Target-dependent considerations for the design of bispecific T-cell engagers

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BACKGROUND

Enhancing efficacy and tolerability could expand the reach of T-cell engagers

T-cell engagers (TCEs) have shown promise at improving patient outcomes in a variety of oncological settings. In hematological tumors, efficient cytotoxicity has been achieved by targeting lineage-specific markers such as CD19, CD20, and BCMA, but limitations in both efficacy and safety have been barriers to realizing their potential for solid tumor indications.

CD3 engagement has been associated with excessive cytokine release in some clinical settings. Reducing this risk could not only improve efficacy by widening the therapeutic window, but also by creating opportunities to further enhance potency through costimulatory modalities such as CD28- and 4-1BB-engaging molecules.[1,2]

It has been known for >20 years that T-cell-mediated tumor-cell killing can be decoupled from excessive release of proinflammatory cytokines,[3] but development of TCEs that achieve this property has proven to be challenging. To address this, we developed a TCE platform that includes novel CD3-binding antibodies that are differentiated from molecules commonly used for TCE development. **We are leveraging this platform to develop TCEs against multiple solid tumor indications, three of which are described here.**

AIM

Generate insights into the design of T-cell engagers for solid tumors

To generate potent TCEs that show minimal cytokine release, we engineer large panels of bispecifics using diverse CD3- and tumor associated antigen (TAA)-binding arms. We vary TCE parameters that impact function, such as binding affinities, geometries, and epitopes, for both CD3 and TAAs. We then apply a suite of high-throughout assessments to identify molecules with desired properties. Data derived from multiple programs provided the foundation for a retrospective analysis of critical properties that impact TCE function. From this analysis, we identified a subset of CD3-binding antibodies that, when paired with TAAs following certain design principles, consistently generate TCEs with potent tumor-cell killing and low cytokine release across tumor targets (Fig. 1).

We applied these lessons across multiple programs and identified molecules that achieve highly potent cytotoxicity with limited cytokine production for solid tumor targets PSMA, 5T4, and B7-H4 (Fig. 2).

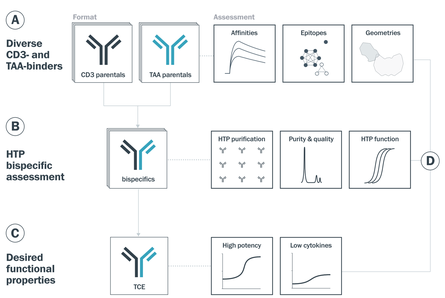

Figure 1. Integration of data from multiple TCE programs. **(A)** Diverse CD3- and TAA-binding antibodies were analyzed and paired to generate hundreds of TCEs. **(B)** Bispecifics were assessed at high-throughput (HTP). **(C)** Molecules with desired properties were identified. **(D)** Data from the parental antibodies and resulting bispecifics were integrated to generate insights into parameters that impact function.

REFERENCES

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2. Claus C, et al. (2019), *Sci Trans Med*. 11(496):eaav5989. doi: 10.1126/scitranslmed.aav5989.
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OUTCOME

CD3 T-cell engagers with potent tumor-cell killing and reduced cytokine release compared to benchmarks for three solid tumor targets

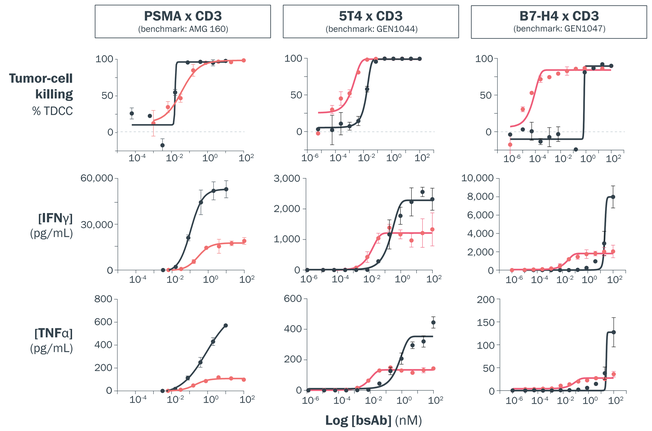

Figure 2. TCEs with functional profiles that are differentiated from clinical benchmarks for three solid tumor targets. Hundreds of TCEs were engineered using diverse CD3- and TAA-binding arms for each target. Functional profiles of AbCellera molecules selected for further assessment are compared to that of clinical benchmarks (produced in-house using sequences from patent literature). Cytokine release and T-cell-dependent cellular cytotoxicity (TDCC) of C4-2 cells, A375 cells, and HCC1954 cells were measured for PSMA, 5T4, and B7-H4, respectively, at ratios of unactivated human T cells to target cells of 5:1, 8:1, and 10:1, respectively, for 72 hours.

Low-affinity CD3-binding antibodies that generate potent T-cell engagers are rare

A set of clonally-related, low-affinity CD3-binders generate highly potent T-cell engagers

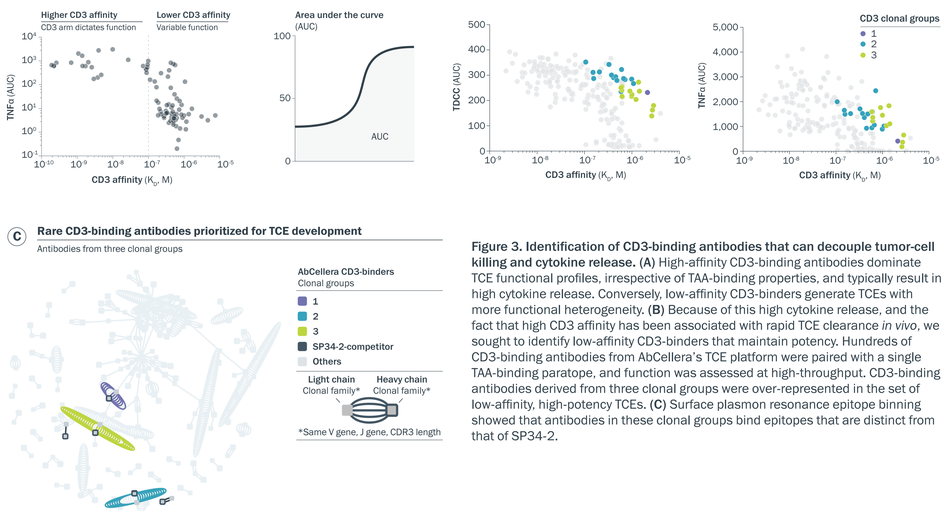

Figure 3. Identification of CD3-binding antibodies that can decouple tumor-cell killing and cytokine release. **(A)** High-affinity CD3-binding antibodies dominate TCE functional profiles, irrespective of TAA-binding properties, and typically result in high cytokine release. Conversely, low-affinity CD3-binders generate TCEs with more functional heterogeneity. **(B)** Because of this high cytokine release, and the fact that high CD3 affinity has been associated with rapid TCE clearance *in vivo*, we sought to identify low-affinity CD3-binders that maintain potency. Hundreds of CD3-binding antibodies from AbCellera's TCE platform were paired with a single TAA-binding paratope, and function was assessed at high-throughput. CD3-binding antibodies derived from three clonal groups were over-represented in the set of low-affinity, high-potency TCEs. **(C)** Surface plasmon resonance epitope binning showed that antibodies in these clonal groups bind epitopes that are distinct from that of SP34-2.

At low CD3 affinity, TAA binding properties impact T-cell engager function

TAA binding affinities and epitopes impact tumor-cell killing and cytokine release

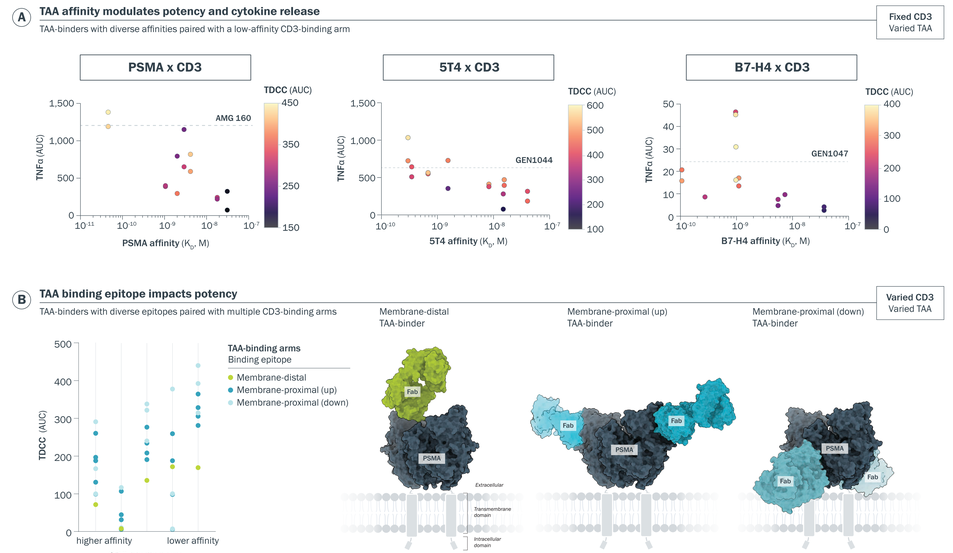

Figure 4. Analysis of the impact of TAA-binding properties on TCE function. Data from our PSMA x CD3 program were used to generate insights into TAA-binding parameters that impact tumor-cell killing and cytokine release. **(A)** TCEs were generated by pairing a single, low-affinity CD3-binder with diverse PSMA-binding antibodies with a range of affinities and epitopes. Higher-affinity TAA-binders were associated with higher tumor-cell killing and cytokine release. **(B)** PSMA-binding antibodies with diverse epitopes were paired with multiple CD3-binding arms. Membrane-distal binding to PSMA typically generated TCEs with lower potency than membrane-proximal PSMA-binders. Antibody-antigen complex structures were generated using electron microscopy and classified into epitope categories by visual inspection. Representative structures for each epitope category are shown.

Application of insights to multiple programs yields T-cell engagers with desired properties

When paired with diverse TAA-binding arms, a unique subset of CD3-binders generate molecules with desired functional profiles across multiple tumor targets

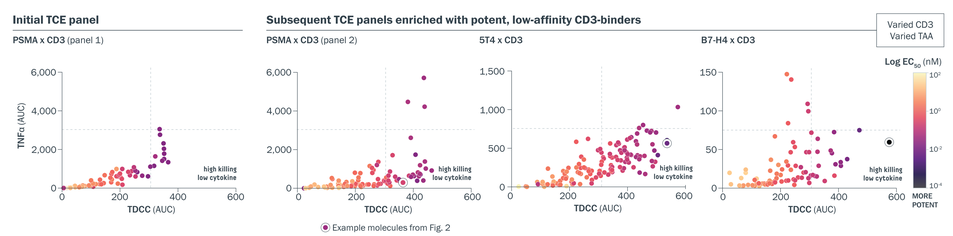

Figure 5. Enrichment of TCE panels with CD3-binding antibodies that can decouple tumor-cell killing and cytokine release. Diverse CD3- and PSMA-binding antibodies were paired to generate an initial panel of PSMA x CD3 TCEs. Following identification of the subset of CD3-binders that can decouple tumor-cell killing and cytokine release shown in Figure 3, along with the insights described in Figure 4, diverse panels of TCEs were engineered for solid tumor targets PSMA, 5T4, and B7-H4. Panels showed enrichment for TCEs with desired functional profiles, enabling identification of the molecules shown in Figure 2.