukaemia (ALL) is the most common cancer in children. The increasing survival rates pose medical challenges of long-term management of late adverse effects of therapies in survivors (sALL). An increased CVD risk later life has been reported in ALL undergoing total body irradiation (TBI) followed by allogeneic haematopoietic stem cell transplantation (HSCT). The aim of this project is to investigate the cardiovascular-immunometabolic phenotype of sALL compared to matched donors.

**Methods:** 14 sALL (disease-free recipients between 3.6 and 21.9 years from TBI-HSCT and immunosuppressive therapy) with their relative healthy sibling Donors (brother/sister) were profiled for medical anamnesis and clinical assessment of cardiovascular/metabolic markers, including intima media thickness of common carotid artery(IMT). Blood was processed for immunophenotyping, proteomics and scRNAseq.

**Results:** Distribution of recipients was 11 M/3 F, while that of donors was 3 M/ 11F, with a median age of 23 and 23.5 years, respectively. No differences in anthropometric characteristics were reported. Significantly higher levels of LDL-C,TG, insulin (and HOMA) and decreased HDL-C were reported in recipients compared to donors. MeTs was diagnosed by adults 5-criteria only in recipients (14.3% prevalence), while regression analysis showed an increased time-dependent slope of IMT in recipients vs. donors (0.0083 vs 0.0034). Blood profile showed an increased levels of Bcells (+52.7%, $p < 0.01$) and decreased $CD34^+$ (-37.5%, $p < 0.05$), while upregulation of acute phase response (CRP, APCS UP) and downregulation of immunoglobulin mediated immune response (CD5L, immunoglobulins DOWN) were observed by plasma proteomics compared to donors. Accelerated telomere shortening was reported in recipient vs. donor PBMC (slope -0.009 vs. -0.0008). scRNAseq on PBMC confirmed the increased frequency of Bcells that were characterized by impaired pathways of metabolism, antigen processing and binding.

**Conclusions:** Our study provides a unique cardiovascular-immunometabolic characterisation of young adult sALL compared to their brother/sister donors, showing a worsening of several CVD risk factors. Ongoing experiments aim to elucidate the molecular causes of the immuno-metabolic reprogramming observed.

## WORKSHOP 10
## T AND B CELLS

### The human bone marrow may offer an IL-15-dependent survival niche for EOMES⁺ Tr1-like cells

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Maintenance of memory T cells in the bone marrow and systemically depends on the homeostatic cytokines IL-7 and IL-15. An immunological memory may also exist for regulatory T cells. EOMES⁺type-1 regulatory (Tr1)-like cells have a rapid in vivo turnover, but if they are short-lived effector cells or are maintained long-term has not been investigated.
EOMES⁺Tr1-like cells expressing GzmK were enriched among CD69⁺Ki67-T cells in the bone marrow of healthy donors, suggesting that they became quiescent and bone marrow-resident. Conversely, CD4⁺GzmB⁺ effector T cells were excluded from the bone marrow-resident fraction. The dichotomy between GzmK⁺ and GzmB⁺T cells was observed both in healthy individuals and in multiple sclerosis patients, and also among CD8⁺ T cells. Intriguingly, bone marrow-resident CD4⁺ memory T cells expressed increased levels of IL-7Rα, while EOMES⁺Tr1-like cells were consistently IL-7Rα$^{lo}$. However, EOMES⁺Tr1-like cells expressed the IL-2/15Rβ-chain, and the latter was induced upon forced expression of EOMES in primary human CD4⁺T cells. Finally, IL-15 rescued EOMES⁺Tr1-enriched populations from death by neglect but was not required for CD4⁺ memory T cell survival. These findings suggest that the bone marrow may provide a survival niche for EOMES⁺Tr1-like cells. The different IL-7 and IL-15 receptor expression patterns of CD4⁺ memory T cells and EOMES⁺Tr1-like cells suggest furthermore that they compete for different homeostatic niches.

### Impairment of innate immunity and depletion of vaccine-induced T cells in the absence of the spleen

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Splenectomy or congenital asplenia is associated with severe reduction of memory B cells and increased risk of fulminant sepsis by encapsulated bacteria. Current guidelines recommend vaccinations against these pathogens before or after splenectomy, but the longevity of immunity acquired after splenectomy has not been determined. The impact of splenectomy on innate immune cells is unknown.
We analyzed frequency, differentiation stage, and function of innate and adaptive immunity in the peripheral blood of adult (n = 41) and pediatric (n = 14) patients splenectomized or born asplenic and in spleens of solid organ donors. Using SARS-CoV-2 vaccination as a model, we show that 1 year after the last immunization, despite normal levels of neutralizing antibodies, memory T cells were significantly reduced. Analysis of post-pandemic spleens shows that spike-specific memory T cells homed to the spleen. We also show a previously unrecognized role of the spleen in the homeostasis of innate NK and Vδ2 T cells. These populations showed altered phenotype and impaired function in the adults, but not in children, suggesting that other tissues may support innate cell development during early life.
The reduced function of innate lymphocytes must be considered as an additional immune impairment and risk factor. These findings emphasize the spleen's irreplaceable role in maintaining immune memory across all ages and suggest that its absence contributes to dysfunctions of innate and adaptive immunity in adults.



## Metabolically active and highly polyfunctional intratumoral VISTA⁺ regulatory B cells are linked to tumor recurrence in early-stage NSCLC

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**Introduction:** Non-small cell lung cancer (NSCLC) is the second most common cancer globally. Surgery remains the standard for early-stage NSCLC, yet recurrence rates range from 30-55%, and 5-year survival is 18.1-22%. While immunotherapy has revolutionized tumor treatment by eliciting or enhancing the response of tumor-infiltrating lymphocytes (TILs), the intricate nature of the tumor microenvironment (TME) frequently impacts immune responses and therapy outcomes.

**Objectives:** To dissect B and T cell landscape of the NSCLC tumor microenvironment, providing insights into their phenotype, metabolic characteristics, capacity to produce cytokines and possible influence on post-surgery cancer recurrence.

**Methods:** A cohort of 48 patients diagnosed with NSCLC across stages IA, IB, IIA, IIB, and IIIA was studied. Using a 21-parameter flow cytometry panel, a 45-parameter mass cytometry panel (single-cell metabolomic profiling, scMEP), and spatial transcriptomics, we examined the complex landscape of B cell phenotypes, their bioenergetics, and interactions with T cells in NSCLC, as predicted by NicheNET. Additionally, prediction analysis (PENCIL) was employed to explore associations between immunological and clinical parameters.

**Results:** Our analysis identified different B cell clusters within the TME, including VISTA⁺ Bregs with unique metabolic and functional characteristics. VISTA⁺ Bregs exhibited high metabolic activity and produced cytokines such as IL-10, TGF-β, IL-6, TNF, and GM-CSF. Spatial analysis revealed colocalization of B cells with CD4⁺/CD8⁺ T cells in the TME. NicheNet computational modeling predicted B-T cell interactions via the VISTA-PSGL-1 axis, supported by colocalization of VISTA⁺ B cells and PSGL-1⁺ T cells. Tumor-infiltrating PSGL-1⁺CD8⁺ T cells demonstrated enhanced metabolism and cytotoxicity. PENCIL analysis linked PSGL-1⁺CD8⁺ T cells and VISTA⁺ Bregs to lung cancer recurrence.

**Conclusion:** Our findings indicate a potential interaction between Bregs and T cells via the VISTA-PSGL-1 axis, which may play a role in NSCLC recurrence.

## Thrombospondin-4 is a component of the supramolecular attack particle shell and is targeted by chronic lymphocytic leukemia for immune evasion

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Supramolecular attack particles (SMAPs) are a newly identified type of particles that together with lytic granules and FasL-containing vesicles contribute to kill infected and tumoral cell by cytotoxic T cells (CTLs) and NK cells. SMAPs are characterized by a cytotoxic core enriched in granzymes and perforin surrounded by a proteinaceous shell, including thrombospondin (TSP)-1. TSP-4 was also detected in bulk analysis of SMAPs released by CTLs; however, its interrelationship and interplay with TSP-1 in SMAPs have not been investigated. We recently validated TSP-4 expression in CTLs, where it was found to display a reciprocal pattern with TSP-1. TSP-4 co-localizes with TSP-1 in a population of lytic granules that correspond to multicore granules (MCGs) and accumulate at the immunological synapse, where TSP-4 is co-released with TSP-1 in association with biologically active SMAPs. Furthermore, we showed for the first time that TSP-4 as well as TSP-1 are required for the killing activity of SMAPs and that both play a role in CTL-mediated cytotoxicity. Of note, we found that leukemic cells from chronic lymphocytic leukemia (CLL) patients produce soluble factors that reduce TSP-4 expression and MCG number as well as compromise SMAP-mediated latent killing activity, suggesting that the SMAP biogenesis program of CTLs is targeted for immune suppression by CLL. Our results not only advance our knowledge of the mechanisms underpinning CTL-mediated killing but, using CLL as a model cancer, establish a novel evasion strategy whereby tumoral cells escape killing by immune cells. This may potentially pave the way for innovative therapies based on soluble, engineered SMAPs to target tumoral and infected cells.

**A humanized transgenic mouse model to investigate CD1c-redirected T cells responses**

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CD1 molecules are non-polymorphic, MHC class I-like proteins that present microbial and self-lipid antigens to CD1-restricted T cells. They are classified into group 1 (CD1a, CD1b, CD1c) and group 2 (CD1d). Group 1 CD1 molecules are expressed on DP thymocytes and antigen-presenting cells (APCs). Under conditions of tissue damage and cellular stress, self-lipid expression can be upregulated, activating self-reactive CD1-restricted T cells to eliminate altered cells, including malignant ones. CD1c is expressed by acute leukemia and can be targeted by human CD1c-restricted, self-reactive T cells specific for the leukemia-associated lipid antigen methyl-lysophosphatidic acid (mLPA). T cells engineered with a selected mLPA-specific TCR (DN4.99) are efficiently redirected against any CD1c$^+$ leukemia cells in vitro and in immunodeficient mice, supporting a “donor-unrestricted” adoptive cell therapy (ACT) strategy to target CD1c$^+$ acute leukemia across MHC barrier. Because group 1 CD1 gene are absent in mice, CD1c transgenic (CD1c Tg) mice were generated to assess efficacy and safety of CD1c-redirected ACT in a fully immunocompetent syngeneic host. Flow cytometry and immunohistochemistry confirmed that CD1c expression in these mice closely resembles human patterns. CD1c$^+$ DP thymocytes and peripheral APCs in CD1c Tg mice were able to select and stimulate DN4.99 TCR T cells. CD1c Tg and C57BL6 mice were similarly susceptible to experimental autoimmune encephalomyelitis (EAE) that was induced to evaluate CD1c self-reactive T cell responses under inflammatory conditions. Furthermore, DN4.99 TCR T cell transfer into sub lethally irradiated CD1c Tg mice did not reveal major systemic toxicity and only transient, mild perturbation of CD1c$^+$ B cell and DC repopulation. Collectively, these results show that CD1c Tg mice are a valuable model to characterize CD1c-restricted T cell response, and support the safety of CD1c-redirected ACT, warranting further investigation for clinical translation.

## WORKSHOP 11
## MUCOSAL IMMUNITY

**A B-cell cross-road between systemic and upper respiratory tract immunity**

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The upper airways are the port of entry for respiratory pathogens. To prevent respiratory infection, immune protection is mostly needed at mucosal sites, particularly in the upper respiratory tract. Despite their great effectiveness in reducing disease severity, SARS-CoV-2 vaccines do not generate sterilizing immunity being unable to prevent infection and block viral transmission. Memory B cells (MBCs) may contribute to the development of sterilizing immunity against respiratory viruses by producing antibodies at the site of viral entry, on the surface of nasopharyngeal epithelia.
By flow-cytometry, single-cell RNAseq, and VDJ analysis, we compared CD27pos B cells in the peripheral blood (PB) with B cells found in nasal and oropharyngeal swabs. We show that systemic antigen-specific activated MBCs increase in response to infection or vaccination. Our single-cell RNAseq identified two clusters of activated MBCs in the PB: CD27_Act_a (FCRL5-T-bet-), which are precursors to circulating plasmablasts, and CD27_Act_b (FCRL5$^+$T-bet$^+$).
The CD27_Act_b pool is transcriptionally, phenotypically, and clonally related to B cells isolated from nasal and oropharyngeal swabs. The latter express markers of tissue residency and are primed for antibody secretion.
Our data indicate that B cells are recruited to the upper respiratory tract (URT) independently of specific antigens but require an inflammatory environment to be recruited and the antigen for their retention. Without inflammation, B cells are absent from URT surfaces. We show that, at mucosal sites, specific B-cell immunity is induced and does not persist when inflammation is resolved thus explaining our inability to induce sterilizing immunity against most respiratory viruses. The CD27_Act_b population bridges systemic and mucosal protection acting in concert with IgG transudate from serum.
Systemic B cells contribute to the upper airway defense only when inflammation drives their recruitment, meaning that B cell migration from the blood to the mucosa of the URT inevitably lags behind the infection.



## Gliadin activated CD4⁺ T cells is a new method to detect activated lymphocytes in the blood of celiac disease patients

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**Introduction:** Gluten-specific CD4⁺ T cells have a key role in celiac disease (CD) pathogenesis. CD diagnosis relies on the detection of serum CD-associated antibodies, followed by the invasive esophagogastroduodenoscopy to assess mucosal damage. Following the diagnosis, CD patients have to follow a restrictive, gluten-free diet (GFD) regimen. We set a new, less invasive diagnostic approach based on monitoring of activated gluten-specific CD4⁺ T cells in the blood of CD patients.

**Methods:** Peripheral blood mononuclear cells (PBMC) from HLA-DQ2.5 adult patients with treated and untreated disease were in vitro stimulated with a pool of gliadin immunodominant peptides or a peptic-tryptic digest of gliadin proteins. After 48 hours, gliadin-specific, responsive CD3⁺CD4⁺ T cells were identified and counted by detecting cells co-expressing OX40/4-1BB markers by multiparametric flow cytometry. Peripheral blood T cell responses to gliadin were also monitored by IFN-γ ELISPOT assay per comparative purpose.

**Results:** We observed that OX40 and 4-1BB activation markers are upregulated on CD4⁺ T cells following the engagement of the HLA-DQ2.5-gliadin complex with the T cell receptor. The frequency of gliadin-specific, CD3⁺/CD4⁺/OX40⁺/4-1BB⁺ cells was significantly higher in untreated patients than in treated and healthy controls and showed a positive correlation with the anti-tTG2 antibody titres. The expression of OX40 and 4-1BB activation markers was independent of the cytokines-secretion profile.

**Conclusion:** This assay, defined as the G.A.T.CD4 (gliadin-activated CD4⁺ T cells) method, might support CD diagnosis, particularly in doubtful cases with very low autoantibody serum titres, and manage the monitoring of disease progression, as the pathogenic T cells expressing OX40 and 4-1BB activation markers are undetectable in the blood of treated patients. We aim to propose the G.A.T.CD4 instead of esophagogastroduodenoscopy to perform accurate and less invasive CD diagnosis.

## Extracellular vesicles from bone marrow-derived mesenchymal stem cells downmodulate the gliadin-induced immunity in celiac disease

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**Introduction:** Extracellular vesicles (EVs) released by bone-marrow mesenchymal stem cells (BM-MSCs) have strong immunomodulant effects potentially useful to treat immune-mediated diseases. We have evaluated the immunoregulatory effects of EVs on gliadin-induced inflammation in *in vitro* models of celiac disease (CeD).

**Methods:** Pure BM-MSCs were obtained from four adult donors and EVs were isolated by ultracentrifugation and characterized by FACS. Gliadin-specific intestinal T cell lines obtained from duodenal biopsies of 6 CeD-patients were cultured for 48-hours with gliadin and EVs at escalating dose (0.2, 4, 8 µg/mL) and IFN-γ production was assessed by ELISA. Intestinal biopsies obtained from 7 treated CeD-patients were cultured for 24-hours in presence of gliadin with/without EVs at 20 µg/mL. Activated CD25⁺ cells were counted in lamina propria by immunohistochemistry whilst IL10, IFN-γ, TGF-β and TNF-α released in organ culture supernatants were measured by ELISA. The EVs-mediated immunomodulatory effect on capability to present gliadin to intestinal T cells was evaluated by FACS in immortalised B-cells from CeD patients cultured for 48-hours with gliadin.

**Results:** EVs treatment induced a significant and dose-dependent reduction of IFN-γ production in response to gliadin in all analyzed T cell lines ($p < 0.05$ at EV concentration of 4 and 8 µg/mL). A marked decrease of CD25⁺ cells density was observed in biopsies incubated with EVs compared to biopsy cultured with gliadin alone (mean ± SD; 58 ± 39 and 80 ± 36, respectively). EVs treatment induced a significant increase of IL-10 in supernatants in gliadin challenged biopsies compared to control cultures with gliadin alone (pg/ml mean ±

SD; 56 ± 46 and 213 ± 163, respectively, p = 0.02). On immortalised B-cells, EVs induced a decrease in the expression of HLA-DQ, HLA-DR, CD80, CD86, CD83.

**Conclusions:** EVs from BM-MSC are able to dampen the gluten-triggered inflammatory response in CeD-experimental models, as mucosal T cell lines and organ cultures, and represent a promising therapeutic tool for celiac patients.

### Investigating caudovirales-induced molecular mimicry in Crohn's disease pathogenesis

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Crohn's disease (CD) has been increasingly linked to imbalances in the gut microbiota, notably through dysregulation in the gut virome. Particularly, elevated levels of caudovirales have been observed in CD patients. Among these, the proteus virus Isfahan species is abundant in CD, suggesting it might trigger an autoimmune response through molecular mimicry. This study investigates whether proteus virus Isfahan proteins share homology with human proteins, potentially initiating autoimmune mechanisms in CD.
Utilizing the IBD transcriptome and metatranscriptome meta-analysis (TaMMA) framework, we analyzed cell populations from intestinal biopsies of CD patients and healthy controls. Immune and non-immune cells were isolated using flow cytometry and underwent transcriptomic analysis. Additionally, we examined the homology of viral proteins to human proteins using NCBI BLAST and iPBA. In vitro experiments involved coculturing T cells with immune cells overexpressing Proteus virus Isfahan protein to explore immune responses.
Our results confirmed increased levels of proteus virus Isfahan, in CD patients, especially in dendritic cells and macrophages. Gene ontology analysis indicated compromised immune responses in dendritic cells and enhanced viral-response pathways in CD macrophages. Notably, the viral protein gp82 showed structural homology with human dCMP deaminase, supporting the molecular mimicry hypothesis. These findings highlight proteus virus Isfahan as a potential activator of autoreactive T cells, exacerbating CD's autoimmunity.
This evidence suggests that proteus virus Isfahan may play a significant role in CD pathogenesis through molecular mimicry, where its protein's similarity to human proteins could trigger immune misrecognition, fostering autoimmunity and perpetuating inflammation in CD. Ongoing experiments aim to further elucidate these interactions, potentially identifying novel therapeutic targets.

### Dual spatial host-mycobacterial gene expression to unveil *Mycobacteria abscessus* pathogenesis in lung tissues

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Understanding the interplay between host responses and pathogen virulence during tissue infections remains a challenge in biomedical research. Co-localizing spatial transcriptomic data from host and pathogen has the potential to revolutionize our understanding of microbial pathogenesis.
To address this, we aimed to demonstrate that customized bacterial probes can be used to simultaneously identify host-pathogen interactions in formalin-fixed paraffin-embedded (FFPE) tissues using probe-based spatial transcriptomics technology.
In previous proof-of-concept work, we designed customized *Mycobacterium probes* targeting the rpoB gene (RNA polymerase β subunit) and the oxidative stress-modulated virulence factor precursor lsr2. We performed spatial gene expression analysis of these bacterial transcripts alongside the host transcriptomic profile in murine lung tissue chronically infected with *Mycobacterium abscessus*, and uninfected. Histological characterization confirmed the presence of granuloma-like structures in infected/inflamed tissues, which were validated by the analysis of spatial transcriptional profiling.
Our analysis revealed distinct host transcriptomic clusters in bacteria-positive and -negative regions, each associated with specific cellular compositions. Notably, rpoB expression correlated with bacterial abundance, while increased lsr2 expression was linked to elevated oxidative stress in lung tissues, demonstrating the feasibility of this dual identification approach.
To improve our assay, we developed an expanded bacterial probe panel targeting over 50 *Mycobacterium abscessus* transcripts relevant to lung infections, including those involved in hypoxic response, virulence factors, and iron and succinate metabolism. This panel is designed for application in infected mice and human explanted tissue.
Preliminary results confirm the presence of all se-



lected genes in murine tissue, and an unbiased cluster analysis is underway. Further investigation is required to assess the relationship between bacterial transcript expression and the host response at the tissue level.
Overall, we demonstrate the potential of dual bacterial and host gene expression assay in FFPE tissues, paving the way for simultaneous detection of host and bacterial transcriptomes in pathological tissues.

# WORKSHOP 12
# TRANSLATIONAL IMMUNOLOGY

## Prognostic role of T cell and macrophage subsets and extracellular vesicle markers in new-onset oligoarthritis patients

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Oligoarthritis is the most common form of juvenile idiopathic arthritis with a variable course. Although most patients follow a benign oligoarticular course, 30-40% progress to severe polyarticular disease (pcJIA), requiring aggressive treatment. Early identification of patients at risk of pcJIA is crucial, requiring new biomarkers. Immune cells are enriched in the joint of oligoarthritis patients, driving inflammation and tissue damage by releasing extracellular vesicles (EVs). Combining immune cell and EV characterization at onset may offer a promising strategy for identifying pcJIA prognostic indicators.
Ninety-four treatment-naïve oligoarthritis patients were enrolled at onset and followed-up for 24 months. Omic-based (miRNomic/proteomic) and immunophenotyping approaches were employed to study EVs and immune cells. EV-miRNA (EV-miR) and EV-protein (EV-Prot) expression profiling was carried-out on plasma (PL) and synovial fluid (SF). PL from 25 age-matched healthy children served as controls. Monocytes/macrophages and T cell subsets were phenotyped in peripheral blood (PB) and SF from 28 patients, and prognostic performance was assessed using ROC curves.
Omic approaches identified a signature of 16 EV-miRs and 152 proteins both systemically and locally, effectively discriminating new-onset patients from controls. Machine learning and WGCNA analysis demonstrated that EV-miR-29a, EV-miR-223, and 16 protein clusters were highly effective in stratifying at onset patients developing different disease courses. Flow-cytometry revealed significant changes in SF T cell composition of patients who would progress to pcJIA, including CD3:CD14 ratio (AUC = 0.8) and HLA-DR$^+$CD4$^+$ cell proportion, and

in HLA-ABC (AUC = 0.8) and CD3 (AUC = 0.9) marker expression on SF-derived EVs. Higher proportion of HLA-DR$^+$ cells in CD4$^+$ (AUC = 0.9) and CD8$^+$ (AUC = 0.9) subsets and of CD4$^+$ (AUC = 0.9) and CD8$^+$ (AUC = 0.9) effector memory cells, and reduced proportions of naïve CD4$^+$ (AUC = 0.8) and CD8$^+$ (AUC = 0.9) subsets were observed in PB of patients progressing to pcJIA.
These findings identify novel prognostic biomarkers for pcJIA, which include EV-miRs, EV-Prots, and immune cells with a strong potential for use in routine laboratory practice to identify patients at risk of pCJIA.

**Sex hormone regulation of hematopoietic regeneration after damage**

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There is growing interest in understanding how the systemic effects of sex hormones influence hematopoietic stem and progenitor cells (HSPCs) function and contribute to overall tissue physiology in steady state conditions and during period of hematopoietic stress. We have previously shown that suppression of sex hormones accelerates thymic and immune regeneration following hematopoietic stem cell transplantation (HCT). Luteinizing hormone (LH) can regulate HSCs, but the exact mechanisms remain largely unexplored. Here, we uncover a previously unrecognized, gender-biased regulation of HSPC recovery after damage mediated by LH.
We assessed the impact of LH on HSPCs in C57BL/6 male and female mice after sub-lethal (SL-TBI) or total body irradiation combined with HCT. Pharmacological inhibition of LH was achieved using a luteinizing hormone-releasing hormone-antagonist (LHRH-Antagonist). Characterization of HSPCs was done by flowcytometry.
Hormonal ablation significantly enhanced not only lymphocyte recovery but also platelet reconstitution after allo-HCT. Platelet recovery was significantly increased in mice after hormonal ablation, and the administration of LH reverted these effects. Exploring the effects of LH on lineage-committed HSCs, we observed sex-specific differences. Female mice treated with LHRH-Antagonist post-SL-TBI showed significantly increased absolute counts of megakaryocyte and all megakaryocyte-biased progenitors. Conversely, LH administration reversed these effects, but similar trend was not observed in male counterpart. Consistent with this, the LH receptor was expressed at higher levels in long-term (LT)-HSCs from females compared to males across different ages.
These findings extend our knowledge on the impact of sex hormones in regulating HSPC function and identify LH as a novel regulator of HSC commitment to the megakaryocyte-lineage. Our study offers the rational for the use of hormonal ablation as therapy to promote not only lymphoid recovery but also megakaryopoiesis in patients receiving HCT as well as other forms of cancer therapies.

**Personalized organ-on-chip model for rheumatoid arthritis treatment**

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Rheumatoid arthritis (RA) is an autoimmune inflammatory disease affecting around 0.5% of the world's population. It is characterized by joint swelling and synovia hyperplasia, leading to impaired mobility and chronic pain. Although many therapeutic options are available on the market, there is still no effective cure nor predictors of treatment response for RA. Consequently, 40% of patients fail to achieve RA remission, which evidences a much-needed change of paradigm regarding innovative trials and new drugs for RA. We aimed to develop a personalized next-generation joint-on-chip (JoC) that hosts RA cells derived from patient biopsies. By accurately replicating the complexity of a RA joint, the JoC model allows for patient-specific clinical trials-on-chip, acting as a predictor of treatment response.
We have designed a 3D microfluidic platform capable of hosting patient-derived cells, encapsulated in an appropriate hydrogel, according to the synovium's physico-chemical characteristics. Our model includes an autologous leukocyte-infiltrated synovial tissue, encapsulated in 5% Gelatin Methacrylate (GelMA), and synovial fluid. RA cells were obtained from synovial biopsies and blood samples, after which synoviocytes were encapsulated and bioprinted in the chip, and cultivated under inflammatory conditions by supplementation with proinflammatory cytokines present in RA joint microenvironment. Then, drug testing was successfully performed, using three of the most commonly administered drugs in RA (methotrexate, celecoxib and anti-TNF-α). Cell viability, proliferation and cytokine secretion were evaluated, as well as lubricin



production by synoviocytes.
Our findings mark a promising start to an ambitious project and represent a significant advancement in RA therapy research. The use of JoC has shown potential to become an invaluable tool in predicting treatment response of RA patients, avoiding submitting patients to various drug cycles until the appropriate therapy is found.

All FLAMIN-GO project (grant agreement No. 953121) partners participated to this work.

## CD300e in metabolic disease: a novel perspective on obesity-related dysmetabolism

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Obesity is a major global health problem and is a contributing factor to the development of cardiovascular diseases, type-2 diabetes, and cancer. It is a multifactorial condition characterized by the remodelling of adipose tissue, which contains an abundance of immune cells thought to be responsible for insulin resistance. A study involving 49 homozygous twin pairs discordant for Body Mass Index (BMI) revealed that mRNA coding for the immune receptor CD300e was upregulated in adipose tissue of the obese twin and downregulated after weight loss. This receptor, primarily expressed by myeloid cells, remains poorly studied and its ligand is unknown. We hypothesized that CD300e might play a role in the adipose tissue remodelling and metabolic dysregulation associated with obesity.
Adipose tissues and liver from wild-type (WT) and CD300e full knock-out (KO) mice, fed a high-fat diet for 16 weeks, were collected and subjected to histological analyses and Real Time PCR to assess inflammation.
The deletion of CD300e resulted in significant adipocyte remodelling, with hypertrophic adipocytes observed in both visceral and subcutaneous adipose tissue of KO mice compared to WT mice. The liver and brown adipose tissue of KO mice exhibited pronounced steatosis and whitening, respectively, less severe in WT mice. The macrophagic infiltrate and inflammation in white adipose tissue were worse in KO mice, and consistently with the notion that obesity-induced inflammation is crucial for glucose dysmetabolism, KO mice exhibited more severe impairments in glucose tolerance and insulin sensitivity compared to WT mice.
Our data support the notion that CD300e may play a compensatory role in mitigating excessive lipid accumulation in adipose tissue and non-adipose tissues, as well as counteracting the metabolic dysfunctions commonly associated with obesity.

## B cell subset dynamics and regulation of serum cytokines during pregnancy and early post-partum in women with multiple sclerosis

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Pregnancy in multiple sclerosis (MS) patients is associated with a reduction of disease relapses due to the down-regulation of inflammation, functional to fetal tolerance. In the post-partum, inflammatory rebound may occur as a result of immunocompetence restoration. B cell subset dynamic, whose role in MS disease pathogenesis is now well established, have been poorly explored during pregnancy and post-partum.
We designed a monocentric non interventional study enrolling untreated pregnant MS women (n = 30) within 8 weeks ± 5 days from last menstrual period. Clinical and immunological data were collected each trimester of pregnancy, as well as two weeks, and three and six months from delivery.
Multi-parametric flow cytometry analysis shows that absolute number and percentage of circulating total $CD19^+$ B cells drop during pregnancy overall and rapidly raised in the first trimester post-partum. This is especially true for those women who experienced a disease relapse post-partum (n = 9).
We also found an important regulation of different B cell subpopulations during and after pregnancy. Interestingly, quantification of serum cytokines by ELLA assay, including the B cell-related factor BAFF and the inflammatory cytokine IL-6, clearly differentiates pregnant MS women who relapse post-partum from those who do not, starting from the beginning of pregnancy.
This study gives novel insights on the B cell immunological adaptation during MS pregnancy identifying biomarkers useful to predict post-partum relapse that may allow individualized timing and modalities of pharmacological intervention for new mothers with MS.

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# POSTER SESSIONS

## POSTER SESSION 1

### IMMUNODEFICIENCIES

**P02**

**Skewing towards effector memory T cells induced by CD70 and 4-1BBL triplosensitivity as mechanism of immune dysregulation**

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Background and Aims: Microduplications of 19p13.3 are associated with immunodeficiency, but the molecular mechanism of the immunologic defect remains unknown. The CD70 and TNFSF9 (4-1BBL) genes reside in this region. Mice overexpressing CD70 or 4-1BBL develop skewing towards effector memory T cells and B cell lymphopenia. We report a novel 19p13.3dup and assess its impact on leukocyte development and functionality.

Methods: Whole exome sequencing was used to acquire genetic data, CGH-array and long-read sequencing to confirm copy number variations. Subpopulations of leukocytes and surface expression of CD70 and 4-1BBL were identified by flow cytometry. Single-cell RNA sequencing was performed on peripheral blood mononuclear cells.

Results: An adult male presenting with common variable immune deficiency (CVID) and severe inflammatory bowel disease (IBD)-like enteropathy since infancy was found to have a complex de novo 830Kb structural variant in the 19p13.3 locus. This variant included two overlapping inv-dups and resulted in a duplication of the CD70 and TNFSF9 genes. Non-immunologic features of FURID19 (facial dysmorphia, urogenital malformation, growth and neurodevelopmental retardation, immunodeficiency, trisomy 19p13) syndrome were absent. B cells were greatly reduced. IgG and IgM levels were low, IgA and IgE undetectable. Surface expression of CD70 and 4-1BBL were comparable to controls in resting cells, however upon stimulation they were significantly increased in the patient. scRNA-seq revealed an expansion of the effectory cell clusters both in $CD4^+$ and $CD8^+$ T cells. Gene set enrichment analysis showed upregulation of inflammatory pathways in the patient.

Conclusions: The region responsible for immunodeficiency in 19p13.3 duplications has been identified, and the presentation expanded to complex CVID-like disease. Skewing of T cells towards an effector memory phenotype was observed, closely mirroring the findings in mouse models. Triplosensitivity of CD70 and TNFSF9 is proposed as a novel mechanism of immune dysregulation in humans.

**P03**

**Cxcr4-R334X mouse: a model to study WHIM syndrome pathogenesis and evaluate innovative gene correction treatment**

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WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome is an immunodeficiency disorder caused by heterozygous autosomal dominant mutations in the gene encoding CXCR4 receptor. The most common mutation (R334X) truncates the intracellular C-tail of CXCR4, resulting in desensitisation to ligand-mediated internalization and myelokathexis. To date, treatment of WHIM Syndrome is conservative and based on plerixafor/mavorixafor and immunoglobulins. We aimed at developing a definitive novel adenine-base-editing (ABE) strategy to correct the most common WHIM

mutation in haematopoietic stem and progenitor cells (HSPCs).
To model the disease and preclinically assess the efficacy of our strategy, we exploited and characterized the murine model carrying the R334X mutation. WHIM mice display peripheral neutropenia and lymphopenia, compared to wild type (WT) controls. Bone marrow (BM) retention of B, T, myeloid, and NK cells is higher in WHIM mice, as well as LK (Lin-Sca1-cKit$^+$) and LSK (Lin-Sca1$^+$cKit$^+$) cell populations, compared to controls.
We are leveraging the ABE protocol on murine HSPCs by exploiting lipid nanoparticles (LNPs) or electroporation as delivery platforms. We identified the culture condition yielding the highest expansion rate of LSK cells from the BM of WT mice. Next, we tested different timing and doses of LNPs, as well as several electroporation programs to efficiently deliver mRNA-GFP in Lin- cells. We monitored cell growth, GFP expression, and HSPCs composition by flow cytometry at 24, 48, 72, and 96 hours after mRNA-GFP delivery. The delivery of LNPs-GFP after three days of culture showed the best outcomes in terms of viability, GFP expression, and stemness, compared to electroporation.
These preliminary results show that murine HSPCs are highly permissive to mRNA delivery by LNPs transfection, ex-vivo. Next, we will apply this protocol to HSPCs isolated from WHIM mice for gene correction of Cxcr4-R334X mutation by testing ABE-machinery and prove the efficacy of this approach in the preclinical disease model.

## P04
## Beyond X-linked agammaglobulinemia: atypical BTK variants and immune dysregulation

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**Background:** Bruton's tyrosine kinase (BTK) is a critical regulator of B-cell development, with loss-of-function variants typically leading to X-linked agammaglobulinemia (XLA), characterized by severe B-cell deficiency and predominant infectious susceptibility over immune dysregulation.

**Case Report:** We report a 2-year-old male with a hemizygous BTK variant, exhibiting a non-classical phenotype dominated by immune dysregulation rather than infectious susceptibility. His clinical history began at 14 months with warm autoimmune hemolytic anemia (wAIHA), which relapsed at 15 months during oral steroid tapering, coinciding with *Bocavirus*, *Rhinovirus*, and *Adenovirus* infections. At 21 months, two months after steroid discontinuation, he experienced another relapse, showing an initial good response to rituximab but recurring upon B-cell immunoreconstitution.
At 17 months, his course was complicated by *P. carinii pneumonia* requiring mechanical ventilation, likely favoured by prolonged systemic steroid use. At 21 months, during his second rituximab cycle, he developed an *A. baumannii* bloodstream infection and norovirus gastroenteritis.
Given the early onset, steroid dependency, and refractory course of wAIHA, the patient underwent a functional and phenotypic immunological assessment, which was normal with adequate immunoglobulin levels. Subsequent clinical exome sequencing revealed a hemizygous missense BTK variant of uncertain significance [c.941A>G; p.(Lys314Arg)].
Interestingly, we previously described a 31-year-old male carrier of a hemizygous BTK variant [c.946A>G; p.(Thr316Ala)] in the same SH2 domain, adjacent to our proband's variant, with infectious susceptibility, late-onset immune dysregulation, mild hypogammaglobulinemia, but normal B cell values in the first years of life. Despite normal BTK expression and phosphorylation, BTK inhibitory phosphorylation was abolished, suggesting a gain-of-function mechanism. Moreover, [c.946A>G; p.(Thr316Ala)] somatic variants have been proven resistant to BTK inhibitors in vitro.

**Conclusion:** We couldn't rule out other genetic or non-genetic factors in the AIHA pathogenesis of our proband. However, this evidence suggests broadening the spectrum of BTK-related disorders beyond classical XLA, emphasizing its role in immune dysregulation.

**P05**

## Inborn errors of immunity meet with real-life clinical settings: a single center experience of genetic profiling in humoral immune defects

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**Background:** Inborn errors of immunity (IEI) entail a diverse group of disorders resulting from hereditary or de novo mutations in single genes, leading to immune dysregulation. This study explores the clinical utility of next generation sequencing (NGS) techniques in diagnosing monogenic immune defects.

**Methods:** Eight patients attending the immunodeficiency clinic and with unclassified antibody deficiency were included in the analysis. Clinical records, immune characteristics, and family histories were reviewed, and a target gene panel (TGP) sequencing was performed to identify pathogenic variants.

**Results:** TGPs identified seven variants in TNFRSF13B (TACI), CARMIL2, STAT1, STAT3, and ORAI1 genes. These findings provided definitive diagnoses and proper prognostic assessment. Patients exhibited a wide range of clinical manifestations, including recurrent infections, autoimmune cytopenias, and organ-specific complications. The genetic diversity observed highlights the importance of genetic testing in diagnosing IEIs and tailoring treatments.

**Discussion:** This study underscores the role of TGPs in diagnosing IEIs, revealing significant genetic heterogeneity and phenotypic variability. They offer a precise tool for identifying underlying genetic defects, facilitating personalized medicine approaches, and improving patient outcomes. The findings emphasize the need for comprehensive genetic testing to uncover novel pathogenic variants, enhancing our understanding of immune system dysfunction.

**Conclusion:** NGS is a critical tool for the management of IEI, enabling precise diagnosis and personalized treatment strategies. Despite resource limitations, the progressive affordability is likely to expand its clinical utility, ultimately improving patient care and advancing the field of immunology. In the meantime, accurate phenotypic assessment is essential for resource optimization and case prioritization.

# IMMUNOTHERAPY

**P06**

## Definition of CTLA4 haplotypes and their worldwide genetic diversity

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The cytotoxic T lymphocyte antigen 4 (CTLA4) gene encodes a critical immune checkpoint molecule. Therapies targeting the CTLA-4 molecule have revolutionized the treatment of several cancer types, including melanoma, lung, and renal carcinomas; however, the variability in therapeutic response highlights the importance of understanding the genetic variability of the encoded gene. This study aimed to define a nomenclature for CTLA4 haplotypes by analyzing single nucleotide polymorphisms (SNPs), exhibiting frequencies above 1% in worldwide populations, encompassing exonic and the candidate regions for cis-regulatory elements. Using data from the 1000 Genomes Project Phase 3, we identified 25 haplotypes across five biogeographic regions. Thirteen haplotypes met the criteria for inclusion in the proposed nomenclature system, which adopts the HLA system designation. For instance, the CTLA4*01 denotes the most common haplotype, while CTLA4*02 indicates a distinct haplotype group presenting a functional mutation at exon 1 (signal peptide), which delays the cell surface expression of the CTLA-4 molecule. This approach lumps together the major relevant functional genetic variations. Notably, the haplotype worldwide distribution revealed significant variability among populations, particularly among the African populations that exhibited the highest CTLA4 haplotype diversity, while European and East Asian populations presented more conserved haplotype profiles. By

grouping critical genetic regions into defined haplotypes, this nomenclature provides a relevant tool for future studies on CTLA4 variability and its impact on immune regulation and in patient response to immunotherapy to anti-CTLA-4 antibodies. This approach pave the way for more precise analysis of the role of the gene in terms of therapeutic outcomes, contributing to advancement in cancer immunotherapy.

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## P07
## Lipid-loaded macrophages promote resistance to immunotherapy in melanoma

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The success of immune checkpoint inhibitors (ICIs) in cancer treatment is closely linked to the ability of T cells to mount an effective immune response. However, various components of the tumor microenvironment inhibit T cell activation, contributing to resistance against therapy. Recent research highlights the significant role of tumor-associated macrophages (TAMs) in modulating ICI efficacy. In this context, we have identified lipid-laden macrophages (LLMs) within tumors, including those of prostate cancer, ovarian cancer, and melanoma. Using single-cell RNA sequencing, we analyzed the immune infiltrate in melanoma models, followed by validation of transcriptional results through multiparametric flow cytometry. To better understand the role of LLMs, we isolated these macrophages from tumor sites and performed bulk RNA sequencing and mass spectrometry, providing insights into their transcriptional profiles. Our findings reveal a correlation between LLM abundance and tumor size, with LLMs promoting cancer progression through mechanisms of immune evasion. We also discovered that these macrophages exhibit dysfunctional autophagy, driven by disruption of the TFEB-dependent CLEAR signaling pathway, which facilitates lipid accumulation. Notably, targeting lipid-loaded macrophages with a TFEB agonist in vivo demonstrates synergy with anti-PD1 checkpoint inhibition in melanoma. Finally, in melanoma patients, LLMs are enriched in tumors that are resistant to ICIs. Collectively, our results uncover a complex interaction between LLMs and cancer cells that contributes to tumor progression and resistance to immunotherapy.

## P08
## Characterization of the molecular mechanisms underlying bone marrow stromal cell-induced multiple myeloma resistance to CAR-T cell therapy

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Multiple myeloma (MM) is a hematological malignancy characterized by the uncontrolled proliferation of plasma cells within the bone marrow (BM). MM is still incurable as most patients relapse or become refractory to treatments. The BM microenvironment is crucial for MM progression and response to therapy. Indeed, BM stromal cells (BMSCs) support MM growth and survival and sustain drug resistance development. CAR-T cell therapy is a promising therapeutic approach for MM, but many patients never respond to therapy or relapse. Several studies show that the BM microenvironment contributes to MM resistance to CAR-T therapy.
This study aims to elucidate the molecular mechanisms underlying BMSC-induced MM resistance to CAR-T cell therapy.
We co-cultured the BMSC cell line HS5, and the MM cell lines RPMI-8226, U266, KMS12-PE, and OPM2 for 1 and 3 days in the presence or absence of a 0.4 µM transwell. Analysis of MM cell viability, proliferation, and CAR-T targets (CD38, CD138, SLAMF7, and BCMA), PD-L1, and FASL expression was performed. The HS5 cell line enhanced MM cell viability in a contact-dependent manner. No changes in MM cell proliferation were observed. HS5 modulated the expression of CAR-T cell targets in a cell-type and molecule-dependent manner. CD138 expression was reduced in all the MM cell lines, whereas HS5 reduced the expression of BCMA and CD38 in RPMI-8226, KMS12PE, OPM2, and U266, respectively. Conversely, HS5 enhances the expression of BCMA in U266 and of SLAMF7 in RPMI-8226 and KMS12PE only. HS5 enhanced the expression of molecules involved in MM immune resistance, such as PD-L1 expression in all MM cell lines and FASL in RPMI-8226 and OPM2.
Our data indicate that HS5-induced modulation of CAR-T targets, PD-L1, and FASL occurs in a molecule- and cell-type-specific manner. Transcriptom-

ic analysis is ongoing to identify common pathways involved in the BMSC-induced resistance of MM cells to CAR-T cell therapy.

**P09**

**Human 3D model of bone metastasis to study the potential therapeutic role of natural killer cells**

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Metastatic cancer is a complex disease caused by a series of simultaneous and partially overlapping events. In particular, the mechanisms of metastasis development have not yet been entirely understood, and the therapeutic control of metastatic progression is far to be achieved. The bone represents a main site for the metastatic spread in different tumor types including breast, prostate and lung. Natural killer (NK) cells represent powerful immune effectors to contrast tumor development and different strategies are being investigated to optimize NK cell efficacy.

The major aim of this study is the development of an in vitro bone metastasis model using human-derived bone fragments. The model will be used to investigate the mechanisms underlying the pre-metastatic niche colonization by tumor cells, as single elements or spheroid aggregates, and the metastasis development. In particular we will focus on: the profiling of tumor cell adaptation to the bone niches, the characterization of microenvironmental changes occurring during the establishment of metastasis, and the description of the impact of NK cells in the metastasis formation.

We generated a metastatic human 3D model in vitro using bone fragments retrieved from donors subjected to hip arthroprothesis and cultured in static or fluid-dynamic systems in the presence of non-small cell lung cancer spheroids, enriched in cancer stem cells. Thanks to the analyses conducted with two-photon microscopy technology and by immunohistochemistry evaluation, we highlighted the presence of metastatic formation in bone fragments reproducing the features of in vivo cancer lesion. In this co-culture system we added also NK cells and we observed that these cells could reach and associate the metastasis formation.

The characterization of the cross-talk between NK cells and metastatic bone marrow and the possible effect of anti-osteolytic drugs in supporting NK cell activity will provide new targets which could be useful to conceive effective therapeutic strategies.

**P10**

**Donor-derived anti-leukemia CTL for the control of leukemia relapse in high-risk children after haploidentical HSCT**

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Adoptive cell therapy (ACT) based on the infusion of donor-derived CTL able to recognize patients' leukemia blasts is a promising approach to control leukemia relapse after allogeneic HSCT. We have developed a procedure for generating donor-derived anti-leukemia CTL able to efficiently lyse both AML and ALL blasts. 26 high-risk relapse (≥ 50%) pediatric patients given haploidentical HSCT (haplo-HSCT) were enrolled for CTL production and 64 batches of advanced therapy medicinal products (ATMP) were produced. The majority of ATMP were $CD3^+/CD8^+$ cells, with a memory/terminal activated phenotype, including T-central memory populations, displaying efficient capacity to lyse patients' leukemia blasts and to secrete IFN-gamma and TNF-alfa. To date, six patients were treated on a non-repetitive basis, while 11 were enrolled in a Phase I/II trial on the safety and preliminary efficacy of anti-leukemia CTL for the prevention of leukemia relapse after haplo-HSCT. This trial is based on the infusion of escalating doses of CTL (from 5 x 104/kg to 8 x 106/kg) every 3 weeks, starting from 45-75 days post-transplant, with a 24-month follow-up period for clinical and immunological evaluation. No serious adverse events, including the occurrence of grade II-IV acute GVHD, were documented during patients' follow-up. 11/17 patients, in many of whom increasing levels of molecular minimal residual disease have been documented in the early post-transplant period, achieved complete and stable remission with follow-up ranging from 6 to 70 months after completion of ACT. Two patients experiencing relapse during ACT were treated on a non-repetitive basis with high-dose CTL, achieved complete remission but later died due to extra medullary relapse. Data obtained so far documented that the ATMP manufacturing protocol allows the production of large numbers of functional anti-leukemia CTL, with a high level of standardization. Preliminary results suggest that CTL may have a role in both the prevention and treatment of leukemia relapse.



## P11
## Characterizing thymic function in pediatric and young adult patients receiving allo-HCT using CD31 and CD38 markers

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Delayed immune recovery in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) raises the risk of infections and poor clinical outcomes. Therefore, understanding thymic contribution to T cell recovery has received significant attention. Recent thymic emigrants (RTEs), newly produced T cells in the thymus, serve as key indicators of thymic function and T cell recovery post-transplant. This study aims to validate thymic function markers, specifically the canonical CD31 and the newly identified CD38, to detect RTEs and predict thymic and immune recovery, while evaluating their association with transplant, donor, and patient variables.

We analyzed 198 patients with hematological malignancies receiving allo-HCT at Memorial Sloan Kettering Cancer Center (46) and Bambino Gesù Children's Hospital (152). To characterize RTEs, CD31 and CD38 differential marker expression was evaluated on naïve CD4 T cells, obtaining the following sub-populations: $CD31^{+}CD38^{-}$, $CD31^{-}CD38^{+}$, $CD31^{+}CD38^{+}$ and $CD31^{+}CD38^{high}$. The quantification of signal joint T cell receptor excision circles (sjTRECs), a molecular assessment of thymic T cell export, was performed on the four sorted populations and we observed the highest sjTREC values within the $CD31^{+}CD38^{high}$ RTE subset, suggesting a more immature phenotype. Both $CD31^{+}$ and $CD38^{+}$ RTE populations exhibited similar recovery kinetics post-transplant, with $CD38^{high}$ RTEs correlating with the latter populations at most timepoints but diverging from day 180 post-allo-HCT, suggesting distinct maturation or turnover rates. Additionally, we demonstrated that recovery of the RTE sub-populations is significantly impacted by transplant platform (T-replete, αβT-cell-CD19-depleted, or umbilical cord HCT) and by the occurrence of grade 3-4 acute Graft-versus-Host-Disease.

We demonstrate that CD31 and CD38 markers reliably measure thymic function after allo-HCT, yielding comparable results. These insights contribute to a better understanding of immune reconstitution and could help guide future strategies aimed at enhancing thymus recovery and improving patient outcomes.

## P12
## Flow cytometric characterization of chimeric antigen receptor T cells before and after infusion in patients with non-Hodgkin's B-cell lymphoma

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**Background:** Chimeric antigen receptor (CAR) T cell are genetically engineered autologous T lymphocytes expressing a chimeric receptor capable of recognizing a specific antigen on target cells, leading to their elimination.

**Aim:** Phenotypic and functional characterization of pre-infusion CAR-T cells and evaluation of their in vivo expansion, to assess their correlation with clinical findings.

**Experimental Design:** A total of 37 patients with non-Hodgkin B-cells lymphoma treated with CD19-CAR-T therapy were enrolled. CAR-T cell infusion products were phenotypically and functionally characterized through multiparametric flow cytometry and compared to T cells from healthy donors. In vivo CAR-T cells kinetics of expansion was evaluated every two days. Data were correlated with treatment response and occurrence of cytokine release syndrome (CRS).

**Results:** CAR-T cell infusion products, compared to T cells from healthy donors, displayed predominantly an effector memory phenotype with a parallel reduction of naïve cells in both $CD4^{+}$ and $CD8^{+}$ T cells. $CD4^{+}$ central memory and $CD8^{+}$ terminally differentiated effector memory were also reduced in CAR-T cells. TIGIT, PD-1, and pro-inflammatory cytokines production was higher in $CD4^{+}$ $CAR\text{-}T^{+}$ cells. Additionally, CAR-T cells of patients who developed CRS were enriched in naïve cells and expressed lower PD-1 compared to CAR-T cells of patients who

did not develop this complication. In vivo expansion peaked between days 7 and 12, with high variability among patients. Two of them, diagnosed with mantle cell lymphoma, exhibited a strong CAR-T cells expansion (detectable in peripheral blood and in bone marrow) but relapsed with a B-cell clone lacking CD19.

**Conclusions:** Pre-infusion analysis helps to correlate cell characteristics to adverse events. Loss of CD19 expression stands out as an escape mechanism of CAR-T cell therapy also in mantle cell lymphoma. This finding highlights the importance of monitoring disease evolution by routine immunophenotyping, especially following biological therapies that selectively target specific antigens.

**P13**

## Zeaxanthins promote anti-inflammatory and neuroprotective effects by aryl hydrocarbon receptor activation

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Goji berries, recently introduced in our diet, are the primary source of Zeaxanthin dipalmitate (ZDP), a nutritional carotenoid that together with the other carotenoids, such as Zeaxanthin (ZEAX), exerts benefit effects such as anti-oxidant and anti-inflammatory for several disease conditions. Several dietary derived molecules are ligands for the aryl hydrocarbon receptor (AhR), which plays crucial role in the control of inflammatory responses.
Based on these evidences, we investigated the potential AhR-mediated immune regulatory properties of Zeaxanthin in bone marrow derived macrophages (BMDMs) and in the in vivo murine model of cerebral ischemia-reperfusion injury.
We found that BMDM activation with the pro-inflammatory cytokine TNF induced the inflammatory marker, nitric oxide synthase (NOS2), which was completely prevented in BMDMs treated with TNF in combination with Zeaxanthin. We confirmed high AhR expression in BMDM that was increased upon treatment with TNF. Moreover, we investigated the ability of ZEAX to activate AhR using a luciferase reporter assay in specific cell lines. We found that ZEAX activated AhR in a dose dependent manner, and its activation was prevented in cells pre-treated with AhR inhibitor. Notably, in *in vivo* model of cerebral ischemia, we demonstrated that systemic pretreatment with ZEAX significantly reduced brain infarct volume and edema providing a significant neuroprotection.
These findings suggest that Zeaxanthins may serve as innovative modulators of immune responses by activating the AhR pathway. They could potentially offer a viable alternative to conventional pharmaceutical drugs.

**P14**

## A polyclonally expanded CD8 T cell repertoire is a strong correlate of tumour regression following Treg-targeted immunotherapy

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The carcinogen 3-methylcholanthrene (MCA) induces fibrosarcomas in-situ enriched with $CD4^+$ T regulatory cells (Tregs), making this the model of choice for studying the impact of Treg depletion. Using FoxP3-DTR transgenic mice, we have shown that Treg depletion can result in a proportion of mice regressing their tumour (herein: responders), correlating with enrichment of T cells in their tumours and clearance of extracellular matrix components. However, the trigger that initiates tumour regression is still unclear. We hypothesised that greater clonal expansion of TILs may be responsible for driving tumour regression following Treg depletion.
We bulk sequenced the αβ TCR repertoire of $CD4^+$ and $CD8^+$ TILs of MCA induced tumours following depletion. Treatment responders displayed a reduced repertoire diversity and increased repertoire clonality compared to non-responding or untreated tumours, a signature present only in the $CD8^+$ but not the $CD4^+$ TIL compartment. This effect was tumour localized since clonal expansion was not observed in tumour draining lymph nodes, suggesting a tumour-specific rather than systemic effect. We then assessed for the presence of a targeted antigen driven response in CD8 TILs using a clustering-based approach but found no difference across treatment groups. This suggests that in MCA-induced tumours, response to Treg depletion correlates with a polyclonal $CD8^+$ T cell response, supporting previous studies that have demonstrated a high number of neoantigens in this model. Future studies will now endeavour to determine if the mutational landscape of MCA tumours is the



trigger for the induction of this polyclonal response in CD8 TILs that is strongly correlative with tumour regression.

**P15**

**Interactions between *E. coli* outer membrane vesicles (OMV) and human dendritic cells (DC) results in DC maturation and engulfment of OMV**

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Recently, a relevant influence of intestinal microbiota in patient's response to immune-therapy against different types of cancers has been reported. However, the mechanism through which the microbiota could affect the immune response remains to be elucidated. The microbiota is a complex system which can release metabolites, molecules and outer membrane vesicles (OMVs), which are small, spherical, bilayered structures released by Gram-negative bacteria during normal growth in response to environmental stressors. Given the abundance of bacteria in human body, OMVs can widely interact with different cell types, including dendritic cells (DCs), equipped to sense microorganism elements.
In this study, we analyzed the effect of two different E. coli OMVs, wildtype and LPS "detoxified", on monocyte-derived dendritic cells (moDCs), obtained from peripheral blood monocytes stimulated with IL-4 and GM-CSF. We showed that even extremely low amount of OMVs can induce maturation of moDCs, as assessed by upregulation of co-stimulatory and HLA molecules. Also, OMV-matured moDCs were protected by natural killer cell killing activity due to the upregulation of HLA-I molecule as previously reported for conventional microbiall activated moDCs.
We then investigated OMV-DC interaction through confocal microscopy: the presence of OMVs can be identified firstly close to cell membrane, then deeper in the cells and some of them fused to lysosomes, suggesting that uptake occurs, at least in part, via endo/phagocytic pathways. Indeed, treating moDCs with Dynasore, a phagocytosis inhibitor, drastically reduced the presence of OMVs inside DCs.
As a whole, these data indicate that E.coli OMV are strong inducers of human DC maturation and can be effectively acquired by endo/phagocytosis.
Further research is needed to explore whether alternative mechanisms, such as membrane fusion, can contribute to OMV internalization also in other human cells, potentially informing the eventual role of bacterial components in regulating cancer patients' response to current immune-therapy.

## NEUROIMMUNOLOGY

**P16**

**The impact of S100B silencing on multiple sclerosis model: the role of glial cells**

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It has been demonstrated that S100B actively participate in neuroinflammatory processes of different diseases of central nervous system (CNS), such as Experimental Autoimmune Encephalomyelitis (EAE), a recognized animal model for Multiple Sclerosis (MS). We previously showed that the inhibition of S100B activity using pentamidine and of S100B astrocytic synthesis using arundic acid determined an amelioration of clinical and pathologic parameters of the disease: the symptoms were milder and delayed.
This study further goes in detail on the role of S100B, and of astrocytic S100B in these neuroinflammatory processes. To this aim we induced EAE in S100 KO mice. Results showed milder clinical disease and lower pathologic scores. To dissect the potential mechanisms that could explain the role of S100B in the development of EAE we sorted, cultured and compared neural subpopulations (astrocytes, microglia and oligodendrocytes) deriving from S100B KO and wild type mice, through flow cytometric panels and ELISA. Neural cells were analysed for proinflammatory molecules showing a significant reduction of TNFβ protein in mice where S100B was silenced. As expected also S100B protein was significantly lower in this strain, although the gene expression of this molecule was not different

from wild type. The possible explanation of this discrepancy resides in the fact that the gene includes a non-coding cassette that allow its encoding but that avoid the generation of a fully functional protein. We also cultured ACSA2$^+$ cells (astrocytes) sorted and enriched from the brains of EAE affected animals, both KO and wild type animals. The usage of S100B inhibitors demonstrate the direct impact of these molecules on specific subpopulation of neural cells, such as astrocytes and microglia.
The present results further individuate astrocytic S100B as a key factor and as a potential therapeutic target for EAE neuroinflammatory processes.

**P17**
**CD103-CD8$^+$ T cells promote neurotoxic inflammation in Alzheimer's disease via granzyme K-PAR-1 signaling**
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The dysregulation of innate and adaptive immunity is a driving force in the development of Alzheimer's disease (AD), the most common form of dementia, affecting more than million people worldwide. Classical AD neuropathology is characterized by β-amyloid (Aβ) deposition, tau hyperphosphorylation, and neuronal loss, but more recent evidence shows that chronic inflammation promoted by local and peripheral immune cells is also a hallmark of AD. CD8$^+$ T cells are one of the most intriguing components of the AD immunological landscape. Most CD8$^+$ T cells in healthy non-lymphoid organs have a tissue-resident memory (Trm) phenotype, fulfill a local protective role, and can rapidly mount immune responses. However, CD8$^+$ T cells also infiltrate the brains of AD patients and equivalent animal models but how the communication between these adaptive immune cells and neural cells promotes disease development is unclear. Here we performed single-cell RNA sequencing in the 3xTg-AD mouse model, which develops both amyloid and tau pathologies, to study how CD8$^+$ T cell subsets may promote the neuropathology of AD. We found that CD8$^+$ Trm cells are strongly dysregulated in AD mice, with an increased number of activated CD103-CD8$^+$ T cells. We observed higher levels of granzyme K (GrK) in the CD103-CD8$^+$ T cells of 3xTg-AD mice and patients with AD compared to controls and discovered a role for GrK in the induction of neuronal dysfunction through the activation of protease-activated receptor 1 (PAR-1). Finally, we demonstrated that GrK-PAR-1 interaction induces tau hyperphosphorylation, revealing a previously unknown immune-mediated neurotoxic axis in AD. Together, our data show that dysfunctional communication between the immune system and central nervous system mediated by CD103-CD8$^+$ T cells and GrK-PAR-1 signaling contributes to the development of AD, identifying new molecular mechanisms that can be targeted to prevent immune-mediated neurotoxic inflammation.

**P18**
**The impact of repeated traumatic brain injuries on the health of professional rugby players, with a focus on the role of circulating mtDNA**
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Rugby is a high-contact sport characterized by repeated traumatic brain injuries (TBIs), especially among forwards (FWD) players, which contribute to systemic inflammation. In this context, mitochondrial DNA (mtDNA) can be released into circulation as a damage-associated molecular pattern, favoring inflammation and activation of innate immune cells. Since the effect of TBIs on the innate immune response and the biological role of mtDNA in contact sports remain unclear, we analyzed mtDNA levels, activation and subset distribution of monocytes.

**Methods:** Thirteen male professional rugby players were monitored over two matches during the 2023-24 season. Venous blood samples were collected before (T0, T2), immediately after (T1, T3) each match, and one month post-season (T4). We extracted DNA from plasma, and quantified mtDNA using Droplet Digital PCR (ddPCR). We assessed monocyte subset distribution (classical, intermediate, non-classical), and expression of activation and adhesion receptors by flow cytometry.

**Results:** MtDNA levels progressively increased from T0 to T4, in both the total population and the FWD players group. Baseline mtDNA levels correlated with key immune cells involved in the inflamma-



tory response at T1 and T3. Flow cytometry analysis showed an increase in the absolute number of monocytes and in the percentage of classical monocytes after the two matches considered (T3), while the percentages of intermediate and non-classical monocytes decreased. Additionally, we found increased levels of adhesion and activation markers in monocytes after each match.

**Conclusion:** Circulating mtDNA increased after each match and progressively over the season, suggesting a cumulative effect. Furthermore, Increased monocyte activation and adhesion in T3 compared to baseline (T0) indicates an increased innate immune response and potential inflammation associated with TBI. Functional studies are ongoing to assess whether increased mtDNA plays a role in the increase of monocyte activation, and if it could serve as an early marker of cell damage and inflammation.

## P20
## How high efficacy therapy modulates antiviral immune responses in multiple sclerosis patients

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with strong associations with viral infections, particularly Epstein-Barr virus (EBV). The allostatic perturbation resulting from viral infection is capable of triggering specific antiviral responses that can cause autoimmune reactions and support neuroinflammation through direct and indirect mechanisms. While antiviral immune responses play an important role in MS pathogenesis, their modulation by disease-modifying therapies is a central issue in neuroimmunology. This study investigates the activation and modulation of antiviral innate and adaptive immune responses in MS, focusing on the effects of anti-CD20 therapy. High-parameter flow cytometry was used to characterize immune cell subsets, innate immune functionality and virus-specific $CD4^+$ and $CD8^+$ T cell responses to EBV, CMV, and SARS-CoV-2. Cytokine and chemokine profiles were assessed using multiplex bead-based assays, and viral seropositivity and neurofilament light chain (NfL) levels, a marker of neuronal damage and of neuroinflammation, were also measured. Anti-CD20 therapy effectively depleted $CD20^+$ B cells but spared antibody-secreting cells (ASCs). A significant reduction in T follicular helper (TFH) cells and EBV-specific $CD4^+$ T cells was observed, with decreased memory and activation markers. In contrast, EBV-specific $CD8^+$ T cells remained generally intact. Notably, immune responses to CMV and SARS-CoV-2 were unaffected. Elevated NfL levels in untreated MS patients suggested ongoing neuroinflammation, which decreased following anti-CD20 therapy. A novel correlation between MCP-3 and NfL, observed at baseline and after EBV stimulation, became non-significant post-treatment, suggesting reduced CNS immune cell trafficking and neuroinflammation after treatment. These findings provide new insights into how anti-CD20 therapy selectively modulates antiviral immunity in MS, particularly affecting EBV-specific $CD4^+$ T cells, while also reducing inflammation and neuronal damage. This research advances our understanding of MS pathogenesis and highlights potential implications for personalized therapeutic strategies.

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## P21
## Study of T cell subsets in peripheral blood of multiple sclerosis patients treated with anti-CD20 mAb therapy

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, and neurodegeneration. Both B and T lymphocytes are crucial in the disease process. Anti-CD20 monoclonal antibody therapies, targeting CD20-expressing B cells, have proven clinically very effective in suppressing MS activity and progression, due to modification of immune responses.
The aim of the study is to assess the frequency and phenotype of T cell populations in the peripheral blood of MS patients undergoing anti-CD20 therapy to understand the long-term influence of these treatments on immune system cells and their rela-

tionship with disease activity and progression.
Peripheral blood samples from MS patients were analyzed before and after anti-CD20 treatment (rituximab, ocrelizumab, ofatumumab) using advanced flow cytometry techniques.
$CD4^+$ and $CD8^+$ T cells demonstrated an enhanced frequency of naive and regulatory subsets and reduced frequencies of effector memory subsets. More surprisingly, γδ T cells, traditionally considered innate-like immune cells, also underwent substantial redistribution across naive and memory compartments, supporting a broader immunomodulatory effect of the therapy.
These preliminary results highlight the complexity of immune alterations induced by anti-CD20 therapy in MS patients and could support the need to develop personalized treatment approaches to optimize the long-term outcomes for MS patients.

**P22**
**Sympathetic neuroimmune communication in lymphoid organs**
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The complex cross-talk between the autonomic nervous system and immune responses has been extensively dissected in several diseases. In hypertension, the noradrenergic tone induces in the spleen the secretion of placental growth factor (PlGF) by alpha-adrenergic receptors. By exerting paracrine effects, PlGF stimulates the egress of $CD8^+$ effector T cells from the spleen to peripheral organs and triggers an inflammatory response, essential for the adaptive cardiac remodeling to withstand hypertensive stress.
Since sympathetic nerve fibers innervate lymphoid organs, we dissected the noradrenergic neuroimmune communication in both murine spleen and lymph nodes. By screening the expression of alpha-adrenergic receptor subtypes, we found that splenocytes, lymph nodes, and dendritic cells express both alpha-1 and alpha-2 adrenergic receptors, suggesting their responsiveness to noradrenaline. Notably, the noradrenaline pre-conditioning of activated splenocytes influences their cytokine profile, increasing interleukin-6 (IL-6) and reducing interleukin-10 (IL-10) production. Surprisingly, these neuroimmune effects are more pronounced in activated lymphocytes, where noradrenaline exposure induces the secretion of PlGF and the skewing of the immune response towards the Th17 subset, consistent with literature describing Th17 cells as PlGF source. Interestingly, the activation of alpha-1 and alpha-2 receptors with selective agonists mimics distinct noradrenaline-mediated effects on activated lymphocytes. While activation of alpha-2 receptor by clonidine increases IL-6 secretion and Roryt expression, the alpha-1 receptor activation by naphazoline reduces IL-10 secretion and Gata3 expression, suggesting distinct signaling pathways mediated by alpha-adrenergic receptors expressed on T cells in response to the sympathetic tone.
In addition, conventional dendritic cells stimulated with noradrenaline acquire a pro-inflammatory phenotype, up-regulating IL-6 expression and reducing production of immunoregulatory kynurenines.
Overall, our findings demonstrate that the noradrenergic tone triggers cell-specific immune responses in different immune cell subsets via activation of alpha-adrenergic receptors, and pave the way for a detailed elucidation of the neuroimmune cross-talk underlying cardiac remodeling in hypertension.

**P23**
**Periprandial high fat diet feeding impacts the blood count of neutrophils via cholinergic receptor nicotinic alpha 7 activation**
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Background: We spend most of our time in "periprandial" state, frequently consuming high fat meals. It is known that high fat diet (HFD) affects the innate immune system and, given the role of the cholinergic receptor nicotinic alpha 7 (α7nAChR) in immune responses, we investigated its involvement during the periprandial metabolic adaptations to HFD.

Methods: We studied the metabolic and immunological periprandial profile of α7nAChR knockout (KO, n = 10) and wild-type (WT, n = 10) mice, fed one week either standard diet (STD) or HFD (60% energy from fats). We profiled the periprandial systemic metabolism via indirect calorimetry, we quantitated periprandial glucose and triglycerides levels in plasma, and we immunophenotyped the whole blood at five zeitgeber times ("ZT", starting by ZT1, corresponding to 8 am, and at the following ZT5,ZT10,ZT17,ZT22).

Results: KO mice consumed a comparable amount of SFD and gained comparable weight versus WT. Vice versa, when fed HFD, KO mice gained more weight and displayed higher periprandial glycemia versus WT. The periprandial triglycerides levels

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and the systemic metabolic behavior of KO mice were however comparable with those of WT. Of note, periprandial circulating Ly6G$^+$ neutrophils increased in KO fed SFD versus WT mice, while this was not observed when they were fed HFD, suggesting a myeloid skewing under different feeding conditions. Upon the hypothesis that this phenomenon could be due to the rapid prandial activation of the vagal signaling, we preliminary immunophenotyped mice re-fed up to 4 hours after an overnight fasting, but we did not recapitulate the higher increase of the blood count of Ly6G$^+$ neutrophils in KO mice in this short-term experimental setting.

Conclusions: α7nAChR impacts the periprandial metabolic phenotype and the blood count of neutrophils. If this phenomenon results from the activation of the receptor on the surface of neutrophils or in other nervous circuitries should be investigated.

## NK CELLS AND ILC

**P24**

### Impact of genetic lysosomal acid lipase deficiency on the immune system: focus on NK cells

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Lysosomal acid lipase (LAL) hydrolyzes cholesterol esters and triglycerides in the lysosome. Genetic LAL deficiency (LAL-D), a rare autosomal recessive lysosomal storage disorder, induces hepatic steatosis and hypercholesterolemia resulting in an increased cardiovascular risk. Enzyme replacement therapy (ERT) with sebelipase alfa showed to reduce liver steatosis and fibrosis and correct dyslipidemia. Little is known about the impact of LAL-D, and consequently of ERT, on other organs and systems. Emerging evidence suggests that LAL-D may affect both innate and acquired immune responses. Whether LAL-D causes immune alterations contributing to cardiometabolic dysfunction and whether ERT is effective in normalizing these alterations remain unclear. Thus, this study aimed to characterize the immunophenotype of ERT-naïve and ERT-treated LAL-D patients versus controls. A trend towards reduced circulating leukocytes in ERT-naïve LAL-D patients was observed, compared to controls. No difference was observed in T lymphocytes frequency. Neutrophils were significantly increased in LAL-D naïve patients, compared to controls, and normalized by ERT. More interestingly, a 75% reduction of NK cells in LAL-D patients was observed, with a shift from the CD56dim (cytotoxic) to the CD56bright (cytokine-releasing) NK subset. Consistently, functional analysis revealed impaired cytotoxicity and degranulation capacity in NK cells from ERT-naïve patients compared to controls. Reduction of NK frequencies persisted even in ERT-treated patients, with a partial correction of subset distribution. These data suggest that LAL-D affects immune cell distribution, particularly NK cells, which seem to be only partially corrected by ERT. Further studies are needed to understand the role of LAL in NK biology and the contribution of the defective immune system to the cardiometabolic consequences of genetic LAL-D. For this purpose, experiments are ongoing on the LAL-KO mouse model, which recapitulates the circulating immunophenotype of LAL-D patients.

**P25**

## Innate lymphoid cell phenotypic and functional alterations in systemic juvenile idiopathic arthritis

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**Objectives:** Systemic juvenile idiopathic arthritis (sJIA) is a chronic childhood disease classically attributed to innate immune cell dysregulation. This study aimed to elucidate the role of innate lymphoid cells (ILCs), including natural killer (NK) cells and helper-ILCs (hILCs), in sJIA during clinically inactive disease (CID) through phenotypic and functional analysis.

**Methods:** Peripheral ILCs from children with sJIA during CID receiving IL-1 inhibitors (n = 34) were analyzed by flow cytometry and compared to 14 patients with unrelated autoinflammatory diseases also on IL-1 inhibitors, as well as 23 healthy children (HC).

**Results:** sJIA patients exhibited a significant reduction in circulating NK cell frequencies compared to HC, with a notable increase in the proportion of CD-56brigh NK cells. Although hILC frequencies in sJIA were similar to those in HC, an increased frequency of ILC1s was observed, which correlated positively with plasma IL-18 levels. Functional assessments revealed that NK cells from sJIA patients during CID had variable IFN-γ production upon IL-18/IL-12 stimulation, inversely correlating with IL-18 levels. Additionally, hILCs from these patients showed a specific impairment in IFN-γ production while maintaining normal IL-13 production, suggesting intrinsic functional defects.

**Conclusion:** This study reveals significant innate immune cell abnormalities in sJIA patients during clinical inactivity, including reduced NK cell frequencies, increased ILC1 frequencies, and abnormal IFN-γ production by ILCs. These findings suggest a persistent, underlying immune dysregulation even in the absence of clinical symptoms, underscoring the potential pathogenic role of distinct immune cell subsets and cytokine interactions in sJIA.

**P26**

## Inhibition of DNAM-1-mediated intracellular signals and cytotoxicity upon NKG2D engagement in human NK cells

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Natural killer (NK) cells are cytotoxic innate lymphocytes representing the first line of defense against viral infections and tumor growth. They act thanks to the expression of a wide array of inhibitory and activating receptors which allow them to lyse transformed cells while preserving healthy cells.
The activating receptors NKG2D and DNAM-1 are both involved in tumor surveillance thanks to their ability to recognize stress-induced ligands. However, the prolonged exposure to NKG2D ligands promotes receptor down-modulation associated to an exhausted phenotype characterized by down-modulation of the cytolytic machinery and upregulation of inhibitory receptors on both human and murine NK cells. On human NK cells, we have recently demonstrated that the sustained engagement of NKG2D by its membrane-bound ligand MICA is also responsible for the functional impairment of DNAM-1. This occurs not only through the upregulation of the checkpoint inhibitory receptor TIGIT -which suppresses DNAM-1-mediated cytotoxicity- but also by a direct inhibition of DNAM-1-related signaling.
With the aim to better evaluate the impact of individual NKG2D crosslinking, primary cultured NK cells were challenged with plate-bound MICA recombinant protein or an agonist anti-NKG2D antibody. We found that both stimuli are able to induce NKG2D endocytosis and lytic granule release but only MICA-mediated NKG2D crosslinking increases TIGIT expression, further confirming a pivotal role for NKG2D/NKG2DL axis in driving NK cell hypo-functionality. We also provided evidence that the agonist antibody, although unable to trigger TIGIT up-regulation, directly inhibits DNAM-1-mediated signal transduction and cytotoxic function through a mechanism that requires NKG2D endocytosis. Unraveling the molecular mechanisms that suppress NK cell activation may pave the way for novel therapeutic anti-cancer strategies aimed at preventing NK cell dysfunction.



## P27
## Macrophages regulate ILC2 responsivity and CTLA-4 expression in the tumor microenvironment

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Chronic inflammation induces lymphocyte dysfunction, characterized by the expression of inhibitory immune checkpoints. For type 2 innate lymphoid cells (ILC2), the acquisition of a state of hypo-responsiveness associated with PD-1 expression has been reported in severe allergic inflammation. Still, the regulation of ILC2 reactivity in the context of cancer is less clear. ILC2 contribution to anti-tumor immune response depends, on the type of tumor and the distinct cellular interplays within the microenvironment. Here, we show that ILC2 in malignant pleural effusions express the immune checkpoints PD-1 and CTLA-4. An in vitro model of ILC2-macrophages interaction demonstrates that this crosstalk is responsible for driving CTLA-4 expression and limiting ILC2 activation. Thus, by preventing ILC2 exhaustion, macrophages maintain ILC2 responsivity to signals from the tissue. These results reveal that, differently from PD-1, CTLA-4 expression on ILC2 is associated with the maintenance of a reactive state during chronic inflammation in the tumor microenvironment.

## P28
## Transcriptional programs of circulating natural killer cells reaching inflamed intestine

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**Background:** Natural killer (NK) cells represent the prototypical cytotoxic subset of the innate lymphocytes, eliciting a potent type 1 immune response through the production of IFN-γ, granzymes, and perforin. These functions are tightly regulated by signal-dependent transcription factors (TFs), such as STATs, which play a crucial role in various pathological conditions. During intestinal inflammation, NK cells can have both pro- and anti-inflammatory functions, however, the molecular mechanisms behind the function of NK cells during colitis remain to be understood.

**Purpose:** This study aims to investigate the transcriptional networks underlying the immunoregulatory functions of NK cells during gut inflammation, by combining genetic and transcriptomic approaches.

**Results:** By integrating transcriptomic analysis and flow cytometry, we observed an increased Stat3 expression and pSTAT3 levels in NK cells from the murine large intestine lamina propria (lilp) during dextran sulfate sodium (DSS)-induced colitis. By employing genetic approaches, we identified a novel mechanism of regulation of Stat3 expression that involves Stat4. Specifically, the conditional deletion of Stat4 in NK cells led to reduced pSTAT3 levels during gut inflammation in the DSS mouse model. To further explore this regulation, we optimized STAT3 activation in response to IL-10 and identified IL-10 target genes in NK cells by bulk RNA-seq analysis.

**Conclusions:** Our findings suggest a role for STAT3 and IL-10 in NK cell regulation during gut inflammation. Understanding these regulatory pathways has the potential to pave the way for novel therapeutic strategies targeting NK cells in inflammatory bowel disease (IBD).

## P29
## Optimization of a CRISPR-Cas9-based genome editing protocol for human and murine innate lymphocytes

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**Backgrounds:** Innate lymphocytes are categorized into cytotoxic natural killer (NK) cells and helper innate lymphoid cells (ILCs). ILCs are tissue-resident cells that act early upon activation by reacting to changes on the cytokine milieu. ILCs are divided into three subsets, ILC1, ILC2 and ILC3, character-

ized by distinct transcription factor expression and polarized cytokine production, mirroring the roles of T helper cells. Precise genetic modification of primary NK cells and other innate lymphocytes has been challenging. This limitation restricts our ability to dissect the mechanisms controlling basic biological processes or innate immune responses in host defence. Previous studies have demonstrated that CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 genome editing offers advantages such as fewer off-target effects, reduced cytotoxicity, and increased editing efficiency.

**Purpose:** To investigate the roles of a wide range of transcription factors and surface markers in innate lymphocytes, we optimized strategies for CRISPR-Cas9 genomic editing of mouse and human primary cells.

**Results:** Herein, we isolated human and mouse innate lymphocytes from peripheral blood mononuclear cells or distinct tissues via enzymatic digestion. Cells were purified using magnetic bead separation or FACS-sorting and kept three days in medium supplemented with specific cytokines. The ribonucleoprotein complex, consisting of Cas9 protein, the selected guide RNA, and electroporation buffer was mixed with NK cells and electroporated using a wide range of electroporation conditions. After 48 hours editing was monitored by flow cytometry. Currently, our optimized protocol enabled precise genome editing of mouse primary ILCs reaching up to 95% of deletion efficiency.

## P30
## Effects of glucocorticoid therapy on immune reconstitution and function upon hematopoietic stem cell transplantation in cancer patients

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Hematopoietic stem cell transplantation (HSCT) represents the only curative option for many hematological malignancies and an efficient immune reconstitution is necessary for a positive outcome in cancer patients. However, HSCT remains a high-risk procedure associated with many complications including graft-versus-host disease, therefore a percentage of HSCT recipients need to be treated with the anti-inflammatory glucocorticoids (GCs). Since GC therapy has been found to regulate innate lymphoid cells function and to inhibit their differentiation from HSCs, a detailed characterization of the effect of GC on immune reconstitution would be beneficial to improve HSCT efficiency. It is known that GCs directly regulate gene expression through their nuclear receptor and that they can induce stable chromatin modifications. Our hypothesis is that GC therapy may generate transcriptional and long-term epigenetic modifications on HSCs, influencing their development towards the different immune cell subsets and hampering the correct immune reconstitution following HSCT. Here we characterized the effects of GC treatment on HSCs differentiation upon HSCT, both in vitro, on cultured HSCs and in vivo, on immunodeficient mice receiving human HSCs. Through RNA- and Assay for Transposase-Accessible Chromatin (ATAC)-sequencing experiments, we characterized the transcriptional signature and the chromatin landscape induced by GC treatment on in vitro cultured HSCs. Furthermore, we evaluated the impact of GC treatment on immune function against leukemia relapse following HSCT in an in vivo model of immunodeficient mice receiving human HSCs and infused with leukemic NALM cells. These data shed light on the molecular mechanism by which GC therapy affects immune reconstitution and represent an important step towards the development of novel strategies to improve HSCT outcome in oncologic patients.

## P31
## Role of placenta specific 8 in the regulation of innate lymphoid cells function

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**Background:** Placenta specific 8 (PLAC8) is a multifunctional protein involved in cell proliferation, differentiation, and immune regulation. However, the possible role of PLAC8 in innate lymphoid cells (ILCs) remains unexplored. ILCs are a family of innate immune cells which control the host defence against infection and cancer and regulate tissue homeostasis. According to their cytokine and transcriptional profiles, ILCs have been divided in five subsets: Natural Killer (NK) cells, ILC1, ILC2, ILC3 and lymphoid tissue-inducer cells (LTi).

**Aim:** To investigate the possible role of PLAC8 in the regulation of ILC functions.

**Results and Methods:** Plac8 expression was analysed in intestinal ILCs in mouse models of colitis and colorectal liver metastasis (CRLM) by single cell RNA sequencing. The impact of tumour microenvironment on Plac8 transcripts was evaluated via tumour intestinal organoids-NK cell coculture sys-



tem and bulk RNA sequencing analysis. Plac8 protein expression and cellular distribution in human NK cells was investigated by flow cytometry and fluorescence confocal microscopy. PLAC8 mRNA expression increased in intestinal ILC1 during colitis but not in Stat4-deficient ILC1. Accordingly, the cytokine IL-12, which is a potent activator of STAT4, upregulated Plac8 transcripts in splenic NK cells. In the tumour context, Plac8 mRNA expression was acquired by NK cells/ILC1 in CRLM and augmented on NK cells co-cultured with intestinal tumour organoids. In humans, PLAC8 is expressed in circulating NK cells and accumulated at immunological synapsis in NK cells contacting tumour target cells.

**Conclusions:** Our data demonstrate that PLAC8 is expressed in human NK cells and that may have a role in the regulation of their activity because of its presence at immunological synapsis. In mice, the expression of PLAC8 in ILCs is modulated by inflammatory cytokines and tumour factors, suggesting that this protein could be a regulator of ILCs in different pathological contexts.

## P32
## Recombinant zoster vaccine in adult primary antibody deficiency patients: responsiveness, modulation of NK cell compartment and Fc-dependent antibody functions

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Recurrent and complicated herpes zoster, caused by the reactivation of latent varicella zoster virus, may occur in immunodeficiency conditions. It is currently unknown if primary antibody deficiency patients respond to adjuvanted glycoprotein E (gE)-based recombinant zoster vaccine (RZV; Shingrix), licensed for aged and immunocompromised subjects. Natural killer (NK) cells contribute to the protective effects of vaccine-induced antibodies (Abs) thanks to the low affinity receptor for IgG, FcγRIIIA/CD16, whose aggregation leads to infected cell killing and IFNγ release, through which they potentiate 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.
We collected blood samples of Common Variable Immunodeficient (CVID, n = 25) and Hypogammaglobulinemic (Hy, n = 12) patients at baseline, 4 weeks after RZV first dose, and 4 weeks after boost. We assessed serologic responses to RZV along with the potential of vaccine-induced anti-gE IgG to activate CD16-dependent functions. While a fraction of CVID and the whole Hy cohort displayed vaccinal responses similar to healthy controls, a large proportion (56%) of CVID patients were unable to produce anti-gE IgG. Vaccine-induced Abs were effective in activating anti-gE-specific CD16-dependent degranulation and IFNγ production of healthy donor NK cells, with a magnitude that correlated with Ab levels. Interestingly, only in CVID cohort, vaccination promoted an expansion of memory NK cells, marked by the lack of FcεRIγ CD16 adaptor, along with a skewing of NK cells towards an activated phenotype, as documented by a transient increase in CD69 activation antigen, and a reduction of CD16 and TIGIT levels.
These data first demonstrated that a fraction of CVID patients respond to RZV, and highlight the capability of RZV-induced Abs to activate robust NK cell-mediated Fc functions. The perturbation of NK differentiation and activation status suggest their potential role in vaccine-induced responses in CVID patients.

## P33
## Multiple myeloma cells shift the fate of cytolytic group 2 innate lymphoid cells (ILC2s) towards TIGIT-mediated cell death

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**Background:** Growing evidence attests to the multifaceted roles of group 2 innate lymphoid cells (ILC2s) in cancer immunity. They exhibit either pro- or anticancer activity depending on tumor type but their function in multiple myeloma (MM) is still not elucidated.

**Methods:** The bone marrow (BM) and peripheral blood (PB) of patients (pts) with MM or precancerous conditions were collected, and specific properties of ILC2 subsets were assessed by flow cytometry.

**Results:** By dissecting ILC2s according to c-Kit marker, we observed that NKp30 and NKG2D were mainly confined to c-Kithi ILC2s, while levels of DNAM-1 was significantly higher in fully mature c-Kitlo cells. Among the total MM-associated ILC2s (MM-ILC2s), we observed a significant increase in c-the Kitlo subset, but the expression of DNAM-1 in these cells was significantly reduced, especially in BM. Interestingly, MM-ILC2s from PB expressed granzyme B (GZMB), but its expression was impaired in BM-ILC2s. Accordingly, MM cells were susceptible to killing by MM-ILC2s derived from PB while eluding ILC2 surveillance in BM. Indeed, in MM-ILC2s derived from BM, the downregulation of DNAM-1 is accompanied by the upregulation of TIGIT, which mediate cell death in ILC2s upon recognition of the cognate ligands expressed by MM cells. These ILC2 changes appeared in clinical precursor conditions and eventually accumulated with disease progression.

**Conclusions:** MM-ILC2s can act as cytolytic immune effectors that are fully competent in PB. However, MM cells shift ILC2 fate towards cell death in BM via the upregulation of TIGIT, thereby representing a potential therapeutic target to restore ILC2 antitumor activity.

## P34
## Evaluation of NK cells in neuropathic pain: correlation between degranulation and μ opioid expression

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**Background and Aim:** Natural killer (NK) cells are the cytotoxic arm of the innate immune system, without any prior activation (unlike cytotoxic T cells or B cells). Chronic pain is a serious disorder that decreases in physical functioning and emotional distress. Recent studies have described modulation of B cells and NK cells expressing opioid receptor μ as a potential marker for measuring pain. Here, we investigate the degranulation and the expression of Interferon gamma of NK cells from mouse models of pain, to study the expression of opioid receptor and the degranulation.

**Material and Methods:** Male mice (24-30 gr) haver been exposed to chronic constriction injuries (CCI) of the sciatic nerve of the right hind leg performed according to the Bennett model. NK cells from C57BL/6 spleen mice are stained with antibodies NK1.1-BV605, CD3-BV886, Ly49C/I BB700, CD107 PE, IFNg BV510; B and T cells are stained with CD45RB780, CD19BV605, CD3 BV786, in combination with anti-Mu Opioid Receptor (MOR), using LSR Fortessa. NK degranulation was assessed with a flow cytometric assay measuring CD107a cell surface mobilization (release of lytic granules) after incubation for 2-3 hours at 37°C and 5% CO2, with or without PMA/Ionomycin stimulation.

**Results:** Here, we observed degranulation performing CD107a cell surface assays revealed higher NK degranulation in CCI mice at 14 and 21 days, compared to sham mice. Furthermore, at the same points, IFNg levels were seen to be modulated in NK cells. Moreover, during allodynia and thermal hyperalgesia evaluated in CCI mice, we observed a modulation of percentage MOR-positive NK, B, and T cells compared to sham mice.

**Conclusion:** We studied degranulation of NK cells and expression of opioid receptors expressed on NK, B and T cells since they are a possible candidate for objective monitoring of pain in patients.

## P35
## Impact of HLA-B -21M/T dimorphism on NK cell anti-leukemia activity in AML patients receiving haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide

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The responsiveness of NK cells to malignant cells is influenced by NK cell education, which depends on inhibitory receptors/self-HLA-I molecules interaction. Key inhibitory receptors include KIRs and CD94:NKG2A, recognizing specific HLA-I molecules and HLA-E on targets, respectively. HLA-E folding depends on peptides derived from HLA-I leader sequences. Only leader sequences of certain HLA-B alleles provide peptides with -21M, enhancing HLA-E folding and surface expression. Consequently, individuals with HLA-B -21M alleles exhibit higher HLA-E surface levels and better-educated NKG2A+ NK cells. We investigated the impact of the HLA-B -21M/T dimorphism on NK cell anti-leukemia activity in AML patients receiving haploidentical-hematopoietic stem cell transplantation (haplo-HSCT)



with post-transplant cyclophosphamide (PT-Cy). Early post-transplant NK cells were CD56bright, showing high CD94:NKG2A expression and low KIR levels, high NKp46, perforin, and granzyme-B indicating a functional potential. However, the CD94:NKG2A/HLA-E inhibitory interaction affects NK cell anti-leukemia activity. NKG2A+ NK cells showed low degranulation against K562-EG loaded with the HLA-B -21M-peptide (VMAPRTVLL). This capacity increased using an anti-CD94 blocking mAb (Y9, IgM). NKG2A+ NK cells from patients with -21M/x donors exhibited significantly higher degranulation against K562-EG pulsed with -21T-peptide (VTAPRTVLL) than those with T/T donors, suggesting the presence of better educated NKG2A+ NK cells with superior degranulation capacity. No difference was observed with VMAPRTVLL-pulsed targets, likely due to the powerful inhibitory signaling mediated by CD94:NKG2A/HLA-E interactions. In a preliminary analysis on 90 haplo-HSCT AML patients, a trend towards improved 1-year LFS in patients with -21M/x donors was observed. This suggests that donor HLA-B -21M/x genotype could enhance NK cell-mediated anti-leukemia responses in the PT-Cy haplo-HSCT, potentially influencing donor selection.

**P36**

## Tissue-resident FcεR1γ⁻ adaptive Natural Killer cells accumulate in Cytomegalovirus-infected carotid plaques

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**Background:** Human cytomegalovirus (HCMV) infects a wide variety of host cells and remains latent following primary infection. HCMV infection is associated with the development of atherosclerosis, although the underlying mechanisms are still limited. This study examines whether the reconfiguration of HCMV-associated Natural Killer (NK) cell compartments is linked to carotid atherosclerotic plaque (CAP) instability.

**Methods:** A total of 85 patients with CAP were classified into high-risk (HR) and low-risk (LR) groups based on clinical criteria. HR patients underwent carotid endarterectomy. HCMV serological status was determined, and NK cell phenotype and functionality were assessed in peripheral blood and CAP samples via flow cytometry. t-SNE and clustering techniques were applied to enhance data visualization. Immunohistochemistry examined the spatial relationship between HCMV-infected cells and NK cells within CAPs.

**Results:** FcεR1γ⁻ adaptive NK cells are enriched in HR HCMV+ patients, showing enhanced antibody-dependent IFN-γ production. These non-conventional NK cells exhibited increased NKG2D expression, potentially amplifying IFN-γ release. Following plaque destabilization, FcεR1γ⁻ NK cells undergo a marked expansion both systemically and within atherosclerotic lesions, acquiring tissue-resident characteristics (e.g., high CD69 expression, lack of CD49e, and CX3CR1). Immunohistochemical analysis revealed a spatial proximity between NK cells and HCMV-infected macrophages within CAP, suggesting that local viral persistence may facilitate NK cell recruitment and activation, contributing to plaque progression.

**Conclusion:** These findings highlight the contribution of HCMV-driven NK cell remodeling to atherosclerotic disease progression, particularly the correlation between antibody-dependent IFN-γ release and FcεR1γ⁻ adaptive NK cells frequency. The reduced response following NKG2D blockade, further underscores a synergistic role of the NKG2D–CD16 axis in NK cell activation. These results suggest that this pathway could play a role in plaque destabilization in HR HCMV+ patients, and that FcεR1γ⁻ NK cells may serve as potential immunological biomarkers for atherosclerotic progression and cardiovascular risk assessment.

## OTHER TOPICS

**P37**

### Evaluation of the antitumor effects of extra-virgin olive oil phenolic compounds extracted by a novel "green" chemistry approach: focus on oxidative genotoxic stress and inflammation

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Despite advancements in early detection and treatment, colorectal cancer (CRC) rates and mortality remain high, underscoring the need for novel preventive and therapeutic strategies. Increasing evidence suggests that extra-virgin olive oil (EVOO) bioactive polyphenols have strong biological effects in various disease models [Di Pietro, M. Front Pharmacol 2022] including antitumor properties. An innovative, eco-friendly method for isolating EVOO polyphenols using Natural Deep Eutectic Solvents (NaDES) has been recently developed [Francioso, A. Molecules 2020]. The present study aims to evaluate the antitumor effects of EVOO polyphenols extracted by NaDES (Poly-NaDES) in a highly aggressive CRC mouse model. At first, the direct antitumor effects of Poly-NaDES were evaluated by MTT assay on a panel of neoplastic and non-neoplastic intestinal epithelial cell lines. Electron Paramagnetic Resonance analysis demonstrated that Poly-NaDES counteracted the ROS production and the consequent phosphorylation of the histone H2AX induced by *E.coli* CNF1, a bacterial toxin with pro-carcinogenic effects [Tozzi, M. J Exp Clin Cancer Res 2025], on intestinal cells, suggesting a protective effect against CNF1-induced DNA damage. In vivo, we used a well-established murine model of colorectal carcinogenesis induced by azoxymethane (AOM) and dextran sodium sulphate (DSS). To accelerate tumor development and increase malignancy, mice received three intrarectal instillations of CNF1. This treatment resulted in the development of highly aggressive adenomas and adenocarcinomas in 100% of mice, as confirmed by histopathological assessment of colon sections. Interestingly, the daily Poly-NaDES administration (5 mg/kg/die) in the drinking water for 60 days, reduced inflammatory infiltrates and revealed the presence of colorectal adenomas with low mitotic index and low expression of 53BP1, a marker of DNA damage, as assessed by H&E staining of colon tissue. Overall, our findings indicate that Poly-NaDES may counteract CNF1-induced pro-tumor effects by reducing oxidative DNA damage, with potential implications for colorectal cancer prevention and treatment.

**P38**

### Immunological alterations in chronic fatigue syndrome: T cell imbalance, cytokine dysregulation, and gender-specific differences

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**Purpose:** To explore fundamental immunological parameters of both cellular and humoral immunity in individuals diagnosed with chronic fatigue syndrome (CFS).

**Methods:** Seventy participants with suspected CFS (mean age: 45.2 ± 3.4 years, 70% female) and 30 healthy controls - HC (mean age: 27.5 ± 12.6 years) were recruited. Participants completed a symptom questionnaire, followed by peripheral blood collection. Flow cytometry immune phenotyping was employed to enumerate absolute number and percentage of T, B lymphocytes, and NK cells. Serum cytokine levels (IFN-γ, TNF-α,I L-2, L-4, IL-6, IL-10, IL-17a) were quantified using a cytometric bead array (CBA) analysis.

**Results:** In CFS, $CD8^+$T cell counts were lower (mean 426.4x109/l)) as compared to HC (mean 636.6x109/l; $p < 0.0001$). Prevalence of $CD4^+$ T cells, and significantly lower NK cell counts were registered in female as compared to male CFS patients ($p < 0.0001$ for both). Significantly elevated levels of TNF-α, IL-10, and IL-4 (mean 2.645, 2.255, and 3.725 pg/ml, respectively) were observed in CFS subjects as compared to HC ($p < 0.0001$ for all). Cytokine concentrations were significantly higher in females as compared to males for IL-10 (4.09 vs.2.64; $p < 0.001$), and IL-2 (0.85 vs. 0.28; $p < 0.05$).

**Conclusions:** Our findings suggest a dysregulation of cellular and humoral immunity in CFS, charac-



terized by altered lymphocyte subsets and cytokine profiles. The observed gender-specific variation in immune parameters emphasizes the importance of sex-specific factors in the pathophysiology of CFS. These findings contribute to a better understanding of the immunological mechanisms underlying CFS and may inform future diagnostic and therapeutic strategies.

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## P39
## A magnifying glass on the immunological properties of lipopolysaccharide (LPS) from the marine Gram-negative bacterium *Rheinheimera japonica*

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Gram-negative marine bacteria have developed striking adaptation strategies to survive and proliferate in the harsh and highly mutable environmental conditions of marine environments. Chemical changes in the structure of lipopolysaccharide (LPS), a key component of their outer membrane, represent interesting examples of adaptive strategies to marine waters. Since marine bacterial LPSs with an unusual chemical structure of lipid A, the glycolipid anchor of LPS to the membrane, often exhibit intriguing immunological properties, in this work we elucidated the structure and the immunological properties of LPS from *Rheinheimera japonica* (*R. japonica*), a Gram-negative bacterium isolated from seashore sediments of the Sea of Japan. *R. japonica* produced a heterogeneous mixture of hypoacylated lipid A species and new chemical structures have been defined. More interestingly, we proved that *R. japonica* LPS does not activate the human Toll-Like receptor 4 (TLR4) in HEK-Blue hTLR4 cells stably transfected with TLR4, myeloid differentiation factor-2 (MD-2) and CD14 genes. Interestingly, competition assays performed on both HEK-Blue hTLR4 cells and phorbol 12-myristate 13-acetate (PMA)-differentiated THP1 cells into M(0) macrophages proved that *R. japonica* LPS acts as an inhibitor of the TLR4-dependent inflammatory signaling, competing with *E.coli* LPS in binding TLR4.
All in all, these findings emphasize the promise of *R. japonica* LPS in developing novel biomolecules for immune-modulatory applications, such as in the design of novel anti-sepsis drugs.

## P40
## Calcineurin-nuclear factor of activated T cells axis in early stem cell commitment: a novel pathway in tissue regeneration

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The nuclear factor of activated T cells (NFAT) is a family of transcription factors composed of five members, four of which (NFATc1-4) are regulated by calcineurin (CN), a calcium-dependent phosphatase. Our group has highlighted additional non-inflammatory roles of NFAT in dendritic cells (DCs).
To investigate the role of the CN-NFAT pathway in early DC differentiation, we transduced a growth factor-dependent splenic DC line with a CN inhibitor (CNi). Inhibiting CN-NFAT signaling increased DC proliferation and altered the cell cycle by prolonging the G2/M phase and shortening the G1 phase. Metabolically, CN-NFAT inhibition induced a pronounced Warburg effect, typical of rapidly proliferating cells and a highly immunosuppressive phenotype. Since this resembled a more “stem-like” state, we extended our focus to adult stem cell (SC) differentiation, studying two tissues with distinct regenerative capacities: the brain and the small intestine/colon.
In vitro, we infected neural stem cells (NSCs) with a lentivirus carrying iCN, while in vivo, we performed intracranial injections targeting the ventricular-subventricular zone. In the intestinal system, we used mice with inducible CN-NFAT inhibition in LGR5$^{+}$ SCs, found in intestinal and colon crypts. We also employed two disease models: Parkinson’s disease (PD) and dextran sulfate sodium (DSS)-induced colitis. NFAT-CN inhibition led to NSC expansion both in vitro and in vivo under resting conditions and in the PD model. A similar expansion of LGR5$^{+}$ cells in the intestine resulted in tissue elongation, mitigating DSS-induced colitis damage. Additionally, we identified NFAT as a regulator of an immune checkpoint phase.
We propose that NFAT plays a key role in early SC differentiation, directly or indirectly controlling this process and coordinating immune system surveillance on activated progenitor cells.

**P41**

## Phenotypical and functional characterization of circulating immune cells in systemic mastocytosis patients

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Systemic mastocytosis (SM) is a rare myeloproliferative neoplasm characterized by uncontrolled proliferation and activation of clonal mast cells. Currently, the diagnosis and treatment of mastocytosis are complex, as well as the feature and role of immune cells other than mast cells, it's not clear. To improve these aspects, 31 SM patients (25 indolent, ISM; 3 aggressive ASM and 3 associated with hematologic neoplasms, AHM) and 12 healthy controls were enrolled. ISM patients were further divided into anaphylaxis (n = 10) and non-anaphylaxis (n = 15).
Peripheral blood samples underwent to complete blood count and immunophenotyping and mononuclear cells were analyzed by flow cytometry to evaluate subsets specific marker and cytokine production after polyclonal stimulation. Finally, we performed Basophil Activation Test on 20 of SM patients and 8 healthy controls.
We found a significant reduction in both frequency and absolute value of lymphocytes and an increase in monocytes in ASM patients compared to the other groups, and a significant increase in $CD4^+$ T lymphocytes in ISM patients. In ASM and AHM patients, there was an increase of the frequency of both $CD4^+$ and $CD8^+$ $CRTH2^+$ cells, whereas $CD4^+CD161^+$ T lymphocytes was significantly increased in the ASM. Frequencies of Treg lymphocyte and activated ILC2 ($KLRG1^+$), respectively, are reduced and increased, in all SM patients compared to controls. Interestingly, an increase in type 2 cytokine-positive cell frequencies was found in ASM patients. Finally, we found a reduced basophils' activation in all SM patients compared to controls, in particular in response to IgE-mediated ex-vivo stimulation.

**P42**

## Modulating leukocyte migration through electric stimulation

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The migration of leukocytes plays a fundamental role in inflammation, host protection, and tissue remodeling. While soluble mediators such as cytokines and chemokines are well-known regulators of leukocyte migration, the role of electrical signals remains largely unexplored.
Despite a growing interest in the usage of electric therapy in immune disorders, there is limited experimental evidence on if and how electric fields specifically affect leukocyte movement.
Here, we present the results of our pilot study EXTRAVOLT, which employs an in vitro microfluidic device along with time-lapse microscopy to analyze how electric stimulation impact on leukocyte migration. We show that alternative electric stimulation patterns can enhance the directed movement of neutrophils toward or inhibit chemotaxis for long periods of observation (up to 2 hours). These results set the basis for future research to elucidate the molecular mechanisms underlying these observations and assess their potential translational applications for inflammatory disorders.

**P43**

## The role of complement component C7 in endometriosis: a decoy for the assembling terminal complement complex?

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One of the most frequently altered immunological pathways in endometriosis (EM) is the complement system(CS). The bioinformatics analysis performed by the EndometDB online database, which collects gene expression data in healthy endometrial tissues and in the peritoneum and ectopic lesions of women affected by EM, revealed differential expression levels of several complement genes



within the ectopic tissue. We focused the analysis on C7, which localisation was described also as a membrane-bound molecule on endothelial cells (ECs). mC7 interacted with the other late complement components to form membrane-bound TCC (mTCC). mC7 acts as a trap for the late complement components to control excessive inflammation induced by its soluble counterpart.
The patients were enrolled at the IRCCS "Burlo Garofolo", Trieste, Italy. The study group consisted of 34 women who underwent laparoscopy to remove EM lesions. During surgery, biopsies of ectopic and endometrial lesions were collected and processed to perform immunohistochemical analysis (fixation in formalin), RNA extraction and cell isolation. Peritoneal fluid and plasma were also collected. The anamnestic and clinical data of the enrolled patients were collected through dedicated clinical reports and stored in the Research Electronic.
We evaluated the C7 gene expression in the tissues and cells isolated from the endometriotic lesions, determining that this gene was locally expressed at high levels in all tissue samples analysed. The C7 component was expressed at high levels by isolated endometriotic cells. Immunohistochemistry confirmed the presence of protein in the epithelial tissue, which showed a high positivity in the glandular-like structures.
In conclusion, ectopic endometrial tissue locally expresses and binds the complement component C7. Its presence at the level of the lesions could also be caused by the activation of complement and therefore due to deposition. The massive production of EM cells could be a decoy against CS activation on ectopic tissue.

## P44
## TROPHOBLAST ORGANOID AS MODEL TO RECAPITULATE COMPLEMENT SYSTEM DYSFUNCTION IN PREECLAMPSIA

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The innate immune system plays a crucial role in pregnancy by promoting maternal tolerance towards the fetus while preserving defense mechanisms against pathogens. Among its components, the Complement system (C) is critically involved at the feto-maternal interface, contributing not only to immune surveillance but also, through its non-canonical functions, to tissue remodelling. However, dysregulation in C activation has been implicated in pregnancy complications, including preeclampsia (PE), recurrent spontaneous miscarriage, and intrauterine growth restriction. Proper control of C activity is essential, and the trophoblast cells of the placenta, in direct contact with maternal blood, express several C regulators. In this study, we investigated the expression of C-related factors in trophoblast cells from preeclamptic and healthy placentas. We evaluated whether the recent 3D trophoblast organoid model can serve as a reliable in-vitro system to recapitulate C dysregulation associated with PE. We analyzed publicly available single-cell RNA sequencing (scRNAseq) data of PE and health at term placentas, identifying altered expression of C regulators across trophoblast subpopulations such as CD46, CD55, CD59, C1QBP, and SERPING1. The functional relevance of these findings was then assessed through immunofluorescence staining on formalin-fixed, paraffin-embedded placental sections. We then examined the expression of these regulators in a 3D trophoblast organoid model derived from first-trimester placentas, which recapitulates key trophoblast subtypes involved in early placentation. All complement regulators were expressed at significant levels. In conclusion, our study highlights the differential expression of C regulators between PE and healthy pregnancies at both transcript and protein levels. Moreover, we demonstrated that trophoblast organoids may serve as a suitable in-vitro model for studying C regulation, providing a valuable tool for investigating the disease mechanisms and identifying potential therapeutic targets.

## TUMOR IMMUNOLOGY

### P45
### Mucosal associated invariant T cells infiltrating resectable non-small cell lung cancer exhibit a tissue resident memory phenotype, enhanced metabolic activation and strong pro-inflammatory properties

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**Introduction:** Circulating mucosal-associated invariant T (MAIT) cells are an innate-like pro-inflammatory and cytotoxic population of effector memory T cells and can represent up to 10% of peripheral $CD8^+$ T cells. Their role in cancer remains controversial. In non-small cell lung cancer (NSCLC) patients, MAIT cells are found within tumor lesions; however, limited data are available on their cell-cell interactions and functional mechanisms within the tumor microenvironment (TME).

**Objectives:** To characterize MAIT cells in NSCLC from both phenotypic and functional perspectives.

**Methods:** We enrolled 37 patients with NSCLC (I, II, IIIA, 8th TNM ed). Multiparameter flow cytometry was employed to assess the phenotype and functional profile of tumor-infiltrating and circulating MAIT cells, focusing on cytokine production (IFN-γ, TNF, IL-2, Granzyme B, and IL-17). Proteome profiling of MAIT cells was conducted using ad hoc isobaric labeling-based multiplexed quantitative proteomics. Additionally, spatial transcriptomics was utilized to localize MAIT cells within the tumor microenvironment (TME).

**Results:** MAIT cells, defined as $CD161^{++}$, $TCR7.2^+$ within $CD8^+$ T cells, are much more represented in blood if compared with those infiltrating the tumor lesion (median ± SEM: 4.90 ± 0.90 vs 2.77 ± 0.87, p = 0.0207). However, MAIT cells infiltrating the TME exhibit a tissue-resident memory (TRM) phenotype (60.35 ± 4.79) and a pronounced polyfunctional profile biased toward Th1 characteristics. A total of 66 proteins are differentially expressed in TME-infiltrating MAIT cells (logFC > 2), primarily associated with metabolic activation, indicating their potential involvement in metabolic and immune reprogramming within the TME.

**Conclusion:** Although less abundant in the TME compared to circulation, the profile of MAIT cells provides new insights into their functional landscape in NSCLC, highlighting their potential as key players in tumor immunity.

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### P46
### Exploring mechanisms of immunotherapy resistance in ovarian cancer

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Immune checkpoint blockade (ICB) has revolutionized cancer treatment, but its effectiveness is limited in many patients, including those affected by ovarian cancer. The tumor microenvironment (TME) plays a crucial role in ICB efficacy, with tumor-infiltrating immune cell subsets, and particularly macrophages, significantly influencing treatment outcomes. However, the precise mechanisms driving ICB resistance in ovarian cancer remain poorly understood. In the present project we utilized samples from platinum-resistant ovarian cancer patients treated with anti-PD1 therapy. We employed advanced high dimensional techniques, including spatial RNA sequencing, single-cell RNA sequencing (scRNA-seq) and Imaging Cytomass (IMC), to dissect the tumor microenvironment (TME) in responding versus non-responding patients. Our analysis revealed significant differences in immune composition and cellular localization between the two cohorts. Notably, we observed an enrichment of macrophages in the TME of non-responding tumors. To investigate this further, we set up an in vitro system in which we exposed macrophages to tumoral condition media and examine them phenotypically and functionality. Additionally, to better characterize macrophage-tumor cell interactions in a 3D model we set up ovarian cancer spheroids. Our data showed that macrophages can shape the transcriptional landscape of tumor cells, driving metabolic rewiring and epithelial-to-mesenchymal (EM) features, which may enhance resistance to therapy. In conclusion, our research suggests a role for TAMs in reducing the efficacy of ICB in ovarian



cancer. Understanding the molecular mechanisms behind these macrophage-mediated interactions may uncover new therapeutic targets to enhance ICB responses and improve patient outcomes.

## P47
## Effect of *Mycobacterium tuberculosis* on human lung macrophages: exploring the role in the context of lung cancer

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**Background:** The relationship between *Mycobacterium tuberculosis* (Mtb) infection and lung cancer remains complex and poorly understood. While some studies suggest Mtb infection as a risk factor, others report an inverse correlation or no association. This study investigates the in vitro effects of Mtb on human lung macrophages derived from non-small cell lung cancer patients (HLMs), exploring the potential role of Mtb in lung cancer development.

**Methods:** HLMs from NSCLC patients were stimulated with increasing concentrations of heat-killed Mtb (HK-Mtb). The resulting supernatants was applied to A549 and HCC4006 lung cancer epithelial cell lines. A549 and HCC4006 cell proliferation was measured, and cytokine release (TNF-α, CXCL8, IL-1β) from HLMs was quantified in the supernatants.

**Results and Conclusion:** Mtb-stimulated macrophage supernatants induced a significant reduction of lung cancer epithelial cell proliferation. In parallel, Mtb exposure enhanced cytokine release from HLMs, suggesting a potential anti-tumorigenic role of Mtb-stimulated macrophages in the context of lung cancer. We hypothesize that this anti-proliferative effect is mediated by the increased cytokine release, particularly TNF-α, which has been shown to exert cytotoxic effects on certain cancer cells. Further experiments will be needed to elucidate the mechanisms underlying this effect, including the specific role of these cytokines, and to validate these findings using live Mtb infection. This research may provide novel insights into the complex interplay between Mtb infection and lung cancer development.

## P48
## Characterization of B cells in murine models of triple-negative breast cancer

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B cells play a pivotal role in the immune response to infections, and growing evidence suggests their significance may extend to the context of cancer. Indeed, several studies have shown that tumor-infiltrating B cells (TIBs) and tertiary lymphoid structures (TLS) containing B cells correlate positively with response to immune check-point inhibitors (ICIs). Recent transcriptomic studies shed light on the high complexity of the B cell compartment in cancer, and suggest that B cells infiltrating tumors (TIBs) might play either a pro-tumor or anti-tumor role depending on the contexts and on their specific functions. However, in contrast to the well-known role of T cells, a comprehensive characterization of B cell subsets and functions in cancer is still lacking. To address this gap, we decided to take advantage of murine models of triple-negative breast cancer (TNBC), a highly complex disease for which the optimal treatment strategy remains a major unmet clinical need. B cells have been reported to serve as favorable prognostic markers for ICI responses for this disease.
We are currently characterizing the diverse B cell populations in two murine TNBC models: the T11-Apobec model in BALB/c mice and the E0771 model in C57BL/6 mice. Preliminary data show that both models exhibit a consistent amount of B cells in tumors and tumor-draining lymph nodes. In particular in this latter compartment we observed a significant expansion of B cells, accompanied by the presence of germinal center reactions. Our preliminary observations suggest that these TNBC tumors constitute a good model where mechanistic studies on B cell functions in cancer can be performed. Indeed, characterizing the heterogeneity, functions, and interactions of B cells with other immune cell populations may unveil novel therapeutic strategies in the field of cancer treatment.

## P49
## Key role of IL-33-activated eosinophils in enhancing tumor immunovisibility and dendritic cell recruitment

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Eosinophils are emerging as key players in cancer exerting diverse roles. The epithelial-derived alarmin IL-33 induces eosinophil activation, promoting their adhesion to tumor cells and direct cytotoxicity through immune synapse formation and degranulation. We recently showed that IL-33-activated eosinophils (Eo33), as opposed to IL-5-stimulated eosinophils (Eo5), can block tumor progression through extracellular vesicle (EV)-driven reprogramming of cancer cells. In this study, we investigated the effects of Eo33 on tumor immunovisibility and recognition by dendritic cells (DC) in mouse and human models. Following co-culture with Eo33, but not with Eo5, tumor cells upregulated key antigen presentation molecules, CD74, H2-DMa, H2-Aa in mouse B16.F10, MCA205, MC38, TC-1 cells and CD74 and HLA-DRA in human A375P tumor cells, indicating enhanced tumor immunovisibility. In transwell co-culture systems, conditioned medium (CM) from mouse and human-Eo33 and tumor cell co-cultures promoted the migration of mouse splenic DC and PBMC, respectively. Interestingly, the immunophenotype of migrated PBMC revealed highest percentage of $CD11^{+}CD14^{-}$ cells towards CM from Eo33-tumor cell co-cultures with respect to Eo5-tumor cell CM. These findings were supported by competitive microfluidic-based migration assays in which PBMC preferentially migrate towards the A375P/Eo33 chamber, compared to the A375P/Eo5 chamber. All of these effects occurred independently of EV, suggesting that they were mediated by soluble factors released following contact of Eo33 with tumor cells. Protein array and ELISAs did not detect any classical DC-attracting chemokines or the ANXA1 find-me-signal selectively in Eo33-tumor cells CM. Conversely, both Eo33 and tumor cells increased their intracellular levels of reactive oxydizing species (ROS) following co-culture with respect to Eo5-tumor cell co-cultures, which may indicate local damage and activating defense mechanisms such as immune cell recruitment. These findings suggest that Eo33 contribute in reshaping the tumor microenvironment by promoting DC recruitment, reinforcing the functional role of IL-33 in modulating eosinophil-mediated anti-tumor responses.

## P50
## Role of the atypical receptor CCRL2 in shaping immune responses in lung cancer microenvironment

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CCRL2 is a non-signaling seven-transmembrane domain receptor that binds chemerin, a protein that promotes chemotaxis of leukocytes, including dendritic cells, macrophages and natural killer (NK) cells. CCRL2 has been shown to regulate the inflammatory response in different pathologic settings, such as inflammatory arthritis, and experimental autoimmune encephalitis [1]. We recently showed that CCRL2 plays a protective role in different models of lung cancer as well as in lung cancer patients [2]. The mechanism underlying this protective phenotype was associated with the selective expression of CCRL2 by alveolar capillary endothelial cells, where CCRL2 plays the role of lung-specific homing molecule for anti-tumor effector NK cells expressing the functional chemerin receptor CMKLR1 [3].
In addition, we observed that CCRL2 deficiency was associated with an increased frequency of tumor-promoting SiglecFhigh neutrophils, a population recently shown to accumulate during cancer progression. This neutrophil subset could be related to the altered distribution of gdT cell subsets observed in lung cancer microenvironment of tumor-bearing Ccrl2 deficient mice.
In this work, we used different experimental approaches, including gene-targeted mice and scRNA sequencing, to investigate the mechanisms underlying the antitumor immune responses shaped by the CCRL2/Chemerin/CMKLR1 axis in the homing of immune cells to the lung.

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## P51
## Transferrin receptor CD71 sustains regulatory T cells in gut homeostasis and promotes effective anti-tumor immunity

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Cancer is a systemic disease influenced by interactions between the gastrointestinal tract, microbes, and the immune system Iron is a critical nutrient not only for tumor cells but also for microbes and immune cells. Regulatory T cells (Tregs), abundant in early life (when microbial colonization occurs) and in tumors, acquire iron via CD71 to support their expansion. We hypothesize that iron supply to Tregs is crucial to maintain gastrointestinal homeostasis but also contributes to immunosuppression in tumors.
First, we characterized the impact of CD71$^+$ Treg reduction on immune homeostasis in tumor-free (TF) mice. Foxp3eGFP-Cre-ERT2 Tfr1 fl/fl inducible conditional KO (Tfr1 icKO) mice, upon treatment with Tamoxifen (TAM), displayed a decreased frequency of CD71$^+$ Tregs especially in the intestinal compartment, with a trend for an increased immune activation status. Then, we assessed the Treg-intrinsic role of CD71 in anti-tumor immunity. In Tfr1 icKO MC38 tumor-bearing (TB) mice, TAM treatment reduced CD71$^+$ Tregs in both colonic lamina propria (cLP) and tumor microenvironment (TME), promoting the expansion of tumor-specific CD8 T cells and reducing tumor growth.
Commensal microbes induce in the intestine the development of RORγt$^+$ Tregs, which migrate to distal tissues to perform repair and immunosuppressive functions. In the TME of breast cancer nodules (obtained with the E0771 cell line), we could detect the presence of RORγt$^+$ Tregs, which expressed higher levels of CD71 compared to Helios$^+$ Tregs, suggesting that RORγt$^+$ Tregs may be mobilized from the colon to the tumor.
In conclusion, our data indicate that iron uptake by Tregs regulates gastrointestinal homeostasis but also mediates their pro-tumor activities

## P52
## Epigenetic control of cancer stem cell immunoevasion: new opportunities from KDM1b

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Cancer is a highly heterogeneous disease which continuously evolves across space and through time. This diversity is currently a major clinical need, limiting the efficiency of most cancer treatments, with the cancer stem cells (CSCs) contributing immensely to the same. Besides being endowed with a marked ability to cope with environmental and therapy-elicited stress, CSCs also evade immune recognition or elimination. In this scenario, epigenetic rewiring is emerging as a driver of tumor evolution, which impacts disease progression and immune evasion. By integrating cutting-edge technologies and clinical data monitoring we provide insights into the role of lysine demethylase 1B (KDM1B) affecting the tumor immune microenvironment by promoting cancer cell stemness and immune escape mechanisms. Our results show that the triggering of KDM1b promotes an adaptive transcriptional rewiring of cancer cells towards stemness, acquired resistance and immune escape. Accordingly, retrospective analysis on breast cancer patient cohorts reveals that tumors expressing high levels of KDM1B are enriched in stemness and immune checkpoint markers as well as poorly immune infiltrated, which strengths our hypothesis on the role of KDM1B in initiating multiple mechanisms of cancer immunoevasion. As we move toward the development of strategies that rationally combine tumor-specific targeting with immunotherapies, the incorporation of epigenetic programs into cancer biology and their clinical implementation are imperative to maximize the potential of anticancer therapies. In this sense, our findings will offer new choices of personalized precision combined epigenetic/immune-based targeted treatments that could be beneficial for hard-to-treat cancer patients.

**P53**

## HFE polymorphism negatively affects pancreatic cancer progression and anti-tumor immune response

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Homeostatic Iron regulator (HFE) is the main altered gene in the hereditary hemochromatosis (HH) disease. Of the two main HFE polymorphisms, H63D rarely causes overt HH symptoms, but has been described as a risk factor for the development of different types of cancer. Little is known about the impact of H63D on pancreatic ductal adenocarcinoma (PDAC), although a great percentage of PDAC patients that can undergo surgery carries this polymorphism. However, they show a reduced survival after resection.

To analyze the impact of H63D on the anti-tumor immune response in the context of PDAC, genetically engineered mice that spontaneously develop pancreatic cancer (so called KC mice) were crossed with mice bearing H67D mutation (KC/H67D), the murine ortholog of the H63D polymorphism. Histological analyses showed that H67D tumors grew faster and metastasized more toward both liver and lungs. Tumor lesions with similar extension in KC mice displayed a strongly reduced lymphoid immune infiltrate when the mutation was present, in favor of a more prominent myeloid one. Accordingly, the strong alteration of the immune response, which was naturally propense toward a type-II regulatory response, led to a reduced survival of these mice.

Regarding the anti-tumor response, H67D-bearing mice showed the ability to mount an antigen-specific immune response and produced greater quantities of antigen-specific antibodies. However, despite the prominent humoral response, tumor still grew faster in both sub-cutaneous and orthotopic settings. Ex vivo analyses highlighted an impaired interferon (IFN)-γ response in KC/H67D mice, in favor of a pronounced interleukin (IL)-4 and tumor necrosis factor (TNF)-α production. Consistently, an important eosinophilia and a greater presence of suppressive cells were detected in KC/H67D tumors.

Altogether, the H67D polymorphism accelerates PDAC progression and strongly affects immune cell activation, representing a promising biomarker that deserves further investigations.

**P54**

## Luspatercept treatment modulates immune-regulatory subsets in subjects with myelodysplastic syndrome with ring sideroblasts

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**Introduction:** Myelodysplastic syndrome (MS) are a hematopoietic disorder with variable leukemia evolution. In MDS with ring sideroblasts (MDS-RS), transfusion-dependent (RBC-TD) anemia represents a major clinical challenge. Luspatercept, a TGF-β pathway inhibitor, has been recently approved for RBC-TD treatment in MDS-RS; the mechanisms underlying its therapeutic activities remain unclear. immune-dysregulation contributes to ineffective hematopoiesis and clonal evolution in MDS. Regulatory T cells (Tregs) and monocytic myeloid-derived suppressor cells (mMDSCs), key immuno-suppressive populations have been implicated in these processes. Given the TGF-β role in the regulation of cell-dependent immune-modulation, we investigated the effect of luspatercept treatment on the immune profile of MDS-RS subjects.

**Methods:** We analyzed peripheral blood from 13 MDS-RS patients with RBC-TD anemia treated with luspatercept and 20 age/sex matched controls. Samples were collected before and after six months of treatment. Immune-fluorescence and multiparametric flow-cytometry was used to assess the amount, activation and proliferation of effector and regulatory subsets. Tregs evaluation included analysis of Foxp3 and of its suppressive isoform Foxp3-Ex2.

**Results:** Luspatercept did not significantly alter circulating $CD3^+CD4^+$, $CD3^+CD8^+$, B and NK lymphocyte amount. The treatment significantly increased the activation and proliferation of the adaptive effectors, as evaluated by their CD54 and ki67 expression. Moreover, a decreased number of $Foxp3^+$ and $Foxp3\text{-}Ex2^+$ Tregs as well as of the mMDSCs was revealed. The ability of luspatercept to significant-



ly reduce in vitro Treg differentiation has been also observed. Higher expression of CD54 on CD8 T cells and lower percentage of mMDSCs, at baseline, has been associated with treatment response, as evaluated according to the IWG 2018 criteria.

**Conclusion:** Our data, as a whole, propose that luspatercept, by affecting TGF-β dependent pathways, might re-shape the immune-microenvironment in MDS-RS, reducing immunosuppression and promoting T cell activation. The possibility to add the immune profile to the prognostic biomarkers of luspatercept treatment efficacy, needs further investigation.

## P55
## Characterization of IDO1H350A mouse model expressing a loss-of function mutant of the enzyme indoleamine 2,3-dioxygenase 1

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Indoleamine 2,3-dioxygenase 1 (IDO1) is a crucial immunomodulatory enzyme that catalyzes tryptophan to kynurenine conversion. Beyond its enzymatic role, IDO1 participates in signal transduction pathways within immune and tumor cells, influencing cellular responses.
To better understand the molecular mechanisms underlying IDO1's dual role, we resorted to a transgenic mouse model expressing a loss-of-function mutant of IDO1 enzyme (IDO1H350A), obtained by the replacement of histidine with alanine at position 350 in the IDO1's catalytic site.
The analysis of Kyn and Trp levels in sera from IDO-1H350A and IDO1WT mice, showed a significant decrease in Kyn/Trp ratio in IDO1H350A compared to IDO1WT mice, confirming the loss of the catalytic activity by the mutated IDO1 protein. Although the level of IDO1 protein increased in response to pro-inflammatory stimuli in splenocytes and conventional dendritic cells (cDCs) isolated from both IDO1WT and IDO1H350A mice, IDO1's enzymatic activity was not detectable in immune cells purified from transgenic mice. Furthermore, IDO1H350A protein in cDCs resulted more stable than IDO1WT protein, undergoing to a slower turnover with a resulting half-life time of 16 hours versus 7.6 hours. Finally, the analysis of the cytokine profile secreted by LPS-stimulated splenocytes and cDCs revealed an exacerbated inflammatory phenotype in IDO-1H350A - expressing immune cells compared to IDO1WT cells.
This preliminary immune characterization of IDO-1H350A mice confirmed: i) the loss of the catalytic activity of IDO1 enzyme in both splenocytes and cDCs; ii) an inducibility of IDO1H350A protein in response to pro-inflammatory stimuli; iii) a slower turnover of IDO1H350A protein in cDCs; iv) an exacerbated inflammatory phenotype of immune cells expressing IDO1H350A.

## P56
## Involvement of innate lymphocytes in the pathogenic mechanisms of colorectal cancer

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Colorectal cancer (CRC) is one of the most common types of cancer globally. Studying the complex immunological microenvironment of CRC patients is crucial both for understanding the immune circuits involved in progression and defense, and for assessing the response to immunotherapeutic treatments. Within the tumor microenvironment, we have focused on innate lymphoid cells (ILC) which belong to "unconventional" lymphocytes of the immune system. A deeper understanding of the role of these subsets could offer advantages for immunotherapeutic approaches and for improving treatments for patients.
Cells were isolated from healthy mucosa, tumor mucosa, and peripheral blood of 10 colorectal cancer patients, to define the immune-phenotype and the frequency of ILC subsets by flow cytometry, following a specific panel directed against surface markers of interest.
The immuno-phenotypic analysis showed heterogeneous distribution of ILC2 and ILC3 subsets in healthy mucosa. In peripheral blood, all three subsets (ILC1, ILC2, ILC3) were uniformly present across patients but exhibited individual variability, suggesting no consistent frequency patterns. In tumor-associated tissue, ILC1 and ILC3 showed uniform distribution without dominant values, while ILC2 were absent, indicating a potential common feature linked to tumor. These results highlight the complex and compartment-specific behaviour of ILC populations in CRC patients and may provide insights into their potential role in tumour immunity. Future developments of this study will include functional assays to evaluate the proliferative capacity, cytotoxic activity, and cytokine production of

ILC populations, aiming to better elucidate immune dynamics in CRC and their clinical implications.

**P57**
**Role of IL17A in defining cancer extracellular matrix composition and as a consequence affecting immune infiltration**
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The tumor microenvironment (TME) is a complex structure shaped by the interplay between tumor cells and normal host cells, manipulated by the tumor to its advantage. Tumor extracellular matrix (ECM), indeed, allow cancer cells to acquire resistance to chemotherapy or target therapy and lastly also immunotherapy. For this, recently, TME and especially matrix are considered promising therapeutic targets.
In this study, we aimed to characterize the tumor microenvironment (TME) in the presence or absence of IL17A and its role in modulating tumor cell invasion. We have previously demonstrated that IL-17A negatively impacts cancer-associated fibroblasts (CAFs) by enhancing their pro-tumorigenic functions and contributing to pancreatic ductal carcinoma (PDAC) progression and ECM remodeling. Mass spectrometry analysis revealed more than 500 proteins differentially present in the tumor scaffolds from IL17A-proficient and deficient mice, which spontaneously develop pancreatic ductal carcinoma (PDAC), the so called KPC. The different ECM composition reflected a different tumor elasticity, as observed through atomic force microscopy (AFM) imaging.
In vitro, CAF isolated from IL17A-proficient and deficient KPC mice displayed a different production of clue ECM proteins as well as an enhanced sprouting capacity in a dense environment. By contrast, in a 3D invasion assay, hetero-spheroids, constituted by PDAC cells WT for IL17A, showed higher invasive growth when in presence of IL17A-proficient CAF compared to those in presence of IL17A-deficient CAF. Quantitative PCR confirmed the differential expression of epithelia-mesenchymal transition (EMT) markers in IL17A-proficient and deficient PDAC cells.
Our results demonstrate a role for IL17A in shaping the TME, particularly through CAF-mediated ECM remodeling, which consequently affects tumor invasion and offers insights for novel combined therapeutic strategies.

**P59**
**Unravelling the spatial and transcriptional evolution of type 1 dendritic cells during lung cancer progression**
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Checkpoint inhibitors enhance anti-tumor T cell activity; however, only one-third of patients benefit, underscoring the need for innovative combination strategies to improve response rates. The role of innate immune cells in shaping effective anti-tumor immunity has been acquiring increased attention—particularly type 1 conventional dendritic cells (cDC1), which are pivotal for the orchestration of antitumoral immunity. This is especially relevant in lung cancer, where cDC1 are both scarce and functionally impaired within the tumor microenvironment.
Here we used a murine model of non-small cell lung cancer (KP model) that faithfully recapitulates the histopathology of the human disease, to investigate the phenotypic and spatial dynamics of intratumoral cDC1 during tumor progression. At early stages, we observed an influx of cDC1 displaying transcriptional signatures consistent with maturation and activation, including cross-presentation markers and cytokines genes. In contrast, late-stage tumors exhibited a reduction in cDC1 numbers and an upregulation of regulatory markers and a decreased proliferation, suggesting functional impairment. Using a cDC1-reporter mouse, we conducted spatial analyses and identified the formation of cDC1-centric immune hubs, which progressively organized into tertiary lymphoid structures as tumors progress.
These findings provide novel insights into the temporal and spatial dynamics of cDC1 within the tumor microenvironment and highlight their potential as therapeutic targets in lung cancer immunotherapy.



## P60
## Generation of Kras/p53 lines to study local immunity in lung cancer

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Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer and a leading cause of cancer-related mortality worldwide. Despite the development of therapies such as immune checkpoint inhibitors, only a limited fraction of patients experiences long-term clinical benefits. Faithful experimental models of NSCLC are key to exploring immune-tumor interactions and are essential for improving therapeutic strategies.
Here we aimed to develop slow progressing transplantable cell lines of the murine KrasLSL-G12D/+/Trp53fl/fl (KP) model of NSCLC to mimic the kinetics of immune-cancer crosstalk in vivo.
Two cell lines were isolated from KP tumors at different stages: one from initial tumor lesions (early cell line, ECL) and the other from established tumors (late cell line, LCL). Both lines were engineered to introduce the model antigen OVA generating ECL-OVA and LCL-OVA. ECL, LCL, and the corresponding OVA lines, were implanted in heterotopic and orthotopic settings to assess tumor growth. We observed that only the ECL-OVA line was rejected, while the others grew progressively. Analysis of OVA specific T cell responses showed equal activation in mice challenged with ECL-OVA and LCL-OVA, suggesting the development of the ability to evade immune control by LCL-OVA.
Orthotopic implantation showed that for both ECL and LCL growth is significantly delayed, compared to other existing transplantable KP lines. Moreover, only ECL-OVA were rejected, confirming previous observations.
Notably, imaging of lung sections highlighted the formation of organized ectopic lymphoid structures comprising B cells T cells and dendritic cells, which are increasingly recognized as important predictors of anti-tumor response efficacy in human cancers.
In summary we have generated a slow progressive model of NSCLC that supports the formation of organized immunity hubs in lung tissues, providing a valuable tool for studying tumor-immune interactions locally in cancer tissues.

## P61
## Overcoming resistance in malignant pleural mesothelioma through EZH2 inhibition and ferroptosis induction

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Malignant pleural mesothelioma (MPM) is an aggressive and treatment-resistant cancer. Although immunotherapy has recently achieved a breakthrough for the non-epithelioid histotype, many patients remain unresponsive due to poor immunogenicity and a highly immunosuppressive tumor microenvironment (TME). Novel therapies are urgently needed to improve outcomes across MPM istotypes. Tazemetostat, an EZH2 inhibitor, showed anti-proliferative potential, but its TME impact is poorly understood. Ferroptosis inducers like RSL3, are emerging as promising strategies to kill resistant tumor cells and enhance immunotherapy efficacy. We posit that tazemetostat-mediated epigenetic reprogramming or RSL3-induced ferroptosis could boost anti-tumor immunity in MPM. To test this, epithelioid, biphasic, and sarcomatoid MPM cell lines were cultured as 2D monolayers or 3D multicellular spheroids (MCS) and treated with tazemetostat or RSL3. Gene expression analysis revealed histotype-specific chemokine profiles, variably enhanced by EZH2 inhibition. Tazemetostat-treated MCS recruited more monocytes, promoting their pro-tumor differentiation and countering the anti-proliferative effects of EZH2 inhibition. Notably, tazemetostat upregulated ferroptosis-related genes (SLC7A11, CHAC1, PTGS2), most prominently in sarcomatoid MCS. Similarly, RSL3 treatment increased ferroptosis-related transcripts and cytotoxicity, particularly in sarcomatoid MPM cells, while monocytes remained resistant. These findings suggest that tazemetostat enhances pro-tumor monocyte recruitment, but also sensitizes MPM cells, especially sarcomatoid subtypes, to ferroptosis. Combining EZH2 inhibition with ferroptosis induction may offer a novel therapeutic strategy for MPM, with potential to reprogram pro-tumor monocytes into anti-tumor effectors.

**P62**

## Dissection of neutrophil heterogeneity in glioblastoma

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Glioblastoma multiforme (GBM) is among the most aggressive brain tumors and is associated with extremely poor prognosis. Its strongly immunosuppressive nature represents a major obstacle to the development of effective immunotherapeutic strategies. Although neutrophilia is frequently observed in cancer patients, the complex interplay between neutrophils and GBM remains poorly understood.
The primary aim of this study was to characterize the phenotype and maturation state of circulating neutrophils, in order to clarify their interactions with the tumor microenvironment and other immune cells. Our findings revealed elevated levels of circulating neutrophils in patients with poorer prognoses. However, beyond total neutrophil count, the maturation and activation states emerged as more informative: patients with a higher proportion of immature neutrophils exhibited longer overall survival.
To further investigate the role of neutrophils in the glioma context, we established an in vivo orthotopic glioma model by injecting the SB28 glioma cell line into both wild-type (WT) and neutropenic (CSF3R knockout) mice. Notably, CSF3R KO mice displayed improved survival compared to WT mice, supporting a pro-tumoral role for neutrophils in this model. Overall, our data suggest that an increased neutrophil count in the blood of patients with poor prognosis does not necessarily predict worse survival outcomes. On the contrary, a shift toward an immature neutrophil phenotype appears to be associated with prolonged survival. Ongoing studies aim to further elucidate the role of neutrophils in the pathophysiology of brain tumors and to explore their potential as therapeutic targets.

**P63**

## Human-in-mice model for studying Natural Killer cell cross-talk with non-small-cell lung cancer bone metastases

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Non-Small Cell Lung cancer (NSCLC) is frequently diagnosed at locally advanced or metastatic stages, with bone localization in 30-40% of cases. There is therefore a need to study the tumor microenvironment in bone metastases from NSCLC and how this could influence and modulate the immune response. Moreover, considering the important role of Natural Killer (NK) lymphocytes in recognizing and killing tumor cells, the development of an in vivo model of bone metastasis to study the interactions between human NK cells and the tumor counterpart is desirable. We present an achievable method to establish a human-in-mice model of bone metastasis consisting of (1) implant of human bone fragment in immunocompromised NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Sug) and (2) injection via caudal artery of NSCLC spheroids, enriched in cancer stem cells. By in vivo bioluminescence imaging and ex vivo immunohistochemical analysis, we observed that tumor cells were able to reach and colonize the bone implant and femurs, with a reduced onset of metastasis in other organs, such as lung and liver. The high rate of metastasis engraftment in the bone implant, the minimal animal handling practices, and the good health conditions of the mice observed during the experiments, make this method reliable and feasible. Moreover, we were able to direct human NK cells to bone metastasis offering the possibility to study their interactions with tumor cells, stroma and immune infiltrate in bone microenvironment. In conclusion, the human-in-mice tumor models, combined with human NK cell transfer, could represent a suitable mean to study the cross-talk of NK cells and NSCLC bone metastasis in a human context.

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# VACCINES

## P65

### Longitudinal study on SARS-CoV-2 mRNA vaccine immunogenicity across individuals with different immunocompromising conditions: heterogeneity in the immune response and impact of Omicron-adapted boosters

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Individuals with primary and secondary immunodeficiencies, being more susceptible to infections, are a priority for vaccination. To determine and compare the immune response elicited by SARS-CoV-2 vaccination across different groups of individuals who are immunocompromised.
In the PatoVac_COV longitudinal prospective single-centre study, the spike-specific B cell and antibody responses to SARS-CoV-2 mRNA vaccination were compared across 5 different groups of individuals with haematological malignancies, hematopoietic stem cell (HCT) or solid organ transplantation (SOT), undergoing haemodialysis, and people living with HIV (PLWH), for a total of 585 participants. Data from participants who were immunocompromised were compared to a group of 123 participants who were immunocompetent . Blood samples were collected before and after each vaccine administration, up to 2 years.
A different immune responsiveness was observed after the first two doses, with haematological, haemodialysis and SOT participants showing reduced responsiveness compared to HCT and PLWH, relative to the comparison group. Spike-specific B cell response was both slower and lower in all groups except in PLWH when compared to participants who were immunocompetent. However, the first booster dose enhanced both the B and the antibody responses in all groups, that persisted up to 2 years after the first vaccine administration. The administration of Omicron-adapted booster vaccines promoted a primary BA.2 RBD-specific B cell response, especially in participants who were immunocompromised. Despite repeated vaccinations, a subset of persistent low-responders, especially among SOT, was identified.
The study highlights the heterogeneous immune response across individuals with different pathologies, the pivotal role of the first booster dose, the primary activation of Omicron-specific B cells elicited by updated variant-adapted vaccines and the persistence of low-responders despite multiple vaccine administrations. These aspects have a clinical relevance for planning vaccination schedules tailored for individuals with different immunocompromising conditions.

## P66

### Evaluation of broadly functional antibodies through the assessment of ADCC against HIV Env and Tat proteins: insights for HIV vaccine development

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Effective immune system activation and the induction of cytotoxic responses are crucial for selectively eliminating infected cells to control viral infections. Antibody-dependent cell-mediated cytotoxicity (ADCC), which utilizes the effector functions of macrophages, neutrophils, and natural killer cells, plays a significant role in HIV infection. In the RV144 Thai trial, ADCC targeting the HIV envelope was associated with protection against infection. Nevertheless, several questions remain, unresolved: i) the effectiveness of ADCC induced by different Env forms, and ii) the role of ADCC against regulatory HIV antigens like Tat.
The capacity of antibodies against Env or Tat to mediate ADCC was assessed using the Rapid Fluorometric assessment of ADCC (RFADCC) assay, using peripheral blood mononuclear cells (PBMCs) from healthy donors as effectors and CEM NK CCR5$^+$ pulsed with Env or Tat and opsonized by specific antibodies, as target cells.
We observed strong ADCC activity (> 40% killing) in target cells pulsed with Tat protein and incubated with hyperimmune rabbit serum or human sera positive for anti-Tat antibodies. ADCC was also

measured in cells pulsed with monomeric gp120 proteins from clade B strains (SF162, JR-CSF, or MN) or a clade C trimeric Env protein lacking the V2 loop (DV2-TV1), incubated with monoclonal antibodies (17b or 48d) or anti-gp140 polyclonal rabbit serum. ELISA showed that 17b binds all monomeric proteins. However, only SF162 was efficiently targeted by 17b in ADCC, while the polyclonal serum showed the highest ADCC activity against DV2-TV1.
Overall, this study demonstrates, firstly, direct killing via ADCC mediated by human anti-Tat antibodies, using RFADCC assay. Additionally, while confirming Env-induced ADCC, data show that different forms of the Env protein induce varying levels of ADCC activity. These observations may have important implications for the development of novel effective HIV vaccines based on Env and/or Tat and to monitor correlates of protection.

**P67**

## On the adjuvanticity of hyaluronan: The case of a SARS-CoV-2 vaccine

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The COVID-19 pandemic emphasized the need for effective and scalable vaccine platforms. While mRNA vaccines have played a crucial role, concerns about their long-term stability and reactogenicity support the exploration of protein-based alternatives. However, due to the poor immunogenicity of protein antigens, the identification of effective adjuvants remains critical.
Hyaluronic acid (HA), a natural polymer widely used in the medical field for its biocompatibility and biodegradability, is gaining attention for its remarkable immunomodulatory properties. Thus, we developed a novel vaccine platform based on the conjugation of the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein to HA (HA-RBD).
The efficacy of the HA-RBD vaccine was evaluated through intramuscular injection of both BALB/c and K18-hACE2 mice, following a two-dose schedule (day 0, 21). Vaccination was well tolerated and elicited high titres of RBD-specific IgGs, including IgG1 and IgG2c subclasses. Moreover, HA-RBD-induced antibodies exhibited potent neutralizing activity against SARS-CoV-2 in vitro.
Compared to formulations with commercial adjuvants such as Quil-A and AddaVax, HA-RBD induced stronger immune responses without triggering local inflammation, highlighting HA's tolerability. HA-RBD vaccination also promoted long-term antibody persistence and the generation of long-lived plasma cells, suggesting the establishment of durable immunological memory.
Most importantly, HA-RBD conferred protection to K18-hACE2 mice following SARS-CoV-2 infection, guaranteeing their survival with respect to untreated mice. Eventually, we also demonstrated that the vaccine remained effective even against sequential infections caused by distinct viral variants, and maintained efficacy several months after the last dose.
While further studies are needed to elucidate HA's adjuvant mechanisms and its role in cellular immunity, these findings support the use of an HA bioconjugate as a promising vaccine platform.

**P68**

## Mode of action of nanoparticles as carrier for bacterial glycoconjugate vaccines

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Protein nanoparticles can increase vaccine immunogenicity by promoting a robust adaptive immune response and long-lasting protective immunity. We exploited nanoparticles, which act as carriers for saccharides presenting multiple copies of target antigens, mimicking the spatial aspect of host-pathogen surface interactions. However, despite the benefits conferred by protein nanoparticles, their mechanism-of-action (MoA) on immune cells remains unclear. Therefore, we performed in-vivo studies to examine the phenotypic characterization of B-cells, the germinal center reaction after a single dose of glyco-nanoparticles compared to traditional glycoconjugate vaccines. Firstly, a set of various NPs (*H. pylori* ferritin, *B. stearothermophilus* transacetylase (1B5S), Thermotoga maritima KDPG aldolase, Qβ, and P22 virus-like particles) was ex-

pressed in *E. coli*, purified, and conjugated with capsular polysaccharides from Group B *Streptococcus* serotype II (PSII). In-vivo results demonstrated that PSII-NPs elicit IgG titers significantly higher than one dose of PSII-CRM and are comparable to two doses of the reference subunit vaccine. Moreover, the antibody response to PSII-NPs increases further 90 days post immunization without boosting. Additionally, the immune response kinetics have been investigated using flow cytometry on lymphocytes isolated from mice immunized with glyco-NPs, showing promising results for next-generation design of multivalent bacterial vaccines. Furthermore, to characterize MoA of NPs, human peripheral blood mononuclear cells were stimulated with glyco-NPs and a classical conjugate. Multi-parametric flow cytometry revealed that glyco-NPs exhibited enhanced interaction with monocytes than PSII-CRM197. It is also worth noting that this type of interaction was observed in B cells. To validate these findings, we stimulated isolated B cells with glyco-NPs. Following a phenotypic characterization of the stimulated B cells, we observed that NPs interacted with naïve B cells, switched CD27-, IgM and not with memory B cells. Overall, these results pave the way for the next-generation design of multivalent bacterial vaccines and provides promising pre-clinical evidence for a single-dose vaccine.

## EPIGENETICS

### P69
### Sex-specific transcriptional and epigenetic reprogramming occurs in peripheral blood neutrophils from chronic obstructive pulmonary disease patients

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Chronic obstructive pulmonary disease (COPD) is a systemic disease marked by high levels of pro-inflammatory molecules and alterations in circulating leukocytes. Although the lung is the primary site of the disease, neutrophilia and altered neutrophil function are recognized as systemic features of COPD. Recent findings indicated that an increased number of circulating neutrophils characterize male rather than female patients. While sex-associated differences in neutrophil numbers have been reported, sex influence on neutrophil transcriptome and epigenome has not been investigated.
This study aims to characterize how sex influences the transcriptional and epigenetic profile of circulating neutrophils in COPD patients.
Neutrophils were purified from whole blood of 21 male and 15 female COPD patients and sex- and age-matched controls. Transcriptomic analysis was performed using the 3' mRNA-seq. Epigenomic analysis of H3K4me3 and H3K27ac was performed using ChIP-seq.
As demonstrated by hierarchical clustering analysis and confirmed by the Principal Component Analysis (PCA), two main clusters of donors can be identified based on the transcriptional profile of circulating neutrophils. Intriguingly, these two clusters differ in donor's sex rather than disease status. PCA results demonstrate that PC4 and PC7 discriminate COPD patients from controls. Sex-disaggregated analysis of PC4 and PC7 reveals a significant difference between male COPD patients and controls however, this difference was not observed among female donors.
PCA of the H3K4me3 and H3K27ac reveals that also the epigenetic profile of circulating neutrophils from COPD patients is influenced by donors' sex. In fact, while PC1 does not significantly discriminate COPD from control nor male from female donors, PC2 significantly differs between COPD and controls in males but not in female donors.
Our data demonstrate that sex-specific transcriptional and epigenetic reprogramming occurs in neutrophils from COPD patients underscoring the necessity for sex-disaggregated epigenetic- and gene expression analysis.

### P70
### Epigenetic control of the C1 complex: insights from C1Q gene expression analysis

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The C1 complex is the initiating component of the classical complement pathway, composed of the recognition protein C1q and the serine proteases C1r and C1s. Its activity is further modulated by binding partners such as C1INH, which extend its roles beyond complement activation. In addition to its classical immune roles, C1q also plays a part in processes like immunomodulation, placental development, and tumorigenesis. C1q is encoded by C1QA,

C1QB, and C1QC genes located on chromosome 1. Unlike most complement proteins synthesized in the liver, C1q is expressed extrahepatically by various cells, including macrophages, monocytes, and immature dendritic cells. The local synthesis of C1q, particularly under inflammatory conditions and during the clearance of altered self, requires tight transcriptional regulation. To explore this regulation, we leveraged publicly available tools and datasets, focusing on the epigenetic mechanisms governing C1Q gene expression. We examined chromatin accessibility and histone modifications across various cell types, at the C1QA, C1QB, and C1QC promoter regions. DNase-seq analysis provided insights into chromatin accessibility, while DNA methylation levels were analyzed from whole-genome bisulfite sequencing experiments. Co-expression analysis revealed tight coordination between C1QA, C1QB, and C1QC genes. Distinct epigenetic patterns emerged across various cell types and tissues, including macrophages, HUVEC cells, and placenta, depending on whether these genes were expressed. Notably, the investigation extended to tumor contexts unveiled potential epigenetic roles in malignancies. The cell type and tumor-specific histone modifications and chromatin accessibility patterns underscore the dynamic nature of epigenetic regulation of C1Q, providing crucial insights into the intricate mechanisms governing the expression of these immunologically significant genes. The findings provide a foundation for future investigations into targeted epigenetic modulation, offering insights into potential therapeutic avenues for immune-related disorders and cancer mediated via C1q.

# POSTER SESSION 2

## AUTOIMMUNITY

**P71**

### New blood markers of microvascular damage in systemic sclerosis

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**Introduction:** Systemic sclerosis (SSc) is a rare disease characterized by microvascular damage, autoimmunity and fibrosis. Severe manifestations are interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), scleroderma cardiopathy and renal vasculopathy. Platelets-to-lymphocytes ratio (PLR), neutrophils-to-lymphocytes ratio (NLR) and monocytes-to-lymphocytes ratio (MLR) are easily available and correlated to vasculopathy in SSc. Despite an unknown pathogenic role, B cell are dysregulated in SSc and specific autoantibodies are necessary for diagnosis. Recently, functional autoantibodies, directed against endothelial targets were described, including anti-angiotensin 2 type 1 receptor (anti-AT1R), related to SSc-PAH and poor outcome.

**Aim:** Detection of anti-AT1R and calculation of PLR, NLR and MLR in a cohort of SSc patients, to explore the role of new markers in predicting microvascular damage.

**Materials and Methods:** In this observational monocentric study, PLR, NLR and MLR were calculated and anti-AT1R measured with a commercial ELISA kit.

**Results:** 83 [71F, age 60 (17) years] patients with a diagnosis of SSc have been enrolled. Thirty-eight (45.8%) with a diffuse cutaneous form (dcSSc) and 45 (54.2%) limited (lcSSc). Ten healthy controls (HC) paired for sex and aged were enrolled. Anti-AT1R levels were higher in SSc patients than HC [7.78 (7.09-9.05) ng/mL vs 3.25 (2.60-4.70) ng/mL, $p < 0.001$]. In SSc, anti-AT1R were higher in patients with ILD [10.97 (7.81-14.15) ng/mL vs 7.38 (6.60-8.16) ng/mL, $p < 0.05$], scleroderma cardiopathy [9.05 (8.04-11.87) ng/mL vs 7.38 (6.60-8.16) ng/mL, $p < 0.05$] and recurrent digital ulcers (DUs) [9.90 (8.04-12.33) ng/mL vs 6.76 (6.11-7.58) ng/mL, $p < 0.01$]. NLR, PLR and MLR showed a positive correlation with new DUs and cutaneous telangiectasia ($p < 0.05$). NLR nega-



tive correlated with tricuspid annular plane systolic excursion/ systolic pulmonary artery pressure ratio (β -7.58, 95%CI -12.50/-2.66, p < 0.01) at echocardiography, MLR positive correlated with renal resistive index (β 0.098, 95%CI 0.401/1.109, p < 0.001) at Doppler ultrasound.

**Conclusion:** Blood markers such as anti-AT1R and cellular ratio are promising markers of microvascular damage in SSc.

## P72
## Peripheral B cell imbalances and T-bet⁺ B cell expansion in autoimmune liver disorders

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Autoimmune liver diseases, including autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and overlap syndrome (Overlap), pose diagnostic and therapeutic challenges due to their variable clinical presentations and potential for progression to cirrhosis and liver failure. The etiopathogenesis and specific triggers of these conditions are not fully understood, which hinder the development of specific therapies. The effectiveness of B-cell depletion therapy, especially in refractory cases, underscores the importance of B cells in immunopathogenesis. In various immunological disorders, such as autoimmunity and chronic infections, B cells with an atypical phenotype, known as T-betpos B cells or autoimmune-associated B cells (ABCs), expand and produce autoantibodies, contributing to immune dysregulation.
This study investigates the frequency of general peripheral B cell distribution and T-betpos B cells in the peripheral blood of patients with AIH, PBC, and Overlap, compared to healthy donors (HD), with a focus on the expression of CD21 and CD11c to define their essential phenotype. Analysis of peripheral B cell distribution revealed a significant decrease in the frequency of Marginal Zone B cells in hepatic disorders (AIH, n = 15; PBC, n = 8; Overlap, n = 11) compared to HD (n = 13). Additionally, PBC exhibited higher proportions of Naive B cells compared to HD. The frequency of circulating T-bet⁺ B cells was significantly higher in hepatic disorders compared to HD. Further characterization of T-betpos B cells revealed distinct subsets based on the expression of CD11c, which was higher in AIH, and CD21, which was higher in PBC. A bimodal distribution of CD11c and CD21 was observed in the overlap condition. Future investigations, including a deeper characterization of circulating T-bet⁺ B cells in all conditions, analysis in liver biopsies, and quantification of serum IL-21 and IFN-γ, will further refine the role of these B cell subsets in liver diseases.

## P73
## study of Immuno-modulatory effect of *Echinococcus granulosus* on the inflammatory response in Algerian patients with Crohn's disease

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**Introduction:** Inflammatory bowel disease (IBD) is an immunologically mediated disease. Notably, it is less common in countries where there is a greater risk of exposure to helminths, such as *Echinococcus granulosus*. These observations support the "hygiene hypothesis", which states that childhood exposure to helminths reduces the risk of developing IBD. Many studies suggest that helminths could be useful in regulating the immune response associated with IBD. In this work, we studied the effect of *Echinococcus granulosus* on inflammatory response induced in patients with Crohn's disease. We focused on evaluating the ex-vivo effect of extracts of this parasite on nitric oxide (NO) and TNF-α production by peripheral blood mononuclear cells (PBMC) from patients with Crohn's disease.

**Methods:** PBMC were recovered from the blood of patients with Crohn's disease. These cells were cultured in the presence or absence of protoscolex excretion-secretion products (PES), lamellar membrane extract or hydatid cyst fluid. The ex-vivo effect of these extracts on the inflammatory response was assessed by measuring NO and TNF-α.

**Results:** The results obtained show a reduction in NO and TNF-α production by PBMC cultured in the presence of parasite's extracts in comparison with non-treated cells.

**Discussion:** The results of this study suggest the establishment of a probable anti-inflammatory effect exerted by *E. granulosus* products on the immune response involved in Crohn's disease.

Keywords: Echinococcus granulosus, Crohn's disease, TNF-α, NO, auto-immunity.

**P74**

## Single-cell RNA and TCR sequencing suggests a key role for tissue-infiltrating EOMES⁺Tr1-like cells and their circulating precursors in autoimmune diseases

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EOMES⁺ regulatory T cells produce IL-10 (EOMES⁺Tr1-like cells) and are involved in several immune-mediated diseases. Here, we analyzed single-cell RNA and TCR sequencing datasets of CD4⁺ T cells from patients with autoimmune diseases. Gene set enrichment analysis (GSEA) identified clusters of EOMES⁺Tr1-like cells in multiple sclerosis (MS) and in juvenile idiopathic arthritis (JIA). They were located in the vicinity of cytotoxic effector T-lymphocyte (CD4⁺CTL) clusters in the UMAPs, suggesting that these cytotoxic clusters may represent distantly related differentiation stages. EOMES⁺Tr1-like cells were enriched and clonally expanded in the cerebrospinal fluid (CSF) in MS and in the synovial fluid in JIA. In MS, an additional cytotoxic cluster of GzmK⁺CCR6⁺T cells was identified. This cluster was neither enriched nor clonally expanded in the CSF, displayed a gene signature of pro-inflammatory Th1/17-cells and was located distantly from EOMES⁺Tr1-like.
Unsupervised sub-clustering of Tr1-like cells identified IL7R$^{hi}$GzmK⁺Th1-like cells that expressed memory-associated genes, in contrast to IL7R$^{lo}$Tr1-like effector subclusters. Cells from the same clones showed interactions between these subclusters, with IL7R$^{h}$Th1-like cells being predominantly present in the blood and L7R$^{lo}$Tr1-like cells being enriched in the target tissue. Furthermore, IL7R$^{lo}$Tr1-like effector cells separated into two major subclusters, suggesting divergent fates, sustained also by trajectory analysis: (i) CTLA4⁺Tr1-like cells, characterized by elevated expression of genes regulating IL-10 expression that represented a terminally differentiation stage and (ii) CHI3L2⁺Tr1-like cells that expressed increased levels of cytotoxic mediators and that were predicted to differentiate further to effector CTLs.
Collectively, these observations suggest a key role for EOMES⁺Tr1-cells and their recirculating precursors in autoimmune diseases. Furthermore, they suggest that some Tr1-like cells might be able to differentiate to conventional CTL, and thus to switch back from regulatory to effector functions.

**P75**

## Psoriasis-related miRNAs activate NK cells by a complex pDC/monocyte/NK cell crosstalk

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Exosomal GU-rich microRNAs (GU-miRNAs) activate plasmacytoid dendritic cells (pDCs) by triggering TLR7. In addition, pDCs enhance natural killer (NK) cytotoxicity and tissue damage in lupus erythematosus. Because both pDCs and NK cells also infiltrate psoriatic lesions, we asked whether deregulated miRNA secretion may foster psoriatic tissue damage by activating pDCs and the crosstalk with NK cells.
In psoriatic skin lesions, the most upregulated miRNAs were miR142, miR146a, miR203 and miR574.
These miRNAs were combined to generate miRNA-mix to reproduce a physiological setting of stimulation.
pDCs but not purified NK cells were fully activated by miRNA-mix. By contrast, when miRNA-mix was used to stimulate total PBMCs, NK cells were able to produce IFN-γ and to kill target cells. Co-culture experiments of purified cell populations revealed that both pDCs and monocytes were required for NK cell activation by miRNA-mix. In detail, miRNA-mix triggered a TLR7/8-mediated release of IFN-α, IL-12 and IL-18 which were responsible for licensing NK cell response to miRNAs.
pDC/monocyte-licensing of NK cell, in response to TLR7/8-ligands adds a further level of complexity to innate immune cell crosstalk.
In conclusion, deregulated exosomal miRNAs potentially activate a tissue-damaging innate immune crosstalk in psoriasis and may represent a novel mechanism involved in pathogenesis.



## P76
## Efficacy and safety of mepolizumab 300 mg in eosinophilic granulomatosis with polyangiitis: a meta-analysis of 8 retrospective studies

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**Background:** Eosinophilic granulomatosis with polyangiitis (EGPA) is a systemic vasculitis driven by eosinophilic inflammation, presenting clinical challenges. While corticosteroids and immunosuppressants have traditionally been standard treatment, the advent of targeted biologics like mepolizumab, a monoclonal antibody against interleukin-5, is reshaping therapeutic strategies by directly addressing the eosinophilic driver.
**Methods:** This meta-analysis, conducted per PRISMA guidelines, synthesizes evidence on mepolizumab efficacy and safety in EGPA, pooling data from eight retrospective studies to overcome the limited number of prospective trials. Key outcomes included oral corticosteroid (OCS) reduction, immunosuppressant discontinuation, remission rates, and adverse events (AEs). Meta-regression was used to explored heterogeneity.
**Results:** Among 165 patients receiving mepolizumab 300 mg monthly, findings were compelling. Mepolizumab significantly reduced OCS use (mean reduction −6.78 mg/day; 95% CI, −9.68 to −3.88), with 30.4% achieving complete withdrawal. The pooled remission rate was 54%, while 30.5% discontinued immunosuppressants. Meta-regression identified baseline cardiac involvement, neuropathy, and concurrent immunosuppressants as predictors of reduced OCS-sparing effect. The safety profile was favorable, with only 19.3% experiencing mild to moderate AEs and no discontinuations due to AEs.
**Conclusions:** Mepolizumab emerges as a transformative therapy in EGPA, reducing corticosteroid dependence, enabling remission, and showing excellent safety. Its capacity to limit long-term toxicity of conventional treatments marks a paradigm shift in EGPA management. However, responses may be weaker in patients with cardiac or neurologic involvement, highlighting the need for individualized treatment strategies. These findings support further prospective studies and standardized remission criteria to clarify mepolizumab role in this evolving therapeutic landscape.

## P77
## Unravelling the impact of targeting IL-17RA/IL-17RC in cystic fibrosis during chronic lung infections

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**Objectives:** Cystic fibrosis (CF) is a rare genetic disease that affects several organs, with major complications observed in the lung. In CF lung IL-17 inflammatory cytokines signal through the IL17RA/RC receptor complex play a critical role in pulmonary defence against CF pathogens. Among CF pathogens, *Pseudomonas aeruginosa* and *Mycobacterium abscessus*, are cause of severe chronic pulmonary disease in people with CF (pwCF). Here, we want to unravel the therapeutic potential of targeting the IL-17 to limit immunopathological responses, without altering host defence during chronic infection with CF pathogens.
**Methods:** We infected il17rc$^{-/-}$ mice and wt controls with *P. aeruginosa* or *M. abscessus* strains, exploiting preclinical mouse model of chronic lung infection. In addition, we are performing in vitro infection on primary CF human bronchial epithelial cells under air-liquid condition (CF-HBE) with/without *M. abscessus* infection exploiting single-cell RNA-seq technologies.
**Results:** We observed that mice lacking il17rc display a higher susceptibility to CF pathogens, including *P. aeruginosa* and *M. abscessus*. Moreover, the depletion of il17rc during chronic infection with *P. aeruginosa* did not alter the percentage of neutrophils and CD4$^{+}$IFN$^{+}$ cellular recruitment, while increased levels of CD4$^{+}$IL-17$^{+}$ cells. We also focused our attention on the role of IL-17RC in modulating immunity during *M. abscessus* chronic infection. We determined that il17rc$^{-/-}$ condition display an altered host resistance to *M. abscessus* infection, while increased pathogenic cells (CD4$^{+}$ IL-17$^{+}$) associated with IL-17 mediated response. Additionally, CF-HBE cells treated with or without IL-17RA/RC ligands, during *M. abscessus* infection, is under investigation.

**Conclusion:** Overall, we expect to identify new mechanisms and provide pre-clinical proof for limiting immunopathological processes occurring during CF chronic infections.

**P78**

## Normothermic machine perfusion and inflammatory mediators adsorption in extended criteria kidney transplantation

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**Background:** Kidney transplantation faces challenges due to the shortage of donor organs, leading to the increased use of Extended Criteria Donor (ECD) organs, including those from elderly donors, donors with comorbidities, and those after circulatory death (DCD). This study focuses on the application of the PerLife system, integrated with the PerSorb device, to modulate inflammatory mediators during normothermic machine perfusion (NMP) of an ECD kidney. The PerSorb device, designed to adsorb inflammatory molecules, demonstrated effective removal of cytokines during the perfusion process.

**Methods:** An ECD donor kidney underwent 320 minutes of normothermic perfusion, allowing detailed organ viability assessments and cytokine modulation, after a total static cold storage time of 715 minutes. To characterize the effectiveness and efficiency of adsorption, perfusate samples were collected, after perfusion parameters stabilization, pre-and post the adsorption cartridge at the following time points: 60 minutes after the perfusion start (T1), 120 minutes after the perfusion start (T2), 320 minutes after the perfusion start (T end), for perfusion dynamics (pressures, flows, vascular resistances, temperature); perfusion solution composition stability for biomolecular and proteomic examination; oxygenation parameters, and lactate levels; urine output.

**Results:** A significant volume of urine output and successful post-transplantation outcomes, with no delayed graft function, highlight the efficacy of this approach. Additionally, the adsorption of inflammatory cytokines was characterized by concentration-dependent removal, suggesting a balanced modulation of both pro- and anti-inflammatory response.

**Conclusions:** The integration of PerSorb into the perfusion process might offer a promising option for evaluating organ viability, and theoretically mitigating ischemia-reperfusion injury. Further studies are needed to validate these findings and explore the long-term clinical impact of this approach on graft survival and function.

**P79**

## Dysregulated cytokine profiles associated with interleukin-17A suppression in B-cell acute lymphoblastic leukemia

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B-cell acute lymphoblastic leukemia (B-ALL) is characterized by the proliferation of immature B-cell precursors arrested at the pre-B-cell receptor checkpoint. The tumor microenvironment in B-ALL comprises a complex network of immune cells and a dynamic cytokine milieu, which play a pivotal role in disease progression and prognosis. Although altered cytokine profiles have been reported in patients with B-ALL, findings remain inconsistent. This study aimed to investigate cytokine profiles in B-ALL patients of African ancestry and to explore their associations with clinical parameters, including liver function, to gain deeper insights into the mechanisms of immune dysregulation in this malignancy.

This cross-sectional study was conducted at Windhoek Central Hospital and Katutura State Hospital in Namibia. Plasma levels of Th1/Th2/Th17 cytokines (IL-2, IL-4, IL-6, IL-10, TNF-$\alpha$, IFN-$\gamma$, and IL-17A) were quantified using a cytometric bead array and analyzed via flow cytometry. Statistical analyses included Mann-Whitney U tests, Student's t-tests, and multiple linear regression to adjust for confounders. Correlation analyses examined cytokine associations with clinical parameters.

We enrolled B-ALL patients (n = 18) and age- and sex-matched healthy controls (n = 18). IL-17A levels were significantly lower in patients with B-ALL compared to healthy controls ($p = 0.02$), while other cytokines showed no significant differences. The reduced levels of IL-17A remained significant ($p = 0.01$) after adjusting for age, sex, and RBC count. Moreover, IL-17A levels were significantly and negatively correlated with serum aspartate aminotransferase (S-AST) levels ($r = -0.62$, $p < 0.001$). Additionally, TNF-$\alpha$ levels showed a significant negative correlation with total bilirubin ($r = -0.60$, $p = 0.01$), suggesting an interaction between immune dysregulation



and altered hepatic function.
The observed reduction in IL-17A in patients with B-ALL highlights its potential value as a biomarker for immune dysfunction. Associations between cytokine levels and liver function markers suggest broader systemic effects of immune alterations in B-ALL.

## DENDRITIC CELLS

### P80
### Multimodal analysis of tissue-resident dendritic cells reveals the immunosuppressive role of DCs type 3 in human NSCLC and a mouse lung tumor model

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Conventional dendritic cells (cDCs) play a pivotal role in activating adaptive immune responses against infectious diseases and impacting cancer progression. cDCs include cDC1s and cDC2s, the latter comprising DC2s (subdivided into DC2As and DC2Bs) and DC3s. DC3s have been implicated in chronic inflammatory conditions such as lupus, psoriasis, and severe COVID-19. Intratumoral cDCs correlate with responsiveness to immune checkpoint blockade (ICB) therapies.
Inflammatory DCs were detected in the ascitic fluid of ovarian or breast tumor patients. DCs expressing CD14 and CD1c - potentially representing the DC3 subtype - identified in the tumor microenvironment (TME) of melanoma patients exhibited immunosuppressive activity.
Given this potential immunosuppressive role, DC3s may contribute to resistance mechanisms hampering the long-term efficacy of ICB therapies in cancer. Non-small cell lung cancer (NSCLC) offers ideal settings to investigate the role of DC3s in tumor progression. NSCLC patients initially respond well to ICB but develop resistance, leading to poor long-term prognosis.
Leveraging tissue multiplexing techniques, CITE-seq mouse datasets, and publicly available scRNA-seq datasets, we discovered that DC3s expand in early stages of NSCLC patients as well as in preclinical mouse models of NSCLC. The DC3s showed a prominent intratumoral localization and were the main T cell interacting tissue DCs. Functionally, DC3s displayed enhanced immunosuppressive and reduced antigen-presentation activities compared to DC2Bs. Cross-species alignment analysis using single-cell transcriptomic data of human and mouse lung validated the markers employed to distinguish murine DC3s from DC2Bs and highlighter their functional conservation.
In summary, this work provides compelling evidence of the existence of human and mouse tissue DC3s in lungs and unveils their pro-tumorigenic function. Single-cell analyses, including cross-species alignment, shed new light on the ontogenetic and functional differences between DC2Bs and DC3s, underscoring the heterogeneity of DCs in cancer. These findings have implications for ICB therapies, which could benefit from targeting immunosuppressive DC3s.

### P81
### Tissue-specific imprinting shapes conventional dendritic cell functionality in tumors and non-malignant tissues

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The tumor microenvironment (TME) is the intricate ecosystem of stromal, vascular and immune cells providing cellular and molecular cues for cancer development and escape from immunosurveillance. Therefore, exploring the heterogeneity and functional state of tumor infiltrating leukocytes has led to groundbreaking therapies aimed to unleash latent intratumor immunity. However, these therapies are effective for 'hot' tumors (high T cell infiltration) but fail for 'cold' tumors, which exclude T cells. As such, converting cold tumors into hot is key to eradicate malignancies, yet it remains an unmet clinical need. In this complex scenario, dendritic cells (DCs) are instrumental to coordinate and initiate proficient antitumor T cell response through recruitment, (cross)-priming and co-stimulation. Nevertheless, while DC manipulation holds great potential for rescuing efficient anti-tumor immunity, clinical efforts have proven disappointing. With the aim of unraveling DCs' adaptations within the TME, we performed an in-depth single cell integrated analysis across human tumors, delineating their

functional states and tissue-specific plasticity.
Here, we show that, comparing the transcriptome of cDC subsets in hot and cold tumors, cDC2s had the highest differentially expressed genes (DEGs), indicating strong TME influence. Specifically, in hot tumors, cDC2s exhibited upregulation of genes involved in chemokine-mediated immune cell trafficking and antigen presentation, likely promoting immune cell recruitment. Furthermore, tissue-specific imprinting influences cDC2 plasticity not only within tumors but also in non-malignant tissues, as they were found to have the greatest number of differentially expressed genes (DEGs) among DC subsets when comparing normal tissues, indicating a non-negligible role of tissue imprinting in shaping cDC2 functionality.
Our findings illuminate cDC adaptations in both tumoral and steady state conditions, highlighting their functional plasticity and role in immune cell recruitment, driven by tissue imprinting and paving the way for tailored immunotherapies. Understanding cDC2-TME interactions could enhance therapies and position cDC2s as fire starters for cold tumors.

**P82**
**Unraveling the role of factor VIII in regulating inflammatory responses in dendritic cell subsets**
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For decades, the immune system and coagulation were considered separate processes. However, growing evidences highlight extensive interactions between these two systems. Among coagulation factors, factor VIII (FVIII) is primarily known for its role in hemostasis. Recent studies indicate that it's expressed in non-hepatic cells, including endothelial cells and monocytes, suggesting a potential FVIII function in immune regulation. Dendritic cells (DCs), key orchestrators of the immune response, exist in distinct subsets, including type 1 (cDC1) and type 2 (cDC2) conventional DCs, each with specialized functions in immune surveillance and activation. Despite the recognized involvement of coagulation factors in immune response, the FVIII role in DC biology remains uncharacterized. This study explores FVIII role in DC subsets, investigating its potential involvement in immune regulation and inflammatory responses.
In vitro experiments using FVIII-deficient DC precursor cells revealed no significant defects in the differentiation of cDCs subsets compared to wild-type controls, suggesting that FVIII isn't essential for DC lineage commitment. We then explored FVIII transcriptional modulation in DCs upon exposure to various inflammatory stimuli. Real-time PCR analyses showed that FVIII expression was upregulated by LPS, IL-6, IL-1β, TNF-α, and PGE2, indicating a dynamic regulation under inflammatory conditions. To assess the functional consequences of FVIII deficiency, we analyzed cytokine production in cDCs subsets following stimulation with TLR ligands. FVIII-deficient DCs exhibited an enhanced ability to produce both pro-inflammatory and anti-inflammatory cytokines suggesting a dysregulated immune response. Finally, we investigated the immunomodulatory effects of recombinant FVIII on DC function. Treatment with KOGENATE led to a modest yet consistent reduction in TNF-α and IL-6 production in response to LPS, suggesting an anti-inflammatory effect of FVIII. Our study uncovers a potential FVIII role in DCs, suggesting that FVIII may influence immune homeostasis and inflammation. These findings offer new perspectives for therapeutic strategies targeting both coagulation and immune-mediated disorders.

**P83**
**Unveiling the role of migratory dendritic cells in liver cancer**
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Liver immune milieu maintains a balance between activation against pathogens and tolerance toward microbiota or food-derived antigens. However, this inherent tolerogenicity can impair the development of effective anti-tumor immunity. Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, with most patients ineligible for surgery and resistant to immunotherapy.
The aim of this project is to elucidate the role of various immune cell populations in shaping anti-tumor responses across different liver cancer settings. Given their dual function in initiating immunity and promoting tolerance, we focus on conventional dendritic cells (cDCs), particularly activated/mature cDCs (mDCs). These cells migrate to lymph nodes, cross-present tumor antigens, and secrete IL-12, orchestrating cytotoxic responses. Our findings indicate that mDCs enhance T cell activation in vitro and mediate the transport of tumor material to the draining lymph nodes in vivo. To investi-



gate their role in HCC, we employ two recently developed gene-targeted mouse models that enable mDC tracking and inducible depletion. We have established autochthonous HCC models in which tumors arise through genetic manipulation or carcinogen treatment. Notably, we are assessing how different HCC genetic backgrounds shape the tumor immune infiltrate. In β-catenin-driven HCC, we observed a reduced frequency of interactions between mDCs and other immune cells, along with their complete exclusion from the tumor mass. Furthermore, we are optimizing a combined protein-RNA scope staining for human mDCs in FFPE HCC samples to correlate mDC abundance and localization with clinical outcomes. The insights gained from this work may inform novel strategies to overcome resistance to current therapies, ultimately improving anti-tumor immunity in liver cancer.

## P84
## Natriuretic peptides as novel regulators of dendritic cell-mediated inflammation

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Natriuretic peptides (NPs), including atrial (ANP) and brain (BNP) types, exert pleiotropic effects in regulating immune responses via the natriuretic peptide receptor-1 (NPR-1), expressed across various immune cells. While NPs are established inhibitors of inflammasome activation and IL-1β secretion in human monocytes, their role in dendritic cells (DCs)-key regulators of innate and adaptive immunity-remains unclear.
Inflammasome activation in DCs can yield both protective and detrimental outcomes depending on the context of the disease, suggesting that modulating this pathway could offer a promising pharmacological strategy for controlling immune responses. This study explored the regulation of the NLRP3 inflammasome by NPs in two conventional DC subsets: cDC1 and cDC2. We found that both subsets express basal levels of the NPR-1 receptor, which increase under inflammatory conditions. Additionally, cDCs themselves produce ANP and BNP during inflammation. Although both subsets express basal levels of NLRP3 inflammasome proteins, cDC2 display a more robust NLRP3/IL-1β activation in response to $LPS^{+}ATP$ stimulation compared to cDC1. Notably, the NPs/NPR-1 axis suppresses NLRP3 activation more effectively in the cDC2 subset by acting at translational and post-translational levels.
These findings highlight NPs as a novel mechanism for controlling the inflammatory phenotype of cDCs and underscores NPs/NPR-1 axis as therapeutic target for immune modulation of DC subsets.

## P85
## Exploring reprogramming of type 1 dendritic cells in response to diverse forms of cell death in the tumor microenvironment

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The immune system's ability to detect and eliminate tumors depends critically on how dendritic cells (DCs) process and present tumor-derived antigens. While the importance of DCs in orchestrating antitumor T cell responses is well-established, the influence of tumor cell death modality; whether apoptotic, necroptotic, or cytotoxic T cell-induced-on DC activation remains poorly defined. We hypothesize that the nature of tumor debris actively shapes the functional state of DCs, particularly conventional type 1 DCs (cDC1). We propose that DCs respond differently to distinct forms of tumor cell death, leading to changes in maturation markers, antigen-presenting capacity and immune-regulatory molecules. To mimic the in-vivo process of uptake and presentation by DCs of tumors we developed an in vitro platform that mimics the in vivo crosstalk between lung tumours and cDC1s. We generated lung tumor derived organoids derived from a Kras/p53 cell line (KP) that expresses both a model antigen (OVA) and a pH-resistant fluorescent protein (ZS-Green), allowing simultaneous tracking of antigen and phagocytosis. In parallel, we developed a protocol to generate bona-fide cDC1 in culture. Here, we induced KP cell death using UV and co-cultured dying cells with cDC1s to assess uptake and phenotypic changes in cDC1. We observed engulfment of cancer cell debris by cDC1 and upregulation of costimulatory markers such as CD40 and the regulatory molecule PD-L1 in cDC1 upon co-culture. Ongoing experiments aim to compare the event triggered in cDC1 upon death induced by cytotoxic T cells, chemotherapy, specific drugs inducing cell death. This platform offers a tractable model to investigate the effects of various tumor cell death mechanisms on cDC1 activation,

providing information on immune regulation and antigen presentation. It improves our comprehension of how DC programming in malignancies is influenced by the antigen acquisition situation.

**P86**
**How lung cancer progression impacts the lineage commitment of type 1 conventional dendritic cells in the bone marrow**
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Conventional dendritic cells type 1 (cDC1) are specialized antigen-presenting cells that engulf exogenous antigens from dead cell bodies, process, and present tumour-associated antigens to naïve $CD8^+$ T cells, orchestrating tumour-specific immune responses. Given their fundamental role, preclinical studies have explored their ability to enhance T cell responses, particularly in tumours that lead to T cell exhaustion and are resistant to immune checkpoint inhibitors.
Preliminary data of our lab using KP (KrasG12D/WT; Tp53) mice, a model of non-small cell lung cancer (NSCLC) that recapitulates the human disease, indicate that cDC1 numbers increase in the lungs of tumour-bearing mice during early tumour development but decline at later stages, mirroring observations in human NSCLC. The underlying causes remain unclear – whether due to reduced DCpoiesis in the bone marrow, impaired precursor migration, or increased cDC1 death within tumour tissues. Additionally, their activity in the tumour microenvironment is impaired in terms of antigen uptake, processing, and T cell activation.
We have established the gating strategies to identify pre-DC in the bone marrow and lung tissues in the KP tumor model. Notably, preliminary analyses of bone marrow revealed an increase in pre-cDCs at 4 weeks post-induction (early stage), followed by a sharp decline at 8 weeks (late stage), suggesting that DCpoiesis may be compromised as tumor progresses. In this project we plan to identify the systemic factors that trigger early remodelling to support increased DCpoiesis at early stages and those that disrupt DCpoiesis at late stages. In parallel, we will explore the potential of the growth factor FMS-like tyrosine kinase 3 ligand (Flt3L) to rescue DCpoiesis and antigen presentation.

## IMMUNE RESPONSE TO PATHOGENS

**P88**
**Salivary immune markers to improve diagnosis and prevention of periodontitis**
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Periodontitis (PD) is one of the most widespread chronic inflammatory diseases of the periodontium, the progressive and irreversible destruction of the tooth-supporting structures, ultimately resulting in tooth loss. Although not fully understood, PD is thought to be caused by an imbalance in the homeostasis between the host and the oral microbiota, resulting in a chronic inflammatory response and improper immune defense. Thus, it is essential to identify specific biomarkers for the early detection of PD, as well as for effectively monitoring and managing periodontal patients.
**Methods:** We studied 10 PD patients (stage III/IV), before and after PD treatment, and 16 healthy subjects as controls. We performed proteomic analysis of the saliva, and evaluate the presence of bacteria in saliva, cell-free mitochondrial DNA (cf-mtDNA) as a damage-associated molecular pattern (DAMP) that contributes to innate immune cell activation, as well as the levels of pro- and anti-inflammatory cytokines.
**Results:** We identified 66 proteins expressed at higher levels in PD patients than controls. 26% were immune-related proteins, including immunoglobulins, polymeric Ig receptors, peroxideroxins, inflammasome induced cytokines, and inflammation-related proteins. At baseline, pro-inflammatory cytokines and cf-mtDNA levels were significantly higher in patients with PD than healthy controls. After treatment, cf-mtDNA levels were significantly reduced in patients with PD, reaching levels like controls. The same trend can be observed for the bacterial DNA.
**Conclusions:** Analysis of the salivary proteome revealed a strong link between severe PD and the overexpression of proteins associated with inflam-



mation. High levels of mtDNA during PD can contribute to maintain a pro-inflammatory status in the periodontal pocket. The significative reduction of cf-mtDNA levels after three-months treatment suggests that this could be a reliable marker of inflammation and could correlate with the severity of periodontitis. Additionally, cf-mtDNA could work as a potential biomarker to monitor the disease progression or resolution.

## P89
## Dynamic immunological responses in SARS-CoV-2 infection: cytokines, autoantibodies, and lymphocyte subsets

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**Purpose:** The emergence of SARS-CoV-2 has sparked an urgent need for comprehensive understanding of its immunological implications. This prospective study aimed to delineate the immunological dynamics in SARS-CoV-2-infected patients, focusing on cytokines, autoantibodies, and lymphocyte subsets.

**Methods:** A prospective study was conducted on 109 SARS-CoV-2-infected patients, recruited from October 2021 to April 2022 in Plovdiv, Bulgaria. Peripheral blood samples were collected on days 1 and 14 post-diagnosis for immunological profiling, including cytokine quantification (IL-28A, IL-10, IL-33, sCD40L - ELISA), autoantibody assessment (ANCA-PR3, ANCA-MPO, AECA - ELISA), lymphocyte subset analysis (T, B, NK cell - flow cytometry), and antibody quantification (IgM and IgG - Turbidimetry). Statistical analyses were performed to assess differences in immune parameters.

**Results:** Elevated levels of IL-10, CD40L, and IL-33 were observed on day 0, with significant declines by day 14, particularly in older patients ($p = 0.0136$, $p = 0.0182$, $p = 0.0002$, respectively). Notably, IL-33 levels exhibited significant differences between severe and moderate COVID-19 cases. Anti-endothelial cytoplasmic antibodies were present in 24.7% and 21.9% of cases on days 0 and 14, respectively. Flow cytometric analysis revealed increased total lymphocytes, T- and B-lymphocyte subsets by day 14 in moderate cases. No significant age-related differences were observed in anti-SARS CoV-2 IgM and IgG levels, although a trend towards lower IgG levels was noted in older individuals. Turbidimetric analysis showed lower IgA levels and higher IgM levels on day 14 in the moderate group.

**Conclusion:** This study provides insights into the immunological landscape of SARS-CoV-2 infection, highlighting the dynamic changes in cytokine profiles, autoantibody presence, and lymphocyte subsets. The observed alterations underscore the complex interplay between viral pathogenesis and host immune responses, offering potential avenues for therapeutic interventions and prognostic markers for disease severity.

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## P90
## Single cell immuno-profile of SARS-CoV-2 positive asymptomatic subjects compared to symptomatic COVID-19 patients

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**Background:** SARS-CoV-2 infected patients exhibited variability in disease manifestations, from the severe to the asymptomatic form. We were able to realize a phenotypical and functional comparison between asymptomatic and symptomatic individuals PBMCs, using SARS-CoV-2 infection as model for different response to the same infectious agent.

**Materials:** Six symptomatic and 6 asymptomatic SARS-CoV-2 positive subjects' peripheral blood was collected. Lymphocytes were tested for specific antigen response, scRNA-seq and TCR/BCR sequencing analysis. Sera were screened for 40 soluble factors via LUMINEX.

**Results:** Symptomatic transcriptomic profile is enriched in markers of cytotoxic components (NK, CTLs) and their pathways of activation (IFN-γ, TNF-α signaling), while the asymptomatyc's T cell compartment is enriched in $CD4^+$ cells than $CD8^+$, more naive than effector for both and globally enriched in genes for immune regulation and quiescence (CD127 high, TIM3, ICOS, PDCD1). The most expanded clones of the CD8 TCR repertoire belong to the symptomatic side. $CD4^+$ T cells TCRs display a comparable variability and expansion between the two groups, with some clones exclusive for one or the other cohort. The B cell cluster is larger in the symptomatic patients, as are the most expanded B cell clones. Flow cytometry evidences B and T an-

tigen specific cells are comparable between the two groups. Interestingly, while gold indicators for systemic inflammation (D-dimer, PCR, LDH) are sharply higher in the symptomatic group, the soluble factors panel shows significant differences only for CXCL13 and IL-6 levels, consistently with existing literature.
**Conclusions:** This immuno-profiling displays an enhanced activation and clonal expansion of the cytotoxic compartment in the symptomatic group, suggesting the aggressive virus counteracting by immunity can also result in severe manifestation. The naive profile of $CD8^+$ and the $CD4^+$ T cell signature is more represented in the asymptomatic, where a regulatory trend is displayed, perhaps indicating a leaner resolution of the infection.

## P93
## Evaluation of the cross-reactivity of circulating $CD4^+$ T lymphocytes in SARS-CoV-2-positive individuals to different viral variants

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**Introduction:** SARS-CoV-2, the virus responsible for the COVID-19 pandemic, is a member of the coronavirus family. This positive single-stranded RNA ($^+$ssRNA) virus encodes various proteins, including the spike protein, which is essential for the virus's ability to enter host cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor. The rapid global spread of the virus has led to the emergence of mutations, particularly in the spike protein, resulting in new variants such as Alpha, Beta, Gamma, Delta, and Omicron. These variants have garnered significant interest due to their increased transmissibility, enhanced virulence, and reduced effectiveness of existing treatments and vaccines.
**Objectives:** This study aims to assess whether circulating $CD4^+$ T lymphocytes from SARS-CoV-2-positive subjects recognize and respond to different viral variants, and to determine if prior infection or vaccination has generated specific immunological memory capable of reacting to new variants.
**Methods:** The study involved 46 patients (11 Alpha, 11 Delta, 13 Omicron BA.1, and 11 Omicron BA.5) categorized by the variant they contracted. After ex vivo antigen-specific stimulation with peptide pools corresponding to each variant, peripheral blood mononuclear cells were analyzed by flow cytometry to evaluate the frequency of activated $CD4^+$ T lymphocytes producing at least three cytokines (IFN-$\gamma$, TNF-$\alpha$, IL-2).
**Results:** No significant differences were observed in the percentages of activated ($CD154^+Ck^+$) $CD4^+$ T lymphocytes among patients in the same variant group. However, it is possible to appreciate the reactivity of SARS-CoV-2-specific $CD4^+$ T lymphocytes against the strain encountered during infection phase and the Omicron BA.1 and BA.5 variants.
**Conclusion:** The findings suggest that $CD4^+$ T lymphocytes induced by prior infection or vaccination recognize conserved regions of the spike protein, and mutations in the variants do not affect antigen recognition. This confirms that SARS-CoV-2-specific $CD4^+$ T cells can activate upon encountering the spike protein, regardless of the variant.

## P94
## Host immunity in schistosomiasis: functional profiling of IL-$22^+$ T cell clones reveals a regulatory axis in macrophage polarization and fibrogenesis

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**Background:** Schistosoma mansoni infection elicits a complex immune response characterized by progressive Th2 polarization to parasite egg antigens, contributing to tissue remodeling and hepatic fibrosis. While effector mechanisms driving granuloma formation have been partially described, less is known about the contribution of other T cell subsets that might counterbalance this process.
**Aim:** To investigate phenotypic and functional characteristics of T cells in S. mansoni-infected individuals, and elucidate their role in modulating innate immune responses and liver fibrogenesis.
**Methods:** PBMCs from patients with confirmed infection were stimulated with soluble egg antigen (SEA). After 5 days, antigen-specific proliferation was assessed by Click-it assay. SEA-responsive T cells were cloned and characterized by cytokine production. To explore cytokine impact in fibrosis, human macrophages (M0, M2) and hepatic stel-

late cells were treated with recombinant IL-13 and/or IL-22. Marker expression and signaling in macrophages were assessed by flow cytometry and western blot. In stellate cells, effects on proliferation and collagen I/III production were evaluated.
**Results:** Analysis of 121 SEA-specific T cell clones revealed a dominant Th2 profile, with all clones producing high IL-13. Some also secreted IFN-γ (n=33) or IL-10 (n=38). Notably, 51 clones co-expressed IL-22 and IL-13, identifying a subset potentially involved in regulation of the fibrotic process. IL-22R was expressed on M0 and M2 macrophages. IL-22 activated p38 MAPK, STAT3, and STAT5, downregulating M2 markers CD163 and CD200R in IL-13-conditioned macrophages. In stellate cells, IL-22 reduced IL-13-induced collagen I/III production and limited their proliferation.
**Conclusions:** IL-22$^+$ T cells modulate immune responses in schistosomiasis by reprogramming macrophages and dampening stromal activation. This regulatory axis may restrain parasite-induced pathology and guide new immunotherapies for helminthic liver disease.

**P95**
**Functional epigenetic remodeling of dysfunctional CD8+ T cells during chronic HBV infection**
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Hepatitis B virus (HBV) is an hepatotropic virus that can establish chronic infection (CHB), ultimately leading to severe liver diseases, including cirrhosis and hepatocellular carcinoma. Worldwide, more than 250 million people are chronically affected by this condition, which remains incurable. The immune response to HBV is predominantly mediated by adoptive immunity, especially CD8+ effector T cells. However, during CHB, antigen-specific CD8+ T cells become dysfunctional, resulting in a persistent viral replication. Therefore, unveiling potential strategies to reinvigorate effector T cells is of considerable interest in the context of HBV treatment.
Emerging evidence from other chronic infections suggests that epigenetic modifications contribute to the maintenance of dysfunctional T cells. Within this framework, the impact of chromatin state alterations and transposable elements activity, especially LINE1 elements, on CD8+ T cells during CHB remains unexplored. To investigate these mechanisms, we employed a well-established model in which HBV-specific CD8+ T cells are adoptively transferred into HBV transgenic mice, where they recognize antigens present on hepatocytes, thus acquiring a state of immune dysfunction typical of CHB.
Our preliminary data suggest that epigenetic remodeling, mainly driven by LINE1 elements, plays a pivotal role in the establishment and maintenance of CD8+ T cell dysfunction in CHB. Indeed, bioinformatic analyses demonstrated that LINE1 elements expression positively correlates with CD8+ T cells dysfunction. In addition, ex vivo treatment of dysfunctional intrahepatic CD8+ T cells with antisense oligonucleotides, specific for murine LINE1 elements, was able to revert their dysfunctional phenotypes, inducing an upregulation of activation markers and antiviral cytokines.
Taken together, these findings highlight the previously unrecognized relevance of LINE1 elements and epigenetic remodeling on CD8+ T cell dysfunction, thereby elucidating a novel mechanism of immune regulation in CHB and opening new opportunities for therapeutic intervention.

## MAST CELLS AND GRANULOCYTES

**P96**
**Single-cell transcriptomic profiling of mast cells in a mouse model of colorectal cancer**
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**Introduction:** Mast cells (MCs) derive from yolk sac and hematopoietic stem cells and terminally differentiate in peripheral tissues. Two main subsets of mature MCs have been described so far: mucosal MCs located in lungs and gastrointestinal epithelia, expressing the mast cell proteases mMCP1 and mMCP2 and connective tissue MCs that are present in intestinal submucosa and peritoneum and express mMCP4-7. In our previous study, we have demonstrated that connective-tissue like MCs accumulate in tumor masses of a conventional colitis-induced colorectal cancer (CRC) mouse model and produce high amounts of the proinflammatory

cytokines. However, their involvement in CRC progression remains poorly investigated. The aim of this study was to identify and characterize novel MC subsets involved in CRC progression.

**Results:** To this aim we used a conventional colitis-induced CRC mouse model (AOM/DSS) and performed single cell RNA-sequencing on c-kit$^+$ and FcεRIα$^+$ single and double positive cells, isolated from tumor masses and unaffected adjacent tissue. Bioinformatic analysis revealed 7 distinct MC clusters present in AOM/DSS treated mice with a heterogeneity in protease expression and different signaling pathways activated. Among them, we have identified MC precursors exclusively present in unaffected tissue and a subset of MCs (cluster 0) highly enriched in tumor lesions that displays a connective phenotype (mMCP6$^+$). Cluster 0 is also characterized by pathways able to regulate a pro-inflammatory response and extracellular matrix disassembly, suggesting a pivotal role in tumor progression.

**Conclusion:** In this study, we identified distinct clusters of intestinal MCs that differ in their maturation states, phenotypes, and signaling pathways in a mouse model of AOM-DSS revealing a previously unrecognized heterogeneity among colonic MCs during CRC progression.

## P97
## Dissecting the role of mast cells in colorectal cancer progression

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**Background:** Mast cells (MCs) are tissue-resident granulated innate immune cells, particularly abundant in skin, lung and gastrointestinal tract. MCs are mainly known for their involvement in IgE-mediated allergic disorders, however their role as double-edged sword in cancer has emerged. Our previous results showed accumulation of activated MCs in a murine model of colitis-induced colorectal cancer (CRC), but MC role in the progression of CRC remains unclear.

**Purpose:** The aim of this study is to dissect the role of MCs during CRC progression by employing MC-deficient murine models.

**Results:** We have initially used a murine model of colitis-induced CRC (AOM/DSS) before and after the intraperitoneal injection of a monoclonal anti-mouse c-kit antibody, known to deplete MCs. MC depletion was accompanied by a milder weight loss and a strong reduction in number of adenomas compared to control mice. Since c-kit is also expressed on colonic tumors, the neutralization of SCF/c-kit axis can be directly responsible for the inhibition of tumor growth. Thus, we generated a constitutive MC-deficient murine model ("Hello Kitty" Cpa3-Cre; Mcl-1$^{fl/fl}$ mice) using Cre/LoxP technology. We first confirmed the depletion of MCs by flow cytometry and immunofluorescence staining and then, we treated Hello Kitty (Cpa3-Cre; Mcl-1$^{fl/fl}$) and control mice (Mcl-1$^{fl/fl}$) with AOM/DSS to induce the onset of CRC. Although we didn't observe any significant difference in tumor burden, a reduction of T cells and neutrophils and a concomitant strong increase of B cells was observed in tumor masses of Hello Kitty mice compared to control mice.

**Conclusions:** Our previous data support a role for MCs in favouring tumor progression. Using the "Hello Kitty" model, we evidenced changes in the composition of immune infiltrate, but further investigations are needed to clarify whether and how a crosstalk between MCs and other immune cells create an environment that contributes to tumor progression.

## P98
## Characterization of interferon dependent lncRNAs in blood neutrophils from chronic obstructive pulmonary disease patients

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Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterized by local and systemic manifestations. COPD is also recognised as a chronic inflammatory disorder. COPD etiology is associated with the complex interaction between environmental and genetic determinants. Numerous genes are involved in the pathogenic process of this disease; among them, long non-coding RNAs have been reported to be involved in COPD molecular mechanisms and classified as potential biomarkers for early diagnosis. This research aims to identify lncRNAs in circulating neutrophils that are reported to play a crucial role in prolonging systemic inflammation.
To this aim, freshly purified blood neutrophils from 48 COPD patients and 45 age and sex-matched



controls were subjected to RNA sequencing analysis. Transcriptomic analysis identified 718 differentially expressed genes upregulated in neutrophils from COPD patients vs controls: 626 protein-coding genes and 92 lncRNAs. Gene ontology (GO) analysis on upregulated genes revealed that upregulated genes are enriched in an IFN/viral response.
IFN signaling is particularly interesting since COPD patients display an increase in IFN response and increased serum IFN levels during exacerbation. On these bases, the sets of genes annotated in the hallmark "IFN alpha" and "IFN gamma" were retrieved from the Molecular Signature Database (MSigDd), and the variation of gene signature associated with each hallmark was evaluated in neutrophils' transcriptome profiles. Interestingly, data showed a significant ($p < 0.05$) increase in the GSVA score in neutrophils from COPD vs controls. Co-expression network analysis (WCGNA R-package) of neutrophil transcriptome led to the identification of 22 modules. GO analysis of all the 22 modules identified one module significantly associated with the IFN response. Five lncRNAs characterize the IFN module. To test the potential role of each identified lncRNA in the IFN response, loss-of-function experiments are currently carried out in HL60 neutrophil-like cell lines and freshly isolated neutrophils.

**P99**

### Investigating mast cells in high-grade serous ovarian cancer from tissue organization to functional behavior

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High-grade serous ovarian cancer (HGSOC) is often diagnosed at advanced stages due to the absence of specific symptoms. In recent years, the impact of tumor microenvironment (TME), particularly the immune components (TIME), has gained attention in disease progression and treatment response. Among the various components of TIME, mast cells (MCs) are also found and beyond their role in allergic responses, they have also been investigated in the tumoral context. However, their function remains poorly understood.
To investigate the role of MCs in HGSOC, we performed IHC on ovarian (pre-neoadjuvant chemotherapy, NACT) and omental (post-NACT) tissue sections to visualize resident MCs (tryptase$^+$) and observed a focal-like organization.
On histological sections from the same patients, we then performed IF to assess the potential colocalization of MCs with proinflammatory mediators. Analysis of these experimental results was extended with clinical data to explore possible correlations with patient outcomes.
Following these observations, we next investigated the functional behavior of MCs in vitro. To this end, we conducted co-culture and culture experiments using conditioned medium (CM) from HGSOC cell lines with HMC-1 to evaluate the effects of the tumor on MCs. In both experimental settings, we observed significant activation of MCs (increased LAMP-1 expression). In addition, this activation correlated with increased transcription of proinflammatory cytokine genes.
Additionally, we generated patient-derived organoids (PDOs) to accurately model patients' disease in vitro and set up cocultures with HMC-1 for drug testing to evaluate the role and significance of MCs in the context of ovarian cancer.
Our findings suggest that MCs could play an important role in HGSOC progression and response to chemotherapy, providing new insights into potential therapeutic targets.

## METABOLISM

**P100**

### Circulating immune and metabolomic landscape of multiple sclerosis patients treated with different immunomodulatory therapy

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**Introduction:** Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system, with relapsing-remitting MS (RRMS) accounting for ~85% of cases. Although disease-modifying therapies (DMTs) reduce relapse rates and slow progression, their impact on immune cell metabolism remains incompletely understood.

**Objectives:** To ascertain the effect on immunomet-

abolic properties of T and B cells of patients with RRMS treated with different DMTs.

**Methods:** Single-cell metabolic profiling (scMEP) using a 45-parameter mass cytometry panel and metabolomics assessed immune metabolism in 43 RRMS patients treated with cladribine, dimethyl fumarate (DMF), fingolimod (FTY), interferon-beta, natalizumab, teriflunomide, or anti-CD20 therapies versus 8 age-matched donors.

**Results:** Unsupervised analysis identified four T cell metabolic states: (1) hyperactivated subsets ($CD38^+$, $Ki\text{-}67^+$) with elevated glycolysis, tricarboxylic acid (TCA) cycle, and pentose phosphate pathway (PPP) activity; (2) memory $CD8^+$ T cells reliant on glycolysis, oxidative phosphorylation (OXPHOS), and amino acid metabolism; (3) memory $CD4^+$ T cells enriched in OXPHOS and fatty acid oxidation (FAO); and (4) quiescent naïve T cells with minimal activity beyond ATP5A and GLUT1. FTY reduced the percentage of naïve and activated memory T cell clusters while preserving glycolysis in memory $CD8^+$ subsets, whereas DMF-treated-patients displayed high percentage of quiescent $CD4^+$ cells. B cell profiling revealed 12 clusters into four classes. Anti-CD20 therapies depleted $CD20^+$ naïve B cells, enriching plasmablasts and atypical subsets. DMF broadly suppressed B cell metabolism, while natalizumab and anti-CD20 therapies induced overlapping signatures. FTY induced B cell shifts. Metabolomics revealed FTY upregulated arginine biosynthesis and alanine, aspartate, and glutamate metabolism compared to DMF ($p < 1 \times 10^{-4}$), suggesting shifts in T cell function, nitric oxide synthesis, and energy dynamics.

**Conclusion:** Therapy-specific immunometabolic remodelling occurs in RRMS-patients, providing a framework for biomarker discovery and precision targeting of immune metabolism in MS.

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## P101
## Growth differentiation factor 15 levels in eating disorders patients

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Growth differentiation factor 15 (GDF15), a stress-responsive TGF-β superfamily cytokine, is increasingly recognized biomarker for regulation of metabolism, energy homeostasis, and body weight but few studies have investigated the connection between GDF15 levels and malnutrition whether by excess or by deficiency in patients with eating disorders (EDs). In humans circulating GDF-15 change with age, smoking, drug abuse, pregnancy and metabolic stresses such as adiposity, starvation, mitochondrial dysfunction, diabetes and cancer. Moreover, in the last few years it was found that GDF15 is exercise-induced hepatokine regulated by glucagon and insulin and this led to greater interest. The broad normal range in human under 40 years-old is 200-1200 pg/mL. In this scenario, we aimed to investigate GDF-15 role measuring its serum concentration in EDs patients because is still poorly understood.

Serum GDF15 concentrations were evaluated using a commercially available ELISA kit in 73 healthy controls and 320 EDs patients, divided in six major groups as described in DMS-V: 98 affected by anorexia nervosa (AN) restrictive subtype, 44 by AN purging subtype, 39 by bulimia nervosa (BN), 84 binge eating disorders (BED), 24 by OSFED, 31 by NOSFED.

No statistical differences were observed in AN patients (both restrictive and purging subtypes) vs healthy control group matching by age and sex. On the contrary BED patients showed high levels of circulating GDF-15 probably due to adipose tissue and high inflammation level.

Moreover GDF-15 was correlated with some critical blood values such as hematocrit, red blood cells and cholesterol suggesting once again its possible therapeutic applications.

## P102
## Aging-related lipid dysregulation impairs T lymphocyte function and immune response

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**Introduction:** Advances in medicine and improvements in public health systems have significantly increased life expectancy, leading to a growing elderly population that is more susceptible to age-re-



lated diseases. Elderly individuals exhibit various lipid dysfunctions at both systemic and cellular levels. It is well established that excessive lipid accumulation at the cellular level predisposes cells to apoptosis. However, whether this phenomenon expands to the immune system and determines the loss of immune functionality associated with aging is still unclear.

**Objectives:** In this study, we investigated whether aging is associated with alterations in lipid metabolism, apoptosis susceptibility, and priming capacity in different subsets of $CD4^+$ and $CD8^+$ T lymphocytes and B lymphocytes.

**Methods:** We analyzed peripheral blood samples collected from two independent cohorts of different nationalities and age groups ranging from 18 to 90 years.

**Results:** We observed that aging is associated with an increase in neutral lipid content in various subsets (including naïve and different subset of memory cell) of $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and B lymphocytes. Furthermore, we found that this increase in lymphocyte lipid content is strongly correlated with blood triglyceride levels, suggesting a close relationship between systemic and cellular metabolism. We then assessed apoptosis susceptibility in different T lymphocyte subsets (naïve and memory) by measuring the levels of active Caspase-3 expression. We observed that with aging, T lymphocyte subsets, particularly within the CD8 compartment, become more prone to apoptosis. These alterations lead to a reduced $CD8^+$ T cell priming capacity in healthy older individuals compared to younger subjects.

**Conclusions:** Our results highlight that with aging, the accumulation of lipid metabolic dysfunctions significantly impairs T lymphocyte functionality in elderly individuals. This may in part explain the reduced vaccine efficacy and increased susceptibility to infections and tumors occurring in older age.

## P103
## Age-related metabolic shifts in T cells impact vaccine response: insights into Immunosenescence

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Immunosenescence is an irreversible and progressive deterioration of the immune system caused by the natural aging process. Vaccines remain the most effective tool to protect older adults, but immune dysfunction associated with aging often results in suboptimal responses. This study explores the metabolic alterations in T lymphocytes that underpin these age-related differences in vaccine responsiveness, with a particular focus on $CD4^+$ and $CD8^+$ T cell subsets.

We analysed immune metabolic profiles in 43 individuals (aged 25-92 years) of healthy donors, stratified into young (YA, 25-49 years), middle-aged (MA, 50-65 years), and older adults (OA, 65-92 years).

PBMCs were collected and assessed via flow cytometry-based metabolic assays, analysing mitochondrial mass, membrane potential ($\Delta\Psi m$), reactive oxygen species (ROS) production, and nutrient uptake; additionally, we evaluated key nutrient receptors (LDL-r, CD98, GLUT-1, CD36) at baseline and following activation via TCR ligation to uncover metabolic constraints linked to T cell responsiveness.

Older adults exhibited higher mitochondrial $\Delta\Psi m$, a hallmark of cellular aging and dysfunctional bioenergetics, despite similar mitochondrial mass and ROS levels compared to young adults. Post-activation, naïve $CD4^+$ and $CD8^+$ T cells from older individuals showed reduced expression of amino acid (CD98) and glucose (GLUT-1) receptors, impairing their metabolic adaptability. This suggests that metabolic readiness is a crucial determinant of immune efficacy. In addition, metabolic changes induced upon activation were more pronounced in younger subjects, whereas older adults showed a lower glycolytic switch, favouring oxidative metabolism, which is less efficient for a rapid immune response.

These findings underscore the metabolic constraints of immunosenescence, revealing that age-driven shifts in T cell metabolism contribute to poor immune responses in old age.

**P104**
**Immunometabolic role of Kupffer cell-derived apolipoprotein E**
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Background and Aim: Apolipoprotein E (ApoE) is a key protein involved in lipoprotein metabolism. While hepatocytes are the primary source of ApoE, myeloid cells - macrophages, as well as Kupffer cells (KCs, liver-resident macrophages) - also contribute to ApoE production. We previously showed that bone marrow transplantation from WT to ApoE KO animals results in normalization of KO plasma lipid levels, with a cholesterol profile resembling that of WT mice. This prompted us to further investigate the contribution of KC-derived ApoE in modulating the immunometabolic response in experimental models.

Methods: Kupffer cell-specific ApoE KO mice were generated by crossing ApoE flox/flox mice with Clec4f-Cre (ApoE KC-KO) mice. Mice were fed a standard chow diet for 12 weeks, then poloxamer assay, FPLC, western blot (WB), 2D-electrophoresis and flow cytometry analyses were performed to characterize their metabolic and immune profiles.

Results: The percentage of KCs and the ratio of pro-inflammatory to anti-inflammatory subsets were not affected by the absence of KC-ApoE, and no other changes in the liver and circulation were reported in immune cell distribution. However, ApoE KC-KO mice showed reduced circulating ApoE levels (-45.2%, p = 0.0214), suggesting that KCs also contribute to the ApoE plasma pool. To assess whether KC-derived ApoE contribute to the total amount of lipoproteins ApoE, a WB on HDL plasma fractions was performed and showed a significantly low level of HDL-ApoE in KO mice, confirmed also by 2D-electrophoresis, and was associated with a shift in the distribution of immature pre-beta HDL particles compared to WT (6.5% vs 2.1%, p = 0.0851).

Conclusions: This study shows that beyond the role of hepatocytes in synthesizing ApoE containing lipoproteins, KCs contribute to systemic ApoE profile and more specifically to ApoE present in HDL. These findings provide insights into the cell-specific functions of ApoE in lipid metabolism, although the underlying molecular mechanisms remain to be elucidated.

## T AND B LYMPHOCYTES

**P105**
**The sterol element binding protein 1C (SREBP1c) preserves immunosuppressive function of regulatory T cells by controlling phospholipid metabolism**
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Aim: Cellular metabolism is recognized as hallmark of immune cells physiologic functions. We aimed at investigating the role of SREBP1c in Treg immunometabolism, given its key role in intracellular lipid homeostasis, that contributes to tolerogenic phenotype.

Material and Methods: A detailed immunophenotyping through flow cytometry and metabolic profiling (metabolomics and seahorse analysis) of isolated Tregulatory ($CD4^+CD25^+$, nTreg) and in vitro induced Treg (iTreg) cells were performed together with in vitro and in vivo assays of Treg function from SREBP1c KO and WT littermates. RNAseq and lipidomics was performed on iTreg.

Results: Srebp1c deficiency reduced suppressive (-21%, $p < 0.01$) and increased migratory function ($^+$40%, $p < 0.05$) of nTreg and iTreg. In vivo, KO mice presented reduced circulating and tissues' level of Treg compared to WT mice (-66%, $p < 0.01$) and nTreg from KO mice worsened EAE progression compared to WT nTreg. We addressed impaired Treg function to metabolic alteration due to Srebp1c deficiency: KO iTreg showed an increased glycolytic potential with preserved mitochondrial function coupled to accumulation of glycolytic metabolites and lactate ($^+$20%, $p < 0.01$) and reduced energy charge (-40%, $p < 0.01$). We associated the switch to anaerobic glycolysis in KO Treg to an impaired lipid metabolism, that was confirmed by RNAseq, that showed a downregulation of lipid metabolic pathways while glycolysis was upregulated and impaired tolerogenic response of KO Treg. In parallel, lipidomics revealed an impaired abundance of lipid species, among all triglycerides (TG), lysophosphatidylcholines (LPC) and phosphatidylcholine (PC), that were associated to a defect in adenosine receptors (ADORA 2A and 2B) pathways. More specifically, KO Treg presented a reduced expression of



ADORA2B receptor, while the expression of phospholipase A2 (PLA2), involved in LPC production, was increased compared to WT cells an effect reverted by in vitro PLA2 inhibition.

Conclusion: By controlling phospholipid metabolism, SREBP1c restrains glycolysis and preserves lipid metabolism and acts as a crucial checkpoint for the immunometabolic suppressive response of Tregs.

## P106
## Polo-like kinase 1 orchestrates cytoskeletal remodeling to promote cytotoxic T lymphocyte migration

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Cytotoxic T lymphocytes (CTLs) play a key role in the elimination of aberrant, tumorigenic or infected cells by recognizing specific antigens and assembling immune synapses (IS) with their targets. The IS acts as a signaling and secretory domain through which CTLs release lytic granules to mediate target cell killing. Effective CTL function requires not only IS assembly, but also efficient migration across tissues to interact with target cells. Migration enables CTLs to move through peripheral tissues, infiltrate tumors, and reach infection sites, ensuring a rapid and effective immune response.
We have recently implicated polo-like kinase 1 (PLK1), a well-known mitotic regulator, in IS assembly. PLK1 promotes transient CTL polarization by regulating the reorientation of the centrosome and microtubule cytoskeleton toward the IS, a process required for lytic granule release. Given its established role in cell migration in other cell types, we investigated whether PLK1 also regulates CTL motility. We demonstrated that PLK1 inhibition impairs CTL migration induced by the C-X-C motif chemokine ligand 12 (CXCL12). PLK1 is phosphorylated on serine 137 upon CXCL12 stimulation and is required for chemokine-induced CTL polarization. Treatment with the PLK1-specific pharmacological inhibitor BI2536 alters both actin and microtubule cytoskeleton dynamics, further supporting its role in CTL migration.
These results demonstrate that PLK1 is critical for CTL migration and indicate that PLK1 inhibition may compromise immune surveillance, potentially facilitating tumor progression and infection persistence.

## P107
## High-grade serous ovarian cancer: characterizing B cells in tumor microenvironment and peripheral blood for personalized medicine

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High-grade serous ovarian cancer (HGSOC), the most lethal gynecological epithelial disease, is diagnosed late-stage in 70% of cases due to late symptoms onset. Immunotherapy has limited benefits, as patients either fail to respond or eventually relapse after an initial improvement.
A thorough understanding of the tumor immune microenvironment (TIME) composition and function is essential for developing effective personalized therapies. To this end, we optimized a multiparametric flow cytometry panel to immunophenotype B cell subpopulations in the TIME and peripheral blood (PB), as their role has not been clarified yet.
Flow cytometry was used to analyze peripheral blood mononuclear cells (PBMCs) as well as cells from peritoneal biopsies of HGSOC patients (FIGO stage III-IV) and healthy controls. Peritoneal biopsies from women undergoing prophylactic oophorosalpingectomy due to genetic predisposition to ovarian cancer were used as controls.
TIME analysis of patients at different FIGO stages revealed high inter-patient variability in $CD19^+$ B lymphocytes, as well as their subpopulations ($CD27^-$/$IgD^-$ double negative, $CD27^-/IgD^+$ naïve, CD27high/CD38high antibody-secreting, and $CD27^+$/CD38-/low memory B cells).
Also, a differential PB subpopulations distribution emerged between controls and patients, with the latter exhibiting higher B cell activity (CD71 positivity). Finally, a general subpopulation redistribution of circulating B cells and their subsets before and after neoadjuvant chemotherapy (NACT) was observed.
To the best of our knowledge, our study is the first extensive flow cytometry characterization of B cell subsets within the TME of HGSOC patients. The evidence in our results could highlight a correlation with the clinical outcome of patients and allow us to identify biomarkers to distinguish patient responsiveness to therapy and relapses.

**P108**

## Immunophenotypic imbalance and metabolic dysfunction of tumor-infiltrating B cells in colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer globally. Conventional treatments, such as chemotherapy, can be combined with emerging immunotherapies, including immune checkpoint blockade-based therapies. However, only specific patient cohorts with particular tumor profiles benefit from these treatments, highlighting the need for more inclusive therapeutic strategies. Therefore, studying the CRC tumor microenvironment (TME) is currently a key focus. While tumor-infiltrating B-cells are consistently present in the TME, their clinical significance has been less explored compared to T cells.

Our study aimed to investigate the B-cell compartment within the CRC TME, both through analysis of human CRC biopsies and in vitro experiments. Analyses of CRC biopsies revealed that tumor-infiltrating B-cells exhibited a diminished response to differentiation stimuli and were enriched in a subpopulation characterized by the absence of surface IgD and CD27, termed double-negative (DN) B-cells (IgD-CD27-), typically representing either B-cell precursors or exhausted B-cells. Notably, we observed a distinct immunoglobulin profile in tumor-infiltrating DN B-cells compared to normal tissue, with a decrease in $IgA^+$ DN B-cells and an increase in $IgG^+$ DN B-cells.

Further investigation showed that these tumor-infiltrating DN B-cells exhibited very low metabolic activity, with levels similar to naïve B-cells, which are known to be metabolically quiescent. To explore the molecular mechanisms behind this low metabolic activity, we analyzed B-cells in vitro in a murine model. Murine B-cells co-cultured with tumoral intestinal organoids displayed significant impairment in both glycolysis and mitochondrial respiration. These metabolic defects were associated with altered expression of key metabolic enzymes and increased mitochondrial dependence.

In conclusion, our findings reveal an imbalance in tumor-infiltrating B-cell subpopulations compared to normal intestinal tissue and shed light on the metabolic alterations B-cells undergo in CRC TME, suggesting that manipulating B-cell metabolism could offer functional advantages in enhancing the anti-tumor immune response.

**P109**

## T cell-derived IFN-γ suppresses T follicular helper cell differentiation and antibody responses

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$CD4^+$ T cells play a critical role in supporting antiviral humoral and cellular immune responses. Although these responses often coexist, one can sometimes dominate at the expense of the other. For instance, lymphocytic choriomeningitis virus (LCMV) induces a strong cellular immune response but generates a suboptimal neutralizing antibody (nAb) response, hindering viral clearance and enabling persistent infection. Recent findings indicate that this skewed preference toward cellular immunity can be observed also during the differentiation of $CD4^+$ T cells. Specifically, subcutaneous (s.c.) LCMV infection predominantly induces T-$bet^+$ T helper 1 (TH1) differentiation while largely neglecting T follicular helper (TFH) differentiation, a key driver of humoral immunity. Here, we investigated the mechanisms responsible for this impaired TFH differentiation. We found that T-$bet^+$ cells induced by s.c. LCMV infection are heterogeneous and encompass a terminally differentiated TH1 subset expressing Granzyme-B (GzmB) and a Tcf-$1^+$ subset that retains the potential for TFH differentiation but that does not express CXCR5 and other mature TFH markers. Interestingly, T cell-derived IFN-γ facilitates the proliferation of the $GzmB^+$ subset and inhibits Tcf-$1^+$ cells' progression into TFH. Consistently, inhibition of IFN-γ enables robust TFH differentiation, formation of germinal centers and increased antibody production. Of note, the suppression of TFH cells by IFN-γ is not directly mediated through $CD4^+$ T cells but rather involves another cell type, possibly dendrit-



ic cells. Our study provides novel insights into the mechanisms directing early CD4+ T cell polarization and affecting cellular and humoral responses to viruses, laying a foundation for the development of more effective vaccine strategies.

**P110**

**Supramolecular attack particles (SMAPs): a new weapon against virus-infected cells**

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Upon antigen recognition on target cells, cytotoxic T lymphocytes (CTLs) assemble a highly organized cell-to-cell contact, known as the immunological synapse (IS), and promote target cell death through either the early release of lytic granules (LGs) and the subsequent activation of the FAS/FASL pathway. Recently, a third cytotoxic mechanism has been identified involving the release at the IS of particles known as SupraMolecular Attack Particles (SMAPs). SMAPs consist of a central core region, containing cytotoxic molecules such as granzymes (Gzms) and perforin (Prf), and an outer glycoprotein shell, with thrombospondin-1 (TSP-1) identified as the most representative component. Here we characterized the expression and intracellular trafficking of TSP-1, in order to exploit this information for TSP-1 engineering to confer SMAP specificity toward virally infected cells. We determined the expression levels of TSP-1 in CD8+ T lymphocytes during their in vitro differentiation to CTLs and we identified, alongside the canonical full-length TSP-1 protein (FL), a protein form of about 60kDa, that is the only expressed in mature CTLs. This form represents the C-terminal portion of TSP-1, likely resulting from the cleavage of the FL protein. We characterized the intracellular localization of TSP-1 FL compared to the 60kDa C-terminal form, demonstrating that 60kDa TSP-1 contains all molecular determinants for proper localization in LGs. Moreover, to establish whether the intracellular trafficking of 60kDa TSP-1 is dependent on glycosylation, we have identified and mutagenized two putative N-glycosylation sites present in the C-terminal portion of the protein and we demonstrated that 60kDa TSP-1 intracellular trafficking is not dependent on glycosylation. These results show that C-terminal portion of TSP-1 is sufficient for the correct intracellular localization of the protein and open the possibility of engineering 60kDa TSP-1 to confer SMAP specificity against specific target cells.

## MONOCYTES AND MACROPHAGES

**P111**

**RIGENERA® biomaterial functionalized by ZnAl-hydrotalcite and gallic acid prevents osteoclast formation in vitro as potential tool for periodontitis treatment**

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Under physiological conditions, bone homeostasis is strictly controlled by dynamic and balanced communication between bone-forming osteoclasts and bone-resorbing osteoblasts. In particular, osteoclasts are macrophage-derived large multinucleated cells whose excessive activity is one of the prominent features of bone loss in chronic inflammatory diseases, such as periodontitis.
Aiming to develop an effective therapeutic product in the rebalance of bone cell turnover, we have prepared and tested a functionalized biomaterial able to release bioactive molecules that act as antiresorptive agents by regulating osteoclast differentiation or activity. Specifically, the RIGENERA® biomaterial, purchased by Biotec S.r.l. in 1-cm blocks, was coated by Prolabin & Tefarm with ZnAl-hydrotalcite intercalated with gallic acid (RIG_HT-GA), a phenolic compound known for its anti-inflammatory and antioxidant properties. Briefly, as an in vitro model for osteoclast analysis, RAW 264.7 macrophages were differentiated by treatment with receptor activator of nuclear factor kappa-B ligand (RANKL) in combination of releasing medium obtained by previous incubation of RIG_HT-GA in culture medium for 24 hours. Therefore, it was evaluated gallic acid kinetic release, the possible cytotoxicity, and the anti-osteoclastogenic effect of RIG_HT-GA. In particular, we observed a significant decrease in both osteoclast number and osteoclast marker gene expression in a dose-dependent manner compared to the non-functionalized control, suggesting the actual release of active gallic acid from RIGENERA® blocks. In conclusion, we observed that the properties of inducing the rapid formation of vital bone, already possessed by RIGENERA® dental implants, can be enhanced by the additional anti-osteoclastogenic effect of gallic acid in a novel formulation of functionalized biomaterial for the treatment of an inflamed environment such as that of the socket of

an extracted or lost tooth.

**P112**
**Monocyte response to mitochondrial damage-associated molecular pattern in elderly subjects: correlation with chronic inflammation and atherosclerosis**

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Elderly people are characterized by a low-grade, chronic inflammation defined "inflamm-aging". Circulating free mitochondrial DNA (cf-mtDNA) triggers inflammation by acting as a damage-associated molecular pattern (DAMP) and promotes activation of innate immune cells, including monocytes. Cf-mtDNA is sensed via Toll like receptor 9 (TLR9), inducing the expression of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IFNα, while NLRP3 inflammasome senses ox-mtDNA and promotes the maturation of IL-1β and IL-18. However, it remains unclear whether the ability of mtDNA to activate a pro-inflammatory response change with aging, potentially modifies the metabolic features of monocytes.

Methods: We isolated monocytes from blood samples of 15 elderly subjects (> 80 yrs) and 15 sex-matched young controls (< 40 yrs) and treated cells with mtDNA, alone or in combination with LPS. We measured immunometabolic parameters of treated monocytes using Seahorse technology, and confocal microscopy to determine the intracellular distribution of TLR9, its colocalization with cytoplasmic DNA, and NF-kB activation. Droplet digital PCR (ddPCR) assay will be used to quantify mtDNA in plasma.

Results: Monocytes from elderly subjects express TLR9 at intracellular level and it colocalizes with cytoplasmic DNA. Treatment with mtDNA alone did not significantly affect NF-kB activation. The addition of LPS increased the nucleus/cytoplasm ratio of NF-kB, suggesting that mtDNA acts as proinflammatory molecule only in presence of additional pro-inflammatory stimuli. Concerning immunometabolic parameters in the same cells, monocytes from elderly subjects exhibited higher basal respiration compared to young controls without treatment. Nevertheless, monocytes from young donors, but not elderly subjects, showed an enhanced response to mtDNA, resulting in increased respiration.

Conclusions: Monocytes from elderly subjects show impaired immunometabolic responses. The ability of mtDNA to promote an inflammatory response is conserved in elderly subjects. However, mtDNA alone did not trigger significant monocyte activation in these subjects.

**P113**
**WASp and nuclear stability: insights into macrophage inflammation and mechanosensitivity**

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The absence of the actin-nucleating factor WASp (Wiskott-Aldrich syndrome protein) induces a proinflammatory state in myeloid cells, whose underlying causes remain poorly defined. Macrophages are among the most abundant population of myeloid cells and constitute the major source of tissue inflammation, but their relevance in the disease is not well defined. Nuclear envelope rupture may trigger inflammatory activation, and cytoskeletal components are emerging as critical to safeguard nuclear dynamics in immune cells in vivo. Here we aim to analyse whether WASp controls nuclear stability in macrophages. First, we observed altered nuclear membrane components, such as LaminA/C and Emerin, in WASp-deficient macrophages. Prompted by this observation, we moved to use microchannels and vertical confiners to mimic the constrictions that cells encounter within tissues. Interestingly, WASp-deficient macrophages underwent increased nuclear blebbing and ruptures when migrating inside constrictions of different sizes. High resolution-imaging of cells under confinement uncovered a differential response in terms of actin polymerization between WT and WKO cells under mechanical forces. Moreover, inflammatory activation was increased in WASp-null cells. To sum up, this evidence suggests that WASp absence renders the nucleus more prone to deformations and ruptures and leads to an inflammatory phenotype under mechanical challenge which may be relevant for the disease.



# MUCOSAL IMMUNOLOGY

## P114
## Quercetin enriched diet improves tissue regeneration in a model of short bowel syndrome

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Short bowel syndrome (SBS) is a malabsorptive condition mostly caused by massive surgical resection of the small intestine and is associated with significant morbidity and mortality, reduced quality of life, and high healthcare costs.
Inflammatory bowel disease (IBD), represent a potential cause leading to an increased risk of SBS with huge clinical consequences. Currently, there are no biomarkers indicating a higher risk to develop SBS or intestinal deficiency in IBD and the therapeutic strategies represents an unmet need for this chronic debilitating disorder.
The overall goal of our study was the development of new therapeutic strategies for IBD patients with SBS and intestinal deficiency and ameliorate patients' quality of life by reducing SBS-associated dysbiosis and promote tissue regeneration.
We developed a murine model of SBS by surgically explanting 10 cm of small bowel from 12-week old mice. 0.5 cm sample was collected from the proximal, medial and terminal part of the explanted intestinal tissue and stored in RNAlater. Mice were kept under a liquid diet for a week. Starting from the second week after the surgery, mice received a conventional solid chow or a 5% quercetin-enriched chow. We collected mice frailty associated parameters (weight, grip strength, open field test) before the surgery (T0) and monitored these parameters every week for 4 weeks following the surgery (T4). Fecal material was collected at T0 and T4. At T4 mice were sacrificed, the intestinal tract explanted and the anastomosis area collected and stored in RNAlater.
Our data indicate that quercetin enriched diet improved general health conditions and changed SBS associated dysbiosis. Future analysis will shed light to the effect potential effects of quercetin-enriched diet for post operative tissue regeneration.

## P115
## Regulation of intestinal epithelial barrier by the atypical receptor CCRL2

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CCRL2 is a seven-transmembrane receptor structurally related to the atypical chemokine receptor family. It is incapable of signaling through either G proteins or β-arrestins. Furthermore, CCRL2 does not scavenge chemerin, a non-chemokine chemotactic protein and the only recognized ligand for CCRL2. CCRL2 is upregulated by pro-inflammatory stimuli and is expressed by leukocytes and barrier cells. Nevertheless, it plays a role in regulating leukocyte recruitment in various inflammatory conditions and cancer. Our preliminary in vivo data indicate that the genetic deletion of CCRL2 results in a more severe phenotype in DSS-induced colitis in mice. This project aims to investigate the role of CCRL2 in the regulation of intestinal epithelial functions in the context of inflammation and tumorigenesis using mouse models of acute colitis and colorectal cancer (CRC), as well as ex vivo 3D intestinal organoid cultures.
Colonic organoids, derived from WT and CCRL2 KO mice, were cultured in vitro and analyzed for their ability to produce pro-inflammatory cytokines. CCRL2 KO organoids exhibited increased expression of IFN-γ-responsive genes. Additionally, Western blotting experiments revealed that CCRL2 deletion enhances IFN-γ-induced STAT1 phosphorylation and activation. Yellow lucifer uptake assays demonstrated increased permeability in CCRL2 KO organoids, although confocal fluorescence microscopy did not reveal any significant differences in the redistribution of tight junction proteins such as ZO-1.
Overall, these findings suggest that CCRL2 plays a protective role in maintaining intestinal homeostasis and regulating inflammation. The deletion of CCRL2 enhances the expression of IFN-γ-responsive genes by promoting STAT1 activation and increases epithelial barrier permeability.

## OTHER TOPICS

### P116
### Targeting the innate immune system with postbiotics: a strategy for controlling intestinal inflammation

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Innate immunity represents the first line of defence against invading pathogens and tissue damage, with neutrophils being main mediators of the innate immune response and playing a central role in the pathogenesis of inflammatory bowel disease (IBD). We showed that neutrophils contribute to intestinal protection and colitis-associated colorectal cancer prevention through the regulation of the intestinal microbiota and by driving a tissue repair pathway dependent on IL-22 production by γδ T cells.
Gut dysbiosis and immune dysfunction contribute to the pathogenesis of Inflammatory Bowel Syndrome (IBS). Postbiotics are microbial-derived products with immunomodulatory properties both locally and systemically with therapeutic potential for IBS treatment.
We investigated the effect of the Lactobacillus paracasei-derived postbiotic on neutrophils and monocytes isolated from the peripheral blood of healthy donors. Postbiotic exposure induced phenotypic and functional modifications in neutrophils, including the modulation of CXCR1, CXCR2, CD10 expression, suggesting a delayed aging process. Furthermore, the postbiotic prolonged in vitro neutrophil survival, enhanced phagocytic function and triggered the secretion of TNF-α, IL-1β and CXCL8, while reducing the release of the granule-associated protein PTX3 compared to LPS-stimulated neutrophils.
On the other hand, monocytes exposed to the postbiotic exhibited increased surface expression of CD80, HLA-DR and PD-L1, along with enhanced secretion of IL-10 and reduced secretion of IL-12p40. These findings highlight the immunomodulatory effects of the L. paracasei-derived postbiotic on innate immune cells, underscoring its potential as a novel therapeutic strategy for inflammatory diseases.

### P117
### Development of a score derived from full blood count parameters to differentiate individuals with active tuberculosis from latent infected

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In 2023, tuberculosis (TB) caused 1.25 million deaths worldwide, making it the second leading infectious cause of death. Diagnosing TB is challenging, as current tests cannot distinguish between active TB (ATB) and latent TB infection (TBI) leading to potential misdiagnosis or delays in treatment. Given that many individuals with ATB and TBI live in developed countries, there is a need for cost-effective and easy-to-use tools to distinguish between these conditions.
Blood cell counts can indicate TB status, with ATB patients showing higher monocyte counts, lower lymphocyte counts, and increased platelet counts compared to TBI subjects.
Research suggests that the monocyte-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, along with absolute counts of various blood cells, could help develop a low-cost and easy-to-use diagnostic tool to distinguish ATB from TBI among IFN-γ release assay (IGRA)-positive subjects without relying on microbiological tests.
We have developed a TB score based on eleven blood parameters that can identify ATB among IGRA-positive subjects with 93% specificity and 71% sensitivity. This study highlights the potential of using specific blood cell counts and derived ratios as biomarkers for distinguishing between ATB and TBI and can be useful in supporting the physician's decisions about the therapeutic strategies to adopt for IGRA-positive subjects.



# SPATIAL AND SINGLE CELL OMICS

## P118
### Single-cell RNA profiling of mature PMN-MDSCs from cancer patients and G-CSF-treated donors

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Human polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs) consist of a population of circulating low-density neutrophils (LDNs) with immunosuppressive properties. Recent studies from our laboratories and others demonstrated that the mature fraction of PMN-MDSCs exerts stronger immunosuppressive functions than its immature counterpart. More recently, by performing bulk RNA sequencing (RNA-seq) experiments, we identified a distinct gene signature shared by mature PMN-MDSCs (mPMN-MDSCs) isolated from non-small cell lung cancer (NSCLC) and head and neck cancer (HNC) patients and mature immunosuppressive neutrophils from GDs (from both the LDN and NDN fractions; GD mLDNs/mNDNs). The latter have been demonstrated to represent reliable cellular models to study cancer mPMN-MDSCs.
For this study, we performed scRNA-seq experiments of either mPMN-MDSCs from NSCLC patients (n = 5) or mLDNs and mNDNs from GDs (n = 6). By data analysis, we have identified a distinct cell cluster of NSCLC mPMN-MDSCs (including > 60% of the total cells and arbitrarily named NSCLC c6), as well as four discrete cell clusters of GD mPMN-MDSLCs (named GD c4-c7 and including > 80% of the total cells), all of them displaying a significant enrichment of our previously identified "mPMN-MDSCs gene signature". In addition to shared genes and TF regulons, activation of the hypoxia signaling pathway and metabolic reprogramming emerged as the most prominent common transcriptomic features of NSCL c6 and GD c4-c7. Notably, by comparing our mPMN-MDSC scRNA-seq data with those of human TANs publicly available, we further defined the common transcriptomic features and TF regulons that are displayed and activated in both circulating mPMN-MDSCs and TANs. Altogether, our data reveal the existence of previously undescribed transcriptomic similarities between circulating mPMN-MDSCs and TANs.

## P120
### *Roseomonas mucosa* as a potential driver of intestinal fibrosis in Crohn's disease: a multi-omic approach

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**Background:** In Crohn's disease (CD), over 50% of cases develop fibrosis, often resulting in strictures. Despite advances in anti-inflammatory therapies, these complications persist due to chronic inflammation, which causes tissue injury and disrupts mucosal functions. Recent studies highlight microbiota dysbiosis as a potential inflammation-independent driver of fibrosis in CD, but specific microbial signatures across fibrosis stages remain underexplored.

**Methods:** We employed the IBD TaMMA framework for a meta-analysis of RNA-seq data from fibrotic CD-associated and healthy tissues. Fibroblast, endothelial, and epithelial populations were isolated via flow cytometry (FACS) and analyzed at the transcriptomic level. Organoids were derived from epithelial layer. Co-culture experiments were performed to explore cellular-bacterial interactions in fibrosis progression, and fibrosis markers (α-SMA and vimentin and FAP) in fibroblasts were assessed via immunofluorescence (IF).

**Results:** Gene ontology analysis revealed activation of antimicrobial immune processes, suggesting a targeted response to microbiota during fibrosis progression. Microbiota analysis showed an increased abundance of *Roseomonas mucosa* in CD patients compared to controls across multiple cell types (endothelial, epithelial, fibroblast, and immune cells). Moreover, fibroblast co-cultures with lysates of *Roseomonas mucosa* (10 ug/mL) displayed elevated fibrosis markers in IF compared to unstimulated controls.

**Conclusion:** Our findings suggest that *Roseomonas mucosa*, or its products, may modulate the transcriptional state of mucosal cells, including fibroblasts, thereby contributing to intestinal fibrosis. To further elucidate the role of bacterial proteins in the transition from healthy to fibrotic tissue, we are conducting spatial transcriptomics analysis on ileocecal resection tissues, followed by RNA-scope with

bacteria-specific probes. This integrative approach aims to map the temporal progression of fibrosis in relation to microbiota, providing deeper insights into its development.

**P121**
**Single-cell RNA-sequencing analysis to identify immune evasion in different histotypes of ovarian cancer**
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Ovarian cancer is one of the most fatal gynecological malignancies with epithelial ovarian cancer (EOC) accounting for 90% of cases. EOC comprises five major histotypes: high-grade serous (HGS), endometrioid, mucinous, and clear cell carcinoma, each with distinct molecular profiles and clinical behaviors.
EOCs frequently recur, metastasize, and resist treatment. Early stages are often asymptomatic, with symptoms appearing in advance stages. The tumor microenvironment (TME) plays a crucial role in tumor progression and therapies resistance by promoting immunosuppression. In advanced stages, high levels of immunosuppressive cells create a hostile microenvironment that limits immune responses. While immune responses in advanced ovarian cancer are well studied, the TME in early-stage EOC remains poorly understood, particularly regarding transcriptional profiles, and cellular composition across histotypes. This study aims to investigate, at single-cell resolution, the TME of early-stage EOC histotypes.
We performed a scRNA-seq on 5 stage-I EOC tumors isolated from patients who had total ovarian resection. $CD45^+$ immune cells and $CD45^-$ tumor/nonimmune were FACS-sorted and analyzed via Chromium (10× Genomics), along with PBMCs from each patient. To investigate the main features, we employed computational techniques for integration, clustering strategies (Scanpy), differential gene expression analysis (Seurat).
Our data revealed significant TME heterogeneity across histotypes, with a prevalence of lymphoid cells in HGS and endometrioid histotypes, while mucinous and clear cell histotypes exhibited a predominance of myeloid cells. $CD4^+$ T cells were abundant in HGS and endometrioid histotypes, with a high prevalence of helper T cells and Tregs. Notably in HGS, Tregs account for 30-40% of $CD4^+$ T cells, exhibiting functional heterogeneity.
This study highlights the heterogeneity of the TME in early-stage EOC, and the central role of $CD4^+$ Treg cells in immunosuppressive dynamics. The presence of Tregs in early disease stages suggest they may be a potential therapeutic target to prevent tumor progression.

## TRANSLATIONAL IMMUNOLOGY

**P122**
**Germline BRCA1 mutations carriers have enhanced type-2 immunity at baseline and stronger response to flu vaccine**
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The breast cancer gene 1 (BRCA1) is a tumor suppressor involved in genome repair, frequently mutated in triple-negative breast cancer. While the impact of germline BRCA1 mutations (gBRCA1m) in breast epithelium has been extensively studied, less is known about their effect on immune cells. Starting from the serendipitous observation of BRCA1 expression in murine and human activated CD4 T cells, we hypothesized that BRCA1 haploinsufficiency might affect the generation of proper immune responses in gBRCA1m carriers.
To test this hypothesis, 28 healthy gBRCA1m heterozygous carriers and 16 age-matched, non-mutated controls were enrolled and vaccinated against influenza (FLU). Blood samples were collected at day 0, 7 and 28 post-immunization to obtain serum and peripheral blood mononuclear cells (PBMCs). Immunoglobulin (Ig) classes dosage, haemagglutination inhibition (HI) assay and multiplex cytokine profiling were performed on serum. Cytokine production was also studied on thawed PBMCs by intracellular flow cytometry. Immune profiling of PBMCs by spectral flow cytometry is currently ongoing, to characterize T follicular helper and plasmablast response to FLU vaccination.
At baseline, clinical data analysis showed higher incidence of allergies in gBRCA1m carriers, consistent with higher frequency of $IgE^+$ subjects in the gBRCA1 cohort, compared to the controls. Cytokine profiling revealed higher serum levels of IL-10 in gBRCA1m carriers, in particular the $IgE^+$ subjects, as compared to the controls. The $IgE^+$ gBRCA1m sub-



jects also showed lower frequencies of memory CD4 and CD8 T cells, which expressed more IL-4.
Only at twenty-eight days post-immunization, the gBRCA1m carriers showed higher concentration of IL-1β and IL-10 in the serum and better response to vaccination, as evidenced by higher HI titers and seroconversion, compared to the controls.
Our study demonstrates that gBRCA1m carriers are characterized by a higher frequency of $IgE^+$ subjects, an immune phenotype polarized toward IL-4-producing Th2/Tc2 subsets, and higher humoral response to FLU vaccination.

## P123
## Systemic accumulation of highly differentiated T cell effectors and their functional link to vascular inflammation in a mouse model of recurrent psoriasis

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Psoriasis is a chronic inflammatory disease associated with systemic comorbidities, including atherosclerosis. In psoriasis patients, skin-primed memory T cells accumulate in peripheral blood and correlate with the severity of the disease, suggesting that T cells activated in the skin play a role in the development of systemic comorbidities. Here, we aimed to investigate in a mouse model of recurrent psoriasis (i) the establishment of $CD4^+$ and $CD8^+$ T cell memory, (ii) whether chronic psoriasis drives the accumulation of T cells expressing markers of activation and terminal differentiation, and (iii) the potential causal link between highly differentiated T cells and vascular inflammation.
In the mouse model, we observed a progressive accumulation of $CD44^+$ memory T cells in the spleen and a reduction of $CD62L^+CD44^-$ naïve T cells in both $CD4^+$ and $CD8^+$ compartments. We obtained similar results in a cohort of patients with psoriatic disease.
In the murine model, chronic inflammation led to a significant increase in highly differentiated $CD4^+$ TEM and CD8 TCM, as well as terminal effector T cells, $TIM\text{-}3^+PD\text{-}1^+$. Memory T cells exhibited an increased expression of CXCR3, with higher proportion of $CXCR3^+$ $CD4^+$ T cell effectors co-expressing the activation marker 4-1BB. Parallel analysis in aorta samples revealed increased expression of endothelial dysfunction (Icam1, Vcam-1) and vascular inflammation (Mcp-1, Olr1) signature genes and a trend towards increased expression of Cxcl10 encoding the ligand of CXCR3. $LFA\text{-}1^+$ $CXCR3^+$ phenotype was markedly enhanced in memory T cells, particularly in the advanced stages of differentiation, indicating a mechanistic link between highly differentiated T cells and endothelial dysfunction and vascular inflammation. These findings highlight the systemic alterations in T cell phenotypes induced by recurrent psoriasis and provide insights into the mechanism of heightened cardiovascular risk in psoriasis patients.

## P124
## Analysis of the transcriptional profile of circulating neutrophils from chronic obstructive pulmonary disease patients

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Chronic obstructive pulmonary disease (COPD) is a systemic inflammatory disease characterized by dysregulated blood neutrophil numbers, frequencies, and functions. COPD is highly heterogeneous disease, in terms of clinical manifestations, severity, frequency of the exacerbation events, comorbidity. Recently it has been suggested that analysis of genomic and transcriptomic data can be useful in dissecting the heterogeneity of the disease. On these bases, this study aims to identify and characterize groups of COPD patients based on the transcriptional profile of circulating neutrophils.
Neutrophils isolated from whole blood of COPD patients (n = 36) were subjected to RNA-seq analysis. Hierarchical clustering analysis of COPD neutrophils' transcriptome identified three groups of patients differing for clinical parameters and patients' sex. 5,356 genes were found differentially expressed (DEGs) between the three groups. Of the 5357 DEGs 3963 are annotated as protein-coding genes, and 545 as lncRNAs. The DEGs arranged into 4 gene clusters that differentiate male from female patients, as well as male patients that differs by disease severity. Gene ontology enrichment analysis revealed that genes involved in the regulation of the IFN-response are more expressed in male patients than in females.
Long non-coding RNAs (lncRNAs) are known to be important in the regulation of innate immune response and to play a role in COPD pathology. Therefore, we focused on the DE lncRNAs, and identified those correlating with the IFN response.
The expression of 16 lncRNAs significantly correlate with the IFN score; 11 out of 16 lncRNAs are more expressed in male donors whereas 5 lncRNAs are more expressed in female patients. Three lncRNAs (namely BISPR, AL139407.1, and LINC00921) are also

differentially expressed in neutrophils from COPD patients compared to sex- and age-matched controls and correlate with COPD clinical features.

**P125**
**Differential in vitro effects of serum mediators from systemic sclerosis and VEDOSS patients on endothelial cells**

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**Background:** Systemic sclerosis (SSc) is an immune-mediated rheumatic disease characterized by microangiopathy, immune dysregulation, and skin and visceral organ fibrosis. The vascular system appears to be the principal target of SSc pathogenic cascade. Endothelial cell (EC) activation, damage, and death are the principal causes of vascular tone dysfunction, ischemia-reperfusion injury, arterial wall remodeling, and decreased capillary blood flow. Very early diagnosis of SSc (VEDOSS) is critical, as the current diagnostic criteria for identifying patients without skin involvement have a low sensitivity, resulting in a large delay in treatment. Thus, in this study, we sought to analyze the differences between VEDOSS and SSc patients, with an emphasis on the role on endothelial cells.

**Methods:** Serum levels of angiogenic factors (VEGF-A, VEGF-A165b, ANGPT1, AGPT2, TGF-β) were measured by ELISA in 16 patients with SSc, 6 VEDOSS and 22 healthy controls (HCs). Human endothelial umbilical vein cells (HUVECs) were cultured in vitro and treated with sera from SSc patients, VEDOSS, or HCs. We assessed the capability of the sera from the two subgroup of patients to induce HUVEC proliferation, survival, tube formation, wound healing, and endothelial-to-mesenchymal transition (EndoMT) compared to HCs sera.

**Results:** SSc patients display higher circulating levels of angiogenic (VEGF-A, ANGPT1, ANGPT2, TGF-β) factors compared to HCs. VEDOSS showed higher serum levels of ANGPT1, ANGPT2 and TGF-β compared to SSc patients. HUVECs treated with SSc patients sera showed an higher proliferation and survival rate, compared to cells cultured with VEDOSS or HCs sera. experiments are ongoing to evaluate the tube formation capacities, wound healing properties, and EndoMT.

**Conclusions:** our preliminary results suggest that the VEDOSS subset of patients is very different from SSc. Further experiments are ongoing to go deeper inside the mechanisms underlying the differential endothelial behavior in SSc and VEDOSS patients.

**P126**
**Bacterial stimuli trigger orofacial granulomatosis pathomechanism trough activating monocytes and neutrophils**

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**Background:** Orofacial granulomatosis (OFG) is a rare chronic inflammatory disorder characterized by persistent or recurrent swelling of the lips and oral mucosa, accompanied by granulomatous inflammation in the orofacial region with limited effective treatment options available. Emerging evidence suggests an immune dysregulation in the development of OFG. Immune cells, including monocytes and neutrophils (PMNs), are involved in inflammatory diseases by releasing pro-inflammatory and immunomodulatory molecules.

**Methods:** In this study we recruited 11 OFG patients and 11 healthy donors (HDs) age and sex matched. PMNs and monocytes from peripheral blood of OFG patients and HDs were purified and then stimulated with LPS, fMLP and PMA, the activation status (percentage of CD62L- PMNs) were evaluated by flow cytometry. The release of monocytes and PMNs-related mediators were measured by ELISA. In addition serum levels of neutrophil-related mediators (MMP-9, MPO and TNF-α) and NET biomarkers (MPO-DNA complexes and Citrullinated Histone H3 (CitH3)) were evaluated by ELISA.

**Results:** Upon stimulation, OFG-derived monocytes displayed a higher release of pro-inflammatory cytokines (CXCL8/IL-8, IL-6, TNF-α, IL-33) compared to HDs. Conversely, OFG-derived monocytes showed a lower release of anti-inflammatory cytokines (IL-10, IFN-γ) compared to HDs. Upon stimulation, peripheral PMNs from OFG patients released large amounts of TNF-α and MPO compared to HDs. In

addition, OFG-derived PMNs showed high percentages of activated PMNs and increased ROS production compared to HDs. Compared to HDs, OFG patients presented higher serum levels of MMP-9, MPO and TNF-α, together with MPO-DNA complexes and CitH3.

**Conclusions:** These data suggest that in presence of various stimuli, monocytes and PMNs of OFG patients displayed an activated phenotype compared to HDs. Unraveling the interplay between bacterial triggers and immune cell function in OFG will be necessary to elucidate mechanisms driving this complex disease and identify novel therapeutic targets for improved management of OFG patients.

## P127

### Human virtual memory CD8⁺ T cells: the role of cytokines on cell development and functionality

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Virtual memory T cells (TVM) are a subset of TCRαβ⁺CD8⁺ T cells recently identified in mice that, despite lacking prior antigen exposure, express typical markers of conventional memory T cells. They are characterized by a high expression of the transcription factor Eomesodermin (Eomes). TVM cells have been recently described also in human peripheral blood. Similar to their murine counterparts, human TVM express high levels of Eomes. Unlinke murine TVM, they are divided into two subsets characterized by mutually exclusive expression of NKG2A and KIR.

While murine TVM development has been extensively studied, little is known about the differentiation of these cells in humans. Evidence suggests that, in mice, IL-4, IL-15, and type I interferons (IFN-I) may play a role in this process. To investigate the impact of these cytokines on human thymic and peripheral TVM development, we cultured human pediatric thymocytes and adult peripheral blood mononuclear cells (PBMCs) in vitro with or without these cytokines and analyzed their phenotype using a 27-color spectral flow-cytometry panel.

TCRαβ⁺CD8⁺Eomes⁺ cells developed among thymocytes only in the presence of IL-4 or IL-4⁺IL-15 after 9 days of in vitro stimulation. Interestingly, these cells were characterized by a naïve phenotype (CD45RA⁺CD27⁺CCR7⁺), lacking the expression of both NKG2A and KIRs. On the other hand, IL-4⁺IL-15 in vitro-stimulated PBMCs resulted in the expansion of two subsets of TCRαβ⁺CD8⁺Eomes⁺ cells characterized by the expression of either KIRs or NKG2A with a TEMRA (CD45RA⁺CCR7-) and TEM (CD45RA-CCR7-) phenotype respectively. Notably, we observed that the stimulation with IL-15⁺IFN-I is necessary by PBMCs to acquire a cytotoxic-potential, evidenced by a high expression of Granzyme B and Perforin.

Overall, our findings indicate that human TVM differentiation is dependent on IL-4⁺IL-15, while cytotoxic-potential of these cells are sustained by IL-15⁺IFN-I stimulation.

## P128

### Engineered human CD34⁺ hematopoietic stem cells as an innovative approach to modulate the immunoinflammatory response during atherosclerosis

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**Aim:** Immune cells are key players in atherosclerosis development, and the growing recognition of their role, coupled with the availability of targeted biologic therapies, highlight the need of models to translate from animals to human the molecular mechanisms and strategies for atherosclerosis immunotherapies. An immune-metabolic characterization of a novel human-immune system mouse on an atheroprone background is presented.

**Methods:** TKO-L (Rag2KO/IL2rgKO/CD47KO-LDLRKO) pups received a hepatic injection of hCD34 either commercial or iPSCs-derived (250,000-300.000 cells/mouse). Humanized HuTKOL were checked for human cells engraftment at 12 weeks and then fed 12-week high-cholesterol diet to investigate immunometabolic phenotype and atherosclerosis, coupled to scRNAseq on bone marrow.

**Results:** HuTKOL presented human hCD45⁺ (24.83%, SE ± 2.94%) in the circulation after engraftment with commercial hCD34⁺ cells, while it's on going the engraftment with iPSCs-derived CD34⁺.In adult HuTKOL, B cells were the most abundant population at 12 weeks, but they decreased over time, compared to T cells that were the predominant population at 24 weeks, similar to human lymphocyte profile.

hCD45⁺ were also successfully engrafted in the thymus (64.58%), spleen (19.89%), bone marrow (27.54%) and liver (32.94%) and atherogenic diet increased circulating memory CD4 T cells and atherosclerosis antigen-related IgM in both sexes. However, a different trend of circulating hCD45⁺ engraftment has been observed between male and female (M:10.84%, F:34.34%). HuTKOL developed dyslipidemia (plasma cholesterol levels: 1137.43 mg/dl, SE± 45.77) and atherosclerosis (% aortic sinus plaque oc-

clusion: 18.24%, SE ± 0.02%) when fed a high-cholesterol diet, despite to a less extend compared to immunocompetent mice. scRNA sequencing of bone marrow revealed that human cells impact myeloid (monocytes/neutrophils) response.
**Conclusions:** HuTKOL mice show a human-like adaptive immune cell profile offering a valuable model for investigating the immunomodulation in atherosclerosis. Interestingly, our data suggest a role of adaptive cells on myeloid response that could affect atherosclerosis development.

**P129**
**Characterization of drug-specific CD4⁺ T cells in patients with chronic inflammatory diseases**
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**Background:** Biologicals are commonly used to treat chronic inflammatory diseases. Treatment failure is a major limitation for the efficacy of biologicals, which can be caused by the development of anti-drug antibodies. The formation of high-affinity anti-drug antibodies suggests the involvement of specific CD4⁺ T cell responses. However, the CD4⁺ T cell reaction against biologicals remains poorly characterized. Therefore, in this study we analyzed CD4⁺ T cells specific to various biologicals and investigated potential differences of drug-specific CD4⁺ T cells in patients with continued remission versus those with secondary nonresponses or allergic reactions.
**Methods:** Peripheral blood samples were collected at baseline and every eight weeks for one year after starting biological therapy. Drug-specific CD4⁺ T cells were analyzed using Antigen-Reactive T cell Enrichment (ARTE) directly ex vivo from human peripheral blood.
**Results:** Of the analyzed biological agents, the chimeric antibody Infliximab showed significantly higher activation of reactive CD4⁺ T cells compared to other humanized or fully human biological agents. Notably, this response was characterized by a Th1 phenotype with evidence of long-term memory. In patients with secondary nonresponses or allergic reactions Infliximab-specific CD4⁺ T cells showed elevated expression of TNF-α, IFN-γ and IL-21.
**Conclusion:** Infliximab exhibits a higher activation of specific CD4⁺ T cell reactions compared to other biologicals, characterized by a Th1 response with long-term memory capabilities. Infliximab is a chimeric antibody consisting of murine variable regions, which likely contributes to its immunogenicity. The increased production of TNF-α, IFN-γ and IL-21 in patients experiencing secondary nonresponses or allergic reactions highlights the potential role of drug-specific T cells in treatment outcomes and formation of anti-drug antibodies. Overall, our data identifies differences in anti-drug CD4⁺ T cell reactions for different biologicals as well as different treatment outcomes which may contribute to enhance drug efficacy and improve long-term success with immunogenic biologicals.

**P130**
**Modulation of mitochondrial dynamics as a novel strategy to overcome T cell exhaustion in the tumor microenvironment**
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The exhaustion of T lymphocytes is an anergic state that occurs during persistent infections and cancer, where PD-1 is a key regulatory protein. Our previous study demonstrated that PD-1 downregulates the activity of Drp-1, a mitochondrial fission key regulator crucial also in several T lymphocyte physiological processes. Although extremely promising CAR-T therapy efficacy towards solid tumours, including neuroblastoma, is still a “goal to be achieved”. Our aim is to identify a strategy to improve the cytotoxic ability of both tumour infiltrating T- and CAR-T cells and to evade PD-1 induced T-cell exhaustion, by modulating mitochondrial dynamics.
We generated two alternative clones of neuroblastoma redirected GD2+ CAR-T cells that constitutively express mutated forms of mitochondria-shaping proteins to unbalance towards fragmentation the morphology of CAR-T cell mitochondria. We first characterized these new CAR-T, by analysing morphology and functionality of their mitochondria. We then performed functional assays revealing an enhanced cytotoxicity of these CAR-T cells against neuroblastoma spheroids.
Thus, the modulation of mitochondrial dynamics might be a promising therapeutic strategy to improve the efficacy of CAR-T cells against sensible targets. Further experiments will be performed to investigate whether these modified CAR-T cells can



overcome PD-1-dependent exhaustion.

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## TUMOR IMMUNOLOGY

### P131
### Distinctive features of tumor-infiltrating γδ T lymphocytes in human glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and deadly primary brain tumor, with a poor clinical outcome which underscores the urgent need for effective therapies. GBM pathogenesis and progression are influenced by the immune microenvironment; hence, a thorough understanding of the immune response within GBM is crucial to better clarify the mechanisms driving tumor development.
This study investigates the role and dynamics of the interaction between tumor cells and γδ T cells in patients with GBM. Flow cytometry and transcriptomic analysis showed that GBM comprised a highly variable rate of tumor-infiltrating lymphocytes (TILs) and 1.3-1.8% γδ T cells, with the majority expressing Vδ2. A similar pattern was detected in the peripheral blood (PB) of GBM patients.
Most Vδ1 and Vδ2 T cells in GBM patients had an effector memory phenotype in both TILs and PB.
Conversely, in meningioma patients (used as controls), Vδ1 T cells predominated in TILs, while Vδ2 T cells in PB. Phenotypical analysis showed that Vδ1 T cells had a predominant central memory phenotype, both in TILs and PB, while effector memory Vδ2 T cells predominated in TILs, and central and effector memory Vδ2 T cells were equally distributed in PB.
The transcriptomic analysis showed a correlation between the expression of TRGC1 and TRGC2 (marking Vδ2 and Vδ1 T cells, respectively) and immune checkpoint genes (PDCD1, HAVCR2, TIGIT, and LAG3) in GBM. These data were partially confirmed by flow cytometry analysis: Vδ1 and Vδ2 T cells from TILs of GBM patients expressed high levels of NKG2A and PD-1 and TIM-3, which were slightly expressed or absent in TILs from meningioma patients. Conversely, only NKG2A was expressed by PB Vδ1 and Vδ2 T cells in GBM patients.
These findings may help to understand the mechanisms of GBM resistance to immunotherapy and identify new therapeutic strategies.

### P132
### Immunotherapeutic efficacy of discoidin domain receptor 2/type 1 collagen inhibition in high-grade serous carcinoma

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**Purpose:** Immunotherapy is aimed at strengthening the immune system to fight tumours. Unfortunately, high-grade serous ovarian carcinoma (HG-SOC) exhibits low response rates to immune checkpoint blockage due in part to the deposition of dense and reticulated collagen that generates a stromal barrier for the infiltration of tumour-specific T cells. Discoidin domain receptor 2 (DDR2) binds type I collagen (Col1) favouring cancer progression and hampering immunotherapy efficacy in several solid tumours. However, the role of DDR2 in HG-SOC response to immunotherapy remains unknown.

**Methods:** HLA-A2$^+$ OVCAR3 cell line was used as a model of HG-SOC. OVCAR3-specific T cell lines (TOVC) were generated by stimulating PBMCs from HLA-A2$^+$ healthy donors with irradiated OVCAR3 cells in the presence of IL-2 and IL-7. After two rounds of stimulation, the efficacy of DDR2/Col1 inhibition on OVCAR3-specific T cell responses was assessed by using the allosteric inhibitor WRG-28 alone or in combination with bispecific antibodies (BsAbs) targeting CD28 and mucin 1 (MUC1), a tumour associated antigen expressed on the surface

of OVCAR3 cells. Surface activation markers associated with tumour-specific T cells (flow cytometry), cytokine production (ELISA), granzyme B (GRZB) and active caspase 3 (Casp3) levels (confocal microscopy) were analysed.

**Results:** The inhibition of DDR2/Col1 interaction by WRG-28 enhances OVCAR3-specific T cell responses by increasing the surface expression of activation markers and both IFN-γ and GM-CSF production. Moreover WRG-28 treatment potentiates the recruitment and the killing capability of tumour-specific TOVC cells by increasing both GRZB release and Casp3 levels. Interestingly, the inhibition of DDR2/Col1 interaction sensitizes tumour cells to CD28xMUC1 BsAbs.

**Conclusion:** This study identifies DDR2/Col1 interaction as a key immune evasion mechanism in HGSOC. Targeting this pathway with DDR2 inhibitors may overcome the stromal barrier improving T cell infiltration and enhancing the efficacy of immunotherapy based on CD28xMUC1 BsAbs.

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## P133
## The potential of a combination therapy comprising chemotherapy, eganelisib and DNA vaccination to impede the progression of pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related mortality in developed countries and has one of the poorest prognoses among all cancers. A proteomic approach was used to identify alpha-Enolase1 (ENO1) as a PDA-associated antigen. Our previous studies have demonstrated that ENO1 DNA vaccination efficiently prolongs the survival of KPC mice, a genetically engineered model that spontaneously develops PDA, and that this effect is potentiated by the favourable environment elicited by PI3K gamma (PI3Kg) ablation. Notably, PI3Kg is responsible for the increase in the recruitment of myeloid-derived suppressor cells (MDSCs) at tumor sites and fibrotic reaction to tumors.
In this study, we hypothesized that MDSCs targeting via pharmacological inhibition of PI3Kg synergizes with second-generation ENO1 DNA vaccine (ENO3PEP) and chemotherapy to elicit strong and sustained immune response against PDA.
KPC mice were vaccinated with ENO3PEP DNA vaccine at weeks 8, 10, 12 and 14. At week 15, mice were treated with Eganelisib (PI3Kg inhibitor) daily for two weeks and received Folfirinox at weeks 8 and 10. At 18 weeks, mice were sacrificed to analyze pancreatic tumor lesions, metastasis and immune infiltration at tumor site. Spleens and sera were also collected to assess INFgamma-secreting T lymphocytes and antibodies response.
Preliminary data indicate that Eganelisib, both alone and in combination with ENO3PEP DNA vaccine, reduces both tumor lesions and metastasis in lung and liver compared to untreated or Folfirinox-treated mice. Both $CD4^+$ and $CD8^+$ T lymphocytes increase at tumor site in mice receiving the combined therapy, along with a higher amount of specific anti-ENO1 antibodies in the sera of treated mice.
In vivo data suggest that combined therapy exhibits superior efficacy in controlling tumor progression compared to control mice. Further studies are required to provide a more detailed characterization of the antitumor response induced by combined treatment.

## P134
## γδ T cells and immune checkpoints in colorectal cancer: a new avenue for immunotherapy

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Colorectal cancer (CRC) is one of the most prevalent and lethal malignancies worldwide, with limited therapeutic options for patients with microsatellite-stable (MSS) tumors, who respond poorly to immunotherapy. γδ T cells play a crucial role in tumor immunity, even though their contribution to CRC progression and response to immune checkpoint blockade remains largely unexplored. This study investigates the infiltration of γδ T cells in CRC and their expression of immune checkpoint receptors, to assess their potential as therapeutic targets. We performed a bioinformatic analysis of single-cell RNA sequencing data from publicly available datasets using R software, followed by an



ex vivo characterization of γδ T cells in CRC patient samples by flow cytometry. We quantified their abundance in the tumor, healthy tissue and peripheral blood, and evaluated the expression of key immune checkpoint receptors, including PD-1, TIM3, TIGIT, LAG3 and CTLA-4.Our results reveal a significant abundance of γδ T cells, displaying strong cytotoxic potential, in tumor tissue, compared to PB and healthy tissue. Moreover, tumor invasiveness correlates with increased γδ T cell infiltration, suggesting a possible role in disease progression. Importantly, we identified a marked reduction in PD-1⁺ Vδ1 γδ T cells with cytotoxic activity in invasive tumors, which may contribute to the poor efficacy of PD-1/PD-L1 blockade in CRC. These findings highlight the potential of γδ T cell-based strategies in combination with immune checkpoint inhibitors to enhance antitumor responses in CRC, paving the way for novel immunotherapeutic approaches.

## P135
## Shared and distinct alterations in mucosal innate lymphoid cells in patients with inflammatory bowel disease

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Innate lymphoid cells (ILCs) are crucial for maintaining intestinal homeostasis and immune responses. Enriched in mucosal tissues, ILCs maintain barrier integrity and regulate inflammation. Dysregulation of ILCs is linked to inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC). Although ILCs have been studied in IBD, their specific role in disease onset and progression remains unclear. This study aims to analyze the distribution and functional characteristics of ILC1 and ILC3 in IBD patients at disease onset and those with chronic, therapy-resistant inflammation. Mucosal biopsies were collected from three groups: onset IBD patients, therapy-resistant chronic IBD patients, and healthy donors (HD). ILC subsets were analyzed using flow cytometry, confocal microscopy and cytokine production from incubated cell suspensions was evaluated using Luminex assays. Additionally, transcriptomic analysis of online datasets was conducted to assess gene expression changes.
Our findings reveal that both ILC1 and ILC3 (NKp44⁺ and NKp44⁻) subsets were increased in IBD patients compared to HD. However, ILC1 frequency was particularly elevated in onset patients, whereas NKp44⁺ and NKp44⁻ ILC3 were more abundant in the chronic condition.
Functional analysis by intracellular staining demonstrated increased production of IFN-γ and TNF-α by ILC1, as well as IL-17 and IL-22 by ILC3 in both IBD patient groups.
Furthermore, the evaluation of ILC-derived cytokine, chemokines and growth factors exhibited significantly elevated levels of several cytokines, including IL-17, IFN-γ, MIP-1β and TNF-α, compared to HD.
Transcriptomic analysis identified the upregulation of inflammatory and ILC-associated genes: RORA, ITGAL, and LEF1, reinforcing the role of ILCs in sustaining inflammation.
Our findings suggest a dynamic transition from ILC1 to ILC3 predominance during IBD progression, which may contribute to chronic inflammation and treatment resistance. These results highlight the potential of targeting ILC subsets as a novel therapeutic approach for modulating immune responses in IBD.

## P136
## Human lung macrophages and circulating monocytes might contribute to bacterial infection susceptibility and prognosis of lung cancer patients

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Background: Non-small cell lung cancer (NSCLC) patients experience heightened susceptibility to infections, often leading to worse prognoses. *Staphylococcus aureus* (SA), *Streptococcus pneumoniae* (SP), and *Pseudomonas aeruginosa* (PA) are frequently involved in pneumonia development and

stand out as significant contributors to infectious complications in cancer patients. Considering the critical role of human lung macrophages (HLMs) in pulmonary host defence, the aim of this study was to investigate the effect of bacterial stimuli (SA, SP, PA, and LPS) on HLMs isolated from lung tissue of NSCLC patients. Due to difficulty in obtaining HLMs from healthy donors (HDs), we compared the response of monocytes from NSCLC patients and HDs to bacterial stimulation, also evaluating TLR2/4 expression.

**Methods:** HLMs and monocytes were isolated and stimulated with heat-killed bacteria or LPS. Cytokine production (CXCL8, IL-6, TNF-α, IL-1β) was measured by ELISA and PCR. Reactive oxygen species (ROS) production was assessed using a spectrophotometric H2DCFDA assay. TLR2/4 expression was evaluated by flow cytometry.

**Results:** Bacterial stimuli induced cytokine production in HLMs via TLR2/4 activation, but did not promote ROS production. Although NSCLC monocytes exhibited reduced basal cytokine levels compared to HDs, bacterial stimulation induced a significantly greater release of pro-inflammatory cytokines in NSCLC patient monocytes compared to those of HDs. A significantly higher percentage of TLR2/4-positive monocytes was observed in NSCLC patients compared to HDs.

**Conclusion:** These findings suggest that both resident lung macrophages and circulating monocytes contribute to the dysregulated inflammatory landscape observed in NSCLC, potentially predisposing these patients to worse outcomes following bacterial infections. While TLR signaling appears to play a role in this heightened response, further investigation is warranted to fully elucidate the underlying mechanisms and identify therapeutic targets to modulate inflammation in NSCLC.

## P137
## Peripheral blood neutrophils and monocytes differentially predict response to anti-PD-1 and BRAF/MEK inhibitors therapy in stage III melanoma patients

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**Background:** Melanoma of the skin displays a rising incidence over the last years. Immune checkpoint (e.g. anti-PD-1) as well as molecules targeting BRAF/MEK axis have revolutionized the therapeutic approaches to melanoma patients (MPs). PD-L1 is expressed on several immune cells, also on human neutrophils and monocytes, and their role in MPs is largely unknown.

**Methods:** 64 stage III MPs were recruited, 42 patients receiving anti-PD-1 therapy and 22 patients BRAF/MEK inhibitors. 55 healthy controls (HCs), sex- and age-matched, were also recruited. Based on their response to therapy, patients were also divided into Progressive Disease (PD) and No Evidence of Disease (NED) groups. Neutrophils and monocytes were isolated from peripheral blood, before and during the therapy, to evaluate their activation state and PD-L1 expression by flow cytometry. Plasma concentrations of neutrophil-related mediators (MMP-9, MPO, CXCL8/IL-8 and GM-CSF) and NET biomarkers (Citrullinated Histone H3 and MPO-DNA complexes) were measured by ELISA.

**Results:** MPs neutrophils displayed increased percentages of $CD16^+$ $CD62L^-$ cells and increased PD-L1 levels compared to HCs. Peripheral blood monocytes of MPs displayed higher percentage of PD-L1 compared to HCs. In the group of patients receiving anti-PD-1 therapy, the subgroup of PD patients showed higher levels of $CD16^+$ $CD62L^-$ and $PD\text{-}L1^+$ PMNs and $PD\text{-}L1^+$ monocytes compared with the NED group. In addition MPs displayed higher plasma concentrations of neutrophil-related mediators and NET biomarkers compared to HCs.

**Conclusions:** Taken together, our data suggest that peripheral innate immune cells can predict clinical response in stage III MPs undergoing anti-PD-1 immunotherapy. Further investigation is needed to dissect a possible role of these cells in disease and therapy response in patients with III stage melanoma.



## P138
## Multiomics profiling reveals a subset of NKG2A$^+$ Vδ2$^+$ T cells associated to response to PD-1 blockade in non-small cell lung cancer

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Lung cancer is the leading cause of cancer-related mortality worldwide. It is one of the most prevalent malignant epithelial neoplasms, second only to hormone-driven tumors. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all cases, and most patients receive a diagnosis at an advanced stage, with a poor prognosis.
The aim of this study was to characterize γδ T lymphocytes within the tumor microenvironment of NSCLC, assessing their frequency and function in both tumor tissue and peripheral blood. Additionally, we analyzed the phenotypic and transcriptomic changes of these cells before and after treatment with PD-1 inhibitors. The study initially involved a bioinformatics analysis of transcriptomic data obtained through single-cell RNA sequencing (scRNA-seq). These data were subsequently validated ex vivo by flow cytometry, evaluating the frequency and phenotype of γδ T lymphocytes in the peripheral blood of patients before and after anti-PD-1 immunotherapy. Our analysis revealed a distinct cluster of γδ T lymphocytes expressing NKG2A [1], likely capable of exerting significant cytotoxic activity in peripheral tissues. This subset was found to be significantly more abundant in healthy tissues than in tumor tissues and exhibited a notable increase in the peripheral blood of NSCLC patients following checkpoint inhibitor immunotherapy.
Our data indicate that γδ T lymphocytes may play a crucial role in controlling tumor progression in lung carcinoma and contribute to the antitumor response induced by the administration of immune checkpoint inhibitors. Furthermore, although preliminary, our flow cytometry analyses suggest that assessing the γδ T lymphocyte population expressing NKG2A in the peripheral blood of NSCLC patients during follow-up could serve as a simple and effective predictive biomarker for response to such therapies.

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## P139
## Peripheral blood neutrophils in advanced melanoma patients undergoing immunotherapy with anti-PD1 immuno-checkpoint inhibitors (ICI)

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**Background:** Cutaneous melanoma is one of the most aggressive forms of skin cancer and a primary cause of cancer-related death. Melanoma treatment has been approved using inhibitors of the PD-1/PD-L1 pathway. However, only a small proportion of patients benefit from monotherapy with anti-PD-1 or anti-PD-L1 drugs. PD-L1 is expressed on several immune cells and can be also expressed on human neutrophils (PMNs). They can play a role in carcinogenesis, and are a heterogeneous cell population. Neutrophil ability to release Neutrophil Extracellular Traps (NETs) contributes to their pro-tumor phenotype. The purpose of this study is to investigate the role of PMNs and NETs in the response to ICI treatment, as well as to evaluate the possible significance of NETs as neutrophil-related biomarkers to predict responses and select patients for suitable treatment.

**Methods:** 7 melanoma patients (MPs) candidate to anti-PD-1 immunotherapy (nivolumab) and 7 healthy controls (HCs) were recruited. PMNs were isolated from peripheral blood before the first therapy administration. They were stimulated in vitro with control medium or PMA to assess ROS production, morphological and kinetic properties, survival and NET release. MPs PMNs as well as HCs PMNs stimulated with melanoma-derived conditioned media (CM) or control media were subjected to RNA sequencing.

**Results:** PMNs from MPs displayed reduced ROS spontaneous release and peculiar morphological properties compared to HCs. Differential basal gene expression was found between MPs undergoing progressive disease and MPs with stable disease. About 40 genes were significantly differentially expressed in PMNs treated with melanoma CM compared to the control.

**Conclusions:** PMNs from MPs display modified morphological characteristics, ROS production, and gene expression. Our preliminary results suggest

a PMN-related gene signature in MPs. Further investigations are needed to go deeper inside PMNs functions during melanoma progression as well as in predicting clinical response to immunotherapy.

**P140**

**Single cells flow cytometry and transcriptomic analysis of different layers of human glioblastoma revealed an impairment of tumor infiltrating lymphocytes function**

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Purpose: Glioblastoma is the most malignant brain tumor in adult population, for which immunotherapy show reduced efficacy. Current knowledge on immunotherapy failure is limited and detailed information about immune infiltrates in glioblastoma are urgently needed to personalized therapeutic strategy.

Methods: We enrolled 33 glioblastoma patients collecting peripheral blood (PB), total tumor resection or tumor samples from the central necrotic area, the intermediate and the marginal tissue through 5-aminolevulinic-acid (5-ALA) assisted surgery. T cells obtained from different samples were evaluated phenotypically, for immune-checkpoints and tissue-residency markers (Trm) expression, and functionally by assessing their cytokine production profile and cytotoxic potential. On additional 3 patients a single-cell-RNA-sequencing (scRNA-seq) analysis was performed. Patients overall survival (OS) were retrospectively assessed and correlated with biological data.

Results: Flow-cytometric analysis showed a significantly higher frequency of T lymphocytes expressing PD-1, CD69 and CD103 in glioblastoma. In particular, we observed a preferential enrichment of CD8 expressing PD-1 and Trm markers in intermediate and marginal tumor areas, in which glioblastoma cells showed an active 5-ALA metabolism. Notably, in the 5-ALA cohort a strong direct correlation between CD103 and OS in the necrotic layer, typically not enriched in Trm effector cells, was observed.
T cells cytokine production results higher in glioblastoma compared to PB samples. Interestingly, the higher frequency of IFN-γ-producing T cells was observed in the intermediate tumor layer.
Preliminary analysis of scRNA-seq data highlight an enrichment of low cytotoxic GZMK$^+$CD8 cells in marginal and intermediate tumor layer, which was further validate at protein level.

Conclusion: In this study we observed a preferential enrichment in CD8$^+$ Trm like T cells expressing PD-1, which showed high cytokine production but low cytotoxic potential, in the intermediate layer. In conclusion, T cells obtained from different GBM layers showed different phenotype and cytokines expression, suggesting new prognostic factors and supporting surgery particle strategy.

**P141**

**Uncovering new targets and pathways to improve the efficacy of combination immunotherapy for lung cancer**

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Lung cancer is a leading cause of cancer-related deaths, with incidence expected to rise, particularly in women. Despite advances in treatment, survival rates for advanced lung cancer remain low. While immunotherapy has shown promise, its efficacy is limited to 25-30% of patients, underscoring the need to understand resistance mechanisms and develop alternative strategies. Combining immunotherapy with chemotherapy or radiotherapy can improve tumor control, but durable responses remain rare. Identifying pathways that enhance tumor immunogenicity and remodel the tumor microenvironment is critical to improving therapeutic outcomes. In this study, we investigate how chemo-immunotherapy modulates immune responses and tumor microenvironment composition. Using a genetically engineered KrasG12D Trp53fl/fl (KP) lung cancer mouse model, tumors are induced via intratracheal Adeno-Cre administration. Mice are randomized into control and treatment groups, with the latter receiving a combination of oxaliplatin, cyclophosphamide, anti-PD-1, and anti-CTLA-4 checkpoint blockade. Following treatment, lungs, lymph nodes, spleens, and blood will undergo deep immune profiling using spectral flow cytometry, scRNA-seq, spatial transcriptomics, and immuno-



histochemistry to characterize immune cell phenotypes, activation states, and cell-to-cell interactions. Our study aims to elucidate key immune networks and inflammatory mediators that influence either response or resistance to therapy. Additionally, by integrating data from primary patient specimens before and after chemo-immunotherapy, we seek to bridge preclinical findings with clinical data. This research will provide insights into the immunomodulatory effects of chemo-immunotherapy and identify novel targets to overcome resistance, ultimately improving outcomes for patients with advanced lung cancer.

## P142
## VS-15 inhibits both the enzymatic and non-enzymatic functions of indoleamine 2,3-dioxygenase 1

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Indoleamine 2,3-dioxygenase 1 (IDO1) is an immune checkpoint protein active in multiple physio-pathological contexts. IDO1 is mostly expressed in dendritic cells, where it induces immunosuppressive responses by relying on both enzymatic and nonenzymatic activity. In its heme-containing form (holo-IDO1), IDO1 exploits the enzymatic activity, which degrades tryptophan and produces kynurenines. In its heme-free form (apo-form), IDO1 exploits the nonenzymatic function where it acts as a signal transducing molecule by binding to tyrosine phosphatase Src homology region 2 domain-containing phosphatases (SHPs) and phosphatidylinositol-3-kinase (PI3K) regulatory subunit p85, anchoring to early endosomes, and in turn modulating gene expression thus promoting the establishment of an immunosuppressive phenotype. In cancer, IDO1 expression represents a critical tumor-escape mechanism. Therefore, IDO1 inhibitors have been developed to be used as anticancer drugs. However, none of these have reached the clinic. One possible explanation is that current inhibitors target only IDO1 enzymatic activity. In this work we aimed to discover a more effective modulator capable of blocking not only the enzymatic but also the signaling-mediated functions of IDO1. By virtual screening, we identified the compound VS-15, which selectively binds to apo-IDO1, inhibits its enzymatic activity, and reduces the IDO1-mediated signaling pathway by negatively interfering with its partnership with SHPs and PI3K p85 as well as with IDO1 anchoring to early endosomes in tumor cells. Moreover, VS-15 counteracts the IDO1-mediated immunosuppressive phenotype in dendritic cells and reduces the inhibition of T cell proliferation by suppressive monocytes from pancreatic cancer patients. Thus, we herein describe the discovery and characterization of a small molecule with an unprecedented mechanism of action, capable of inhibiting both the enzymatic and non-enzymatic functions of IDO1. These results pave the way for the development of next-generation IDO1 inhibitors with a competitive advantage over the current modulators.

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## P143
## Turning "cold" tumors "hot": ERAP1 inhibition remodels the tumor microenvironment of melanoma

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In recent years, immunotherapies like immune checkpoint blockade (ICB) have revolutionized the field of cancer treatment. However, the number of patients who can benefit and have a long-term response to treatment is still limited, raising the need to find new synergies to improve the clinical response of these patients.
In this context, we proposed the modulation of ERAP1, a key enzyme of the antigen processing and presentation pathway, that recently has been proven to positively influence the response to ICB-based therapy and the levels of tumor-infiltrating lymphocytes in a murine model of neuroblastoma.
Here, we investigated the extent to which ERAP1 shapes the tumor microenvironment (TME) of "cold" and "hot" murine melanoma models. We found that inhibition of ERAP1 makes melanoma cells more susceptible to immune cell-mediated killing by inducing an important change on the stability of the peptide-MHC-I complex and the immunopeptidome of melanoma, increasing the activation state of T cells and NK cells. In "hot" melanoma tumors, ERAP1 inhibition reduces tumor growth by recruiting activated NK cells to tumors; while in

"cold" melanoma models, the absence of ERAP1 is not sufficient to control tumor growth but can reshape the infiltration in the TME by recruiting more immune cells inside the tumor core. These results confirm that ERAP1 inhibition is a promising strategy to turn "cold" tumors into "hot" tumors, highlighting the potential of this non-toxic approach for combination with immunotherapies and the need to identify biomarkers of response to ERAP1 inhibition.


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