PROGRAM & ABSTRACT BOOK

French Dendritic Cell Society biennial meeting - 2025

"Unveiling the future of Dendritic Cell and Macrophage biology in health and disease"

December 4th – 5th 2025, Institut Curie, Paris, France

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Institut Curie, Paris France

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VENUE

The biennial French DC Society meeting of 2025 will take place in Paris

Location : Institut Curie

Address : 12 rue Lhomond, 75005 Paris

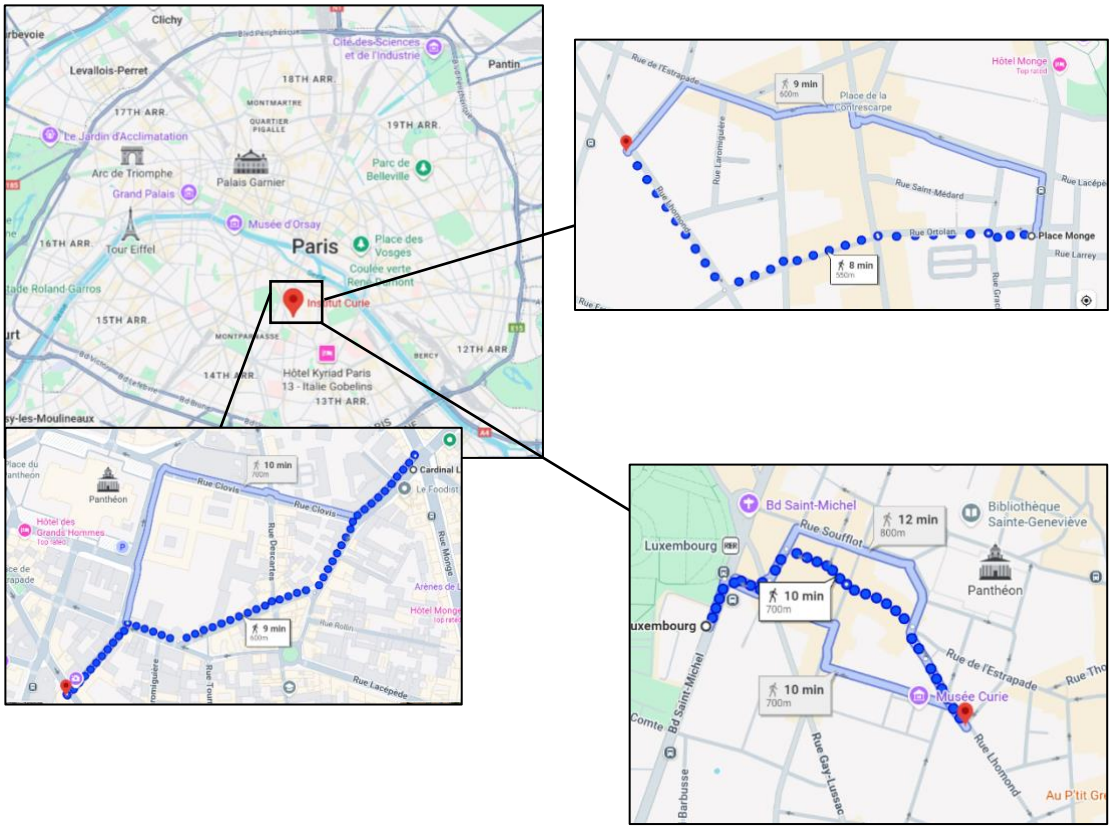
How to come:

By subway **Stop at Place Monge then~ 8 min by walk.**

Stop at Cardinal-Lemoine then~ 9 min by walk.

By RER: RER B – Stop at trainstation Luxembourg then ~10 min by walk

By Bus: Lines 21, 24, 27, 38, 47, 82, 84, 85, 89 (Size sorted by stop proximity)



PROGRAM

Thursday December 4th, 2025

8:00 **Opening of the registration desk**
8:45 **Welcome address** *Julie Helft*

9:00-9:45 KEYNOTE LECTURE I
Chrysothemis BROWN (MSKCC, New York,USA)
Thetis cell ontogeny, transcriptional regulation and function

SESSION I – Emerging Dendritic Cell lineages
Chairs: Florent Ginhoux and Jérôme Martin

9:45-10:15 **Rabi UPADHYAY (New York University, New York, USA)**
Towards targeting unique dendritic cell functions
10:15-10:45 **Helena PAIDASSI *(CIRI, Lyon, France)***
Back in the spotlight: Dendritic cell subsets and the maintenance of intestinal tolerance

10:45-11:15 Coffee Break 

11:15-11:45 **Filipe PEREIRA *(Lund University, Lund, Sweden)***
In Vivo Dendritic Cell Reprogramming for Cancer Immunotherapy
11:45-12:15 **Simon YONA (*The Hebrew University, Israel*)**
Human DC kinetics in health and inflammation

12:15-13:00 **Selected oral presentations**
12:15. Anfei HUANG *(Wurzburg University, Germany)*
Refractory dendritic cells initiate CD8 T cell exhaustion
12:30. Margaret JACKSON *(Institut Curie, France)*
A transcriptional coordinator of dendritic cell maturation and activation states
12:45. Nagham ALOUCHE *(UNIL, Lausane Switzerland)* *Architects of Immunity: How crosstalk between fibroblasts and dendritic cells shapes the T cell niche*

13:00-13:30 **Sponsors Lunch talks**:
13:00 MILTENYI
13:15 PROTEINTECH

13:00-15:00 **LUNCH and Poster viewing**

SESSION II – Dendritic Cells and macrophages in tissues
Chairs: Vanja Sisirak and Julie Helft

15:00-15:30 **Emmanuel GAUTIER *(Sorbonne Université, Paris, France)***
Kupffer cells in health and disease
15:30-16:00 **Andreas SCHLITZER *(LIMES - Institut, Bonn University, Germany)***
Spatial regulation of chronic inflammation by dendritic cells

16:00-16:30 **Elena TOMASELLO** (***CIML, Marseille, France***)
The role of plasmacytoid DCs during viral infections and beyond

16:30-17:00 Coffee Break 

17:00-17:30 **Marc BAJENOFF (*CIML, Marseille, France*)**
Myeloid cell-Stroma interactions

17:30-18:15 **Selected oral presentations**
17:30 Claire CENAC *(Toulouse university, France)*
Aging and lung niche drive the constitutive escape from X chromosome inactivation of Tlr7 in female alveolar macrophages
17:45 Chloe ADMANE *(Sanger Institute, UK)*
Multiomics dissection of Immune-Skin interactions in macrophage-bearing skin organoids
18:00 Darawan TABTIM-ON *(Institut Curie, Paris, France)*
Aryl hydrocarbon receptor controls the macrophage repair program across tissues

18:15 **Welcome Cocktail at Institut Curie**

20:00 **Speaker Dinner**

Friday December 5th, 2025

8:30 Opening of the registration desk

9:00-9:45 KEYNOTE LECTURE II
Yasmine BELKAID (Institut Pasteur, Paris, France)
Dendritic cell control of multi kingdom interactions

SESSION III – Metabolism and Cellular biology of Dendritic Cells and macrophages
Chairs: Johan Garaude and Fabien Blanchet

9:45-10:15 **Joel HAAS** (***Institut Pasteur de Lille, Lille, France***)
Defining the role of hepatic dendritic cells in the development of MASLD

10:15-10:45 **Gaëtan BARBET *(Rutgers University, New Brunswick, USA)***
Early Signals and Adaptation of Dendritic Cell upon Pathogen Recognition

10:45-11:15 Coffee Break 

11:15-11:45 **Johan GARAUDE (*Immunoconcept, Bordeaux, France*)**
Metabolic recycling of phagocytosed bacteria defines microbial viability-specific immunity

11:45-12:15 **Selected oral presentations**
11 :45 Vasco RODRIGUES (*Institut Curie, Paris, France)*
GAS7 couples constitutive fluid uptake with intrinsic resistance to viral infection in macrophages

12:00 Tamara CARVALHO *(IRIM, Montpellier, France)*

Modulation of lipid metabolism and related-proteins in human blood-derived myeloid cells upon dengue virus infection

12:15 Barbara SCHRAML *(LMU Munich, Germany)*

Periweaning diet-induced activation of an IFNgamma-mediated regulatory circuit promotes the homeostasis of CD8+ T cells

12:30-14:30 LUNCH and Poster viewing

SESSION IV – Dendritic Cells and macrophages in Cancer and Inflammation
Chairs: Paula Michea and Dorothée Duluc

14:30-15:00 Evangelos GIAMPAZOLIAS *(Cancer Research UK, Manchester, UK)*

Decoding host-microbiome interactions in Cancer Immunity. Killing cancer with a gut instinct

15:00-15:30 Damya LAOUI *(VIB, Ghent, Belgium)*

Dissecting the cDC heterogeneity and spatial immune organization in the lung tumor microenvironment

15:30-16:00 Pierre GUERMONPREZ *(Institut Pasteur, Paris)*

Tumor-infiltrating Dendritic Cells: challenges and opportunities for immunotherapy

16:00-16:30 Coffee Break

16:30-17:00 Federica BENVENUTI *(ICGEB, Trieste, Italy)*

Decoding type1 DCs across tumor stages in genetic models of non-small cell lung cancer

17:00-17:30 Mariola KUROWSKA-STOLARSKA (*University of Glasgow, Glasgow, UK*) Synovial tissue dendritic cells: their role in pathology and disease remission

17:30-18:15 <u>Selected oral presentations</u>

17:30 Livia LACERDA-MARIANO (*Institut Curie, Paris, France)*

Tumor Stage Drives Metabolic Reprogramming of Dendritic Cells

17:42 Aliki VASILAKOU (Immunoconcept, Bordeaux, France)

The role of DNASE1L3 in cancer immunity

17:54 Kevin MULDER *(Gustave Roussy, Villejuif, France)*

DC subsets and states unravelled across human juxta-tumoral and malignant tissues

18:06 Jean MONATTE *(CRCM Marseille France)*

Myeloid-driven immune ecosystem stratification in Triple-Negative Breast Cancer: from preclinical discovery towards clinical translation

18:20 Award ceremony and concluding remarks

18:30 End of the meeting

SHORT TALK ABSTRACTS

SESSION I – Emerging Dendritic Cell lineages

Short Talk - 1

Refractory dendritic cells initiate CD8 T cell exhaustion

Anfei Huang[1], Milas Ugur[1], Deeksha Seetharama[1], Tsuneyasu Kaisho[2], Martin Vaeth[1], Yinming Liang[3], Georg Gasteiger[1] and Wolfgang Kastenmüller[1]

[1]Würzburg Institute of Systems Immunology, Max Planck Research Group at the Julius-Maximilians-Universität Würzburg; Würzburg, Germany. [2]Department of Immunology, Institute of Advanced Medicine, Wakayama Medical University; Wakayama, Japan. [3]Center of Disease Model and Immunology, Hunan Academy of Chinese Medicine, Changsha, China.

CD8 T cell exhaustion that is observed during their chronic activation prevents exuberant tissue damage but is a key factor limiting anti-tumor immunity. An important cause of this adapted function is the continuous activation of T cell receptors promoting cell-intrinsic negative feedback signaling for example through PD-1. However, the signals that induce T cell exhaustion on a T cell-extrinsic level are unclear. By eliminating the effector molecule perforin in CD8 T cells, an otherwise acute viral infection became chronic allowing us to determine the timing and underlying mechanism of T cell exhaustion. Unexpectedly, five days after both acute and chronic infections, we observed the emergence of refractory dendritic cells (DC) that responded poorly to pathogen-associated molecular patterns. Refractory DC that presented viral antigens reprogrammed activated T cells towards T cell exhaustion. Mechanistically, we found that the development of refractory DC depended on inflammatory signaling in preDC, which subsequently upregulated DUSP-1 that suppressed their further activation. DUSP-1 was also upregulated in DC from convalescent COVID-19 patients indicating a conserved mechanism in humans. Together, we revealed a feedback loop that initiates T cell exhaustion by modulating DC development which could be exploited for therapeutic strategies to treat chronic infections and cancer.

Short Talk - 2

A transcriptional coordinator of dendritic cell maturation and activation states

Margaret Jackson (1), Patrick Tran Van (1), Xavier Lahaye (1), Nicolas Manel (1)

(1) Institut Curie, INSERM U932, Paris, France

Short Talk - 3

Architects of Immunity: How crosstalk between fibroblasts and dendritic cells shapes the T cell niche

Nagham Alouche (1), Hélène Cannelle (1), Stéphanie Favre (1), José A. Villegas (1), Marija Jokic (1), Sandra Maréchal (2,3), Clint De Nolf (2,3), Maeva Delacrétaz (1), Alexandros Sifis (1,13,14), Chloé Chapuis (1), Allison Burns (4,5), Giovanna Ambrosini (4,5), Isabelle Dupanloup (6,15), Jun Abe (7), Ute Koch (8), Nicolas Guex (4,5), Jens V. Stein (7), Ivan Maillard (9,10), Tom Cupedo (11), Burkhard Ludewig (12), Freddy Radtke (8), Sophie Janssens (2,3), Sanjiv A. Luther (1).

1 Department of Immunobiology, University of Lausanne (UNIL), Lausanne, Switzerland. 2 Laboratory for ER Stress and Inflammation, VIB Center for Inflammation Research, Ghent, Belgium. 3 Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium. 4 Bioinformatics Competence Center, University of Lausanne, Lausanne, Switzerland. 5 Bioinformatics Competence Center, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. 6 Swiss Institute of Bioinformatics, Lausanne, Switzerland. 7 Department of Oncology, Microbiology and Immunology, University of Fribourg, Fribourg, Switzerland. 8 Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Life Sciences, Swiss institute for Experimental Cancer Research, Lausanne, Switzerland 9 Division of Hematology-Oncology, Department of Medicine, University of Pennsylvania, Philadelphia, PA. 10 Division of Hematologic Malignancies, Department of Medicine & Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 11 Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands 12 Institute of Immunobiology, Cantonal Hospital St.Gallen, St.Gallen, Switzerland 13 Present address: Division of Thoracic Surgery, Department of Surgery, CHUV, Lausanne University Hospital, Lausanne, Switzerland 14 Present address: Agora Cancer Research Center Lausanne, Lausanne, Switzerland 15 Present address CANSEARCH Research Platform for Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, Faculty of Medicine, University of Geneva, Geneva, Switzerland.

Compartmentalization of secondary lymphoid organs is orchestrated by various types of fibroblastic reticular cells (FRCs) which guide immune cell migration, interactions, and thereby innate and adaptive immunity. Yet, the pathways driving FRC differentiation into functionally distinct subsets remain poorly understood. Here, we identify Notch2 signaling in lymph node (LN) FRCs as a key regulator of their specialization, particularly for T zone FRCs (TRCs). Using mouse genetics, single-cell transcriptomics, and multiplexed protein imaging, we show that mature CCR7^{+} dendritic cells expressing the Notch ligand Jagged1 initiate and sustain CCL19hi TRC identity. This promotes CCL19 gradient formation and the organization of central T zone niches enriched in Xcr1^{+} DCs and CD8^{+} T cells. Both the spatial organization and Notch2-Jagged1 signaling activity are conserved in human LNs. Disrupting T zone segregation via Notch2 inactivation in FRCs impairs the generation of CD8+ T cell memory precursors. These findings reveal a conserved Notch2-dependent TRC programming by DCs that organizes distinct T cell niches essential for adaptive immunity.

SESSION II – Dendritic Cells and macrophages in tissues

Short Talk - 4

Aging and lung niche drive the constitutive escape from X chromosome inactivation of Tlr7 in female alveolar macrophages

Claire Cenac (1), Jade Capot (1), Sophie Laffont (1) and Jean-Charles Guéry (1)

(1) Univ Toulouse, INSERM, CNRS, Infinity – Toulouse Institute for Infectious and Inflammatory Diseases, Toulouse, France

Toll-like receptor 7 (TLR7) is a sensor of single-stranded RNA encoded on the X chromosome and is a major component of the antiviral immune response. In female mammals, inactivation of one of the 2 X chromosomes (XCI) balances dosage of X-linked genes between sexes. However, ~20% of human X-linked genes escape XCI. Using a genetic fate-mapping model with skewed-XCI in female Tlr7-reporter mice, we found that alveolar macrophages (AM) were the main immune cell population in which Tlr7 escapes XCI in the lung. We investigated whether reactivation of Tlr7 from the Xi was dependent on the origin of AM and could be influenced by sex hormones. Our data show that AM with biallelic expression of Tlr7 accumulate in the lung of female mice with aging with >80% of penetrance. This phenotype was similarly observed when AM were from hematopoietic origin, in both male or female suggesting that the lung cellular niche, rather than sex hormones and progenitor origin is critical to drive biallelic expression of Tlr7 in AM. Finally, we showed that Tlr7 biallelic AM exhibit enhanced expression of TLR7 protein compared to Tlr7 monoallelic cells, as well as higher proinflammatory cytokine production upon TLR7 stimulation. Together our results demonstrate that, Tlr7 escapes X chromosome inactivation specifically in tissue-resident AM with aging. This could contribute to the better viral resistance in females compare to males, especially in aging populations.

Short Talk - 5

Multiomics dissection of Immune-Skin interactions in macrophage-bearing skin organoids

Chloe Admane (1,2), Elias Farr (1), Fereshteh Torabi (1), Kim Evans (1), Maryna Panamarova (1), Nusayhah Hudaa Gopee (2), Elena Winheim (1), Kwasi Kwakwa (1), Cameron Collins (1), Emily Smith (1), Benjamin Rumney (1), Luca Verger (3), Diana Adão (1), Christine Hale (1), Vicky Rowe (1), Jiyoon Lee (4), Karl Koehler (3), Vijaya Baskar Mahalingam Shanmugiah (1), Emily Stephenson (1,2), Fränze Progatzky (3), April Rose Foster (1), Muzlifah Haniffa (1,2)

[1] Wellcome Sanger Institute, Hinxton, Cambridgeshire, United Kingdom. [2] Newcastle University Biosciences Institute, Newcastle upon Tyne, Tyne and Wear, United Kingdom. [3] Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom. [4] Boston Children's Hospital, Boston, MA, United States.

Macrophages are among the first immune cells to seed human prenatal skin, emerging in a largely pathogen-free environment long before immune defence is required. Their loss in Csf1op/op mice and CSF1R-deficient humans disrupts normal development, highlighting a fundamental morphogenetic role beyond immunity. Current understanding of skin morphogenesis mostly stems from animal models that do not fully capture human biology. iPSC-derived hair-bearing skin organoids (SkO) offer a sophisticated 3D model that mimics human skin development, yet lack immune components.

We benchmarked SkO against human prenatal skin across gestation (7-17 PCW) using single-cell-RNA sequencing and spatial transcriptomics and revealed reduced endothelial heterogeneity in SkO. Angiogenic factor analysis suggested macrophage-derived signals, absent in SkO, are critical for endothelial development, supported by spatial co-localization of macrophages and endothelial cells in prenatal skin.

For the first time, we established long-term SKO co-cultures with autologous iPSC-derived macrophages. We generated an atlas using single-nucleus-RNA-seq and spatial transcriptomics across multiple timepoints over 130 days, profiling over three million cells. Selection of organoids with and without vasculature allowed exploration of reciprocal effects of macrophages and vasculature on neural and stromal maturation. Whole-mount immunostaining of SkO revealed that macrophages enhance vascular network remodelling. Single-cell analyses indicated the influence of macrophages on neural heterogeneity and specification, suggesting broader impacts on skin morphogenesis.

This platform provides an unprecedented window into human prenatal skin development, revealing how macrophages drive vascular and neural networks and shape tissue architecture. Macrophage-bearing SkO offers a powerful system for dissecting immune-skin crosstalk in development and disease.

Short Talk - 6

Aryl hydrocarbon receptor controls the macrophage repair program across tissues

Darawan Tabtim-On (1), Alba de Juan (1), Mathieu Vetter (2), Gaëtan Juban (3), Estelle Remion (4), Léa Guyonnet (5), Coralie Martin (4), Bénédicte Chazaud (3), Elodie Segura (1,2)

[1] Institut Curie, PSL Research University, INSERM, U932, Paris, France, [2] Institut Necker Enfants Malades, Université Paris Cité, INSERM, U1151, Paris, France, [3] Institut NeuroMyoGène, University of Lyon, INSERM, U1217, CNRS, UMR 5310, Lyon, France, [4] Muséum National d'Histoire Naturelle, MCAM, UMR7245, Paris, France, [5] Cytometry Platform, CurieCoreTech, Institut Curie, Paris, France

Macrophages are essential for orchestrating tissue repair, yet the transcriptional control of their repair program remains poorly understood. Here we identify an essential role in this process for the Aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. In a mouse model of skin wound healing, we find that AhR deficiency in macrophages leads to delayed repair with defective formation of new tissue. Kinetic analysis of skin wounds shows that AhR deficiency in macrophages prolongs inflammation and disrupts angiogenesis. Combining in vivo and in vitro repair assays, we further demonstrate that AhR in macrophages is also involved in the production of soluble repair mediators and the cross-talk with fibroblasts and keratinocytes, in both mouse and human macrophages. Transcriptomic analysis indicates that, during tissue injury, AhR signaling is specifically activated in macrophages by efferocytosis. Finally, we show that the role of AhR in macrophages is conserved in lung repair after parasite injury and in muscle regeneration after sterile injury. Our results indicate that AhR is a key regulator of the macrophage repair program across tissues, coordinating both the timely resolution of inflammation and the production of pro-repair mediators.

SESSION III – Metabolism and Cellular biology of Dendritic Cells and macrophages

Short Talk - 7

GAS7 couples constitutive fluid uptake with intrinsic resistance to viral infection in macrophages

Anaël Hanouna (1), Pierre-Grégoire Coulon (1), François-Xavier Gobert (1), Mabel San Roman (1), Sarah Taheraly (1), Mathieu Maurin (1), Philippe Benaroch (1) and **Vasco Rodrigues** (1)

(1) Institut Curie, PSL University, Inserm U932, Immunity and Cancer, 75005 Paris, France

Tissue surveillance is a major function of macrophages and requires the constitutive uptake of extracellular fluid via macropinocytosis. However, this process may be exploited by viral pathogens that evolved the ability to enter cells in endocytic compartments. It is unclear how macrophages cope with the need to constantly scan their environment without becoming a vehicle for viral propagation. Here, we demonstrate that the constitutive uptake of fluid by human macrophages is coupled to a cell state that restricts viral replication. The underlying mechanism is centred on Growth Arrest-Specific-7 (GAS7). GAS7 is required for an optimal macropinocytic flux in macrophages by sustaining the activation levels of the small GTPase Rac1. Consequently, GAS7 is essential for membrane ruffling that precedes macropinosome formation. Remarkably, GAS7 is also critical for macrophage control of the replication of a wide range of DNA and RNA viruses. Despite their increased internalization of extracellular fluid, GAS7-expressing macrophages demonstrate resistance to productive viral infection. We found that the antiviral function of GAS7 is independent from its role in macropinocytosis and occurs downstream of viral fusion. In the absence of GAS7 the rates of protein translation in macrophages are increased, ultimately promoting viral replication. Our work identifies GAS7 as factor enhancing the sentinel function of macrophages, by boosting macropinocytosis, while also limiting the risk of increased viral replication that may arise in cells actively engaged in tissue surveillance.

Short Talk – 8

Modulation of lipid metabolism and related-proteins in human blood-derived myeloid cells upon dengue virus infection

Tamara Carvalho (1), Soléna Rossi (1), Raphaëlle Lopez (1), Aude Boulay (1), Lise Holsteyn (1), Laura Picas (1), Tineke Cantaert (2), Dorothée Missé (3), Laurence Briant (1) and Fabien P. Blanchet (1)

[1] Institut de Recherche en Infectiologie de Montpellier, University of Montpellier, CNRS UMR9004, Montpellier, France [2] Immunology Unit, Institut Pasteur du Cambodge, Pasteur Network, Phnom Penh, Cambodia [3] MIVEGEC, University of Montpellier, CNRS, IRD, Montpellier, France

Severe manifestations of dengue virus (DENV) infection, like hemorrhagic fever and shock syndrome, represent major global health challenges. Development of effective therapies becomes essential to decipher how host metabolism contributes to disease progression. Macrophages and dendritic cells are critical and early mediators of host immune response, and recent evidence suggests that DENV co-opts lipid metabolic pathways in these cells to facilitate replication. Neutral lipid reservoirs such as lipid droplets (LDs) coated by perilipin (PLIN) proteins are emerging as key components of this process. DENV was reported to exploit host fatty acid synthase recruitment, autophagy-mediated degradation of lipid droplets, and interconnect viral capsid proteins with LDs to fuel viral particle assembly. Using high-throughput imaging content and lipidomics, we analyzed neutral lipid profiles, LD content, and PLIN expression during infection of human blood-derived myeloid cells. Our findings revealed altered expression and localization of PLIN2 and PLIN3 in MDM and a reduced neutral lipid content in both myeloid cell types. Treatment of MoDC with lipid metabolism inhibitors further decreased lipid content and affected infection levels, highlighting a functional link between lipid regulation and DENV replication. Lipidomic analyses confirmed that viral infection reshapes the lipid landscape of MoDC, with significant changes in fatty acids, triglycerides, and ceramides. Together, our data place human myeloid cells as central players in DENV pathology, with PLIN and LD metabolism being central to infection and potentially to downstream immune dysregulation. Targeting lipid homeostasis in these cells may offer a promising route for therapeutic intervention to tackle dengue-mediated severe symptoms.

Short Talk – 9

Periweaning diet-induced activation of an IFNgamma-mediated regulatory circuit promotes the homeostasis of CD8+ T cells

Ramin Shakiba (1,2,†), Doğuş Altunöz (1,2,†), Kaushikk Ravi Rengarajan (1,2) Hamsa Narasimhan (1,2,) Nikos E. Papaioannou (2,3), Jessica Vetters (4,5), Maria L. Richter (6), Denise Messerer (7), Sabine Schwamberger (8), Andreas Goschin (9), Dimitrios Starfas (1), Tobias Straub (10), Michele Proietti (9), Katrin Böttcher (11), Maria Colomé-Tatché (6), Dirk Haller (8,12), Sophie Janssens (4,5), Christian Schulz (7,13), Anne B. Krug (1), **Barbara U. Schraml** (1,2*)

1 Institute for Immunology, Biomedical Center, Faculty of Medicine, LMU Munich; Planegg-Martinsried, Germany. 2Institute of Cardiovascular Physiology and Pathophysiology, Biomedical Center, Faculty of Medicine, LMU Munich, Germany. 3 Current affiliation: Immune Regulation Laboratory, Center of Basic Research, Biomedical Research Foundation Academy of Athens; Greece. 4 Laboratory for ER Stress and Inflammation, VIB-UGent Center for Inflammation Research, Ghent, Belgium. 5 Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium. 6 Biomedical Center, Physiological Chemistry, Faculty of Medicine, LMU; Munich, Germany. 7 Department of Medicine I, LMU Klinikum, Ludwig Maximilian University (LMU) Munich, Munich, Germany. 8 Core Facility Gnotobiology, ZIEL Institute for Food & Health, TUM; Freising, Germany. 9 Institute for Immunodeficiency (IFI), Medical Center and Faculty of Medicine, University of Freiburg; Germany. 10 Core Facility Bioinformatics, Biomedical Center, LMU; Munich, Germany. 11 Department of Internal Medicine II, University Hospital Rechts der Isar, TUM School of Medicine and Health, Department Clinical Medicine, Munich, Germany. 12 Chair of Nutrition and Immunology, School of Life Sciences, Technical University of Munich, Freising, Germany. 13 Department of Immunopharmacology, Mannheim Institute for Innate Immunoscience (MI3), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany. † contributed equally

Balancing pathogen defence with maintaining tolerance to benign antigens in the neonatal period is essential for survival and the establishment of life-long immune homeostasis. Instructed by environmental signals type 1 conventional dendritic cells (cDC1) drive either T cell tolerance or immunity. Here, we uncover an interferon (IFN)-γ-driven regulatory circuit in early life that relays dietary cues to spleen cDC1. Loss-of-function demonstrates that IFNγ-mediated STAT1-signaling induces an immunogenic maturation program in spleen cDC1 that instructs cDC1 to expand effector memory $CD8^+$ T cells. This program emerges during weaning, when IFNγ production from lymphocytes rises in response to chow, it occurs in germ-free mice and remains responsive to dietary intervention in adult mice. Thus, IFNγ production from lymphocytes relays dietary information during weaning to spleen cDC1, allowing cDC1 to recalibrate the T cell pool at the moment of nutritional independence.

SESSION IV – Dendritic Cells and macrophages in Cancer and Inflammation

Short Talk – 10

Tumor Stage Drives Metabolic Reprogramming of Dendritic Cells

Livia Lacerda Mariano (1), Mabel San Roman (1), Helene Moreau (1), Christel Goudot (1), Ana-Maria Lennon (1)

(1) Institute Curie, INSERM U932

Dendritic cells (DCs) orchestrate anti-tumor responses by capturing tumor antigens and presenting them to T cells in the draining lymph nodes. Upon antigen uptake, DCs initiate a maturation program characterized by the upregulation of activation markers and the CCR7 receptor, which guides their migration through lymphatic vessels. Recent studies have shown that DCs lose their ability to migrate to lymph nodes and exhibit an “exhausted” phenotype in late-stage tumors. We hypothesized that temporal changes in the tumor microenvironment impair the immunogenic maturation of DCs. Using a colon adenocarcinoma (MC38) tumor model, we evaluated changes in DC biology from early (day 8) to late tumor stage (day 15). In early-stage tumors, all DC subsets showed enhanced expression of interferon-stimulated genes (ISGs), increased phagocytosis of tumor cell debris, and elevated production of reactive oxygen species (ROS). As the tumor progressed to late stages, DCs underwent a metabolic shift, upregulating antioxidant genes, increasing mitochondrial mass, and downregulating ISGs. During this metabolic transition, DCs lost their capacity to migrate to lymph nodes, reduced phagocytosis and protein translation, and increased expression of efferocytosis genes. These changes were at least partially driven by the tumor microenvironment, as bone marrow-derived DCs injected into tumors at different stages exhibited phenotypes similar to endogenous DCs. In conclusion, temporal changes in the tumor microenvironment influence the antigen uptake strategy by DCs and affect their maturation state within the tumor.

Short Talk – 11

The role of DNASE1L3 in cancer immunity

Aliki Vasilakou [1] [2]*, Pauline Santa [1]*, Séverine Loizon [1], Anne Garreau [1], Anaïs Roubertie [1], Vanja Sisirak [1]§, Dorothée Duluc [1]§

[1] ImmunoConcEpT, CNRS UMR5164, University of Bordeaux, 33000 France [2] Cancer Biology Graduate Program, UB Grad 2.0, University of Bordeaux
**co-first authors; §co-last authors*

Tumor-derived DNA is a key driver of anti-tumor immune responses by activating dendritic cells (DCs). Upon sensing DNA through TLR9 or cGAS, DCs produce type I interferons (IFN-I) that promote cytotoxic T lymphocyte (CTL) activation. Conversely, tumor DNA-mediated cGAS activation contributes to the recruitment of immunosuppressive cells, that limit anti-tumor immune responses. In addition, tumor-associated neutrophils undergo NETosis, releasing DNA that forms neutrophil extracellular traps (NETs), which shield tumor cells from CTLs. Thus, accumulated DNA shapes the balance between immune activation and suppression. Despite the importance of DNA in the regulation of anti-tumor immunity, the mechanisms controlling DNA abundance and immunostimulatory remain poorly described in this context. We identified DNASE1L3, a DC-derived endonuclease that digests extracellular DNA and limits immune activation. Although DNASE1L3 has emerged as a modulator of anti-tumor immunity, its role during therapy is unknown. Using Dnase1l3-deficient mice with spontaneous or orthotopic mammary tumors, we found that loss of DNAse1L3 impaired chemotherapy (CT) efficacy, indicating its requirement for optimal CT-induced immunity. Tumor cells killed by doxorubicin released partially fragmented DNA, which DNASE1L3 fully digested, enhancing DNA sensing by TLR9 and cGAS. Supplementing CT- or radiotherapy (RT)-treated tumor supernatants with DNASE1L3 further increased TLR9 and/or cGAS pathway activation. Because DNASE1L3 is selectively secreted by DCs, we examined its regulation in the tumor microenvironment. Tumor supernatants upregulated DNASE1L3 in DCs, while Dnase1l3-deficient or inhibitor-treated DCs showed reduced IFN-I secretion upon TLR9 stimulation. Together, these findings identify DNASE1L3 as a tumor-regulated enzyme essential for DC activation and therapy-induced anti-tumor immunity and we are currently exploring the underlying mechanisms involved in these processes.

Short Talk – 12

DC subsets and states unravelled across human juxta-tumoral and malignant tissues

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Dendritic cells (DCs) are professional antigen-presenting cells (APCs). While plasmacytoid DCs (pDCs) are poor APCs at a steady state, myeloid progenitor-derived DCs (mDCs) that com-prise DC1s, DC2s and DC3s are specialised in T-cell priming. To generate unbiased human DC atlases, we integrated DCs from 13 tumour tissues across 40 datasets to generate a pDC+mDC-VERSE (DC-VERSE) and an mDC-VERSE scRNA-seq compendium. We characterised DC sub-sets and “states” across these tissues. Most studied tumours contained CD207+ DCs, a subset of CD1c+ DCs, whose expansion inversely correlated with tumour CD8+ resident memory T-cells, T-cell clonality and survival of patients treated with immune checkpoint inhibitors. Similarly to CCR7+ mDCs - a common “state” of DC1s, DC2s and DC3s, we found that CD207+ DCs were a common "state" of DC2s and DC3s. Spatially-resolved single-cell transcriptomic and immunohis-to-fluorescence of human carcinomas demonstrated that lymphocytes and most DCs were enriched within tumour stroma, while CD207+ DCs were mostly embedded within tumour nests. These DC-VERSEs provide a robust resource available to the scientific community on DCs in health and pathology.

Short Talk - 13

Myeloid-driven immune ecosystem stratification in Triple-Negative Breast Cancer: from preclinical discovery towards clinical translation

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Background: Triple-negative breast cancer (TNBC) exhibits heterogeneous responses to immunotherapy, despite being the most immunologically active breast cancer subtype. While tumor-infiltrating lymphocytes predict outcomes, myeloid cells, particularly neutrophils and macrophages, dominate the immune landscape and fundamentally shape therapeutic responses. However, current biomarker approaches fail to capture this myeloid heterogeneity, thereby limiting the implementation of precision immunotherapy.

Experimental approach: Using the immunocompetent MMTV-R26Met TNBC model, we performed integrative multi-omics profiling, including histological, genomic, transcriptomic, proteomic, and immune analyses, to define immune-molecular subtypes. Single-cell RNA sequencing of four syngeneic grafts from spontaneous tumors enabled detailed analysis of the myeloid compartment and ligand-receptor interactions. Therapeutic studies evaluated epirubicin ± anti-PD-1 efficacy in neutrophil-enriched (S37) versus macrophage-enriched (S39) orthotopic syngeneic MMTV-R26Met models. Clinical validation was performed in a cohort of 125 stage II-III TNBC patients treated with neoadjuvant pembrolizumab-chemotherapy.

Results: Unsupervised clustering identified four TNBC subtypes: (1) dendritic cell-enriched/proliferative, (2) macrophage-enriched/mesenchymal, (3) T-cell enriched/mixed, and (4) neutrophil-enriched/OXPHOS-high, characterized by persistent splenomegaly. These clusters paralleled human TNBC immune subtypes, with lymphocyte-enriched tumors associated with superior survival compared to myeloid-dominant profiles. Single-cell RNAseq analysis revealed distinct myeloid programming across subtypes. Tumor-derived cell lines confirmed these distinct signaling landscape by qPCR. In vivo, neutrophil-enriched S37 tumors showed minimal response to anti-PD-1 monotherapy whiel achieved 33% complete responses with epirubicin + anti-PD-1 combined treatment, associated with increased CD8+ T cells and reduced neutrophil infiltration. In contrast, macrophage-enriched S39 tumors responded to anti-PD-1 monotherapy while exhibited resistance to combination therapy. We are currently investigating systemic myeloid biomarkers in clinical cohorts to identify predictors of immunochemotherapy response. Preliminary analysis suggests that imaging and hematologic parameters reflecting systemic myeloid states may correlate with treatment responsiveness, supporting their potential clinical implementation for patient selection.

Conclusions: This work establishes myeloid ecosystem (neutrophil-enriched or macrophage-enriched) stratification as a fundamental principle in TNBC immunotherapy. Ongoing clinical validation of myeloid biomarkers aims to enable precision patient selection for pembrolizumab-chemotherapy regimens.

POSTER ABSTRACTS

Thursday December 4th, 2025

Poster – 1

A tumor-activated program maintains conventional type 2 dendritic cells in an immature epithelial state in the tumor micro-environment

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Dendritic cells (DCs) are sentinel cells of the immune system controlling the development of adaptive immunity. Depending on the cues instructing their terminal differentiation, DCs can either spark the onset of adaptive immune responses or tolerance. Immune suppression is a hallmark of cancer, with multiple mechanisms orchestrated by tumor-derived factors precluding the development of efficacious adaptive immunity. Here, we have decided to characterize DC populations within the KrasG12D p53-/- model of lung adenocarcinoma. Tumor progression is associated with a marked expansion of DCs in the TME, particularly CD11b+ DC2/3, which progressively acquire an epithelial-like phenotype characterized by expression of CD103 and cell adhesion molecules (Epcam, Cldn1, Cdh1), whose ligands are expressed by tumor cells. These epithelial-like DCs (EpDCs), found in close proximity to tumor cells, originate from conventional DC2 progenitors, suggesting that the EpDC phenotype represents an acquired functional state of DC2 within the TME. EpDC differentiation occurs locally in the TME in response to tumor-derived factors (GM-CSF, TGF-b, CCL20), rather than at the level of bone marrow progenitors. In vivo lineage-tracing experiments further indicate that EpDCs have enhanced retention and survival within the TME. This EpDC phenotype is also observed in other murine tumor models (breast, pancreas) and in some human non-small cell lung cancers (NSCLC). Although their exact function remains to be elucidated, our findings suggest that EpDCs may represent a therapeutic target, particularly through blockade of tumor-induced inhibitory signals that sustain their interaction with tumor cells.

Poster – 2

Donor Corticosteroid Therapy Modulates Macrophages to Improve Early Lung Graft Immunity

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Background: Preclinical studies have recently revealed the critical role of innate immunity in determining lung transplantation outcomes. Although the International Society for Heart and Lung Transplantation recommends high-dose corticosteroid administration to donors, this practice is inconsistently applied worldwide. Investigating its impact on the donor lung's innate immune response – an unexplored area - could provide valuable evidence to support adoption of donor preconditioning with corticosteroids, beyond their traditional administration to recipients.

Method: We used a cross-circulatory pig platform that consists of a donor lung placed extracorporeally and connected to the circulation of a recipient pig whose leukocytes are fluorescently labeled.

Results: Donor preconditioning - compared to recipient's treatment alone - reduced the presence of $CD3^{pos}$ T-cell in the graft from both the donor and recipient, and enhanced the anti-inflammatory profile of alveolar macrophages, at least during the first 10 hours of donor-recipient interaction. The alveolar macrophages isolated from corticosteroid-preconditioned pig lungs exhibited decreased gene expression of T-cell attracting chemokines during the 10-hour reperfusion period, correlating with the reduced T-cell infiltration. Similarly, human lung macrophages showed lower expression of these T-cell-attracting chemokines and higher anti-inflammatory profiles upon corticosteroid treatment.

Conclusion: Our results show that the early immune status of lung grafts is improved by treating donors with corticosteroids through macrophage-targeted mechanisms. This finding provides an immunological rationale for expanding the implementation of donor preconditioning with corticosteroids.

Poster – 3

Intra-tumoral delivery of FLT3L with CXCR3/CCR5 ligands promotes XCR1+ DC1 infiltration and activates anti-tumor immunity

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An important factor limiting the development of anti-tumor immunity lies in the extent of tumor infiltration by immune cells, including conventional dendritic cells (cDCs). cDCs rely on FLT3L for their differentiation from bone marrow progenitors, expansion in periphery and survival. Systemic administration of FLT3L expands cDCs but might disturb hematopoiesis and support Treg expansion. Our aim is to develop a cell-based local intervention to spark tumor infiltration by cDCs and activate the cancer immunity cycle. We report that intra-tumoral engraftment of autologous mesenchymal stromal cells engineered to express the membrane bound form of FLT3L (eMSC-FLT3L) induces anti-tumor immunity when combined with the poly(I:C) adjuvant. eMSC-FLT3L + poly(I:C) enhanced intra-tumoral expansion of cDC1s together with the infiltration of activated, tumor-specific T cells. Mice deficient in cDC1s, T and NK cells do not respond to eMSC-FLT3L + poly(I:C) immunotherapy. In vivo blockade of CXCR3 and CCR5 demonstrate that poly(I:C)-induced chemokines (CXCL9 and CCL5) are required for the therapeutic effect of eMSC-FLT3L + poly(I:C). Delivery of eMSC-FLT3L-CCL5-CXCL9 bypasses the requirement of poly(I:C) and is sufficient to induce anti-tumor immunity, pre-cDC1s and cDC1s infiltration and activated T and NK cells recruitment. Furthermore, we showed that eMSC-FLT3L-CCL5-CXCL9 stimulate the infiltration of cDC1s in a T cell independent maner. Finally, we provide evidence that eMSC-FLT3L-CCL5-CXCL9 engraftment stimulates the local accumulation of XCR1+CLEC9A+ human cDC1s in the skin of humanized immunodeficient mice. Altogether, these data support the therapeutic potential of an intra-tumoral engraftment of engineered stromal cells to stimulate cDC1s infiltration, local expansion and cDC1-dependent anti-tumor immunity.

Poster – 4

Kinesin-1 coordinates crosstalk between microtubule and actin cytoskeletons during dendritic cell migration

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Dendritic cells (DCs) are professional antigen (Ag)-presenting cells that excel in initiating adaptive immune responses by continuously scanning peripheral tissues for Ags. To facilitate efficient DC migration, constant crosstalk between actin and microtubules is required to coordinate cytoskeletal networks and actomyosin contractility, but the related mechanisms have not been extensively characterized. We show that mouse DCs lacking Kif5b (the heavy chain of kinesin-1) exhibit a major impairment in cell migration in vivo and in vitro. Mechanistically, kinesin-1 coordinates cytoskeletal crosstalk between actin and microtubules during DC migration by modulating negatively RhoA activity through its interaction with GEF-H1, thereby limiting GEF-H1's availability in the cytosol. The same mechanism operates in human primary-monocyte-derived DCs and regulates efficient migration in a confined environment. Thus, our results highlight kinesin-1 as a key regulator of DC migration, through its coordinated control of cytoskeletal dynamics.

Poster – 5

Development of a pathotype-specific therapeutic strategy targeting synovial macrophages in osteoarthritis

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Osteoarthritis (OA), the most prevalent rheumatic disease, affects over 500 million people worldwide and remains incurable. It is characterized by cartilage degradation, bone remodelling, and synovitis, a key driver of disease onset and progression. Our group recently identified three histological synovial pathotypes with distinct molecular signatures, representing relevant disease endotypes. Macrophages, the most abundant immune cells across these pathotypes, play a central role in OA pathophysiology. We hypothesize that characterizing macrophage subpopulations could guide the development of pathotype-specific strategies, advancing personalized therapies.

The diversity and spatial organization of macrophages in OA synovial tissues from patients undergoing total knee replacement were analysed by single-cell RNA sequencing (scRNAseq, n=3 per pathotype, 2 controls) and spatial transcriptomics (n=2 per pathotype and controls) using a custom panel combined with an immuno-oncology panel. An in vitro model was established to study macrophage subsets of interest, and a lipoplexe-based strategy is being developed for their targeted modulation.

scRNAseq and spatial analyses confirmed marked heterogeneity among synovial macrophages. Notably, a deep lining subset expressing CCL chemokines, corresponding to previously described TREM2low macrophages, was enriched in OA, particularly in the lympho-myeloid pathotype. To functionally characterize this subset, we developed CD14+-derived macrophages mimicking this phenotype. Based on differential gene expression, lipoplexes carrying siRNA mixes were designed to reduce target expression. Their penetration into the synovium was validated ex vivo by light sheet microscopy, and further studies will evaluate therapeutic benefits.

This study ultimately aims to demonstrate that a pathotype-specific macrophage modulation could help limit OA progression.

Poster – 6

Perivascular tracks support migration of dendritic cells in the spleen upon activation

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The migration of Type I conventional dendritic cells (cDC1s) to and within the spleen plays a central role in promoting an effective adaptive immune response. However, the spatio-temporal dynamics of cDC1 movement and maturation in the spleen and how this correlates with their interactions with T cells remain underappreciated. Our study uses intravital and confocal microscopy, as well as flow cytometric strategies, to show that precursors of cDC1s arrive in the spleen primarily in the red pulp, where they finish developing into cDC1s. Systemic inflammation induces fast and synchronous migration of cDC1s into the white pulp, with quick loss of ability to take up antigen. This migration follows perivascular tracks, which harbor specific stromal populations that physically support cDC1 migration along the pathway. Functional blood circulation is essential to form a soluble gradient of the chemokines CCL19 and CCL21, which signal through CCR7 to allow long distance directional track usage. Functionally, disruption of cDC1 migration into the white pulp by specific deletion of CCR7 in cDC1s strongly hampers T cell activation in response to Listeria monocytogenes infection. Collectively, our data adds to the understanding of cDC1 dynamics and how spleen architecture supports cDC1 migration and function during inflammation.

Keywords: Dendritic cells, spleen, migration, dendritic cell maturation, inflammation.

Poster – 7

Heterogeneity of myeloid cells in Anaplastic Thyroid Cancer: when “niches” matter

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Anaplastic thyroid cancer (ATC) is a very aggressive and ultrarare undifferentiated thyroid cancer with one of the poorest prognoses in oncology. Macrophages can constitute up to 70% of ATC tumor microenvironment, which may account for therapeutic resistance and aggressiveness. This project investigates the spatial architecture of ATC tumors and the contribution of myeloid cells in the aggressive behavior of anaplastic tumor cells. Compared to differentiated thyroid cancer characterized by a low number of macrophages, this study confirms that ATC tumors are highly enriched in tumor-associated macrophages. Our spatial-omics analysis also revealed distinct immune niches within ATC tumors, each associated with specific macrophage subsets that could be further described by scRNA-seq analysis.Importantly, our data also showed that T cells are abundant in the tumor microenvironment. Spatial transcriptomic and proteomic analyses further uncovered a specialized immune hub characterized by close interactions between ATC-associated macrophages, $CD8^+$ exhausted T cells, and $CD4^+$ regulatory T cells. Interestingly, T cells within this hub secrete CSF1, likely sustaining ATC macrophage recruitment and function. Finally, using 3D tumoroid models and in vitro co-cultures, we reproduced ATC-specific macrophage program and demonstrated its capacity to promote tumor cell invasion. Together, these findings provide the first spatially resolved framework of the ATC immune microenvironment and suggest that macrophage–T cell crosstalk is a central driver of tumor aggressiveness.

Poster – 8

Intratumoral delivery of lipid nanoparticle-formulated mRNA encoding IL-21, IL-7, and 4-1BBL induces anti-tumor immunity

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Therapeutic mRNA demonstrated remarkable potential in tackling various diseases including cancer. Local delivery of mRNA-based immunotherapy offers a promising avenue as it enables the production of specific immunomodulatory proteins that can stimulate the immune system to recognize and eliminate cancer cells while limiting systemic exposure and toxicities. Here, we developed and employed lipid-based nanoparticles (LNPs) to intratumorally deliver an mRNA mixture encoding the cytokines interleukin (IL)-21 and IL-7 and the immunostimulatory molecule 4-1BB ligand (Triplet LNP). IL-21 synergy with IL-7 and 4-1BBL led to a profound increase in the frequency of tumor-infiltrating CD8+ T cells and their capacity to produce granzyme B and IFN-γ, leading to tumor eradication and the development of long-term immunological memory. Mechanistically, we found that within the immune cells, the mRNA transfection efficiency of intratumorally-delivered LNPs was highest in macrophages, monocytes and cDC2s, inducing the activation and migration of the latter to the tumor-draining lymph nodes (tdLNs). Differential Nichenet analysis on the scRNA-seq datasets of Triplet LNP-treated and control tumors predicted that upon therapy, mainly cDC2s and migDCs induced cytotoxic CD8+ T cells in the tdLNs. Moreover, we showed that the efficacy of the Triplet LNP was dependent on tdLN-tumor CD8+ T-cell trafficking and that the presence of CD39+ CX3CR1+ tumor-specific CD8+ T cells in the blood could be used as a prognostic factor for the response to Triplet LNP therapy. Finally, we highlight the therapeutic potential of the Triplet LNP in multiple murine tumor models and its superior therapeutic efficacy to immune checkpoint blockade. Ultimately, the expression of these immunomodulators was associated with better overall survival in cancer patients.

Poster – 9

Clarifying Dendritic cell terminology in the context of single-cell diversity

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Over the last years advanced single cell studies have expanded out knowledge of Dendritic cell (DC) biology, defining novel populations, progenitors and developmental processes. However, this heterogeneity has also created the need to refine our naming conventions and provide a structured framework for new discoveries, which is especially important to integrate now discoveries from single-cell sequencing experiments.
DC undergo constant replenishment from the bone marrow. Their ontogeny derives from either lymphoid or myeloid origin, depending on the type of DC. For the lymphoid lineage common lymphoid progenitors (CLP) develop into pre-plasmacytoid dendritic cells (pre-pDC), subsequently differentiating into plasmacytoid dendritic cells (pDC). For the myeloid lineage common myeloid progenitors (CMP) differentiate into monocyte-DC progenitors (MDP), which further differentiate into common DC progenitors (CDP), Ly6C+ MDP, and common monocyte progenitors (cMoP). CDP then develop into pre-DC1 and pre-DC2, which eventually give rise to DC1 and DC2, respectively. Ly6C+ MDP differentiate into pro-DC3 and from there into DC3, while cMoP give rise to monocytes that potentially develop into monocyte-derived DCs (moDCs), which can be identified in certain inflammatory conditions. We propose to term these differentiated DC populations ‘DC subsets’, which exit the bone marrow to disseminate into tissues. When DC are however characterized in tissues, they show a complex heterogeneity, even at the steady state (such as selective expression of CD103, CD301b, CX3CR1, CD11b, etc.), suggesting local adaptation to the microenvironment. These characteristics we propose to term ‘phenotypes’, which can additionally acquire ‘states’ (such as the upregulation of costimulatory molecules) once they become activated by local immune contexts or pathogen sensing.
The use of a unified terminology will help integrate novel studies faster into the growing field of DC biology and help build specialized annotation tools to automatically define these populations in single-cell sequencing datasets.

Poster – 10

The liver microenvironment regulates hepatic dendritic cell function

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Liver diseases are often characterized by dysregulated immune responses. In a mouse model of hepatitis B (HBV) pathogenesis, HBV-specific CD8+ T cells are abnormally primed by hepatocytes, resulting in improper activation. This dysfunction can be reverted by liver-resident macrophages Kupffer cells (KC) but not hepatic dendritic cells (DC), despite DC's known ability to induce strong effector responses. To investigate how the liver microenvironment shapes the function of hepatic DC, we characterized their phenotype by flow cytometry in mouse models of liver inflammation.

We found that DC strongly upregulate co-stimulatory molecules in response to inflammatory stimuli, challenging the dogma of the tolerogenic hepatic DC. To evaluate whether hepatic DC can be stimulated to efficiently prime HBV-specific CD8+ T cells, we used an Xcr1-IFNa fusion protein to deliver IFNa directly to cross-presentation competent DC1. Unexpectedly, this did not result in functional effector CD8+ T cells but specifically induced an expansion of activated regulatory T (Treg) cells in the liver. Treg may actually play an important role in driving the function of DC1 in the liver, since the expression of maturation markers on hepatic DC1 was significantly increased shortly after Treg depletion in FoxP3-DTR mice.

We now plan to deplete Treg during HBV pathogenesis to assess their impact on the capability of hepatic DC1 to induce effector HBV-specific CD8+ T cells. The mechanisms behind the DC1-induced intrahepatic Treg expansion will also be investigated by RNA sequencing and validated through in vivo blocking strategies in models of HBV pathogenesis as well as hepatocellular carcinoma.

Poster – 11

Molecular characterization of pathways controlling mregDC differentiation

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Dendritic cells (DCs) are the main antigen presenting cells and the orchestrators of immune responses. Although DCs arise from hematopoietic progenitors by distinct ontogenies, there is accumulating evidence that DCs express a conserved “maturation” program across different tissues and especially in tumors. These cells have been called mregDC, migDC or activated DCs and identified in several tumor types expressing both genes of maturation/migration and tolerance /immunosuppression. However, it is still unclear which intrinsic molecular pathways drive mregDC development. Here, we integrated publicly available single-cell RNA sequencing dataset from several human tissues to find conserved programs and states of mregDCs. These states expressed differential levels of CD206 and CCR7, and were recapitulated in ovarian cancer samples. Then, we stablished an in vitro culture system to produce mregDCs from human CD34+ hematopoietic progenitors to experimentally address our questions. By scRNAseq analysis at different time points, we identified molecular pathways that could drive the “regulatory” program. We further characterized the kinetic and phenotype of the cells across time. Finally, we validated the non-canonical NFkB pathway as a key regulator of mregDC development using genome-editing tools (CRISPR/Cas9) and chemical inhibitors. Our results provide a new understanding about mregDC development in humans, which is a prerequisite for manipulating this immunosuppressive program for therapeutic purposes.

Poster – 12

FceRIneg DC3 subset accumulates in the urine of patients with nephrotic syndrome (NS) in complete remission: a potential role in the resolution of NS?

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Nephrotic syndrome (NS) is a glomerular disease characterized by increased permeability of the filtration barrier. In order to understand the underlying immune mechanism, the aim of this study is to characterize different subsets of DCs in the urine of patients with NS. Urine and blood samples were collected from children with NS (n=6) and compared with controls (n=9). Cells were analyzed by FACS with markers identifying DC subsets, as well as T cells, NK, neutrophils and macrophages. Flow cytometry data, analysed by PCA, allowed the discrimination of NS patients from controls, suggesting that the FACS panel used was relevant in identifying a cellular signature of NS in this study. Preliminary data show that, although the frequencies of CD3T-cell populations in urine are very low (<0.05% total cells), the HLA-DR population may reach up to 2% of live cells. Among the DC subtypes, the DC3 (CD1c+CD14+) population was significantly increased in the urine of NS patients compared to controls. Screening the composition of DC3 population, a higher frequency of DC3 not expressing the FceRI-receptor accumulates in the urine of patients with complete remission compared to patients in remission but still on treatment or in relapse. These results suggest that the FceRInegDC3 population may develop during patients' recovery and open the question of whether it is involved in the resolution of NS. These findings demonstrate for the first time that a DC subset can be found in urine in pediatric patients with NS and may be related to the remission status.

Poster – 13

Dendritic cells as key mediators of viral-induced molecular mimicry in Crohn's Disease

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Background: Dendritic cells (DCs) play a pivotal role in maintaining intestinal immune tolerance and orchestrating adaptive responses to the gut microbiota. In inflammatory bowel diseases (IBD), including Crohn's disease (CD), dysregulated DC activation contributes to chronic mucosal inflammation. Beyond bacterial dysbiosis, recent evidence highlights the gut virome — particularly the expansion of Caudovirales bacteriophages — as an emerging immunomodulatory factor in IBD pathogenesis.
Aim: We investigated how DCs respond to viral components of the gut virome, focusing on Proteus virus Isfahan, a Caudovirales species enriched in CD mucosa, to elucidate its potential role in triggering molecular mimicry and loss of tolerance.
Methods: Transcriptomic and metatranscriptomic profiling of intestinal immune populations identified Proteus virus Isfahan enrichment in DCs from CD patients. Proteus virus Isfahan viral protein gp82, showing structural homology to human dCMP deaminase, was overexpressed in monocyte-derived DCs via lentiviral vectors. We analyzed DC phenotype, and capacity to activate T cells, followed by co-culture with intestinal epithelial cell line to assess barrier integrity and immune-mediated damage.
Results: Data reveal altered antiviral gene expression and impaired tolerogenic function in CD-derived DCs, suggesting their inability to mount proper immune responses to bacteriophage components. gp82-overexpressing monocyte-derived DCs induced T-cell activation and inflammatory cytokine production, supporting a potential viral-driven autoimmune loop.
Conclusion: Our findings propose DCs as central mediators linking gut virome dysbiosis to autoimmune mechanisms through molecular mimicry in Crohn's disease. Understanding this DC–virus interaction may open novel therapeutic avenues targeting immune–virome crosstalk in IBD.

Poster – 14

Regulatory Networks Governing Human Tumor-Associated Macrophages Uncovered by Humanized Models

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Tumor-associated macrophages (TAMs) are central drivers of resistance to conventional and immune-based therapies. Recent single-cell studies have uncovered an unexpected heterogeneity of macrophage states in human cancers, moving beyond the simplistic “M1–M2” paradigm. However, progress in understanding human TAM biology has been hampered by the lack of physiologically relevant in vivo models. Here, we leverage next-generation humanized mouse models implanted with cell line xenografts (CDX) to dissect the transcriptional and epigenetic programs that shape monocyte-to-macrophage differentiation within the tumor microenvironment. We show that these immuno-CDX models faithfully reproduce the phenotypic and molecular hallmarks of TAMs observed in patient tumors. By integrating single-cell transcriptomic and epigenomic profiling with high-dimensional co-expression and enhancer-driven regulatory network analyses, we reconstruct a comprehensive framework of human TAM states. Furthermore, CRISPR-mediated gene editing of hematopoietic stem cells prior to humanization identifies MafB as a key regulator of TAM identity. Our findings establish humanized models as a powerful platform to study TAM biology in vivo, uncover fundamental regulators of macrophage plasticity, and highlight new therapeutic targets for next-generation cancer immunotherapies.

Poster – 15

Antigen presentation for B-cell activation by dendritic cells: exosome-free control and antagonistic regulation by glucocorticoids vs LPS.

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Dendritic cells (DCs) are professional antigen-presenting cells that capture antigen (Ag) in the periphery and migrate to lymph nodes to activate T cells. Previously, we showed that DCs are Ag transporters and B-cell activators in vivo and in vitro, through a new mechanism of extracellular release of native Ag. However, the mechanisms of control and regulation of Ag presentation for B-cell activation remain poorly understood. By using an in vitro DC–MD4 B-cells co-culture system we investigated the role of small GTPases and exosomes in the control of Ag presentation, as well as its regulation by glucocorticoids or LPS. Strikingly, we showed that extracellular release of Ag consists of an exosome-free mechanism, contrasting with the exosome-dependent extracellular T-cell activation. We showed that glucocorticoids, anti-inflammatory steroid hormones, inhibit both Ag display on the cell surface and Ag release by DCs in vitro. In contrast, bacterial LPS, a potent inducer of DC maturation, enhances native Ag release and potential B-cell activation. We then visualized by confocal microscopy the transfer of fluorescent Ag by DCs, underlying the induced B-cell activation. We observed co-localization of Ag with B-cells in co-culture in vitro. Furthermore, purified lymph node-resident cDCs are also able to capture and release native Ag in vitro, suggesting a potential role in Ag transfer and B-cell priming. Thus, our study provides new mechanistic insights into the modes of Ag delivery for B-cell activation by DCs and suggests a promising approach for drug modulation of the DC-elicited Ag-dependent B-cell responses and humoral vaccination.

Poster – 16

Phenotypic changes and kinetics of steady-state gut cDC migration

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Conventional dendritic cells (cDCs) are key antigen presenting cells which link innate and adaptive immunity by transferring antigenic information from peripheral organs to T cells in lymph nodes (LNs). However, despite their central role in the induction of adaptive immune responses, the kinetics and molecular regulation of the cDC life cycle and migration remain poorly understood. Using a variety of in vivo techniques, we examine the kinetics of cDC turnover in the intestine and address the molecular changes throughout the various stages of the cDC life cycle – from tissue entry and differentiation to CCR7 upregulation and subsequent migration into draining LNs. Our data demonstrate that the life cycle of gut cDCs is highly dynamic, characterised by continuous alterations in transcriptome, protein expression and proliferation rates. These progressive changes culminate in cDC homeostatic activation and migration resulting in a resource-intensive daily turnover of up to a quarter of intestinal cDCs and an almost complete daily replacement of the migratory cDC compartment in the mesenteric LN. This high turnover rate ensures that the mesenteric LN maintains an accurate reflection of the intestinal immunological state, supporting rapid adaptation to emerging immune challenges.

Poster – 17

Dendritic cell diversity shapes immune organization and clinical outcome in neuroblastoma

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Neuroblastoma (NB) is a complex disease with varying presentations, accounting for 15% of childhood cancer deaths. Spontaneous regression in low-risk NBs associated with opsoclonus-myoclonus-ataxia paraneoplastic syndrome (OMAS) possibly implies immune mechanisms that are not yet completely understood. Using a multimodal approach combining transcriptomic and cytometric analyses on fresh tumor samples as well as in situ analysis on a large retrospective cohort of patients with NB, we reveal that OMAS NB harbor dense infiltrates of both innate and adaptive immune cells, particularly an enrichment of type 1 conventional dendritic cells (cDC1) and the formation of mature tertiary lymphoid structures (TLS) with germinal centers, evocative of ongoing antigen presentation triggering an orchestrated localized immune response. We also observed a significantly higher expression of the CCL2 chemokine and total DC gene signature, in NB with OMAS compared to NB with MYCN amplification. Furthermore, DC infiltrate positively correlated with CCL2 expression in NB. Importantly, the presence of TLS, CD8 T cells as well as cDC1, cDC2, and mature DC, but not pDC, correlated to improved survival, including in the high-risk group of NB patients. We also observed an enrichment of mature DC after chemotherapy in patients with favorable clinical outcome. These results provide new insights into the diversity of DC subsets and its link with immune architecture in NB, impacting NB patients clinical outcome, and open new avenues for understanding pediatric tumor immunity.

Poster – 18

Dynamics in osteoclasts, the multinucleated phagocytes: a single-cell perspective on fusion and fission

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Osteoclasts (OCLs) are multinucleated, bone-resorbing phagocytes derived from fusion of myeloid precursors. They also undergo fission into osteomorphs, daughter cells that recycle into new OCLs. We previously showed that OCLs are also antigen-presenting cells, but their functional diversity remains poorly characterized. We investigated this heterogeneity using scRNAseq of BM-derived OCLs and functional assays.

After differentiation, mononucleated cells (1N), OCLs with 3–4 (3–4N) and 5–6 nuclei (5–6N) were sorted for scRNAseq. We identified 7 clusters (C0–C6), whose proportions changed with multinucleation.

Velocity analysis revealed two main lineages originating from cluster C2 (enriched in 1N), progressing through C1, C3, and C4 (enriched in 3–4N) associated with immune-related genes, including Fcgr2b/3. The trajectory continued to C0 (enriched in 5–6N), linked to bone-resorption markers such as CD200, and then split into C5 and C6, both exclusive to multinucleated OCLs. C6 displayed high resorption association, while C5 associated with osteomorph markers.

These findings were validated using FACS and analysis of spatial transcriptomics data of bone (Xenium). In functional assays, CD200+ OCLs showed high resorption but low antigen-presentation activity, while CD16/32+ OCLs exhibited opposite profile and strongly activated CD4+ T cells. C5 had intermediate resorptive activity and retained fusion potential.

Our study uncovers new insights into OCL heterogeneity and dynamics, suggesting early-fused OCLs retain immune function, and switch toward resorption specialization with further fusion and that only part of them recycles into osteomorphs. Identification of different OCL subsets may help to develop of new therapeutic options for OCL-related diseases.

Poster – 19

Autophagy deficiency in dendritic cells impacts immunoscenecence

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Aging is associated with a progressive dysregulation of the immune system, a process referred to as immunosenescence. Dendritic cells (DCs), which are key orchestrators of the immune response, exhibit functional alterations with aging that can significantly impact the immune system. Our study investigates the contribution of autophagy, a major pathway in cellular homeostasis, to DCs' function and its impact on the adaptive immune system. While autophagy declines with age its contribution to immunosenescence has not yet been fully characterized. Accordingly, we followed a cohort of old mice lacking autophagy in their DCs (Atg14flox/flox /CD11c-Cre+) and their littermate controls. We monitored their weight loss, survival and systemic signs of inflammaging by measuring cytokine levels, screening histologically peripheral organs and performing an extensive immunophenotyping of the T cell compartment. In parallel we analyzed the phenotype and function of aged DCs lacking autophagy.

Results: Our results reveal a critical contribution of autophagy in DCs to inflammaging. Atg14flox/flox /CD11c-Cre+mice show reduced survival and increased weight loss that correlate with an enhanced inflammatory status. Single-cell RNA sequencing analysis of the CD4 T cell compartment shows a skewed distribution of CD4T cell subsets with an imbalance in the naïve/memory ratio as well as an increase in the diversity of their TCR repertoire. In parallel, we find a proinflammatory transcriptional profile in autophagy-deficient DCs with a modification of their MHC Class II immunopeptidome.

Perspectives: Ongoing analysis on the phenotype and function of aged DCs will reveal how autophagy impacts immunosenescence, opening new avenues for therapeutic interventions.

Poster – 20

Human skin myeloid programmes across life stages and niches

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The human skin is the largest immune organ and plays a critical role as a barrier with the external environment. It shifts from contact with sterile amniotic fluid in utero to constant microbial exposure after birth. Macrophage and dendritic cell (DC) networks support tissue morphogenesis and pathogen defence, and their functions vary with microenvironment, ontogeny and tissue state. However, how macrophage and DC programmes change across life in healthy human skin remains poorly defined.

In prenatal skin, we previously showed that macrophages are among the earliest immune cells to seed the skin, where they align with endothelium and express pro-angiogenic gene programmes. To place these prenatal observations in a life-span context, we assembled a multi-omic single-cell atlas of prenatal, pediatric and adult human skin including ~2 million cells and performed Xenium in pediatric skin (n=19). We delineate DC (Langerhans, cDC1/cDC2/DC3, plasmacytoid, LAMP3^{+}CCR7^{+} migratory) and macrophage subsets (LYVE1^{+}, HLA-DRhigh, TREM2^{+}) and quantify broad module scores for antigen presentation, interferon responsiveness, matrix programmes and angiogenic support. Spatial mapping recovers cellular ecosystems and canonical niches: Langerhans cells are found at the epidermis, lip mucosa and sebaceous glands, and LYVE1^{+} macrophages along capillary beds.

We find prenatal macrophages are transcriptionally distinct from postnatal pediatric counterparts. We observe a shift from tissue-supportive profiles toward defence- and stress-adapted programmes, with increased antigen-presentation signatures postnatally.

By resolving life-stage and microenvironment-linked myeloid programmes in human skin—and highlighting pediatric skin as a key window of immune programming—this atlas provides a human reference for interpreting age-biased disease patterns.

Poster – 21

RIG-I-like receptor-dependent type I Interferon regulates antigen dose and activation in yellow fever vaccine 17D-infected antigen presenting cells

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The live-attenuated yellow fever vaccine 17D-204 (YF17D) activates robust innate immune responses followed by rapid induction of adaptive immunity resulting in long-lasting protection. YF17D triggers the production of type I interferons (IFNs) which have a dual role in antigen presenting cells regulating their infection and contributing to their activation. Infection with YF17D was detected in primary human blood monocytes and conventional dendritic cells (DCs) and in monocyte-derived DCs but was highly restricted by type I IFN. Blocking IFNAR signaling in YF17D-infected PBMC from vaccinated donors resulted in increased activation of YF17D-specific CD8+ T cells. Consistently, peak IFN-alpha□plasma levels correlated inversely with the CD8+ T cells response in YF17D vaccinees. Loss of function experiments demonstrated a dominant role of retinoic acid inducible gene I (RIG-I)-like receptors (RLRs) and mitochondrial antiviral signaling protein (MAVS) for type I IFN induction and restriction of YF17D. The type I IFN response was mediated by 5’ tri- or diphosphate dsRNA intermediates that are formed during YF17D infection. In vivo proximity labelling (IPL) of RIG-I and next-generation sequencing confirmed interaction of RIG-I with YF17D-dsRNA in infected cells. Thus, YF17D-triggered RLR-signaling restricts viral replication through type I IFN and thus limits the production of viral antigens that can be presented to T cells.

Poster – 22

Investigating the impact of modulating the migration of monocytes on their differentiation into tumor-associated macrophages

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Tumor-associated macrophages (TAMs) have emerged as major orchestrators of antitumor immune response. They mainly derive from monocytes, which extravasate from blood vessels and then migrate and differentiate within the tumor, and can play pro or anti-tumoral roles, depending on mechanisms that are not fully understood. Previous work from our lab has identified the lysosomal calcium channel TRPML1 as a positive regulator of myeloid cell migration. Remarkably, we found that this activating this channel within the macrophage compartment enhances the efficiency of check-point blockade on large tumors. This result suggests that increased migration of differentiating monocyte within tumors might help their differentiation into anti-tumor TAMs, which was supported by results obtained from single-cell RNAseq. These data prompted us hypothesizing that by enhancing monocyte migration, TRPML1 might modify their capacity to interact with tumoral niches, thereby modulating their differentiation program. To test this hypothesis, we built an experimental pipeline that combines flow cytometry and live-imaging experiments of tumor explants to link monocyte differentiation to their migration properties and capacity to interact with their environment (cells and extracellular matrix). We foresee that rerouting TAM differentiation by acting on monocyte migration through TRPML1 could be a promising way to enhance the efficiency of check point blockade and tumor rejection by the immune system.

Poster – 23

Expression of CD74 in myeloid cells protects the colon from DSS-induced colitis

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Ulcerative colitis, a chronic inflammation of the distal colon, is marked by a massive infiltration of myeloid cells into the lamina propria and substantial alterations in the CD4 and CD8 T cell compartments. The precise role of DCs and antigen presentation in UC remains unclear. Using public scRNA-sequencing data, we identified a population of activated DCs that emerge in the colon upon DSS-induced colitis in mice and express CD74, the MHC class II-associated Invariant Chain. CD74 is known to play a pivotal role in the capacity of DCs to process and present antigens to T cells as well as to migrate to lymph nodes. To investigate whether CD74 expression in DCs impacts on colitis, we generated CD74 conditional knockouts (cKO) using CD11c-Cre (targeting both DCs and macrophages). Remarkably, we found that these animals displayed exacerbated DSS-induced colitis, as evidenced by colon shortening as well as increased weight loss and immune cell infiltration. This phenotype was not observed when deleting the CD74 gene using LysM-Cre, indicating that it indeed involves colonic DCs rather than macrophages and neutrophils. These results suggest that CD74 expression in DCs is protective in DSS-induced colitis, suggesting that these cells may be a critical factor in UC pathogenesis.

Poster – 24

Dual-signaling CAR macrophages to reprogram the Tumor Micro-Environment and boost local anti-tumor immunity

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Despite major advances in cancer immunotherapy, many solid tumors remain refractory to treatment. These tumors are characterized by an immunosuppressive microenvironment that dampens effective immune activation and promotes tumor escape. Notably, solid tumors are often infiltrated by large numbers of monocytes and macrophages, making them attractive vehicles for therapeutic reprogramming.

We developed genetically engineered macrophages expressing Chimeric Antigen Receptors (CARs) specific for tumor antigens, incorporating intracellular domains derived from CD40 and a truncated form of STING (stimulator of interferon genes). Engagement of CD40 on antigen-presenting cells activates the NF-κB pathway and enhances expression of MHC class II and co-stimulatory molecules, while STING activation drives robust type I interferon (IFN) responses.

Our optimized construct, CAR-CD40-STING, triggers simultaneous activation of NF-κB and type I IFN pathways upon antigen recognition in vitro, leading to strong cytokine secretion and a shift toward a pro-inflammatory macrophage phenotype. These cytokines have the potential to remodel the tumor microenvironment and promote local immune activation.

Beyond direct tumor reprograming, we engineered CAR-CD40-STING macrophages as a vaccination platform, capable of presenting tumor-derived peptides on MHC class I molecules to stimulate antigen-specific T-cell responses within the tumor. This dual-function strategy aims to ignite a local inflammatory milieu and boost adaptive anti-tumor immunity, offering a versatile approach for treating solid cancers resistant to current therapies.

Poster – 25

A tumor-induced CD103$^+$EpCAM$^+$ DC2 subset mirrors human Langerhans cells and associates with immunotherapy response

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Dendritic cells (DCs) play central roles in anti-tumor immunity, yet their functional diversity and adaptation within the tumor microenvironment remain incompletely understood. To better characterize DC heterogeneity and tumor-driven adaptation, we analyzed CD11b$^+$ DC2 populations in the KP (KrasG12D, p53-/-) model of lung adenocarcinoma by single-cell RNA sequencing (scRNAseq) and flow cytometry. This analysis revealed a marked tumor-induced expansion of a CD103$^+$EpCAM$^+$ epithelial-like subset, which we refer to as EpDCs. The presence of EpDCs was validated in an independent public KP DC dataset. Functionally, EpDCs exhibited limited egress to tumor-draining lymph nodes and persistent retention within the TME in a CCR7$^-$ immature state. Moreover, the engraftment of human NSCLC tumors induced the differentiation of human EpDCs in humanized mice. Comparative transcriptomic analyses revealed that EpDCs closely resemble human CD207$^+$CD1a$^+$ Langerhans cells (LC) DCs, which are similarly enriched in human tumors. Application of an LC-like DC gene signature to a cohort of patients treated with anti–PD-L1 therapy showed that enrichment of this signature correlated with improved survival in a subset of patients with high tumor mutational burden suggesting a role in anti-tumor immunity against neo-epitopes. Collectively, our findings identify EpDCs as a tumor-induced, immature DC subset analogous to LCs DCs, with potential implications for anti-tumor immunity and response to immune checkpoint blockade.

Poster – 26

Impact of skin fibrosis on the immune response of dendritic cells

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We have recently shown that the deformation events dendritic cells (DCs) experience when migrating in constrained environments lead to the upregulation CCR7, enabling their migration to lymph nodes. This is dependent on the lipid metabolism enzyme cPLA2, which is activated upon stretching of the nuclear membrane of DCs, highlighting the mechanosensitivity of these cells. The aim of this study is to investigate the reciprocal relationship between DC mechanosensing and tissue fibrosis that results from excessive deposition and cross-linking of extracellular matrix components, notably collagen, which profoundly alters tissue mechanical properties. We aim at understanding how DCs not only respond to fibrotic changes but also contribute to the establishment of a fibrotic stage associated with immune exhaustion and increased susceptibility to pathology. We used the bleomycin-induced skin fibrosis model, in which mice received subcutaneous injections five days per week for two weeks. Samples were collected at multiple time points to monitor fibrosis progression and DC migration. Our observations reveal that dermal thickening coincides with extensive remodeling of white adipose tissue, where adipocytes are replaced by an aligned collagen network. DC migration to draining lymph nodes becomes progressively impaired and is nearly abolished by day 21. Interestingly, CCR7+ DCs accumulate within fibrotic skin in two distinct niches: (i) adjacent to Lyve1^{+}CD31^{+} vessels near hair follicles in the dermis, and (ii) near Lyve1^{-}CD31^{+} vessels within remodeled white adipose tissue. Using cPLA2flox/flox*CD11c-Cre mice, we further found that deletion of cPLA2 in the CD11c compartment partially rescues DC migration to draining lymph nodes. Unexpectedly, cPLA2 deletion in this compartment also abolished dermal fibrosis. These findings suggest a spatiotemporal regulation of DC migration during fibrosis and highlight a potential mechanistic link between DC mechanosensing and fibrotic progression.

POSTER ABSTRACTS

Friday December 5th, 2025

Poster – 27

Skin TSLP acts on transitional dendritic cell-derived tDC2 to promote GATA3-expressing effector regulatory T cells

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Originally identified as a pro-Th2 cytokine, thymic stromal lymphopoietin (TSLP) has been recently reported to promote via dendritic cells (DC) the generation and accumulation of GATA3-expressing effector regulatory T cells (eTreg) in the context of cutaneous melanoma. In this study, employing an experimental mouse model with induced TSLP expression by epidermal keratinocytes combined with genetic tools, we deciphered TSLP-triggered DC-T cell axes, revealing that TSLP drives GATA3+ eTreg through a specific migratory DC population where the costimulatory molecule OX40L is crucially required. By conducting studies from transcriptomic identity, lineage-traced ontogeny, surface marker expression to functionality, we identified and characterised this DC population. Our data demonstrated that TSLP acts on tDC2 derived from transitional DC (tDC) to promote GATA3+ eTreg, thus uncovering a previously unrecognised tolerogenic axis in promoting immune suppression, which is likely conserved in human, in pathophysiological contexts such as inflammation and cancer.

Poster – 28

Senescent cells upregulate immune checkpoint PD-L1 to evade immune surveillance

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The accumulation of senescent cells promotes aging and age-related diseases, but molecular mechanisms that senescent cells use to evade immune clearance and accumulate in tissues remain to be elucidated. Here, I report that p16-positive senescent cells upregulate the immune checkpoint protein programmed death-ligand 1 (PD-L1) to accumulate in aging and chronic inflammation. I show that p16-mediated inhibition of cell cycle kinases CDK4/6 induces PD-L1 stability in senescent cells via downregulation of its ubiquitin-dependent degradation. p16-expressing senescent alveolar macrophages elevate PD-L1 to promote an immunosuppressive environment that can contribute to an increased burden of senescent cells. Treatment with activating anti-PD-L1 antibodies engaging Fc-gamma receptors on effector cells leads to the elimination of PD-L1 and p16-positive senescent cells in aged and chronically inflamed lungs. Our study uncovers a molecular mechanism of p16-dependent regulation of PD-L1 protein stability in senescent cells and reveals the potential of targeting PD-L1 to improve immunosurveillance of senescent cells and ameliorate senescence-associated damage and systemic inflammation.

Poster – 29

The extracellular matrix of oral squamous cell carcinoma modulates the spatial distribution and phenotypes of tumor-associated macrophages in OSCC mouse model

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Oral squamous cell carcinomas (OSCC) are cancers of the oral cavity and represent the sixth most common cancer worldwide. Despite therapeutic advances, only 50% of patients survive five years after diagnosis due to relapse and loco-regional spread. Recent studies have identified Tenascin-C (TNC), a major extracellular matrix component, as an immunosuppressive factor upregulated in OSCC that promotes tumor progression. However, its role in modulating tumor-associated macrophages (TAMs), key players of the OSCC immune microenvironment, remains poorly understood. TAMs contribute to tumor growth and metastasis by secreting pro-inflammatory cytokines and promoting angiogenesis, thereby sustaining an immunosuppressive microenvironment. Using spectral flow cytometry and multiparametric imaging, our laboratory identified a heterogeneous macrophage population in human OSCC, including a subset expressing both CD206 and CD11c, predominantly localized within TNC-rich regions. Our goal is to perform a comprehensive characterization of macrophage subsets, focusing on $CD206^{+}CD11c^{+}$ macrophages, to clarify their specific role in driving tumor progression in murine models of OSCC. Two complementary models are used: a 4-nitroquinoline-1-oxide (4-NQO)-induced carcinogenesis model and an orthotopic mEERL95 tumor model established in TNC wild-type and knock-out mice. Spectral flow cytometry revealed macrophage heterogeneity and an enrichment of $CD206^{+}CD11c^{+}$ macrophages in TNC-expressing tumors. Spatial analysis confirmed their accumulation within TNC-enriched regions, with increased maturation and phagocytic activity. Importantly, pharmacological blockade of macrophages using an anti-CSF1R treatment led to a reduction in tumor volume, supporting their functional role in OSCC progression.

Poster – 30

Bidirectional Crosstalk Between Oncofetal Cells and SPP1 Macrophages Drives Fetal-Like Reversion in CRC

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Colorectal cancer (CRC) is rising in young adults, with fetal-like reversion implicated in tumor development. SPP1 macrophages and fetal cells appear central to this process. This study investigates what drives fetal-like reversion and how fetal cells reciprocally influence macrophage differentiation.

Spatial transcriptomics from CRC patients were used to map SPP1 macrophages and ANXA1+ fetal-like epithelial cells. Single-cell data was aggregated into normalized pseudo-bulk profiles and analyzed with ssGSEA, followed by CellChat to evaluate cell–cell communication. In vitro, KPN, AK and A organoids were stimulated for 24h with IFNγ,IL-6,TNFα or TGFβ1, and fetal-like status was assessed by flow cytometry (LY6A). Organoids were co-cultured for 48h with M0, M1 or M2 macrophages to evaluate fetal-like reversion and macrophage polarization (CD80,CD86,CD163,CD206).

Our analyses showed SPP1 macrophages closely localized with oncofetal epithelial cells, forming a rim around anxa1^{+} regions, with MIF–CD74 identified as a key interaction axis. Single-cell data revealed four tumor clusters enriched for fetal-like signatures, separating into high-IFN and intermediate-IFN groups. High-IFN clusters displayed activated cGAS/STING and STAT1 pathways with enhanced antigen presentation, while intermediate-IFN clusters showed increased TGFβ,STAT3,TNFα and IL-6 signaling. In vitro, IFNγ,IL-6,TNFα and TGFβ1 elevated Ly6a expression in organoids. Fetal organoids induced immunosuppressive macrophage polarization, whereas stem-like organoids promoted pro-inflammatory states; reciprocally, immunosuppressive macrophages drove fetal-like reversion in organoids.

SPP1 macrophages and oncofetal cells form a mutually reinforcing niche driving fetal-like reversion in colorectal cancer. This bidirectional loop between epithelial state and macrophage polarization may represent a key mechanism of tumor progression and a promising therapeutic target.

Poster – 31

Breaking the Wall of Immunotherapy Resistance in MSS Colorectal Cancer Liver Metastases

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Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide, with liver metastases in advanced stages markedly worsening prognosis due to poor responses to immune checkpoint blockade. Our data indicate that CRC progression involves coordinated reprogramming of epithelial, stromal, and innate immune compartments, forming “bad niches” that shape treatment outcomes. In a mouse model with intracolonically injected AKPT tumor organoids, genetic ablation of monocyte-derived macrophages markedly reduced tumor growth and improved survival under Fc-enhanced anti-CTLA-4/anti-PD-1 therapy, highlighting the critical role of macrophages in mediating immunotherapy efficacy. Notably, this therapeutic mechanism relies on macrophage IFN signaling, suggesting that specific myeloid subpopulations may remain unresponsive and contribute to resistance. Supporting this, $SPP1^{+}$ macrophages, characterized by low IFN activity, have been shown to mediate anti–PD-1 resistance via TGF-β signaling, and metastatic lesions exhibit similarly low IFN levels, further underscoring the importance of the myeloid compartment in CRC metastases. We hypothesize that niche evolution from primary tumors to liver metastases involves extensive reprogramming of hepatic myeloid populations. Leveraging comprehensive human spatial omics, we show that the organization and functional states of myeloid cells differ between primary tumors and liver metastases, highlighting tissue-intrinsic determinants of therapy response. Deep profiling of myeloid populations and their role in shaping immunosuppressive niches will be essential to identify combinatorial strategies to overcome therapy resistance and improve outcomes in metastatic CRC patients.

Poster – 32

Evidence for a myeloid dendritic cell subset that modulates CD123 expression in kidney transplant recipients.

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Dendritic cells (DC) are key mediators of antigen presentation, particularly in kidney transplantation. Yet, their roles in this setting remain underestimated. To investigate these roles, peripheral blood mononuclear cells (PBMC) from healthy donors and kidney-transplant recipients were analyzed using multiparametric flow cytometry.
We characterized seven DC subsets: AS-DC, pDC, cDC1, DC2, DC3, moDC and tolDC. These PBMC were stimulated with a cocktail of TLR-ligands (R848, CpGA, PolyI:C) to assess changes in the expression of immune checkpoints (ICP) across the different dendritic populations. In a subset of patients, we identified an unexpected cell population Lin (CD3/19/56/203c)- /DR+ /CD14-, co-expressing CD11c and CD123/IL-3R. In-depth analysis indicates that these cells correspond to DC4 (CD11c+ CD1c- BDCA-3- CD16+), previously described by Villani et al. ICP profiling revealed a significant decrease in CD80 (-69%), CD86 (-42%) and ICOS-L (-41%) in these cells, whereas CD40 and OX40-L were increased following stimulation (+28 and (13% respectively). Expression of CD123 appeared to be associated with a distinct activation profile. Indeed, DC4 CD123+ displayed a 32% reduced expression of OX40-L compared to DC4 CD123-.

Poster – 33

Deciphering the Tissue Instruction of Plasmacytoid dendritic cell functions in homeostasis and during viral infections

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In most systemic viral infections, plasmacytoid Dendritic Cells (pDCs) are the main source of type I interferon (IFN-I). During systemic viral infection by mouse cytomegalovirus (MCMV), a natural rodent pathogen, most of circulating IFN-I are produced by a minor fraction of splenic pDCs, while pDCs residing in others organs are unable to exert this function (Zucchini et al. Int Immunol 2008). Moreover, the ability of pDCs to produce IFN during systemic viral infection appears mainly restricted to pDCs residing in lymphoid organs, suggesting that organ-specific cell type(s) named “niche” can instruct the pDC functions. We previously showed at the single-cell level that, during MCMV infection, splenic IFN-producing pDCs sequentially acquire five distinct functional activation states that are spatiotemporally regulated (Abbas et al. Nat Immunol 2020). Indeed, at the peak of IFN production IFN-producing pDCs localize in splenic marginal zone (MZ), where they interact with MCMV-infected cells, while later on, once they have ceased IFN production, ex-IFN+ pDC express CCR7, migrate to the T-cell zone (TCZ) and acquire antigen-presenting functions. By using intersectional genetics, we recently generated a new mouse model, the SCRIPT mice, that allows a specific identification of pDCs by fluorescence and discriminates within pDC IFN-producing vs non-producing cells (Valente et al. Nat Immunol 2023). Indeed, in MCMV-infected SCRIPT mice only IFN-producing pDC can migrate to TCZ, while non-producing pDC accumulate in the MZ. We hypothesize that infection-induced signals mediated by niche-cells located in the MZ vs TCZ, respectively, instruct pDC to exert IFN production vs antigen presentation.

Poster – 34

Tumor stage-dependent remodelling of lung-resident cDC1 in a non-small cell lung cancer model

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Cancer progression challenges cDC1-mediated immunosurveillance by impairing their differentiation, function, and survival. However, the progressive adaptation of the cDC1 compartment in slowly developing endogenous tumor models has been poorly investigated.
Here we crossed the KP (KrasG12D/+; Trp53fl/fl) model of non-small cell lung cancer to a cDC1 reporter (XCR1-venus) to capture the evolution of lung resident cDC1 in normal lungs, early tumors and established adenocarcinoma.
We found that tumor progression induces accumulation of cDC1, paralleled by an increase in the number of preDCs seeding the lung and an expansion of committed preDC1 in the bone marrow. Bulk RNA-seq of lung cDC1 suggested early upregulation of maturation genes, cytokines, and genes controlling cross-presentation, whereas late-stage tumor cDC1 express high level of regulatory genes and reduced proliferative genes. scRNA seq identified four distinct cDC1 clusters corresponding to early committed cDC1, resting cDC1, homeostatically activated (h.a.) cDC1, and ISG-activated cDC1, plus a fifth, less abundant cluster corresponding to mature regulatory cDC1 (CCR7+/Fscn1+/LAMP3+). Intriguingly, advanced tumors were largely depleted of resting and h.a. cDC1 and enriched in early cDC1 and ISG–activated cDC1.
Spatial analysis of control lungs and tumor tissues showed cDC1 organized in clusters that preferentially localize around vessels. cDC1-centric immune hubs containing T and B cells formed near vessels and progressively organized into tertiary lymphoid structures as tumors develop. Ongoing analyses aim to localize diverse cDC1 subsets within tumor tissues and probe their activity.
Altogether, this study illuminates the dynamics of cDC1 reprogramming in a model that reflect disease progression in humans.

Poster – 35

Targeting the stromal cell compartment of tumors for the induction of tertiary lymphoid structure to activate anti-tumor immunity

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Rationale: Tertiary lymphoid structures (TLS) are ectopic immune cell aggregates that recapitulate the structural organization of secondary lymphoid organs (SLOs) and arise postnatally within peripheral, non-lymphoid tissues, particularly in contexts of chronic inflammation. TLSs have been widely observed in multiple cancer types, where their presence correlates with improved patient prognosis. This supports the hypothesis according to which therapeutic intervention activating TLS formation and persistence could support the development of anti-tumor effects. Objective: The aim of our project is to develop cell-based strategies targeting the tumor stromal compartment to promote the formation of TLSs. Approaches and results: Our team is developing a stromal cell-based targeted therapy purposed to generate TLS in cancer models, through the targeted activation of the lymphotoxin signaling pathway. Indeed, there is substantial in vivo genetic evidence that activation of this signaling pathway is a core component of the organogenesis of both SLO and TLS through the activation of mesenchymal stromal cells and the differentiation of vascular cells into high endothelial venules (HEV)-like cells. Here, we report our first attempts in recapitulating this differentiation processes of stromal cells supporting TLS formation by screening multiple signaling pathways synergizing with the lymphotoxin pathway in mediating TLS-relevant stromal cell activation both in vitro and in vivo.

Poster – 36

Immune checkpoint inhibition immunotherapy promotes the expansion of early hematopoietic progenitors expressing dendritic cell markers

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Dendritic cells (DCs) are short-lived cells that need to be constantly renewed during hematopoiesis in the bone marrow (BM). Given their important contribution to the success of immunotherapies with immune checkpoint inhibitors (ICI), we hypothesized that these therapies might promote their generation.

OBJECTIVES: Here, we investigate the regulation of DC development in a model of productive anti-tumor response activated by ICI therapy.

METHODS: We analyzed DCs and their progenitors in the grafted melanoma mouse model Yumm1.7-OVA, which is responsive to anti-PD1 and anti-CTLA4 antibodies.

RESULTS: Through flow cytometry, we first demonstrated that ICI induce the expansion of BST2+ SIGLECH+ CX3CR1+ plasmacytoid DC-like cells in the spleen. A single-cell RNA sequencing revealed dynamic changes in early hematopoietic progenitors within the BM. Notably, we identified an ICI-induced cluster of Lin- C-KIT+ SCA1+ multipotent progenitors (MPP) expressing DC markers and IFN-stimulated genes. Administration of anti-IFNAR and, to a lower extent, anti-IFNGR blocking antibodies during ICI therapy prevents the accrual of this cluster. Accordingly, administration of recombinant IFNa/b, but not IFNg, is sufficient to upregulate DC markers in the MPP population.

CONCLUSION: Altogether, we demonstrated that a transient IFN signalling during the early stages of ICI immunotherapy remodels early hematopoietic progenitors to express multiple ISGs and DC-associated genes. The immunological consequences of these ICI-induced changes for tumor control are currently under investigation. Our findings have important implications for designing novel interventions that target early myeloid progenitors undergoing DC commitment, potentially addressing primary resistance to ICI immunotherapy.

Poster – 37

ETV6 controls microbiota-driven type I interferon production in monocytes and is a target for improving the efficacy of anti-PD1 therapy in cancer

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Cells of the monocyte–macrophage lineage play a crucial role in shaping anti-tumor immunity and resistance to treatment. Recent studies have demonstrated that tumor-infiltrating monocytes can promote anti-tumor responses through type I interferon (IFN-I) secretion within the tumor microenvironment. A better understanding of the underlying molecular mechanisms is essential for harnessing this phenomenon for therapeutic interventions. Here we find that deletion of the transcription factor ETV6 in monocytes unleashes microbiota-driven IFN-I production. Mechanistically, we demonstrate that ETV6 acts as a transcriptional repressor of STING. Using the MCA101-OVA fibrosarcoma model, we show that ETV6 deletion in monocytes increases the efficacy of anti-PD1 treatment and promotes increased cytotoxic responses in the tumor microenvironment. Altogether, our findings identify ETV6 as a potential therapeutic target to reprogram monocytes and enhance anti-tumor immunity in combination with checkpoint blockade therapy.

Poster – 38

Characterization of IL-23 producing cells during Klebsiella pneumoniae pulmonary infection

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Pneumonia caused by antibiotic-resistant bacteria poses a significant threat to public health. Developing strategies to reinforce anti-infectious innate immune defenses against these infections represents an innovative therapeutic approach. Dendritic cells detect microbial signals and convert them into immunological signals, thus playing a central role in controlling the anti-infectious immune response, particularly the production of interleukins 17 and 22 (IL-17/22). These interleukins play a key role in the lung's defense against extracellular pathogens such as Klebsiella pneumoniae. Despite the establishment of the crucial role of Interleukin-23 (IL-23) in the modulation of IL-17/22 production, the cellular source of IL-23 remain unclear, especially in the lung. Using a murine model of K. pneumoniae pulmonary infection, we observe a peak in inflammatory mediator expression (Il6, Il12b, Il23a, …) in lung tissue at 21h post-infection. This transcriptional response is associated with an influx of immune effectors like neutrophils or inflammatory monocytes. Concerning the dendritic cells (DCs) population, only number of inflammatory DCs is increased during early stage of K. pneumoniae infection. Then, an analysis of the transcript of various lung's dendritic cells subsets suggest that mainly inflammatory DCs express Il23a and Il12b during infection. Finally, a first single-cell RNA analysis revealed a distinct population of dendritic cells exhibiting an inflammatory profile that expresses Il23a mRNA. Our findings delineate a subset of lung inflammatory dendritic cells that promote antimicrobial immunity, presenting a promising target for the development of innovative immunotherapy strategies to combat multidrug-resistant infections.

Poster – 39

Targeting Non-Canonical NF-κB Signaling to Modulate mregDC Function

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Dendritic cells (DCs) are professional antigen-presenting cells and the orchestrators of immune responses. Upon maturation in cancer settings, DCs can acquire an immunoregulatory profile, referred to as “mature DCs enriched in immunoregulatory molecules” or mregDCs. These cells express signature genes of maturation/migration (like LAMP3, CD40, CD86 and CCR7) and tolerance (including PDL1, TIM3 and CD200). As this dual role has a potential effect on immune responses in the tumour context, in-depth insights into mregDC origins and the mechanisms governing their differentiation are necessary. We generated an in vitro model of DC differentiation from human bone marrow CD34+ progenitors, without a tissue or inflammatory context. We characterized phenotypically the kinetics of different maturation states of DCs, associated with variable expression of new markers (like IL-7R and GM-CSFR). Furthermore, these states of maturation were conserved across the three subsets of DCs (conventional DC1, DC2 and DC3). Moreover, we investigated the molecular pathways controlling mregDC development. The non-canonical NF-κB pathway is a major signaling cascade that regulates immune responses in a stimulus-specific manner. Using agonistic antibodies, chemical inhibitors and gene editing by CRISPR-Cas9, we demonstrated the implication of the non-canonical NF-κB pathway in driving the mregDC state. We further found the transcription factor STAT3, known for orchestrating immunosuppressive responses in cancer, as a negative regulator of the mregDC programming. These findings contribute to a better understanding of mregDC development in humans, with potential implications for immunotherapy.

Poster – 40

Spatiotemporal Profiling of Dendritic cells in Pancreatic Ductal Adenocarcinoma

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The processing and presentation of tumor-derived antigens by dendritic cells (DCs) constitutes an integral part of the anti-tumoral immune response and is required to prime tumor-specific T cells. Pancreatic ductal adenocarcinoma (PDAC) is characterized by an immunosuppressive tumor microenvironment (TME), but how it modulates DC dynamics and their antigen presentation machinery is not yet fully understood. Here, we aim to characterize DC heterogeneity and function in a spatiotemporal manner throughout PDAC onset and progression. Using an murine orthotopic tumor model, we show that DC1, DC2, DC3 subsets and their respective precursors infiltrate the tumor early on, but reduce in numbers throughout PDAC progression. This was accompanied by similarly low levels of migratory CCR7+ DCs. While MHCII protein levels were unchanged on DCs from the TME compared to healthy tissue, they displayed an increased occupancy of MHCII by the Class II-associated invariant chain peptide (CLIP) that blocks the MHCII binding groove until replacement by a processed antigen. Moreover, disruption of antigen presentation by the removal of the MHCII invariant chain CD74 accelerates PDAC tumor growth and worsens survival. In the next steps, interaction networks of DCs with other TME-resident immune cells, as well as their gene expression and spatial localization will be investigated in order to further understand TME-mediated DC paucity and dysfunction.

Poster – 41

Identification of a Cxcl9+ cDC1 subpopulation increasing at early stages of tumor development that colocalize with T cells

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While tumor immune evasion mechanisms are now well characterized in mammary tumors, the very early events implicated in the immune sensing of preneoplastic cells remain poorly understood. To characterize the molecular and cellular mechanisms of early immune surveillance in triple-negative breast cancer (TNBC), we used a spontaneous sporadic mammary tumor model in mice which recapitulates the different stages of development (Healthy, Pre-neoplasia and invasive carcinoma). Given the important role of dendritic cells (DC) in the initiation of anti-tumor immune response, we hypothesize they could play a central role in the anti-tumor immune surveillance. We performed in-depth analyses of DC types sorted from mammary tissues at the different stages by single cell RNA sequencing (scRNAseq) and single cell spatial transcriptomics. Preliminary analysis revealed DC heterogeneity (e.g., presence of mature DCs) and different activation states. Based on their role in anti-tumor immunity, we focused on cDC1 and highlighted 4 subpopulations with a specific increase of Cxcl9+/Ccr7- cDC1 cluster from preneoplastic stage likely representing an intermediate state between immature and mature cDC1. Using spatial transcriptomics, we discovered immune aggregates involving mature DC in preneoplastic areas and confirmed that the Cxcl9+/Ccr7- cDC1s colocalize with T cells withing those aggregates. By integrating spatial and scRNAseq datasets, these observations will be pursued through ligand receptor interaction analyses between Cxcl9+ cDC1 and interacting cells in the objective to understand the mechanisms of early immune surveillance of DC.

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Targeting inhibitory receptors on tumor infiltrating epithelial DCs in lung cancer

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Dendritic cells (DCs) orchestrate T cell–mediated immunity against lung cancer, yet their activation and migratory programs are often impaired. While CCR7high DCs can support CD8+ T cell priming through IL-12 production, tumor-associated DCs often remain in an immature, regulatory state. Using the KrasG12D; p53-/- (KP) model of non-small cell lung cancer, we analyzed DC heterogeneity by flow cytometry, single-cell RNA-seq, and spatial imaging. We identified a distinct population of CD103+ DC2s enriched in tumor-bearing lungs, localized near proliferating tumor cells. Transcriptomic profiling revealed an epithelial-like gene signature (Epcam, Cdh1, Cldn1) and reduced interferon response pathways. Functionally, these CD103+ DC2s were CCR7low and poorly mature, suggesting impaired capacity to prime T cells. We hypothesize that inducing activation of these epithelial-like DCs (EpDCs) might unleash the activation of tumor-specific T cells. Here, we report that EpDCs express ITIM-bearing inhibitory receptors and that antibody-mediated blockade of these myeloid checkpoints limits tumor growth while enhancing the expansion of EpDCs and tissue-resident memory (Trm) $CD8^+$ T cells. Specifically, targeting the ITIM-bearing receptor SIGLEC-E, a member of the Siglec family, reduced tumor load, consistent with improved antitumor immunity.

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The antiviral immune function of the alarmin S100A9 is governed by ion-chelation activity in human myeloid cells

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Although alarmin-driven inflammatory conditions can exert proviral effects, we and others have recently unveiled an antiretroviral function of the alarmin S100A9 in human myeloid cells. We now further established that Ca2+/Mg2+-binding motifs are essential for S100A9-mediated antiviral function which occurs at the HIV-1 reverse transcription (RT) step. Indeed, chelation-defective S100A9 mutants failed to restrict HIV-1 RTase activity. Modulation of Ca2+ or Mg2+ levels abrogated S100A9 WT-mediated HIV-1 restriction while having no effect on S100A9 mutants. This effect was further supported by experiments done in human primary MoLC expressing endogenous S100A9. Biochemical experiments uncovered the presence of S100A9-containing complexes correlating with HIV-1 restriction phenotype. Finally, immunofluorescence confocal imaging revealed an unexpected co-localization of S100A9 with lipid membrane remodelling proteins galectin-3 and ATG9. Interestingly, S100A9 appeared excluded from HIV-1-containing compartment, especially at virological synapses between infected myeloid cells and target CD4+ T cells. This may explain the increased viral transmission rate to target cells observed upon coculture with myeloid cells deficient for S100A9. Therefore, our findings uncovered a novel chelation-dependent mechanism driving the intracellular antiretroviral function of human S100A9 and highlighted the existence of potential intracellular “alarmin-hubs” which might contribute to host innate immunity.

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Regulation of tissue-resident memory CD8$^+$ T cells by antigen-presenting cells in the tumor microenvironment

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Tissue-resident memory CD8+ T cells (CD8+TRM; CD103+CD69+CD49a+) represent a non-circulating subset of immune cells that permanently reside within peripheral tissues. High CD8+TRM density in the tumor microenvironment (TME) has been associated with improved patient survival in breast and lung cancers. However, the mechanisms underlying their activation and regulation within the TME remain unclear. Notably, positive correlations between CD8+ TRM and several antigen-presenting cell (APC) subsets including cDC1, DC3, FOLR2+ macrophages and Langerhans cells, as well as their spatial proximity within tumors suggest that distinct APC populations may play a key role in CD8+TRM regulation.

My PhD project aims to understand the role of APCs in regulating CD8+TRM antitumor functions within the TME of breast and lung cancer patients. Using spectral flow cytometry, we identified heterogeneous CD8+TRM populations, including a proliferative (Ki67+) subset expressing multiple chemokine receptors. Further, we are currently characterizing APC subsets with a particular focus on those expressing E-cadherin (CD103 ligand) and chemokines potentially involved in CD8+TRM localization and maintenance.

In addition, spatial analyses using high-plex tissue imaging revealed direct physical contacts between CD8+TRM and APCs, suggestive of immunological synapse formation and local reactivation. Our ex-vivo live imaging analysis further confirmed that CD8+TRM establish dynamic synapses (kinapses) with monocyte-derived dendritic cells (MoDCs), leading to their activation.

Together, these findings highlight the phenotypic diversity of CD8+TRM and the complexity of their crosstalk with APCs. They provide new insights into the mechanisms driving local CD8+TRM reactivation within tumors and identify potential clues for therapeutic modulation of their antitumor activity.

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HTLV-1-Infected T Cells and Dendritic Cells: A Reciprocal Manipulation?

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Immune dysfunctions, particularly the poor responsiveness of dendritic cells, have been repeatedly reported in HTLV-1 carriers, including asymptomatic individuals. Our team previously showed that in vitro exposure of monocyte-derived dendritic cells (MDDCs) to HTLV-1 impairs their maturation and responsiveness to TLR stimulation, independently of viral capture or infection, a phenotype reminiscent of innate immune tolerance. This impaired responsiveness results from cross-talk between MDDCs and HTLV-1–infected T cells, mediated by soluble factors. To identify these mediators, we analyzed the transcriptomes of HTLV-1–exposed MDDCs and of infected T cells cocultured with MDDCs. This analysis revealed an upregulation of genes involved in lipid biosynthesis in MDDCs, and of genes associated with lipid transport and catabolism in infected T cells. Consistent with these findings, our functional assays suggest a transfer of lipids from MDDCs to infected T cells, which may subsequently trigger the production of soluble inhibitory mediators by the latter. To further characterize the factors underlying this cross-talk, we are conducting omics analyses of the co-culture supernatant to assess its lipid and protein composition, which may be altered during co-culture. Overall, our work highlights the complex interactions between MDDCs and HTLV-1–infected T cells, ultimately contributing to the virus's escape from dendritic cell–mediated innate functions.

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Monocyte engineering to promote anti-tumor immunity

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Despite significant progress, immunotherapies remain largely ineffective against solid tumors. Current strategies, including immune checkpoint blockade, CAR T cells, and T cell engagers, primarily target T cell activities. Meanwhile, extensive research has revealed the abundance and pro-tumoral activity of myeloid cells within the tumor microenvironment (TME) across various cancer types. However, effective tools to harness these cells for anti-tumor responses are still lacking.

Here, we describe a novel approach to reprogram the TME by locally activating myeloid cells through NF-κB and interferon signaling. Our therapeutic strategy relies on the genetic modification of autologous monocytes prior to their reinfusion into the patient. To this end, we engineered a chimeric antigen receptor (CAR) incorporating a STING-derived intracellular signaling domain, termed CAR-STIZ.

Monocytes from healthy donors are transduced with lentiviral vectors to generate CAR macrophages (CAR-M), which expressed the receptor at the plasma membrane. The STING-based CAR-STIZ can signal at the plasma membrane of CAR-M independently of the endogenous STING. Upon tumor antigen recognition, CAR-STIZ activates IRF3 and NF-κB pathways, leading to the secretion of inflammatory cytokines and upregulation of co-stimulatory molecules by CAR-M . In functional assays, CAR-M control tumor growth in a 3D spheroid model via TNFα-dependent mechanisms and importantly, cooperate with autologous T cells to eliminate tumor cells in co-culture. In an immunodeficient mouse model reconstituted with autologous PBMCs, intratumoral injection of CAR-M results in partial tumor control.

Our study introduces a novel strategy for myeloid cell engineering by leveraging a STING-derived intracellular domain that promotes macrophage activation and inflammatory signaling. Unlike previous CAR-M designs focused mainly on phagocytosis, CAR-STIZ macrophages remodel the TME and enhance T cell-mediated anti-tumor responses.

This platform provides a promising complement to existing immunotherapies, particularly for solid tumors resistant to T cell–centric approaches.

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Role of FOLR2+ macrophages in the recruitment of lymphocytes

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We recently identified a distinct subpopulation of macrophages in human and mouse breast tumors that expressthe folate receptor 2 (FOLR2). FOLR2+ macrophages localize within lymphoid aggregates, and their abundance is associatedwith increased CD8+ T cell infiltration. We therefore hypothesized that FOLR2+ macrophages could contribute to anti-tumor immunity via the recruitment of lymphocytes. To investigate this, we used a pre-clinical lung cancer model to characterize the spatial distribution of FOLR2+macrophages and their cellular interactions. We found that FOLR2+ macrophages are localized around bronchovascularbundles and at the level of the pleura lung lining, but are largely excluded from tumor islets. FOLR2+ macrophages are in close proximity with CD3+ T cells and MHCII+ antigen-presenting cells, suggesting potential roles in local immune regulation. The two distinct localizations of FOLR2+ macrophages lead us to hypothesize that FOLR2+ macrophages might be an heterogenous population. Reanalysis of published scRNA-seqdata revealed that FOLR2+ macrophages are a heterogeneous population comprising distinct subsets (CX3CR1+FOLR2int and TIM4+LYVE1+FOLR2+ (TLF)). In addition, TLF macrophages can be subdivided into two transcriptionally distinct populations, whose spatial organization remains to be determined. To assess the contribution of FOLR2+ macrophages in anti-tumor immunity, we developed a genetic model allowing inducible depletion FOLR2+ macrophages. We found that the distinct FOLR2+ subsets follow different kinetics of repopulation after depletion, suggesting distinct ontogenetic origin and turnover. In sum, our findings reveal spatially restricted heterogeneous subsets of FOLR2+ macrophages with potential roles in shaping the immune microenvironment. Ongoing studies will elucidate their function in anti-tumor immunity.

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The role of FOLR2+ macrophages in anti-tumor immunity

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We have recently identified a subpopulation of macrophages infiltrating human and mouse breast tumors and characterized by the specific expression of folate receptor 2 (FOLR2). Building on the close proximity between FOLR2+ TAMs and tumor-infiltrating $CD8^+$ T cells, we hypothesized that FOLR2+ macrophages could contribute to anti-tumor immunity by supporting T-cell infiltration or maintenance. We developed a genetic model allowing the inducible depletion of FOLR2+ macrophage in a pre-clinical breast cancer model. We confirmed that depletion of FOLR2+ macrophages leads to an increase in tumor growth associated with a decrease in tumor specific CD8+ T cells infiltration and activation. We also hypothesized that FOLR2+ TAMs could be therapeutically harnessed for in situ activation of $CD8^+$ T cells by targeting tumor-antigens to the endocytic receptor FOLR2. We designed an anti-FOLR2 antibody coupled to MHC-I and MHC-II-restricted ovalbumin immunodominant peptides. We validated that FOLR2+ macrophages can uptake and present OVA peptides to antigen-specific T cells in vitro. We also validated their specific targeting in vivo. Finally, we showed that FOLR2+ macrophages targeting in vivo in a mouse model of breast cancer, fosters the tumor infiltration of adoptively transferred OVA specific T cells and their persistence. Taken together, our results show that FOLR2+ macrophages are important for effective anti-tumor immunity by promoting T-cell infiltration and activation. We also showed that targeted delivery of antigens to FOLR2+ macrophages could enable specific activation of antigen specific T cells, thereby optimizing anti-tumor immunity.