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INTRODUCTION

MEDI-538 (MT103/blinatumomab) is a bispecific single-chain, T cell-engaging (BiTE) antibody that binds CD19+ human B lymphoma and normal B cells, and recruits CD3+ human T cells. Transient linkage of B and T cells by MEDI-538 mediates T cell lysis of B cell targets without an apparent need for costimulation and induces T cells to proliferate, secrete cytokines, and upregulate surface activation markers. CD19 is expressed through most stages of B cell development; pro-B cells up to the plasma cell stage, then down-regulate CD19 as they reach their end stage of plasma cell differentiation. In ongoing Phase I clinical trials, MEDI-538 administered by continuous intravenous (IV) infusions induces objective clinical responses and clears bone marrow infiltration in heavily pre-treated, CD19+ B cell, non-Hodgkin's lymphoma patients.

OBJECTIVE

In an effort to explore alternative routes of administration, we utilized preclinical models to assess the serum bioavailability, pharmacodynamic effects, and anti-tumor potency of MEDI-538 following subcutaneous (SC) administration.

RESULTS

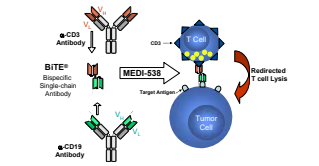
Pharmacokinetics: Serum bioavailability of MEDI-538 was determined following a single IV or SC bolus injection to cynomolgus monkeys and mice. These animals represent pharmacologically non-relevant species models for evaluating off-target activity of MEDI-538 based on its lack of specific binding to B and T cells in these species. A highly sensitive pharmacokinetic (PK) ELISA assay based on electroimmunofluorescence was utilized to measure the amount of MEDI-538 present in each serum sample. Following SC delivery in mice, MEDI-538 concentrations reached a peak at 4 hours, exhibited dose-proportionality, and was cleared from the blood at a similar rate as compared to IV administration of the drug ($t_{1/2}$ of 4-6 hours). Dose optimization studies showed that varying the dose concentration or volume for a given dose level did not effect the concentration of MEDI-538 in the serum after SC administration. Serum bioavailability of MEDI-538 delivered via a single SC dose in the mouse was 20-30% based on the mean area under the curve (AUC). In similar studies, PK parameters and serum bioavailability in cynomolgus monkeys was comparable to that measured in mice.

Efficacy: MEDI-538 also provided anti-tumor efficacy following SC administration in NOD/SCID mice that were engrafted with human B lymphoma cells mixed with human PBMC and that were capable of demonstrating human T cell-mediated killing of tumor cells. Pharmacodynamic effects were observed following SC administration of a hybrid mouse surrogate form of MEDI-538 (anti-human CD19 x anti-mouse CD3; hys103) to immunocompetent human CD19 transgenic knock-in mice. Hys103 recruits murine T cells for redirected lysis of human CD19 expressing mouse B cells. Treatment caused a reduction in B cells and an increase in activated T cells in the spleen as measured by flow cytometry.

CONCLUSIONS

These results demonstrated that SC delivery of MEDI-538 (1) yielded comparable PK characteristics in multiple animal models, (2) provided sufficient systemic levels for efficacy against tumor challenge in xenograft mouse models, and (3) depleted B cells in an immunocompetent mouse model. Taken together, our data suggest that the SC route of administration may be feasible as an alternative method for delivery of MEDI-538.

MEDI-538 MECHANISM OF ACTION



PHARMACOKINETIC PARAMETERS

In CD-1 mice, SC administered MEDI-538 was 20-30% bioavailable and exhibited serum concentrations that were dose-proportional, reached a peak at 4 hrs, and had a similar terminal half-life as IV administered drug

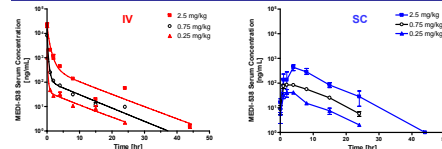


Figure 1. The mean MEDI-538 concentration versus time profile of serum samples collected from CD-1 mice after IV and SC administration of 0.5 mg/kg (0.75 circles), 2.5 (squares) mg/kg of MEDI-538. After injection of the indicated doses of MEDI-538, serum samples were collected at 5 min, 1, 2, 4, 8, 15, 24, and 44 hrs. Serum concentrations of MEDI-538 were measured by a specific ELISA method (LLOQ=0.2 ng/mL) and reported here as the arithmetic mean for each sample at each time interval after injection. A two-phase exponential decay, non-linear regression model was used for IV treated mice.

Table 1. Pharmacokinetic parameters of MEDI-538 in CD-1 mice

Route (Dose)	N	C _{max} (ng/mL)	T _{max} (hr)	t _{1/2} (hr)	AUC _{0-∞} (ng·hr/mL)	AUC ₀₋₂₄ (ng·hr/mL)	CL (mL/hr/kg)	Bioavailability
IV (0.25 mg/kg)	2	2,130	0.081	4.7	12,700	7,962	1,706	1.00
IV (0.75 mg/kg)	2	11,500	0.041	5.1	5,441	3,752	4,680	1.00
IV (2.5 mg/kg)	2	3,080	0.081	4.2	4,470	4,786	3,780	1.00
IV (2.5 mg/kg)	2	3,080	0.081	4.2	4,470	4,786	3,780	1.00
SC (0.25 mg/kg)	2	107	4	5.1	4,105	2,445	14,505	0.25
SC (0.75 mg/kg)	2	277	4	5.2	2,040	2,099	14,400	0.25
SC (2.5 mg/kg)	2	151	4	4.7	2,530	3,190	10,340	0.24
SC (2.5 mg/kg)	2	156	4	4.2	343	354	6,900	0.27

N listed for each dataset. C_{max}, peak serum concentration; T_{max}, time to peak serum concentration; t_{1/2}, half-life of serum concentration; AUC_{0-∞} are under the serum concentration-time curve up to the last measurable sampling time; AUC₀₋₂₄ area under the serum concentration-time curve to infinity; CL, total body plasma clearance estimated as Dose/AUC_{0-∞} after intravenous administration; Bioavailability, absolute bioavailability estimated as AUC_{0-∞}(SC)/AUC_{0-∞}(IV).

Following SC administration in cynomolgus monkeys, MEDI-538 was 22% bioavailable and exhibited serum concentrations that reached a peak at 4 hrs, and had a similar terminal half-life as IV administered drug

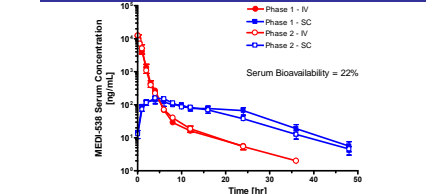


Figure 2. The mean MEDI-538 concentration versus time profile of serum samples collected from cynomolgus monkeys after IV and SC administration of 0.5 mg/kg of MEDI-538. A cross-over design with a one week interval was utilized. Phase 1 - IV then SC, Phase 2 - SC then IV. After injection of the indicated doses of MEDI-538, serum samples were collected over a one week period. Serum concentrations of MEDI-538 were measured by a specific ELISA method (LLOQ=0.2 ng/mL) and reported here as the arithmetic mean for each sample at each time interval after injection.

Table 2. Pharmacokinetic parameters of MEDI-538 in cynomolgus monkeys

Route	C _{max} (ng/mL)	T _{max} (hr)	t _{1/2} (hr)	AUC _{0-∞} (ng·hr/mL)	AUC ₀₋₂₄ (ng·hr/mL)	CL (mL/hr/kg)	Bioavailability
IV	12,400 (1,700)	0.1 (0.09)	4.3 (1.1)	12,700 (2,100)	12,800 (2,100)	40 (5.9)	1.00
SC	150 (25)	4.3 (0.8)	7.5 (1.3)	2,660 (220)	2,700 (230)	166 (17)	0.22 (0.051)

N=6 (3 M, 3 F). C_{max}, peak serum concentration; T_{max}, time to peak serum concentration; t_{1/2}, half-life of serum concentration; AUC_{0-∞} are under the serum concentration-time curve up to the last measurable sampling time; AUC₀₋₂₄ area under the serum concentration-time curve to infinity; CL, total body plasma clearance estimated as Dose/AUC_{0-∞} after intravenous administration; Bioavailability, absolute bioavailability estimated as AUC_{0-∞}(SC)/AUC_{0-∞}(IV).

IN VIVO ANTI-TUMOR EFFICACY

Two In Vivo Anti-Tumor Mouse Models		
Human tumor and T cells	SC model	IV model
MEDI-538 Administration	SC, opposite flank	SC
Endpoint	Tumor volume	Paralysis; survival
NOD/SCID		

SC treatment with MEDI-538 completely inhibits the growth of SC engrafted CD19+ Namalva B-lymphoma cells mixed with human CD3+ T cells in NOD/SCID mice

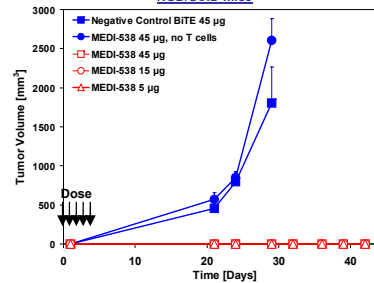


Figure 3. Cohorts of NOD/SCID mice were inoculated SC with 10⁶ Namalva cells in the absence (without T cells) or presence of 2x10⁶ human CD3+ T cells isolated from healthy donors. The daily doses of MEDI-538 as indicated or a negative control BITE were administered by intravenous injection via the tail vein on the indicated days following tumor cell engraftment. Mean values of tumor growth curves are shown. Error bars, standard error of the mean.

SC treatment with MEDI-538 prolongs the survival of NOD/SCID mice engrafted IV with CD19+ Ramos B Lymphoma cells and human CD3+ T cells

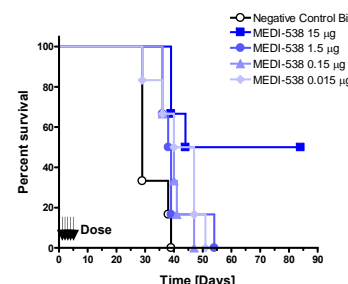


Figure 4. Cohorts of NOD/SCID mice were inoculated IV with 10⁶ Ramos cells mixed with 2x10⁶ human CD3+ T cells isolated from healthy donors. Five daily doses of MEDI-538 as indicated or a negative control BITE were administered by subcutaneous injection on the indicated days following tumor cell engraftment. The number of mice alive versus time after treatment is graphed. Animals were euthanized when overt signs of paralysis were observed.

PHARMACODYNAMIC EFFECTS

Pharmacodynamic effects were evaluated using a hybrid mouse surrogate form of MEDI-538 (anti-human CD19 x anti-mouse CD3; hys103) and immunocompetent human CD19 (huCD19) transgenic knock-in mice

huCD19 transgenic mice:

Established by Tom Tedder et al. (Zhou et al., Mol Cell Biol 1994; 14:3884-3894)

- Expression of huCD19 restricted to B cells
- Expression of huCD19 comparable to expression on normal human B cells
- Functional characteristics of huCD19 T cells comparable to function of T cells from the background mouse strain (C57BL/6) of mice

Hys103 mediates mouse T cell lysis of a B cell lymphoma line (NALM-6)

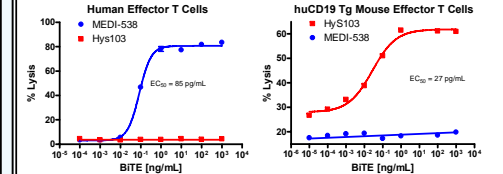


Figure 5. CD3+ human T cells or CD3+ mouse T cells from huCD19 transgenic mice (spleen) were combined with DIOC₁(3)-labeled human CD19+ NALM-6 target cells in the presence of the indicated concentrations of MEDI-538 or hys103. After 18 hrs for the human T cells and 42 hrs for the mouse T cells, specific cell lysis was determined by means of a flow cytometry-based assay. EIT ratio was 5:1. EC₅₀ values are indicated. Error bars, standard deviation of the mean.

Dose-dependent reduction of splenic and bone marrow B cells in huCD19 transgenic mice following SC treatment with a single dose of hys103

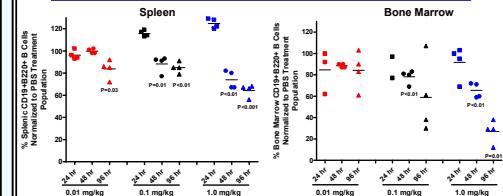


Figure 6. Four huCD19 transgenic mice were administered a single SC dose of hys103 at the indicated dose levels. Hys103-induced effects on the spleen and bone marrow CD19+ B cell populations were monitored by flow cytometry analysis at the indicated times following treatment, and reported as the percentage of B cells following treatment normalized to the number of B cells present in PBS treated mice. Bars, arithmetic mean of replicates; P values, significant difference between experimental and vehicle control as determined by T-test.

Dose-dependent activation (CD69) of splenic and bone marrow T cells in huCD19 transgenic mice following a single SC administration of hys103

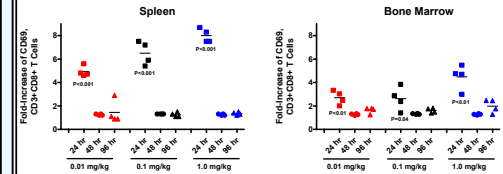


Figure 7. Four huCD19 transgenic mice were administered a single SC dose of hys103 at the indicated dose levels. Hys103-induced effects on the spleen and bone marrow CD3+CD8+ T cell populations were monitored by flow cytometry analysis at the indicated times following treatment, and reported as the fold increase of the mean fluorescence intensity (MFI) of CD69 following hys103 treatment as compared to the MFI of CD69 on T cells. PBS treated mice evaluated at the same time interval. Similar levels of CD69 activation were observed with CD3+CD4+ T cells. Bars, arithmetic mean of replicates; P values, significant difference between experimental and vehicle control as determined by T-test.

ACKNOWLEDGEMENT

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