Identifying T-cell engagers with optimal potency & cytokine release profiles

with differentiated and developable CD3-binding antibodies

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#1886

THE PROBLEM

Most T-cell engagers are built from sub-optimal CD3-binders

CD3 T-cell engagers have the potential to be powerful cancer treatments, but the small number of available CD3-binding antibodies and limited multispecific engineering technologies have been barriers to development.

Approximately 80% of T-cell engagers in clinical trials are engineered with CD3-binding antibodies derived from SP34-2, a mouse antibody discovered in 1985 that binds an unstructured linear epitope on the N-terminus of CD3ε[1]. SP34-2 and other CD3-binders such as OKT3 and UCHT1 are often heavily engineered to reduce the risk of cytokine release syndrome and to improve developability properties.

THE GOAL

Build optimal T-cell engagers from novel CD3-binding antibodies

Identifying T-cell engagers that balance anti-tumor potency with potential toxicities, such as cytokine release syndrome, requires simultaneous tuning of both the CD3- and tumor-binding arms. Pairs of antibodies that achieve this balance are rare, creating a need for diverse and developable antibodies that can be combined and tested at scale to identify optimal clinical candidates.

To streamline T-cell engager development, we discovered and characterized novel CD3-binding antibodies with binding and functional properties that are distinct from commonly used CD3-binders, including SP34-2.

To validate our CD3-binding antibodies, we used our bispecific engineering platform, OrthoMab™, to generate proof-of-concept T-cell engagers targeting either EGFR or PSMA. We then compared tumor cell killing and cytokine release profiles to benchmark-derived molecules across different tumor cell lines.

THE OUTCOME

T-cell engagers with high potency and low cytokine release

We used our high-throughput T-cell engager platform to characterize the resulting bispecific antibodies. The results demonstrate that:

- T-cell engagers engineered from AbCellera's portfolio of differentiated CD3-binding antibodies have functional profiles superior to benchmark molecules
- OrthoMab™-engineered T-cell engagers have favorable developability and PK properties
- The optimal CD3-binder depends on several factors, including tumor target and target density

Together, these studies illustrate that combining novel CD3-binding antibodies, clinically validated multispecific engineering technologies, and an integrated discovery and development engine streamlines identification of optimal T-cell engagers for diverse tumor targets.

T-cell engagers with optimal functional properties

We generated proof-of-concept T-cell engagers with high potency and low cytokine release.

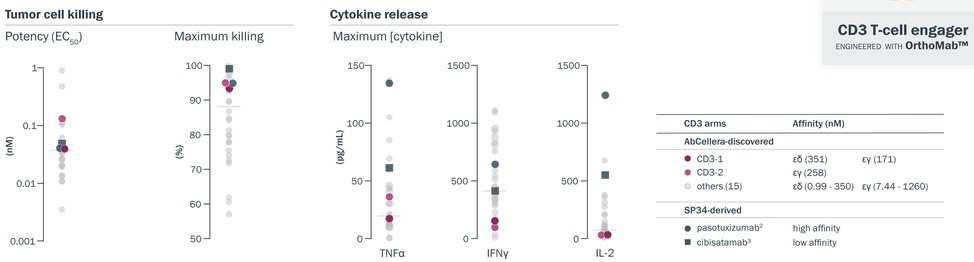


Figure 1. T-cell engagers engineered using diverse CD3-binding antibodies have broad tumor cell killing and cytokine release profiles. We generated bispecific antibodies with a fixed EGFR-binding arm (matuzumab) and varied CD3-binding arms (AbCellera-discovered or SP34-derived) using our multispecifics engineering platform, OrthoMab™. T cell-mediated killing of HeLa cells was measured using unactivated human T cells incubated with target cells at a ratio of 10:1. Cell killing and cytokine concentrations were measured 48 hours following addition of the bispecific antibodies. Potency (EC_{50}) is the concentration of each bispecific antibody needed to induce 50% of the maximal T cell-mediated killing of HeLa cells.

We identified T-cell engagers with optimal functional profiles.

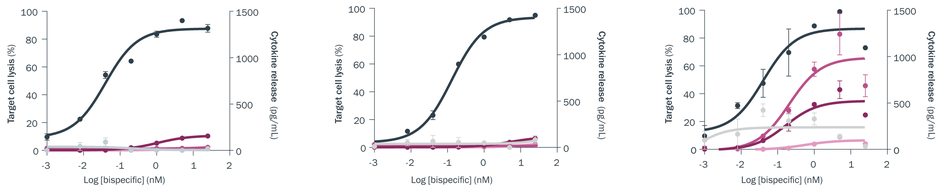


Figure 2. Functional properties of T-cell engagers engineered with AbCellera's CD3-binding antibodies are superior to benchmark-derived molecules. Tumor cell killing and cytokine release curves were evaluated for CD3 x EGFR antibodies engineered using a fixed EGFR-binding arm (matuzumab) and CD3-binding arms from AbCellera's panel (CD3-1, CD3-2) or pasotuxizumab (SP34-derived) using the assay described in Figure 1.

OrthoMab™-engineered bispecifics with favorable developability properties.

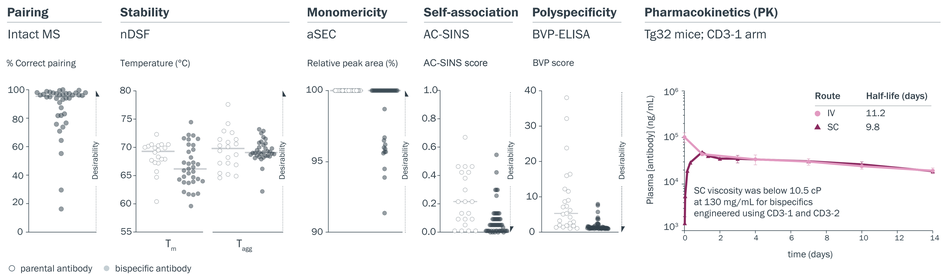


Figure 3. Most OrthoMab™-engineered bispecifics have developability properties comparable to parental antibodies. The fractional percentage of correctly paired vs. incorrectly paired bispecifics was measured by intact mass spectrometry (MS). Stability, monomericity, self-association, and polyspecificity were measured by nDSF, aSEC, AC-SINS, and BVP-ELISA, respectively.

Figure 4. An exemplary CD3 x EGFR bispecific antibody with favorable PK properties. Following a single 5 mg/kg dose in Tg32 mice (human FcRn transgenic, n=4), concentrations were quantified using an electro-chemiluminescence assay.

Fixed EGFR-binding arm

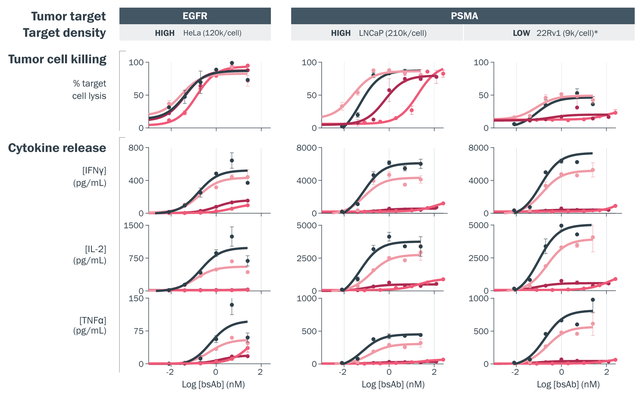
Varied CD3-binding arm

CD3 T-cell engager
ENGINEERED WITH **OrthoMab™**

Custom-built for different tumor targets

We identified optimal CD3-binding arms for different tumor targets.

T-cell engager function across different tumor targets and target densities



*Approximately 30% of 22Rv1 cells do not express PSMA (data not shown)

Figure 5. The optimal CD3-binding arm is dependent on tumor target and target density. We compared the functional profiles of CD3 x PSMA and CD3 x EGFR T-cell engagers across tumor targets and cell lines. Bispecifics were engineered using CD3-binding arms from AbCellera's panel (CD3-1, CD3-2, CD3-3) or pasotuxizumab, and either a fixed EGFR-binding arm (matuzumab) or a fixed PSMA-binding arm (TNB-585). Functional properties for CD3 x EGFR bispecifics were measured using the assay described in Figure 1. Tumor cell killing and cytokine release for CD3 x PSMA bispecifics were measured 48 hours and 72 hours following addition of the bispecific antibodies for LNCaP and 22Rv1 cells, respectively.

From novel CD3-binding antibodies

Fully human CD3-binding antibodies that are distinct from commonly used molecules.

Antibody sequence diversity overlayed with epitope data

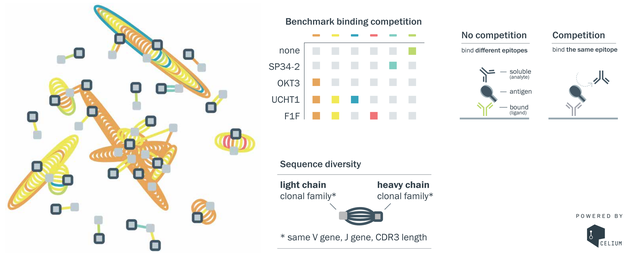
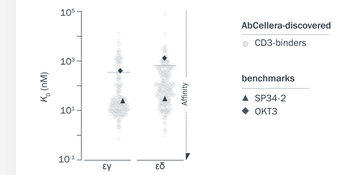


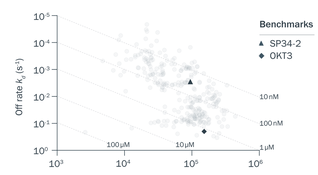
Figure 6. Proprietary immunization protocols using humanized mice yielded CD3-binding antibodies with diverse sequences that bind different epitopes. Antibody sequence diversity was visualized using our proprietary data visualization software, Celium™. Clusters represent clonal families based on V genes, J genes, and CDR3 lengths. The plot is colored using data from high-throughput surface plasmon resonance (SPR) epitope binning, demonstrating that the majority of our CD3-binding antibodies bind epitopes that are distinct from that of SP34-2.

Novel CD3-binding antibodies with diverse binding properties

Broad binding affinities
Human CD3



A range of on and off rates
Human CD3εδ



Human + cyno cross-reactive binders
Subunit and species specificities

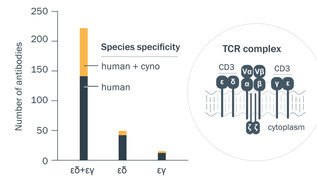


Figure 7. High-throughput assessment of binding properties revealed diverse binding kinetics and specificities. (A) Binding affinity to human CD3 εγ and εδ subunits was measured by SPR-coupled kinetic analysis.

(B) On and off rates were measured by SPR-coupled kinetics and are plotted along the X and Y axes with affinities displayed diagonally.

(C) Subunit specificity was assessed by high-throughput SPR and species specificity was measured by binding to CHO cells expressing either human or cyno CD3.

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2. Friedrich, M. et al. 2012. Molecular cancer therapeutics. 11(12):2664-2673.
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