Affinity tuned XmAb® 2+1 PSMA x CD3 bispecific antibodies demonstrate selective activity in prostate cancer models

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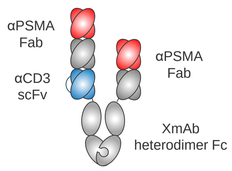
Introduction

- Prostate specific membrane antigen (PSMA) is an intriguing prostate cancer (PC) target as its expression can increase in higher grade tumors, metastasis, and with androgen deprivation therapy.
- A type II integral membrane protein, PSMA has long generated interest as a therapeutic antibody target, demonstrated by clinical-stage efforts with T-cell engaging bispecific antibodies and radioconjugates.
- Unlike targets for hematopoietic cancers, solid cancer targets like PSMA are not tumor-restricted and can exhibit basal levels of expression on normal cells.
- Normal tissue expression of PSMA has been described on the secretory epithelium of prostate tissues, small intestine, proximal renal tubules, and salivary glands.
- To create a more selective T-cell engaging antibody for PC, we extended our XmAb heterodimeric Fc platform to create the 2+1 Fab_2-scFv-Fc format, which is bivalent for PSMA and monovalent for CD3.

A XmAb 2+1 Fab_2-scFv-Fc format enables selective tumor targeting

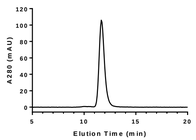
① **XmAb heterodimeric Fc platform allows for well-behaved, high-yielding, and easily manufactured 2+1 bispecific antibodies**

- Modified Fc domain eliminates FcγR affinity
- Preserved FcRn affinity can be enhanced with Xtend Fc technology to promote even longer half-life
- Fc substitutions promote heterodimer formation and facilitate purification by standard antibody techniques such as Protein A + ion-exchange chromatography

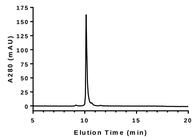


2+1 PSMA x CD3

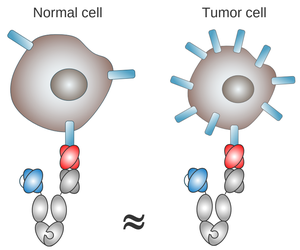
Favorable solution properties (SEC)



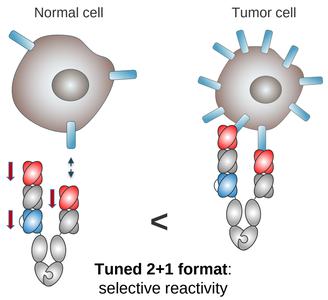
High purity bispecific (IEX)



② **Affinity tuned 2+1 bispecific antibodies allow for selective engagement of high-expressing tumor target cells over low-expressing normal cells**



1+1 format: broad reactivity

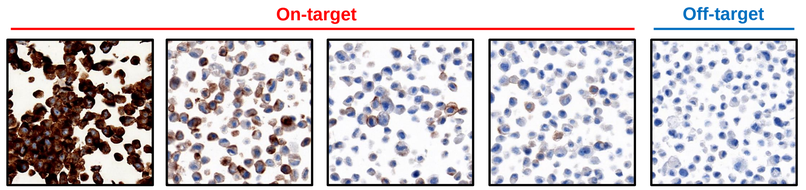


Tuned 2+1 format: selective reactivity

B Cell line proxies for "on-target" and "off-target" tissue identified by IHC

- PSMA prevalence was measured by IHC scoring of 160 PC and 93 normal tissue FFPE cores
- PC3 cells were stably transfected and sorted to create a gradient of hPSMA-expressing lines for downstream studies
- Antigens/cell on various cell lines ranged from ~140K to ~3K, and were correlated against tumor and normal tissues by IHC

		High	Med	Low	Absent
	(n)	% (n)	% (n)	% (n)	% (n)
Prostate Cancer	160	55% (88)	28% (45)	14% (23)	3% (4)
Normal Tissue	93	6% (6)	7% (7)	19% (18)	67% (62)



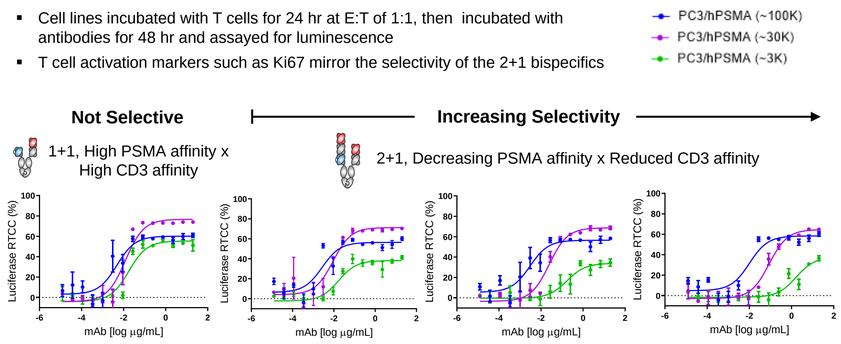
C Bivalent 2+1 format retains binding despite reduced monovalent affinity

- Cell binding performed on PC3/hPSMA (~30K)

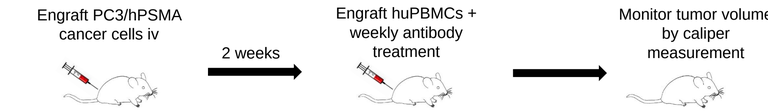
Decreasing PSMA affinity

D Tuned 2+1 bispecifics selectively kill high expressing cell lines in vitro

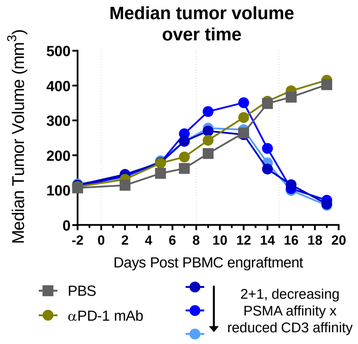
- Cell lines incubated with T cells for 24 hr at E:T of 1:1, then incubated with antibodies for 48 hr and assayed for luminescence
- T cell activation markers such as Ki67 mirror the selectivity of the 2+1 bispecifics

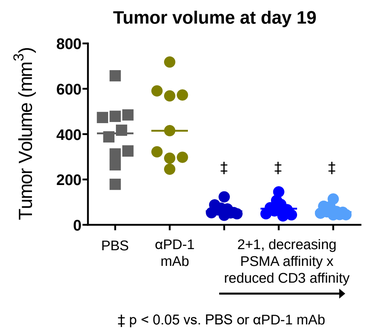


E XmAb 2+1 bispecifics reverse tumor growth of "on-target" cell line in mice



- PC3/hPSMA (~100K) tumors in engrafted mice (n=10 per group) maintained high PSMA expression in vivo





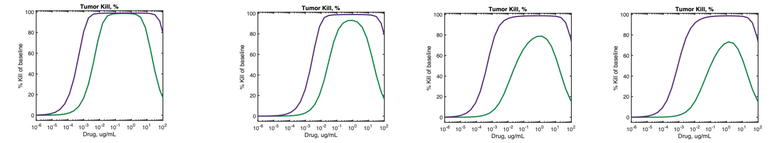
F Modeling predicts tumor-selective killing under clinical conditions

- Semi-mechanistic model of trimer formation (T cell, bispecific antibody, tumor cell)
- Assumed in vivo conditions for "cold" prostate tumors (E:T 1:100)

PC3/hPSMA (~30K)
PC3/hPSMA (~3K)

Not Selective
1+1, High PSMA affinity x High CD3 affinity

Increasing Selectivity
2+1, Decreasing PSMA affinity x Reduced CD3 affinity



Summary

Tuned XmAb 2+1 PSMA x CD3 bispecific antibodies:

- Are humanized, well-behaved, and efficiently purified and manufactured.
- Feature a human Fc domain, which can be modified with Xtend technology.
- Effectively recruit T cells to kill PSMA+ cancer cell lines in vitro.
- Induce anti-tumor activity in human PBMC-engrafted NSG mice.
- Are predicted to have strong anti-tumor activity with an improved safety profile.

These results support clinical testing of a 2+1 PSMA x CD3 bispecific antibody as a therapeutic option for patients with prostate cancer.

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