SHARED FRAMESHIFT NEOANTIGENS ARE EXPRESSED THROUGHOUT MISMATCH REPAIR DEFICIENT CANCER DEVELOPMENT AND ARE RECOGNIZED BY TISSUE INFILTRATING T CELLS THAT ARE DYSREGULATED IN ADVANCED LESIONS

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Background Patients with Lynch syndrome (LS), characterized by germline inactivation of one mismatch repair (MMR) gene allele, have an increased risk (up to 80%) of developing colorectal (CRC) and endometrial cancers with high microsatellite instability (MSI-H). Increased T cell infiltrate into the normal mucosa of LS patients is correlated with a delayed onset of CRC. Therefore, the precancerous microenvironment (PCME) of LS patients provides a unique window into the earliest stages of antitumor immunosurveillance potentially able to be targeted by immunoprevention strategies. We have identified i) highly immunogenic frameshift (fs)-neoantigen peptides shared among MSI-H patient tumors and ii) T cell receptors (TCRs) used to track fs-specific T cell trafficking to the primary tumor and metastases of an LS patient. We hypothesized that fs-neoantigen expression occurs early in MMR deficient (MMRd) tumor development, but fs-specific T cell function is partially abrogated by chronic antigen stimulation and immunosuppressive PCMEs/tumor microenvironments (TMEs).

Methods We analyzed peripheral blood mononuclear cells (PBMCs), MMRd normal tissue precancerous lesions, and MSI-H tumors from the colon and endometrium of LS/sporadic MSI-H patients. Multiplexed immunohistochemistry and spatial transcriptomic analysis were utilized to assess MMR loss and the immune landscape of the PCME and TME. Whole-exome and bulk RNA sequencing were undertaken to analyze fs-neoantigen expression and quality. To functionally assess T cell specificity and phenotype, we leveraged an in vitro neoantigen-specific T cell expansion and stimulation assay combined with ex vivo flow cytometric analysis and single-cell RNA/TCR sequencing of PBMCs/MMRd lesions.

Results Highly immunogenic, shared fs-neoantigens are expressed in MMRd normal mucosa, hypermutated adenomas, and tumors of LS patients. The precancerous fs-neoantigen landscape also contains a significant number of neoantigens absent in the tumors sequenced, despite possessing a comparable overall fs-load; Fs-specific T cells are detectable in peripheral blood, precancerous tissue, and tumors of LS patients. However, fs-specific T cells in MMRd tissue relative to peripheral lymphocytes show reduced antigen responsiveness, weakened ability to proliferate in vitro, and increased expression of immune dysregulation (PDCD1, HAVCR2, LAG3, TOX) programs.

Conclusions Our study provides a preliminary map of the fs.neoantigen landscape and TCR repertoire in MMRd tumor development. This dataset will also enable further study of fs-specific T cell differentiation and interactions with myeloid, epithelial, and stromal compartments in our single-cell PCME/TME atlas of LS patients to better understand immune escape. Lastly, these results highlight the importance of fs-neoantigen quality and evolution in vaccine design and biomarker selection for immunoprevention efforts.

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REFERENCES

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