SCIENTIFIC CASE STUDY

Development of PSMA x CD3 T-cell engagers using an integrated, functional approach

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

BACKGROUND

PSMA-targeted bispecifics for prostate cancer

CD3 T-cell engagers (TCEs) targeting prostate-specific membrane antigen (PSMA) have emerged as a promising approach for the treatment of metastatic castration-resistant prostate cancer.[1] However, dose-limiting toxicities have been a barrier to bringing them to patients.[2]

Identifying TCEs that balance anti-tumor potency with potential toxicities requires simultaneous tuning of both the CD3- and tumor-binding arm. To develop optimal TCE candidates, we engineer hundreds of bispecific molecules from highly diverse parental antibodies, and employ a high-throughput process to identify molecules with desired functional properties.[3]

AIM

Fine-tune T-cell engagers to decouple tumor-cell killing and cytokine release

Here, we show a function-first approach to address the clinical challenges observed in PSMA-targeted bispecifics. Starting with a diverse panel of more than 180 PSMA x CD3 bispecifics,[3] our aim was to identify TCEs against PSMA that:

- show potent tumor-cell killing, with low levels of cytokine release
- induce T-cell properties associated with anti-tumor immune responses, including proliferation of $CD4^+$ and $CD8^+$ T-cell subsets
- have favorable developability profiles

OUTCOME

PSMA x CD3 T-cell engagers that induce potent tumor-cell killing with reduced cytokine release

Starting with highly diverse CD3- and PSMA-binding antibodies, we used a functional approach to identify promising TCE candidates. These TCEs show significantly lower cytokine release than clinical benchmarks, while maintaining potent killing of PSMA-expressing cancer cell lines *in vitro*.

In parallel, we are investigating novel CD3-binding arms that can further decouple tumor-cell killing and cytokine release.

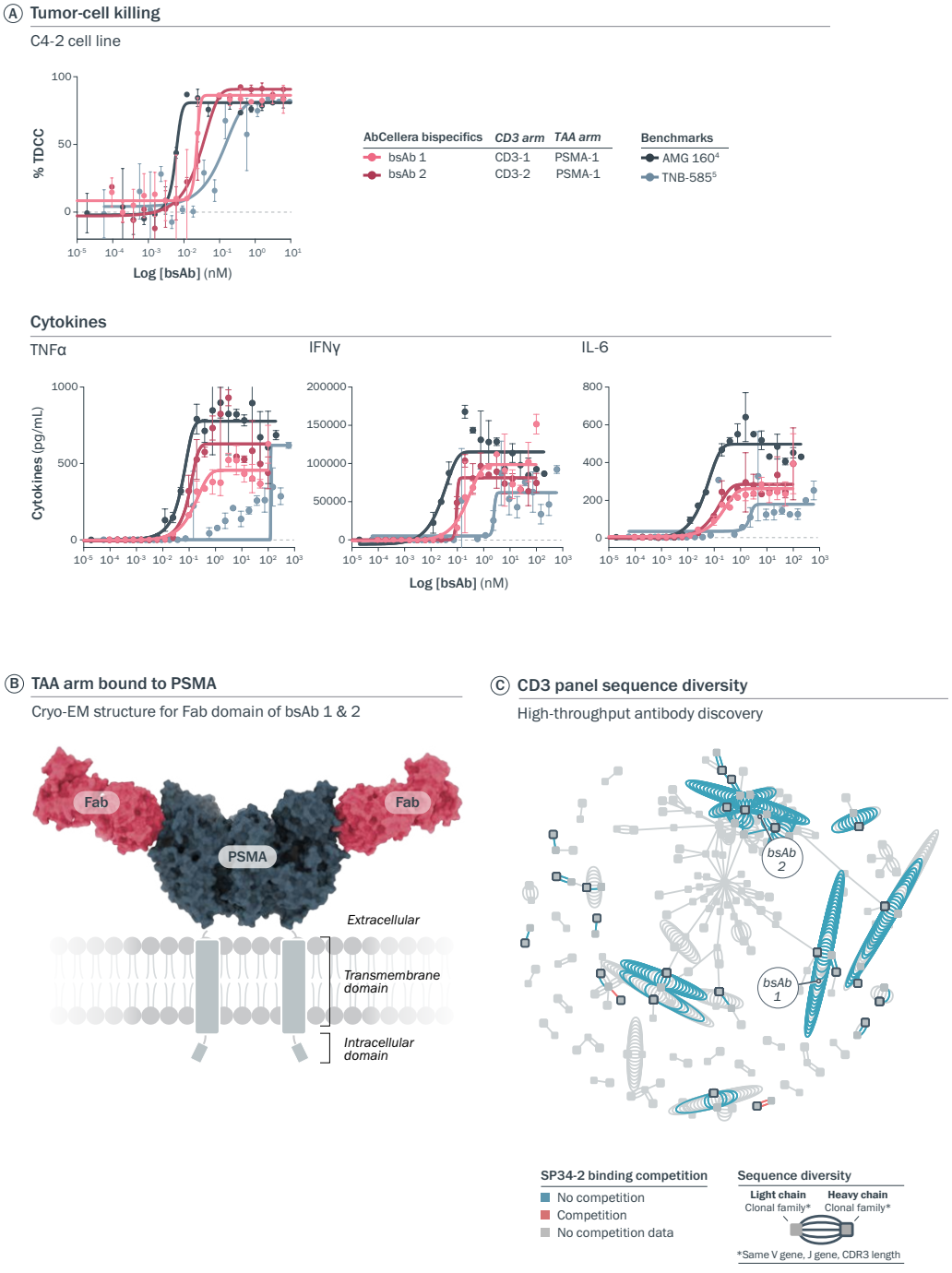
Additional preclinical characterization will determine the potential of these PSMA-targeted bispecifics as therapeutics for prostate cancer.

PSMA x CD3 T-cell engagers that are differentiated from clinical benchmarks

We used our antibody discovery and development engine to generate over 180 diverse PSMA x CD3 bispecifics. Using high-throughput T-cell dependent cellular cytotoxicity (TDCC) and cytokine release assays, we identified molecules with the desired functional profile — activity that falls between the range of clinical benchmarks AMG 160[4] and TNB-585.[5] Two bispecifics (bsAb 1 and 2) showed significantly lower cytokine release than AMG 160, while maintaining potent tumor-cell killing activity (Fig. 1A). The PSMA-binding arm shared by these bispecifics shows membrane-proximal binding (Fig. 1B) and the CD3-binding arms bind epitopes distinct from that of SP34-2 (Fig. 1C).

FIGURE 1. Selection of PSMA x CD3 bispecifics with functional activity between the range of two clinical benchmarks.

(A) Two PSMA x CD3 bispecifics (bsAb 1 and 2) show max tumor-cell killing that is comparable to clinical benchmark AMG 160,[4] with reduced cytokine release. Function was assessed with a high-throughput TDCC assay using human peripheral blood mononuclear cells (PBMCs) incubated with target cells at a 10:1 ratio for 72 hours. **(B)** The TAA arm shared by bsAb 1 and 2 shows membrane-proximal binding to PSMA. The structure was generated using a size-exclusion chromatography-purified complex and cryo-electron microscopy. **(C)** CD3-binding arms were selected from AbCellera's diverse CD3 panel, which is visualized using Celium™ and colored by binding competition with the commonly used CD3-binding antibody SP34-2. The CD3 arms used for bsAb 1 and 2 bind epitopes that are distinct from that of SP34-2.

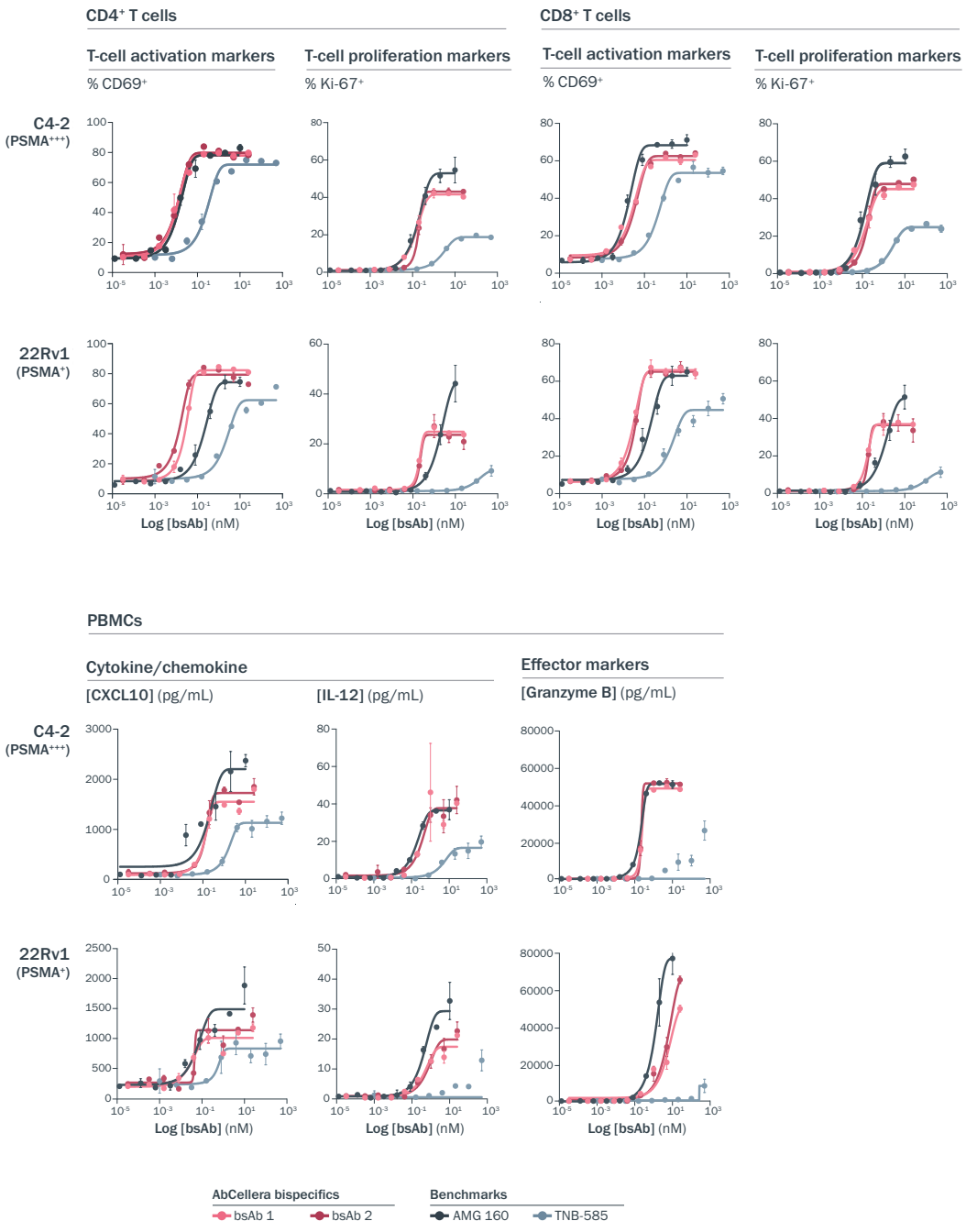


PSMA x CD3 T-cell engagers induce T-cell activation, proliferation, and granzyme production

To understand the anti-tumor properties of our bispecifics, we performed T-cell profiling in cancer cell lines C4-2 and 22Rv1, which express high and low levels of PSMA, respectively. Our bispecifics showed robust anti-tumor immune responses, with proliferation of CD4⁺ and CD8⁺ T-cell subsets, markers of T-cell activation (CD69, IL-12) and redirection (CXCL10), and release of granzyme B (Fig. 2).

FIGURE 2. T-cell profiling in cancer cell lines with high and low PSMA expression.

bsAb 1 and 2 induce proliferation of CD4⁺ and CD8⁺ T-cell subsets, production of T-cell redirection chemokine (CXCL10) and activation cytokines (IL-12), and release of granzyme B from lymphocytes, at levels comparable to AMG 160. PBMC effector and target cells were incubated at a ratio of 10:1 for 48 hours, with markers measured using Meso Scale Discovery® and flow cytometry. Results from a single representative PBMC donor are shown.

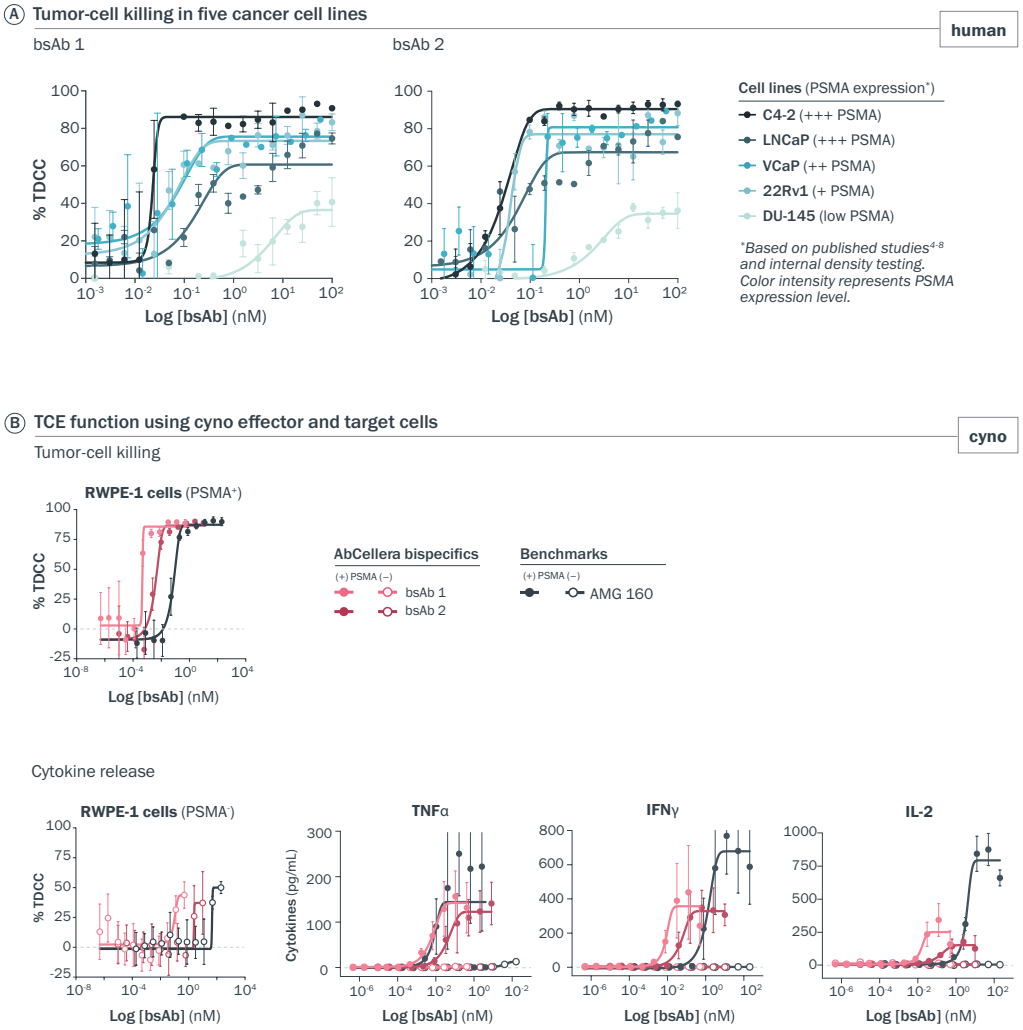


PSMA x CD3 T-cell engagers show potent tumor-cell killing and low cytokine release across cell lines and species

We assessed potency of our bispecifics across high- and low-expressing target cells, demonstrating that bsAb 1 and 2 showed consistent and potent tumor-cell killing activity across varied levels of PSMA expression (Fig. 3A). Further, our bispecifics demonstrated species cross-reactivity, with dose-dependent killing of cyno PSMA-expressing cells (Fig. 3B). Maintaining TCE functional profiles across conditions and a non-human primate model positions these molecules favorably for future IND-enabling studies.

FIGURE 3. Tumor-cell killing in human and cyno PSMA-expressing cancer cells.

(A) Tumor-cell killing activity of bsAb 1 and 2 is consistent across varied levels of PSMA expression in multiple human cancer cell lines. **(B)** bsAb 1 and 2 show dose-dependent killing of cyno PSMA-expressing cells with lower cytokine release than AMG 160. Cyno pan T-cell donor cells were incubated with target cells at a 10:1 ratio for 48 hours. Data not shown for IL-6, which showed low concentration across bsAb 1/2 and benchmarks.



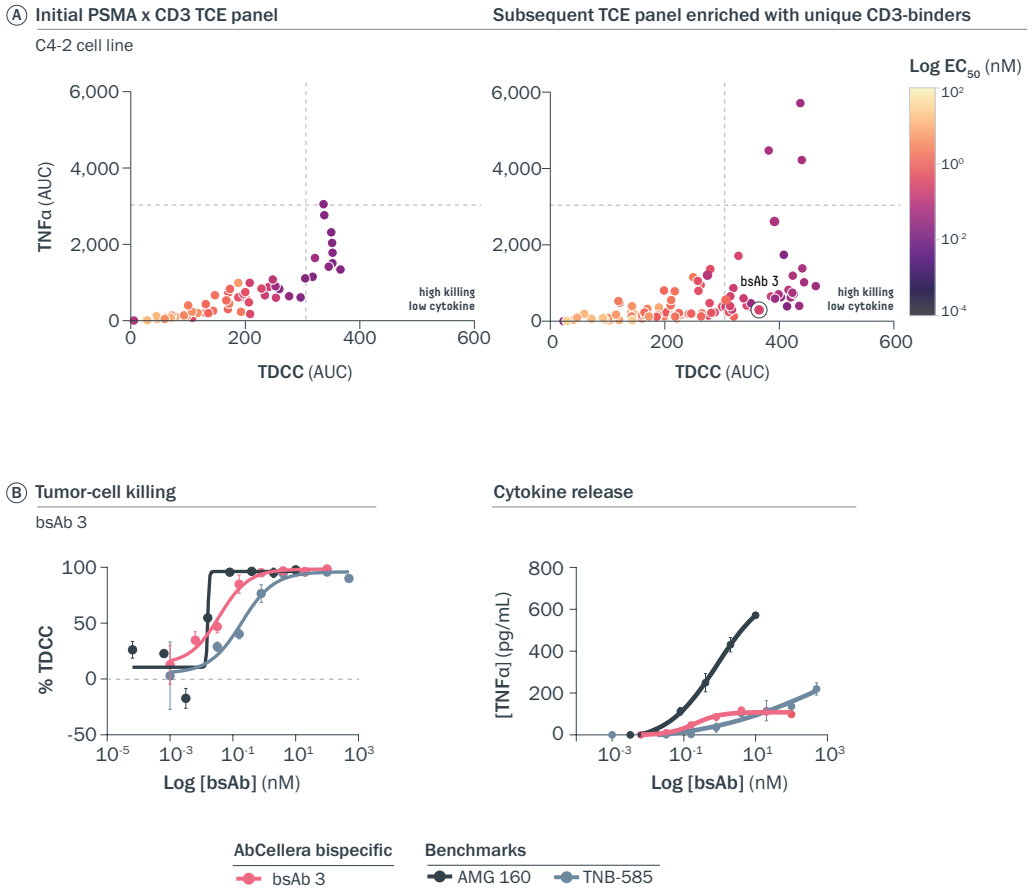
NEXT STEPS

A T-cell engager panel enriched for desired properties

As part of our TCE platform development, we identified a subset of unique, low-affinity, high-potency CD3-binders.[9] In a subsequent panel of PSMA x CD3 TCEs, we enriched our PSMA x CD3 TCE panel with unique CD3-binders and observed an increase in molecules with desired functional properties (Fig. 4A). Bispecifics from the enriched panel that show clear differentiation from clinical benchmarks (Fig. 4B) will be assessed in further preclinical characterization in parallel with those presented in Figures 1-3.

FIGURE 4. Enrichment with unique CD3-binders further decouples tumor-cell killing and cytokine release.

(A) We identified a subset of CD3-binding antibodies that generate TCEs with high potency and reduced cytokine release across multiple tumor targets. These were combined with three PSMA-binding antibodies and assessed by high-throughput TDCC. Our panel with unique CD3-binders shows enrichment for TCEs with desired functional profiles; several candidates will be assessed in parallel with bsAb 1 and 2. **(B)** Tumor-cell killing and cytokine release for an example bispecific (bsAb 3) from this panel is shown.



CONCLUSION

PSMA x CD3 TCEs are a promising therapeutic for the treatment of advanced prostate cancer, yet dose-limiting toxicities have hindered their path to the clinic. We aimed to address this challenge by generating TCEs that achieve potent cell-killing despite low cytokine production.

The data shown here demonstrate our function-first approach to the identification of promising TCE candidates. By beginning with diverse pairs of parental antibodies and screening at scale, we were able to fine-tune T-cell responses and identify molecules that show decoupling of tumor-cell killing and cytokine release. Importantly, we identified TCE candidates with clear differentiation from clinical benchmarks and the potential to widen the therapeutic window for this drug class.

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.

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