Immunotherapy with anti-PSMA × anti-CD3 bispecific antibody stimulates potent killing of a human prostate cancer cell line and target-mediated T cell activation in monkeys: A potential therapy for prostate cancer

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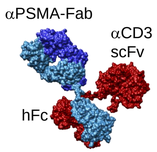
Introduction

- Prostate-specific membrane antigen (PSMA) is over-expressed in prostate cancer and represents an attractive immunotherapy target.
- Due to the highly selective expression of PSMA on prostate cells, anti-PSMA antibodies have been used as imaging and therapeutic agents in prostate cancer, and toxin-conjugated antibodies are also under development. However, such antibodies do not induce T cell-mediated killing of PSMA$^+$ cancer cells.
- To exploit the potent mechanism of a T cell immunotherapy in prostate cancer, we designed humanized anti-PSMA × anti-CD3 bispecific antibodies possessing a full Fc domain to maintain long serum half-life and facilitate efficient manufacturing.
- We also generated anti-PSMA bispecifics with different affinities for CD3 to explore the relationship between PSMA$^+$ target cell depletion, acute T cell activation, and its associated cytokine release syndrome (CRS).

A Fab-scFv-Fc bispecific antibodies are readily produced & purified

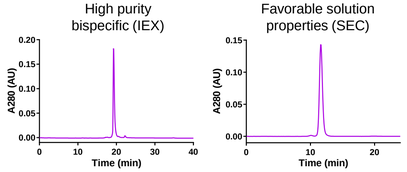
① Portable T cell-recruiting bispecific antibody design via Fab-scFv-Fc format

- Modified Fc domain eliminates FcγR affinity but preserves FcRn affinity for antibody-like half-life.
- Fc substitutions promote heterodimer formation and facilitate purification by standard methods.
- An existing αPSMA-Fab was humanized and plugged into the platform w/o further reformatting.



Fab-scFv-Fc

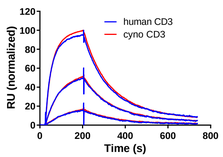
- High yielding stable cell lines (1-3 g/L)
- Protein A + Ion exchange chromatography

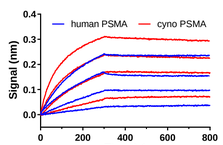


Analytical chromatography (XmAb14484)

② Cyno cross-reactive αCD3 domain is optimized and affinity-engineered for potency modulation and paired with αPSMA-Fab for bispecific antibodies

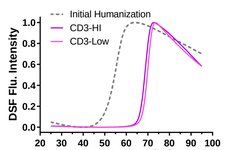
Similar human and cyno affinity





α-human-capture of bispecific antibodies with 2x serial dilutions of PSMA (50 to 0.8 nM)

Anti-CD3 scFv stability engineering

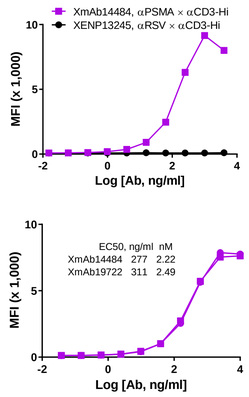


Human and cynomolgus PSMA binding affinities

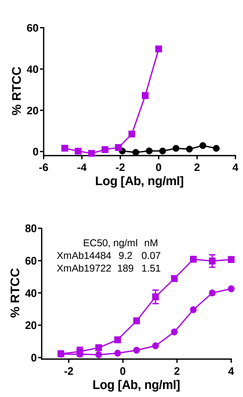
K_D (nM)	Human	Cyno
XmAb14484	0.4	0.2
XmAb19722	0.7	0.4

B Bispecifics bind prostate cancer cells and mediate RTCC killing in vitro

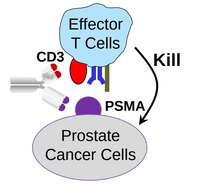
① LNCaP prostate cell binding

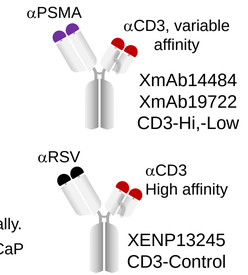


② LNCaP RTCC, hPBMC



Redirected T Cell Cytotoxicity (RTCC)



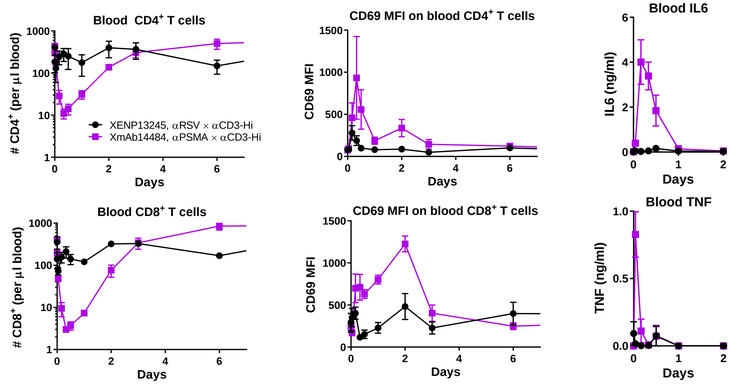


- αCD3 affinity variants bind to PSMA on LNCaP prostate cancer cells equally.
- αCD3 affinity variants mediate different potency and efficacy in killing LNCaP prostate cancer cells.

C T cell response and CRS is dependent on the engagement of target cells in monkeys

① αPSMA x αCD3 but not αRSV x αCD3 bispecific induces T cell activation and CRS

- Cynomolgus monkeys (n = 3): 0.03 mg/kg XmAb14484, 3 mg/kg XENP13245; single dose i.v.

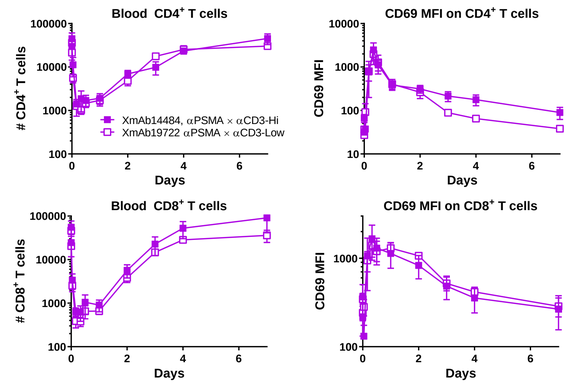


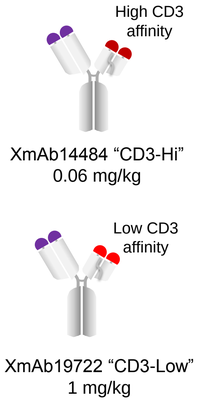
- Peripheral T cell margination, activation and CRS serve as surrogate biomarkers for immunotherapy against PSMA$^+$ (solid tumor) target cells.
- T cell engagement in the absence of target cells even at very high doses of αRSV × αCD3 bispecific antibody does not induce T cell activation and is inactive in monkeys.

D Anti-PSMA bispecific with reduced CD3 affinity shows similar T cell activation but lower CRS in monkeys

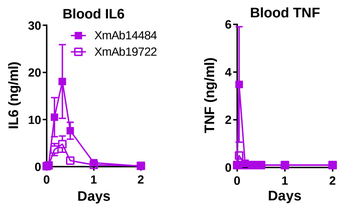
- Cynomolgus monkeys (n = 3): 0.06 mg/kg XmAb14484, 1 mg/kg XmAb19722; single dose i.v.

① αPSMA bispecifics with different CD3 affinities mediate similar T cell activities





② Lower CD3 affinity αPSMA bispecific variant induces less CRS



- Anti-PSMA bispecifics with different αCD3 affinities show similar T cell margination and activation.
- Lower CD3 affinity correlates with reduced cytokine release even at higher dose levels.

Summary

The anti-PSMA × anti-CD3 bispecific antibodies XmAb14484 & XmAb19722:

- Are humanized and contain a human Fc domain for long serum half-life.
- Effectively recruit T cells to kill PSMA$^+$ prostate cancer cell lines in vitro.
- Stimulate PSMA target-dependent T cell activation, T cell margination, and cytokine release in monkeys at doses from 0.03 to 1 mg/kg.
- Induce similar levels of T cell responses, while reduced CD3 affinity leads to lower CRS.
- Are efficiently manufactured using standard antibody production methods.

These results support clinical testing of anti-PSMA × anti-CD3 bispecific antibodies in prostate cancer.

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