Tackling the specificity challenge in the generation of peptide-MHC binding fragments for CAR-T-based therapeutics

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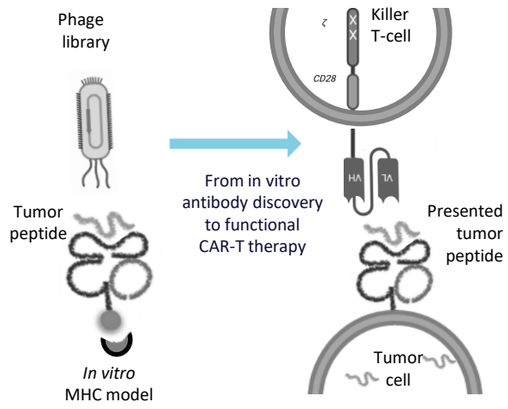
Introduction

MHC class I molecules play a crucial role in the immune system by presenting peptides, including ones derived from tumor antigens, to cytotoxic T cells enabling recognition and elimination of cancer cells by the immune system. Leveraging this mechanism, an attractive therapeutic approach is developing antibodies specific for tumor peptide loaded MHC class I complexes to subsequently engineer the T-cell receptor to generate CAR-T cells for enhanced cancer immunotherapy.

Advancements in computational analyses of multi-omic data sets has accelerated the identification of tumor-specific peptides presented in MHC class I molecules and as such are of interest for CAR-T approaches. However, targeting tumor peptides presented in MHC complexes is a challenging task from a specificity perspective as the presented peptide is a small epitope in a bulky complex. Additionally, the difference between a tumor peptide and a normal peptide might be only one amino acid.

Phage display is a powerful technology to tackle the challenges associated with the identification of antibodies that specifically recognize tumor peptides in complex with MHC molecules. Here we show how a significant diverse set of such antibodies was isolated from AVS Bio's proprietary human scFv phage display libraries by applying smart panning strategies. Subsequent functional verification of identified scFvs in a CAR-T model revealed CAR-T cells that could kill tumor cells in cytotoxicity assays, highlighting the value of our advanced human phage display technology for development of CAR-T-based therapeutics.



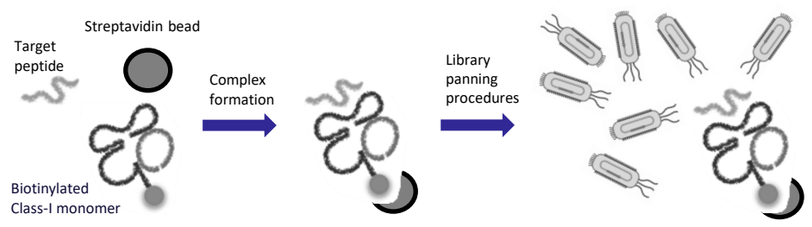
Smart selection strategies using high quality human scFv repertoires advances the identification of highly specific and functional tumor peptide MHC binding fragments

Total timeline: 11-17 weeks

Target and Tools

1-2 weeks

1. Production of MHC complexes loaded with (off-)target peptides
2. Magnetic bead preparation for library pannings

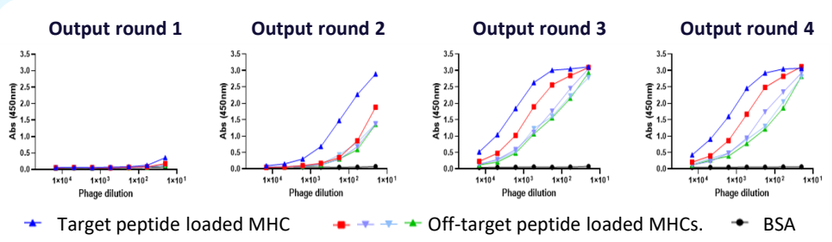


Target peptide was selected via multi-omic analyses of human tumors and healthy tissues. Off-target peptides were designed based on homologous peptides found in omic data sets of healthy tissue. In silico tools were used for MHC molecule binding prediction of the target peptide. Next, recombinant MHC molecules were loaded with the target or off-target peptides for use in phage selections, depletions and screenings.

Polyclonal Phase

1-2 weeks

1. Rescue phage display library
2. Four rounds of library panning:
   - Deselection on off-target peptide MHC complexes
   - Selection on target peptide MHC complex
3. Polyclonal phage screening



The results of the polyclonal screening show discriminating binding between target and off-target loaded MHC complexes. No binding towards irrelevant protein BSA was observed. Output rounds 2 and 3 were selected for monoclonal screening, based on a trade off between target reactivity and sequence diversity.

Monoclonal Phase

2-3 weeks

1. Monoclonal scFv production and screening
2. Monoclonal sequencing

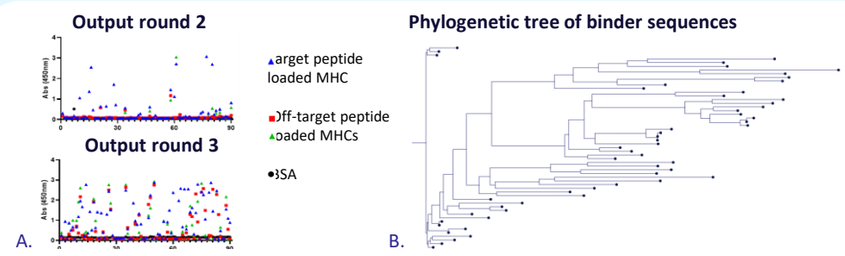
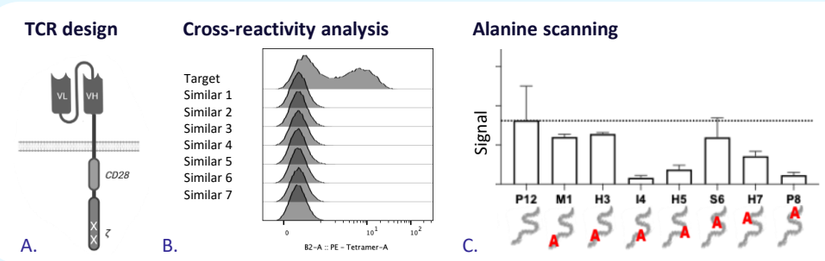


Figure A shows the reactivity of 90 monoclonals from both output round 2 and 3. Output round 2 yielded clones with higher specificity compared to round 3. However, output round 3 yielded more reactive, but non-specific clones. These data are consistent with the polyclonal screening. In total, ***42 CDR3-H unique sequences*** *were obtained. Figure B shows the phylogenetic tree of the 42 clones.*

Binder Characterization

4-6 weeks

1. Re-engineering of selected scFvs for CAR-T generation
2. CAR-T cross-reactivity analysis
3. Validation of CAR-T footprint

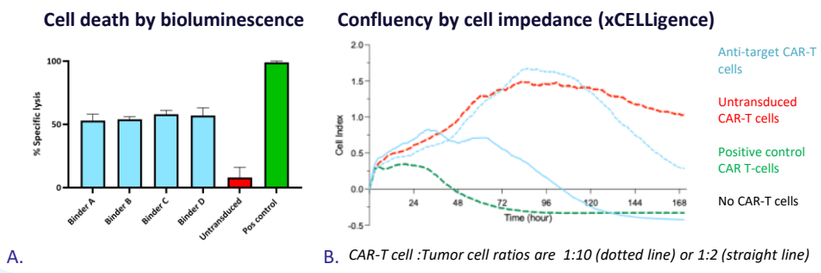


Selected antibody sequences were re-engineered to Chimeric Antigen Receptor (CAR)-formats (either in VH-VL and VL-VH orientation) and expressed in T cells (Figure A). Cross-reactivity of each CAR-T towards MHC complexes loaded with similar peptides was determined (exemplified in Figure B) as well as the antigen footprint by peptide alanine scanning (exemplified in Figure C).

Cytotoxicity Analysis

3-4 weeks

1. Short term cytotoxicity assays on endogenously expressing cells
2. Long term cytotoxicity assays on endogenously expressing cells



Tumor killing potential of CAR-T cells was assessed by incubating the CAR-T cells with target-expressing tumor cells and monitoring cell death of the tumor cells using bioluminescence readout after 24 hours of coculture (Luciferase expressing tumor cells, n=3 primary donors) (Figure A) and cell impedance-based tumor cell confluency measurements after 7 days using the xCELLigence (Figure B).

Rapid development of functional CAR-Ts driven by a premium human phage display approach

Conclusion

Our ready-to-use human scFv repertoires and sophisticated phage display technology are advancing identification of functional binders against tumor peptides loaded in MHC complexes, exhibiting high target specificity and potential to eradicate tumors cells in CAR-T format.