Engineered toxin bodies targeting PD-L1 to alter tumor immunophenotypes and deliver broad antigenic diversity and patient coverage

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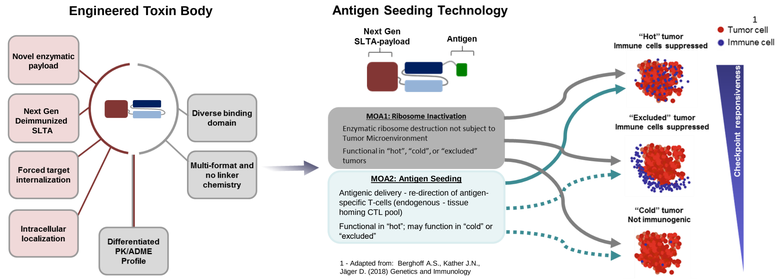
Abstract 1628
AACR 2021

MOLECULAR TEMPLATES

Targeting PD-L1 with ETBs to Overcome Checkpoint Resistance



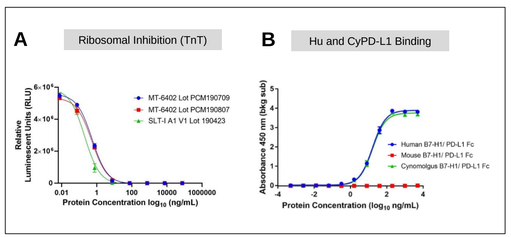
PD-L1 targeted ETBs provide distinct benefits for overcoming clinical challenges of checkpoint non-responsive tumors

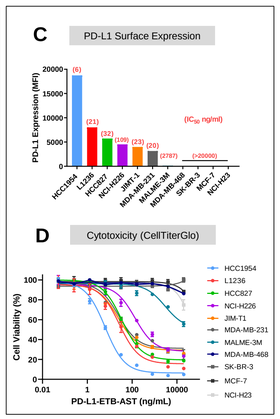
- Deliver irreversible ribosomal inhibition and deliver antigen payload from a single fused protein – target independent of immune environment
- Proprietary deimmunized Shiga Like Toxin A (SLTA) to reduce CLS – increased safety profile
- MOAs dependent on C_{MAX} not AUC – coupled to short $T_{1/2}$ -built for increased potency and limited toxicity
- Unique biology of ETBs with Antigen Seeding Technology allows for dual MOA

MT-6402 is a Potent PD-L1 Targeted ETB Entering the Clinic

MT-6402 - potent ribosomal inhibition, Human and Cynomolgus PD-L1 binding, Reduced Checkpoint Blockade

(A) MT-6402 inhibits ribosomes in a cell free assay (TnT) with similar IC_{50} to SLTA (DI SLTA-1)
(B) MT-6402 binds recombinant Human (Hu) and Cyno (Cy) PD-L1 protein (ELISA) with high affinity and similar IC_{50}





MT-6402 elicits potent cytotoxicity on PD-L1 positive and clinically relevant tumor cell lines

(C) mAb detection on Hi, Mid, and Lo PD-L1 expressing cell lines from clinically relevant indications

(D) Cytotoxicity assay (CellTiter-Glo) – MT-6402 can target Hi, Mid, and Lo clinically relevant PD-L1 expressing targets for cytotoxicity (CellTiter-Glo)

Functional MOA	MT-6402	ng/ml	
Scaffold	Deimmunized (DI) SLTA-1-scFv	N/A	
Reactivity	Human/Cyno (NHP)	N/A	
Ribosome Inhibition	(+)	0.52 (IC50)	
Target Binding (PD-L1)	(+)	Hu PD-L1 Kd	16.45
		Cy PD-L1 Kd	16.28
Cellular Cytotoxicity	(+)	HCC1954, L-1236*, HCC827, NCI-H226, JIMT-1*, MDA-MB-231, MALME-3M, MDA-MB-468, SK-BR-3, MCF-7, NCI-H23 *L-1236 and JIMT-1 were purchased from the DSMZ repository	5, 21, 32, 109, 23, 20, 2787, >10,000, >10,000, >10,000, >10,000 (IC50)

Delivering Antigen Seeding Technology to the Clinic

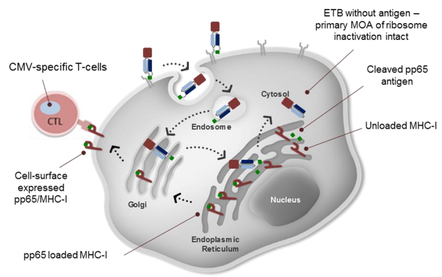
Antigen Seeding Technology (AST) – built within MT-6402 for seeding antigenic peptide and T cell response

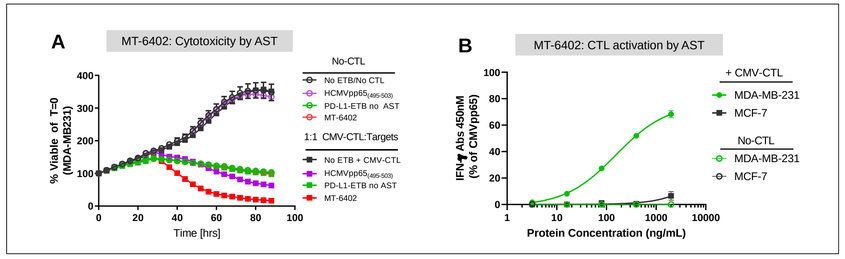
MT-6402 contains an HLA:A02 restricted antigen from Human Cytomegalovirus (HCMV). MT-6402 "seeds" CMV-restricted MHC-I peptide response for redirection of endogenous CTLs against tumor cells.

MT-6402 delivers peptide antigen for potent dual MOA cytotoxicity profile

(A,B) Co-culture model with PD-L1/HLA:A02 matched targets and CMV-restricted CTLs (1:1, E:T ratio)
(A) Dose dependent cytotoxicity is enhanced with dual MOA of MT-6402 as detected by enumeration of % viability (live cell imaging – Incucyte-S3)
(B) T cell activation profile; AST response is coupled to activation of CMV- restricted CTL response and dose-dependent release of IFN-γ

MT-6402-AST for Antigen Seeding Technology (AST)





MT-6402 derived ETBs for Broadening of the Patient Population

HLA: A02 is the most prevalent MHC Haplotype in the United States, covering about 1/3 of the population

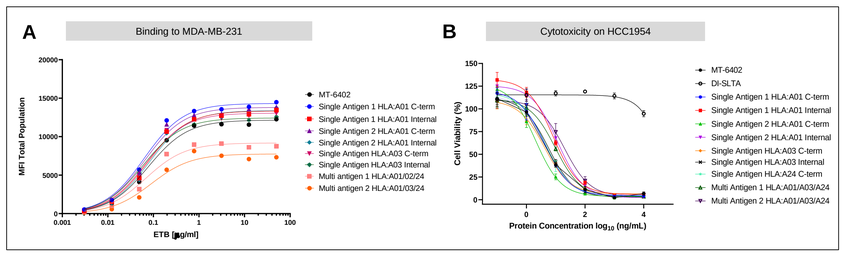
Single and multi-antigen ETB design

Single antigen
DI-SLTA αPD-L1 HLA:A01
DI-SLTA HLA:A01 αPD-L1

Multi-antigen
DI-SLTA αPD-L1 HLA01/03/24

ETBs with single or multiple antigens retain both binding and *in vitro* cell kill (MOA1 activity) compared to MT-6402

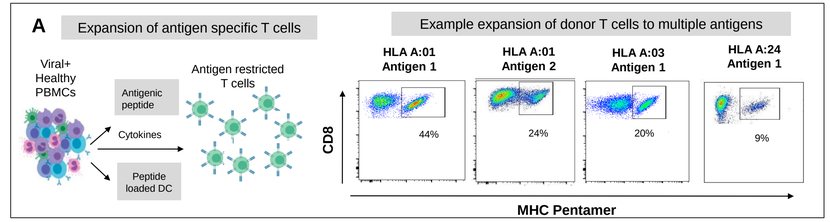
(A) Single and multi-antigen containing ETBs retain binding to PD-L1 target cells.
(B) Single and multi-antigen containing ETBs retain MOA1 activity compared to MT-6402 on human cell lines

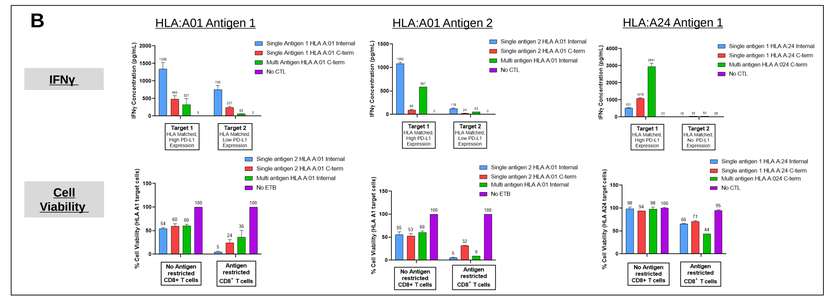


MT-6402 derived ETBs Demonstrate Dual MOA Activity

ETBs with single or multiple antigens retain AST activity *in vitro* (MOA2 activity)

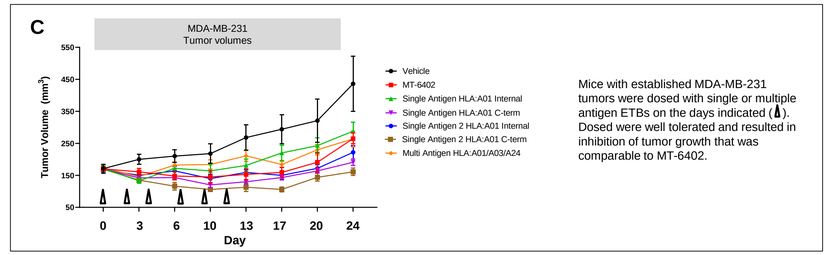
(A) CTLs that recognize antigens delivered by these ETB are prevalent in donor PBMCs and capable of being expanded.
(B) *In vitro* delivery of antigens to PD-L1 target cells activate antigen specific donor CTLs, resulting in IFNγ release and T-cell mediated cell kill.





ETBs with single or multiple antigens retain efficacy (MOA1 activity) compared to MT-6402

(C) *In vivo* efficacy prevents tumor outgrowth comparable to MT-6402 with each ETB design.



Mice with established MDA-MB-231 tumors were dosed with single or multiple antigen ETBs on the days indicated (▲). Dosed were well tolerated and resulted in inhibition of tumor growth that was comparable to MT-6402.

Conclusions

- MT-6402 is a PD-L1 targeted ETB with activity against cell lines from clinically relevant indications
  - Designed to deliver a unique and dual MOA approach for directly targeting PD-L1 expressing tumors
  - Does not require pre-existing or "hot" tumor microenvironment and may reactivate "cold" or "excluded"
  - IND filing has been accepted by the FDA with Phase I trials slated to begin in 2021
- MT-6402's Antigen Seeding Technology covers the largest MHC haplotype in the US.
  - Delivery of antigens that are restricted to additional MHC haplotypes could broaden the patient population that could benefit from AST.
  - ETBs based on MT-6402 that deliver additional antigens retain expected potency and target binding, while also activating donor CTLs with matched haplotypes.
  - ETBs delivering antigens to a broader population are under investigation for *in vivo* safety, efficacy and function.

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