Engineered Toxin Body Targeting TIGIT Depletes Tregs in the Tumor Microenvironment and Reduces Tumor Burden in Mice

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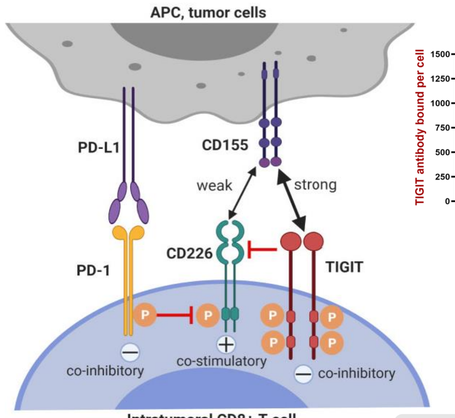
MOLECULAR TEMPLATES

TIGIT is a Promising Immuno-Oncology Target

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a promising new target for cancer immunotherapy but, to date, monoclonal antibody approaches have shown little efficacy as a monotherapy or in combination with αPD-L1 mAb in clinical studies [1-2]. TIGIT is known to be over-expressed on Tregs in the tumor microenvironment (TME) of multiple solid tumors and functions as an immunological checkpoint. TIGIT is often co-expressed with PD-1 on Tregs and CD4⁺ and CD8⁺ T cells in the TME [3-5]. Here we describe a wholly new approach to targeting TIGIT through direct cell kill of TIGIT positive (and TIGIT/PD-1 co-expressed) cells in the TME.



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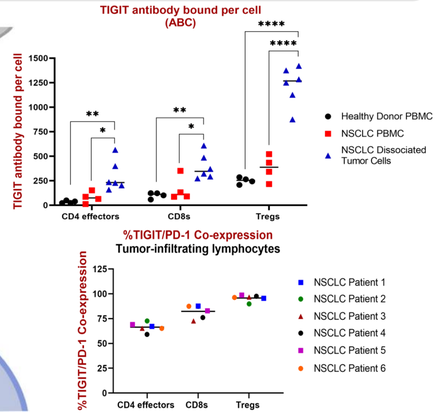
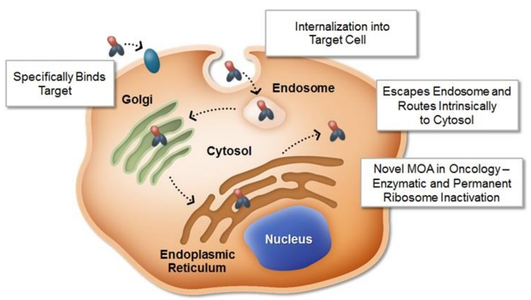


TIGIT expression is significantly elevated on TILs specifically Tregs from NSCLC tumors and there is a high frequency of TIGIT/PD-1 co-expressing Tregs and CD8⁺ TILs. ABC = Antibody Bound per Cell (receptor density)

TIGIT Targeted ETBs are Designed to Deplete TIGIT Expressing Cells

Engineered Toxin Bodies (ETBs) are comprised of a proprietary engineered, de-immunized (DI) form of the Shiga-like toxin A subunit (SLTA) genetically fused to antibody-like binding domains. TIGIT targeting ETBs can bind to both human and cynomolgus TIGIT. Contrary to mAbs, which function by steric hinderance of the TIGIT-CD155 axis, TIGIT ETBs function by direct cell kill of TIGIT expressing cells and represent a novel approach to targeting TIGIT expressing cells in cancers.



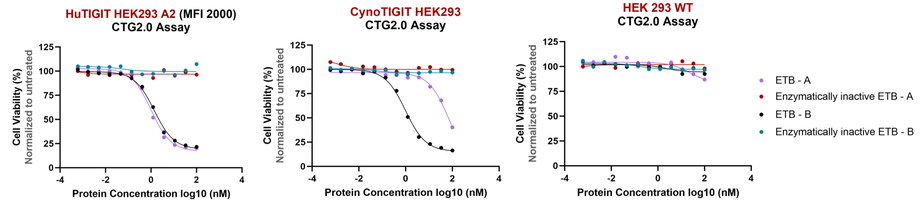
TIGIT targeted ETBs can deplete TIGIT expressing cells including those co-expressing TIGIT and PD-1 through:

- **Specificity for TIGIT:** Activity on only TIGIT expressing immune cells including Tregs
- **Potency:** Direct cell-kill (high pM potency) of primary Tregs via irreversible inactivation of ribosomes and subsequent apoptosis

Here we provide proof of concept for ETBs as a novel modality for the depletion of TIGIT-expressing immune cells *in vitro* and *in vivo* using mouse model system.

Cytotoxicity on TIGIT Overexpressing Cell Lines

TIGIT Targeted ETBs Show High pM Potency and Induce Cytotoxicity in a Target-Dependent Fashion

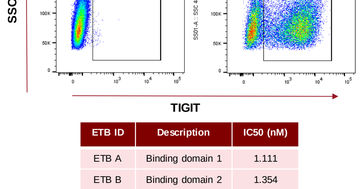


ETB	Description	HEK293 (IC50 nM)		
		HuTIGIT	CynoTIGIT	WT
ETB-A	Binding domain 1	0.994	72.53	NC
ETB-B	Binding domain 2	1.375	0.983	NC

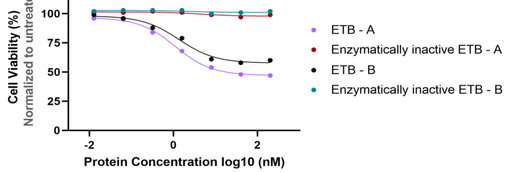
Viability of TIGIT expressing stable overexpression and parental cell line was measured 96 hours after ETB addition to cells using Cell Titer-Glo® 2.0 (Promega). IC50 values reported in nM. NC = No Cytotoxicity. The ETBs tested had low nM potency on the human TIGIT overexpressing cell line tested. The human TIGIT overexpressing line tested in this assay expressed similar TIGIT expression as Tregs in the TME.

Induction of Apoptosis on TIGIT Expressing Primary Tregs

TIGIT ETBs Show High pM Potency on TIGIT Expressing Primary Tregs



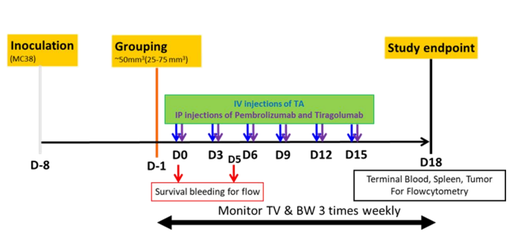
ETB ID	Description	IC50 (nM)
ETB A	Binding domain 1	1.111
ETB B	Binding domain 2	1.354

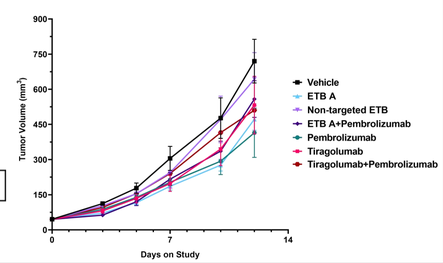


Primary Tregs were isolated from healthy PBMC using magnetic bead-based cell sorting, cultured in the presence of αCD3/CD28 beads and Human IL-2 for 5 days and treated with different concentrations of ETBs for 48 hours. Cells were stained with Apotracker Green™ and viability dye to determine ETB based cytotoxicity.

In vivo Efficacy of TIGIT Targeting Agents in MC38 Tumor-Bearing B-hPD-1/hTIGIT Knock-in Mice

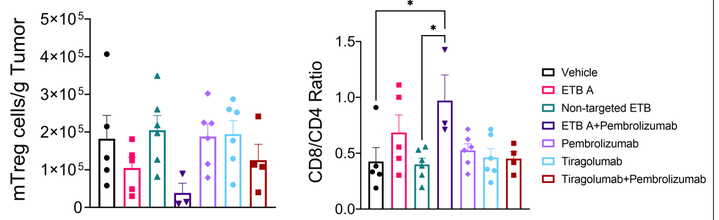
TIGIT ETB Monotherapy Shows Best Overall Reduction in Tumor Burden and is Comparable to the αPD-1 mAb Group



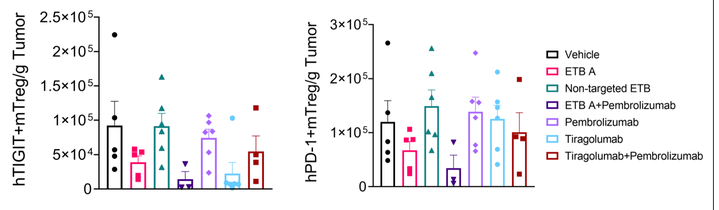


In vivo PD Effects of TIGIT Targeting Agents in MC38 Tumor-Bearing B-hPD-1/hTIGIT Knock-in Mice

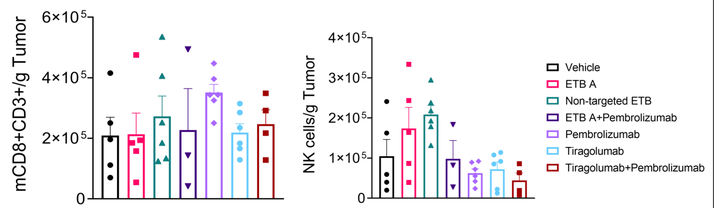
TIGIT ETB Alone or in Combination with αPD-1 mAb Reduces Tregs and Increases CD8:CD4 T-Cells Ratio in the TME



TIGIT Targeted ETB Specifically Depletes TIGIT and PD-1 Expressing Tregs in the TME



CD8 T cells and NKs in the TME are Spared by TIGIT Targeted ETB



CONCLUSIONS

- TIGIT targeted ETBs deplete human TIGIT over expressing cells and ex-vivo cultured primary Tregs in a target dependent manner and show high pM potency.
- TIGIT targeted ETB specifically depleted TIGIT-expressing tumor-infiltrating Tregs in dissociated tumor cells from NSCLC patient.
- Targeting TIGIT using our Engineered Toxin Body platform promotes tumor regression through elimination of TIGIT/PD-1 co-expressing immune cells within the TME.
- Our data supports using ETB as a monotherapy to target TIGIT and represents a wholly novel approach for modulating TIGIT within the TME.

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