DEFINING AND THERAPEUTICALLY TARGETING A FUSION-DERIVED PUBLIC NEOANTIGEN IN DESMOPLASTIC SMALL ROUND CELL TUMOR USING T-CELL RECEPTOR GENE THERAPY

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Background Sarcomas are a heterogenous group of malignancies of mesenchymal cell origin that are difficult to treat with often poor prognoses. Approximately 30% of sarcomas are characterized by expression of fusion proteins that function as oncogenic drivers. Desmoplastic small round cell tumor (DSRCT) is a prototypical fusion-driven sarcoma defined by a pathognomonic EWSR1-WT1 fusion event. The resultant EWSR1-WT1 oncogenic fusion protein contains a shared junctional amino acid sequence divergent from normal self-proteins. We hypothesized that clonally conserved fusion proteins might yield an immunogenic subset of shared, or public, neoantigens (NeoAgs) potentially serving as targets for novel immunotherapeutic approaches.

Methods and Results Using an HLA-immunoprecipitation/mass spectrometry (HLA-IP/MS) screen, we identified a 9-amino acid peptide sequence (SSYGQQSEK) derived from the junction of the EWSR1-WT1 fusion protein and presented in the context of HLA-A*03 and -A*11, two prevalent HLA alleles that frequently bind homologous peptides. We confirmed that the same peptide sequence is physiologically presented by HLA-A*03+ DSRCT cells. Using fluorophore-conjugated HLA-multimers (dextramers) loaded with the MS-identified NeoAg, we detected circulating T cells that bind the fusion NeoAg in a subset of HLA+ DSRCT patients, confirming immunogenicity. We subsequently used in vitro antigen-directed clonal expansion of fusion NeoAg-specific T cells to isolate $n=3$ HLA-A*03-restricted and $n=1$ HLA-A*11-restricted fusion NeoAg-reactive clones. Using single-cell sequencing, we retrieved the TCRab gene sequences of the T cell receptors (TCRs) expressed by these T cells and cloned them into retroviral expression vectors. Polyclonal CD8+ T cells transduced with the retrieved TCR genes bound fusion NeoAg-loaded dextramers but not viral peptide-loaded control dextramers. Additionally, CD8+ T cells expressing candidate TCRs robustly upregulated TNFα after coculture with cells expressing the requisite HLA allele and EWSR1-WT1 fusion and specifically lysed HLA+ DSRCT cells. Interestingly, we identified one unique TCR which binds the fusion NeoAg in a peptide-centric manner that can specifically lyse both HLA-A*03+ and -A*11+ DSRCT cells, but did not engage fusion NeoAg presented in the context of HLA-A*02. This implies that a single TCR therapeutic could cover >36% of all North American DSRCT patients.

Conclusions Our data establishes that the junction of the recurrent EWSR1-WT1 fusion is naturally processed and presented in the context of prevalent HLA alleles by DSRCT cells. These findings establish proof-of-principle that fusion-derived public NeoAgs are an actionable source of therapeutic targets for fusion-driven malignancies. This work establishes a foundation for the clinical translation of a new class of T cell-based therapies targeting EWSR1-WT1 and other recurrent oncogenic fusions.

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