Results The results show precise expression levels for each of the 60 markers in the assay in each individual cell in the sample, maintaining spatial information about each cell. Dozens of immune cell subtypes were identified and quantified based on protein expression profiles. Spatial analysis of the samples reveals quantifiable heterogeneity of immune cell infiltration within the tumor samples, demonstrating the utility of the ChipCytometry platform for in-depth immune profiling in clinical samples.

Conclusions The ChipCytometry platform enables simultaneous detection of multiple protein markers on a single tissue section for deep immune cell profiling in the tumor microenvironment. Combined with the single-cell spatial information, such data sets provide an opportunity for the discovery of new complex multiplexed biomarker signatures to inform therapeutic development.

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HOW PROTON PUMP INHIBITORS BLUNT IMMUNE CHECKPOINT INHIBITORS EFFICACY: A ROLE OF THE MICROBIOME?

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Background While Immune checkpoint inhibitors (ICIs) are revolutionizing the management of many advanced cancers, several studies have reported that the gut microbiota composition may have an impact on ICI response. Antibiotics have been shown to alter the efficacy of immunotherapies, but other commonly used medications known to interact with the microbiota might also impact the clinical benefit of these treatments. Clinical studies revealed that patients treated with ICI could be stratified into responder (R) or non-responder (NR) according to their microbiota composition. In a total of 635 patients with advanced cancer treated with anti PD-1, anti PD-L1 or anti CTLA-4 between 2015 and 2017 in Bordeaux, M. Kostine et al. described an association between the baseline use of co-medications, including proton pump inhibitors (PPIs), and a significantly shortened overall survival.1 Our objectives are a) to address whether PPIs impact the ICI response, and b) to understand if the underlying mechanism involves the gut microbiota.

Materials and Methods We first explored the impact of the PPI omeprazole on the composition of the microbiota in different segments of the gut. This was conducted in mice after long- or short-time exposure to omeprazole. In parallel, we explored omeprazole-induced changes in the intestinal transcriptome using bulk RNA sequencing of gut tissue segments. In other experiments, we interrogated the impact of omeprazole on anti-PD-1 efficacy in mice transplanted with different cancer cell lines. Using 16S rDNA sequencing, we characterized both the gut as well as the local tumor microbiomes of R and NR mice.

Results Our results revealed that omeprazole treatment resulted in a decrease of bacteria associated with a healthy gut and an expansion of oral bacteria and environmental pathobionts, consistent with published studies.2 3 Notably, omeprazole administration led to a striking reduction in Lachnospiraceae spp., which are enriched in the ‘microbiotype’ of ICI-responders patients.4 Multi-omics integration of the gut microbiome and transcriptional data sets using weighted gene co-expression network analysis (WGCNA) identified omeprazole-induced transcriptional modules in the colon significantly associated with depletion or enrichment of specific microbiota components. From this integration, we will reconstruct the bacterial and host metabolic networks towards identifying metabolic signals linked to impaired anti-tumor immunity.

REFERENCES

Investigating the Roles of IL-25 and ILC2s in APC-Mutation-Mediated Colorectal Cancer

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Background Colorectal cancer (CRC) is the second leading cause of cancer-related death. The majority of CRC cases are caused by mutations in the adenosomatous polyposis coli gene (APC), and are resistant to current cancer immunotherapies. Most immunotherapeutic strategies rely on utilising adaptive T cells, and their lack of efficacy in CRC suggests there may be immunosuppressive signals in the tumour microenvironment. Using a mouse model of APC-mutation-driven CRC, recent work by our lab has shown that interleukin 25 (IL-25)-activated group 2 innate lymphoid cells (ILC2s) promote intestinal tumourigenesis through activation of monocytic myeloid-derived suppressor cells (M-MDSCs) and the resulting suppression of CD8+ T cells.1 However, the impact of IL-25 and ILC2s on individual intestinal tumour morphology is not completely understood. In this study, we use histological techniques to investigate the impact of genetic ablation of IL-25 or ILC2s on the morphology of APC-mutation-driven mouse intestinal tumours.

Materials and Methods The mouse strains used were Apc1322T/Il25tm+Il7tacr+Rorafox on a C57BL/6Ola genetic background.1 Apc1322T/+ Il25tm+/Il7tacr+ and Apc1322T/+ Rorafox/fox Il7tacr+ mice were used to investigate the effect of IL-25 deficiency on CRC2a defining IL-25 deficiency, respectively, on intestinal tumours. Small intestinal tissue was fixed in 4% formaldehyde for 24 h and dehydrated. Intestines were embedded in paraffin and sectioned at 4 μm for histology, or 7.5% gelatin + 10% sucrose in PBS, followed by cryosectioning at 20 μm for

immunohistochemistry. Paraffin sections were stained with haematoxylin 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<sup>1322T</sup> mice lacking IL-25 had smaller tumours and showed less dysplasia than Apc<sup>1322T</sup> 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<sup>1322T</sup> mice lacking IL-25 appeared larger, indicating a more robust anti-tumour immune response.

Likewise, Apc<sup>1322T</sup> mice lacking ILC2s had smaller, less dysplastic tumours. TATLS in these mice were bigger than mice with ILC2s but smaller than Apc<sup>1322T</sup> 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, in the tumours of Apc<sup>1322T</sup> 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<sup>−</sup> panTCR<sup>β</sup> CD19<sup>+</sup> 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 experiment-susing 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 transcriptions). 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, agranulocytic (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 TREM<sup>1</sup>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 sparse 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