Engineered toxin body targeting CTLA-4 (MT-8421) depletes Tregs in the tumor microenvironment and synergizes with αPD-1 to enhance T cell immunity

Abstract 817

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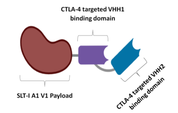
MT-8421 represents a wholly differentiated approach for targeting CTLA-4

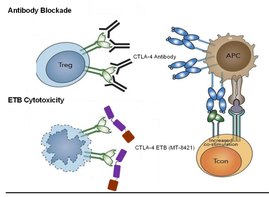
Engineered Toxin Bodies (ETBs) are fusion proteins consisting of an antibody fragment fused to a genetically engineered (de-immunized (DI)) form of the Shiga-like toxin A subunit (SLTA). Once the antibody fragment portion of the ETB binds its target, the SLTA portion of the ETB induces internalization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

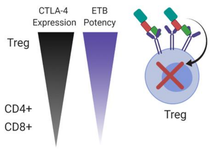
MT-8421 is a CTLA-4 targeted ETB comprised of biparatopic VHH domains linked to a de-immunized SLTA A1 subunit



MT-8421 characteristics	
CTLA-4 affinity	Sub-nM
Treg Potency	Sub-nM
Size	55 kD
In vivo half-life	~3 hrs

MT-8421 Mechanisms of action

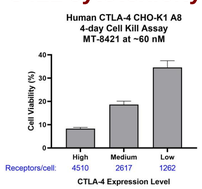




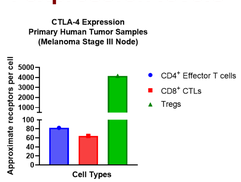
MT-8421 cell kill has been fine tuned to induce cell kill ONLY in cells with high expression of CTLA-4 like that seen-on T regulatory cells found within the tumor microenvironment.

Antibody mediated CTLA-4 blockade	ETB-mediated CTLA-4$^+$ cell clearance
Steric hinderance of CTLA-4 ligand interactions	Targeted removal of CTLA-4$^+$ immune cells
Potent inhibition of ALL immune cells	Cell killing fine-tuned for high CTLA-4 receptor occupancy (tumor microenvironment only)
Peripheral effects include re-awakening CTLA-4+ CD8 T cells	Peripheral effects minimal, no alteration of systemic CTLA-4$^+$ CD8 T cells
Tumor Tregs remain in the TME (extra-CTLA-4 suppressive functions still possible)	Tumor-resident Tregs killed/removed (independent of peripheral T effector functions)
Half-life is approximately 3 weeks	Half-life is approximately 3 h (washout period shorter); intracellular MOA of cell death is enzymatic and permanent

MT-8421 cytotoxicity varies with CTLA-4 expression levels

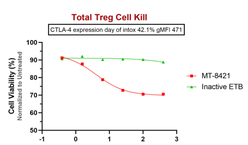


MT-8421 cell death is dependent on CTLA-4 receptor density.

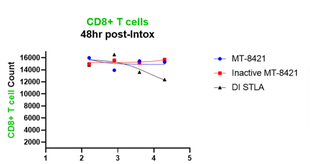


CTLA-4 receptor levels in tumor-resident Tregs are high in stage III melanoma patients

MT-8421 preferentially kills T regulatory Cells



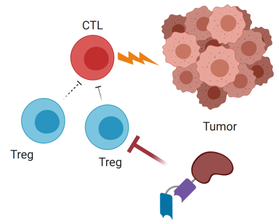
MT-8421 potently kills T regulatory cells (sub-nM IC50)

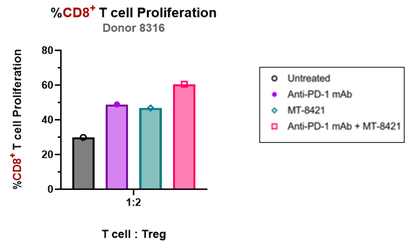


MT-8421 has minimal cell kill on peripheral CD8$^+$ T cells isolated from healthy individuals

PBMCs from healthy individuals were exposed to increasing levels of MT-8421 or its inactive (SLTA-dead) analogue and measured for evidence of cell death by apotracker (Tregs) or CTG (CD8$^+$). Apotracker was used for Treg evaluations due to the low numbers of Tregs in PBMCs. Note: the frequency of CTLA-4 positive Tregs was 42% with an MFI of 471.

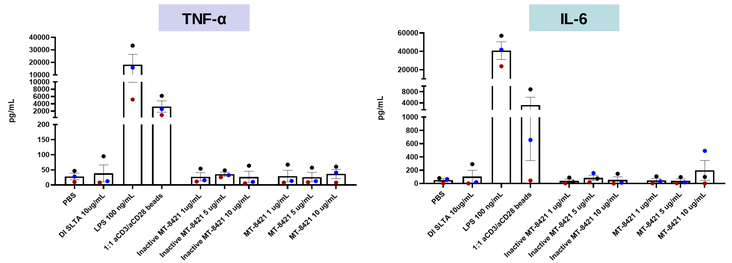
MT-8421, alone or in combination with anti-PD-1 antibody, inhibits Treg-mediated T cell suppression





- Selective depletion of CTLA-4 expressing Tregs promotes proliferation of CD8$^+$ T cells (CTL), which are known to be functionally suppressed by Tregs.
- The combination of CTLA-4 targeted ETB and PD-1 blockade further enhances CD8$^+$ T Cells proliferation better than either treatment alone.
- ***MT-8421 reduction of Treg cell suppression in this co-culture setting is further evidence of selective killing of Tregs over CD8$^+$ T cells***

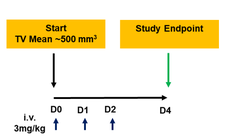
MT-8421 does NOT result in pro-inflammatory cytokine release

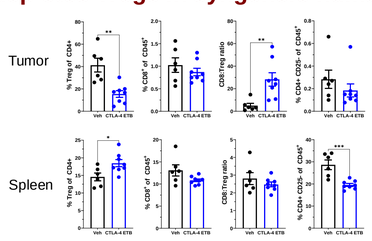


In vitro cytokine release by stimulated PBMC samples was examined at 24 hours following incubation with MT-8421 (1, 5, and 10 ug/mL).

- No generalized innate immune cytokine secretion after treatment of PBMCs with MT-8421
- * Each colored dot on the bar graph represents individual donor PBMCs
- ***IL-6 has been shown to correlate with αCTLA-4 mAb toxicity and drive tumor growth; IL-6 blockade can de-couple toxicity from anti-tumor responses (Hailemichael et al., 2022)***

MT-8421 selectively depletes Tregs in syngeneic humanized mouse model





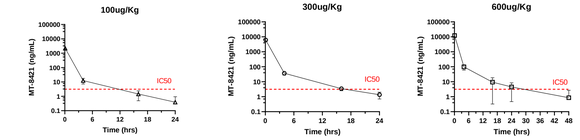
MT-8421 preferentially kills tumor resident Tregs in mouse xenograft model

Human CTLA-4 knock-in HuGEMM mice (Biocytogen) were inoculated with MC38 tumors. ETB was dosed at 3 mg/kg for 3 consecutive days. On day 4, the tumors and spleens were harvested and processed for immunophenotyping.

MT-8421 4-week GLP IND-enabling NHP Study

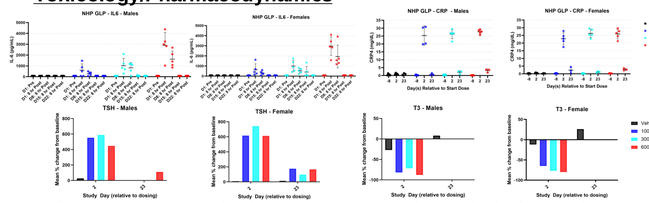
Dose level (µg/kg)	ROA	Dosing Day	Number of Animals, Main Study		Number of Animals, Recovery	
			Males	Females	Males	Females
0	IV	1, 8, 15, 22	3	3	2	2
100	IV	1, 8, 15, 22	3	3	2	2
300	IV	1, 8, 15, 22	3	3	2	2
600	IV	1, 8, 15, 22	3	3	2	2

Pharmacokinetics



- On Day 1, mean MT-8421 C_{max} increased proportionally to dose
- C_{max} hundreds of fold above the primary Treg IC50
- No accumulation of MT-8421 occurred on Day 8

Toxicology/Pharmacodynamics



- GLP Nonhuman primate study has been completed, no test article related effects on mortality, body weights, gross obs, organ weight, or histology were observed
- Transient and reversable changes in TSH, IL-6, and CRP were observed upon initial dosing and may represent target-mediated pharmacodynamic effects
- Highest non-severely toxic dose (HNSTD) established at 600 µg/kg

Phase 1 Study

A Phase I first-in-human study with MT-8421 is expected to initiate in mid-2023 and will assess safety, PK/PD, TME changes, and efficacy as monotherapy with a potential combination study with a PD-1 antibody also planned.

Conclusions

MT-8421 represents a wholly differentiated approach for CTLA-4 targeting with the real possibility of driving efficacy with reduced systemic toxicities

- Traditional CTLA-4 monoclonal antibodies are limited by significant peripheral toxicities, thought to be driven by release of peripheral T effector function
- MT-8421 functions by killing CTLA-4$^+$ immune cells in a receptor density dependent manner
- MT-8421 shows no activity on peripheral CD8$^+$ T cells (thought to mediate AEs associated with mAb CTLA-4 inhibition)
- MT-8421 can eliminate (not just block) Tregs in the TME in a mouse model system, thereby fundamentally altering the tumor microenvironment through direct elimination of suppressor cells (thought to drive efficacy in current marketed CTLA-4 targeted mAbs).
- MT-8421 is well tolerated in non-human primates at doses hundreds of fold above the primary Treg IC50

Hailemichael et al., 2022. Interleukin-6 blockade abrogates immunotherapy toxicity and promotes tumor immunity, Cancer Cell, 40, 509-523