**Background**

Immune checkpoint inhibitor (ICI) colitis is among most prevalent immune-related adverse events (irAEs). To develop evidence-based treatment, profound pathophysiological understanding is required.

Tissue-resident memory Tcells appear key inflammatory effectors in ICI-colitis based on single cell RNA-seq studies, but these lack tissue architectural insight.1 3

**Novelty of our approach**

Simultaneous interrogation of phenotypical abundance, spatial distribution enabling epithelium-lamina propria comparisons, and immune cell functionality in ICI-colitis.

**Patients recruited from UNMC cohort**

Biobank study open to UMC Utrecht patients undergoing a first ICI regimen for solid malignancies. Blood and stool are collected at baseline, on-treatment and upon irAEs, along with biopsies.

**We combined high-resolution DAPI imaging and high-dimensional imaging mass cytometry to obtain single-cell spatial data on 36 immunological markers**

FFPE colitis tissue from n=23 patients

**MATRISSE pipeline**

Epithelium (left) and single-cell (right) annotated plot

**1. ICI incites colon mucosal CDB T cells to increase granzyme B production, particularly in combi-ICI colitis**

Graftase B production was highest in combi-ICI colitis, both in colon CDB-T cells measured by IFN-γ (left) and serum levels measured by the multiplex Olink® Target 55 Immuno-Oncology panel in a partially overlapping cohort (right).

**ACTA-4 and combi-ICI colitis span a spectrum of T helper skewing from Th1 to Th1 dominant disease; Th17 dominant CDA T cells were identified based on cytokine concentration (IL-17+/IFNγ+) and median IL-17 (left). CombI-ICI cell and IFNγ positive Th17 cytokine serum levels (Olink®) were noted (right).**

**2. Activated tissue-resident CDB T cells become main granzyme B producers**

Mixed-effects model summarizing phenotypical, spatial and clinical characteristics' effect on CDB granzyme B production

**3. Especially combi-ICI colitis is Th1-polarized disease**

**Conclusions & future Outlook**

- Not epithelial localization of CDB T cells itself, but activated tissue-resident phenotype – more common in epithelium – mainly explains cytokoty in the predominantly Th1-polarized environment of ICI-colitis.

- Tissue T cells in ICI-colitis highly express PD-1, thus present as likely direct targets of ICI. Th1-polarization may contribute to cause, and/or be consequence of Th1-mediated activation by ICI.

**4. Tissue T cells in ICI-colitis highly express PD-1**

Some patient as left figure: colon mucosa T cells highly express PD-1 (orange). Staining with both anti-PD-1 and anti-IgG (blue) showed no binding of reovirus to PD-1.

**References**