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MT-3724, an engineered toxin body targeting CD20 for non-Hodgkin's lymphoma

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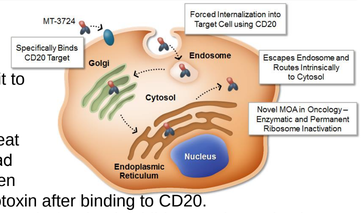
BACKGROUND

Molecular Templates has developed 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 destroy target expressing cells via a novel mechanism of action in oncology - ribosome inactivation.

CD20 is a well characterized target for agents used to treat non-Hodgkin's lymphoma (NHL). Molecular Templates' lead compound, MT-3724, is a CD20-targeted ETB that has been engineered to force the rapid internalization of the immunotoxin after binding to CD20.



Once MT-3724 is delivered to appropriate cells, the Shiga toxin A subunit inhibits protein synthesis and promotes apoptosis of tumor cells. The difference in MOA allows for activity in the refractory setting, where resistance to other treatments has emerged, and may also allow for combination therapy. Pre-clinical studies with immunomodulatory drugs (IMiDs), PI3K and Bcl-2 inhibitors, which are used in treatment of NHL, have shown promising results when combined with MT-3724.

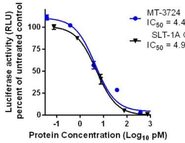
MT-3724 has potent direct cell kill activity on CD20 positive lymphoma cells and is the first immunotoxin to CD20 to enter the clinic. The on-going phase I study being conducted at Memorial Sloan-Kettering Cancer Center, MD Anderson Cancer Center, and New York University Langone Medical Center has shown promising safety and efficacy in highly refractory NHL patients. (Trial NCT02361346, see also AACR poster CT049 in Poster Section 13 , Phase I study presentation).

MT-3724 MECHANISM OF ACTION

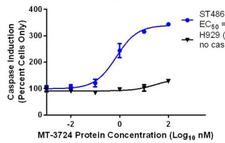
Engineered toxin bodies have a mechanism of action that is unique to oncology

MT-3724 inhibits protein translation by irreversibly and enzymatically inactivating ribosomes, leading to ribotoxic stress , caspase activation and apoptosis. MT-3724 specifically targets and potently kills CD20 expressing tumor cells, and demonstrates minimal cytotoxicity on cells that lack CD20 surface expression.

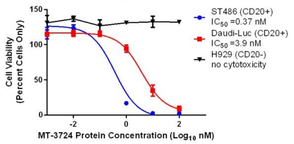
Protein Synthesis Inhibition: Potent inhibiton in Vitro



Caspase 3/7 Assay: Specific activity on CD20+ve cells



Cell Viability Assay: Specific cytotoxicity on CD20+ve cells



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 (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: Daudi cells were treated with a dilution series of each test agent (A or B) individually or in combinations with a fixed ratio of effective concentrations of each (ie 1:1, 3:1, 1:3). For experiments with Lenalidomide, cells were treated with the IMiD then after 24hrs, MT-3724 was added. For all other inhibitors, the treatment was concurrent. Cell viability was measured with CellTiter-Glo. Ki was determined as the sum of ratios $C_{50,A}/IC_{50,A}$ and $C_{50,B}/IC_{50,B}$.

Internalization: ST486 (CD20+) cells were incubated with 100 nM of ^{131}I labeled MT-3724 or Rituxan mAb for 30, 60 or 120 minutes. Cells were harvested, washed, and then the surface bound proteins were stripped with acid buffer. Percent internalization was determined by dividing the CPM after stripping (internal) by the cell associated CPM (total bound and/or internalized).

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-3724 was administered IP at a dose of 1.2mg/kg/dose for 5x/week every other week for three weeks (10 doses total). Whole body luminescence signal was taken throughout the study.

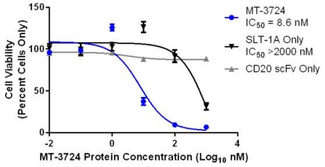
Refractory setting: PET scan of a patient treated at 5 mcg/kg/dose of MT-3724 by IV infusion for 5 cycles. Each cycle includes 6 doses over 2 weeks.

IN VITRO ACTIVITY OF MT-3724

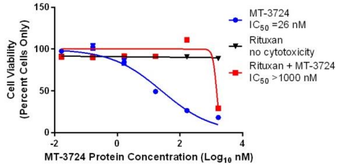
MT-3724 specifically targets then potently and directly kills CD20 expressing tumor cells.

The specificity of MT-3724 is conferred by the scFv and the MOA is conferred by the SLTA domain of the ETB. MT-3724 binds the same epitope as Rituxan, but targeted cell kill is direct rather than immune mediated. MT-3724 rapidly internalizes to CD20+ve target cells as compared to Rituxan.

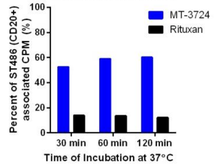
Cell Viability Assay: Targeted, Direct Cell Kill by MT-3724



Cell Viability Assay: Direct cell kill by MT-3724 is abrogated by Rituxan



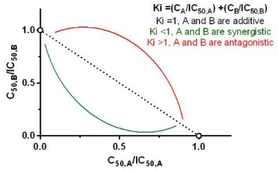
Rapid Internalization of radiolabeled MT-3724



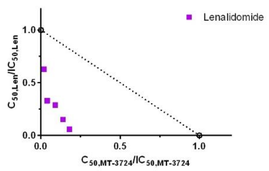
MT-3724 works in synergy with multiple classes of inhibitors and immunomodulatory agents (IMiDs)

MT-3724 added to CD20+ Daudi cells pre-treated (IMiD) or co-administered (inhibitors) with other NHL therapies acts in synergy to kill the target cells, indicating the potential for combination therapy with different mechanisms of action.

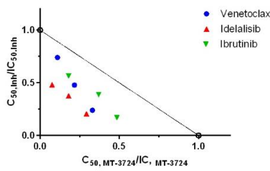
Isobologram to visualize the effect of combination of two test agents



Synergistic cytotoxicity of MT-3724 on cells pre-treated with Lenalidomide



Synergistic cytotoxicity by combination of MT-3724 with inhibitors



Combination testing:
Ki determination: For each test agent, the concentration to achieve 50% cell kill ($C_{50,A\text{ or }B}$) is divided by the concentration to achieve 50% cell kill when the single agent is used alone ($IC_{50,A\text{ or }B}$) to determine the effect of combination.
Isobologram: Ratios $C_{50,A}/IC_{50,A}$ vs $C_{50,B}/IC_{50,B}$ are plotted as an XY graph.

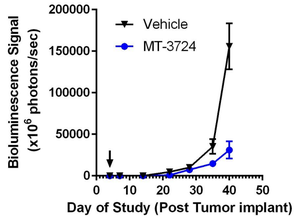
Test Agents		Combination with MT-3724		
Compound	Class	Inhibitor treatment	Average Ki	Indication
Venetoclax	BCL-2 inhibitor	concurrent	0.71	Synergy
Idelalisib	PI3K inhibitor	concurrent	0.60	Synergy
Ibrutinib	BTK inhibitor	concurrent	0.68	Synergy
Lenalidomide	IMiD	24 hrs prior	0.40	Synergy

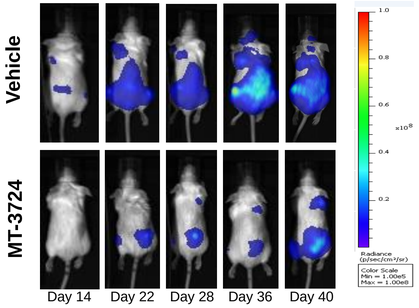
IN VIVO ACTIVITY OF MT-3724

Decreased tumor growth in Daudi-Luc Xenograft by MT-3724

CB17 SCID mice were treated with MT-3724 starting four days post injection of Daudi-Luc cells. MT-3724 treatment given five times a week, on weeks one and three, showed reduced tumor burden (T/C of 11% on Day 40).

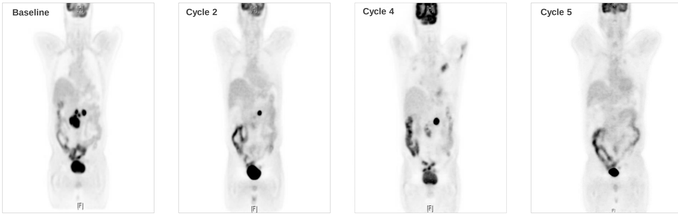
Average BLI Signal





Response to MT-3724 in a Refractory Patient Setting:

Patient History Prior to study::Diagnosed 2009 with Follicular Lymphoma, (stage 2) treated with Bendamustine + rituximab, IFRT (partial response); 2013 stage 3A , refractory to 5 cycles of R-CHOP with biopsy of transformed DLBCL.
MT-3724 Clinical Trial: Patient treated with MT-3724 at (5 mcg/kg for 5 cycles). Response observed.



ENGINEERED TOXIN BODIES PLATFORM TECHNOLOGY

- Molecular Templates' lead ETB, MT-3724, is an scFv fused to a proprietary form of SLTA that can induce CD20 internalization and cell death via ribosome inactivation
- MT-3724 shows promising activity in an ongoing Phase I trial in relapsed/ refractory NHL patients (Poster CT049)
- Unique MOA in cancer and synergy in vitro with inhibitor/ immunomodulatory agents indicates potential of combination therapy
- Other ETBs in development include CD38, PD-L1 and HER2 targeted immunotoxins on a next generation scaffold (Poster 595)

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