CTLA-4 targeted engineered toxin bodies designed to deplete regulatory T cells (Tregs)

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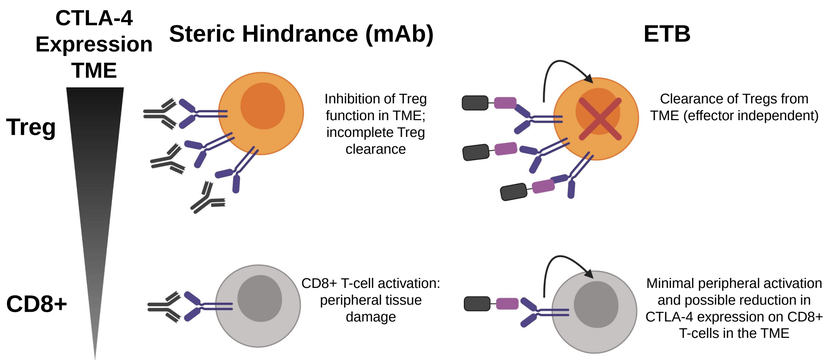
CTLA-4 Targeted ETBs Designed to Deplete Regulatory T cells

Engineered Toxin Bodies (ETBs) are fusion proteins consisting of an antibody fragment genetically fused to a proprietarily de-immunized (DI) form of the Shiga-like toxin A subunit (SLTA). Once the antibody fragment portion of the ETB binds its target, the SLTA portion of the ETB induces internalization into the cell, routing to the cytosol, and cell kill through enzymatic and irreversible ribosomal destruction.

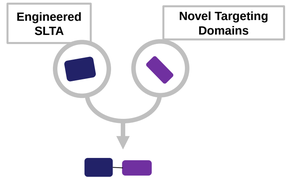
CTLA-4 targeted ETBs are designed to deplete CTLA-4 positive regulatory T cells in the tumor microenvironment (TME) through:

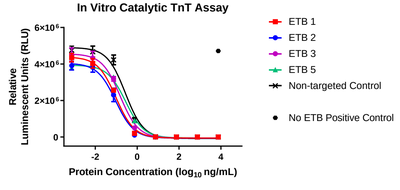
- **Specificity for CTLA-4**: Preferential activity against Tregs vs CD8+T-cells based on receptor density
- **Potency**: Direct cell-kill of Tregs via enzymatic and irreversible inactivation of ribosomes
- **Small size (44-55 kDa)**: Increased tumor penetration



ETB Mechanism of Action

ETB Candidates Demonstrate Similar Catalytic Activity *in vitro*



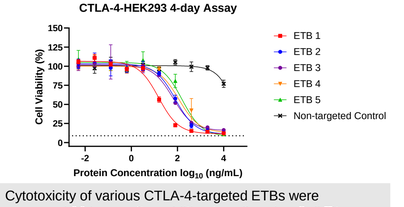


Protein synthesis inhibition by CTLA-4-targeted ETBs measured with TnT® T7 Quick transcription/ translation system (Promega) in vitro luciferase assay

Cytotoxicity Assays on CTLA-4 Overexpression Cell Lines Demonstrate Target-dependent Cell Kill

ETB	CHO-K1			HEK293T	
	Human	Monkey	WT	Human	WT
ETB 1	188	214.2	NC	15.23	NC
ETB 2	1225	1528	NC	82.2	NC
ETB 3	488.3	1611	NC	63.33	NC
ETB 4	963.4	2170	NC	112.2	NC
ETB 5	1331	1544	NC	156.8	NC

ETB cytotoxicity to various CTLA-4 stable overexpression and parental cell lines. IC50 values reported in ng/mL. NC= no cytotoxicity



Cytotoxicity of various CTLA-4-targeted ETBs were measured 96 hours after addition to cells using Cell Titer-Glo®(Promega)

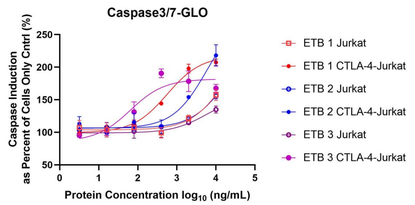
CTLA-4 ETBs Induce Apoptosis of CTLA-4 Expressing Jurkat T-cells

CTLA-4 ETBs Induce Caspase-mediated Cell Death by Targeting CTLA-4 Without Binding CD28

Binding (Kd) ELISA

ETB	Human	Monkey	Mouse	hCD28
ETB 1	13	18	NB	NB
ETB 2	217	108	NB	NB
ETB 3	21	22	NB	NB
ETB 4	67	1595	NB	NB
ETB 5	20	27	NB	NB

ETB binding to various species of CTLA-4 protein and CD28 was measured by ELISA. Kd values reported in ng/mL. NB= no binding



Caspase activity was measured after 48 hours after ETB addition with the Caspase 3/7-Glo®(Promega) method

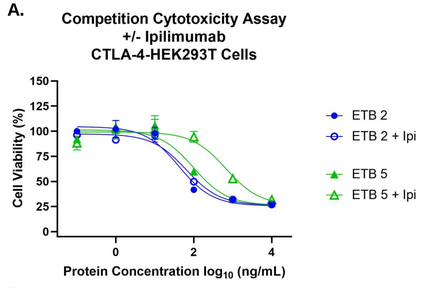
ETB Candidates Engage Blocking and Non-blocking CTLA-4 Epitopes

Lead Candidates Selected for Differentiated and Diverse CTLA-4 Binding Properties

ETB	Antagonized by Ipilimumab	Blocks CTLA-4-B7
ETB 1	Yes	minimal
ETB 2	No	No
ETB 3	partial	Yes
ETB 4	Yes	No
ETB 5	Yes	minimal

A. Ipilimumab IgG was added to cells at a final concentration of 10ug/mL. 1hr later, representative CTLA-4-targeted ETBs were added, and cell viability measured 96 hours after ETB addition using Cell Titer-Glo®(Promega)
B. CTLA-4 Blockade Bioassay (Promega) was used to measure the ability of ETB to block the interaction of CTLA-4 with its ligands in a cell system. ETB was added to CTLA-4-Jurkat cells, aAPC/Raji cells were added, then signal readout after an 8h incubation.

A.



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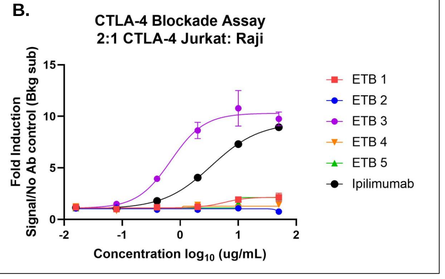
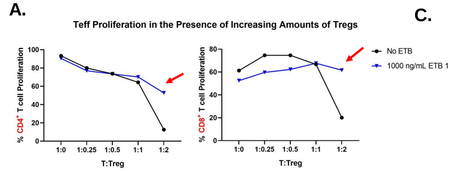


CTLA-4 ETBs Relieve Treg-mediated Suppression in Primary Human PMBC Assays

CTLA-4 ETBs Relieve Suppression of T Effector (Teff) Cells Co-cultured with Tregs

A.



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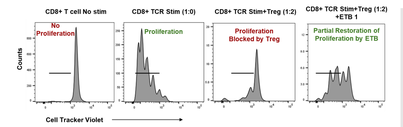

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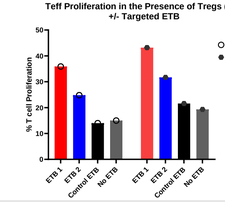
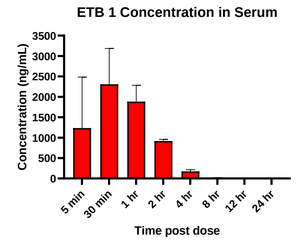


A. Teff cells were stained with Cell Tracker Violet and co-cultured with αCD3/CD28 beads and isolated Tregs at various ratios for 4 days. The % of cells that show proliferation (loss of tracker die) are quantified.
B. Histogram depiction of CD8+ Teff proliferation as described in (A, right panel, arrow), 1:2 ratio of Teff:Treg and ETB 1 at 1000 ng/mL
C. Suppression assay at a 1:2 Teff:Treg ratio with ETBs 1 and 2 at 1000 ng/mL. % T cell proliferation was measured as the % cells undergoing 3+ rounds of proliferation in the co-culture model.

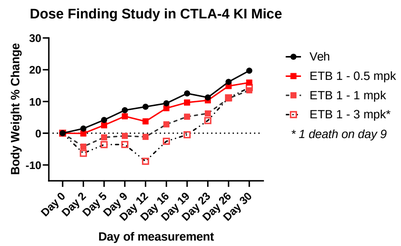
CTLA-4 ETB 1 Displays a Short Serum Half-life and Is Well Tolerated in Vivo

CTLA-4 ETB 1 Displays a Short Serum Half-life



C57BL/6 mice were dosed 2 mg/kg, i.p., with representative ETB 1. Serum samples were taken through 24 hours and analyzed by ELISA. n=3 mice per time point.

CTLA-4 ETB 1 is Tolerated in Humanized Mice



Vehicle (Veh) or representative ETB 1 was dosed every third day i.p., three different doses, when CTLA-4 humanized knock-in (KI) mice were 4-6 weeks of age. Body weight measurements were performed through day 30.

CONCLUSIONS

- CTLA-4-targeted ETBs are designed to preferentially deplete regulatory T cells in the TME to improve efficacy and reduce the toxicity associated with CTLA-4 targeted antibodies
- CTLA-4 ETB candidates directly bind and kill CTLA-4 positive cells
- CTLA-4 ETB candidates that bind diverse CTLA-4 epitopes are being developed to evaluate efficacy independent of blocking function as an additional strategy to reduce adverse events
- CTLA-4 ETBs antagonize Treg suppression of T-effector proliferation in co-culture models
- The short half-life of ETBs allow for target engagement and irreversible cytotoxicity to target cells paired with rapid clearance to mitigate the duration of adverse events
- In vivo efficacy studies are underway
- A CTLA-4 ETB is expected to enter the clinic in 2021

References:
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