Structural principles of peptide-centric Chimeric Antigen Receptor recognition guide therapeutic expansion

[1,2]**Sagar Gupta**†, [2]Yi Sun†, [2]Tyler J. Florio†, [2]Michael C. Young, [3]Quinlen F. Marshall, [2]Samuel E. Garfinkle, [2]Georgia F. Papadaki,[2]Hau V. Truong, [3]Emily Mycek, [3]Peiyao Li, [3]Alvin Farrel, [4]Nicole L. Church, [4]Shereen Jabar, [4]Matthew D. Beasley, [4]Ben R. Kiefel, [3]Mark Yarmarkovich, [2]Leena Mallik, [3]John M. Maris, [2]Nikolaos G. Sgourakis

[1]College of Arts and Sciences (2024), University of Pennsylvania
[2]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania
[3]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia
[4]Myrio Tx, Melbourne, Victoria, Australia

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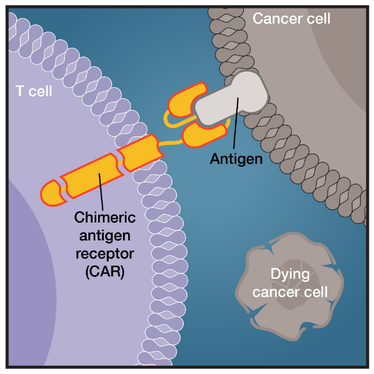
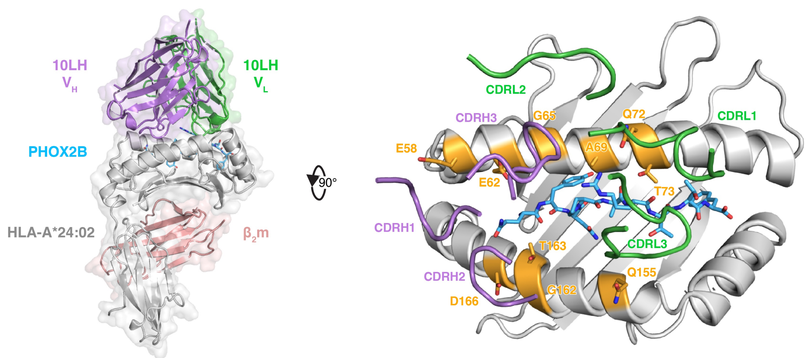
sagarg@sas.upenn.edu

Introduction



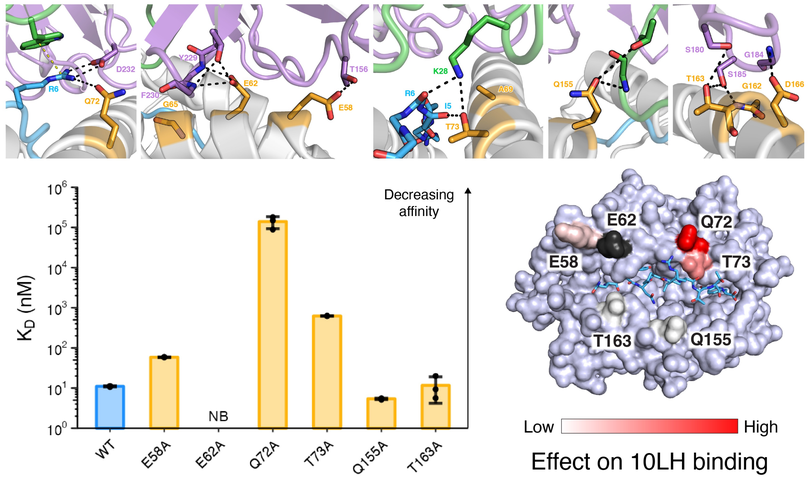
- Chimeric Antigen Receptors (CARs) targeting cell surface antigens have achieved remarkable efficacy for liquid tumors such as B-cell leukemias[1].
- CAR T-cell therapy for solid tumors faces issues such as lack of a sustained response.
- Tumor-specific antigens considered "undruggable" by traditional CAR T-cell therapy can be targeted after being presented by major histocompatibility complex (MHC) class I molecules as peptide/MHC-I complexes[2].
- We recently reported the development of a peptide-centric CAR (PC-CAR) 10LH targeting a neuroblastoma-enriched, unmutated peptide (QYNPIRTTF) derived from the PHOX2B oncoprotein presented on HLA-A*24:02, a human MHC allotype[3].
- Here, we leverage insights from our crystal structure of a 10LH:PHOX2B/HLA-A*24:02/β_2m complex to expand the patient cohort amenable to CAR T-cell therapy.

Crystal structure of the 10LH complex



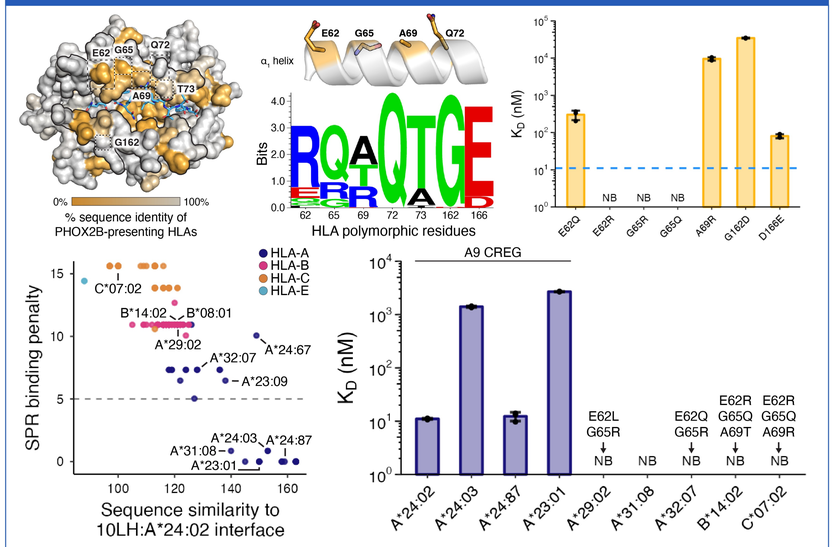
- We determined the crystal structure of the 10LH:PHOX2B/HLA-A*24:02/β_2m complex at a resolution of 2.1 Å.
- The PC-CAR engages the N- and C-termini of the MHC-I peptide bindng groove via its CDR3 loops from the heavy and light chain, respectively.

Alanine scanning of key HLA framwork residues



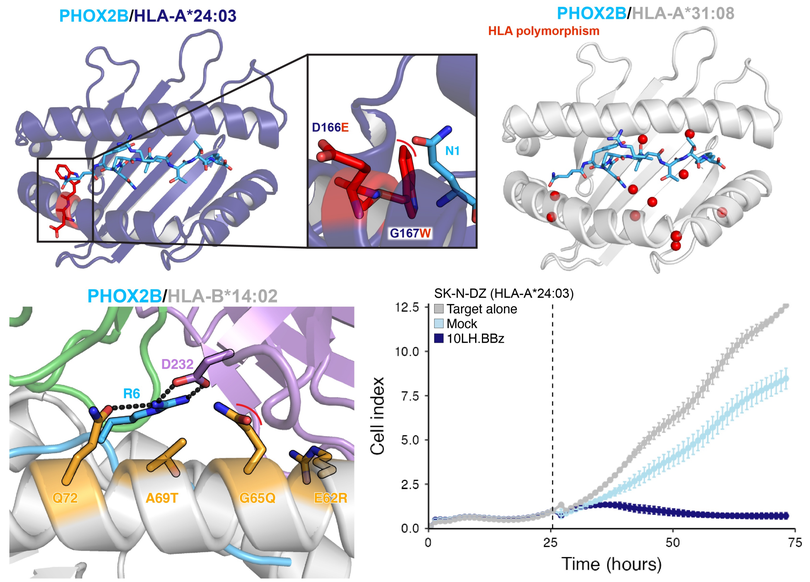
- High-affinity complex formation requires framework residues on A*24:02, most notably those residues that are adjacent to R6 of PHOX2B.

HLA allotype expansion



- Biochemical analysis of framework residue polymorphisms identifies a set of PHOX2B-presenting HLA alleles that can cross-react with 10LH.

Structural modeling of non-A24 interactions



- Our results provide strong support that 10LH can bind to several additional allotypes from the A9 CREG (25.2% of the American population).

Conclusions

- Through a combination of structural, biochemical, and functional approaches, we have characterized the molecular basis for peptide-centric recognition of HLA molecules by the 10LH PC-CAR.
- Our structure provides a basis for expanding the scope of peptide targets presented by HLA-A*24:02 and other HLAs through the rational design of the CDR loops.

References and Acknowledgments

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