Engineered Toxin Bodies (ETBs) targeting Trop2

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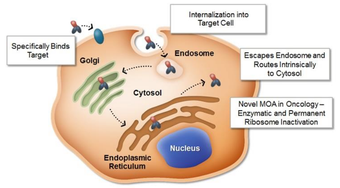
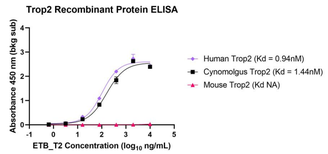
Background

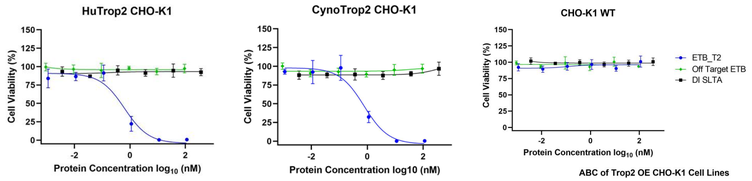
Engineered Toxin Bodies (ETBs) are comprised of a proprietarily engineered form of Shiga-like Toxin A subunit (DI SLTA) genetically fused to antibody-like binding domains. ETBs work through novel mechanisms of action and are capable of forced internalization, undergoing retrograde translocation to the cytosol, and inducing potent cell-kill via the enzymatic and permanent inactivation of ribosomes, resulting in the inhibition of protein synthesis and induction of apoptosis through ribotoxic stress mechanisms.



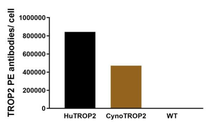
Trop2 ETB Mechanism of Action and Specificity

ETBs are being developed to target cell surface receptors expressed on solid tumors including tumor-associated calcium signal transducer 2 (Trop2). Trop2 is a clinically validated target of antibody drug conjugate (ADC) therapy in metastatic triple-negative breast cancer (mTNBC) and other cancers. Trop2 targeted ETBs retain catalytic activity of the engineered SLTA subunit and demonstrate potent specific and direct cell kill. Trop2 targeted ETBs are specific for Trop2 and capable of demonstrable cross-reactivity with human and cynomolgus Trop2 in both recombinant protein ELISA and concentration dependent cytotoxicity assays with overexpression (OE) CHO-K1 cell lines.

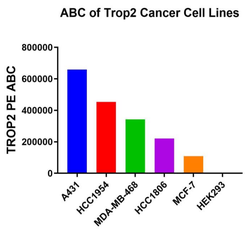


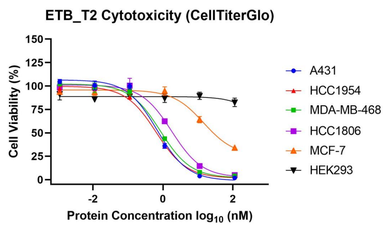


Cell Line	Overexpression	Trop2 Density (ABC)	IC50 (nM)
HuTrop2 CHO-K1	Human Trop2 (Accession NP 002344.2)	842736	0.64
CynoTrop2 CHO-K1	Cynomolgus Trop2 (Accession XP 005543292.1	471584	0.77
CHO-K1 WT	NA	0	NA



Cytotoxicity on Trop2 Positive Cancer Cell Lines



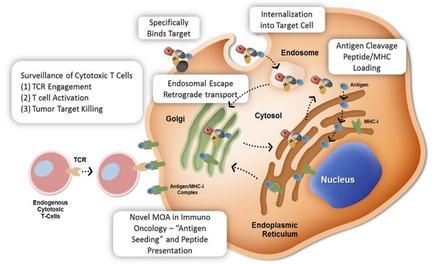


Cell Line	Disease	Trop2 Density (ABC)	IC50 (nM)
A431	epidermoid carcinoma	659513	0.69
HCC1954	breast; ductal carcinoma	452913	0.64
MDA-MB-468	breast; adenocarcinoma	343212	0.84
HCC1806	breast; acantholytic squamous cell carcinoma	220818	1.78
MCF-7	breast; adenocarcinoma	108185	14.52
HEK293	NA	0	NA

- Dose dependent cytotoxicity demonstrated on multiple clinically relevant tumor cell lines including triple negative breast cancer (TNBC) cell lines, MDA-MB-468 and HCC1806.

Antigen Seeding Technology (AST)

Antigen Seeding Technology (AST) incorporates the fusion of an immunodominant antigenic peptide to an ETB scaffold allowing for the delivery of intracellular peptide for subsequent MHC-I loading, surface presentation, and re-direction of an endogenous memory T cell response against the tumor. AST is a novel mechanism of action for re-directing immune responses to tumors. MTEM's first AST enabled ETB in clinical development, MT-6402, targets PD-L1+ tumors and delivers the HLA-A*02 restricted peptide from the CMV pp65 tegument protein. Additional MHC class I restricted antigens can be added to ETBs to match HLA-A haplotype (ex. HLA-A*01, HLA-A*24, etc) and broaden the patient population that could benefit from AST.

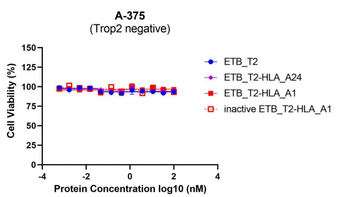


AST Enabled ETBs Retain Direct Cell Kill Potency

AST enabled Trop2 targeted ETBs retain potency on Trop2+ cells via internalization and inactivation of ribosomes (Direct Cell Kill). Site specific mutagenesis of known catalytic residues in the DI SLTA subunit inactivate direct cell kill in ETBs. Both active and inactive ETBs are capable delivering viral antigen and inducing cytokine secretion and T-cell mediated killing (AST) in a co-culture assay of Trop2 target cells with antigen matched HLA type and antigen specific cytotoxic T-cells (CTL).

			Activity on Trop2+ Cells	
Test Article	Target	HLA Target	Direct Cell Kill	AST
ETB_T2	Trop2	NA	YES	NO
ETB_T2-HLA_A24	Trop2	HLA-A*24	YES	YES
ETB_T2-HLA_A1	Trop2	HLA-A*01	YES	YES
inactive ETB_T2-HLA_A1	Trop2	HLA-A*01	NO	YES

Direct Cell Kill on Transgenic A-375 Cells expressing Human Trop2

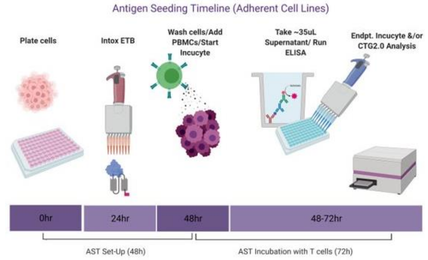


- No cytotoxicity observed on Trop2 negative cell line

Test Article	IC50 (nM)
ETB_T2	0.066
ETB_T2-HLA_A24	0.068
ETB_T2-HLA_A1	0.091
inactive ETB_T2-HLA_A1	NA

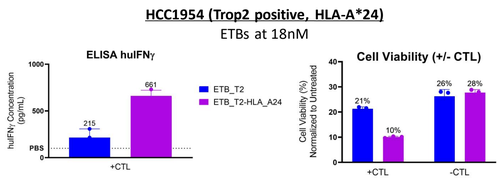
Trop2 Mediated Antigen Seeding Technology (AST)

The general workflow for performing AST assays involves treating and incubating target cells with test agent (ex. ETB or positive control peptide), co-culturing with antigen restricted T-cells isolated from HLA matched PBMCs, collection of supernatant for detection of human interferon gamma (huIFNγ) via ELISA (huIFNγ MAX ELISA, Biolegend) and cell viability assessment by ATP quantification (CTG2.0, Promega).



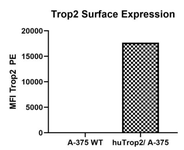
HLA-A*24 Trop2 AST Model with HCC1954 Cell Line

- **huIFNγ ELISA:** CTLs specific for HLA-A*24 antigen released huIFNγ at >3-fold higher levels (pg/mL) when exposed to HCC1954 cells previously treated with an ETB harboring an HLA-A*24 AST antigen (ETB_T2-HLA_A24) compared to an ETB without AST antigen (ETB_T2).
- **Cell Viability:** An additional 2-fold reduction in the viability of residual (direct cell kill surviving) HCC1954 cells was observed in samples previously exposed to ETB_T2-HLA_A24 compared to ETB_T2 and when co-cultured to HLA-A*24 restricted CTLs.
- **Conclusions:** These results support Trop2 mediated AST and delivery of HLA-A*24 antigenic peptides to Trop2 positive target cells, allowing antigen presentation and specific T-cell recognition.



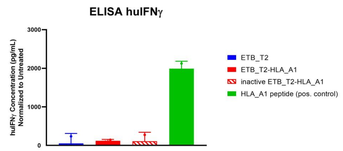
HLA-A*01 Trop2 AST Model with Transgenic A-375 Cell Line

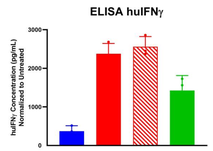
AST activity assays require HLA matched target positive cells and antigen specific CTLs derived from donor PBMCs. A-375 cells (malignant melanoma) are HLA-A*01 and readily accommodate transgenic engineering, presenting a useful system for preparing AST models. A-375 cells were transduced with human Trop2 lentivirus, and selected clones evaluated for surface expression (huTrop2/ A-375) .

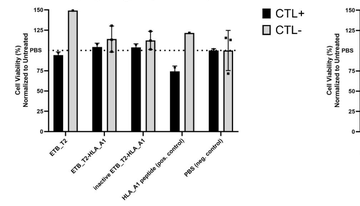


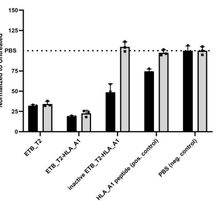
A-375
(Trop2 negative; HLA-A*01)
ETBs at 25nM; peptide at 250nM

huTrop2/ A-375
(Trop2 positive; HLA-A*01)
ETBs at 25nM; peptide at 250nM









- **huIFNγ ELISA:** CTLs specific for HLA-A*01 antigen released huIFNγ at >6-fold higher levels (pg/mL) when exposed to A-375/huTrop2 cells previously treated with an ETB harboring an HLA-A*01 AST antigen (ETB_T2-HLA_A1) or catalytically inactive version incapable of direct cell kill (inactive ETB_T2-HLA_A1) compared to an ETB without AST antigen (ETB_T2).
- **Cell Viability:** A >50% reduction in viability of A-375/huTrop2 cells was observed in samples previously exposed to inactive ETB_T2-HLA_A1 when co-cultured to HLA-A*01 restricted CTLs (CTL+) compared to without addition of CTLs (CTL-).
- **Conclusions:** These results support Trop2 mediated AST and delivery of HLA-A*01 antigenic peptides to Trop2 positive target cells, allowing antigen presentation and specific T-cell recognition.

Conclusions and Future Plans

- **Trop2 targeted ETBs show in vitro target specific picomolar potency on Trop2 positive tumor cell lines**
- **AST enabled Trop2 targeted ETBs retain direct cell kill potency and alter tumor immunophenotype to allow for antigen specific T-cell recognition**
- **Final lead selection based on additional targeting domains and AST antigens underway**

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