immunohistochemistry. Paraffin sections were stained with hematoxylin and eosin (H&E) or Ki67 according to standard methods. For immunohistochemistry, cryosections were stained overnight at 4°C with fluorescent antibodies.

Results H&E images confirmed that transgenic *Apc*^{1322T}mice lacking IL-25 had smaller tumours and showed less dysplasia than *Apc*^{1322T} mice with normal IL-25 expression. Ki67 staining showed that tumours express higher Ki67 levels than adjacent normal intestinal tissue. The tumour-associated tertiary lymphoid structures (TATLS) of *Apc*^{1322T} mice lacking IL-25 appeared larger, indicating a more robust anti-tumour immune response.

Likewise, *Apc*^{1322T}mice lacking ILC2s had smaller, less dysplastic tumours. TATLS in these mice were bigger than mice with ILC2s but smaller than *Apc*^{1322T} mice lacking IL-25, indicating that IL-25 may act via additional protumourigenic cell types.

Immunohistochemistry confirmed the presence of ILC2s, as well as MDSCs the tumours of *Apc*^{1322T}mice, suggesting that these cells create an immunosuppressive niche.

Conclusions This pilot study confirms that genetic ablation of either IL-25 or ILC2s promotes anti-tumour immune reactions and decreases tumour size, correlating with reduced intestinal tumour proliferative capacity and dysplasia. Mice lacking IL-25 or ILC2s had larger TATLS, which are known to be associated with improved prognosis in patients. This study, along with previous data, highlights the potential therapeutic benefit of targeting the IL-25-ILC2 axis for colorectal cancer. Further studies are required to bring this from bench to bedside.

REFERENCE

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P02.07 INNATE IMMUNITY ATLAS OF HEPATOCELLULAR CARCINOMA UNRAVELS THE DIFFERENTIATION HIERARCHY OF MYELOID NK CELLS AND MDSCS

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Background Hepatocellular carcinoma (HCC) environmental risk factors include viral infection, alcohol abuse and the metabolic syndrome. While there is evidence that boosting the activity of tumor-specific T cells might benefit patients with HCC, the underlying liver soil (cirrhosis, NASH) renders this cancer's tumor microenvironment somewhat unique. Despite a significant therapeutic advance in the treatment of advanced HCC, ~75% of patients do not respond to immunotherapies for unclear reasons. Such a heterogeneous response highlights the need to further explore etiology- and organ-specific immunity towards improved patient stratification and the development of new combination therapies.

Materials and Methods With the objective to characterize the innate immunity landscapes of HCC, we employed droplet-based 3' scRNA-seq of CD45$^+$ panTCRαβ$^-$ CD19$^-$ cells, freshly isolated from tumors or adjacent non-tumoral livers of 10 HCC patients. In parallel, we used spatial transcriptomics (10x Genomics, Visium platform) to localize identified cell populations with respect to tumor and tissue features. Functional validation was carried in *ex vivo* co-culture experimentsusing patient-derived cells and *in vivo* using mouse models.

Results We present the most comprehensive atlas to date of hepatic innate immunity cells (~100,000 single cell transcriptomes). Besides describing the remarkable diversity of innate immunity cell states, our study identified and functionally characterized previously unexplored subsets of cytotoxic cells with myeloid features (myeNK) and novel myeloid-derived suppressor cell (MDSC) differentiation states. We computed signaling entropy at the single-cell level to characterize the differentiation hierarchy of these poorly annotated cells and show that myeNK cells are highly differentiated and exhibit potent lytic activity against cancer cells. Our analysis also distinguished three main MDSC lineages, a granulocytic (G-MDSC), a monocytic (M-MDSC) and an 'immature' subset with TAM-like features. This latter lineage presents the highest entropy and is the most immunosuppressive. Finally, we identify a discriminatory expression of the inflammatory receptor TREM-1 on MDSCs, particularly in NASH setting, and unravel this receptor as a potential therapeutic target in HCC.

Conclusions Our data support the stratification of patients according to etiology to define optimal therapeutic regimens and identify TREM1^{high} MDSC as deleterious effectors of HCC.

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P02.08 THE ROLE OF THE INFLAMMASOME IN THE SPATIOTEMPORAL EVOLUTION OF THE IMMUNE CELL LANDSCAPE IN POST-RESECTION GLIOBLASTOMA

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Background Glioblastomas (GB) are the most severe and deadliest brain tumors in adults. Survival is estimated < 15 months after diagnosis and with a relapse rate > 95%. The current standard-of-care involves surgery, when possible, and radiotherapy coupled with chemotherapy. Two characteristics might underlie the high relapse rate in GB: 1) the infiltrative capacity of tumor cells that spread out of the hypoxic and acidic tumor core, and 2) the unique composition of the tumor immune microenvironment (TME) that is sparce in T lymphocytes and natural killer (NK) cells but dominated by glioma-associated macrophages (GAMs). Although surgery is a standard treatment in GB, it fails to remove infiltrative tumor cells and causes an inflammatory and immunosuppressive trauma that might promote GB recurrence by altering the TME. However, the post-resection diversity of immune cells

in GB and the pathways that determine their functions in primary growth versus post-resection recurrence remain largely unknown. In this project, we characterize the immune landscape of GB before and after surgical resection and explore the role of the inflammasome in its dynamics and regulation.
Materials and Methods GL261-GFP-GLuc mesenchymal-type GB cells were orthotopically injected in WT or inflammasome-deficient(*Ice*$^{-/-}$) mice. On day 18 post-implantation, tumors and adjacent parenchyma tissue were collected from the unresected group (group 1). In parallel, tumor resection was performed on a second group of mice (group 2). 10 days later, tumors and adjacent parenchyma tissue were collected from group 2. Following tissue dissociation, immune cells were FACS-sorted from GB tumor-bearing mouse brains. Sorted immune cells were multiplexed using barcoaded lipid indices into 6 different pools and ScRNAseq (10x Genomics) was performed. For the scRNAseq, 30,000 cells/pool, corresponding to 7,500 viable cells/sample were loaded on the 10x chip.
Results Following putative doublet removal and exclusion of stressed or dead cells, we analysed the transcriptomes of ~61,000 single immune cells. Following data integration with Seurat, community detection, non-linear dimension reduction and graph clustering, 23 Louvain clusters were identified, including 15 from the myeloid lineage and 8 from the lymphoid lineage. we observed a significant depletion of microglia (MG)/MG-TAM from the tumor compared to the adjacent non-tumoral parenchyma, which was accompanied by a significant influx of bone-marrow-derived (BM)-TAM and monocytes as already known. Little differences were observed between WT or *Ice*$^{-/-}$mice before resection. However, post-resection remodelling of the GB TME was regulated by the inflammasome. Notably, monocytes, dendritic cells and regulatory T cells (Treg) subsets increased post resection in the adjacent non-tumoral tissue in WT but not *Ice*$^{-/-}$mice. Similarly, the intra-tumoral influx of Treg and the compositional changes of BM-TAMs observed in WT mice were blunted in inflammasome-deficient conditions. These TME differences correlated with faster tumor regrowth and decreased survival rates in WT mice compared to inflammasome-deficient mice.
Conclusion Our data reveal a significant impact of GB resection on TME remodeling and implicate the inflammasome in post-resection recurrence.

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P02.09 INTEGRATED SINGLE-CELL PROFILING DISSECTS CELL-STATE-SPECIFIC ENHANCER LANDSCAPES OF HUMAN TUMOR-INFILTRATING T CELLS

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Background Despite extensive studies on the chromatin landscape of exhausted T cells, the transcriptional wiring underlying the heterogeneous functional and dysfunctional states of human tumor-infiltrating lymphocytes (TILs) is incompletely understood.
Materials and Methods We use single-cell chromatin profiling and integrate publicly available single-cell RNA-seq data of TILs from several patients over four cancer entities to study gene-regulation in T cell (dys-)function.
Results We identify gene-regulatory landscapes in a wide breadth of CD8$^+$ TIL functional states. Our analysis predicts enhancer-promoter interactions in human TILs and prioritizes key elements by super-enhancer analysis. We define a human common chromatin trajectory to T cell dysfunction and determine involved key enhancers, transcriptional regulators, and deregulated target genes in this process. Finally, we validate enhancer regulation at immunotherapeutically relevant loci by targeting non-coding regulatory elements with potent CRISPR activators and repressors.
Conclusions Our study provides a framework for understanding and manipulating cell-state-specific gene-regulatory cues from human tumor-infiltrating lymphocytes.

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P03 Vaccine therapy

P03.01 DEVELOPMENT OF A MULTI-TUMOUR ANTIGEN VACCINE FOR HARD-TO-TREAT CANCERS

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Background Glioblastoma (GMB), advanced prostate cancer (PCa) and triple negative breast cancer (TNBC) are hard-to-treat cancers with 5-year survival rates of 5%, 12% and 30% respectively in their metastatic stage. With surgery, chemotherapy, and radiotherapy (and hormone therapy for PCa) being the only feasible treatment options, novel therapies are urgently required. Adjuvanted peptide vaccines based on tumour antigens hold promise in prophylactic and therapeutic settings, especially when there is residual disease following treatment. Research suggests that the cancer/testis antigens HAGE and NY-ESO-1 as well as the tumour associated antigen WT1 (antigens of interest) are good candidates for peptide vaccine development, being expressed at various levels in GBM, PCa and TNBC tissues. Additionally, the expression of these antigens can be upregulated by treatment with low-dose DNA methyltransferase inhibitors like decitabine (DAC), offering the possibility to maximise the detection and destruction of residual cancer cells by vaccine-induced T cells.
Materials and Methods A panel of GBM, PCa and TNBC cell lines was treated with 1μM, 5μM and 10μM DAC and tested for antigens of interest using qPCR and western blot, aiming to validate them as immunotherapeutic targets. Peptide sequences derived from these antigens, including a mutated NY-ESO-1-derived sequence, were selected for vaccine development using *in silico* prediction algorithms (SYFPEITHI and IEDB