In vivo efficacy of a PD-L1 targeted Engineered Toxin Body (ETB) comprised of direct cytotoxicity and T-cell mediated tumor targeting

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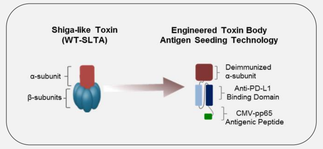
Engineered Toxin Bodies – Unique dual mechanism of action approach to target PD-L1 expressing solid tumors

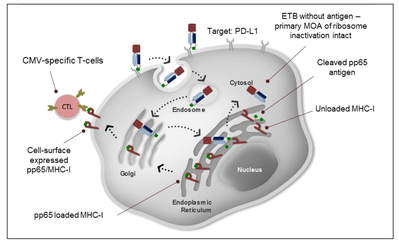
ETBs are comprised of a proprietarily engineered form of Shiga-like Toxin A subunit (SLT-A) genetically fused to an antibody-like binding domain to deliver potent cell-kill via the enzymatic and permanent inactivation of ribosomes to targeted tumor cells

MTEM's **PD-L1-ETB-AST**, targets PD-L1 with a unique and dual cytotoxic approach to maximize response against solid tumors

(i) Direct cell-kill via permanent inactivation of ribosomes

(ii) Delivery of antigenic peptide to re-direct endogenous CMV-specific T-cells to the tumor – Antigen seeding technology (AST)





PD-L1-ETB-AST is designed for PD-L1 expressing solid tumor indications

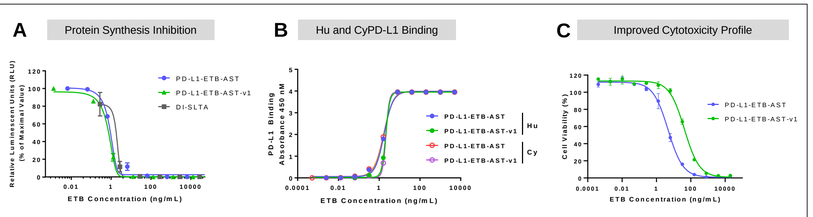
- Delivers potent and selective cytotoxicity to PD-L1 expressing tumors
- Limited targeting of peripheral blood mononuclear cell populations
- Recruitment of potent endogenous HCMV-specific CTL response
- Dual MOA allows for activity regardless of tumor immune status

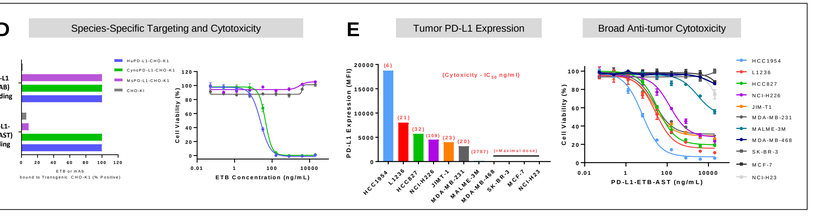
Optimized PD-L1 Targeting ETB delivers potent cytotoxicity to tumor target cells

PD-L1-ETB-AST: Optimized for specificity and maximal anti-tumor effect

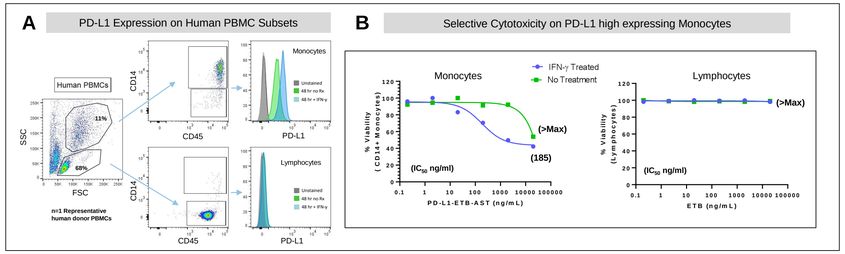
- (A) PD-L1-ETB-AST retains the potent protein synthesis inhibition of DI-SLTA
- (B) PD-L1-ETB-AST effectively binds Human (Hu) and Cyno (Cy) PD-L1 and drives potent-targeted cell kill against transgenic lines (D)
- (C) PD-L1-ETB-AST is optimized for cytotoxic potency vs previous versions (i.e. PD-L1 ETB-ASTv1)
- (D-F) PD-L1-ETB-AST is highly selective and targets clinically relevant tumor cells with a range of surface PD-L1 expression

Key Attributes	Profile	Potency (ng/ml)
Protein Synthesis Inhibition	(+)	IC_{50}: 0.36
Target Binding (Species Specific PD-L1)	(+)	Kd: Human: 1.8 Kd: NHP: 1.7
Cellular Cytotoxicity (PD-L1 + Tumor Cells)	(+)	IC_{50}: 1-110
Cellular Cytotoxicity (PD-L1 + Monocytes)	(+)	IC_{50}: 2-200
Antigen Seeding – Cell Kill	(+)	IC_{50}: 20-250



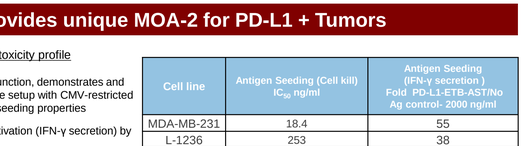


PD-L1-ETB-AST Selectively targets subsets of PD-L1 positive immune cells



Higher PD-L1 Expression on immune subsets is necessary for PD-L1-ETB-AST Cytotoxicity

- (A) IFN-γ increases PD-L1 expression on Monocytes but not other immune subsets derived from human PBMCs
- (B) Cytotoxicity against immune cells is restricted to IFN-γ treated monocytes expressing higher levels of PD-L1
- (C) Correlation of PD-L1 expression and PD-L1-ETB-AST Cytotoxicity across human PBMC donors

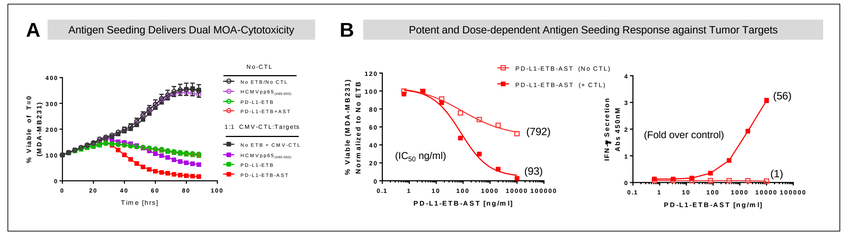


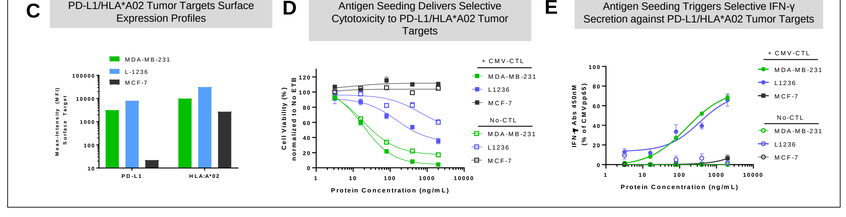
PD-L1-ETB-AST provides unique MOA-2 for PD-L1 + Tumors

PD-L1-ETB-AST delivers antigen for potent dual MOA cytotoxicity profile

- (A) PD-L1-ETB-AST, but not a control ETB without AST function, demonstrates and enhanced cell kill of PD-L1/HLA:A02 targets in a co-culture setup with CMV-restricted effector CTLs present driven by PD-L1-ETB-AST antigen seeding properties
- (B) Dose-dependent enhancement of cell kill and T cell activation (IFN-γ secretion) by PD-L1-ETB-AST observed in the presence of CTLs
- (C- D) PD-L1 and HLA:A*02 expression on tumor target cells and co-culture assays demonstrate that HLA:A02 expression is required but is not sufficient, without PD-L1 target expression, CTL activation and CTL mediated cytotoxicity by PD-L1-ETB-AST

Cell line	Antigen Seeding (Cell kill) IC_{50} ng/ml	Antigen Seeding (IFN-γ secretion) Fold PD-L1-ETB-AST/No Ag control- 2000 ng/ml
MDA-MB-231	18.4	55
L-1236	253	38
MCF-7	> 20000	6.6



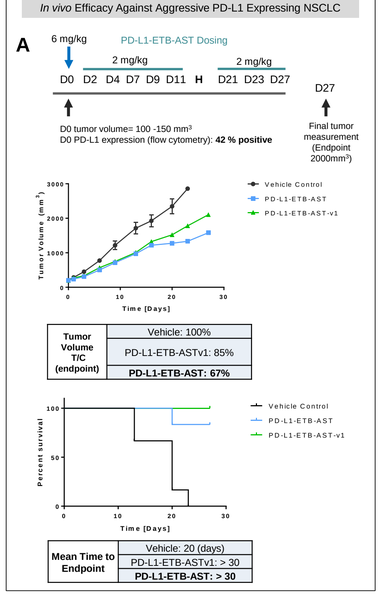


PD-L1-ETB-AST Displays activity against human tumors *in vivo*

PD-L1-ETB-AST is effective against Human Patient Derived Xenografts (PDX) *in vivo*

- (A) –Model A: PD-L1-ETB-AST restricts tumor growth and prolongs endpoint survival against an aggressive NSCLC PDX Model
- (B) – Model B: PD-L1-ETB-AST promotes tumor regression and increased endpoint survival against a low PD-L1 expressing NSCLC Model

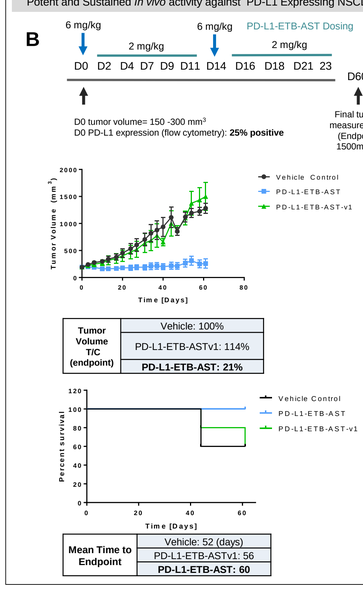
In vivo Efficacy Against Aggressive PD-L1 Expressing NSCLC



Tumor Volume T/C (endpoint)	Vehicle: 100%
	PD-L1-ETB-ASTv1: 85%
	PD-L1-ETB-AST: 67%

Mean Time to Endpoint	Vehicle: 20 (days)
	PD-L1-ETB-ASTv1: > 30
	PD-L1-ETB-AST: > 30

Potent and Sustained *In vivo* activity against PD-L1 Expressing NSCLC



Tumor Volume T/C (endpoint)	Vehicle: 100%
	PD-L1-ETB-ASTv1: 114%
	PD-L1-ETB-AST: 21%

Mean Time to Endpoint	Vehicle: 52 (days)
	PD-L1-ETB-ASTv1: 56
	PD-L1-ETB-AST: 60

PD-L1-ETB-AST Safety and Pharmacodynamics in NHPs

- An exploratory study (n=2/ dose level) was conducted to inform a GLP toxicology study in the pharmacologically relevant cynomolgus monkey.
- Intravenous administration of PD-L1-ETB-AST once per week for 4 weeks was tolerated in monkeys up to the highest tested dose in this study, 450µg/kg. Minimal changes in clinical observations and serum testing were not considered adverse.
- Indication of T cell activation by PD-L1-ETB-AST was observed as a test article related increase in type I cytokines (TNF-alpha, IFN-gamma and IL-2) in 1 of 2 animals in each of the two higher dose cohorts.

CONCLUSIONS

- PD-L1-ETB-AST is a PD-L1 targeted ETB with activity against clinically relevant cell lines
  - Designed to deliver a unique and dual MOA approach for directly targeting PD-L1 expressing tumors
  - Targeting of peripheral PBMCs limited to a small subset of PD-L1 expressing monocytes – limiting toxicity profile
  - Targeting high expressing PD-L1 immune cells may provide benefit at tumor site when these cells are present
  - Dual MOA allows for activity irrespective of tumor immune status – potential for hot and cold tumor efficacy
- PD-L1-ETB-AST displays safe and efficacious profile *in vivo*
  - Well tolerated in NHP at doses relevant to predicted potency
  - De-immunized ETB scaffold reduces CLS profile associated with immunotoxin class
  - Displays tumor growth and survival benefits in PDX in vivo models
- PD-L1-ETB-AST is slated for clinical development in 2020

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