In vivo efficacy of a PD-L1 targeted, antigen seeding engineered toxin body

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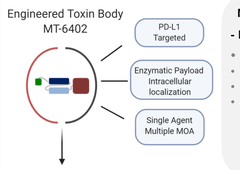
MT-6402 is a unique single agent approach to exploit the full benefit of PD-L1 targeting

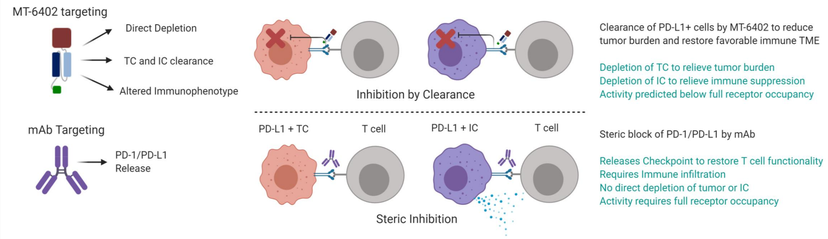


MT-6402 is a cytotoxic fusion protein designed for **depletion of *PD-L1* Tumor (TC) and immune cells (IC)**

- Delivers cell kill via Shiga-like toxin payload and alters immune phenotype of TC for recognition by T cells

- **Specificity**: Specific antibody fragment domain targets to PD-L1 on the surface of TC and IC
- **Payloads**: Proprietary and deimmunized Shiga-like toxin A (DI-SLTA) and CMV restricted peptide antigen
- **Routing and cell-kill** via enzymatic and irreversible inactivation of ribosomes mediated by DI-SLTA
- **Altered immune phenotype**: Delivery of a non-self antigenic peptide to recruit a CMV-reactive CD8+ T-cell response to tumors

Potency: Dual mechanism of action in a single fusion molecule – **Depletes TC and IC directly - A unique single agent effect not achieved with current monoclonal antibody therapies**



Clearance of PD-L1+ cells by MT-6402 to reduce tumor burden and restore favorable immune TME

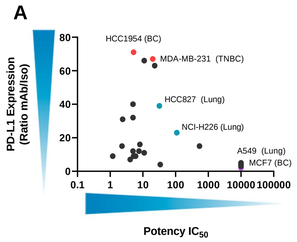
Depletion of TC to relieve tumor burden
Depletion of IC to relieve immune suppression
Activity predicted below full receptor occupancy

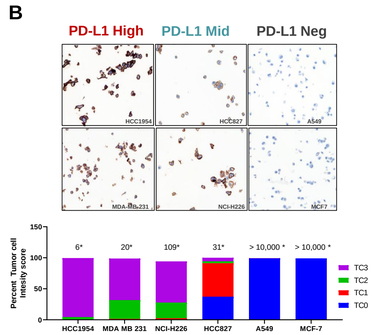
Steric block of PD-1/PD-L1 by mAb

Releases Checkpoint to restore T cell functionality
Requires Immune infiltration
No direct depletion of tumor or IC
Activity requires full receptor occupancy

MT-6402 targets PD-L1 tumor cells for depletion via distinct modes of action

MT-6402 delivers direct tumor cell cytotoxicity across a range of PD-L1 expression profiles



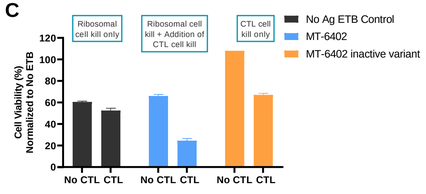


(A) 29 representative Lung, Skin, Breast, and Ovary tumor cell lines were evaluated for surface PD-L1 expression (flow cytometry) and cytotoxic sensitivity to MT-6402 *in vitro*

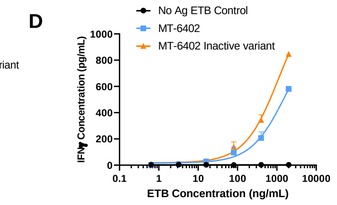
- Colored dots represent PD-L1 hi (red), mid (blue), and low (purple) tumors

(**B**) Tumor cell lines were evaluated for surface PD-L1 expression by IHC (22C3) and scored for intensity as TC 0 -3

SLTA-mediated (ribosomal inactivation) and CTL mediated kill of MT-6402 treated PD-L1/HLA*A02 target cells



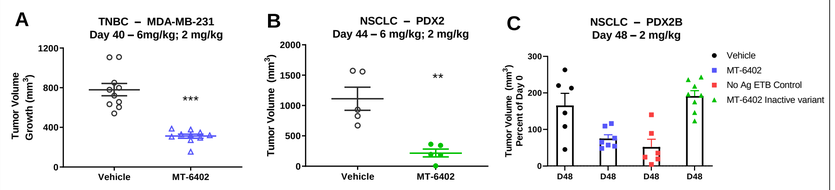
MT-6402 stimulates CMV-specific CTLs to secrete IFN-gamma against PD-L1/HLA*A02 target cells presenting viral antigen



MDA-MB-231 human tumor cells expressing PD-L1 and HLA:A02 were co-cultured with MT-6402 (both cytotoxic MOAs), targeted-SLTA with no antigen (No Ag ETB control), or an inactive PD-L1-targeted antigen containing ETB (MT-6402 inactive variant) for 5 days in the presence or absence of CMV-HLA:A02 restricted T-cells and evaluated for (**C**) viability or (**D**) T-cell activation via IFN-gamma secretion.

MT-6402 restricts PD-L1 positive tumors growth *in vivo*

MT-6402 restricts tumor growth in vivo across indication relevant tumor models and activity is dependent on Shiga-like Toxin direct tumor cell depletion

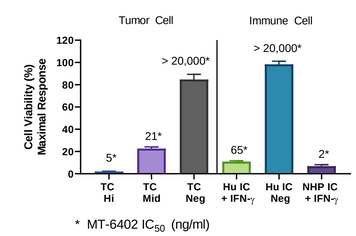


Human tumor and PDX cell lines were implanted subcutaneously in immunodeficient mice and treated with MT-6402. MT-6402 displayed significant control of tumors with limited toxicity in a human TNBC, MDA-MB-231 (**A**) and human NSCLC patient derived xenograft models (**B,C**). Additionally MT-6402 activity was compared in the NSCLC model to related molecules lacking either the viral antigen (No Ag ETB control) or direct cell kill ability (MT-6402 inactive variant) to demonstrate the direct SLTA-mediated TC effect of the molecule *in vivo* (**D**). Model and efficacy endpoints are summarized in the table.

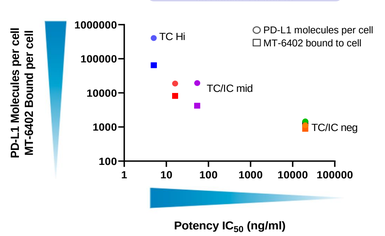
Response	MDA-MB-231	PDX2	PDX2B
Dose	6 mg/kg (dose 1) 2m/kg (3 x week)	6 mg/kg (dose 1) 2m/kg (3 x week)	2 mg/kg (3 x week)
Treated/Control - response	60%	21%	43%
Mean to endpoint	Vehicle: 49 MT-6402: > 50	Vehicle: 52 MT-6402: > 60	Vehicle: 59 MT6402: > 65

MT-6402 binds to and targets TC and IC with similar potency profiles *ex vivo*

MT-6402 potently depletes tumor and immune cell subsets from human and NHP donors



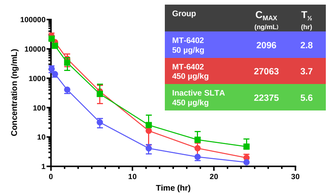
MT-6402 potency correlates to PD-L1 surface expression and ETB binding on Human TC and IC



Representative PD-L1 high (HCC1954/TC hi), mid (MDA-MB-231/TC mid), and neg (MCF7/TC neg) tumor cells were compared to isolated human CD14+ monocytes (Hu IC neg) or human or NHP CD14+ monocytes treated with IFN-gamma to induce PD-L1 (Hu/NHP IC + IFN-γ) and evaluated for potency of MT-6402 cytotoxicity by Cell Titer-Glo® (Promega) (**A**). PD-L1 surface molecules and MT-6402 binding to Human target cells was determined on TC and IC subsets by flow cytometry and plotted against MT-6402 IC_{50} for cellular cytotoxicity to evaluate the relationship between target expression, MT-6402 target binding and potency (**B**).

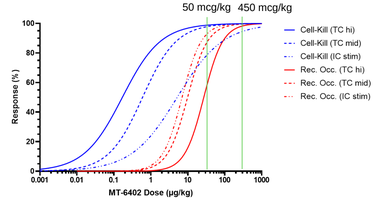
MT-6402 is predicted to target PD-L1 tumor and immune cells for depletion *in vivo*

Pharmacokinetic Curves
MT-6402 - Day 1 of NHP Study



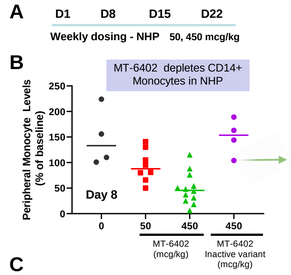
Group	C_{MAX} (ng/mL)	$T_{\frac{1}{2}}$ (hr)
MT-6402 50 µg/kg	2096	2.8
MT-6402 450 µg/kg	27063	3.7
Inactive SLTA 450 µg/kg	22375	5.6

Simulated Human MT-6402 Cell-Kill and Receptor Occupancy vs Simulated Human Dose



MT-6402 or the MT-6402 inactive control were administered in non-human primates (NHP) at 50 µg/kg (MT-6402) or 450 µg/kg (MT-6402 and inactive control) weekly for 4 doses (**A**). Human serum exposure was simulated from NHP PK data then correlated with the in vitro IC_{50} potency values and receptor binding data to predict maximum cell-kill and receptor occupancy for TC and IC subsets (**B**).

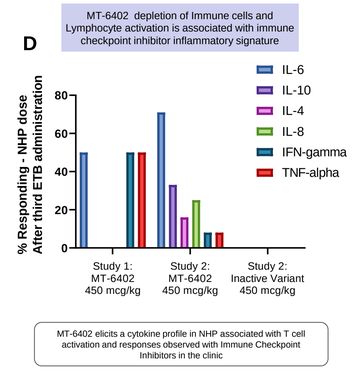
MT-6402 elicits a distinct irAE profile in NHP predictive of clinical benefit



MT-6402 or the MT-6402 inactive variant control were administered weekly for 4 weeks in non-human primates (**A**). Immune cell subsets were evaluated from circulation by flow cytometry (**B,C**). Serum cytokine responses were evaluated across two independent NHP studies (**D**). The data is displayed as percent of responder for study 1, n=2 NHP and study 2 n=8 NHP (MT-6402) and n=5 (iMT-6402 inactive variant). Data represents cytokine responses after repeated weekly dosing (weeks 3 and 4).

Dose dependent Direct IC cell kill Requires functional SLTA

Dose dependent Immune activation is associated with direct cell kill activity



MT-6402 elicits a cytokine profile in NHP associated with T cell activation and responses observed with Immune Checkpoint Inhibitors in the clinic

MT-6402 is designed to deplete PD-L1 positive IC and TC in the TME. Targeting of IC in patients has shown clinical benefit (1,2) and immune activation and immune related adverse event (irAE) development in humans is correlative to favorable response (3,4). irAE development in NHP is not observed with single agents only with ICI in combination(5). **Targeting cells for depletion with MT-6402 may lead to unique benefits in the clinic not observed with antibody treatment which mediates activity through steric block alone.**

MT-6402 depletes immune cells expected to express PD-L1, stimulates immune activation and a pharmacodynamic and irAE profile consistent with combination but not single agent checkpoint inhibitors in NHP (5). Responses are dose dependent and mediated by STLA direct cell kill and not steric inhibition as the inactive control does not trigger immune activation or PD response *in vivo*. ***MT-6402 is a unique therapeutic approach with the ability for potent responses in humans as a single agent therapy.***

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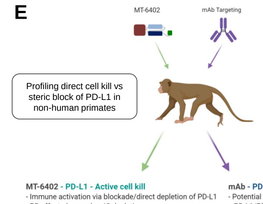

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Group	Dosing	Immune Effects	Pharmacodynamic Response
MT-6402	50 mcg/kg	↑Monocyte depletion ↑T cell, B cell, Eosinophil expansion increased with sequential dosing	Minimal Troponin-I release; no significant cardiac findings
MT-6402	450 mcg/kg	↑↑Monocyte depletion ↑↑↑T cell. B cell, NK, Eosinophil expansion; increased with sequential dosing Cytokine induction IL-2, IFN-γ, TNF-α, IL-6, IL-10	Troponin-I release associated with CD3+ T cardiac infiltration minimal monocyte and B cell infiltration[1] resolving with dose cessation; evidence of skin inflammation[2]
SLTA-inactive	450 mcg/kg	No remarkable findings	No Remarkable findings

1,2 – myocarditis and dermatitis are common immune-related adverse events (irAE) observed predominantly with combination immune checkpoint inhibitors in clinical settings and are associated with beneficial therapeutic response (3,4).

CONCLUSIONS

MT-6402 is a unique agent designed to deplete tumor and repressive immune cells in the TME

- MT-6402 specifically and directly kills PD-L1+ **tumor** cells
  - Potent *in vitro* activity against a variety of PD-L1+ tumor cells
  - MT-6402 treatment results in tumor growth delay and survival benefits in NSCLC PDX *in vivo* model
- MT-6402 can alter the immunophenotype of the tumor and allow for recognition by effector T cells by delivering a viral antigen in complex with MHC-I on the HLA*A02+/PD-L1+target cell surface
- MT-6402 specifically and directly kills PD-L1 + **immune** cells
  - Potent *in vitro* activity against PD-L1+ human or non-human primate immune cells
  - MT-6402 elicits an immune mediated irAE profile in NHP that is associated with clinical benefit in patients treated with immune checkpoint inhibitors - irAEs have not be seen in NHP models with monotherapy mAb checkpoint inhibitors and irAE response by MT-6402 may suggest benefit in patients as a unique PD-L1 targeted single agent
- MT-6402 targets PD-L1 with multiple unique mechanisms of action that may provide greater potency than is seen with current PD-L1 antibodies; MT-6402 is slated for clinical development in 2020

References:
1. Kowanetz, M., et al. Differential regulation of PD-L1 expression by immune and tumor cells in NSCLC and the response to treatment with atezolizumab (anti-PD-L1) PNAS 15,43 (2018)
2. Tang, F., Zheng, P. Tumor cells versus host immune cells: whose PD-L1 contributes to PD-1/PD-L1 blockade mediated cancer immunotherapy?. Cell Biosci 8, 34 (2018).
3. Das, S., Johnson, D.B. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. j. immunotherapy cancer 7, 306 (2019).
4. Changhua, J. et al. Myocarditis in Cynomolgus monkeys following treatment with immune checkpoint inhibitors. Translational Cancer Mechanisms and Therapy, (2019).
5. Nakamura. Biomarkers for Immune checkpoint inhibitor-mediated tumor response and adverse events. Frontiers in Medicine. 6: 119 (2019)

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