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MT-5111: A Novel HER2-Targeting Engineered Toxin Body in Clinical Development

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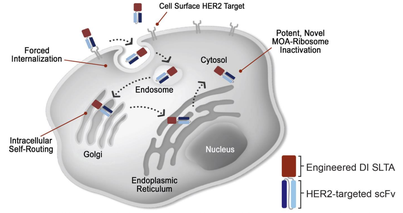
BACKGROUND

- Engineered toxin bodies (ETBs) are comprised of a proprietarily engineered form of Shiga-like toxin subunit A (SLTA) genetically fused to antibody-like binding domains
- ETBs work through novel mechanisms of action and are capable of forcing receptor internalization, self-routing through intracellular compartments to the cytosol, and inducing potent cell kill via the enzymatic and permanent inactivation of ribosomes (**Figure 1**)
- MT-5111 is a de-immunized (DI) ETB targeting human epidermal growth factor receptor 2 (HER2) for solid tumors
  - MT-5111 works through a novel mechanism of direct cell kill via SLTA-mediated enzymatic ribosome inactivation, and may not be subject to resistance mechanisms that exist for tyrosine kinase inhibitors, antibody-drug conjugates, or antibody modalities
  - MT-5111 binds an epitope on HER2, distinct from trastuzumab or pertuzumab, that may provide for combination potential with other HER2-targeting agents
  - MT-5111 is a 55-kilodalton protein and may have improved tumor penetration capability in solid tumor settings
  - MT-5111 has reduced antidrug antibody development and improved tolerability in mice relative to a control ETB without the de-immunization mutations in the SLTA domain

Figure 1. MT-5111 Mechanism of Action



DI SLTA = de-immunized Shiga-like toxin subunit A; HER2 = human epidermal growth factor receptor 2; MOA = mechanism of action; scFv = single-chain variable fragment.

MT-5111 Demonstrates Activity on Gastric Cancer Cell Lines That Express HER2

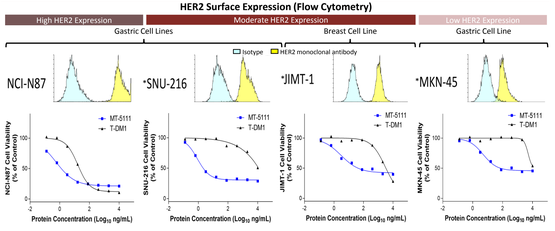
- Cell surface HER2 expression was evaluated by flow cytometry in a cell line panel that included 47 different cell lines and was reported as HER2-specific monoclonal antibody/isotype control signal (S/I). The cytotoxicity of MT-5111 and ado-trastuzumab emtansine (T-DM1) after 96 hours of treatment was also tested in this cell line panel by CellTiter-Glo® (Promega)
- A total of 7 gastric cell lines were included in the 47-cell line panel
  - NCI-N87 had high HER2 expression (S/I≥100)
  - SNU-216 had moderate HER2 expression (S/I>10 and <100)
  - MKN-45, MKN-1, SNU-1, SCH, and Hs 746T had low/negative HER2 expression (S/I≤10) (**Table 1**)
- Of 9 cell lines with moderate to high HER2 expression, of which two were gastric (NCI-N87, SNU-216), MT-5111 effectively killed 8 of the 9: half maximal inhibitory concentrations (IC_{50}s) were ~1 to 3 ng/mL (**Table 1**)
  - The activity of MT-5111 was similar or better than T-DM1 in MT-5111-sensitive cell lines
  - No cytotoxicity was observed on multiple HER2-negative cell lines
- T-DM1-resistant cell lines with moderate surface HER2 expression (SNU-216 gastric cancer, JIMT-1 breast cancer) were sensitive to MT-5111, but were not effectively killed by T-DM1 (**Figure 2**)
  - As a protein, MT-5111 is not expected to be a substrate of drug efflux transporters such as MDR1, which has been demonstrated to be one of the primary mechanisms of resistance to T-DM1
- Of gastric cell lines with low HER2 surface expression, MT-5111 showed dose-dependent activity in 3 (MKN-45, Hs746T, and SCH) of 5 cell lines (**Table 1**)

Table 1. Cytotoxic Activity on Select Cancer Cell Lines

Indication	Cell Line	IC_{50} (ng/mL) MT-5111	IC_{50} (ng/mL) T-DM1	HER2 S/I Ratio
Stomach	NCI-N87	0.6	17.0	264.2
Stomach	SNU-216	0.9	>10,000.0	24.8
Stomach	MKN-45	5.0	5,100.0	7.5
Stomach	Hs746T	13.0	2,300.0	2.0
Stomach	SCH	66.0	2,300.0	2.5
Stomach	MKN-1	>10,000.0	>10,000.0	5.6
Stomach	SNU-1	>10,000.0	>10,000.0	4.7
Ovary	SK-OV3	0.4	7.1	163.9
Breast	HCC1569	0.3	39.0	138.3
Breast	HCC202	0.8	7.1	95.5
Breast	EFM-192A	2.0	8.0	220.6
Breast	SK.BR-3	2.6	3.4	162.0
Breast	JIMT-1	3.1	3,000.0	25.3
Breast	MDA-MB-453	>10,000.0	170.0	34.9
Breast	MDA-MB-468	>10,000.0	2,800.0	1.2
Breast	BT-20	>10,000.0	>10,000.0	1.0

HER2 = human epidermal growth factor receptor 2; IC_{50} = half maximal inhibitory concentration; I = isotype; S = specific; T-DM1 = ado-trastuzumab emtansine.

Figure 2. Cell Kill Comparison of MT-5111 to T-DM1 in a High HER2 Expression Gastric Cancer Cell Line (NCI-N87), 2 Moderate HER2 Expression and T-DM1 Resistant Gastric and Breast Cancer Cell Lines (SNU-216, JIMT-1), and a Low HER2 Expression and T-DM1 Resistant Gastric Cancer Cell Line (MKN-45)

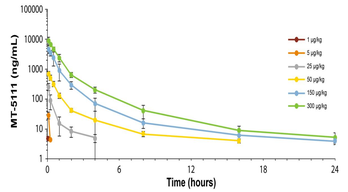


HER2 = human epidermal growth factor receptor 2; T-DM1 = ado-trastuzumab emtansine.
*T-DM1-resistant cell line.

First-in-Human Doses of MT-5111 Expected to Achieve MT-5111 Exposure Above That Needed for *In Vitro* Cellular Cytotoxicity of HER2-Positive Tumor Cells, as Indicated by Good Laboratory Practice (GLP) Studies in Non-Human Primates (NHP)

- The cynomolgus monkey is the pharmacologically relevant species and was used for the evaluation of toxicity of MT-5111 (**Table 3**)
  - MT-5111 was administered at a more frequent dosing schedule (3x/week) in the NHP toxicity study than the phase 1 study (weekly)
  - Dose-dependent toxicity observed in primates included:
    - Increased circulating troponin-I levels at ≥25 µg/kg; minimal at 25 µg/kg
    - Increased ECG findings (atrioventricular block) at ≥50 µg/kg
    - Increased myocardial degeneration/damage at ≥150 µg/kg
  - The highest non-severely toxic dose (HNSTD) was 5 µg/kg
- NHP GLP pharmacokinetics (PK) study results are provided in **Figure 5**
  - Less than dose-proportional PK observed at doses <150 µg/kg based on dose-normalized area under the curve and maximum concentration values
  - In NHP, the MT-5111 half-life was about 2 to 5 hours
- Simulated human PK
  - Simulations were based on the 25 µg/kg NHP PK data using the Dedrick model
  - This modelling suggests that MT-5111 can be administered at doses in humans above the IC_{50} required for HER2-specific cellular cytotoxicity *in vitro* (**Figure 6**)

Figure 5. NHP GLP PK Studies



Dose (µg/kg)	1	5	25	50	150	300
AUC_{last} (hr*ng/mL)	–	5	95	613	3,995	8,395
AUC/D	–	1	4	12	27	28
$T_{1/2}$ (hr)	–	–	2.4	3.5	4.7	3.1
C_{max} (ng/mL)	5	25	236	713	4,725	8,805
C_{max}/D	5	5	9	14	32	29

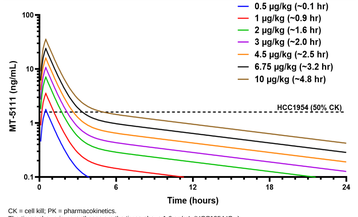
AUC = area under the curve; C_{max} = maximum concentration; D = dose; GLP = Good Laboratory Practice; NHP = non-human primates; PK = pharmacokinetics; $t_{1/2}$ = half-life.

Table 3. GLP Toxicology Study of MT-5111 in NHP

Group	Test Material	Dose (µg/kg)	Dosing Days (Route)	Number of Animals	
				Main	Recovery
1	Vehicle	0	1, 3, 5, 8, 10, 12 (IV)	3	2
2	MT-5111	1		3	2
3	MT-5111	5		3	2
4	MT-5111	25		3	2
5	MT-5111	50		3	2
6	MT-5111	150		3	2
7	MT-5111	300		3	2

GLP = Good Laboratory Practice; IV = intravenously; NHP = non-human primates.
Study design is a combination of 2 GLP studies in NHP.

Figure 6. Simulated Human PK

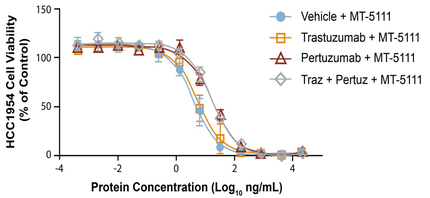


CK = cell kill; PK = pharmacokinetics.
The times shown in parentheses are the times above 1.6 ng/mL (HCC1954 IC_{50}).

MT-5111 Activity *In Vitro* is Retained in the Presence of Approved HER2-Targeted Antibodies

- HER2-positive HCC1954 cells, known to be resistant to trastuzumab, were pretreated with vehicle or one or two HER2-targeted monoclonal antibodies (100 µg/mL each) for 1 hour prior to addition of MT-5111. The cytotoxic activity of MT-5111 on these cells was measured by CellTiter-Glo® (Promega) 120 hours after protein addition
- MT-5111 demonstrated effective cell killing *in vitro* against trastuzumab-resistant HCC1954 cells (**Figure 3**)
- Regardless of the presence of either traztuzumab, pertuzumab, or both in combination, the cytotoxicity of MT-5111 on the HCC1954 cells was minimally affected (IC_{50} within 5x of control) (**Table 2**)

Figure 3 . Cytotoxicity of MT-5111 on HCC1954 Cells in the Presence of Excess Trastuzumab and Pertuzumab Pretreatment



Pertuz = pertuzumab; Traz = trastuzumab.

Table 2. MT-5111 IC_{50} in the Presence of Excess Trastuzumab and Pertuzumab

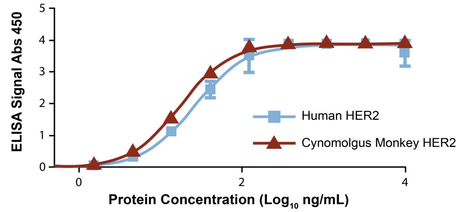
Pre-Treatment	MT-5111 IC_{50} (ng/mL)
Vehicle (no monoclonal antibody)	3.9
Trastuzumab 100 µg/mL	5.4
Pertuzumab 100 µg/mL	18
Trastuzumab 100 µg/mL + pertuzumab 100 µg/mL	16.4

IC_{50} = half maximal inhibitory concentration.

MT-5111 Binds to Human and Non-Human Primate HER2 Protein

- An enzyme-linked immunosorbent assay (ELISA) using HER2 protein from human and cynomolgus monkey sequences and an anti-toxin monoclonal antibody was used to determine the species cross-reactivity of MT-5111
- MT-5111 binds to cynomolgus monkey and human HER2 protein with similar affinity; thus, the cynomolgus monkey is a relevant model for MT-5111 toxicology studies (**Figure 4**)
  - MT-5111 K_d:
    - 18 ng/mL for cynomolgus monkey HER2 protein
    - 26 ng/mL for human HER2 protein

Figure 4. MT-5111 Binding to Human and Cynomolgus Monkey Recombinant HER2 Protein

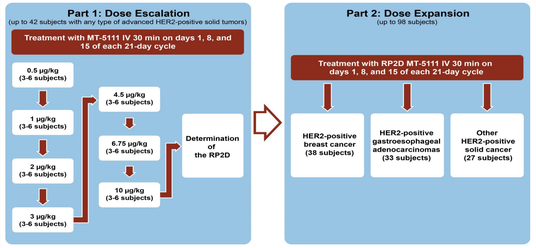


Abs = absorbance; ELISA = enzyme-linked immunosorbent assay; HER2 = human epidermal growth factor receptor 2.
The signal was normalized to the signal in the background control samples.

An MT-5111 Phase 1 Study (NCT04029922) Is Enrolling Subjects

- A first-in-human, phase 1, open-label dose-escalation and dose-expansion study of MT-5111 is enrolling subjects (**Figure 7**)
  - Key inclusion criteria consist of subjects ≥18 years old with a histologically confirmed, unresectable, locally advanced or metastatic solid cancer, including breast cancer, gastric or gastroesophageal adenocarcinomas, and other solid cancer that is HER2-positive and the malignancy is relapsed, refractory to, or intolerant of existing therapy(ies)

Figure 7. Phase 1 Study Design



IV = intravenously; RP2D = recommended phase 2 dose.

CONCLUSIONS

- MT-5111 is a novel HER2-targeted therapy with potential to be used in the treatment of HER2-positive cancers, including breast, gastric, and other solid cancers
- MT-5111 comprises a novel mechanism of action that could potentially overcome mechanisms of tumor resistance to currently available HER2-targeted therapies
  - With a protein cytotoxic payload, MT-5111 is not predicted to be a substrate of drug efflux transporters that have been reported to limit efficacy of antibody-drug conjugates
- Toxicology studies in the primate model, using a 3x more frequent dosing schedule than the phase 1 study, identified the HNSTD for MT-5111 was 5 µg/kg
  - MT-5111 binds to cynomolgus HER2, thus this is a pharmacodynamically relevant model
  - Pharmacodynamic effects on cardiac function are noted at the higher doses tested, consistent with a HER2-mediated effect
- Modelling suggests that MT-5111 will be administered at doses in humans above the IC_{50} required for HER2-specific cellular cytotoxicity *in vitro*
- Dosing has initiated in a phase 1, first-in-human, open-label study with MT-5111 (NCT04029922)
  - Subjects with HER2-positive solid tumors, including gastric malignancies, whose disease has progressed after treatment with other approved therapies are eligible to enroll

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Disclosures

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