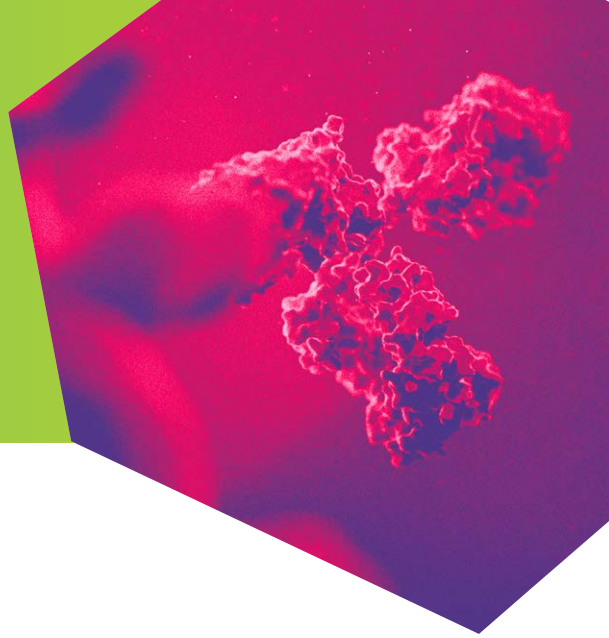
SCIENTIFIC CASE STUDY

T-cell engagers targeting pMHCs



The following data was presented as a poster at the **Society for Immunotherapy of Cancer Annual Meeting 2023**.

INTRODUCTION

TCEs targeting pMHCs could expand the reach of cancer immunotherapies

Bispecific T-cell engagers (TCEs) induce T-cell-mediated tumor-cell killing by simultaneously binding tumor-associated antigens (TAAs) and CD3, a T-cell activating protein. However, the target repertoire of most TCEs is restricted to TAAs on the surface of tumor cells, which constitute less than 15% of cellular proteins.[1,2]

Accessing intracellular TAA peptides displayed on MHC class I (pMHCs) would greatly expand the TCE target pool, particularly for solid tumor applications.[3] TCEs have the potential to overcome limitations associated with other pMHC-targeting modalities, such as soluble T-cell receptors (TCRs), by eliminating the need for extensive engineering of endogenous TCRs to produce molecules with the requisite affinity and specificity.[4]

Developing pMHC-targeting TCEs with high potency and specificity

To be effective, TCEs targeting pMHCs must have a high degree of specificity to a small (~10 amino acid) peptide while avoiding substantial interactions with the MHC to minimize the risk of off-target toxicities. In addition, they need to bind to tumor cells expressing low levels of the target while working in concert with the CD3-binding arm to induce tumor-cell killing.

We have integrated our TCE platform, which includes hundreds of diverse CD3-binding antibodies, with our antibody discovery, engineering, and development capabilities to build a platform capable of overcoming the challenges that have limited development of pMHC-targeting TCEs.

OUTCOME

Functional TCEs against a pMHC target

In this case study, we present data demonstrating how we leveraged our platform to discover functional and specific TCE molecules targeting the pMHC melanoma-associated antigen 4 (MAGE-A4).[5]

We identified MAGE-A4 x CD3 TCEs in a 1x1 format that show:

- Potent *in vitro* functional activity with comparable tumor-cell killing and cytokine release to a clinical-stage 2x1 TCE
- Diverse binding orientations and high specificity for MAGE-A4-pMHC with no binding to closely related peptides

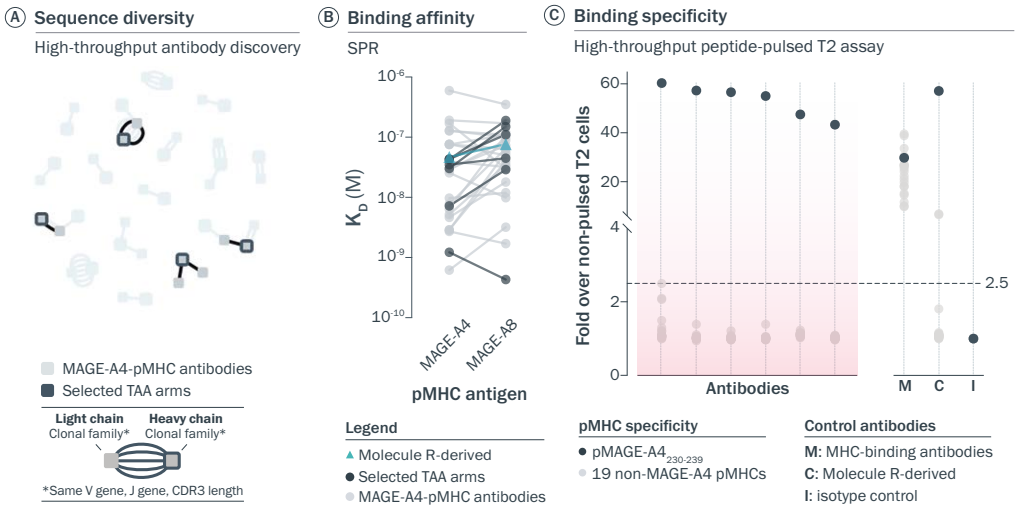
By combining our TCE platform with our discovery and protein science capabilities, our technology is enabling discovery of antibodies that are highly specific to pMHCs, expanding the accessible target space for TCEs in solid tumors.

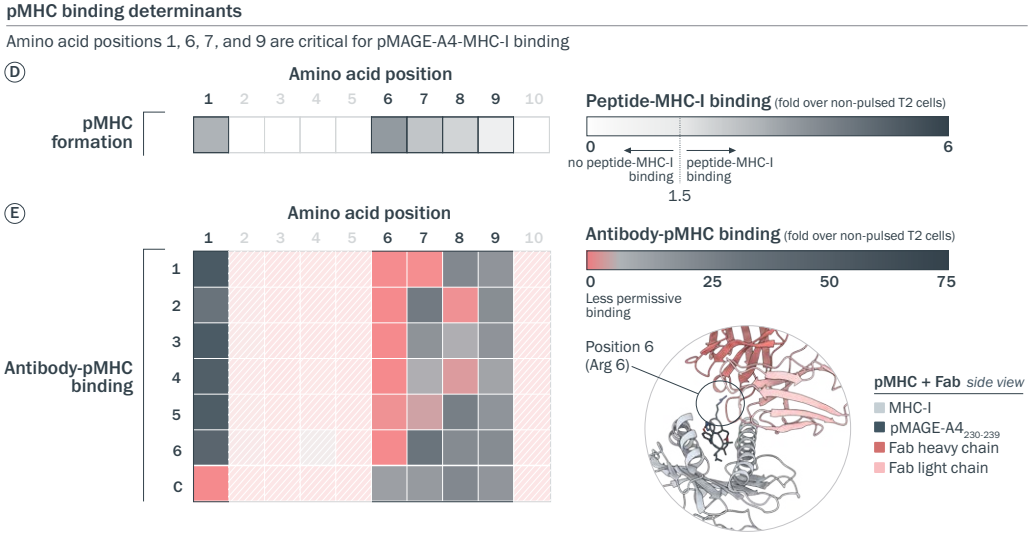
Specific and developable pMHC-binding antibodies for TCE development

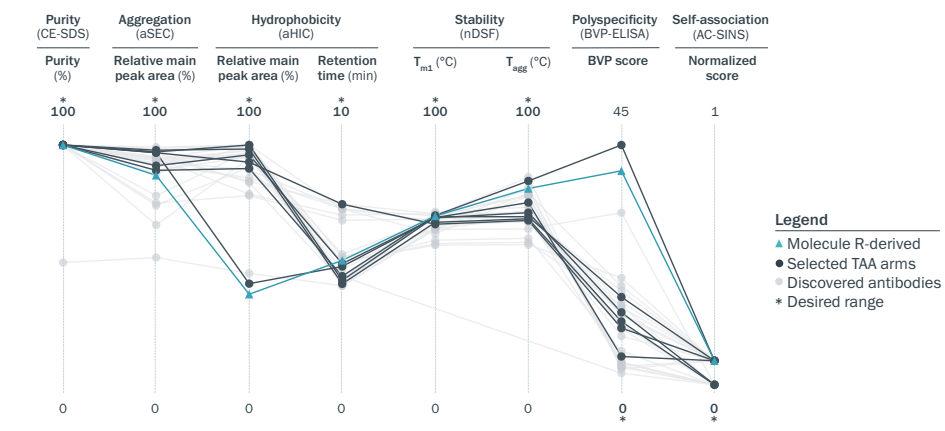
Proprietary immunization strategies and high-throughput single-cell screening was used to discover antibodies against pMAGE-A4$_{230\text{-}239}$ displayed on HLA-A:02*01 (Figure 1A). Six antibodies with high affinity (Figure 1B) and specificity (Figure 1C) to MAGE-A4-pMHC were selected for pairing with 19 CD3-binders from our TCE platform to generate more than 200 MAGE-A4 x CD3 bispecifics in a 1x1 format.

FIGURE 1. Selection of diverse, high-affinity, and specific MAGE-A4-pMHC antibodies for TCE engineering

(A) 45 antibodies that were discovered from humanized mice using high-throughput single-cell screening were expressed. Antibody sequence diversity was visualized using Celium™. The six MAGE-A4-pMHC-binders that were selected for bispecific engineering following characterization are highlighted. **(B)** Antibody binding kinetics to MAGE-A4-pMHC and MAGE-A8-pMHC were assessed using SPR. Binding affinities of selected TAA arms ranged from 7 nM to 15 µM. **(C)** pMHC binding specificity was assessed using a high-throughput peptide-pulsed T2 assay with MAGE-A4-pMHC and 19 other closely related pMHCs. Selected TAA arms show high specificity for MAGE-A4-pMHC with low to no binding to the other pMHCs tested. **(D)** Each amino acid of pMAGE-A4$_{230\text{-}239}$ was replaced with every possible amino acid to create an X-scan peptide library of 192 peptide variants. To determine relative binding of each peptide to HLA-A:02*01, the accumulation of pMHCs on the surface of peptide-pulsed T2 cells was assessed by flow cytometry using an anti-HLA-specific antibody. Values below 1.5 were considered negative for pMHC formation. The median value of HLA accumulation is shown. **(E)** The median values of antibody-pMHC binding is shown for the six selected TAA arms and a clinical-stage antibody 'C', which is the TAA-binding arm of Molecule R (monospecific IgG format).[6] The side chain of pMAGE-A4$_{230\text{-}239}$ is fully solvent-exposed at the Arginine 6 position. **(F)** Selected TAA arms have favorable developability profiles, including desired purity, stability, aggregation, relative surface hydrophobicity, self-association, and polyspecificity.







Functional and specific MAGE-A4 x CD3 TCEs

We performed high-throughput functional, structural, and specificity assessments of more than 200 MAGE-A4 x CD3 TCEs alongside a clinical-stage molecule in a 2x1 format.[6] Structural analyses of the TAA-binding arms revealed diverse Fab-pMHC binding orientations (Figure 3A), which translated into a broad range of tumor-cell killing (Figure 2A,B) and cytokine release (Figure 2C) profiles. We assessed T-cell-mediated killing of MAGE-A4$^+$ tumor cells and selected molecules that displayed potent, target-specific tumor-cell killing properties for further assessment.

FIGURE 2. Identification of functional MAGE-A4 x CD3 TCEs for further assessment

(A) T-cell-mediated killing of wild-type and MAGE-A4 knockout cells was measured in a high-throughput T-cell-dependent cytotoxicity (TDCC) assay using unactivated human T cells incubated with target cells at a ratio of 10:1. Tumor-cell killing and cytokine concentrations were measured 48 hours following addition of the TCEs. We assessed more than 200 1x1 MAGE-A4 x CD3 TCEs alongside Molecule R (057D03 paired with CD3-binder V9 in a 2x1 bispecific format, Roche).[6] Twelve TCEs with desired functional activity that were selected for further assessment are highlighted. **(B)** Potency (EC_{50}) is the concentration of each TCE needed to induce 50% of the maximal T-cell-mediated killing of A375 cells. **(C)** Cytokine release profiles of TCEs were assessed using a TDCC assay.

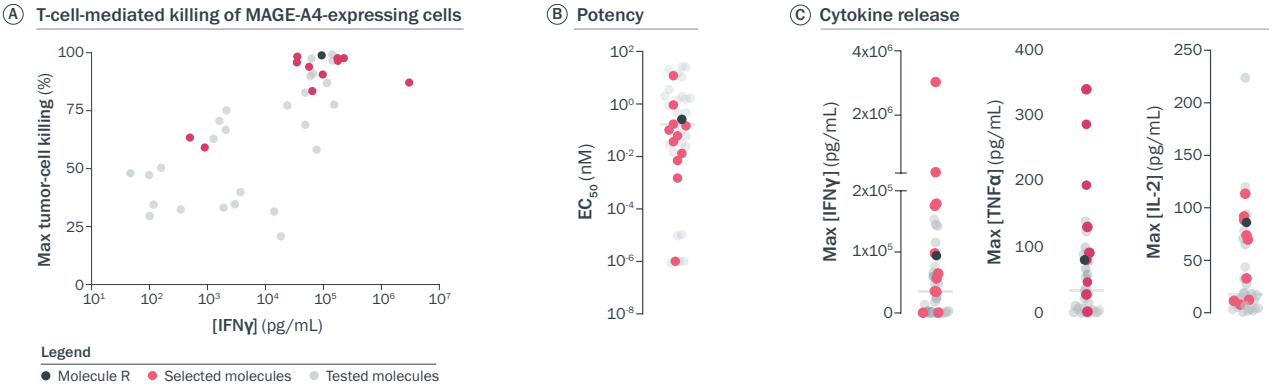
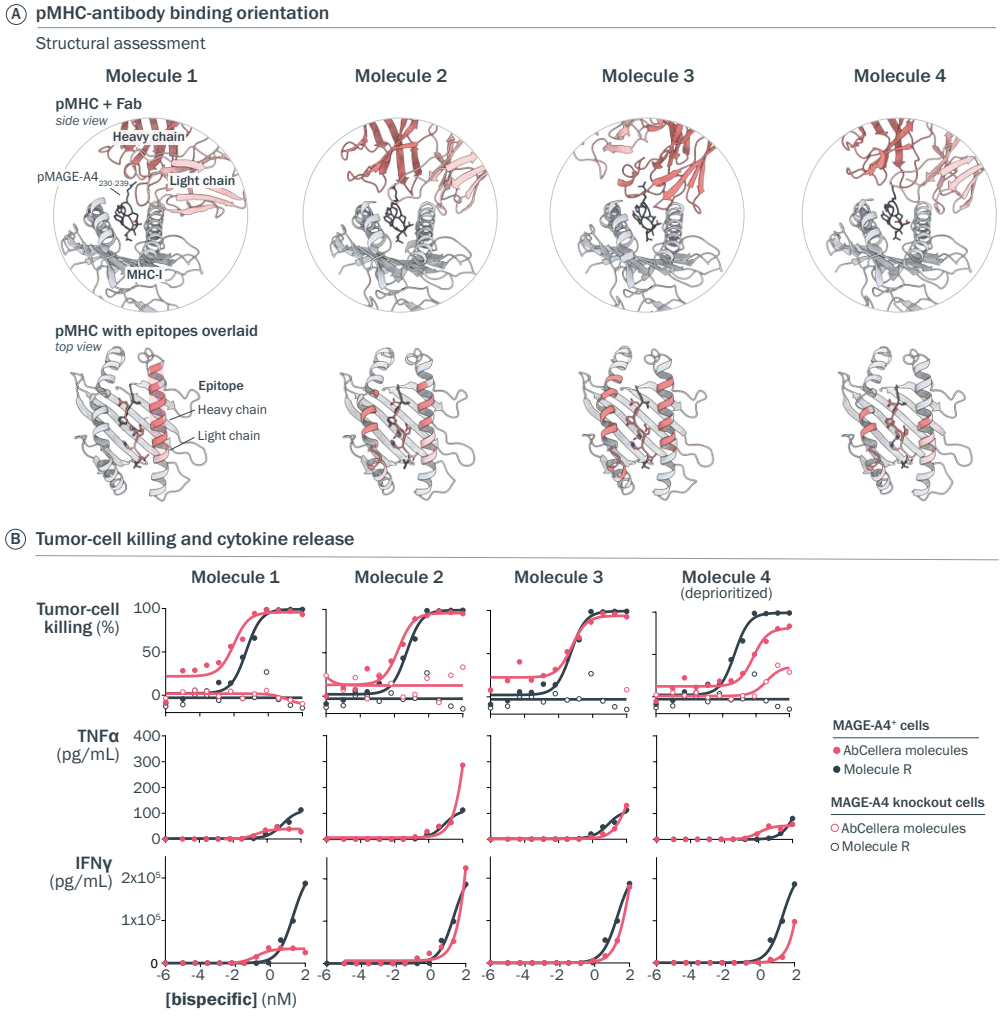


FIGURE 3. Structures and functional activity of example MAGE-A4 x CD3 TCEs

(A) We assessed the structures of antibody Fabs bound to pMAGE-A4$_{230-239}$ displayed on MHC-I (HLA:02*01) using X-ray crystallography and cryo-electron microscopy at 2.7 to 3.3 Å. The TAA arms show peptide-centric binding to MAGE-A4-pMHC at diverse angles and orientations. **(B)** T-cell-mediated killing of MAGE-A4-expressing A375 cells and A375 MAGE-A4 knockout cells was measured in a high-throughput TDCC assay using unactivated human T cells incubated with target cells at a ratio of 10:1. The release of TNFα and IFNγ at increasing antibody concentrations are shown. Functional activity of Molecule R (057D03 paired with CD3-binder V9 in a 2x1 bispecific format, Roche)[6] is also shown. Molecule 4 is an example of a TCE that does not meet the desired tumor-cell-killing profile due to killing of MAGE-A4 knockout cells at higher antibody concentrations.



CONCLUSION

We used our antibody discovery and development engine to identify diverse and developable antibodies that bind with high affinity and specificity to a pMHC target. These antibodies were paired and tested with our previously described, fully human CD3-binding arms to generate hundreds of TCEs in a 1x1 format. Structural assessments and X-scan binding assays revealed diverse pMHC-binding determinants, while high-throughput functional analyses demonstrated that the binding diversity observed resulted in a broad range of functional profiles. We identified molecules with potent and specific killing of target-expressing tumor cell lines that rival that of a clinical-stage molecule in a 2x1 format.[6] Together, these data demonstrate how our TCE platform, combined with our antibody discovery and protein science capabilities, is unlocking this high-value target class to expand the reach of immuno-oncology therapeutics.

REFERENCES

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