DEFINING T CELL RECOGNITION OF A HIGHLY
CONSERVED FUSION NEOANTIGEN IN FIBROLAMELLAR
CARCINOMA

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Background Fibrolamellar carcinoma (FLC) is a rare and fatal liver malignancy that primarily affects otherwise-healthy adolescents and young adults. No curative therapies are currently available, so novel treatments are needed to improve patient outcomes. In 2014, Honeyman et al.1 identified the key genetic event in FLC tumorigenesis: a recurrent deletion in chromosome 19 that fuses DNAJB1 to PRKACA. The resulting fusion protein, now known to be the essential driver mutation in FLC, is present in all cases and has an identical amino acid sequence in more than 90% of patients. We believe this highly conserved fusion protein is a potentially-ideal neoantigen target for T cell-based immunotherapy, and therefore sought to define fusion-specific T cell responses in FLC patients.

Methods To identify functional T cell responses to the DNAJB1-PRKACA fusion, tumor infiltrating lymphocytes (TILs) from an FLC patient tumor were expanded ex vivo, then stimulated with fusion peptides predicted to be presented by the patient’s class I HLA alleles. We then used single cell gene expression profiling and T cell receptor (TCR) sequencing of patient TILs, and antigen-specific expansion2 and peptide-HLA tetramer staining of patient peripheral blood T cells, to identify two fusion-specific TCRs. Both TCRs were reconstructed and expressed in cells, and their specificity and functionality were validated in vitro using peptide-HLA tetramer staining, intracellular cytokine staining, and killing assays. Finally, using a xenograft model in immunodeficient mice, we tested the ability of these TCRs to control the growth of fusion-expressing tumors in vivo.

Results We observed a small but robust T cell response to fusion peptide EIFDGYGEEV among patient TILs, and subsequently identified two TCRs in the patient that recognize this fusion peptide presented on HLA-A*68:02. These TCRs specifically bind their cognate tetramer and mediate both cytokine production and killing of fusion-expressing target cells in vitro, while sparing wild-type-expressing controls. Treating mice bearing fusion-expressing tumors with TCR-engineered T cells resulted in transient tumor clearance and significantly extended survival compared to mice treated with vehicle or mock-transduced T cells.

Conclusions We have identified, to our knowledge, the first reported endogenous T cell response to the DNAJB1-PRKACA fusion in an FLC patient. Further, we have defined two fusion-specific T cell receptors that hold promise for development in novel cell-based immunotherapies for FLC.

REFERENCES