SCIENTIFIC CASE STUDY

Functional and specific T-cell engagers for a peptide-MHC tumor target

The following data was presented as a poster at the **American Association for Cancer Research® (AACR) Annual Meeting 2024.**

BACKGROUND

pMHCs could expand therapeutic opportunities for T-cell engagers

T-cell engagers (TCEs) are among the most promising new modalities in cancer therapy. However, their target repertoire has been restricted to tumor-associated antigens (TAAs) that are expressed on the cell surface, which make up <15% of cellular proteins.[1,2] Accessing intracellular peptides displayed on MHC class I (pMHCs) would greatly expand the target pool for TCEs.[3]

There are multiple modalities that target pMHCs, including soluble T-cell receptor (TCR)-based molecules and TCR-mimic bispecific TCEs. Soluble TCRs must be extensively modified to enhance affinity, which can lead to promiscuous binding independent of the peptide.[4,5] In contrast, antibodies bind pMHCs with affinities in the nanomolar to picomolar range,[6] reducing the engineering required to generate potent molecules. However, structural studies reveal that while TCRs typically bind along the peptide's core,[7] antibodies often bind with a bias towards the termini.[8] This can reduce specificity and necessitates extensive specificity screening to avoid off-target toxicities.

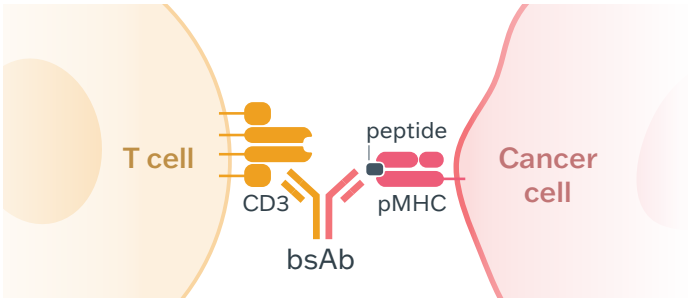


FIGURE 1. Bispecific antibodies (bsAbs) that target pMHCs could expand therapeutic opportunities for TCEs.

AIM

Generate potent, specific MAGE-A4 x CD3 T-cell engagers

We generated CD3 TCEs targeting melanoma-associated antigen 4 (MAGE-A4)-pMHC, a tumor-specific antigen expressed by many solid tumors, but not by most healthy tissues.[9] We paired six pMHC-binding arms with our diverse CD3-binders[10] and assessed bispecific function. We implemented an *in vitro* and *in silico* workflow to assess specificity of MAGE-A4 x CD3 TCEs across hundreds of pMHCs, and integrated the results with structural data to identify a highly specific molecule that is differentiated from a clinical benchmark.

OUTCOME

A MAGE-A4 x CD3 T-cell engager that is differentiated from a clinical benchmark

We identified a potent, highly specific MAGE-A4 x CD3 TCE that is ready for further preclinical assessment and shows:

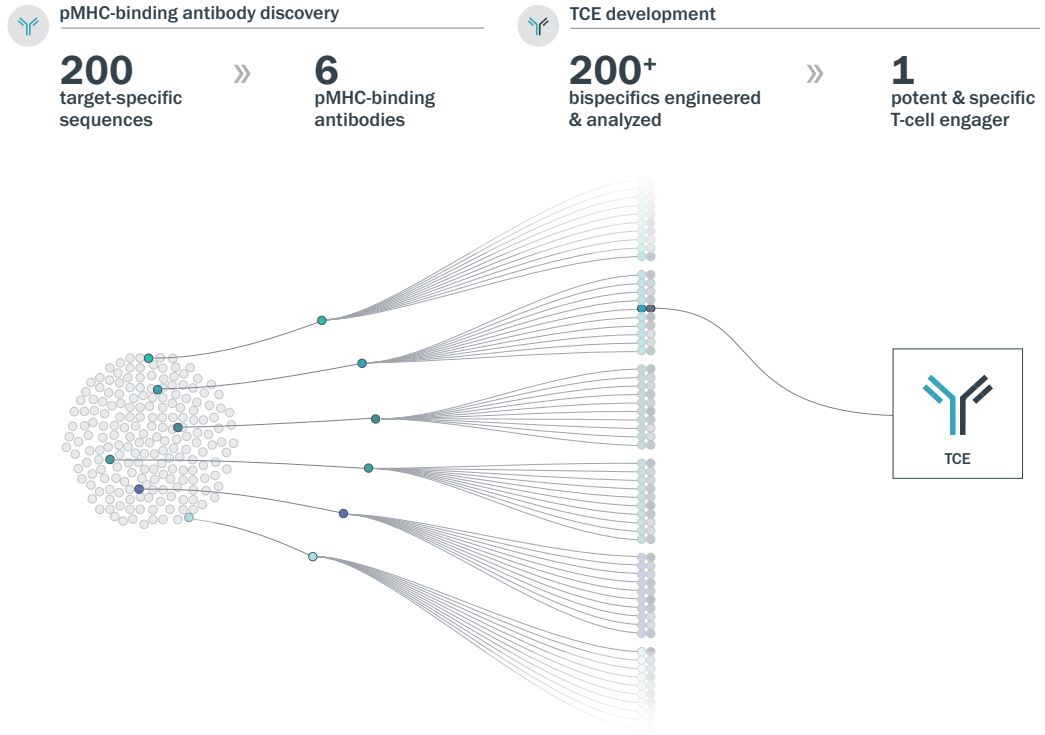
- Potent activity across multiple MAGE-A4-expressing-cell lines with no activity against MAGE-A4-negative cells
- Highly specific binding to MAGE-A4-pMHC with no binding to more than 180 non-MAGE-A4 pMHCs
- Binding predominantly to the central residues of the MAGE-A4 peptide

These data demonstrate how our TCE platform, combined with our antibody discovery capabilities, can generate highly specific TCEs for pMHC targets.

Generating rare potent and specific T-cell engagers for pMHC targets without extensive protein engineering

We used our proprietary single-cell screening platform to interrogate 1.5 million single cells. We identified 200 unique MAGE-A4-pMHC- binding antibody sequences, six of which had specificity and developability profiles suitable for TCE engineering. We leveraged our TCE platform, which includes novel CD3-binding antibodies that are differentiated from molecules commonly used for TCE development, to generate more than 200 1x1 bispecific TCEs. Following high-throughput functional and biophysical analyses, 12 were selected for in-depth assessment. Antibodies were produced at mid-scale for rigorous *in vitro* assessment, and one molecule with the potency and specificity required to target MAGE-A4-pMHC was identified for further preclinical assessment.

FIGURE 2. Deep screening of immune repertoires, high-throughput *in vitro* assessments, and a robust TCE platform enable identification of rare TCEs with high potency and specificity for pMHC targets.

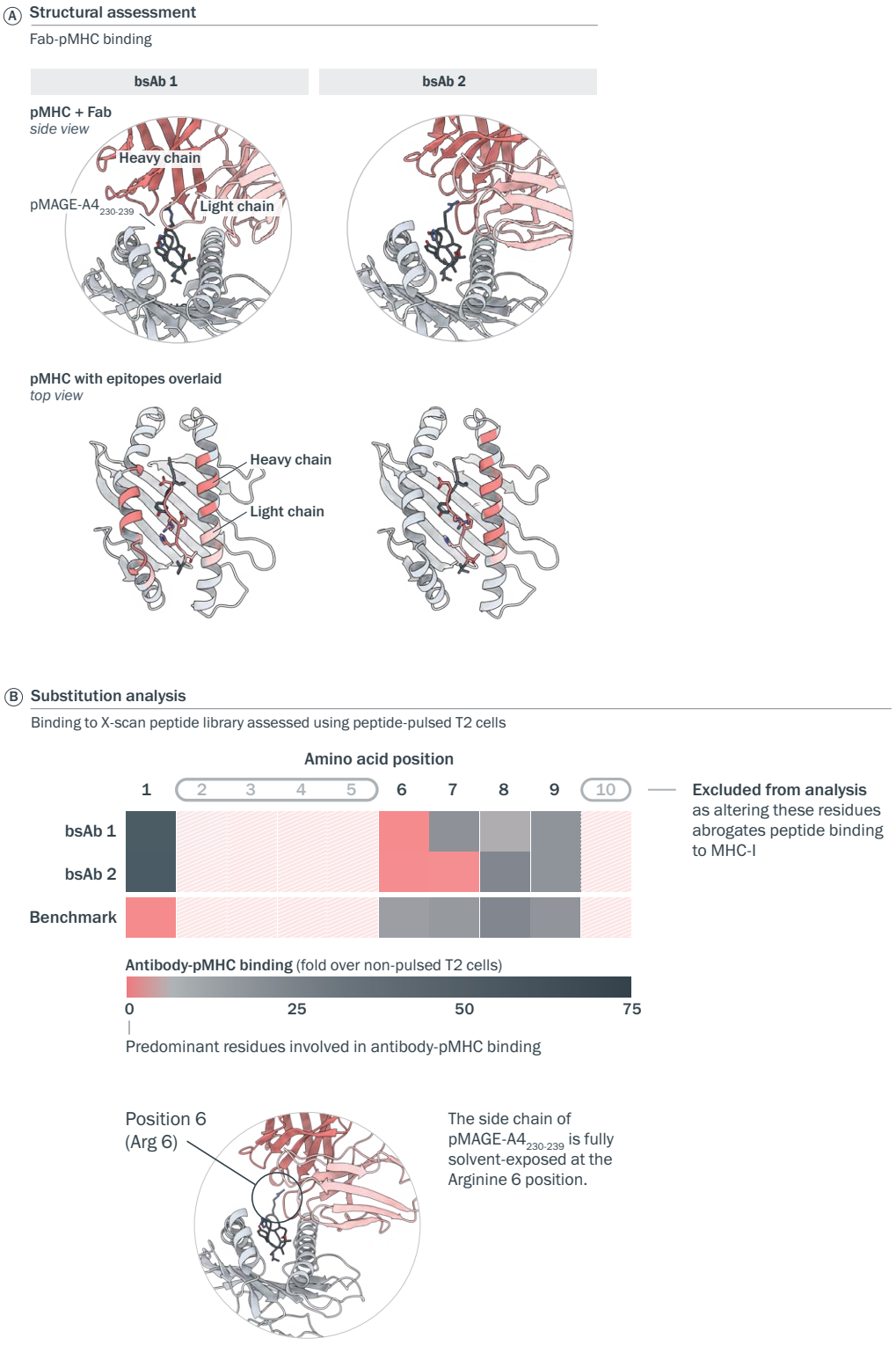


pMHC-binding profiles that are differentiated from a clinical benchmark

To elucidate antibody-pMHC interactions, we profiled pMHC-binding antibodies using structural and substitution analyses. Data demonstrated a high degree of diversity in Fab-pMHC binding orientations. Two TAA-binding arms that were used to generate the TCEs shown in Figure 4 are highlighted, revealing peptide-centric binding (Fig. 3A) and predominant binding residues that differ from that of a clinical benchmark (Fig. 3B).[11]

FIGURE 3. pMHC binding properties show differentiation from a clinical benchmark.

(A) Antibody Fabs bound to pMAGE-A4$_{230-239}$ displayed on MHC-I (HLA-A*02:01) were assessed by cryo-electron microscopy at 2.7 to 3.3 Å and show peptide-centric binding. **(B)** Each amino acid of pMAGE-A4$_{230-239}$ was replaced with every possible amino acid to generate 190 variants. Substitutions that abrogated peptide binding to MHC-I (assessed by flow cytometry) were excluded from the analysis. The median values of antibody-pMHC binding compared to the benchmark (Molecule R, monospecific IgG format) [11] are shown.

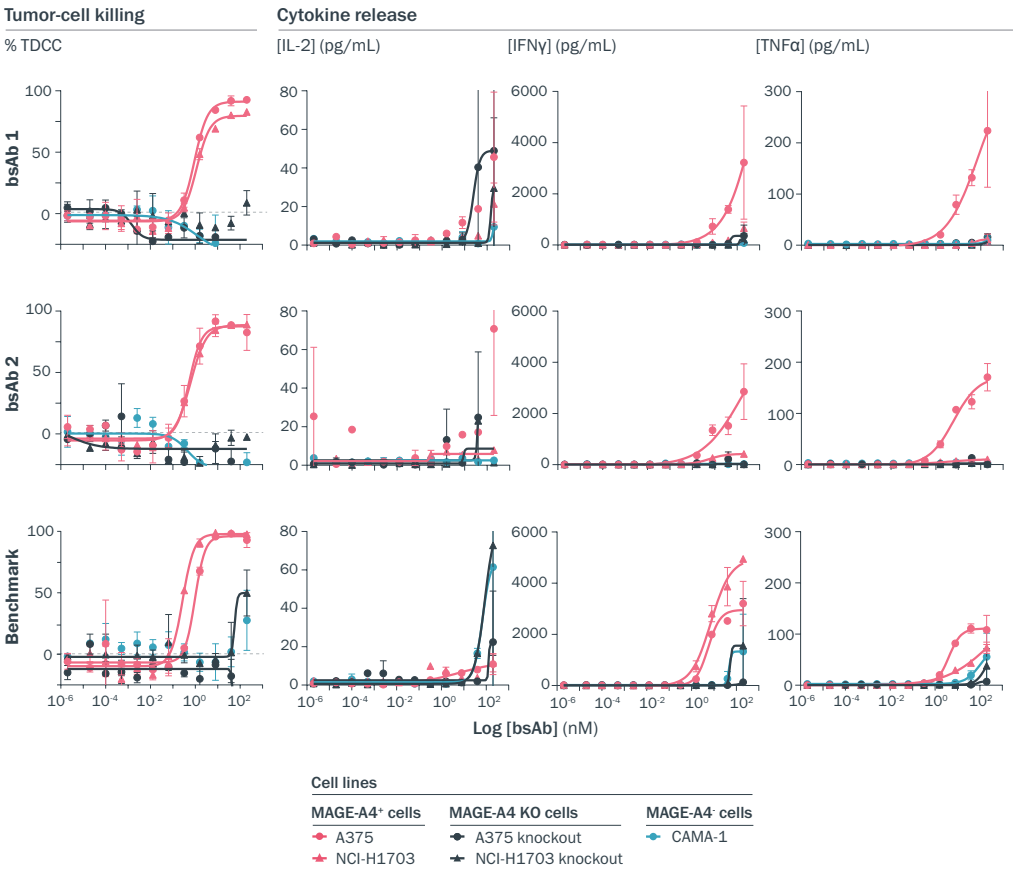


MAGE-A4 x CD3 T-cell engagers with potent and specific tumor-cell killing

Following high-throughput functional and preliminary specificity assessments of 200+ 1x1 TCEs, a subset of functional molecules was produced at mid-scale for further characterization. Two molecules, bsAb 1 and bsAb 2, demonstrated highly specific activity against cell lines expressing MAGE-A4, with potencies comparable to a 2x1 clinical benchmark (Fig. 4).[11]

FIGURE 4. MAGE-A4 x CD3 TCEs show functional profiles comparable to a clinical benchmark.

Functional profiles for two molecules selected for further assessment are compared to that of a clinical benchmark[11]. Cytokine release and T-cell-dependent cellular cytotoxicity (TDCC) of MAGE-A4+, MAGE-A4 knockout (KO), and MAGE-A4− cell lines were measured using unactivated human T cells incubated with target cells at a ratio of 10:1 or 20:1 for 72 hours.

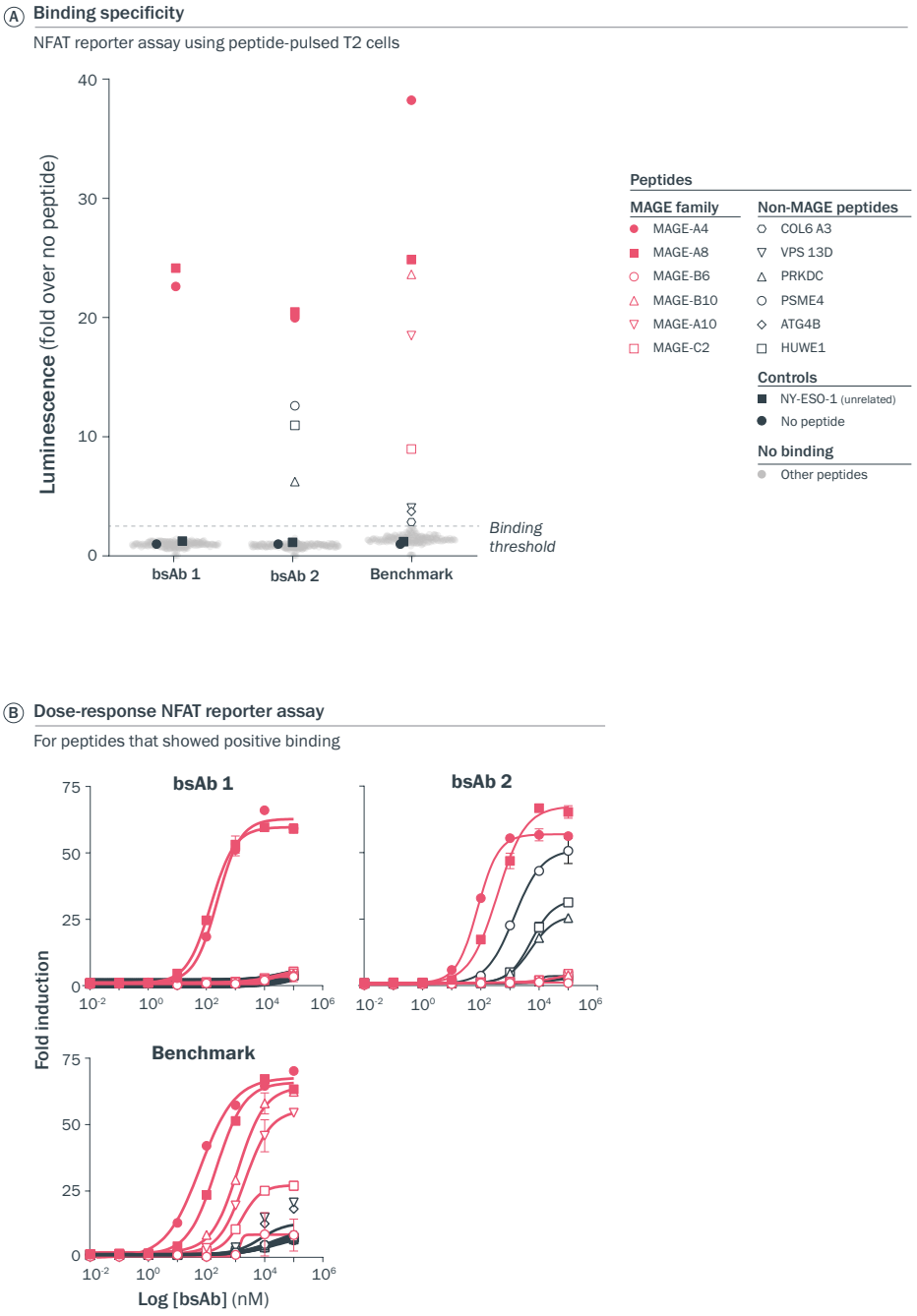


A T-cell engager with high specificity for MAGE-A4-pMHC

To assess specificity of CD3 x MAGE-A4 TCEs, antibody-pMHC binding data from X-scan and structural analyses (Fig. 3) were integrated into the CrossDome package[12] to select 180+ peptides, and binding specificity was assessed using an NFAT report assay. Data reveal that bsAb1 binds with high specificity to MAGE-A4- and MAGE-A8-pMHCs with no binding to any of the peptides tested (Fig. 5).

FIGURE 5. Specificity assessment shows that bsAb 1 specifically binds tumor-associated peptides from MAGE-A4- and MAGE-A8-pMHC but not to any other peptides tested.

(A) T2 cells were pulsed with relevant peptides and co-cultured with bispecifics and Jurkat NFAT reporter cells. Activation of the NFAT response element indicates positive binding to the peptide-pulsed T2 cells. **(B)** Dose-response curves were generated for peptides showing a signal greater than 2.5-fold over the no peptide control to validate single-point data shown in Fig. 5A.



CONCLUSION

TCEs are among the most promising new modalities in cancer therapy, but limitations in efficacy and safety have been barriers to realizing their potential for solid tumor indications. To address these challenges, we developed a TCE platform that includes novel CD3-binding antibodies to widen the therapeutic window for this modality, costimulatory building blocks to enhance efficacy for difficult-to-treat cancers, and discovery capabilities to broaden the range of TCEs to complex peptide-MHC tumor targets.

Data shown here demonstrate how our TCE platform could unlock pMHCs as a target class for this modality by generating molecules with a high degree of potency and specificity. By combining out antibody discovery capabilities with our TCE platform, we were able to identify a rare CD3 x MAGE-A4 pMHC that showed potent and specific tumor-cell killing and no binding to 180+ non-MAGE peptides.

Using this platform, we are focused on unlocking the full potential of this modality by advancing internal programs and by engaging in strategic partnerships to bring powerful new cancer medicines to patients.

REFERENCES

1. Weekes MP, et al. (2010). J Biomol Tech. 21(3):108-115. PMID: 20808639.
2. Hong CW, et al. (2014). FEBS Lett. 588(2):350-355. doi: 10.1016/j.febslet.2013.10.025.
3. Chandran SS, et al. (2019). Immunol Rev. 290(1):127-147. doi: 10.1111/imr.12772.
4. Zhao Y, et al. (2007). J. Immunol. 179:5845–5854. doi: 10.4049/jimmunol.179.9.5845.
5. Border EC, et al. (2019). OncoImmunol. 8:e1532759. doi: 10.1080/2162402X.2018.1532759.
6. Foote J, Eisen HN. (1995). Proc. Natl Acad. Sci. USA. 92:1254–1256. doi: 10.1073/pnas.92.5.1254.
7. Rossjohn J, et al. (2015). Annu. Rev. Immunol. 33:169–200. doi: 10.1146/annurev-immunol-032414-112334.
8. Holland CJ, et al. (2020). J. Clin. Invest. 130:2673–2688. doi: 10.1172/JCI130562.
9. Schooten E, et al. (2018). Cancer Treat Rev. 67:54-62. doi: 10.1016/j.ctrv.2018.04.009.
10. de Puyraimond V, et al. (2023). Journal for ImmunoTherapy of Cancer. 11(Suppl 1):A1523. doi: 10.1136/jitc-2023-SITC2023.1367.
11. Weinzierl T, et al. (2021). (International Publication No. WO/2021/122875). World Intellectual Property Organization.
12. Fonseca AF, et al. (2023). Font Immunol. 14:1142573. doi: 10.3389/fimmu.2023.1142573.