SCIENTIFIC CASE STUDY

Designing T-cell engagers to widen the therapeutic window

The following data was presented as a poster at the **American Association for Cancer Research® (AACR) Annual Meeting 2024.**

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 these challenges, we developed a TCE platform that includes novel CD3-binding antibodies that are differentiated from molecules commonly used for TCE development. Here, we demonstrate how our platform can generate TCEs with high potency and reduced risk of excessive cytokine release across multiple solid tumor targets.

AIM

Generating 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 (Fig. 1). 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 (Fig. 2).

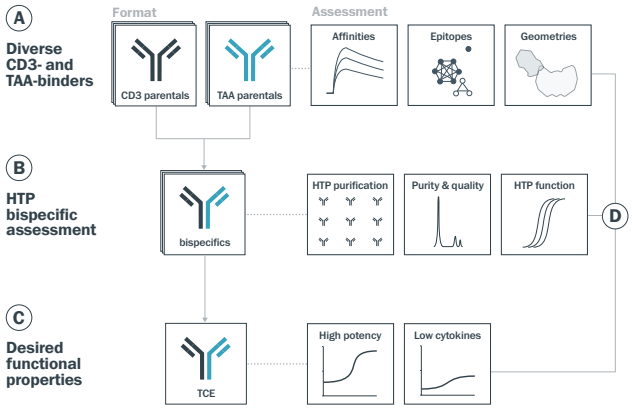


FIGURE 1. Integration of data from multiple TCE programs.

(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.

OUTCOME

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. 5).

A subset of rare, low-affinity CD3-binders that generate highly potent T-cell engagers

We used our discovery and development engine to discover hundreds of novel CD3-binding antibodies. Assessement of these antibodies as bispecific TCEs demonstrated that high-affinity CD3-binders typically result in high cytokine release, irrespective of TAA-binding parameters (Fig. 2A). Conversely, low-affinity CD3-binders result in TCEs with functional heterogeneity. However, those that generate TCEs with high potency are rare. This, along with the rapid clearance of high-affinity TCEs observed *in vivo*, was the impetus for identifying rare, low-affinity CD3-binders that maintain high potency across multiple tumor targets (Fig. 2B,C).

FIGURE 2. Identification of CD3-binding antibodies that can decouple tumor-cell killing and cytokine release.

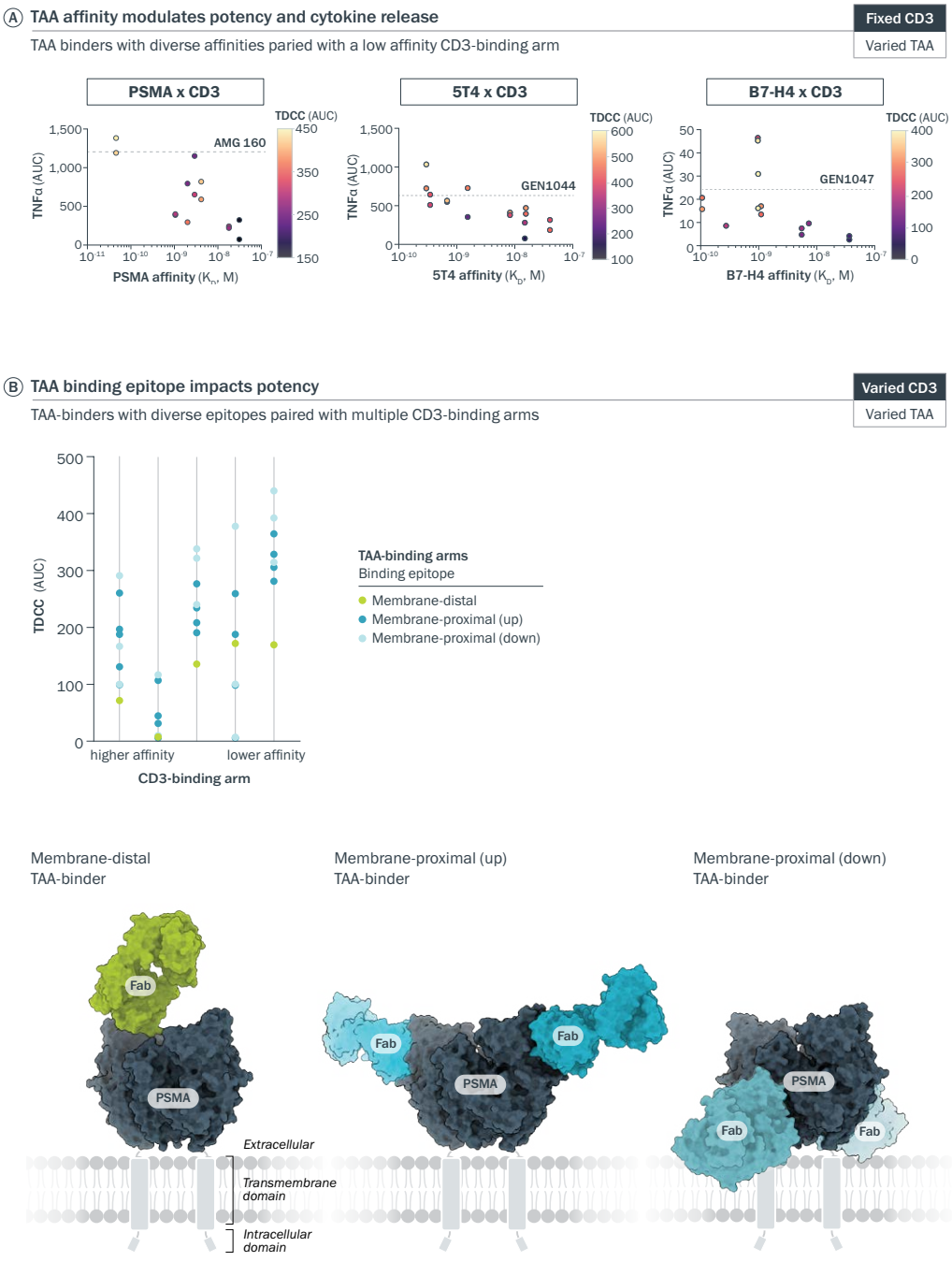
(A) In an intitial panel of TCEs, diverse CD3-binding antibodies from our platform were paired with diverse tumor-binding arms generated using our discovery engine. Antibodies were assessed at high-throughput, and functional profiles were compared to CD3 binding affinity. **(B)** To identify low-affinity CD3-binders that maintain potency when engineered as TCEs, we paired hundreds of our CD3-binding antibodies with a single TAA-binding paratope and assessed function 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

We analyzed antibodies from our PSMA x CD3 program to generate insights into TAA-binding parameters that impact tumor-cell killing and cytokine release. When paired with low-affinity CD3-binding antibodies, both TAA binding affinity (Fig. 3A) and epitope (Fig. 3B) were found to modulate TCE potency and cytokine release.

FIGURE 3. Analysis of the impact of TAA-binding properties on TCE function.

(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 yielded potent T-cell engagers with desired properties for three solid tumor targets

Prior to identification of low-affinity CD3-binders that can promote decoupling of tumor-cell killing and cytokine release (Fig. 2), we paired diverse CD3- and PSMA-binding antibodies to generate an inital panel of TCEs (Fig. 4A). While this panel did generate molecule with desired functional properties, those with high potency, reduced cytokine release, and low affinity were rare. Subsequently, we generated a second panel of TCEs targeting PSMA, along with panels for solid tumor targets 5T4 and B7-H4, that were enriched with our low-affinity, high-potency CD3-binders, resulting in a higher density of molecules with desired functional properties (Fig. 4B). As shown in Figure 5, our approach enabled identification of potent TCEs with functional profiles that are differentiated from clinical benchmarks across three solid tumor targets.

FIGURE 4. Enrichment of TCE panels with CD3-binding antibodies that can decouple tumor-cell killing and cytokine release.

Diverse panels of TCEs were engineered for solid tumor targets PSMA, 5T4, and B7-H4. Following identification of low-affinity CD3-binders that can generate potent TCEs (Fig. 2), panels showed enrichment for molecules with desired functional profiles. Select molecules from these panels are shown in Figure 5.

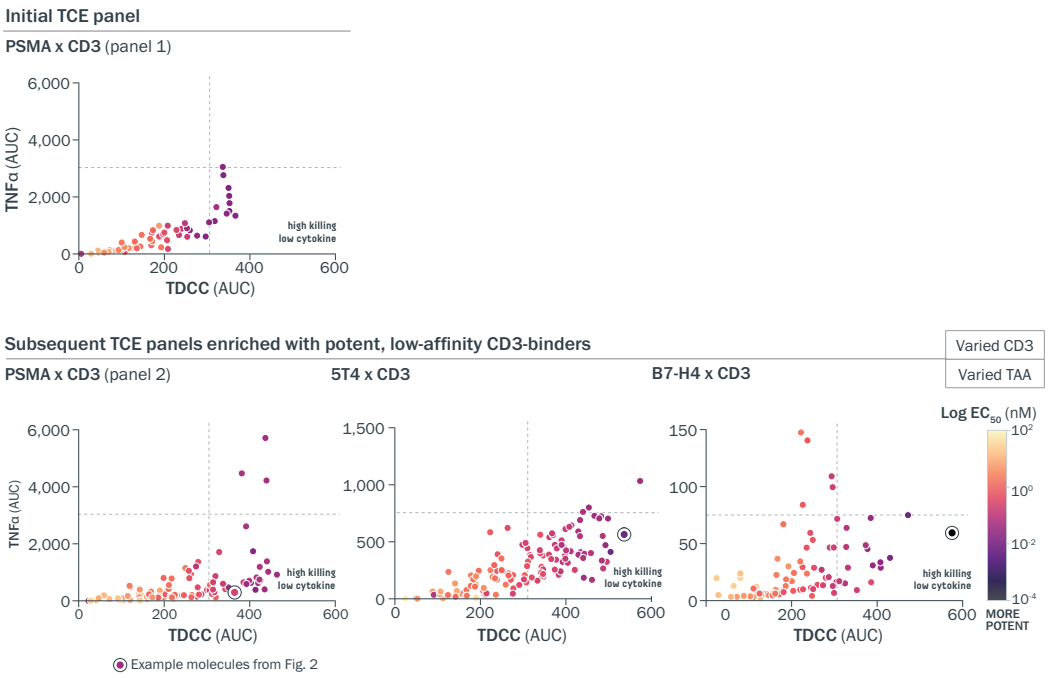
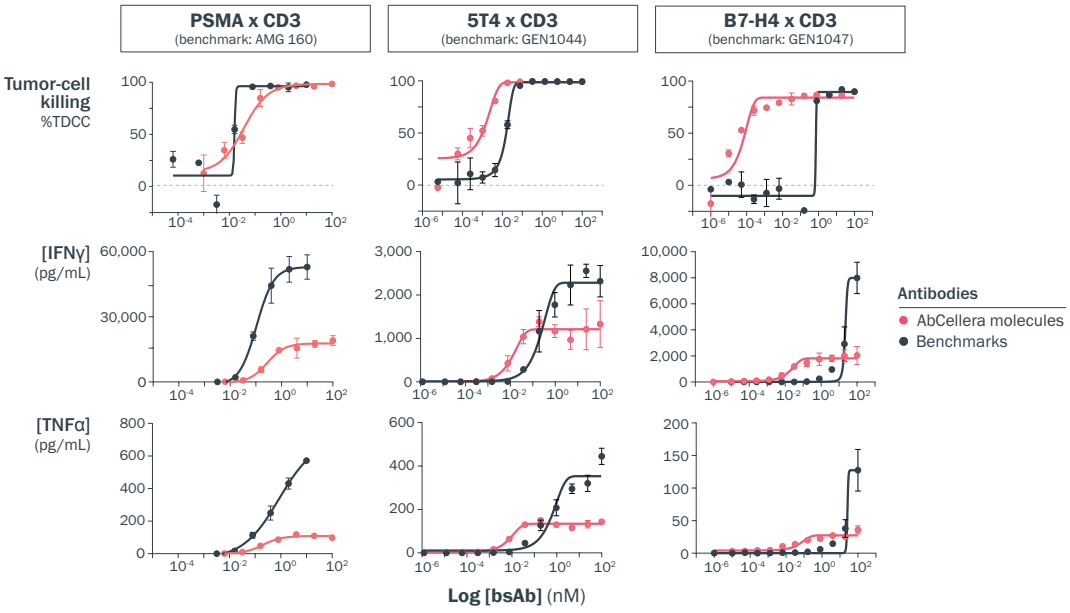


FIGURE 5. TCEs with functional profiles that are differentiated from clinical benchmarks for three solid tumor targets.

Hundreds of TCEs were engineered using multiple CD3- and TAA-binding arms for each target (Fig. 4). 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.



CONCLUSION

TCEs are among the most promising new modalities in cancer therapy, but limitations in efficacy and safety have been barriers to realizing their potential for solid tumor indications. To address these challenges, we developed a TCE platform that includes novel CD3-binding antibodies to widen the therapeutic window for this modality, costimulatory building blocks to enhance efficacy for difficult-to-treat cancers, and discovery capabilities to broaden the range of TCEs to complex peptide-MHC tumor targets.

Data shown here illustrate that we can repeatedly generate TCEs that maximize tumor-cell killing without inducing excessive cytokine release. Reducing the risk associated with CD3 engagement could improve efficacy both by widening the therapeutic window and by creating opportunities to further enhance potency through costimulatory modalities.

Using this platform, we are focused on unlocking the full potential of this modality by advancing internal programs and by engaging in strategic partnerships to bring powerful new cancer medicines to patients.

REFERENCES

1. Skokos D, et al. (2020). Sci Trans Med. 12(525):eaaw7888. doi: 10.1126/scitranslmed.aaw7888.
2. Claus C, et al. (2019). Sci Trans Med. 11(496):eaav5989. doi: 10.1126/scitranslmed.aav5989.
3. Faroudi M, et al. (2003). Proc Natl Acad Sci USA. 100(24):14145-14150. doi: 10.1073/pnas.2334336100.