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Next-generation engineered toxin bodies: CD38, PD-L1 and HER2 targeted ETBs

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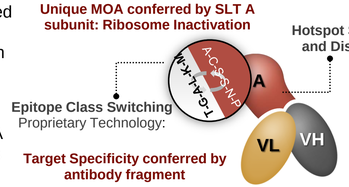
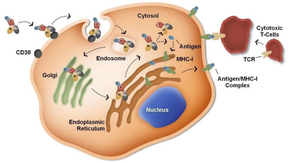
BACKGROUND

Molecular Templates is developing engineered toxin bodies (ETBs), potent recombinant immunotoxins that combine the specificity of an antibody fragment with the powerful direct cytotoxicity of the Shiga-like toxin A subunit to specifically kill target expressing cells. Once delivered to appropriate cells, the Shiga toxin A subunit enzymatically inhibits protein synthesis and promotes apoptosis of tumor cells.



Our next-generation ETB scaffold has been modified through genetic engineering to systematically and comprehensively reduce B and CD4+T cell epitopes. Molecular Templates has developed a proprietary epitope class switching technology designed to both reduce the anti-drug response and promote the anti-tumor response by replacing naturally occurring CD4+ T cell epitopes with CD8+ T cell epitopes; when combined with surface remodeling, powerful reductions in the anti-drug response against ETBs after repeat administration is demonstrated. Additionally, the engineering of CD8+ T cell epitopes on the ETB scaffold can allow for foreign antigen presentation in complex with MHC class I on the tumor cell surface.



This de-immunized, next generation scaffold has been combined with multiple binding domains, including scFv and VHH antibody fragments. ETBs targeting CD38, PD-L1 and HER2 are being developed. The potency, reduced immunogenicity, unique mechanism of action and immune modulating activities of ETBs in this second generation scaffold allows for these agents to be effective in a refractory setting as well as in combination with other agents in the growing field of immuno-oncology.

ETBS IN DEVELOPMENT

ETB	Target	Oncology Indication	Status
MT-3724	CD20	Non-Hogkin's Lymphoma	Phase 1/1b ongoing
MT-4019	CD38	Multiple Myeloma	IND-enabling studies
mt-17000	PD-L1	Hodgkin's lymphoma, melanoma, lung, breast	pre-clinical
mt-2000	HER2	HER2+ breast cancer	pre-clinical

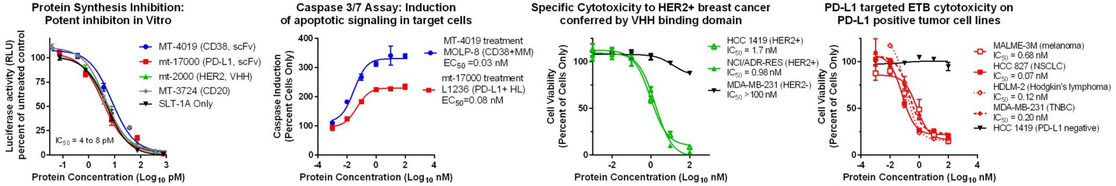
METHODS

Protein Synthesis Inhibition: Serially diluted proteins were added to the TnT quick (Promega) master mix which includes the luciferase DNA plasmid. Luciferase protein synthesis was measured by light output (RLU) after adding the luciferase substrate (Promega). Luciferase activity is displayed as a percentage of untreated controls.
Cell based Assays: Serially diluted proteins combined with cells were incubated for 20 (Caspase) or 72-120 (Cell Viability) hours prior to measurement with Caspase-Glo 3/7 or CellTiter-Glo (Promega). Caspase activity or cell viability is shown as a percentage of untreated, cells only controls.
Combination Studies: H929 cells were treated with a dilution series of each test agent individually with a fixed ratio of effective concentrations of each (ie 1:1, 3:1, 1:3). Cells were treated with the IMiD, then after 72hrs, MT-3724 was added and cell viability was measured 48 hours later with CellTiter-Glo (Promega). Ki was determined as the sum of ratios $C_{50,A}/IC_{50,A}$ and $C_{50,B}/IC_{50,B}$. Data shown for three experiments as indicated by different symbols.
Antigen Seeding Technology: PD-L1 targeted ETBs containing either CMV or FLU HLA-A2 immunodominant antigens were produced and incubated with L1236 cells for 18-24 hrs. The MHCI/CMV complex was detected by flow cytometry using a PE-labeled TCR multimer (Altor). Functional presentation of MHC-I/FLU complex is shown by activation of the NFAT-luciferase reporter construct stably transfected into a FLU-specific TCR reporter cell line.
Relative Immunogenicity: BALB/C mice were administered MT-4019 or the un-modified mt-4000 IP at 0.25 mg/kg/dose 3x/week for a total of 12 doses over 5 weeks. Serum was collected, diluted and incubated with the ETB, then the immune complex was captured on CD38 protein coated ELISA plates. Anti-drug antibodies (ADA) were detected with an HRP conjugated anti-mouse IgG antibody. Signal is shown with the serum dilution factored in.
Tolerability: BALB/C and C57BL/6 mice were administered up to 5 mg/kg/dose of MT-4019 IP 3x/week for two weeks and weights measured throughout.
Xenograft: Daudi-Luc cells were inoculated via tail vein to CB17 SCID mice. Four days later, mice were randomized into groups with matched average BLI signal and treatment began. MT-4019 was given IP at 0.6 mg/kg/dose, 5x/week for two weeks (10 doses total) and BLI monitored throughout.

MECHANISM OF ACTION

Engineered toxin bodies have a mechanism of action that is unique to oncology

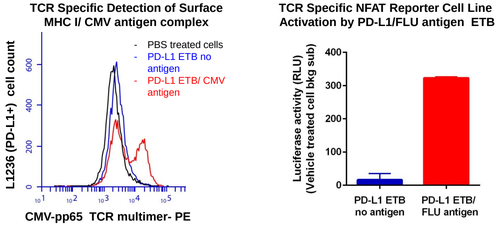
ETBs inhibit protein translation by irreversibly and enzymatically inactivating ribosomes, leading to ribotoxic stress, caspase activation, and apoptosis. This MOA is conserved with MT-3724, Molecular Template's CD20 targeted ETB, which is in clinical trials for NHL. ETBs directed by VHH or scFv binding domains to specific targets have potent cytotoxicity against HER2+ cells, including the T-DM1 resistant NCI/ADR-RES cell line. PD-L1 ETBs show robust activity on PD-L1 + cell lines from a variety of cancer types, including melanoma, NSCLC, breast cancer and Hodgkin's lymphoma.



ANTIGEN SEEDING TECHNOLOGY

Viral Antigen delivery via ETB for MHC-I surface presentation

PD-L1 targeted ETBs that contain viral epitopes show surface expression (CMV pp65 antigen) and functional activation of TCR signaling (FLU M1 antigen) in two different assays.

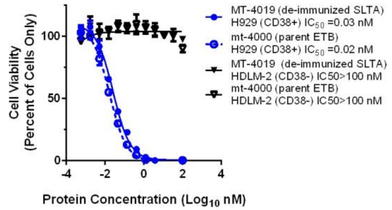


CD38 TARGETED ETB MT-4019: POTENT ACTIVITY, REDUCED IMMUNOGENICITY

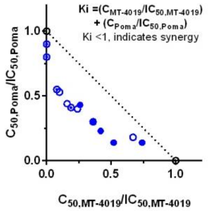
MT-4019 has potent, specific activity suited for combination with IMiDs

MT-4019 has similar potency to the parent ETB (same scFv, unmodified SLTA) and specifically targets CD38 expressing cells, with minimal effect on CD38 negative cell lines. Pre-treatment of H929 myeloma cells with Pomalidomide shows synergistic cytotoxicity with MT-4019.

MT-4019 retains potency and specificity with a de-immunized SLTA catalytic domain



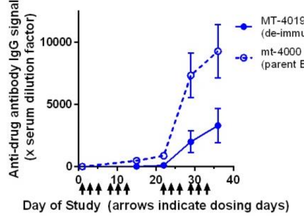
Synergistic cytotoxicity of MT-4019 on cells pre-treated with Pomalidomide



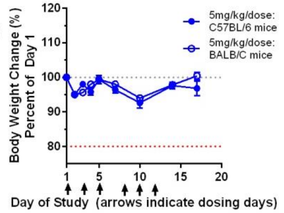
MT-4019 has reduced antibody generation and is well tolerated

BALB/C mice dosed repeatedly with MT-4019 (12 doses of 0.25 mg/kg over 5 weeks) show delayed and reduced ADA formation. Both BALB/C and C57BL/6 mice tolerate high doses of MT-4019 (5mg/kg/dose) with minimal body weight loss or clinical signs.

Reduced Immunogenicity of MT-4019 after repeat dosing

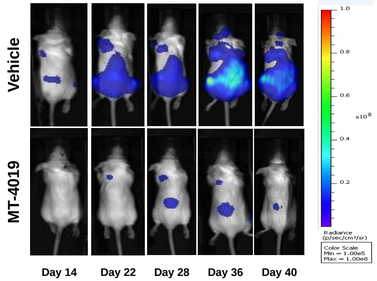


Tolerability of MT-4019 in Immunocompetent Mice



Decreased tumor growth in vivo

CB17 SCID mice treated with MT-4019 (0.6 mg/kg/dose; 5x/week for 2 weeks) starting four days post injection of Daudi-Luc cells reduced tumor burden (T/C of 11% on Day 40).



ENGINEERED TOXIN BODIES PLATFORM TECHNOLOGY

- Unique MOA for ETBs and synergy in vitro with inhibitor/ immunomodulatory agents indicates potential for combination therapy
- Development ETBs include CD38, PD-L1 and HER2 targeted immunotoxins on a next generation scaffold that has reduced immunogenicity
- Molecular Templates lead compound, MT-3724, targets CD20 and shows promising activity in an ongoing Phase I trial in relapsed/ refractory NHL patients
  - Posters at AACR 2016 1483 (pre-clinical) and CT049 (clinical)

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