Screening for Cross-Reactivity and Validation of Peptide-Centric CAR-T Cells for Neuroblastoma using MHC Dextramer®

Yarmarkovich, M., Marshall, Q.F., Warrington, J.M. *et al.*
Targeting of intracellular oncoproteins with peptide-centric CARs. *Nature* 2023.
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Background

Neuroblastoma (NB) is an aggressive paediatric cancer. Yamarkovich *et al.* discovered that the NB immunopeptidome is enriched with intracellular peptides derived from essential oncoproteins. These antigens cannot be targeted using traditional Chimeric Antigen Receptor (CAR)-T cells limited to surface antigens. Since NB is driven by epigenetically deregulated transcriptional networks rather than mutations, unmutated self-peptides presented on tumor cells were also included in the antigen-discovery process. The authors engineered peptide-centric (PC)-CARs with a single-chain antibody variable fragment (scFv) region, capable of binding MHCs presenting intracellular peptides. The resulting PC-CARs induced potent tumor killing across multiple HLA alleles in NB cells in vitro, and complete tumor regression in mice (**Fig. 1**).

Study description

The unmutated peptide QYNPIRTTF on HLA-A*2402 encoded by PHOX2B was identified by immunopeptidomics and selected for further studies based on pMHC binding affinity expression data. Normal donor-matched CD8+ T cells were pulsed with peptide, enriched using MHC Dextramer® reagents, and incubated with pulsed dendritic cells. Expanded T cells were validated for antigen-specificity by staining with NB-specific MHC Dextramer® reagents and analyzed by flow cytometry. Candidate PC-CARs were screened with predicted cross-reactive peptides to minimize off-target effects against MHC or the normal immunopeptidome.

Results

Staining with MHC Dextramer® showed significant cross-reactivity to the MHC in first generation PC-CARs (**Fig. 2**). Cross-reactive binding was later abrogated using saturation mutagenesis. Following selection via protein display, and further cross-reactivity screening, PC-CARs capable of selective binding were successfully engineered.

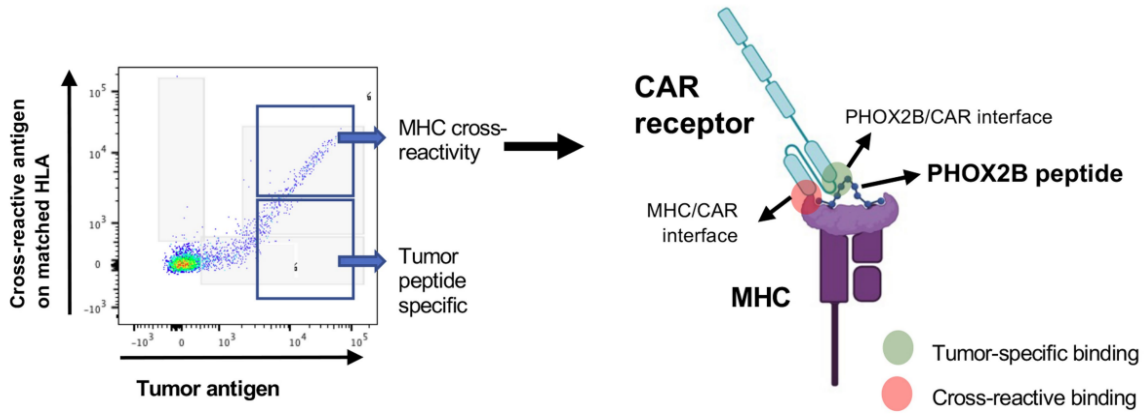

Fig. 1: Cross-reactive binding of first-generation PC-CARs identified using MHC Dextramer®. Flow cytometry of Jurkat cells transduced with A7 CAR stained with PHOX2B MHC Dextramer® on x-axis and mismatched PBK peptide on matched HLA-A*2402 on y-axis.

Conclusions

- MHC Dextramer® reagents were successfully used to characterize target interaction, peptide cross-reactivity, and allele specificity of PC-CAR T cells, leading to complete tumor regression in mice.
- PC-CARs provide a roadmap to target non-immunogenic intracellular oncoproteins in cancer.
- PC-CARs recognized identical peptides on multiple HLA allotypes, expanding the patient population that could potentially benefit from PC-CAR T-cell therapy.