Altering tumor immunophenotypes with PD-L1 engineered toxin bodies

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MOLECULAR TEMPLATES

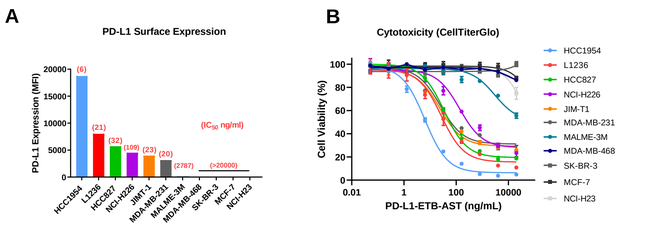
Targeting PD-L1 with dual MOA ETBs to Overcome Checkpoint Resistance

Direct cell kill by ribosomal destruction

MT-6402 elicits potent cytotoxicity on PD-L1 positive and clinically relevant tumor cell lines, independent of HLA-A serotype

(A) PD-L1 detection on High, Medium, and Low PD-L1 expressing cell lines from clinically relevant indications.

(B) Cytotoxicity assay (CellTiter-Glo®) – MT-6402 can target High, Medium, and Low (clinically relevant) PD-L1 expressing targets for cytotoxicity (CellTiter-Glo®)



*L-1236 and JIMT-1 were purchased from the DSMZ repository

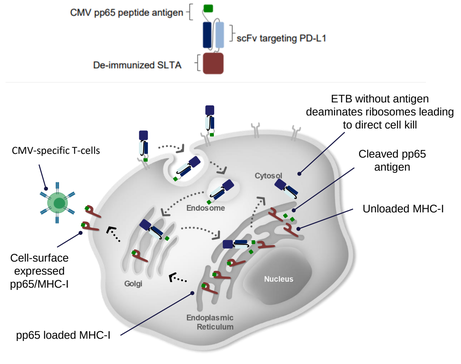
Altering the tumor immunophenotype to redirect CMV specific T cells

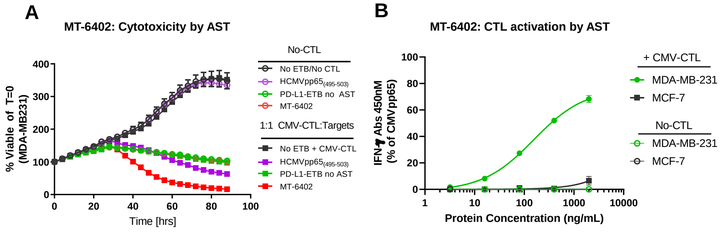
Antigen Seeding Technology (AST) – built within MT-6402 for seeding antigenic peptide and T cell response

MT-6402 contains an HLA-A*02 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 AST delivery by 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

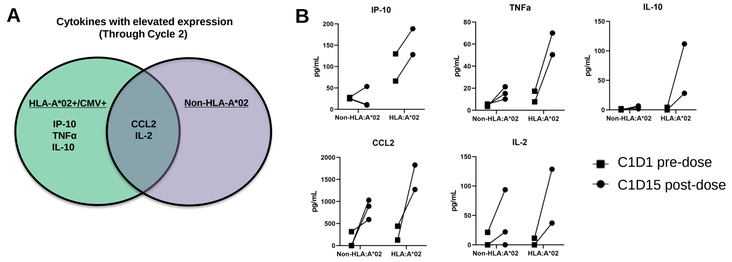




MT-6402 cytokine release in HLA-A matched vs HLA-A unmatched patients

Treatment for the first 5 patients that completed cycle 1 (one month) resulted in differential cytokine signatures for patients that could benefit from AST

(A) Venn diagram of overlapping cytokine release in HLA-A*02 and CMV+ versus Non-HLA-A*02 patients.
(B) Cytokines levels (pg/ml) present in the periphery in patient serum pre-dose (C1D1 pre-dose) vs cycle 1 day 15 post dose (C1D15 post-dose) separated by HLA:A*02 and non-HLA-A*02 serotype.

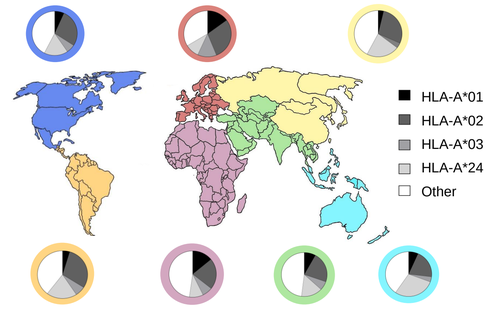


For more information on this trial (NCT04795713), see our AACR 2022 MT-6402.Abstract control # 7936, ID:CT152

MT-6402 derived ETBs for Broadening of the Patient population

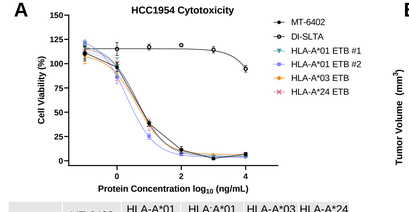
HLA-A*02 is the most prevalent MHC Haplotype in the United States, covering about 1/3 of the population

Additional MHC class I restricted antigens specific for HLA-A*01, HLA-A*03, and HLA-A*24 were selected from the 59 known HLA-A serotypes to broaden the patient population that could benefit from AST.
These selections will ensure coverage of majorities from every region indicated. The "other" fraction contains all remaining HLA-A serotypes present in each location. Graphs made from averaged country data in the Allele Frequency Net Database and are estimates from each indicated region (www.allelefrequencies.net)



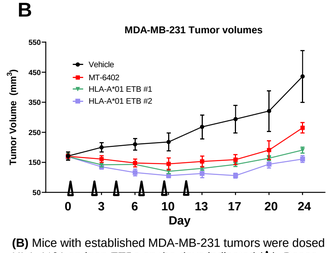
MT-6402 derived ETBs retain direct cell kill activity *in vivo*

ETBs with other antigens retain efficacy compared to MT-6402



	MT-6402	HLA-A*01 ETB #1	HLA:A*01 ETB #2	HLA:A*03 ETB	HLA-A*24 ETB
IC50 (ng/ml)	4.57	4.13	2.05	4.07	4.49

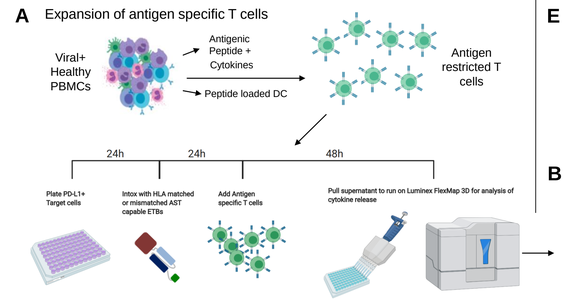
(A) *In vitro* direct cell kill potency is retained compared to MT-6402 on HCC1954 cells.

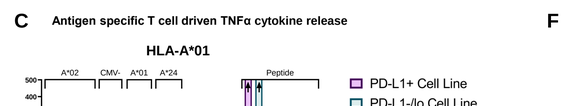


(B) Mice with established MDA-MB-231 tumors were dosed HLA-A*01 antigen ETBs on the days indicated (▲). Doses were well tolerated and resulted in inhibition of tumor growth that was comparable to MT-6402.

PD-L1 engineered toxin bodies alter immunophenotypes and induce predictable cytokine signatures

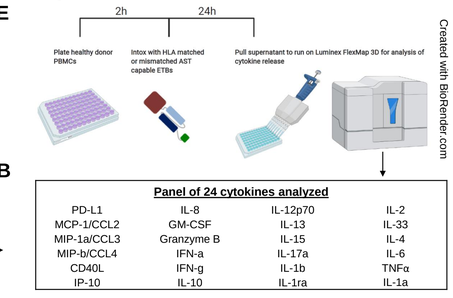
AST assay followed by Luminex cytokine analysis





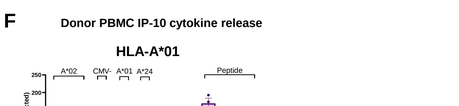
1. Enzymatically inactive MT-6402
2. MT-6402
3. MT-6402 without HLA-A*02 antigen
4. HLA-A*01 ETB
5. HLA-A*24 ETB
6. DI-SLTA only
7. PBS
8. HLA-A*01 restricted CMV antigen
9. HLA-A*02 restricted CMV antigen
10. HLA-A*24 restricted CMV antigen

Cytokine release assay after ETB intoxication of healthy PBMCs



Panel of 24 cytokines analyzed

PD-L1	IL-8	IL-12p70	IL-2
MCP-1/CCL2	GM-CSF	IL-13	IL-33
MIP-1a/CCL3	Granzyme B	IL-15	IL-4
MIP-b/CCL4	IFN-a	IL-17a	IL-6
CD40L	IFN-g	IL-1b	TNFα
IP-10	IL-10	IL-1ra	IL-1a



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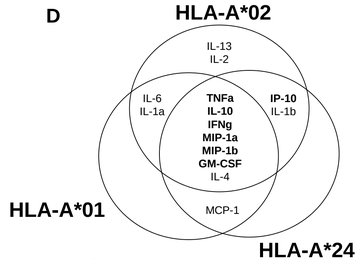

MT-6402 derived ETBs induce cytokine release in healthy PBMC donors AST assays independent of HLA type

(A) Workflow for AST assay timeline followed by **(B)** cytokine analysis
(C) Antigen specific T cell driven TNF**α** cytokine release from HLA-A*01 and HLA-A*02 co-culture assays 48h post intoxication with ETBs carrying matched or mismatched antigens. Purple bars are HLA matched PD-L1hi target cells. Blue bars are HLA matched PD-L1$^{-/lo}$ target cells.
(D) Venn diagram of overlapping cytokines released in HLA matched co-cultures for HLA-A*01, HLA-A*02, and HLA-A*24
(E) Workflow for PBMC cytokine release assay timeline and **(B)** cytokines analyzed
(F) IP-10 cytokine release from HLA-A*01 and HLA-A*02 donor PBMCs intoxicated with ETBs carrying matched or mismatched antigens.
(G) Cytokine summary table from the AST assay, the PBMC assay, and clinical data in an HLA matched setting, where Green boxes indicate cytokine release.

G	AST assay	PBMC assay	MT-6402 Trial
TNFα			
IL-10			
IP-10			
IFNγ			
MIP-1α			
MIP-1β			
GM-CSF			
IL-6			
IL-4			

Conclusions

- MT-6402 is a PD-L1 targeted ETB with activity against cell lines from clinically relevant indications
  - Designed to deliver a unique dual MOA approach for targeting PD-L1 expressing tumors for direct cell kill, or by altering their immunophenotype to redirect CMV reactive CTLs to tumors
  - MT-6402 is currently in the clinic in a phase I dose escalation study (NCT04795713) and has shown early PD effects (See AACR 2022 Abstract Control #7936, ID: CT152)
- MT-6402's Antigen Seeding Technology allows for HLA-A*02 restricted antigen delivery and CMV specific T cell activation.
  - Delivery of antigens restricted to additional MHC haplotypes will broaden the patient population that could benefit from AST.
  - Two different model *in vitro* cytokine release assays indicate overlapping cytokine signatures that are seen in the clinic, supporting translatability of targeting additional HLA subtypes.