P1-18-35

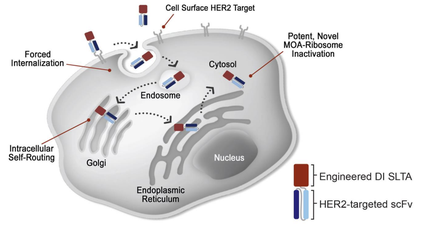
MT-5111: A Novel HER2-Targeting Engineered Toxin Body Under Clinical Development to Overcome Mechanisms of Resistance to Existing HER2-Targeted Therapies

Jack P. Higgins, PhD[1], Asis Sarkar, PhD[1], Eric T. Williams, PhD[1], Aimee Iberg, PhD[1], Roger Waltzman, MD, MBA[1], Erin K. Willert, PhD[1] | [1]Molecular Templates, Austin, TX, USA.

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 the solid tumor settings
  - MT-5111 has reduced antidrug antibody and improved tolerability in mice relative to a control ETB without the deimmunization mutations in the SLTA domain

Figure 1. MT-5111 Mechanism of Action

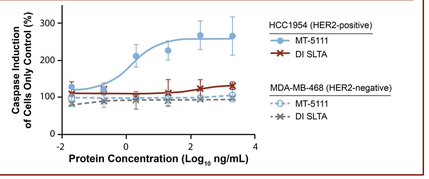


DI SLTA = de-immunized Shiga-like toxin A; HER2 = human epidermal growth factor receptor 2; MOA = mechanism of action; scFv = single-chain variable fragment.

MT-5111 Activates Caspase Activity on Target Cells, Consistent with Delivery of the SLTA Subunit

- Shiga-like toxin is known to induce apoptotic cell death through activation of caspases
- Caspase activity was measured after 20 hours after addition of MT-5111 with the Caspase 3/7-Glo® (Promega) method
- MT-5111 induces caspase activation when incubated with HER2-positive HCC1954 cells (**Figure 2**)
  - Caspase activation by MT-5111 was not observed on target negative MDA-MB-468 cells
  - Caspase activation by the DI SLTA subunit alone was not observed on either cell line

Figure 2. Caspase 3/7 Activity of MT-5111



MT-5111 Has Potent and Specific Activity on HER2-Positive Cell Lines

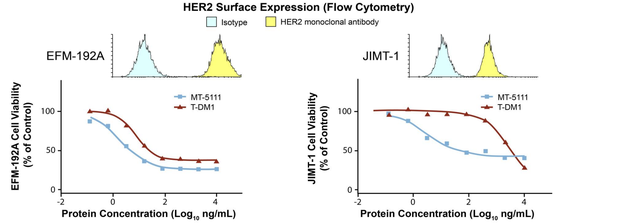
- A cell line panel consisting of 47 distinct cell lines was evaluated for HER2 surface expression by flow cytometry and reported as HER2-specific monoclonal antibody/isotype control signal (S/I). The same panel was tested for cytotoxicity activity by Cell Titer-Glo® (Promega) of HER2-targeted agents, MT-5111 and ado-trastuzumab emtansine (T-DM1) with a viability measurement 96 hours after protein addition
  - From the 47 cell lines tested, HER2 surface expression was high (S/I≥100) in 5 cell lines, moderate (SI>10 and <100) in 4 and low/negative (S/I≤10) in the remaining cell lines
  - MT-5111 demonstrated potent cytotoxic activity in 8 of 9 cell lines with moderate to high HER2 expression (half maximal inhibitory concentration [IC_{50}] of ~1-3 ng/mL) (**Table 1**)
    - In cell lines sensitive to MT-5111, the activity is similar or better than T-DM1
    - In HER2-positive cell lines, with the exception of the MDA-MB-453 cell line, the IC_{50} was >500-fold lower as compared to HER2-negative cell lines
  - The T-DM1-resistant cell line with moderate cell surface HER2 expression (JIMT-1 breast cancer) was sensitive to MT-5111, but was not effectively killed by T-DM1 (**Figure 3**)

Table 1. Cytotoxic Activity on Select Cancer Cell Lines

Indication	Cell Line	IC_{50} (ng/mL) T-DM1	IC_{50} (ng/mL) MT-5111	HER2 Spec/Iso Ratio
Stomach	NCI-N87	17.0	0.6	264.2
Breast	EFM-192A	8.0	2.0	220.6
Ovary	SK-OV3	7.1	0.4	163.9
Breast	SK.BR-3	3.4	2.6	162.0
Breast	HCC1569	39.0	0.3	138.3
Breast	HCC202	7.1	0.8	95.5
Breast	MDA-MB-453	170.0	>10,000.0	34.9
Breast	JIMT-1	3,000.0	3.1	25.3
Stomach	SNU-216	>10,000.0	0.9	24.8
Breast	MCF-7	5,000.0	>10,000.0	4.2
Breast	MDA MB 231	>10,000.0	>10,000.0	3.2
Breast	MDA MB 468	2,800.0	>10,000.0	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; iso = isotype; spec = specific; T-DM1 = ado-trastuzumab emtansine.

Figure 3. Cell Kill Comparison of MT-5111 to T-DMI in a High HER2 Expression Breast Cancer Cell Line (EFM-192A) and a Moderate HER2 Expression and T-DMI-Resistant Breast Cancer Cell Line (JIMT-1)

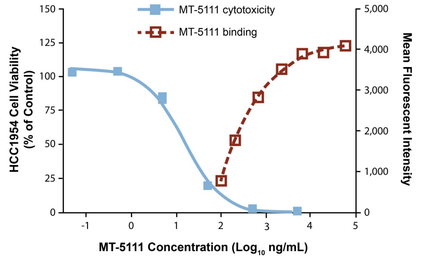


HER2 = human epidermal growth factor receptor 2; T-DM1 = ado-trastuzumab emtansine.

MT-5111 Kills HER2-Positive HCC1954 Cells at Concentrations Below Levels of MT-5111 Needed for Receptor Saturation

- Binding of MT-5111 (1 hour, on ice) to HER2-positive HCC1954 cells was measured with a flow-based assay using a labeled anti-toxin monoclonal antibody for detection and reported as mean fluorescence intensity as a function of protein concentration
  - Saturation of MT-5111 binding to HER2 on the cell surface was observed at concentrations >6,700 ng/mL (**Figure 4, right axis**)
- Cytotoxicity of MT-5111 was measured 96 hours after addition to a high-density assay format of HCC1954 cells using Cell Titer-Glo® (Promega)
  - MT-5111 kills target cells in this assay format with an IC_{50} of 15 ng/mL, with 80% killing observed at 50 ng/mL (**Figure 4, left axis**)

Figure 4. Binding and Cytotoxicity of MT-5111 to HER2-Positive HCC1954 Cells

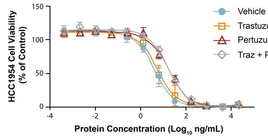


HER2 = human epidermal growth factor receptor 2.

MT-5111 Effectively Kills Trastuzumab-Resistant Cells and Demonstrates Cytotoxicity in the Presence of Approved HER2-Targeted Antibodies *In Vitro*

- HER2-positive HCC1954 cells are reported to be insensitive to trastuzumab. HCC1954 cells (low density format) were either pre-treated 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 Cell Titer-Glo® (Promega) 120 hours after protein addition.
  - MT-5111 has potent activity on the HCC1954 cells (**Figure 5** and **Table 2**)
  - Cytotoxicity of MT-5111 on HCC1954 cells is minimially affected (IC_{50} within 5x of control) in the presence of either traztuzumab, pertuzumab, or in the presence of both in combination (**Figure 5** and **Table 2**)

Figure 5. MT-5111 Cytotoxicity on HCC1954 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

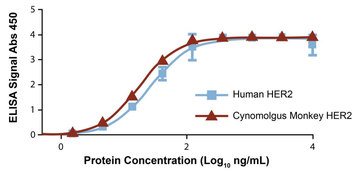
Pre-Treatment	MT-5111 IC_{50} (ng/mL)
Vehicle (no monoclonal antibody)	3.9
Trastuzumab 100 µg/mL	5.4
Pertuzumab 100 µg/mL	18
Trastuzumab 100 µg/mL + pertuzumab 100 µg/mL	16.4

IC_{50} = half maximal inhibitory concentration.

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

- The affinity of MT-5111 was measured by enzyme-linked immunosorbent assay (ELISA) using recombinant extracellular domain proteins and was similar for human and cynomolgus monkey HER2
  - K_d: 26 ng/mL for human HER2 and 18 ng/mL for cynomolgus monkey HER2 (**Figure 6**)
- The cynomolgus monkey is a relevant model for MT-5111 toxicology studies

Figure 6. Binding of MT-5111 to Human and Cynomolgus Monkey Recombinant 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.

MT-5111 Good Laboratory Practice Studies in Non-Human Primates Indicate Planned First-in-Human Doses Will Achieve MT-5111 Exposure Above Levels Needed for *In Vitro* Cellular Cytotoxicity of HER2-Positive Tumor Cells

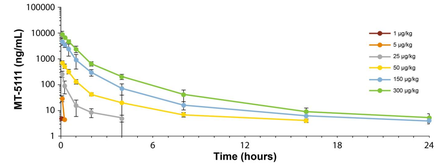
- Good Laboratory Practice (GLP) toxicology studies of MT-5111 in non-human primates (NHP) is outlined in **Table 3**
- Pharmacokinetic (PK) data was measured after the first intravenous dose using a Meso Scale Discovery-based assay and is shown in **Figure 7**
  - Based on dose-normalized area under the curve and maximum concentration values, less than dose-proportional PK observed at doses <150 µg/kg
  - The MT-5111 half-life in NHP was approximately 2 to 5 hrs
- The simulated human PK using the Dedrick model is shown in **Figure 8**
  - Post-infusion time above 1.6 ng/mL (mean IC_{50} on HCC1954 cells from multiple experiments) was calculated to be 0.1 to 4.8 hours
    - ~0.1 hours (0.5 µg/kg), ~0.9 hours (1 µg/kg), ~1.6 hours (2 µg/kg), ~2 hours (3 µg/kg), ~2.5 hours (4.5 µg/kg), ~3.2 hours (6.75 µg/kg), ~4.8 hours (10 µg/kg)
  - This modeling suggests that MT-5111 can be administered at doses in humans above the IC_{50} required for HER2-specific cellular cytotoxicity *in vitro*

Table 3. MT-5111 GLP Toxicology Study in NHP

Group	Test Material	Dose (µg/kg)	Dosing Days (Route)	Number of Animals: Main	Number of Animals: 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 = nonhuman primates. Study design is a combination of 2 GLP studies in NHP.

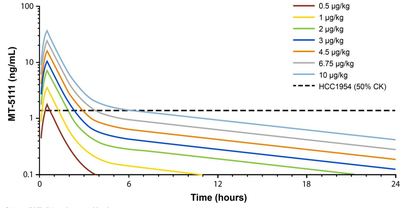
Figure 7. PK from NHP GLP Studies



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 = nonhuman primates; PK = pharmacokinetics; $t_{1/2}$ = half-life.

Figure 8. Simulated Human PK

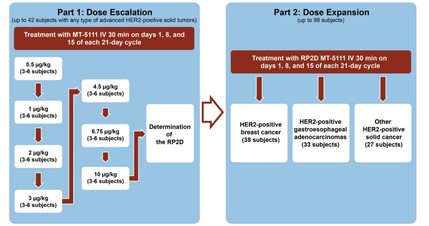


CK = cell kill; PK = pharmacokinetics.

A Phase 1 Open-Label Study of MT-5111 in HER2-Positive Solid Cancers, Including Breast Cancer or Gastric or Gastroesophageal Adenocarcinomas, Is Currently Enrolling Subjects (NCT04029922)

- Study design is shown in **Figure 9**
- Adults ≥18 years are eligible if they have a histologically confirmed, unresectable, locally advanced or metastatic solid cancer that is HER2-positive and the malignancy is relapsed, refractory to, or intolerant of existing therapy(ies)

Figure 9. Phase 1 Study Schema



IV = intravenously; RP2D = recommended phase 2 dose.

CONCLUSIONS

- MT-5111 represents a novel HER2-targeted therapy that could provide benefit in subjects with HER2-positive breast cancers
- MT-5111 has a novel mechanism of action that has the potential to overcome mechanisms of tumor resistance to existing HER2-targeted therapies, and binds to a distinct epitope on HER2, allowing for the possibility of combination with other HER2-targeted therapies
- 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 dose escalation and expansion study of MT-5111 (NCT04029922) in subjects with HER2-positive solid tumors whose disease has progressed after treatment with other approved therapies

Acknowledgments

The study was sponsored by Molecular Templates, Inc. Funding to support the development of this poster was provided by Molecular Templates to MedVal Scientific Information Services, LLC, Princeton, NJ.

Disclosures

JPH, AS, ETW, AI, RW, and **EKW** are Molecular Templates employees.

Presented at the San Antonio Breast Cancer Symposium, December 10-14, 2019, San Antonio, Texas, USA

The study was sponsored by Molecular Templates, Inc.