An Improved Tri-specific Antibody (TsAb) Platform for Optimally Engaging T Cells to Treat Solid Tumor Malignancies

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Background

Although T-cell recruiting bispecific antibodies have shown clinical success in hematological malignancies, their application in solid tumors has not been realized due to several challenges, including a narrow therapeutic window due to cytokine release syndrome (CRS)-induced toxicity, peripheral T-cell sink, suboptimal activation of T cells, and an immunosuppressive tumor microenvironment (TME). We describe the development of a novel tri-specific antibody (TsAb) platform designed to optimally engage T cells in the TME by leveraging both CD3 and CD28 co-stimulation (Fig. 1). TsAbs activate T cells only in the presence of a tumor associated antigen (TAA), leading to specific tumor cell killing and low systemic cytokine release. Our Targeted Tumor modulation (ToTeM) platform can potentially generate durable and safer T cell engagers for a range of solid tumors.

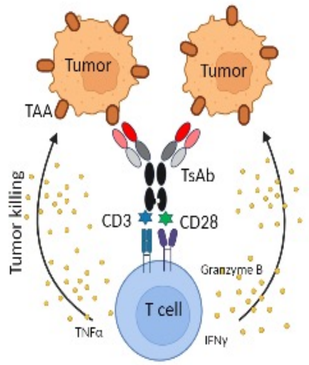
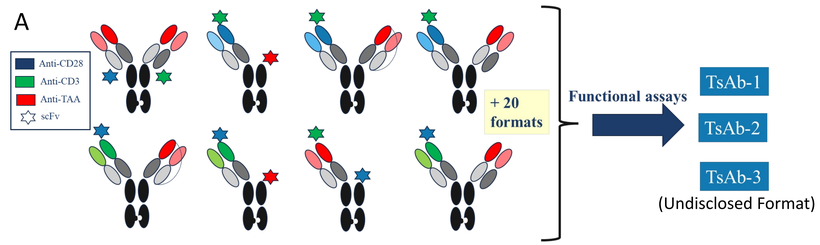


Figure 1: Schematic representation of Tumor targeted mechanism of action of T cell engager TsAb (Representative Format)

Structure and Screening of TsAbs



B

Key Attributes	TsAb-1	TsAb-2	TsAb-3
Complexity	Low	Low	Low
Affinity (TAA)	High	High	High
Affinity (CD3)	Medium	High	Low
Affinity (CD28)	Medium	High	Medium
KIH Heterodimerization	Yes	Yes	Yes
Fc Silencing	Yes	Yes	Yes
Off-target cytokine response	Low-medium (only at high conc)	Low-medium (only at high conc)	Nil
Off-target activation	No-low	No-low	No
Monomer % after Protein A	~60-70%	~60-70%	~70-80%

C

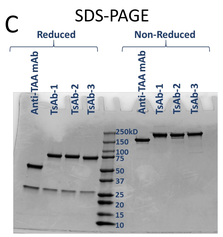
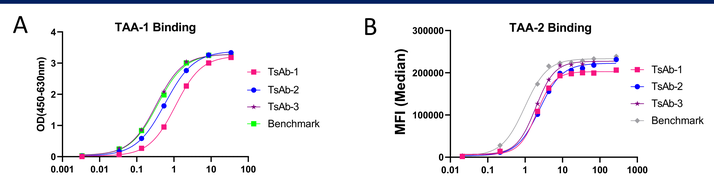


Figure 1: Structure of TsAb formats (A) Key attributes of TsAb formats (B) SDS PAGE image (reducing and non-reducing) for TsAb formats (C)

TAA binding of TsAbs is comparable to benchmark antibodies

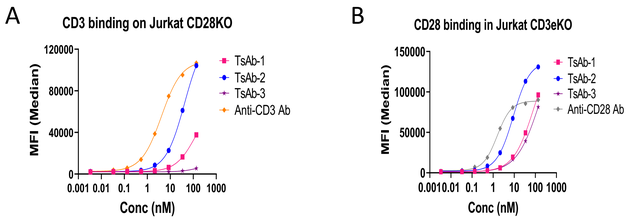


	TsAb-1	TsAb-2	TsAb-3	Benchmark
EC50	1.097	0.5775	0.3356	0.3642

	TsAb-1	TsAb-2	TsAb-3	Benchmark
EC50	2.032	2.416	1.908	0.9727

Figure 2: TAA-binding of TsAb assessed by ELISA (A) or flow cytometry-based assay on TAA over expressing cancer cell line (B)

TsAbs are designed to have reduced affinity for CD3 and CD28

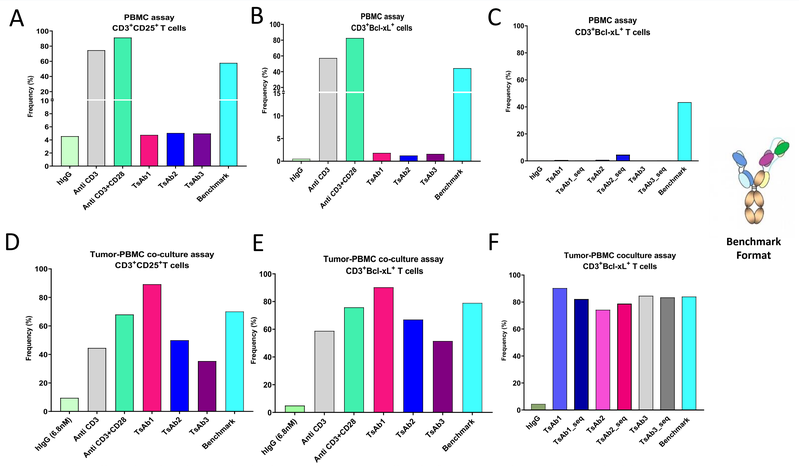


CD3 affinity: TsAb2 >TsAb1> TsAb3

CD28 affinity: TsAb2 >TsAb1=TsAb3

Figure 3: Flow cytometry-based assessment of CD3 and CD28 binding in CD28KO (A) and CD3KO Jurkat cells (B)

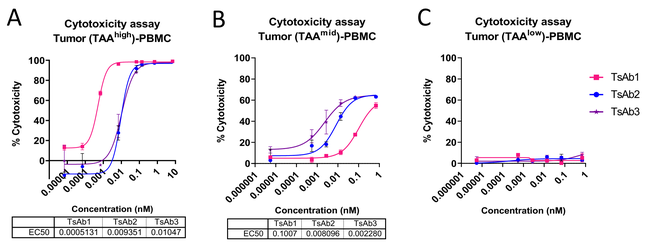
TAA engagement is a prerequisite to T-cell activation and Bcl-xL induction by TsAb



T cell activation by TsAbs is dependent on antibody format and not CD3 or CD28 sequence.

Figure 4: Flow cytometric assessment of T-cell activation (CD3+CD25+) and Bcl-xL induction (CD3+Bcl-xL+) using human PBMCs (A-C) and tumor-PBMC coculture assays (D-F) by TsAbs at 0.068nM or 0.68nM concentration. TsAb_seq has different CD3 and CD28 sequence.

TsAbs show TAA expression dependent tumor cytotoxicity in PBMC co-culture assay



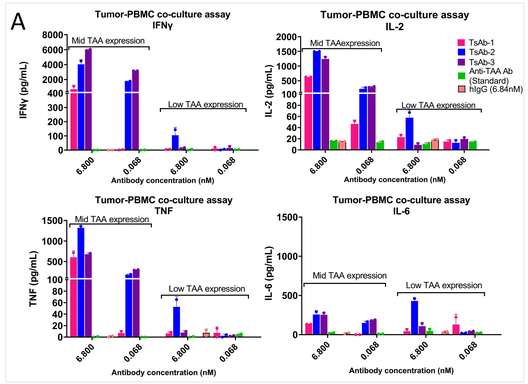
	TsAb1	TsAb2	TsAb3
EC50	0.0005131	0.009351	0.01047

	TsAb1	TsAb2	TsAb3
EC50	0.1007	0.008096	0.002280

Dose-dependent cytotoxicity was observed in TAA^{high} and TAA^{mid} expressing cell lines and not in the TAA^{low} cell line with TsAb-1, TsAb-2 and TsAb-3

Figure 5: BioGlo assessment of tumor cytotoxicity in TAA^{high} (A), TAA^{mid} (B) and TAA^{low} (C) expressing cell lines co-cultured with human PBMC. Cytotoxicity data presented is normalized to human IgG

TsAbs induce cytokine release in PBMCs only in the presence of TAA-expressing tumor cells



- TsAb has the potential to limit systemic toxicity or peripheral cytokine release by T cells.
- Cytokine release in presence of TAA correlates with tumor cytotoxicity.

Figure 6: Assessment of cytokine release by TsAbs 1-3 by TAA^{high} and TAA^{low} expressing cell lines and human PBMC coculture assay (A). Cytokine release by plate coated and soluble antibodies using human PBMCs (B)

TsAb3 enhances effector and central memory T cell differentiation after TAA engagement

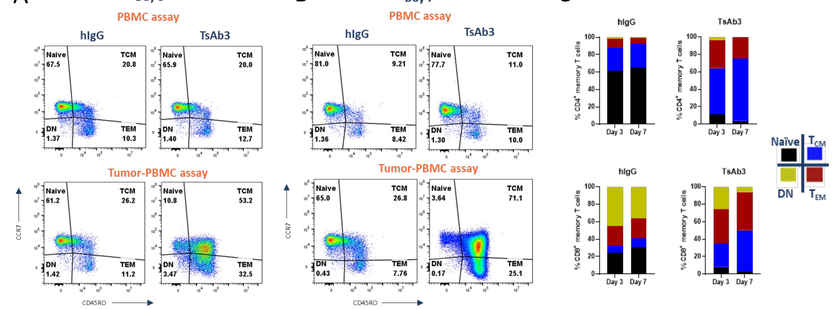


Figure 7: Flow cytometric assessment for induction of central (T_{CM}) and effector memory (T_{EM}) T cells by human IgG and TsAb3 on day 3(A) and day 7(B), at 4.994nM concentration (Scatter plots), using PBMC alone and tumor-PBMC co-culture assay. C shows bar graphs of scatter plots

Conclusions

- We have identified three lead formats with good expression, purity, functional attributes, and potential to limit "on-target off-tumor toxicity". Lead TsAb formats simultaneously engage TAA, CD3 and CD28 leading to T-cell activation only in the presence of TAA.
- TsAbs retain strong TAA-binding which is prospectively critical for tumor-targeting of the TsAb.
- TsAbs show different levels of attenuation in CD3 and CD28 binding, which is format-driven and not sequence dependent.
- Formation of TAA-TsAb-PBMC synapse activates T cells, induces Bcl-xL expression, and cytokine release, leading to tumor cell killing activity.
- We have successfully validated our ToTeM platform across three different TAAs. These versatile formats can be utilized not only in T-cell engager therapeutics, but also in rationale combinations with immune checkpoint inhibitors for the treatment of solid tumors.