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

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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.

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 MAGE-A4-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.

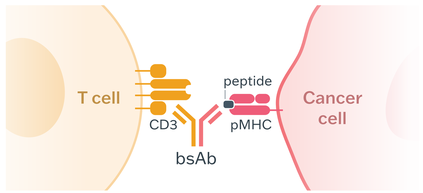

Figure 1. Bispecific antibodies (bsAbs) that target pMHCs could expand therapeutic opportunities for TCEs.

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 for further preclinical assessment that 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

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

Bispecific 1 and 2 show target-specific tumor-cell killing across multiple cell lines

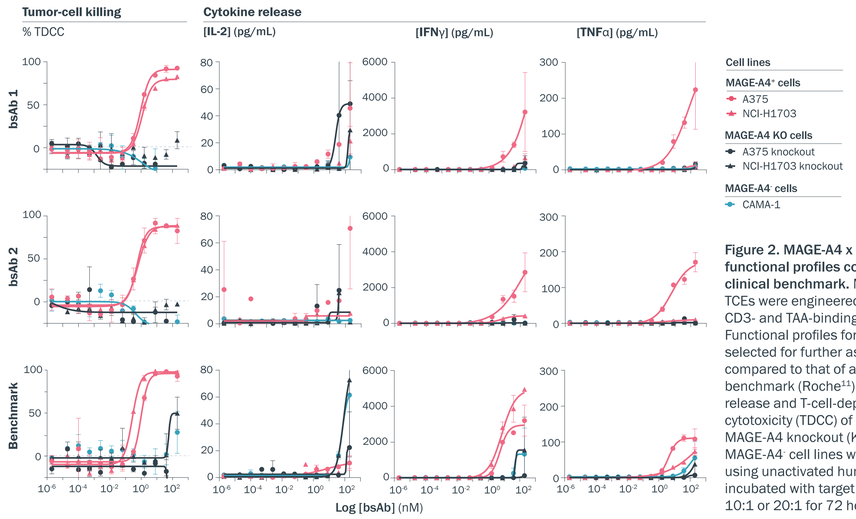

Figure 2. MAGE-A4 x CD3 TCEs show functional profiles comparable to a clinical benchmark. More than 200 TCEs were engineered using diverse CD3- and TAA-binding arms. Functional profiles for two molecules selected for further assessment are compared to that of a clinical benchmark (Roche[11]). Cytokine 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

Bispecific 1 does not show binding to any of the 180+ non-MAGE peptides tested

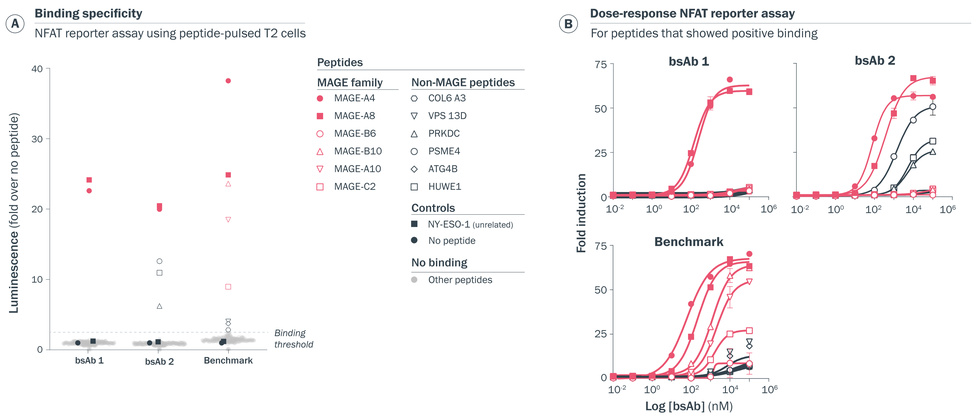

Figure 3. Specificity assessment shows that Bispecific 1 specifically binds tumor-associated peptides from MAGE-A4- and MAGE-A8-pMHC but not to any other peptides tested. Antibody-pMHC binding data from X-scan and structural analyses (Fig. 4) were integrated into the CrossDome package[12] to select peptides for antibody specificity assessment. **(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. 3A.

pMHC-binding profiles that are differentiated from a clinical benchmark

Antibody-pMHC interactions profiled using structural and substitution analyses

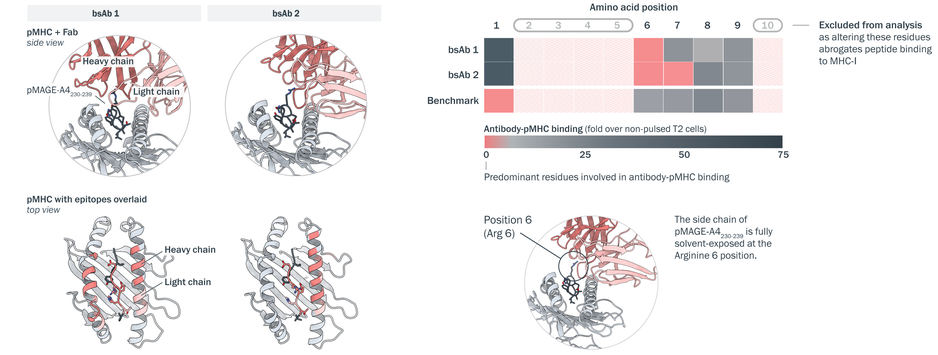

Figure 4. pMHC binding properties show differentiation from a clinical benchmark. (A) Antibody Fabs bound to pMAGE-A4$_{230-239}$ displayed on MHC-I (HLA-A*02:01) were assessed by cryo-electron microscopy at 2.7 to 3.3 Å and show peptide-centric binding. **(B)** Each amino acid of pMAGE-A4$_{230-239}$ 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.

Generation of rare potent and specific T-cell engagers for pMHC targets

A high-throughput platform generated pMHC-targeting T-cell engagers without extensive protein engineering

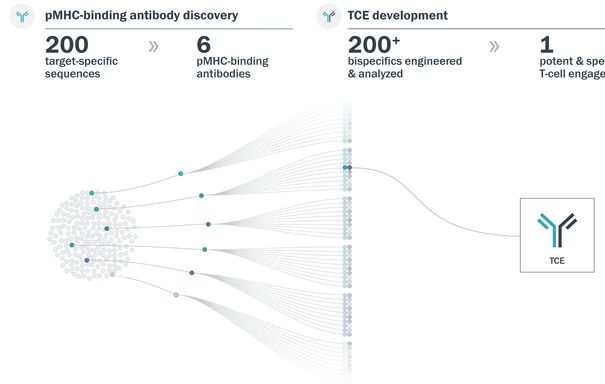

Figure 5. Deep screening of immune repertoires, high-throughput *in vitro* assessments, and a robust TCE platform enable identification of rare TCEs with high potency and specificity for pMHC targets. We used our proprietary single-cell screening platform to interrogate 1.5 million single cells. We identified 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.

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. Weinjoint 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.

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AbCellera's T-cell engager platform

We paired **six MAGE-A4-pMHC binders** with **19 antibodies from AbCellera's TCE platform** to generate and assess 200+ bispecifics in a 1x1 format.

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