Background Patients with Lynch syndrome (LS), characterized by germline inactivation of one allele of the mismatch repair (MMR) genes, have an increased risk (up to 80%) of developing cancers with high microsatellite instability (MSI-H).\(^1\) MSI-H tumors account for 15–30% of all colon, endometrial, and gastric cancers.\(^2\) Due to unrepaired frameshift (fs) mutations, these cancers possess a high tumor mutational burden which has enabled microsatellite instability to emerge as a biomarker of immune checkpoint blockade (ICB) response. However, there is still a significant population of MSI-H patients unresponsive to ICB treatment.\(^3,4\) Therefore, novel strategies to prevent LS-associated tumor development and predict ICB resistance are urgently needed. T cell surveillance in LS is evidenced by (i) elevated fs-loads in MMR-deficient (MMRd) nonneoplastic tissue\(^5\) and (ii) a correlation between increased T cell infiltration into normal mucosa of LS patients and delayed onset of colorectal cancer.\(^6\) We previously characterized unique immunogenic fs-peptides shared in MSI-H cancers\(^7\) and identified T cell receptors (TCRs) specific to MSI-H-associated fs-peptides. Utilizing TCR sequencing, we demonstrated that fs-specific T cells were present in the primary tumor, draining lymph nodes, and metastases of an LS patient. However, tumor growth in the presence of these fs-specific T cell clones suggests suboptimal T cell surveillance.

Methods Our hypothesis is that MMRd precancerous and malignant lesions expressing fs-neoantigens escape immune surveillance due to suppression of T cell infiltration and activity. We investigated whether this is the result of immunosuppressive activity from innate immune cell populations and/or T cell dysfunction. Using normal, precancerous, and tumor tissue collected from our cohort of LS and sporadic MSI-H cancer patients we are leveraging whole-exome sequencing, spatial transcriptomics, and multiplexed immunohistochemistry.

Results This has allowed us to begin to define the spatiotemporal landscape of fs-neoantigen expression, innate immune cell localization, and T cell dysfunction in the course of MSI-H tumor development to explain how MMRd lesions escape T cell surveillance. Additionally, our analysis of MSI-H tumors from The Cancer Genome Atlas (TCGA) with high fs-neoantigen loads but low cytotoxic T lymphocyte (CTL) signatures (assessing granzyme and perforin expression) have revealed lower immune-stimulatory macrophage and higher activated mast cell-related gene signatures. We have also observed high frequencies of exhausted (PD-1+TIM-3+) tumor infiltrating lymphocytes in advanced MSI-H tumors assessed by flow cytometry.

Conclusions This work supports the development of fs-neoantigen-based vaccination/immunomodulation strategies to prevent LS-associated tumor development and identifies potential ICB resistance biomarkers for advanced MSI-H tumors.

REFERENCES


Ethics Approval The study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board, approval numbers: STUDY-21–01317 and STUDY-19–00936-CR001. Informed consent was given by all participants in this research study.