Engineered Toxin Body Mediated Depletion of TIGIT Expressing Immune Cells for Cancer Immunotherapy

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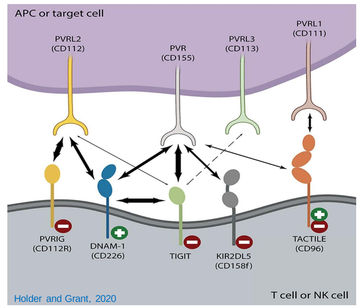
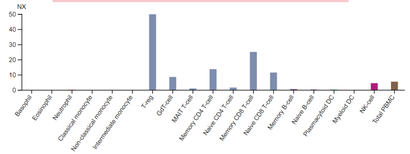
TIGIT is a Novel Immuno-Oncology Target

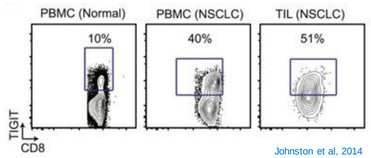
TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an exciting novel target for immuno-oncology which functions as an immune checkpoint on multiple immune cell types including memory CD8+, CD4+ Treg, and memory CD4+ cells. TIGIT upregulation on tumor infiltrating lymphocytes (TILs) has been observed in multiple cancer types and contributes to an immunosuppressive tumor microenvironment (TME). Interestingly, TIGIT is commonly co-expressed with PD-1 on Tregs in the TME, tumor antigen specific CD8+ T cells and CD8+ TILs, leading to weakened anti-tumor immune responses. To date, TIGIT inhibiting monoclonal antibodies (mAb) have shown little activity as a monotherapy in clinical and preclinical studies.



TIGIT RNA expression on immune cells



proteinatlas.org



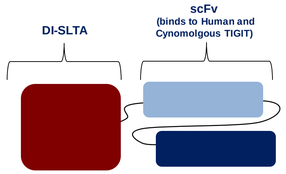
Johnston et al, 2014

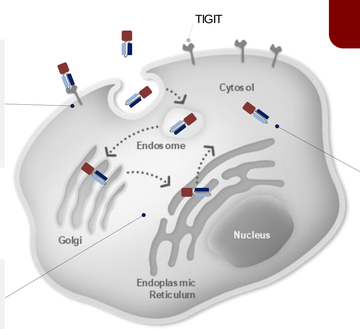
Ligand/receptor affinity (nM)	TIGIT	CD226	CD96	CD112R
CD155	1-3	114-19	37.6	-
CD112	NA	0.31-8.97	-	88

TIGIT expression is significantly elevated on tumor infiltrating lymphocytes and PBMC from cancer patients

TIGIT Targeted ETBs are Designed to Deplete TIGIT Expressing Immune Cells

Engineered Toxin Bodies (ETBs) are proteins that consist of an antibody fragment genetically fused to a proprietary de-immunized (DI) form of the Shiga-like toxin A subunit (SLTA).





- Internalization
  - SLTA induces receptor independent, clathrin-dependent internalization
  - SLTA suppresses membrane fluctuation and induces toxin clustering
- Intracellular Routing
  - SLTA adept at escaping early endosome and self-routing to Golgi, ER, and to cytosol
- Ribosome Destruction
  - SLTA is an RNA N-glycosidase that enzymatically depurinates adenine 4324 in mammalian 28S rRNA
  - SLTA activates Caspases 6/9 and triggers cleavage of BID and PARP

TIGIT targeted ETBs represent a wholly new approach to targeting TIGIT expressing cells including those co-expressing TIGIT and PD-1 through:

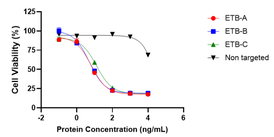
- **Specificity for TIGIT:** Activity on TIGIT expressing immune cells including Tregs
- **Potency:** Direct cell-kill of primary Tregs via irreversible inactivation of ribosomes

Here we provide proof of concept for ETBs as a novel modality for the depletion of TIGIT-expressing immune cells

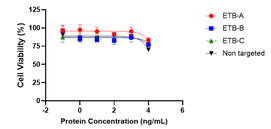
Cytotoxicity in Overexpressing Cell Systems

TIGIT ETBs Show Sub nM Potency and Induce Cytotoxicity in a Target-dependent Fashion

Hu TIGIT HEK293 Clone C2



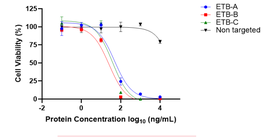
HEK293 Wild Type



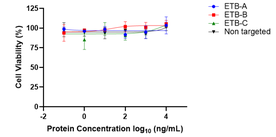
ETB	HEK293T (IC50 nM)		CHOK1 (IC50 nM)	
	Human	WT	Human	WT
ETB-A	0.12	NC	0.87	NC
ETB-B	0.09	NC	0.46	NC
ETB-C	0.23	NC	0.68	NC

Viability of various TIGIT expressing stable overexpression and parental cell lines was measured 96 hours after ETB addition to cells using Cell Titer-Glo® (Promega). IC50 values reported in nM. NC= no cytotoxicity. The ETBs tested had sub nM potency on both human TIGIT overexpressing cell lines tested

Hu TIGIT CHOK1 Clone C5

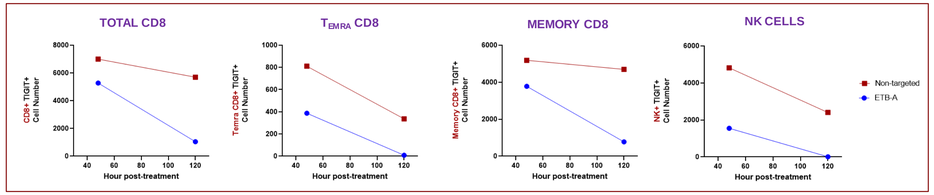


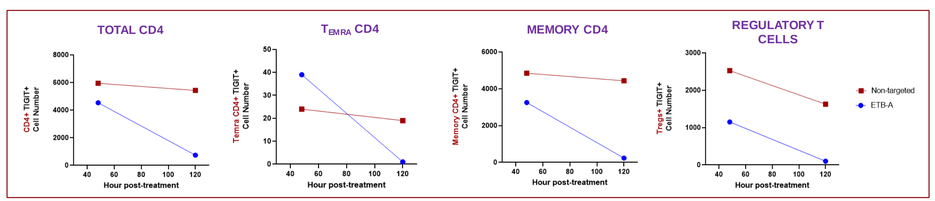
CHOK1 Wild Type



Ex-Vivo Depletion of TIGIT Expressing Immune Cells in Periphery

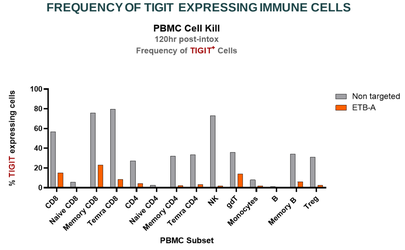
TIGIT ETB Can Specifically Kill TIGIT Expressing Immune Cells



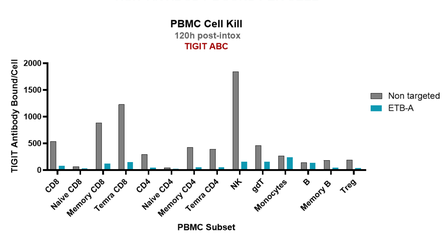


PBMC from normal donor was rested overnight in PBMC media in presence of aCD3/CD28 beads. Next day, the cells were treated with or without TIGIT targeting ETB for 48h and 120h. After treatment, the cells were harvested, beads removed from the cells using a magnet and the cells stained with antibodies for flow cytometry acquisition.

FREQUENCY OF TIGIT EXPRESSING IMMUNE CELLS



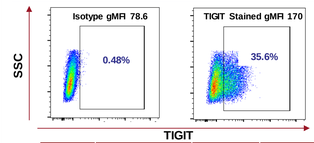
TIGIT ANTIBODY BOUND PER CELL



Most of the TIGIT expressing immune cell subsets in PBMC are depleted by TIGIT targeting ETB after 120h of treatment. The decrease in frequency of TIGIT expressing cells correlates to a decrease in the number of TIGIT receptors present on these cells.

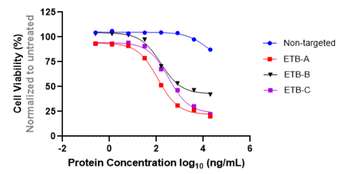
Induction of Apoptosis on TIGIT Expressing Primary Tregs

TIGIT ETB Candidates Induce Apoptosis in TIGIT expressing Primary Human Tregs



ETB ID	Description	IC50 (ng/mL)	IC50 (nM)
ETB A	Binding domain 1	115.6	2.1
ETB B	Binding domain 2	329.7	6.0
ETB C	Binding domain 3	154.8	2.8

Primary Tregs from healthy donor
Apotracker-based apoptotic cell kill
Total TIGIT+ cell kill

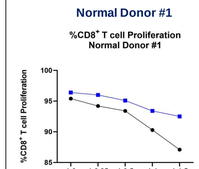


Primary Tregs were isolated from healthy PBMC using magnetic beads, cultured in the presence of αCD3/CD28 beads for 5 days and treated with different concentrations of ETBs for 48 hours. Cells were stained with Apotracker and Live/dead dye to determine the apoptosis by ETBs.

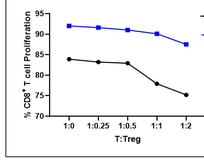
TIGIT ETB Increases The Proliferation of T Cells

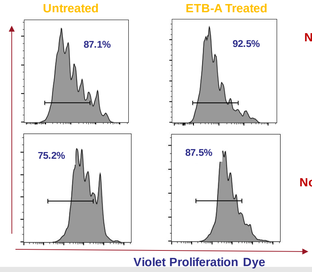
TIGIT Targeting ETB Reverse the Treg Mediated Suppression of T cell Proliferation

Normal Donor #1



Normal Donor #2





Tregs and T cells were magnetically isolated from PBMC of a healthy donor. The T cells were co-cultured with autologous Tregs at various ratios and were either treated with or without TIGIT ETB for 4 days. The cells were stimulated with aCD3/CD28 beads to stimulate the proliferation of T cells. After 4 days of incubation, the aCD3/CD28 beads were removed from the cells and the cells were surface stained with aCD8 antibody and acquired on a flow cytometer.

CONCLUSIONS

- TIGIT-targeted ETBs are designed to preferentially deplete TIGIT expressing immune cells, preferentially Tregs in the TME to improve efficacy
- Multiple PoC ETBs have been identified with the ability to induce cytotoxicity on human TIGIT-expressing cell lines
- TIGIT targeting ETBs induce apoptosis in TIGIT expressing, ex-vivo cultured Tregs
- TIGIT targeted ETBs could specifically deplete TIGIT expressing immune cells including Tregs and NK cells in PBMC from normal donor
- ETBs targeting TIGIT positive Tregs and T cells result in less immunosuppression and the promotion of T cell proliferation
- Overall, these preclinical data support the use of ETB technology as a monotherapy to target TIGIT including TIGIT and PD-1 co-expressing cells
- Tolerability and efficacy study in mice is underway to understand the efficacy of TIGIT ETBs as monotherapy

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