In vivo efficacy of a CD38-specific Engineered Toxin Body

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Abstract

The cell surface glycoprotein CD38 is highly expressed on the surface of several B cell lineage cancers, such as the plasma cell malignancy multiple myeloma, some lymphomas and chronic lymphocytic leukemia (CLL), where it is a marker of unfavorable prognosis. CD38 is a transmembrane receptor and ectoenzyme that is absent or expressed at a low level on most resting leukocytes under normal conditions, making this an appealing target for directed therapy for hematological malignancies. Recent clinical trials with antibodies to CD38 have shown promise in multiple myeloma. While these antibodies rely on the recruitment of an immune response for cytotoxicity, we have developed an engineered toxin body (ETB) comprising a CD38 binding scFv and a modified Shiga-like Toxin A subunit capable of specifically recognizing and directly killing CD38 expressing cells. The CD38 targeted ETB has a different mechanism of action than current treatments for multiple myeloma (such as immunomodulatory agents, protease inhibitors and chemotherapies). Our CD38 targeted ETB has shown to be well tolerated in mice and displays dose dependent efficacy in a CD38 positive tumor setting. The CD38 targeted ETB significantly reduced tumor burden and increased survival over a 40-fold dose range in a disseminated Daudi xenograft, early treatment model. In this setting, the mean tumor burden at day 28 was reduced to 29% of control for the lowest dose group (0.05 mg/kg) and less than 1% of control for the two higher doses (0.5 and 2 mg/kg). Median survival of the control, untreated group was 34 days; the low dose group had extended median survival to 59.5 days and, at the day 60 study end, 90 or 100% of mice were alive in the higher two dosing groups. Our results show that the CD38 targeted ETB is a promising targeted therapeutic agent against CD38 positive cancer cells and is currently under further development.

Introduction

Engineered toxin bodies (ETBs) are targeted biological therapeutics derived from the ribosome-inactivating alpha subunit of Shiga-like toxin 1, SLT-1A. ETBs retain the ability of the parent toxin to induce internalization, escape the endosome, translocate to the cytosol, and enzymatically shutdown protein synthesis. SLT-1A has no intrinsic ligand and is instead targeted to cells by the associated SLT-1B subunit. ETBs have been proprietarily engineered such that each contains a unique target binding domain fused to a modified SLT-1A protein which allows for specific delivery to a cell surface target. Upon binding specifically to cells expressing the receptor, the intrinsic properties of SLT-1A allow the ETB to internalize, route to the cytosol, halt protein synthesis and kill the cell.

MT-4007 is an ETB targeted to CD38 via a specific single chain variable fragment (scFv). In a murine dose finding study, the MTD was not reached at the highest tested dose of 2 mg/kg, indicating that MT-4007 is well tolerated in vivo. Proprietary modification of the SLT-1A protein has identified variants, including MT-4007-D, that have reduced recognition by antibodies. MT-4007 and MT-4007-D show potent in vitro direct cell kill activity and good efficacy in vivo. Two xenograft models using Daudi lymphoma (early treatment, disseminated model) and H929 multiple myeloma cells (established, subcutaneous model) have been responsive to CD38 directed ETBs. Ongoing work to further de-immunogenize the CD38 targeted ETB while retaining potency is ongoing.

MOLECULAR TEMPLATES

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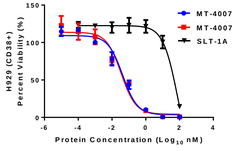
In vitro properties of MT-4007 and MT-4007-D

Specific Cytotoxicity: CD38 +ve cells | **Reduced Immunodetection:**

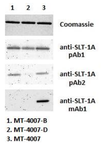
A. H929 (CD38+, multiple myeloma) Cell Kill



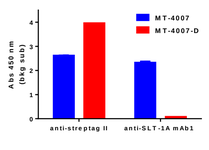
B. Cytotoxicity on CD38+ malignant human B-cell lines

Cell Line	CD38	MT-4007	MT-4007-D	SLT-1A
H929	+	0.043	0.035	80
Daudi	+	0.172	0.259	106
ST486	+	0.219	0.112	351
Raji	+	5.03	6.13	2394
U266	-	NC	NC	218
SKBR3	-	NC	NT	145
HCC1954	-	NC	NT	57

C. Denaturing conditions: Western blot



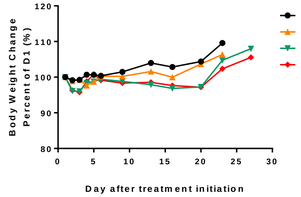
D. Native conditions: ELISA



Specific Cytotoxicity: Potent specific cytotoxicity of MT-4007 and MT-4007-D on **A.** CD38 expressing H929 (multiple myeloma) cells and on **B.** multiple CD38 positive but not CD38 negative human cancer cell lines. Cell viability was measured by the CellTiter-Glo® assay (Promega) after 72 hr incubation with the ETBs. Non-linear regression analysis was performed with Graphpad Prism software. IC_{50} values (nM); NC = not cytotoxic, an IC50 could not be calculated; NT= not tested.
Reduced immunodetection: C. Western Blot: Equivalent amounts of MT-4007, MT-4007-D and an earlier variant, MT-4007-B were probed with anti-SLT-1A polyclonal or monoclonal antibodies; MT-4007-D shows reduced immunodetection by both Ab under denaturing conditions. **D. ELISA**: Both ETBs bind recombinant CD38 in an ELISA assay as detected with an antibody to the fused streptag II. MT-4007-D is not detected with the anti-SLT-1A mAb under native conditions.

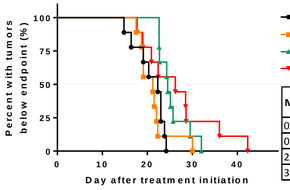
MT-4007-D provides dose- dependent delay in tumor growth: H929 subcutaneous xenograft

A. Stable Body weight during MT-4007-D treatment



MT-4007-D	BW Nadir	Day
0 (Control)	-0.90%	2
0.5 mg/kg	-2.30%	4
2.0 mg/kg	-3.90%	3
3.0 mg/kg	-4.20%	3

B. MT-4007-D delays H929 Tumor Growth (TTE)

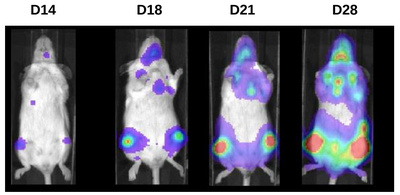


MT-4007-D	Deaths (n=9)	Median TTE	P value
0 (Control)	9	22.3	
0.5 mg/kg	9	21.2	NS
2.0 mg/kg	9	24.5	0.004
3.0 mg/kg	9	26.2	0.04

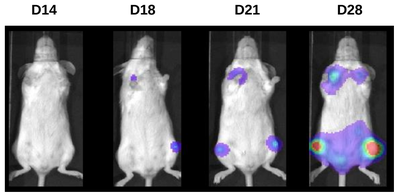
Dose dependent efficacy in a H929 subcutaneous, late treatment xenograft model: 1x10^7 H929 human multiple myeloma cells were injected SC into C.B.17 SCID mice. 16 days post engraftment, mice were randomized into groups with an average tumor burden of 135 mm^3 and treatment was initiated (D1). Mice were administered MT-4007-D IP at 0.5, 2 or 3 mg/kg on Days 1, 3, 5, 8, 10 and 12. **Tolerance: A. Body Weight:** Mice in all treatment groups had stable body weight, indicating that MT-4007-D is well tolerated. **Efficacy: B. Tumor Growth delay:** Efficacy in mice treated with ≥ 2 mg/kg MT-4007-D is shown by extended time to endpoint (TTE, endpoint tumor volume of 2000mm^3). P values by Mantel –Cox test compared to the vehicle control group.

Decreased tumor burden with MT-4007 : Daudi-Luc Disseminated Xenograft

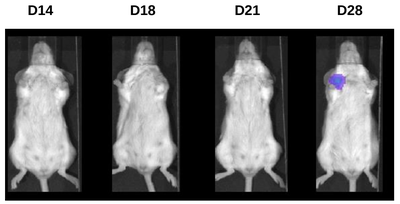
A. Vehicle control whole body BLI



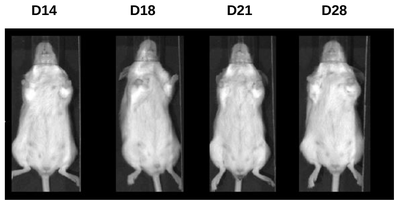
B. MT-4007 0.05 mg/kg whole body BLI

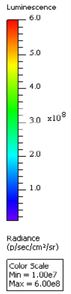


C. MT-4007 0.5 mg/kg whole body BLI

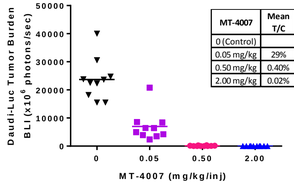


D. MT-4007 2 mg/kg whole body BLI





E. Tumor burden (BLI) at Day 28

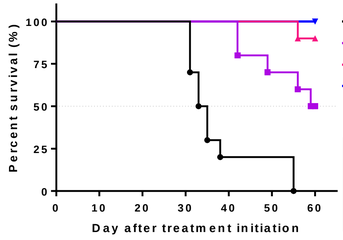


MT-4007	Mean T/C	Mean BLI	P value
0 (Control)		23676	
0.05 mg/kg	29%	6957	< 0.0001
0.50 mg/kg	0.40%	99.29	< 0.0001
2.00 mg/kg	0.02%	4.13	< 0.0001

Daudi-Luc disseminated xenograft early treatment model: C.B.17 SCID mice were implanted IV on Day 1 with 2.5x10^6 Daudi-Luc cells/mouse and whole body bioluminescent imaging (BLI) confirmed tumor take. One hour after treatment, MT-4007 was administered IP at increasing doses and dosing was repeated on Days 3, 5, 8, 10 and 12. Tumor burden was assessed by BLI. Median treated/ control (T/C) values for mice treated with ≥ 0.5 mg/kg were significant on all tested days. Tumor growth delay was observed for all treated groups. **A-D. Representative Images:** One mouse per group per day represents the dose dependent delay in tumor growth after treatment with MT-4007 on days 14, 18, 21 and 28 post tumor implant and treatment initiation. **E. Tumor burden on D28:** graphical representation of BLI data for all mice. Mean tumor burden at D28 for all treated groups was significantly different from control. T/C values show the dose dependent decrease in tumor volume. P values calculated by 1 way ANOVA with Dunnett's test for multiple comparisons to the control group.

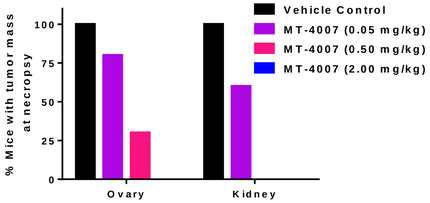
MT-4007 extends survival and reduces tumor incidence in murine xenograft model

A. Daudi-Luc Disseminated Xenograft: extended survival with MT-4007 treatment



MT-4007	Deaths (n=10)	Median survival	P value
0 (Control)	10	34	
0.05 mg/kg	5	59.5	0.0002
0.50 mg/kg	1	Undefined	< 0.0001
2.00 mg/kg	0	Undefined	< 0.0001

B. Daudi-Luc Disseminated xenograft- reduced tumor mass incidence at terminal necropsy with MT-4007 treatment



Dose dependent increase in survival and decrease in tumor incidence: A. Extended survival in the Daudi-Luc disseminated xenograft early treatment model: Mice in all treatment groups had a statistically significant increase in lifespan. Mice were euthanized due to >20% body weight loss or clinical signs (i.e. hind limb paralysis). All mice in the highest treatment group survived to the study end. P values by Mantel –Cox test compared to the vehicle control group. **B. Incidence of tumor masses found at terminal necropsy:** Mice euthanized due to disease progression or at study end were examined for tumor masses. Fewer mice with masses in the ovary or kidney were found in all treatment groups; no mice in the high dose group had tumor masses at terminal (study end) necropsy.

Conclusions

- MT-4007 and MT-4007-D display specific cytotoxicity towards CD38-positive human cancer cells in vitro including H929 (multiple myeloma) cells.
- Genetic Engineering of MT-4007 identified a variant, MT-4007-D, which retains potent and selective toxicity toward CD38+ve cells but has reduced immunogenic potential as evidenced by deceased recognition by polyclonal and monoclonal antibodies under denaturing and native conditions.
- MT-4007 and MT-4007-D are well tolerated in vivo. Treatment of mice with MT-4007-D, 6 doses over 12 days of up to 3 mg/kg, resulted in minimal weight loss.
- MT-4007 showed potent, dose- dependent efficacy in an early treatment setting, significantly reducing the tumor burden and increasing lifespan of mice injected with Daudi-Luc tumor cells in a disseminated model of disease. MT-4007 was also efficacious in the established H929 tumor model (data not shown).
- MT-4007-D showed efficacy when treating after multiple myeloma tumors were established, delaying tumor growth and extending the time to endpoint (survival) in a dose dependent manner.
- **MT-4007-D is a promising CD38 targeted therapeutic and is undergoing further development in pre-clinical studies.**