Breaking barriers to access intracellular targets with T-cell engagers

Discovery of diverse, developable, and ultra-specific antibodies against a MAGE-A4 pMHC

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THE OPPORTUNITY

Unlock intracellular tumor targets

Immunotherapies are transforming cancer treatment by mobilizing the immune system to fight cancer. T-cell engagers — a type of immunotherapy — eliminate cancer cells by simultaneously binding tumor targets and the T-cell activating protein, CD3. Because T-cell engagers cannot access intracellular targets, their use is limited to targets expressed on the tumor cell surface.

T-cell engagers against peptides displayed on major histocompatibility complexes (pMHCs) are a promising approach for unlocking previously inaccessible, high-value, intracellular tumor targets[1].

One such target is melanoma-associated antigen 4 pMHC (MAGE-A4-pMHC), a tumor-specific antigen expressed by many solid tumors, but not by most healthy tissues[2].

THE CHALLENGE

Finding rare antibodies against pMHCs

MAGE-A4-pMHCs are challenging immunotherapy targets because (a) proteins within the MAGE-A family are highly homologous[2], (b) MAGE-A4 is often mutated in tumor cells[3], and (c) there is very low expression of MAGE-A4-pMHCs on tumor cells[4].

Antibodies that bind to MAGE-A4-pMHC with the specificity and affinity needed to minimize the risk of off-target binding are rare. Those that do bind and have favorable developability profiles are even more rare, making them difficult to identify using traditional approaches.

Furthermore, T-cell engager development requires tumor-binding antibodies that function as bispecifics, creating a need for diverse antibodies that can be paired with CD3-binders and tested for optimal function.

THE SOLUTION

Start with diverse panels of pMHC-binding antibodies

We used our antibody discovery and development engine to discover and characterize human MAGE-A4-pMHC-binding antibodies with favorable binding and developability profiles.

Lead MAGE-A4-pMHC antibodies will be selected and paired with our panel of previously described CD3-binding antibodies using our bispecific engineering platform, OrthoMab™, to streamline the development of MAGE-A4-pMHC T-cell engagers.

THE OUTCOME

We identified a panel of antibodies against MAGE-A4-pMHC with:

- **diverse sequence** and binding profiles
- **ultra-specific, high-affinity** binding
- **favorable developability** profiles

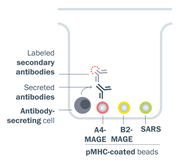
45 diverse antibodies against a MAGE-A4 peptide-MHC target

Antibody discovery

(A) Sequence analysis

Single-cell screening

(B) Multiplexed bead-based screening assay



Sequence diversity

(C) CDR3 length

V gene usage

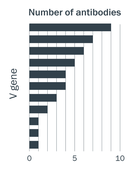
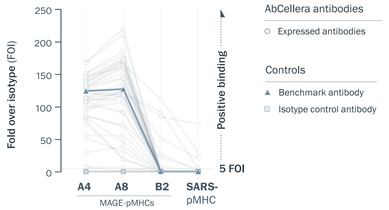


Figure 1. Proprietary immunization technologies and high-throughput screening strategies were used to amplify and capture antibody diversity. (A) Humanized mice were immunized with human pMAGE-A4$_{230-239}$ presented on MHC-I (HLA:02*01). **(B)** Multiplexed bead-based single-cell screening assays were used to screen 1.5 million single B cells and identify antibodies that bind to MAGE-A4-, but not MAGE-B2- or SARS-pMHC (an unrelated peptide control). **(C)** 45 MAGE-A4-pMHC-binding antibodies from 23 clonal families with sequence diversity were selected for high-throughput expression and further characterization.

Qualitative binding assessment

(A) Bead-based binding assay



Binding kinetics

(B)

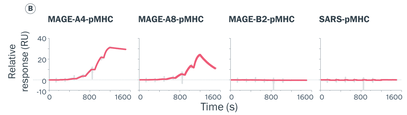
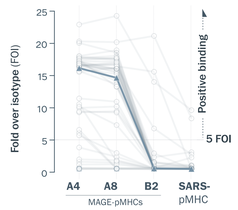


Figure 2. MAGE-A4-pMHC-binding antibodies were identified. **(A)** Antibody binding was assessed using a multiplexed bead-based binding validation assay with MAGE-A4-pMHC and closely related pMHC antigens. Binding (> 5 FOI) was compared to a MAGE-A4-pMHC-binding benchmark antibody and detected using flow cytometry. **(B)** Sensorgrams generated from Surface Plasmon Resonance (SPR) kinetic analysis of a selected target-binding antibody show relatively strong binding to MAGE-A4-pMHC compared to MAGE-A8-pMHC. No binding was observed to MAGE-B2- or SARS-pMHCs.

(C) Peptide-pulsed T2 cell-binding assay

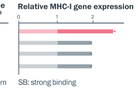


Peptide panel for binding assessment

(D)

Peptide	Amino acid sequence	Predicted peptide affinity to MHC-I[b]
A4	G V Y D G R E H T V	SB
A8	G L Y D G R E H S V	SB
B2	G V Y D G E E H S V	SB
SARS	R L Q S L Q T Y V -	SB

Relative MHC-I gene expression



D exposed surface residue ☐ residue differs from pMAGE-A4$_{230-239}$ SB: strong binding

(C) Antibody binding was further assessed in a cell-based peptide-pulsed T2 assay with MAGE-A4-pMHC and closely related pMHCs. Binding (> 5 FOI) was detected using flow cytometry. Over 70% of antibodies tested bound to both MAGE-A4-pMHC and MAGE-A8-pMHC (a closely related peptide also expressed on tumor cells) in both assays. **(D)** Relative MHC-I gene expression levels in T2 cells pulsed with different peptides (as a measure of positive peptide binding to MHC-I) are shown.

Developable antibodies with favorable biophysical properties

Biophysical assessment

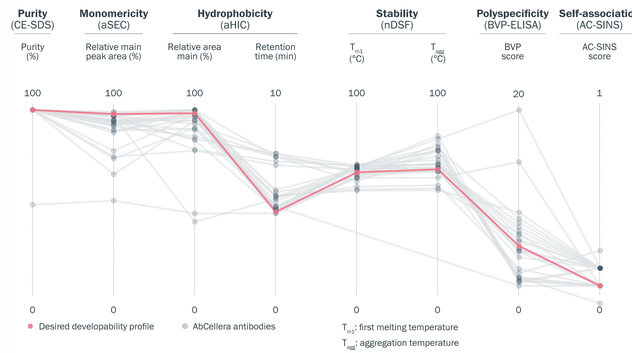


Figure 4. MAGE-A4-pMHC-binding antibodies have favorable developability profiles with high purity measured by capillary gel electrophoresis using sodium dodecyl sulfate (CE-SDS), low aggregation measured by absolute size exclusion chromatography (aSEC), low relative surface hydrophobicity measured by analytical hydrophobic interaction chromatography (aHIC), high stability measured by nano differential scanning fluorimetry (nDSF), low polyspecificity assessed by baculovirus particle enzyme-linked immunosorbent assay (BVP-ELISA), and low self-association measured by affinity-capture self-interaction nanoparticle spectroscopy (AC-SINS) with scores normalized to high and low controls.

Ultra-specific, high-affinity MAGE-A4-pMHC binders

Expanded peptide panel for binding assessment

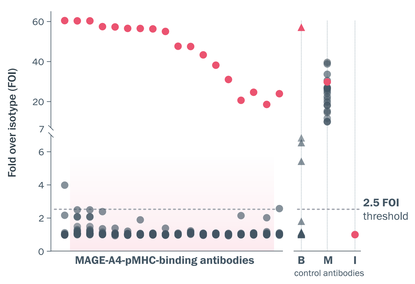
(A)

MAGE family	Amino acid sequence	Predicted peptide affinity to MHC-I[b]	MHC-I relative gene expression
A4	G V Y D G R E H T V	SB	
A10	G L Y D G M E H L I	SB	
B3	R I Y D G K K H F I	WB	*
B5	Q I Y D G K K Y Y I	WB	*
B6	G I Y D G I L H S I	SB	
B10	G L Y D G I E H F M	SB	
C2	G V Y A G R E H F V	WB	*excluded due to weak peptide-MHC-I binding
sCRAP pMHC			
CELSR3	G L S D G Q W H T V	SB	
LAMA1	L L Q D G K W H T V	SB	
FLNC	G L S E G H T F Q V	SB	
CYP4F3	F M F E G H D T T A	SB	
VPS13B	G L M D G S P H F L	SB	
DYNC1H1	F L S D P Q V H T V	SB	
FASN	A L L D G R L Q V V	SB	
MMP9	F T I F E G R S Y S A	WB	
PIGR	L L N D T K V Y T V	SB	
TSPAN1	L L Y D I R T N A V	SB	
TTN	F L H D G Q E Y T L	SB	
MALL	K L L D P R I Y Y I	SB	
SARS	R L Q S L Q T Y V -	SB	
non-pulsed	- -	- -	control

D exposed surface residue ☐ residue differs from pMAGE-A4$_{230-239}$ SB: strong binding WB: weak binding

Cell-binding assessment

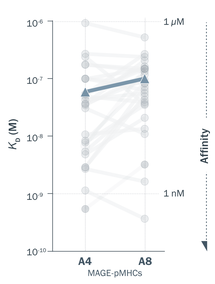
(B) Peptide-pulsed T2 cell assay



(B) Highlighted MAGE-A4-pMHC-binding antibodies show ultra-high specificity for MAGE-A4-pMHC with low to no binding to the other pMHCs tested.

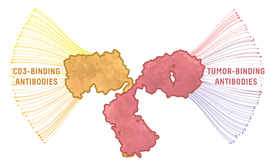
Binding affinity

(C) SPR



(C) SPR kinetic analysis of antibody binding to MAGE-A4-pMHC and MAGE-A8-pMHC. No binding was observed to MAGE-B2- or SARS-pMHCs (data not shown).

Conclusions & next steps



With AbCellera's antibody discovery and development engine, we identified a panel of diverse and developable antibodies that:

- bind with high affinity and high specificity to MAGE-A4-pMHC, assessed by recombinant protein and cell-based binding
- are specific for MAGE-A4 over multiple other peptides that bind to MHC-I with high affinity
- have favorable developability profiles

These antibodies will be paired with our previously described fully human CD3-binders to generate T-cell engagers for further development. We will further assess the antibodies using AbCellera's capabilities in functional, developability, and specificity assays including:

- tumor cell killing and cytokine release assays
- bispecific antibody developability assessments
- peptide-pulsed T2 binding assays with a rationally designed screening library[7]
- a large pool of pMHCs in a non-biased system to investigate potential off-target cross-reactivity[8]
- high-resolution structural assessments of antibody-pMHC interactions[9]

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