Characterization of a Tumor-targeting and Activatable T-MASK Platform to Enhance Tumor Accumulation and Tolerability of Potent Immune Modulators

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I BACKGROUND

T-MASK Platform Technology

T-MASK (Targeted Metallo/protease Activated SuperKine) platform involves fusion of a dual IL-13 tumor-targeting/masking domain to an IL-2 superkine via a matrix metalloprotease (MMP) sensitive linker (PSL), to offer the following unique features:

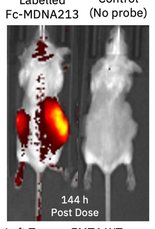
- Tunable blockade of IL-2R agonism to reduce peripheral immune stimulation for enhanced tolerability
- Tumor targeting to IL-13Rα2 highly expressed in a broad range of cancer indications but not normal tissues
- Cleavage and release of IL-13 tumor-targeting/masking domain by MMPs to restore IL-2R agonism within the tumor microenvironment (TME)

MDNA213 is an IL-13Rα2 Specific Superkine for Masking and Tumor Targeting

MDNA213 binds the decoy IL-13Rα2 with high affinity (K_D = 0.8 nM) and selectivity (no binding to the functional IL-13Rα1)

- IL-13Rα2 is overexpressed in a wide range of solid tumors, including 'cold tumors'
- IL-13Rα2 is a tumor-associated antigen with minimal to no expression in normal tissues
- IL-13Rα2 expressing tumors have abundant MMPs in the TME thereby driving invasion.
- IL-13Rα2 expression is associated with poor clinical outcome in multiple tumor types

MDNA213 to IL-13Rα2 Expressing Tumors



Left Tumor: EMT6 WT
Right Tumor: EMT6/ectopic IL-13Rα2

IVIS analysis following a single dose of VivoTag800 labelled Fc-MDNA213 (2 mg/kg; IV)

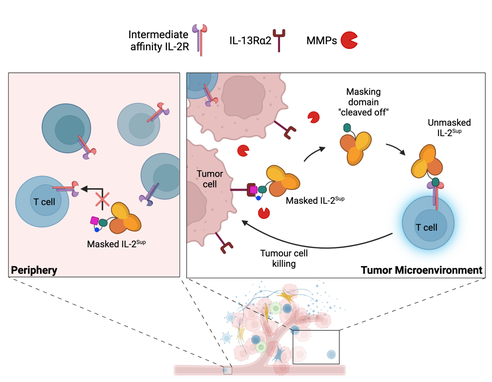
IL-13Rα2 Positive Cancers: Annual World-Wide Incidence > 2M

Liver Cancer **82%** Hou et al., J Cancer Res & Clinical Oncol (2009)	Breast Cancer **75%** Papageorgis et al., Br Cancer Res (2015)	Glioblastoma **75%** Joshi et al., Cancer Res (2000)	Ovarian Cancer **75%** Kioi et al., Cancer (2006)
Pancreatic Cancer **71%** Shimamura et al. Clin Cancer Res (2010)	Colon Cancer **66%** Barderas et al., Cancer Res (2012)	Kidney Cancer **53%** Kang et al., J Per Med (2021)	Mesothelioma **50%** Oncomine Cancer MicroArray (OMCA Database)
Prostate Cancer **47%** Nagai et al., Cancer Reports (2023)	Lung Cancer **44%** Xie et al., Oncotarget (2015)	Head & Neck Cancer **33%** Kawakami et al., Clin Cancer Res (2003)	Melanoma **32%** Beardi et al., Clin Cancer Res (2013)

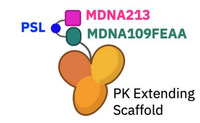
Based on RNA and/or protein expression

II RESULTS

T-MASK of a Long-Acting IL-2 Superkine



T-MASK IL-2^{Sup}

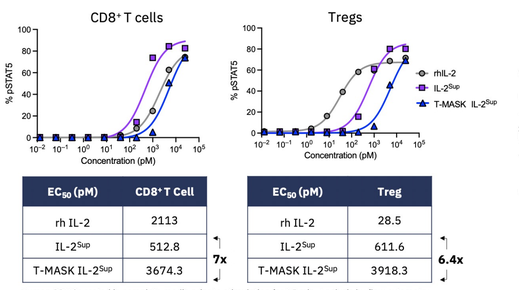


MDNA213:
- IL-13 superkine selective towards IL-13Rα2 on tumors

MDNA109FEAA:
- not-alpha, beta-enhanced IL-2 Superkine

PSL:
- protease-sensitive linker; susceptible to MMP cleavage

Attenuated Potency in Human CD8$^+$ T Cell pSTAT5 Signaling



EC_{50} (pM)	CD8$^+$T Cell
rh IL-2	2113
IL-2^{Sup}	512.8
T-MASK IL-2^{Sup}	3674.3

7x

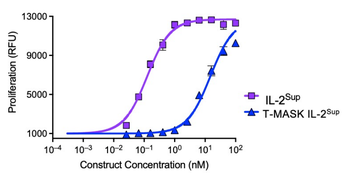
EC_{50} (pM)	Treg
rh IL-2	28.5
IL-2^{Sup}	611.6
T-MASK IL-2^{Sup}	3918.3

6.4x

Human PBMCs rested in complete media prior to stimulation for 15 min. Analysis by flow cytometry.
Average from 3 PBMC donors

T-MASK IL-2^{Sup} Shows Reduced Capacity to Induce CTLL-2 Cell Proliferation

CTLL-2 is a murine cytotoxic T cell line that depends on IL-2 to proliferate

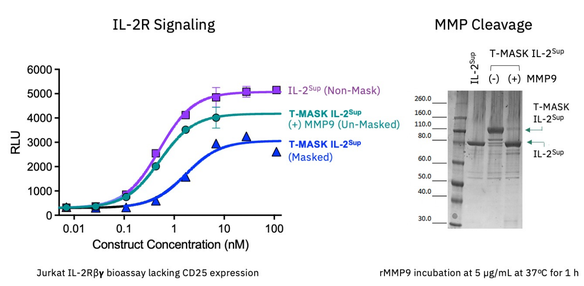


	EC_{50} (pM)
rh IL-2	93.3
IL-2^{Sup}	125.8
T-MASK IL-2^{Sup}	14,280

113x

CTLL2 treated with the indicated construct for constructs for 48 hours.
Cell number quantified using Cell Titer Blue viability reagent
(RFU: relative fluorescence unit)

MMP9 Cleavage of T-MASK IL-2^{Sup} Restores IL-2R Signaling Potency



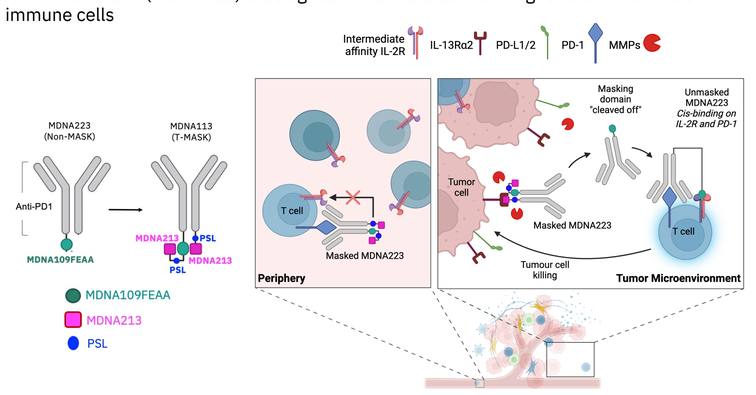
Jurkat IL-2Rβγ bioassay lacking CD25 expression

rMMP9 incubation at 5 µg/mL at 37°C for 1 h

III RESULTS

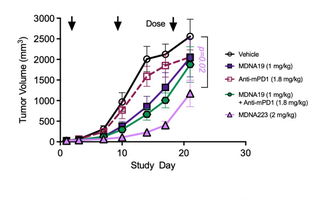
T-MASK of an Anti-PD1-IL-2^{Sup} Immunocytokine

Anti-PD1-IL-2^{Sup} (MDNA223) is designed to facilitate cis—binding to IL-2R and PD1 on immune cells



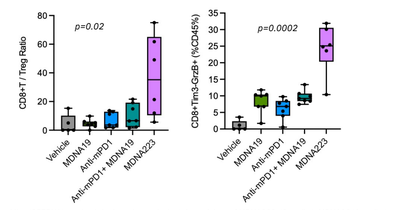
MDNA223 is Superior to Co-administration of Long-acting IL-2^{Sup} + Anti-PD1

Efficacy in B16F10 Melanoma Model



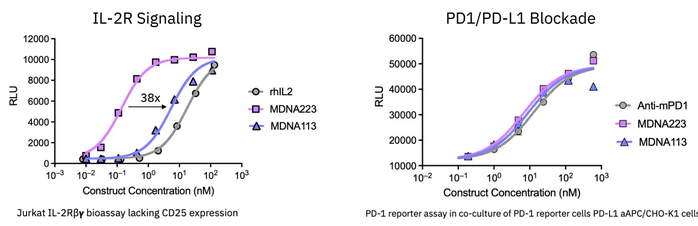
IP treatment QWx3, Avg tumor size at initiation of dosing: 30 mm^3
MDNA19, anti-mPD1 and MDNA223 used at equal molar dose
MDNA19 is a Fc fused MDNA109FEAA long-acting superkine
Statistical analysis by the non-parametric Mann Whitney test (efficacy)

Enhanced Tumor Infiltration of Cytotoxic CD8$^+$ T Cells Expressing Granzyme B



Mice (C57Bl/6) received single molar equivalent dose of dose of MDNA223 (2 mg/kg), MDNA19 (1 mg/kg) and anti-mPD1 (1.8 mg/kg) by IP and samples were collected on Day 7 for analysis
Statistical analysis by Kruskal Wallis Test

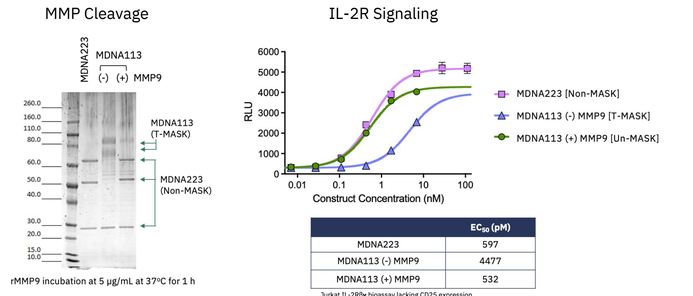
MDNA113 Exhibits Reduced IL-2R Signaling While Maintaining PD1/PDL-1 Blockade



Jurkat IL-2Rβγ bioassay lacking CD25 expression

PD-1 reporter assay in co-culture of PD-1 reporter cells PD-L1 aAPC/CHO-K1 cells.

Proteolytic Cleavage of MDNA113 Restores IL-2R Agonism



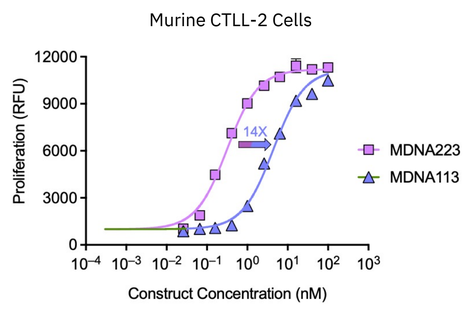
rMMP9 incubation at 5 µg/mL at 37°C for 1 h

	EC_{50} (pM)
MDNA223	597
MDNA113 (-) MMP9	4477
MDNA113 (+) MMP9	532

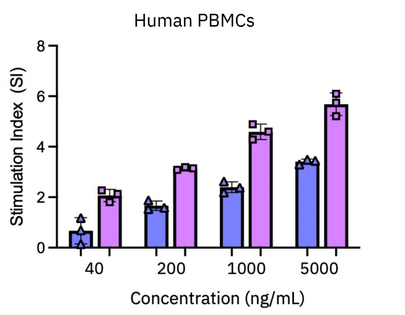
Jurkat IL-2Rβγ bioassay lacking CD25 expression

IV RESULTS

Reduced IL-2 Mediated Proliferation of Mouse and Human Immune Cells



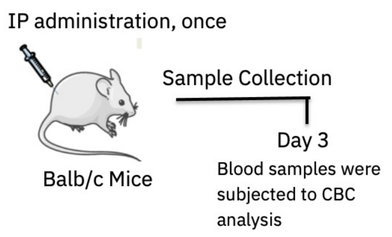
CTLL2 treated with the indicated construct for constructs for 48 hours.
Cell number quantified using Cell Titer Blue viability reagent
(RFU: relative fluorescence unit)

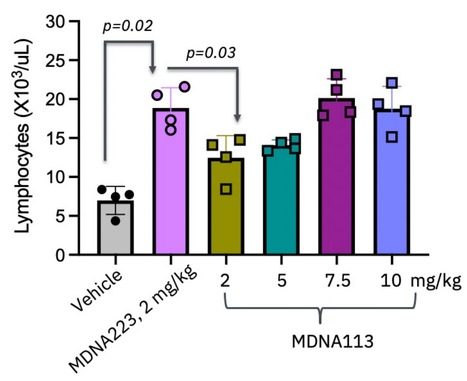


Human PBMCs cultures treated with constructs for 48-hours.
Proliferation measured based on BrdU incorporation by ELISA

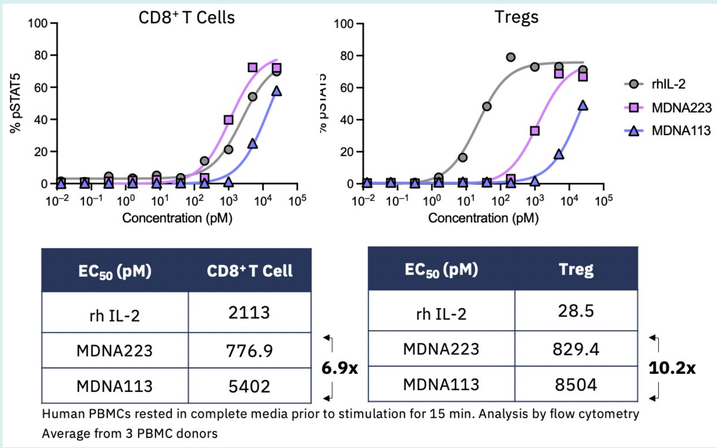
Reduced Stimulation of Lymphocyte Expansion in Mice

Partial blockade of lymphocyte expansion can be rescued by increasing dosage of MDNA113





Reduced pSTAT5 Signaling in Human CD8$^+$ T Cells



EC_{50} (pM)	CD8$^+$T Cell
rh IL-2	2113
MDNA223	776.9
MDNA113	5402

6.9x

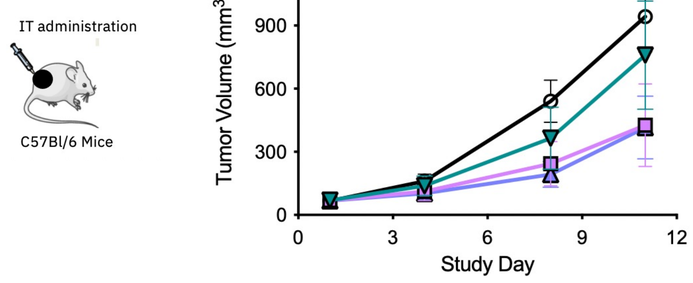
EC_{50} (pM)	Treg
rh IL-2	28.5
MDNA223	829.4
MDNA113	8504

10.2x

Human PBMCs rested in complete media prior to stimulation for 15 min. Analysis by flow cytometry
Average from 3 PBMC donors

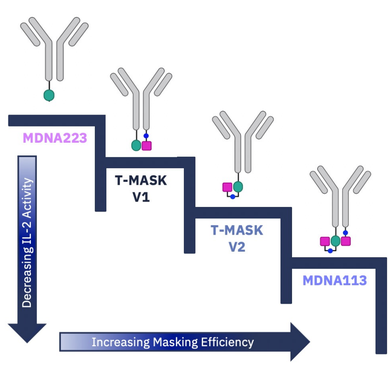
MDNA113 Achieves Similar Efficacy as MDNA223 in MC38 Tumor Model

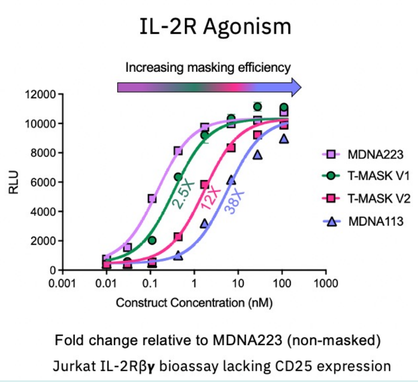
Unmasking of MDNA113 in the TME restores its potency



Avg tumor volume of 40 mm^3 at initiation of dosing;
Dose of 15 ug/tumor by IT injection

Leveraging T-MASK Versatility to Fine-Tune IL-2R Agonism





Fold change relative to MDNA223 (non-masked)
Jurkat IL-2Rβγ bioassay lacking CD25 expression

V SUMMARY

- T-MASK platform integrates 'tumor targeting' with 'conditional activation' to maximize anti-tumor efficacy and minimize systemic toxicity
- MDNA113 (T-MASK of anti-PD1-IL-2^{Sup}) shows reduced IL-2R agonism with no change to PD1/PDL-1 blockade
- MMP cleavage of MDNA113 and T-MASK IL-2^{Sup} restored IL-2R signaling
- MDNA113 (T-MASK) reduces systemic lymphocyte expansion
- MDNA113 (T-MASK) is as effective as non-masked MDNA223 in tumor models
- T-MASK platform offers opportunity to target and fine-tune immune cell stimulation in TME to improve therapeutic index