**MREGDC/T HELPER NICHEs ENABLE LOCAL REACTIVATION OF CD8 T CELLS UPON PD-1 BLOCKADE**

**Background** While T cell accumulation in tumors is associated with response to immune checkpoint blockade (ICB), many T cell-rich tumors fail to respond to ICB.

**Methods** Here we leveraged a large neoadjuvant PD-1 blockade trial in hepatocellular carcinoma (HCC) to search for correlates of ICB response within T cell-rich tumors.

**Results** Paired single-cell RNA and TCR sequencing of nearly one million immune cells revealed that anti-tumor responses to ICB correlated with significant clonal expansion of intratumoral CXCL13⁺CH25H⁺IL-21⁺TCF7⁺ CD4 T helper cells (CXCL13⁺ Th) and GranzymeK⁺PD-1⁺ CD8 effector-like T cells, whereas terminally exhausted CD39ʰ⁰TOXʰ⁰PD-1ʰ CD8 T cells dominated in non-responders. Most T cell clonotypes that expanded post-treatment were found in pre-treatment biopsies, suggesting local reactivation of antigen-experienced T cells by PD-1 blockade. Notably, PD-1⁺TCF-1⁺ CD8 T cells with features of progenitor-exhausted cells were found in tumors of responders and non-responders. Strikingly, tumors from responders were highly enriched in mregDCs, a DC state triggered by capture of tumor debris, which formed physically interacting cellular triads with CXCL13⁺ Th cells and PD-1⁺TCF1⁺ progenitor-like CD8 T cells. Receptor-ligand analysis revealed unique interactions within these triads that may promote the differentiation of progenitor-exhausted CD8 T cell into effector-like cells upon PD-1 blockade.

**Conclusions** These results suggest that discrete intratumoral niches that include mregDCs and CXCL13⁺ Th cells control the differentiation of tumor-specific progenitor-exhausted CD8 T cell clones into effective anti-tumor T cells in patients treated with ICB.

**Trial Registration** NCT03916627

**Ethics Approval** Samples of lymph node, tumor and non-involved adjacent liver were obtained from surgical specimens of patients undergoing resection at Mount Sinai Hospital (New York, NY) after obtaining informed consent in accordance with a protocol reviewed and approved by the Institutional Review Board at the Icahn School of Medicine at Mount Sinai (IRB Human Subjects Electronic Research Applications 18-00407) and in collaboration with the Biorepository and Department of Pathology.

The single-arm, open-label, phase 2 trial of HCC patients with resectable tumors was registered on ClinicalTrials.gov (NCT03916627, Cohort B). 21 patients were enrolled and received two cycles of Cemiplimab before surgical resection as described in (Marron et al., 2022a).