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

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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 bispecifics that are differentiated from clinical benchmarks

T-cell engagers with optimal pairing of CD3- and PSMA-binders induce potent tumor-cell killing and low cytokine release

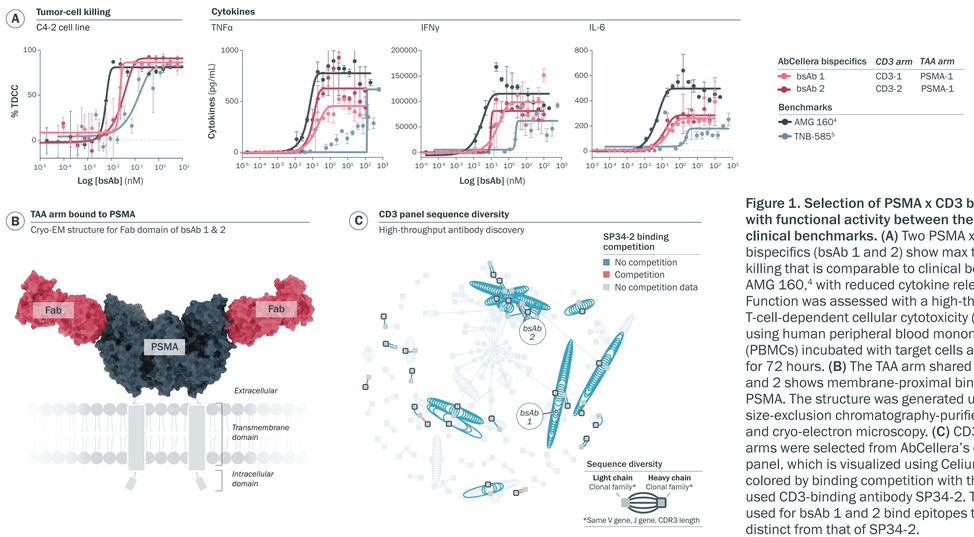

Figure 1. Selection of PSMA x CD3 bispecifics with functional activity between the range of two clinical benchmarks. (A) Two PSMA x CD3 bispecifics (bsAb 1 and 2) show max tumor-cell killing that is comparable to clinical benchmark AMG 160,[4] with reduced cytokine release. Function was assessed with a high-throughput T-cell-dependent cellular cytotoxicity (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.

Robust T cell-mediated immune responses

T-cell engagers induce T-cell activation, proliferation, and granzyme production

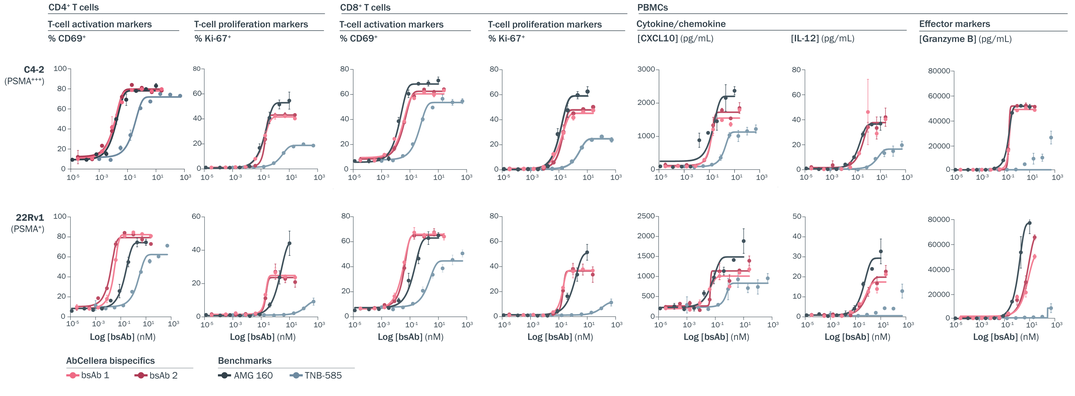

Figure 2. T-cell profiling in cancer cell lines with high and low PSMA expression. bsAb 1 and 2 induce proliferation of CD4⁺ and CD8⁺ T-cell subsets, production of T-cell redirection chemokine (CXCL10) and activation cytokines (IL-12), and release of granzyme B from lymphocytes, at levels comparable 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.

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T-cell-mediated lysis of PSMA-expressing prostate cancer cells

Potent tumor-cell killing and reduced cytokine release is consistent across cell lines and donors

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)** Cytokine release and tumor-cell killing profiles were consistent across cell lines and between PBMC donors, measured using the assay described in Figure 1A with a 72–96 hour incubation. Max cytokines (pg/mL) were standardized per condition (donor and cell line) by subtracting the mean and dividing by the standard deviation. Dose-dependent response curves from a representative donor are shown in Figure 1A. **(C)** 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

Find out more about our CD3 program by scanning the QR code above.

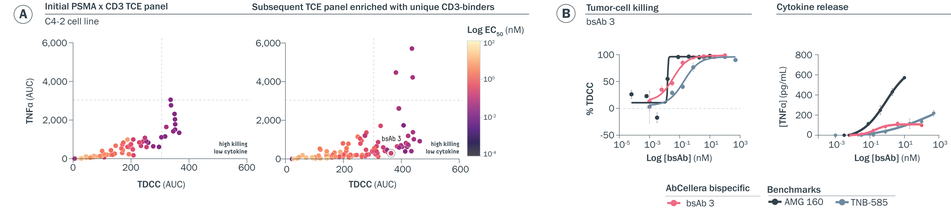

Figure 4. Enrichment with unique CD3-binders further decouples tumor-cell killing and cytokine release. (A) We identified a subset of CD3-binding antibodies that generate TCEs with high potency and reduced cytokine release across multiple tumor targets. These were combined with three PSMA-binding antibodies 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.