

## ANALYSIS OF T-CELL RECEPTOR SEQUENCES IDENTIFIES SIGNATURES ASSOCIATED WITH NEOANTIGEN EXPOSURE IN PERIPHERAL BLOOD OF LYNCH SYNDROME PATIENTS

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**Background** Lynch Syndrome (LS) is the most common inherited colorectal cancer (CRC) syndrome. It constitutes the perfect model to understand DNA mismatch repair deficient (dMMR) carcinogenesis, which underlies 15% of early-stage CRC.<sup>1</sup> LS patients develop dMMR tumors with high loads of shared neoantigens, which are recognized by the immune system.<sup>2</sup> Elucidating the role and molecular landscape of public T-cell receptors (TCRs), provides potential as a diagnostic strategy for exposure to viruses and other immune-related phenotypes<sup>3–5</sup>. This study aims to characterize the TCR beta chain (TCRB) landscape of LS patients and to identify the presence of cancer-specific TCRB clones in the peripheral blood of LS carriers.

**Methods** A total of 122 PBMCs and 29 colorectal tissue samples have been collected from patients enrolled during their routine screening colonoscopy to an ongoing IRB-approved biospecimen protocol (MDACC IRB# PA12–0327). All TCRB repertoires were sequenced using the Immunoseq TCRB assay (Adaptive Biotechnologies). Bioinformatics analyses were performed using the immunarch R package<sup>6,7</sup> and the immuneML software<sup>8</sup> by comparing the TCRB repertoires among LS cancer survivors, LS healthy carriers (with no cancer history), and controls with no cancer and no family history of LS. To validate some of the identified TCRBs, a viral peptide and a cancer neoantigen predicted to be recognized by these public TCRBs were used to isolate peripheral T-cells from healthy human donors using pMHC-tetramer assays and single-cell TCR sequencing.

**Results** Our data show that LS cancer survivors have less diverse TCRB repertoires compared to LS healthy carriers due to the presence of hyper-expanded TCRBs in the group of survivors. Our results also show that a set of highly expanded cancer-specific public TCRBs is detectable in the peripheral blood of LS cancer survivors after annotation to the McPAS-TCR database<sup>9</sup>. We observe an overlap of TCRBs between the tissue (precancers and cancers) and PBMCs in patients for which both types of samples were collected at the same time-point. Finally, with our pMHC-tetramer assays, we are capable of isolating TCRBs that were initially identified with bulk-TCR sequencing.

**Conclusions** Overall, our data suggest that the T-cell response of LS patients against developing cancers is not entirely restricted to their tumor microenvironment, with expanded cancer-specific public TCRBs being detectable in the peripheral blood of LS cancer survivors. This is the first step toward identifying a TCR signature that serves as a biomarker of early cancer detection in LS patients.

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**Ethics Approval** All patients included in this study provided written informed consent at The University of Texas MD Anderson Cancer Center (MDACC). All samples were obtained from study participants through an Institutional Review Board (IRB) approved protocol at MDACC (Protocol PA12–0327).

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