MDX2004, a novel immune rejuvenator targeting CD3, CD28, and 4-1BB, augments tumor immunity in preclinical animal models

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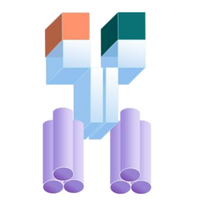
Abstract: 1156

BACKGROUND

MDX2004: A multispecific immune rejuvenator



▪ MDX2004 design

- Cancer immunotherapy has demonstrated significant clinical success; however, only a subset of patients benefit and show durable responses. Limited efficacy is often attributed to suboptimal antitumor T cell generation and impaired function of immunological memory
- MDX2004, a first-in-class trispecific antibody-fusion protein recognizing CD3, CD28, and 4-1BB (CD137) on human T cells, is a novel immunotherapeutic candidate designed to overcome such hurdles by increasing stem-like and memory T cell responses
- MDX2004 engages 4-1BB through its natural ligand, via two 4-1BBL homotrimers engineered on the C-terminal end of the molecule
- MDX2004 is designed to rejuvenate cellular immunity, thereby enhancing antitumor immunity
- MDX2004 is currently being evaluated in a Phase 1/2, first in human, clinical study in patients with advanced tumors (NCT07110584)

RESULTS

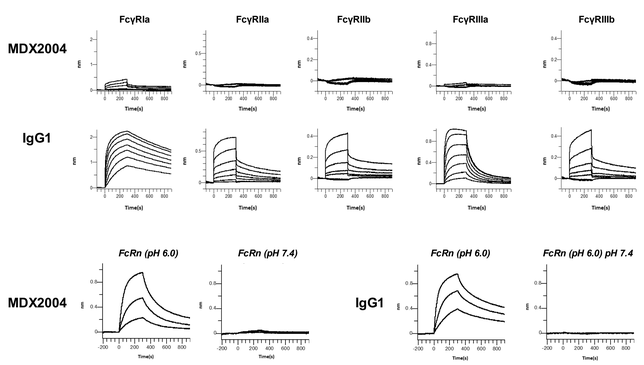
1. Binding profile of MDX2004

A)

	K_D (nM)				
	Human	NHP	Dog	Mouse	Rat
CD3	653.37	N.B.	N.B.	N.B.	N.B.
CD28	2.47	2.47	N.B.	N.B.	N.B.
4-1BB	≤ 0.01[a]	≤ 0.01[a]	≤ 0.01[a]	N.B.	N.B.

[a] The Octet R8 (Sartorius) biolayer interferometry instrument has a K_D determination lower bound of 0.01 nM.

B)



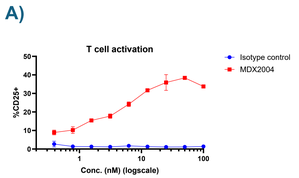
C)

Figure 1. A) Binding affinity of MDX2004 to its human targets and animal species orthologs was determined using biolayer interferometry (BLI). Table shows binding affinities (equilibrium dissociation constants (K_D)) of MDX2004 to human and animal ortholog targets. KD=equilibrium dissociation constant; N.B.=no binding; NHP=non-human primate. **B)** Binding of MDX2004 or IgG1 control (2-fold titration from 1000 to 15.6 nM) to human FcγRs was analyzed by BLI. **C)** Binding of MDX2004 or IgG1 control (titrated 50 to 12.5 nM) to human neonatal Fc receptor (FcRn) analyzed at acidic pH (6.0) or neutral pH (7.4) by BLI.

- MDX2004 binds to human T cell targets CD3, CD28 and 4-1BB.
- MDX2004 does not bind to CD3, CD28, 4-1BB from mouse and rat, and it only binds to 4-1BB in dog.
- While MDX2004 binds NHP CD28 and 4-1BB, it does not bind NHP CD3.
- MDX2004 shows abrogated or significantly reduced binding to Fc-γ receptors
- MDX2004 retains the physiologic pH-dependent binding dynamic with human neonatal Fc receptor

2. MDX2004 induces T cell activation with moderate cytokine secretion *in vitro*

A)



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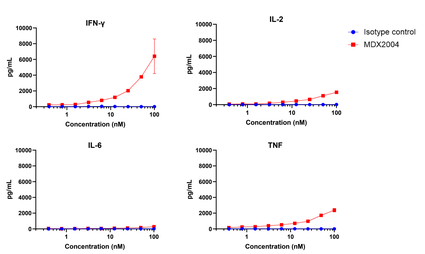
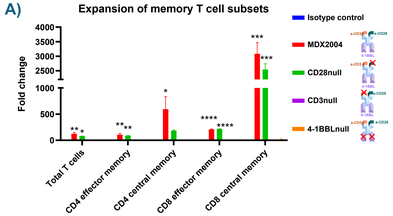


Figure 2. Human PBMCs were treated *in vitro* with MDX2004 or isotype control for 3 days. Representative donor (1 of 6) is shown. **A)** Percentage of T cells expressing CD25 activation marker was measured by flow cytometry. Graph depicts mean ± standard deviation. **B)** Concentration of cytokines in the supernatant was measured using a multiplexing assay. Graphs depict mean ± range.

- MDX2004 demonstrates dose-dependent T cell activation of primary human T cells from all six donors. Concentration dependent MDX2004-mediated cytokine release was highest for IFN-γ, IL-2, and TNF, with moderate release for IL-6.

3. Expansion of memory and stem-like T cells by MDX2004 *in vitro*

A)



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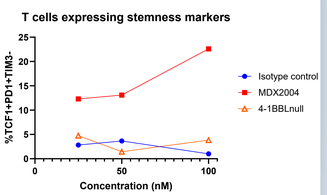
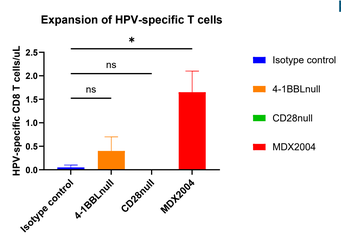


Figure 3. Human PBMCs were treated *in vitro* with MDX2004, MDX2004 variants (CD28null, CD3null, and 4-1BBLnull), or isotype control. Representative data from 1 out of 3 donors. **A)** After 10 days, T cell memory subsets were analyzed by flow cytometry. Results are expressed as fold change in cell numbers for each cell subset, compared to pretreatment values (mean + range of experimental duplicates). Statistics are calculated relative to the isotype control in each subpopulation by a one-way ANOVA for each cell subset with a Dunnett's multiple comparison test. *p ≤ 0.05; **p ≤ 0.005;***p ≤ 0.001; ****p ≤ 0.0001. **B)** After 7 days, stem like T cells were detected by flow cytometry as TCF1+PD1+TIM3-

- MDX2004 induces significant expansion of T cell memory subsets, in particular CD8 central memory. Engagement of 4-1BB and CD3 is required for this effect
- MDX2004 expands stem-like T cells in vitro through 4-1BBL

4. MDX2004 induces the expansion of antigen-specific T cells and drives potent cytolytic activity against tumor cells *in vitro*

A)



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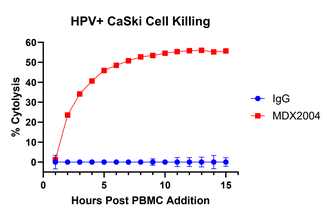


Figure 4. Human PBMCs from donors seropositive for HPV were treated *in vitro* with MDX2004, MDX2004 variants (CD28null, 4-1BBLnull), or isotype control for 7 days. **A)** HPV-specific T cells were detected by flow cytometry using MHC tetramers. Results are expressed as mean + range of experimental duplicates. Statistical significance was determined by 1-way ANOVA followed Dunnett multiple comparison test against isotype control (*p < 0.05). **B)** MDX2004 and isotype-treated treated PBMCs were washed and subsequently cocultured with an HPV+ tumor cell line, and tumor cell killing was measured by real-time cell analysis. Results are expressed as single data points for MDX2004 and mean + range for isotype control.

- MDX2004 Expands Antigen-specific CD8 T Cells
- MDX2004 Induces T cell Mediated Killing of Virally-induced Cancer Cells

5. MDX2004 surrogate expands memory and stem-like T cells *in vivo* in NHP

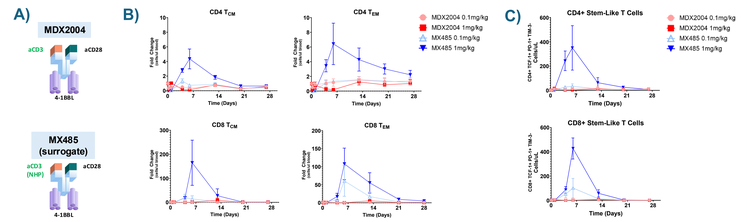
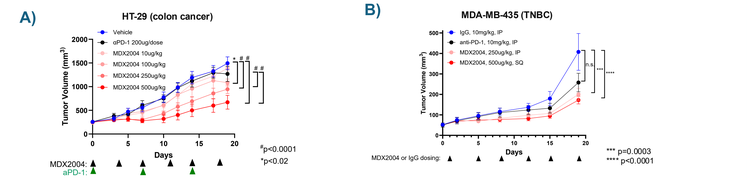


Figure 5. Cynomolgus macaques received a single i.v. dose of MDX2004, or its NHP surrogate MX485. Peripheral blood was collected at the indicated timepoints and analyzed by flow cytometry. Total T cells were defined as live CD2+ population and live CD2+ CD4+ or CD2+CD8+ to identify T cell subsets. Precision count beads were used to calculate the number of cells per µl of blood. Fold change in cell numbers was calculated compared to pre-dose values. Results are shown as mean ± SD of three animals per group. **A)** MX485 is identical to MDX2004 but with an NHP-cross-reactive CD3 binder **B)** CCR7 and CD45RA were used to define memory subsets: central memory (T_{CM}, CD45RA-CCR7+) and effector memory (T_{EM}, CD45RA-CCR7-). **C)** Stem-like T cells were identified as TCF1+ PD1+ TIM3-.

- MDX2004 surrogate (MX485) induced expansion of peripheral stem-like and memory T cells consistent with its activity in vitro. Engagement of CD3 is critical for these effects on T cells, as MDX2004, which does not cross-react to NHP CD3, shows abrogated or very diminished activity

6. MDX2004-mediated tumor growth inhibition correlates with increased clustering and proliferation of intratumoral CD8+ T Cells



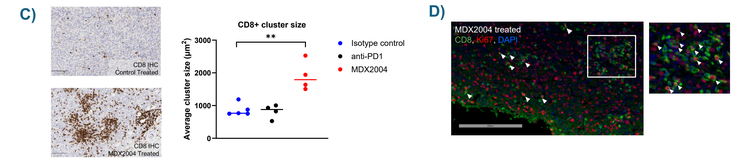


Figure 6. A) NSG mice reconstituted with human PBMC and implanted s.c with HT-29 tumor cells on day -7. On day 0, mice were dosed i.v. with MDX2004, anti-PD1, or vehicle. **B)** NCG mice reconstituted with human cord blood CD34+ cells were implanted s.c. with MDA-MB-435 tumor cells and 7 days later dosed i.p. or s.c. with MDX2004, anti-PD1, or isotype control. A) and B) Graphs depict the mean of each group with SEM error bars (n=5 mice/group). Statistical significance was determined by 2-way ANOVA with Tukey's multiple comparisons. **C)** CD8 IHC analysis of tumors isolated from HT-29 study (A). Left: representative pictures of CD8 IHC. Right: images were evaluated with QuPath. Cluster size was averaged for each mouse; each dot represents the average size of clusters in one animal. One-way ANOVA followed by Tukey's test (** p<0.005). **D)** HT-29 tumor samples from MDX2004-treated animals (A) were analyzed by Ki67/CD8 immunofluorescence. Representative image from 1 animal.

- MDX2004 demonstrated significant inhibition of tumor growth in two humanized mouse models
- Tumor growth inhibition by MDX2004 correlated with increased clustering and proliferation of intratumoral CD8 T cells

CONCLUSIONS

MDX2004 is a novel multispecific immune rejuvenator that engages CD3, CD28 and 4-1BB. MDX2004 induces robust T cell responses, including the 4-1BB-dependent expansion of stem and memory CD8 T cells, and inhibits tumor growth with acceptable tolerability in animal models. These results provide preclinical proof-of concept to support the evaluation of MDX2004 as an immunotherapeutic agent for the treatment of cancer.

1. ModeX Therapeutics, Weston, MA, USA. An OPKO Health Company