ALTERED INTERACTIONS BETWEEN CIRCULATING AND TISSUE-RESIDENT CD8 T CELLS WITH THE COLONIC MUCOSA DEFINE CHECKPOINT INHIBITOR COLITIS

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Background Antibodies targeting immune checkpoint inhibitors (ICIs) CTLA-4 and PD-1/PD-L1 have revolutionized the treatment of metastatic solid tumors. However, their use is limited by a high incidence of immune-related adverse events. The colon is a frequent target of this immune attack seen in up to 45% of patients on dual PD-1 and CTLA-4 blockade. We leveraged a multi-omics strategy to further our understanding of the cellular and molecular drivers giving rise to ICI-associated colitis and nominate treatment solutions that spare antitumor immune response.

Methods We collected paired endoscopic colon mucosal biopsies and blood specimens from 13 irColitis patients, 8 healthy individuals, and 8 controls on ICIs, and analyzed them with single-cell/nuclei RNA sequencing with paired TCR and BCR sequencing, multispectral fluorescence microscopy, and secreted factor analysis.

Results Analyses of over 300,000 single epithelial, mesenchymal, and immune single cells revealed that patients with irColitis showed expanded mucosal Tregs, ITGAEhi CD8 tissue-resident memory T cells expressing CXCL13 and Th17 gene programs. We also identified two circulating ITGB2hi CD8 T cell populations associated with irColitis – a CX3CR1hi population predicted to be intravascular and an EOMEShi KLRG1hi population. Comparison of dual anti-PD-1/CTLA-4 versus anti-PD1 monotherapy revealed expansion of those two circulating ITGB2hi CD8 T cell populations, as well as a cytotoxic GNLYhi CD4 T cell subset, and endothelial cells associated with hypoxia gene programs. Cell-cell communication analysis predicted crucial roles for ICAM and CXCR3 ligand-mediated recruitment and retention of these two circulating T cell populations by epithelial, endothelial and myeloid cells during active colitis. In irColitis, we also observed significant epithelial turnover marked by fewer LGR5+ stem cells, more transit amplifying cells, and upregulation of apoptotic and DNA-sensing programs. Mature epithelial cells with top crypt genes upregulated interferon-stimulated pathways, CD274 (PD-L1), anti-microbial genes, and MHC-class II genes, and down-regulated aquaporin and solute-carrier gene families, likely contributing to epithelial cell damage and absorptive dysfunction. Transcriptional programs associated with irColitis were distinct from those in the tumor microenvironment, which may have important therapeutic implications. Finally, by examining many drugs in clinical trials for inflammatory bowel disease, we expand the putative therapeutic options for treating irColitis reported to-date.

Conclusions This multi-omics approach nominates novel irColitis therapeutic targets and redefines irColitis as a disease not simply marked by the aberrant expansion of CD8 T cells but rather altered global interactions between immune cells and the colon mucosal epithelial or mesenchymal cells.

Ethics Approval Informed consent was obtained from all patients in accordance with protocols obtained from the Mass General Brigham and/or DANA- Farber/Harvard Cancer Center Institutional Review Boards (DFCI/HCC 11-181 and 13-416, Mass General Brigham 2015P001333).