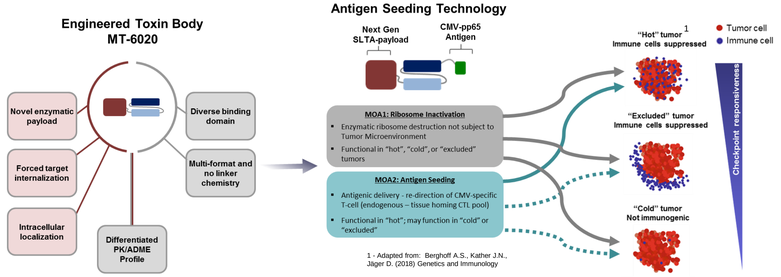
The safety and efficacy profile of a PD-L1 directed Engineered Toxin Body, as a novel targeted direct-cell kill approach for the treatment of PD-L1 expressing cancers

Hilario J. Ramos, Asis Sarkar, Sara LeMar, Brigitte Brieschke, Joseph D. Dekker, Veronica Partridge, Pablo Maceda, Michaela Sousares, Garrett L. Robinson, Aimee Iberg, Shaoyou Chu, Jensing Liu, Jack P. Higgins, Erin K. Willert - Molecular Templates Inc., Austin, TX.

Abstract 3900
AACR 2019

MOLECULAR TEMPLATES

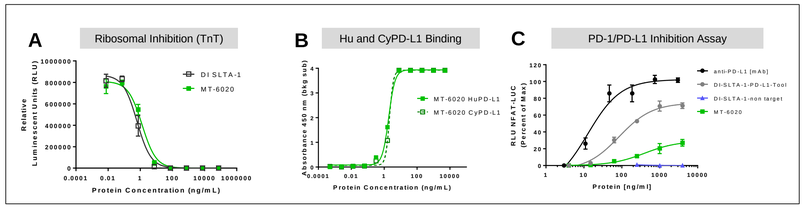
Targeting PD-L1 with ETBs to overcome checkpoint resistance



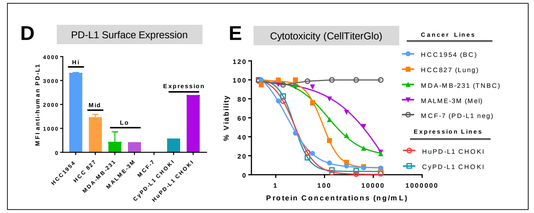
- PD-L1 targeted ETBs provide distinct benefits for overcoming clinical challenges of checkpoint non-responsive tumors
  - Deliver irreversible ribosomal inhibition and deliver antigen payload from a single fused protein – target independent of immune environment
  - Proprietary deimmunized Shiga Like Toxin A (SLTA) to reduce ADA and 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 allows for dual MOA activity independent of immune status/checkpoint resistance of PD-L1

MT-6020 is a Potent PD-L1 Targeted ETB with NHP Cross-reactivity

- MT-6020 - potent ribosomal inhibition, Human and Cynomolgus PD-L1 binding, Reduced Checkpoint Blockade
  - (A) MT-6020 inhibits ribosomes in a cell free assay (TnT) with similar IC_{50} to SLTA (DI SLTA-1)
  - (B) MT-6020 binds recombinant Human (Hu) and Cyno (Cy) PD-L1 protein (ELISA) with high affinity and similar IC_{50}
  - (E) MT-6020 elicits potent direct cytotoxicity on both Hu and Cy PD-L1 expressing CHOKI cell lines
  - (C) MT-6020 displays > 500 fold reduction in activity and reduced maximal ability to block T cell activation (NFAT reporter assay)



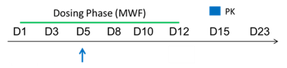
- MT-6020 elicits potent cytotoxicity on PD-L1 positive and clinical relevant tumor cell lines
- (D, left) mAb detection on Hi, Mid, and Lo PD-L1 expressing cell lines from clinically relevant indications
- (D, right) PD-L1 expression with mAb detection on Hu and Cy PD-L1 CHOKI
- (E) Cytotoxicity assay (CellTiter-Glo) – MT-6020 can target Hi, Mid, and Lo clinically relevant PD-L1 expressing targets for cytotoxicity (CellTiter-Glo)



Functional MOA	MT-6020	Potency (IC_{50}) ng/ml	
Scaffold	Deimmunized (DI) SLTA-1-scFv	N/A	
Reactivity	Human/Cyno (NHP)	N/A	
Ribosome Inhibition	(+)	2.8	
Target Binding (PD-L1)	(+)	Hu PD-L1	1.9
		Cy PD-L1	2.2
Cellular Cytotoxicity	(+)	HuPD-L1 CHOKI, CyPD-L1 CHOKI	5.9, 5.7
		HCC1954, HCC827, MDA-MB-231, MALME-3M, MCF-7	3, 84, 139, 1555, >10,000
PD-1/PD-L1 Blockade	Reduced	MT-6020	>10,000
		α-PD-L1 mAb	19

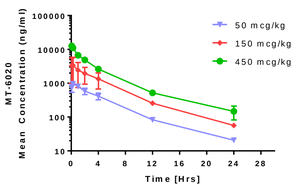
MT-6020: Favorable PK profile and safety window in NHP

Schematic: Dosing and PK schedule



A

Pharmacokinetics and Serum $T_{1/2}$ MT-6020 - Day 5 of study



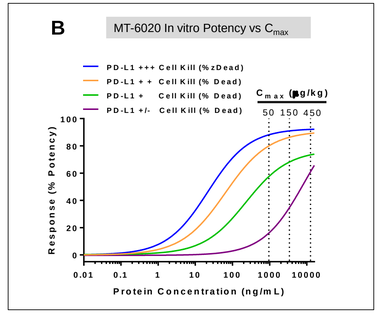
Group (µg/kg)	$T_{1/2}$ (hr)	AUC_{0-24hr} (hr*ng/ml)	C_{max} (ng/ml)
50	4.5	5160	959
150	4.57	16800	3410
450	4.4	39600	12700

MT-6020 Study Design

Species	Administration of MT-6020	Duration of Dosing	Dose Level (µg/kg)	No Observable Adverse Effect Level (NOAEL)
Cynomolgus Macaque F, n=2 / group	Intravenous injection MT-6020 (PBS pH 7.4)	3 X per week; 2 weeks for total of 6 doses	50, 150, 450	450 µg/kg

B

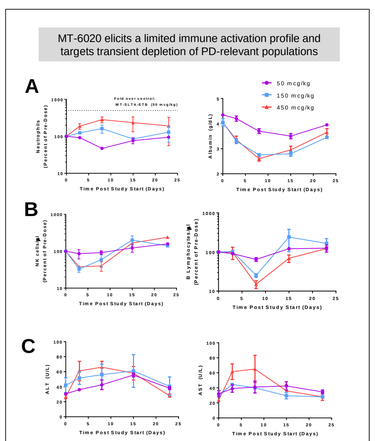
MT-6020 In vitro Potency vs C_{max}



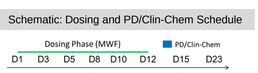
- MT-6020 demonstrates a dose-dependent exposure profile in NHP
  - (A) Serum exposure after low, mid, high dose i.v administration of MT-6020
  - Kinetic profile on day 5; at 5 min, 15 min, 2, 4, 12, 24 hr post dose 3
  - (Table) Calculated $T_{1/2}$, Area under the curve, and maximal exposure values for MT-6020
- Potency vs Toxicity Window Profiling
  - IC_{50} cytotoxicity; Hi +++ (HCC1954, BC), Mid ++(HCC827, Lung), Lo+ (MDA-MB-231, TNBC) and Lo +/- (MALME-3M; Mel) PD-L1 targets
  - Calculated serum concentration (ng/ml) for 50, 150 and 450 mcg/kg (dashed lines) based on C_{MAX} values
  - NOAEL for MT-6020 identified at 450 mcg/kg (~ 12,000 ng/ml)
  - IC_{50} Potency window ranges from 200 – 2000 fold over maximal exposure
  - MT-6020 Shows favorable profile expected to safely target a range of PD-L1 expressing cancers

MT-6020: Well tolerated with limited immunogenicity

MT-6020 elicits a limited immune activation profile and targets transient depletion of PD-relevant populations



Schematic: Dosing and PD/Clin-Chem Schedule



Summary: NOAEL PD and Clin-Chem Findings

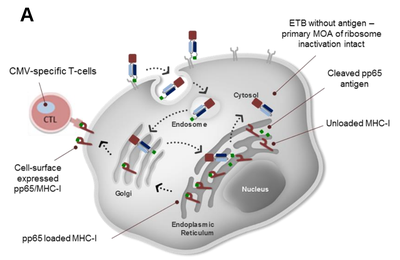
NOAEL	Cytokine	Pharmacodynamics/PBMC Profile				Clinical Chemistry		
		Target	Effect	Fold (maximal 450 mcg/kg)	Fold (maximal 50mcg/kg)	Target	Effect	Max Fold
450 mcg/kg	No effects on serum IL-2, IFN-γ, IL-6, IL-8, or TNF-α	Neutrophil	Transient Increase	3.4	<1	AST	Transient Increase	3.3
		B Lymphocyte	Transient Decrease	5.4	2	ALT	Transient Increase	3.1
		NK Cell	Transient Decrease	3.5	1.2	Albumin	Transient Decrease	1.5

- MT-6020 displays limited toxicity profile in NHP
  - (A) Neutrophil expansion is low level and transient compared to WT-SLTA scaffold (see dashed line – 1st generation WT ETB scaffold)
  - Albumin level profile consistent with innate immune activation and restoration to homeostasis
  - (B) Kinetic profile of immune subsets reveals transient depletion of B and NK cell subsets
  - (C) Kinetic profile of mild and transient hepatotoxic response as measured by AST and ALT levels
  - (Table) **NOAEL identified at 450 mcg/kg** in NHP with limited Immune activation or clinical chemistry concerns

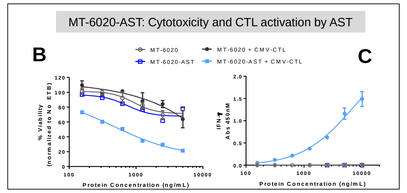
Delivering Antigen Seeding Technology for Clinical Development

- Antigen Seeding Technology (AST) – built on MT-6020 scaffold to seed antigenic peptide and T cell response
  - MT-6020 fused with the NLVPMVATV – HLA:A02 restricted peptide from Human Cytomegalovirus (HCMV) (**MT-6020-AST**)
  - MT-6020-AST retains potency and specificity profile of MT-6020
  - MT-6020-AST "seeds" CMV-restricted MHC-I peptide response for redirection of endogenous CTL response against tumor
- MT-6020-AST delivers peptide antigen for potent dual MOA cytotoxicity profile
  - (B,C) Co-culture model with PD-L1/HLA:A02 matched targets and CMV-restricted CTLs (1:1, E:T ratio)
  - (B) Dose dependent cytotoxicity is enhanced with dual MOA of MT-6020-AST as detected by enumeration of % viability (live cell imaging – Incucyte-S3)
  - (C) T cell activation profile; AST response is coupled to activation of CMV-restricted CTL response and dose-dependent release of IFN-γ

MT-6020-AST for Antigen Seeding Technology (AST)



MT-6020-AST: Cytotoxicity and CTL activation by AST



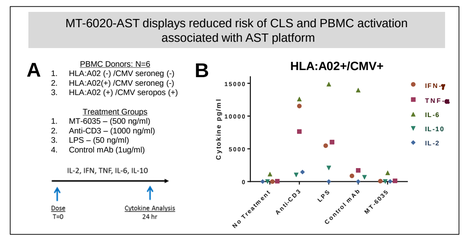
Target Cell line (MT-6035)	Cell Kill IC_{50} (ng/ml) (CTG 2.0)
HumanPD-L1	36
CynoPD-L1	17
HCC1954	35
HCC827	38
MDA-MB-231	115
MALME-3M	1479

ETB	AST IC_{50} (Fold Cell kill ; cytokine) over no Ag control)	
MT-6020-AST	2.7	30
MT-6020	1	1

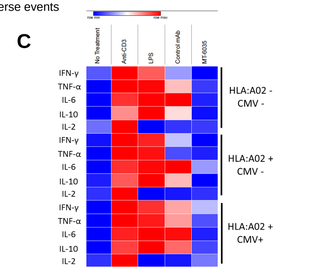
MT-6020-AST displays a safe and reduced CLS profile

- MT-6020-AST triggers limited cytokine response associated with AST platform
  - (A) HLA:A02/CMV positive or negative PBMC donors were treated with MT-6020-AST or control agents for 24hrs
  - (B) Cytokine profile identified by Luminex Bead array – HLA:A02 and CMV positive donors (average of n=6)
  - (C) MT-6020-AST elicits minimal cytokine response vs. controls – no major association to HLA or CMV positivity groups (Heat map)
  - MT-6020-AST and AST platform show minimal risk for cytokine mediated adverse events

MT-6020-AST displays reduced risk of CLS and PBMC activation associated with AST platform



C



CONCLUSIONS

- MT-6020 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"
  - Does not act as a checkpoint inhibitor
- MT-6020 Tolerability and exposure profile in NHP demonstrates a broad and favorable safety window
  - De-immunized ETB scaffold reduces CLS profile associated with immunotoxin class
  - MT-6020 is easily coupled with AST (MT-6020-AST) while retaining expected safety and efficacy profiles
  - MT-6020-AST is slated for clinical development in early 2020

Contact: hilario.ramos@mtem.com

mtem MOLECULAR TEMPLATES