Targeting Neoantigens Derived From the ZRSR2 Mutation in Hematologic Malignancies

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Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are two types of hematologic malignancies with especially poor prognoses and limited therapeutic options. Notably, mutations in RNA splicing factors are commonly found in these cancer types and concentrated in four main genes, one of which is ZRSR2, responsible for splicing highly conserved minor introns. For this project, both in silico and in vitro analyses were performed to identify and test mis-splicing-derived neoantigens from ZRSR2 mutations that could serve as novel immunotherapeutic targets. Potential neoantigens derived from ZRSR2 mutations were predicted based on RNA-Seq splicing analysis of MDS and AML patient datasets. In silico prediction of major histocompatibility complex (MHC) class I binding identified numerous peptides of interest using MHCFlurry and NetMHCPan. Putative peptides were validated for their MHC class I binding in vitro using T2 binding assays. T-cell and antigen-presenting cell populations were differentiated and expanded through immunogenicity assays to perform ELISpot Assays and Flow Cytometry in order to examine T-cell activation. Several splice variant peptides, including DERL2_2, KRTCAP2_1, and TMEM33_2, demonstrated viability as potential targets through MHC class I presentation and stimulation of CD8+ T-cell cytokine secretion. Dextramer staining with the DERL2_2 and KRTCAP2_1 peptides identified tumor antigen-specific CD8+ T-cells. RT-PCR with ZRSR2 knockout demonstrated robust intron retention, validating the role of the ZRSR2 mutation in transcriptome-wide dysregulation. Thus, two immunogenic neoantigens, prevalent across blood cancers, were found and ultimately can serve as T-cell therapy and cancer vaccine targets through a novel therapeutic route.

Awards Won:

Third Award of $1,000