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Targeting intracellular tumor antigens to fight cancer

Discovery and development of functional and specific T-cell engagers against a MAGE-A4 pMHC.

AUTHORS

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FURTHER READING

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THE OPPORTUNITY

Targeting intracellular tumor antigens to fight cancer

Bispecific T-cell engagers (TCEs) mobilize the immune system to fight cancer by simultaneously binding a tumor-associated antigen (TAA) and CD3, a T-cell activating protein. TCEs that target peptides displayed on major histocompatibility complexes (pMHC) can unlock previously inaccessible intracellular TAAs[1].

For example, melanoma-associated antigen 4 (MAGE-A4) is expressed by many solid tumors, including melanoma, bladder, head and neck, and gastroesophageal cancers, but not by most healthy tissues[2].

THE CHALLENGE

Peptide-MHCs are difficult targets

To be effective, TCEs against MAGE-A4-pMHC targets should:

- have **high target-binding affinities**, as tumor cells express very low levels of MAGE-A4-pMHC[3]
- bind to MAGE-A4-pMHC with the requisite specificity to **minimize the risk of off-target binding**[4,5]
- have TAA- and CD3-binding arms that **function optimally as a pair**
- and have **favorable developability** profiles.

Identifying parental antibody pairs that meet these criteria has been challenging using conventional antibody discovery and development approaches[6].

THE SOLUTION

Integrated technologies streamline TCE development

To address these challenges, we developed an integrated TCE platform that includes hundreds of diverse, fully human, CD3-binding antibodies and capabilities for antibody discovery, engineering, and functional screening.

THE OUTCOME

12 MAGE-A4 x CD3 bispecific TCEs for further assessment

We assessed hundreds of TCEs to identify 12 molecules with:

- **functional activity** against MAGE-A4$^+$ tumor cells
- **diverse binding orientations** to MAGE-A4-pMHC

Selection of diverse, specific, and developable pMHC-binders for TCE development

TAA arms

We strategically selected six TAA arms to generate hundreds of bispecific MAGE-A4 x CD3 TCEs

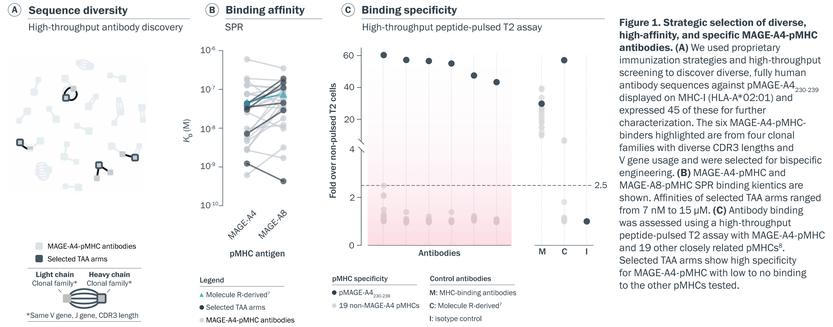
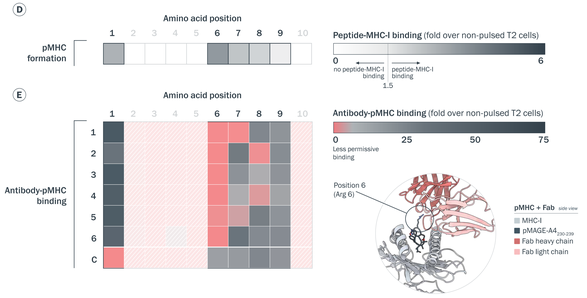


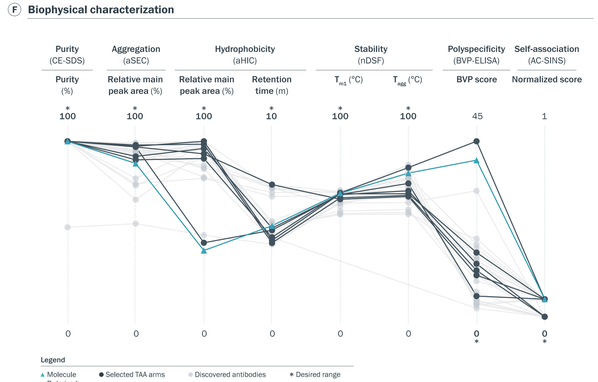
Figure 1. Strategic selection of diverse, high-affinity, and specific MAGE-A4-pMHC antibodies. (A) We used proprietary immunization strategies and high-throughput screening to discover diverse, fully human antibody sequences against pMAGE-A4$_{230-239}$ displayed on MHC-I (HLA-A*02:01) and expressed 45 of these for further characterization. The six MAGE-A4-pMHC-binders highlighted are from four clonal families with diverse CDR3 lengths and V gene usage and were selected for bispecific engineering. **(B)** MAGE-A4-pMHC and MAGE-A8-pMHC SPR binding kientics are shown. Affinities of selected TAA arms ranged from 7 nM to 15 µM. **(C)** Antibody binding was assessed using a high-throughput peptide-pulsed T2 assay with MAGE-A4-pMHC and 19 other closely related pMHCs[8]. Selected TAA arms show high specificity for MAGE-A4-pMHC with low to no binding to the other pMHCs tested.

Peptide-MHC and antibody-pMHC binding profiles

Identification of pMAGE-A4 residues critical for antibody binding by substitution analysis



(D) Each amino acid of pMAGE-A4$_{230-239}$ 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', TAA-binding arm of Molecule R (monospecific IgG format)[7]. The side chain of pMAGE-A4$_{230-239}$ 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.

Bispecific Engineering

We paired **six diverse, specific, and developable MAGE-A4-pMHC binders** with **19 anti-bodies from AbCellera's CD3 platform** that have diverse kinetic profiles, affinities, and subunit specificities, and favorable developability profiles.

We engineered and assessed 200$^+$ bispecific TCEs in a 1x1 format using our multispecific engineering platform, OrthoMab™.

See more data on our CD3 program

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Identification of 12 functional MAGE-A4 x CD3 TCEs

bispecifics

We assessed hundreds of TCEs for antibody-induced killing of MAGE-A4$^+$ tumor cells

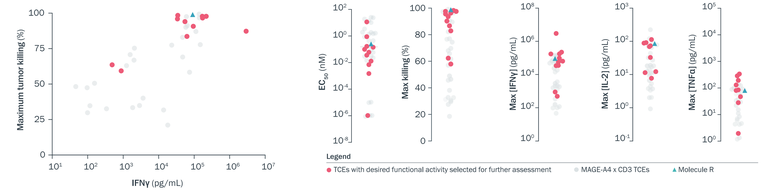


Figure 2. Identification of 12 functional MAGE-A4 x CD3 TCEs for further assessment. (A) T-cell-mediated killing of MAGE-A4-expressing and MAGE-A4 knockout A375 cells was measured in a high-throughput assay using unactivated human T cells incubated with target cells at a ratio of 10:1 for 48 hours. We assessed 200$^+$ 1x1 TCEs alongside Molecule R (057D03 paired with CD3-binder V9 in a 2x1 bispecific format)[7]. 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 (IFNγ, IL-2, and TNFα) of TCEs were assessed.

We explored antibody-antigen interactions and the associated functional profiles

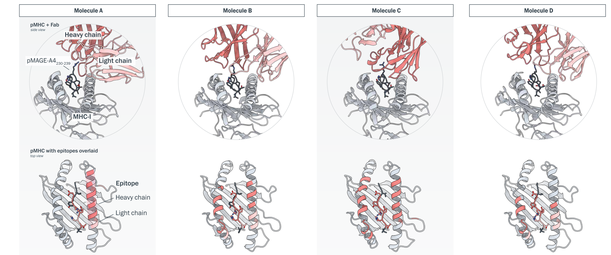
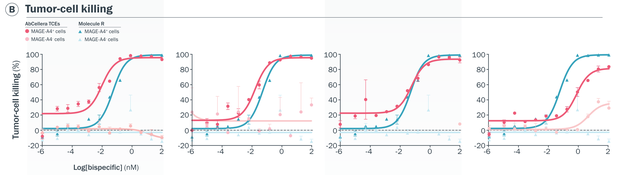
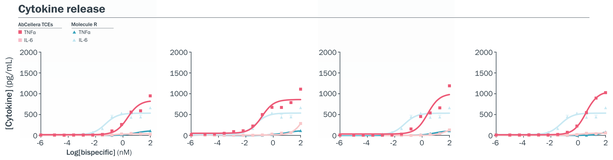


Figure 3. Structures and functional activity of example MAGE-A4 x CD3 TCEs. (A) Structures of antibody Fabs bound to pMAGE-A4$_{230-239}$ displayed on MHC-I (HLA-A*02:01) were assessed by cryo-electron microscopy at 2.7 to 3.3 Å. The TAA arms show peptide- centric binding to MAGE-A4-pMHC at diverse angles and orientations.



(B) Tumor-cell killing and cytokine release curves from the assay described in Figure 2 are shown. Molecule D 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.



CONCLUSIONS & NEXT STEPS

We used our antibody discovery and development engine to identify diverse and developable antibodies that bind with high affinity and specificity to MAGE-A4-pMHC. These antibodies were paired and tested with our previously described, fully human, CD3-binding arms to generate hundreds of 1x1 TCEs. We integrated multiple binding parameters, including target specificity, kinetics, and affinity, with TCE functional properties to identify molecules for further assessment.

We demonstrated effective and specific killing of target-expressing tumor cell lines *in vitro*, and performed high-resolution structural assessments and X-scan binding assays to assess specificity to MAGE-A4-pMHC.

Next Steps:

- Additional specificity assessments guided by SPR and pulsed T2 cell X-scan assays with *in silico* predictions to identify molecules with low risk of off-target cross-reactivity
- *In vitro* functional activity assays using additional MAGE-A4-expressing cell lines
- High-resolution functional assessments, including T-cell activation, proliferation, and exhaustion
- High-resolution developability assessments
- *In vivo* efficacy studies

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