AACR 2017
Abstract 2659

MT-4019: a de-immunized engineered toxin body targeting CD38 for multiple myeloma

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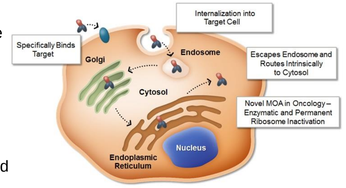
Background

ETB Technology

- Engineered Toxin Bodies (ETBs) are potent recombinant immunotoxins that combine the specificity of an antibody fragment with the powerful direct cytotoxicity of the Shiga-like toxin A subunit (SLTA)
- ETBs induce their own internalization, route through the cell in a predictable manner, enzymatically and irreversibly destroy ribosomes to shutdown protein synthesis and induce apoptosis in tumor cells



- The mechanism of action expands the target universe and is distinct from other therapeutics, including ADCs, making ETBs an attractive treatment for patients
- MT-3724 is a 1st-gen ETB with nM affinity for the B-cell marker CD20 with monotherapy activity and safety in heavily treated DLBCL patients with low rates of neutralizing antibodies
- However, a more potent ETB scaffold with reduced immunogenic potential would be desirable to enhance efficacy in solid tumor indications where immunogenicity may be more relevant
- 2nd generation ETBs change construct orientation and use proprietary modifications to de-immunize the SLTA domain
- These changes comprehensively reduce B cell and CD4+T cell epitopes and dampen the innate immune response while having >10x more potency than 1st generation ETBs

MT-4019 Therapeutic Potential

- CD38 is a clinically validated surface receptor expressed on malignant plasma cells; expression persists after failure of antibody treatment
- MT-4019 is a CD38-targeted 2nd generation ETB
- Pre-clinical studies in rodents and non-human primate models have demonstrated that MT-4019 is well tolerated, with decreased anti-drug antibody (ADA) and innate immune reponses
- *In vitro* data shows picomolar efficacy against CD38 expressing cell ines, including cell lines that are insensitive to Daratumumab

ETB Construct Description

- MT-3724 – 1st generation ETB, targets CD20, binds both NHP and human CD20
- MT-4019 – 2nd generation ETB, targets human CD38, does not bind NHP CD38
- mt-4040 – control ETB, targets CD38, binds both NHP and human CD38; has improved potency of 2nd generation construct but 1st generation SLTA with engineered furin resistance

Methods

Cytotoxicity: ETBs were incubated with cells for 48-72 hours, then cell viability was measured with CellTiter Glo 2.0 (Promega) ATP detection reagent. For competition assays, cells were incubated with MT-4019, vehicle, or Daratumumab (Dara) for indicated times at 37C, then the second compound was added and incubation continued.
Mouse *in vivo*: ETBs were diluted in PBS and administered at 1mg/kg/dose by IP to mice (n= 5 or 6/group) for one cycle (days 1, 3, 5, 8, 10, 12) at Charles River Laboratories. Serum tested for ADA 10 days post last dose (Day 22) by ELISA.
Cynomolgus (cyno) *in vivo*: ETBs were diluted in PBS and administered by IV to NHP (n= 2 or 3/group) for two cycles at MPI Research. Peripheral blood samples were collected (pre-dose if on dosing days) for clinical chemistry panels and flow cytometry of cell populations was run at MPI. Serum exposure was measured by ELISA; ETBs bound to plates coated with recombinant human CD38 protein and were detected with polyclonal anti-SLTA antibodies and anti-rabbit HRP secondary.
IgG ADA ELISA: Serum samples were diluted and incubated with ETB overnight to form immune complexes, then were captured on an ELISA plate coated with recombinant human CD38 protein or CD20 peptide. Species specific IgG-HRP was used for detection.
TLR-4 ELISA: SLTA proteins were incubated with recombinant human TLR-4. Polyclonal anti-SLTA antibodies and anti-rabbit HRP secondary was used for detection for the subunit ELISA and protein-L HRP was used for detection for the ETB ELISA.
SLTA descriptions: WT= wildtype; DI= de-immunized and furin resistant; WT-FR= furin resistant but otherwise wildtype
All graphical analysis and non-linear regression was done with GraphPad Prism software.

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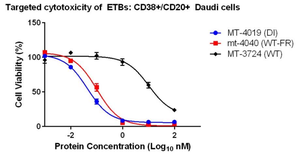
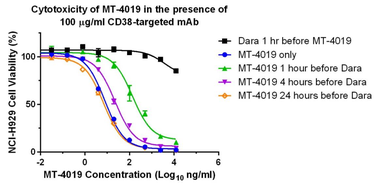
MT-4019 has potent activity *in vitro*

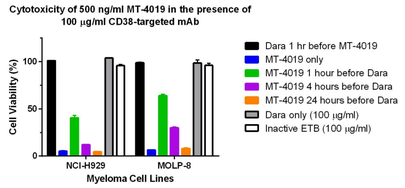
- On cell lines with similar CD20 and CD38 receptor expression (Daudi and ST486), MT-4019 and mt-4040 have increased potency compared to 1st generation MT-3724
- CD38-targeted ETBs MT-4019 and mt-4040 can kill cells that express CD55 and CD59, complement inhibitory proteins (CIP) that restrict CDC activity of CD38-targeted monoclonal antibodies (mAbs) and are associated with Daratumumab insensitivity

Cell Line	Surface Target		Surface CIP		IC_{50} (pM)		
	CD38	CD20	CD55	CD59	MT-4019	mt-4040	MT-3724
NCI-H929	**47,859**	182	**18,800**	**138,287**	5	19	Did not kill
MOLP-8	**170,317**	161	**26,635**	**207,860**	81	94	Did not kill
ST486	**97,046**	**152,602**	9,330	5,516	37	6	302
Daudi	**116,577**	**121,164**	8,468	1,230	44	102	10,370
JM-1	**24,417**	995	14,325	4,927	276	Not tested	Did not kill
Jurkat	5,276	26	8,821	31,445	Did not kill	Not tested	Did not kill



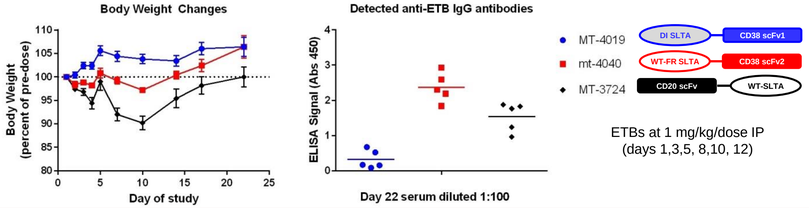
- Binding and activity of MT-4019 is blocked by prior incubation of the cells with Daratumumab; however the rapid internalization of MT-4019 allows for cytotoxicity when the ETB is provided to the cells prior to Daratumumab addition
- Catalytic inactivation of the SLTA subunit (inactive ETB) abolishes cytotoxicity.





MT-4019 has reduced ADA response in mice

- Compared to MT-3724 or mt-4040, which contain wild type SLTA, MT-4019 (de-immunized SLTA) shows much lower ADA response

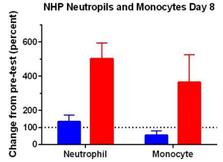


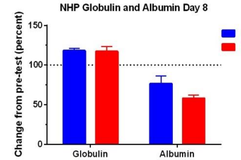
MT-4019 has reduced immunogenicity in NHPs

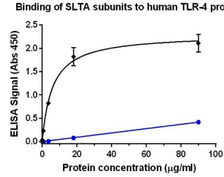
- ETBs were administered for two cycles at 0.05 mg/kg/dose IV with a two week break between cycles (days 1,3,5,8,10,12; 29,31,33,36,38,40).
- No adverse body weight effects or clinical observations were directly attributable to the test articles.

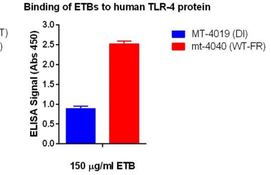
Innate response and Toll-like receptor 4 (TLR-4) binding to ETBs and SLTA subunits

- ETBs generate inflammatory/immune response in NHPs. The severity was less for animals receiving MT-4019 than mt-4040
- MT-4019 inflammatory/immune response:
  - Increased globulin and IgA concentrations
- mt-4040 inflammatory/immune response:
  - Increased neutrophil and monocyte counts
  - Presence of reactive monocytes
  - Increased globulin, IgA and IgG concentrations
- Decreases in albumin were numerically less for animals receiving MT-4019 than mt-4040
- Binding of the catalytic SLTA subunit to recombinant human TLR-4 is reduced by introducing de-immunization mutations
  - MT-4019 binding signal to TLR-4 is lower than that of mt-4040



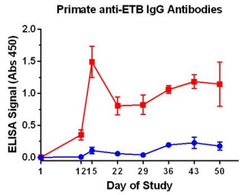


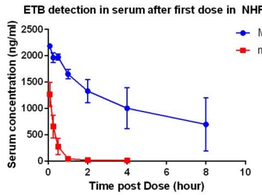




Serum exposure, target cell depletion and IgG ADA response in non-human primates

- In NHPs, there is greater serum exposure to MT-4019 than mt-4040
  - Decreased serum exposure after first dose of MT-4040 compared to MT-4019 is presumed due to target-mediated clearance
  - MT-4019 is detected after the 6th dose but mt-4040 is below the limit of quantitation
- CD38+/CD3- populations are depleted after a single dose of mt-4040, but rebound by day 8, likely due to the development of ADAs
- Even though there is higher serum exposure, MT-4019 has reduced IgG ADAs in NHPs throughout the study





Conclusions

- The 2nd generation ETB scaffold displays lower innate and adaptive immune responses *in vitro* and in animal models
- MT-4019 has picomolar efficacy against CD38+ cells *in vitro* including cell lines that do not respond to Daratumumab; it will enter human clinical studies in 2018
- Additional 2nd generation ETBs under development target HER2, PD-L1 and other oncology targets
- 3rd generation ETBs include 2nd generation features plus the ability to deliver foreign MHC class I antigens for presentation on the tumor surface (antigen seeding)