Preclinical characterization of a novel CTLA-4-targeted ETB for direct Treg depletion

Swati Khanna, Caleigh Howard, Lilia A. Rabia, Alvaro Aldana, Jay Zhao, Asis Sarkar, Eric Williams, Banmeet Anand, Betty Chang, Chris Moore, Hilario J. Ramos, Aimee Iberg -- Molecular Templates Inc., Austin, TX.

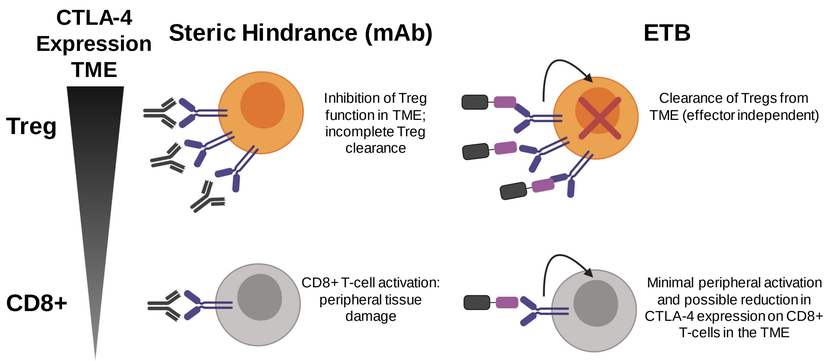
AACR Annual Meeting 2021
Abstract Number 1627

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:

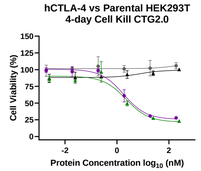
- **Specificity for CTLA-4**: Preferential activity against Tregs vs CD8+T-cells based on receptor density
- **Potency**: Direct cell-kill of Tregs via enzymatic and irreversible inactivation of ribosomes
- **Small size (40-55 kDa)**: Increased tumor penetration



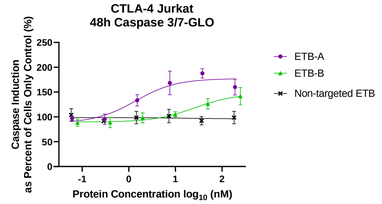
Cytotoxicity in Model Cell Systems

CTLA-4 ETB Candidates Induce Cytotoxicity in a Target-dependent Fashion

ETB	CHO-K1				HEK293T	
	Human	Monkey	Mouse	WT	Human	WT
ETB-A	3.0	1.5	NC	NC	8.3	NC
ETB-B	6.5	5.2	NC	NC	7.8	NC



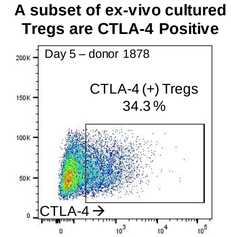
Viability of various CTLA-4 stable overexpression and parental cell lines was measured 96 hours after ETB addition to cells using Cell Titer-Glo®(Promega). IC50 values reported in nM. NC= no cytotoxicity

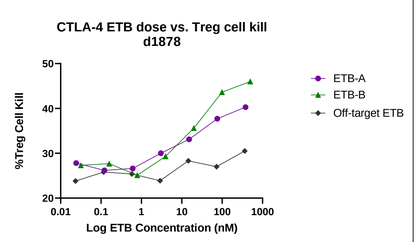


Caspase activity was measured 48 hours after ETB addition with the Caspase 3/7-Glo®(Promega) reagent.

Induction of Apoptosis on Primary ex-vivo Tregs

CTLA-4 ETB Candidates Induce Apoptosis in Primary Human Tregs

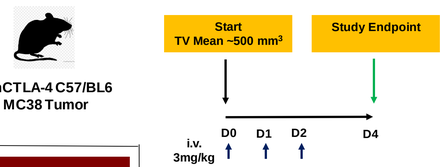


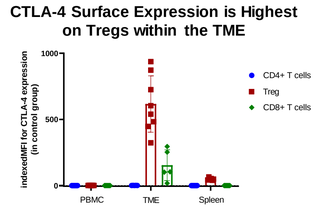


Tregs at day 6 post isolation were treated with different concentrations of ETBs for 48h and the cells were stained with Annexin V antibody and 7-AAD to measure apoptotic cells.

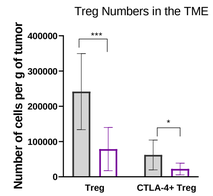
CTLA-4 ETB-A depletes Tregs in the TME and induces Peripheral T cell proliferation

T cell Immunophenotyping in a Tumor-bearing Syngeneic humanized Mouse Model

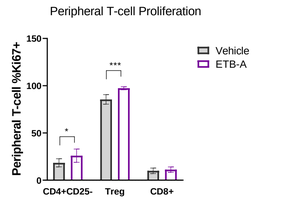




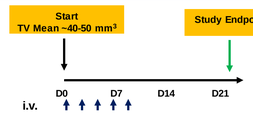
CTLA-4 ETB-A Depletes Tregs in the TME



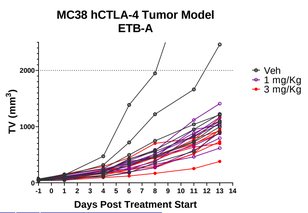
CTLA-4 ETB-A Results in proliferation of CD4+ T-cells in the periphery (blood)



Modest Tumor Growth Inhibition Observed with ETB-A Treatment



CTLA-4 ETB-A treatment in a fast-growing tumor model designed for CTLA-4 blocking mAbs. (ns)= not significant



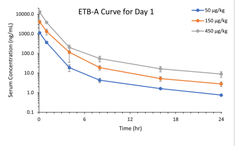
Gps	Treatment	N	% TGI (Day 13)
1	Veh Control	7	-
2	ETB-A 1.0 mg/kg	8	22.39% (ns)
3	ETB-A 3.0 mg/kg	8	36.54% (ns)

CTLA-4 ETB-A is Well-tolerated in NHPs

Study Design

Group	Test Article	Dose (ug/kg)	Dosing Days	# of Females
1	Vehicle	0	1, 8, 15, 22	2
2	ETB-A	50	1, 8, 15, 22	3
3	ETB-A	150	1, 8, 15, 22	3
4	ETB-A	450	1, 8, 15, 22	3

PK Analysis

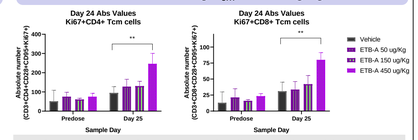


- Dose-proportional TK was observed with mean half-lives between 5.8 and 8.6 hours
- Early serum levels exceed concentrations required for activity on ex-vivo Tregs

Toxicology Summary for ETB-A

- **No mortality was observed.**
- On Day 2, clinical signs of limited usage of hind limb (1/3 animals at 50 µg/kg) and mild to moderate swelling of hind limb (1/3 at 450 µg/kg) were observed.
- Liver enzyme increase was found immediately after the first dose and maxed, after D15 gradually subsiding.
- **Overall, little to no histopathological differences were observed in liver, heart, colon, stomach, kidney and adrenal gland.**
- 450 ug/kg is the highest dose administered in the study, which was tolerated

Increased Proliferating T_{cm} at 450 ug/kg ETB-A



To determine absolute counts of cell populations, blood samples were stained with Abs to marker antigens, red blood cells lysed, and aliquots of remaining intact cells analyzed utilizing the BD LSR Fortessa X20 flow cytometer.

In Vitro Binding Properties

CTLA-4 ETB Candidates bind human and cynomolgus CTLA-4

Binding (K_d) ELISA

ETB	Human	Monkey	Mouse	hCD28
ETB-A	0.38	0.35	NB	NB
ETB-B	0.58	0.51	NB	NB

ETB binding to various species of CTLA-4 protein and CD28 was measured by ELISA. K_d values reported in nM. NB= no binding

Binding (K_d) Octet

ETB	K_a	K_{dis}	K_d
ETB-A*	1.3e5	3.9e-5	0.03
ETB-B	4.5e5	1.1e-3	2.4

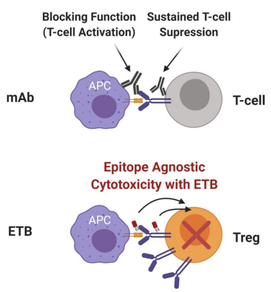
ETB binding kinetics to recombinant human Fc-CTLA-4 was measured on the Octet platform. Kinetic values reported in nM. *Avidity effects expected in the measurement for ETB-A

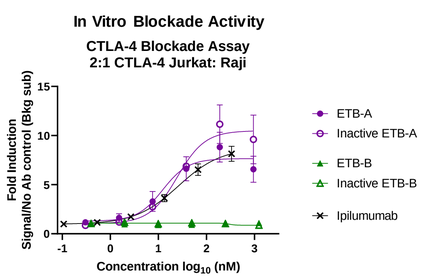
Binding (K_d) On-cell Flow

ETB	Human CTLA-4
ETB-A	2.2
ETB-B	6.9

ETB binding to hCTLA-4-Jurkat cells was measured with flow cytometry using an ETB detection antibody. K_d values reported in nM.

CTLA-4 ETB Candidates Possess Different In Vitro Blockade Activities





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. Inactive ETBs harbor mutations in the SLTA domain that render the ETB unable to inactivate ribosomes.

Inactive ETB	EC50 (nM)
ETB-A	24.7
ETB-B	No signal
Ipilimumab	25.9

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
- Multiple ETB candidates have been identified with the ability to bind human and cynomolgus CTLA-4 and to induce cytotoxicity on CTLA-4-expressing cells.
- CTLA-4 ETB candidates induce apoptosis in ex-vivo cultured Tregs that express CTLA-4.
- In a transgenic mouse model expressing human CTLA-4 and bearing syngeneic subcutaneous tumors, CTLA-4 expression was highest on the Treg cells within the tumor microenvironment compared to other T cell populations and compartments
  - In the mouse transgenic model, we observed that ETB treatment depletes Tregs in the TME, supporting our overall hypothesis. Peripheral CD4+ T cell proliferation was observed in response to ETB treatment.
- Initial tox assessment was performed in a NHP model. ETB candidate A was well tolerated up to 450 ug/kg. A modest increase in proliferating CD4+ and CD8+ central memory T cells was observed and is a potential pharmacodynamic effect.
- Overall, these preclinical data support the use of ETB technology to deplete immune repressive regulatory T cells to allow immune reactivation to tumor.

Website: www.mtem.com; Contact: bd@mtem.com, aimee.Iberg@mtem.com

This poster was sponsored and prepared by Molecular Templates. All authors are employees of Molecular Templates.