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Combination of MT-3724 with sirolimus reduces anti-drug antibody response and prolongs drug exposure

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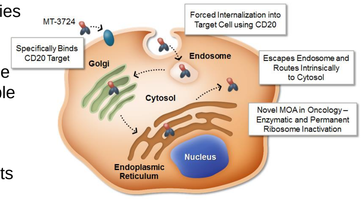
Background

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. ETBs can induce their own internalization, route through the cell in a predictable manner, enzymatically and irreversibly destroy ribosomes to shutdown protein synthesis and induce apoptosis of tumor cells. This mechanism of action is distinct from that of other therapeutics, making ETBs an attractive treatment for patients who have become resistant to chemotherapy and other treatment modalities.



MT-3724 is Molecular Templates' first-generation ETB targeting CD20, a surface receptor that is highly expressed on malignant B cells in hematological malignancies. Pre-clinical and clinical studies have shown promising results for non-Hodgkin's lymphoma cell lines and patients; however anti-drug antibodies are formed in experimental animals and patients after repeat dosing. Sirolimus (rapamycin) is a macrolide compound approved to prevent organ rejection and treatment of LAM, and has been used in tolerization protocols with replacement enzymes. In pre-clinical studies, we have co-administered sirolimus in combination with MT-3724 in rodents as well as in a non-human primate model in order to reduce the anti-drug antibody response to MT-3724. These studies demonstrated that the combination of sirolimus with MT-3724 decreased anti-drug antibody response, prolonged serum exposure of MT-3724, and improved B-cell depletion as compared to MT-3724 alone.

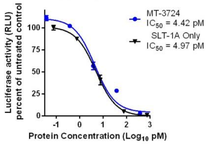
The combination of sirolimus with MT-3724 is an attractive and feasible regimen which could be further explored in clinical studies.

MT-3724 Mechanism of Action

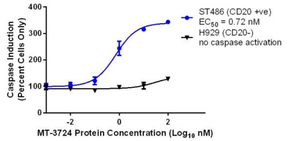
Engineered toxin bodies have a mechanism of action that is unique to oncology

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.

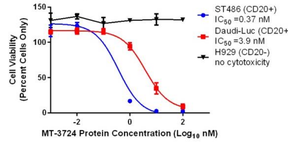
Protein Synthesis Inhibition: Potent inhibiton in Vitro



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



Cell Viability Assay: Specific cytotoxicity on CD20+ve cells



Methods

Method for detection of anti-drug antibodies (ADA) in human and primate serum: Using a Meso Scale Discovery System (MSD) ADA responses to MT-3724 determined by three step process of Screening, Confirmation & Titer Assay. Screening assay combined defined ratios of biotinylated MT-3724 and Sulfo-Tag MT-3724 and diluted (10-fold) sample, incubated at room temperature (RT), added to streptavidin-gold MSD plate, incubated at RT, MSD Read Buffer added and plate read on MSD instrument. Samples with signal over screening assay cut off are considered positive. Positive screening samples are confirmed by addition of unlabeled MT-3724 and incubated at 37C then the screening assay is conducted as above. For confirmatory assay positive samples, a dilution series is prepared, the screening assay conducted & the greatest dilution with signal above screening assay cut point is reported.

Method for detection of ADA in murine serum: Serum samples are diluted 1:1000 and combined with MT-3724 to form immune complexes, incubated at 2-8C, added to ELISA plates coated with CD20 polypeptide, washed, incubated with anti-mouse IgG HRP at RT, washed, TMB ultra added, A450 detection.

Method for detection of MT-3724 in primate serum: Serum samples diluted 1:10, added to CD20 peptide coated MSD plate, incubated at RT, anti-SLTA1 antibody added, incubated at RT, goat-anti-mouse Sulfo-Tag antibody added, incubated at RT, MSD Read Buffer added and plate read on MSD instrument. Standard curve of MT-3724 diluted in normal primate serum used to calculate sample concentration of MT-3724.

Development of anti-drug antibodies in primates & patients

Anti-MT-3724 antibodies develop after repeat dosing in non-human primates (NHP) & relapsed/refractory Non-Hodgkin's Lymphoma (NHL) subjects

- One Cycle of MT-3724 is 6 doses over 12 days via IV bolus (primates 75 mcg/kg/dose) or IV infusion for NHL subjects
- After one MT-3724 cycle ADA responses were detectable in 100% of primates and 38% of NHL subjects
- Not all ADA responses in NHL subjects were neutralizing
- ADA responses in NHL subjects limit MT-3724 exposure

ADA responses to MT-3724 likely develop more rapidly in primates due to greater immune function, compared to delayed ADA responses observed in NHL Subjects

Figure 1. MT-3724 ADA Responses (All NHL Subjects)

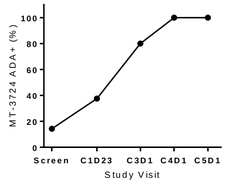


Figure 2. MT-3724 ADA titers in NHPs over time

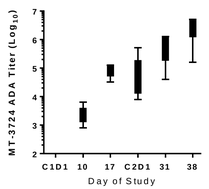


Table 1. Serum Cmax & ADA responses over time for MT-3724 NHL subjects completing five cycles

NHL Sub-Type	Best Response	MT-3724 mcg/kg/dose	Serum Cmax MT-3724 (ng/mL)							Anti-MT-3724 Antibody Response (Titer)					
DLBCL	PR	5	24	22	29	24	37	<LLOQ	<LLOQ	0	0	0	0	320	5,000
DLBCL	CR	20	87	N/D	56	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0	40	40	100	625,000	4,000
DLBCL	SD	75	579	630	510	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0	2,000	1,000	8,000	8,000	8,000
FL	SD	10	55	34	92	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0	1,000	1,000	25,000	125,000	125,000
FL	SD	10	47	34	20	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0	1,000	1,000	125,000	128,000	256,000
Study Visit			C1D1	C1D5	C1D12	C2D1	C3D1	C4D1	C5D1	Screen	C1D23	C2D1	C3D1	C4D1	C5D1

Neutralizing antibody response detected by cellular cytotoxicity assay

Sirolimus reduced MT-3724 anti-drug antibody response in mice

Optimal oral sirolimus treatment delays onset and reduces MT-3724 IgG ADA response in mice

- Optimal reduction of ADA response after Cycle 1 and 2 when sirolimus loading and maintenance dose provided during Cycle 1 (Table 3, Group 3)
- No additional reduction of ADA response when sirolimus also provided during Cycle 2 (Table 3, Group 4)
- Sirolimus treatment without loading dose reduces ADA response after 1st MT-3724 Cycle, but not Cycle 2 (Group 2)
- Sirolimus administration during first three MT-3724 doses provides minimal reduction of ADA response (Fig. 3, Day 17)

Sirolimus treatment of mice during MT-3724 Cycle 1 or 1&2 numerically reduced ADA response after Cycle 1 (Day 17) with significant reduction observed after Cycle 2 (Day 38)

Figure 3. IgG ADA responses from MT-3724 treated mice

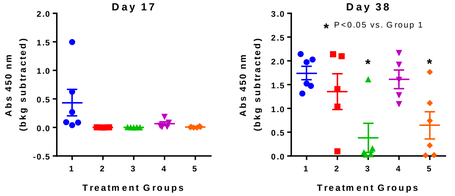


Table 2. BALB/c treatment groups

G #	Sirolimus Treatment Description	Sirolimus Loading 0.3 mg PO (days)	Sirolimus Maintenance 0.1 mg PO (days)	MT-3724 0.25 mg/kg IP (days)
1	Vehicle Control	--	--	3, 5, 7, 10, 12, 14, 24, 26, 28, 31, 33, 35
2	Cycle 1, no LD	--	1-14	
3	Cycle 1, w/ LD	1	2-14	
4	Week 1, w/ LD	1	2-7	
5	Cycle 1 & 2, w/ LD	1, 22	2-14, 23-35	

Table 3. IgG ADA Responses as % of Group 1

Group	Day of Study			
	17	24	31	38
1	100%	100%	100%	100%
2	0%	2%	135%	78%
3	0%	3%	23%	22%
4	15%	43%	166%	93%
5	2%	7%	11%	37%

Sirolimus reduced ADA responses in non-human primates and improved PK/PD profile

Oral sirolimus treatment reduced ADA responses and prolonged PK/PD response in primates

- Sirolimus significantly reduced ADA responses after three and six doses of MT-3724 (Fig. 4, Day 10 & 17, respectively)
- Measurable serum MT-3724 levels observed on Day 14 in 5/6 sirolimus treated animals compared to 0/6 in Group 1 (Fig. 5)
- Sirolimus treatment animals demonstrated extended PD response (Nadir Day 17 vs. Day 10) and greater decrease (-93% vs -79%) in CD20+hi B-cells compared to Group 1 (Fig. 6).
- Elevated body temperature observed in one Group 1 animal and five sirolimus treated animals; NSAID and antibiotics provided

Table 4. Primate treatment groups

G #	Sirolimus Loading 3 mg/kg PO (days)	Sirolimus Maintenance 1 mg/kg PO (days)	MT-3724 0.075 mg/kg IV (days)
1	--	--	3, 5, 7, 10, 12, 14, 24, 26, 28, 31, 33, 35
2	1	2-14	

Figure 4. ADA responses in non-human primates measured by MSD bridging assay

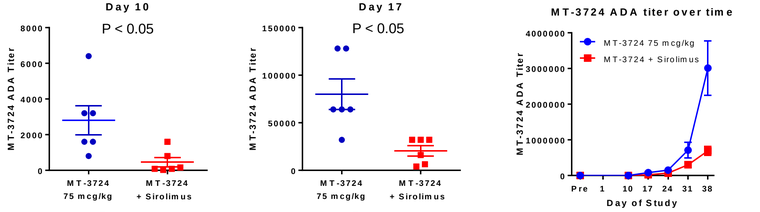


Figure 5. MT-3724 Cmax over time

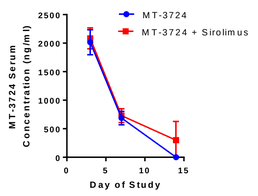
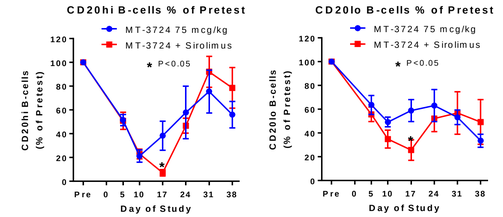


Figure 6. Peripheral B-cell levels in non-human primates



Conclusions

- In primates, sirolimus reduced ADA levels enabling longer serum exposure and prolonged PD response of MT-3724
- The combination of sirolimus with MT-3724 is an attractive and feasible regimen which could be further explored in clinical studies

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