Beyond bispecifics: MDX2001, a novel tetraspecific antibody targeting T lymphocyte activation and survival enhancing receptors (LASER) directed to TROP2 and c-MET in solid tumor malignancies

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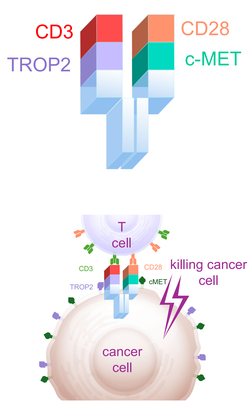
Abstract: 1287

BACKGROUND

MDX2001: A Lymphocyte Activation and Survival Enhancement Receptor Antibody (LASER)

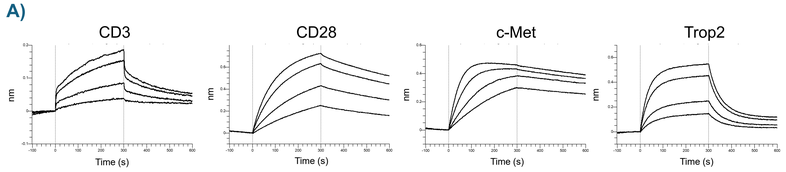


- MDX2001 is a first-in-class tetraspecific LASER recognizing CD3 and CD28 on human T cells and c-MET and TROP2 on tumor cells.
- MDX2001 is designed to:
  1. optimize T cell activation/survival by dual signaling through CD3 and CD28
  2. overcome target expression heterogeneity and treatment escape/resistance by binding to two different tumor antigens.
- MDX2001 is engineered with knob-into-hole mutations to promote Fc heterodimerization, and an Fc-null format to prevent triggering of Fc-dependent immune activation
- MDX2001 is currently being evaluated in a Phase 1/2a, first in human, clinical study in patients with advanced solid tumors (NCT06239194)

RESULTS

1. Binding of MDX2001 to CD3, CD28, c-MET and TROP2

A)



B)

Species / Target	Binding affinity (KD) (nM)			
	CD3	CD28	c-MET	TROP2
Human	27.2	13.8	3.13	46.3
Mouse	N.B.	N.B.	N.B.	N.B.
Rat	N.B.	N.B.	N.B.	N.B.
Dog	N.B.	N.B.	N.B.	N.B.
NHP	N.B.	13.8	3.84	37.8

Figure 1. Binding affinity of MDX2001 to its human targets and animal species orthologs was determined using biolayer interferometry (BLI), with MDX2001 (titrated 120, 80, 40, and 20 nM) binding to ligand (CD3, CD28, c-MET, or TROP2) loaded onto a biosensor. A) Binding profiles of MDX2001 to human targets. Each line represents a concentration of MDX2001. B) Binding affinities (equilibrium dissociation constants (K_D)) of MDX2001 to human and animal ortholog targets.
KD=equilibrium dissociation constant; N.B.=no binding; NHP=non human primate

- MDX2001 binds to human T cell targets CD3 and CD28 and human tumor targets c-MET and TROP2.
- MDX2001 does not bind to targets (CD3, CD28, c-MET, and TROP2) from mouse, rat, and dog.
- While MDX2001 binds NHP CD28, c-MET, and TROP2, it does not bind NHP CD3.

2. Binding of MDX2001 to Fc-γ receptors and FcRn

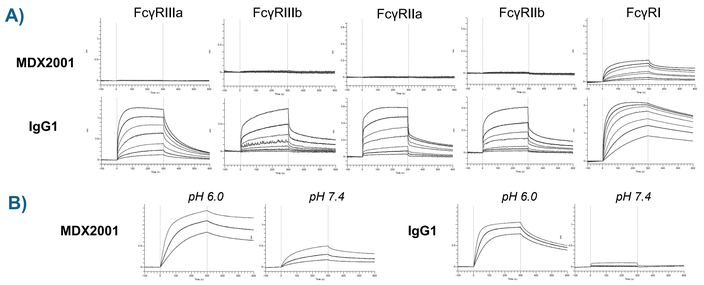


Figure 2. A) Binding of MDX2001 or IgG1 (2-fold titration from 1000 to 15.6 nM) to human FcγRs was analyzed by BLI.
B) Binding of MDX2001 or IgG1 (titrated 62.5, 31.3, and 15.6 nM) to human FcRn analyzed at acidic pH (6.0) or neutral pH (7.4)

- MDX2001 shows abrogated or significantly reduced binding to Fc-γ receptors
- MDX2001 retains the physiologic pH-dependent binding dynamic with human neonatal Fc receptor

3. MDX2001 induces potent T cell activation in the presence of target tumor cells

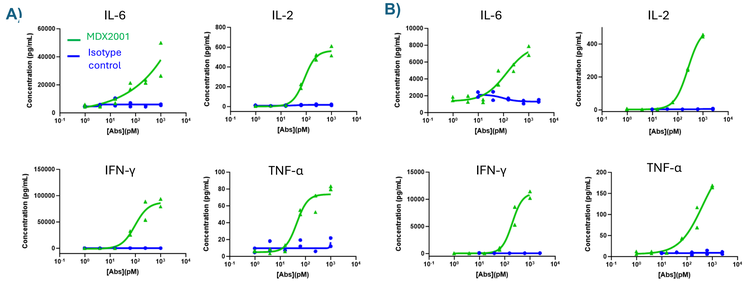


Figure 3. Human PBMC were co-cultured with H1975 (A) or HCC1143 (B) tumor cell lines in the presence of MDX2001 (green lines) or isotype control (blue lines). Cytokine concentrations in the supernatants were measured using Luminex-based multiplex assay.

- MDX2001 induces the secretion of IL-6, IFN-γ, IL-2, and TNF-α when added to co-cultures of PBMCs and tumor cells

4. MDX2001 induces minimal T cell activation in the absence of target tumor cells

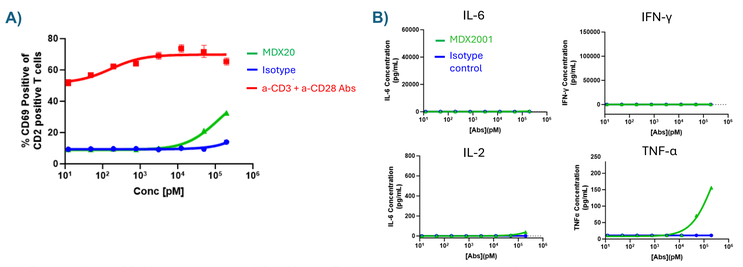


Figure 4. Human PBMC were treated with MDX2001 and T cell activation was measured by flow cytometry (A) and cytokine release (B). Green lines: MDX2001. Blue lines: isotype control. Red lines: anti-CD3/anti-CD28 mAb treatment.

- MDX2001 induces minimal T cell activation at concentrations below 10 nM when target tumor cells are not present.

5. MDX2001 triggers robust in vitro tumor cytolytic activity

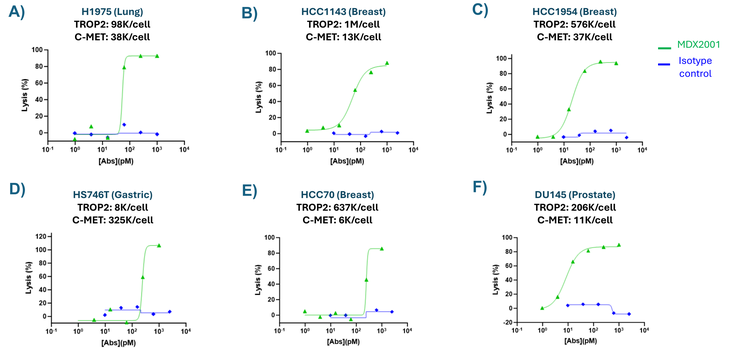


Figure 5. Human PBMC were co-cultured with H1975 (A), HCC1143 (B), HCC1954 (C), Hs746T (D), HCC70 (E), DU145 (F) tumor cell lines in the presence of MDX2001 (green lines) or isotype control (blue lines). Expression of TROP2 and c-MET (molecules per cell) indicated on the top of each graph.

- MDX2001 induces potent anti tumor cytolytic activity against a panel of 6 different human tumor cell lines expressing various levels of TROP2 and c-MET.

6. MDX2001 inhibits tumor growth in a humanized mouse model

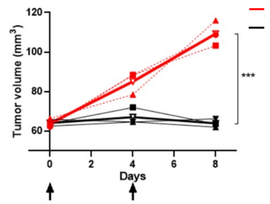


Figure 6. Immunodeficient NSG mice were implanted with HCC1954 breast tumor cells orthotopically and engrafted with human T cells from a healthy donor. MDX2001 or vehicle were intravenously administered starting 3 to 4 hours after mouse humanization, with a second dose administered 4 days later. Black arrows indicate dosing days. Individual mouse values are shown in thin lines; group mean values are shown in thick lines. Mean tumor volume at Day 8 was compared between groups using unpaired t-test. ***: p<0.001.

- MDX2001 induced significant inhibition of tumor growth relative to vehicle in a humanized mouse model

CONCLUSIONS

- MDX2001 binds to human CD3, CD28, c-MET and TROP2 with nanomolar affinity.
- MDX2001 demonstrates abrogated or significantly reduced binding to Fc-γ receptors while retaining the physiologic pH-dependent binding dynamic with the human neonatal Fc receptor.
- When incubated with human PBMCs and tumor cells expressing TROP2 and c-MET, MDX2001 induces T cell activation at concentrations as low as 15 pM.
- In contrast, MDX2001 induces only marginal T cell activation in human PBMCs in the absence of tumor cells.
- MDX2001 induces potent in vitro anti-tumor cytolytic activity by human PBMCs against human tumor cell lines expressing various levels of TROP2 and c-MET.
- MDX2001 induces significant inhibition of tumor growth relative to vehicle in a humanized mouse model.

MDX2001 is novel tetraspecific T cell engager that demonstrates potent antitumor activity *in vitro* and *in vivo*, with minimal T cell activation in the absence of tumor cells.

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