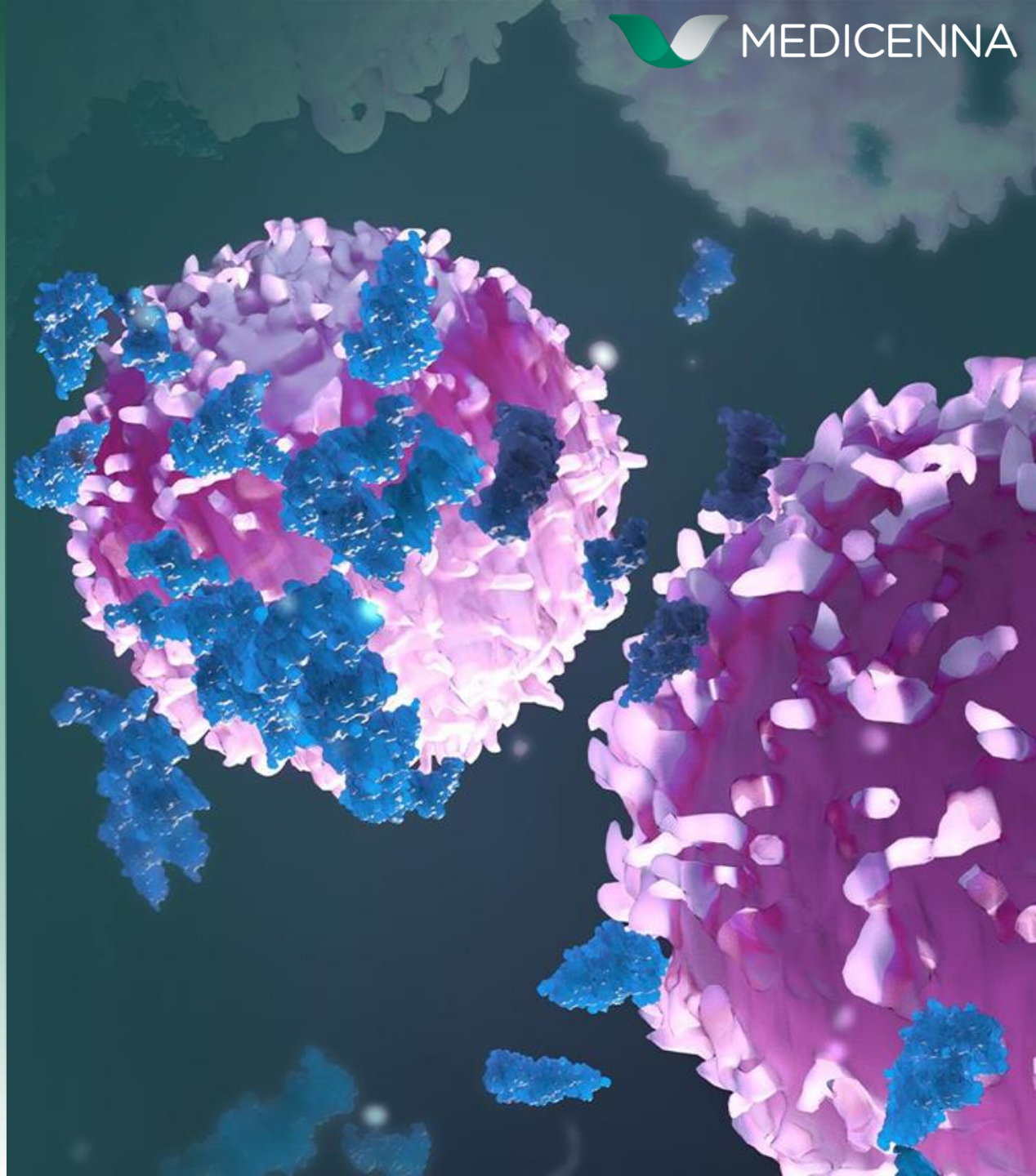


Characterization of MDNA113, a Tumor-Targeting Anti-PD1-IL-2SK Immunocytokine with Conditional Activation to Increase Tolerability and Maximize Efficacy

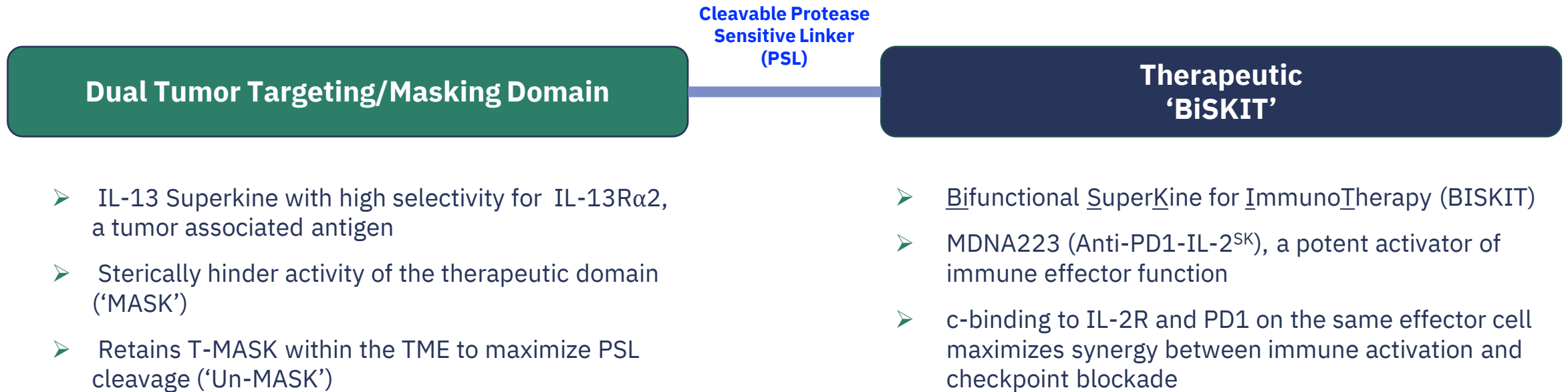
Aanchal Sharma, Minh D. To, Qian Liu, and Fahar Merchant



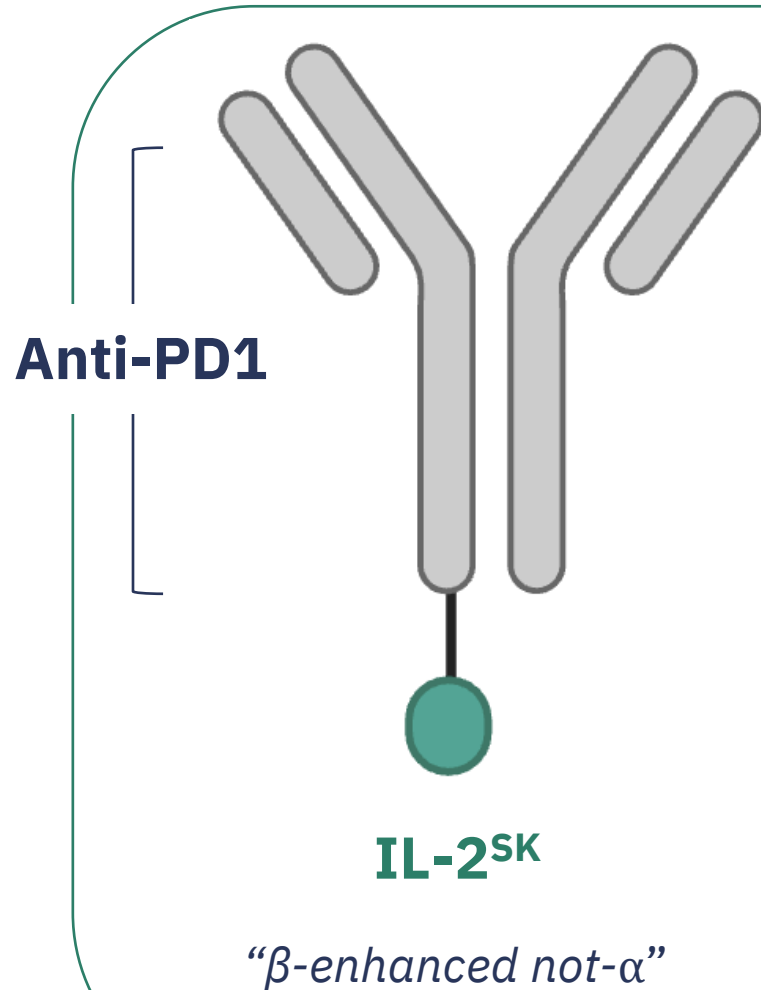
Distinctive Features of the T-MASK Platform

T-MASK (Targeted Metallo/Protease Activated SuperKine) designed to:

- Minimize risk of systemic toxicity
- Maximize therapeutic activity at the tumor site



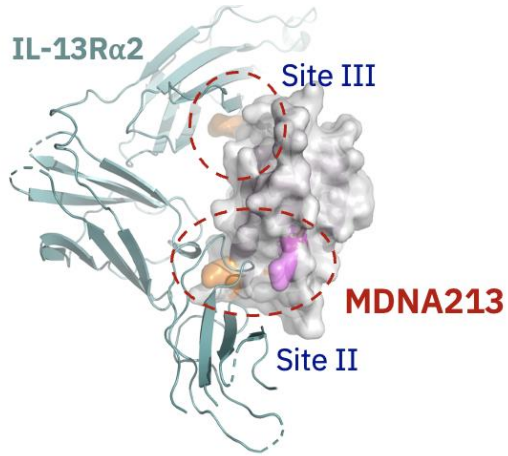
MDNA223, an Anti-PD1-IL-2^{SK} BiSKIT



- engineered to promote cis-binding to IL-2R and PD-1 receptors on the same effector immune cell
- designed to maximize synergy between IL-2R agonism (potentiates immune response) and PD1/PDL-1 immune checkpoint blockade (prevents immune exhaustion)
- combining 2 potent therapeutics into one immunotherapy

Preferentially activates CD8⁺ T and NK cells with limited Treg increase

MDNA213, an IL-13^{SK} with Highly Selective Affinity for IL-13R α 2



MDNA213

An IL-13 Superkine Targeting IL-13R α 2 for Tumor Targeting and Masking

- High affinity and selectivity for IL-13R α 2 (a tumor associated antigen on a broad range of tumors)
- Acts as a masking domain to hinder engagement of T-MASK with its cognate receptor, thereby limiting signaling
- Retention of T-MASK in IL-13R α 2 expressing tumors promotes cleavage by therein proteases to fully activate its immune stimulatory activity

IL-13R α 2 Positive Cancers Annual World-Wide Incidence > 2M

IL-13R α 2 expression is associated with unfavorable clinical outcomes in multiple cancers; limited expression on normal tissues

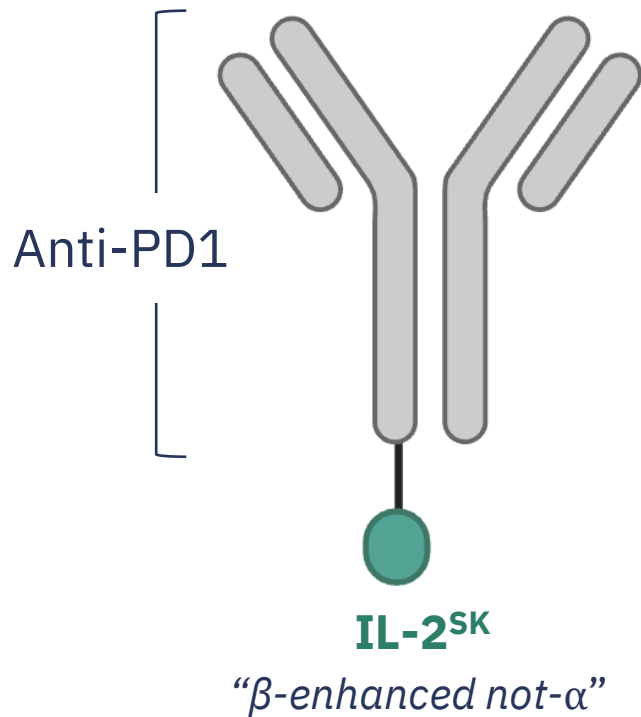
Liver Cancer 82% Hou et al., J Cancer Res & Clinical Oncol (2009)	Breast Cancer 75% Papageorgis et al., Br Cancer Res (2015)	Glioblastoma 75% Joshi et al., Cancer Res (2000)	Ovarian Cancer 75% Kioi et al., Cancer (2006)
Pancreatic Cancer 71% Shimamura et al. Clin Cancer Res (2010)	Colon Cancer 66% Barderas 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)

MDNA113 is a Masked IL-13^{SK} Tumor Targeting Anti-PD1-IL-2^{SK}

T-MASK (Targeted Metallo/Protease Activated SuperKine)

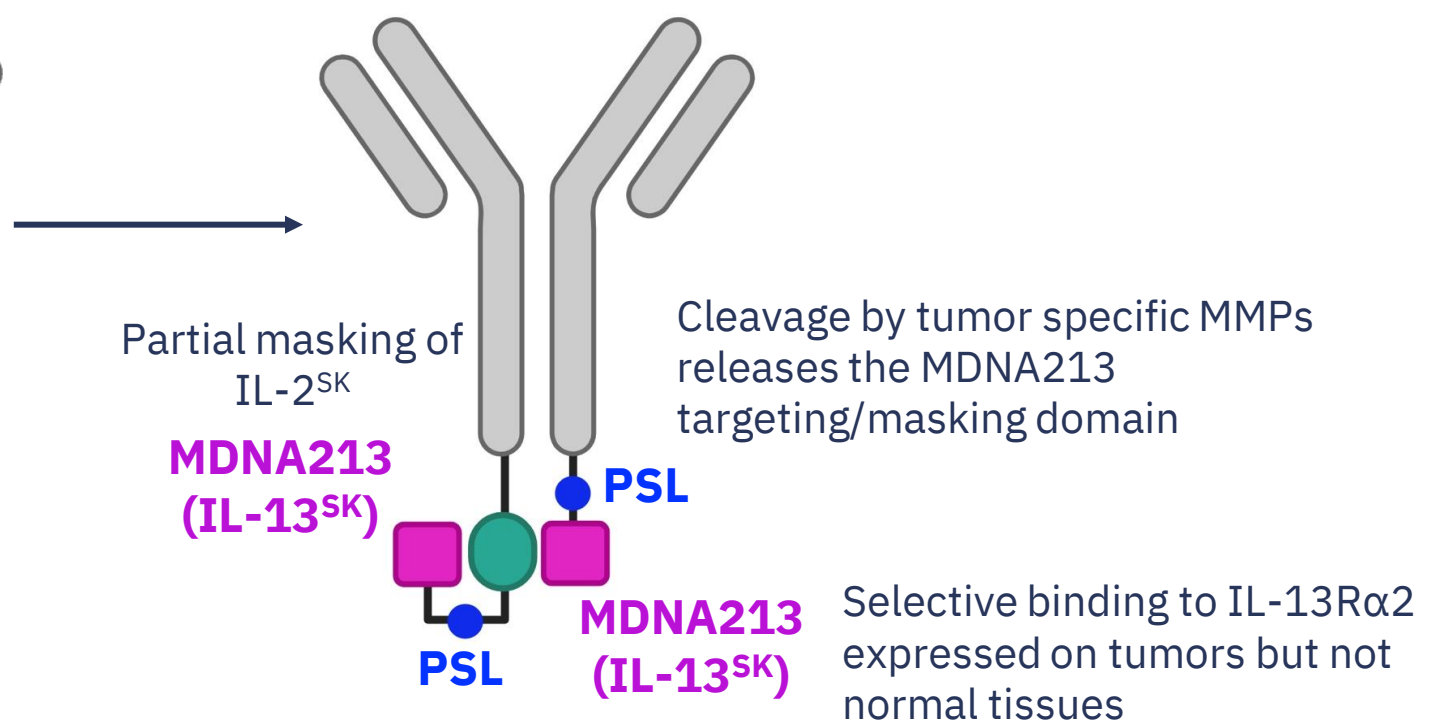
cis-Binding to
PD-1 and IL-2R

MDNA223
(Non-Masked)



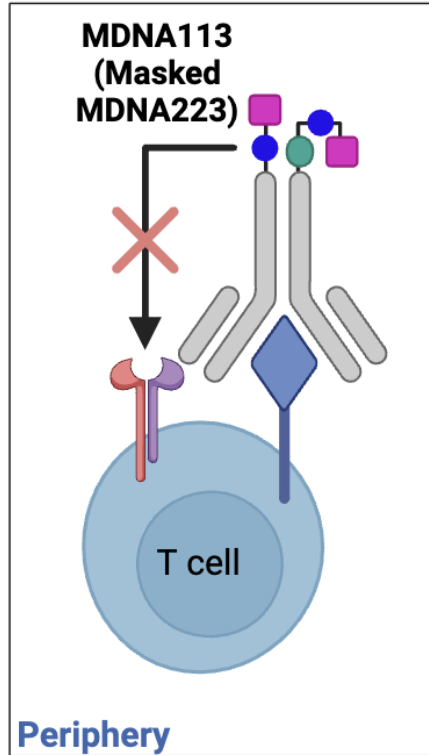
MDNA113
(T-MASK)

*Tumor targeting and
conditional activation*

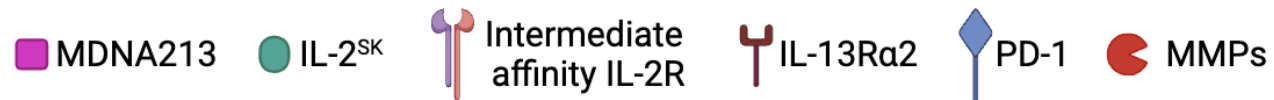
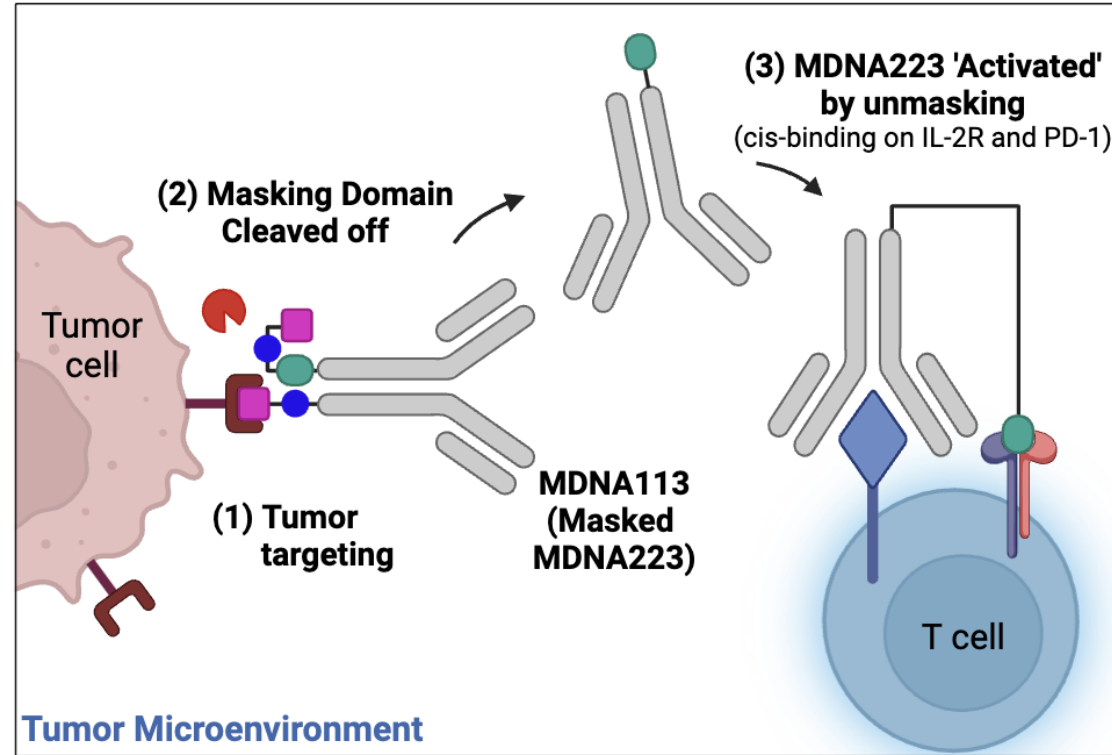


Mechanism of Action

Attenuated IL-2R Stimulation

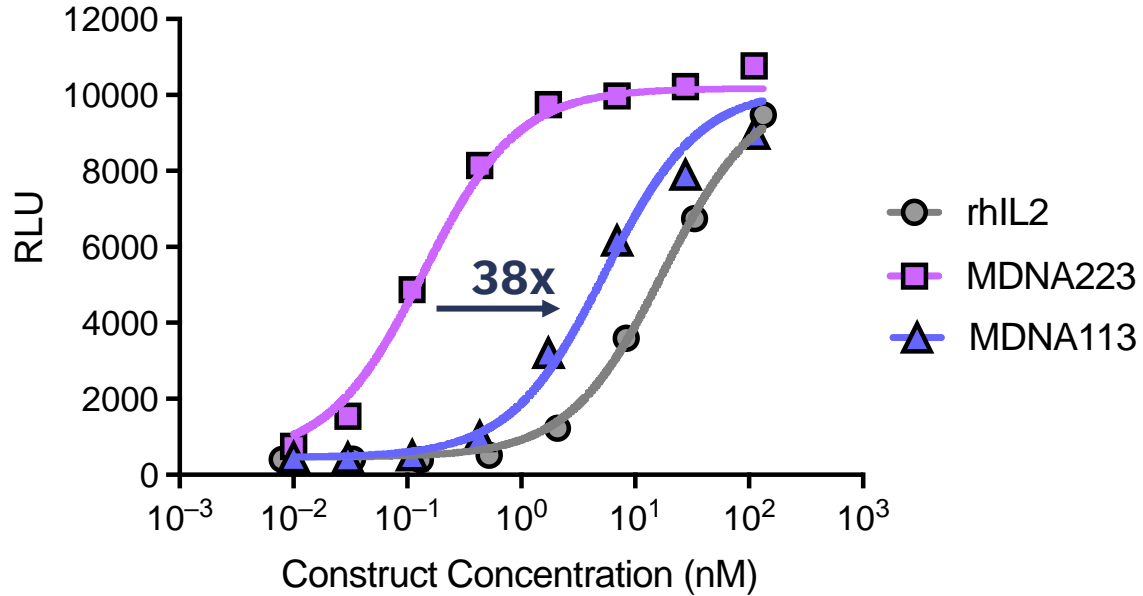


Fully Restored IL-2R Stimulation



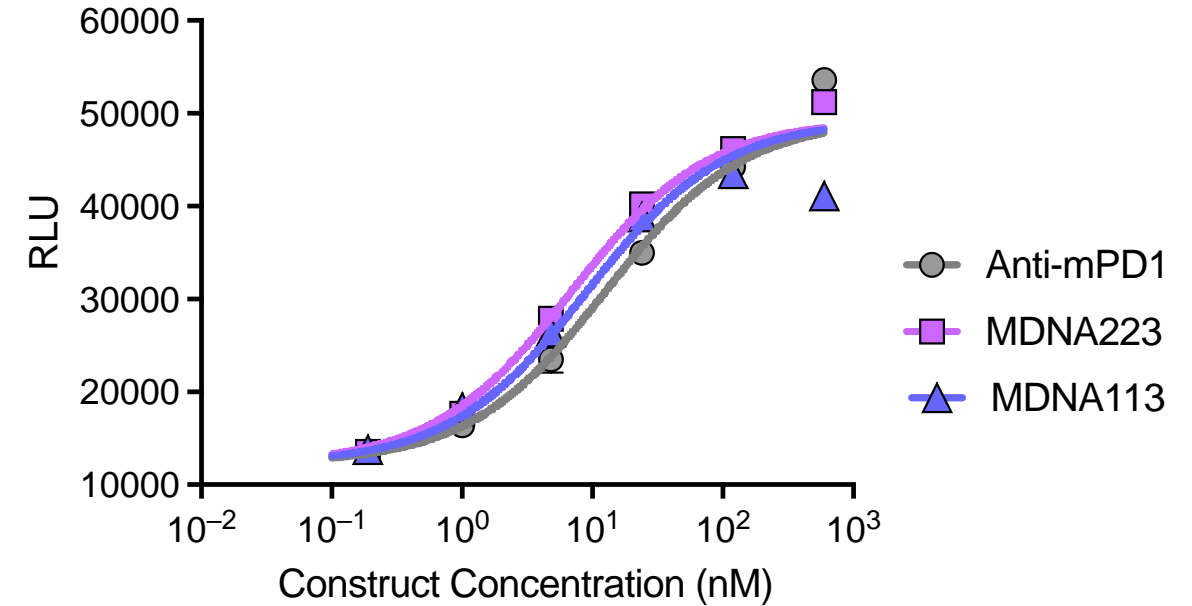
MDNA113: Attenuated IL-2R Signaling with Intact PD1/PDL-1 Immune Blockade

IL-2R Agonism is Attenuated



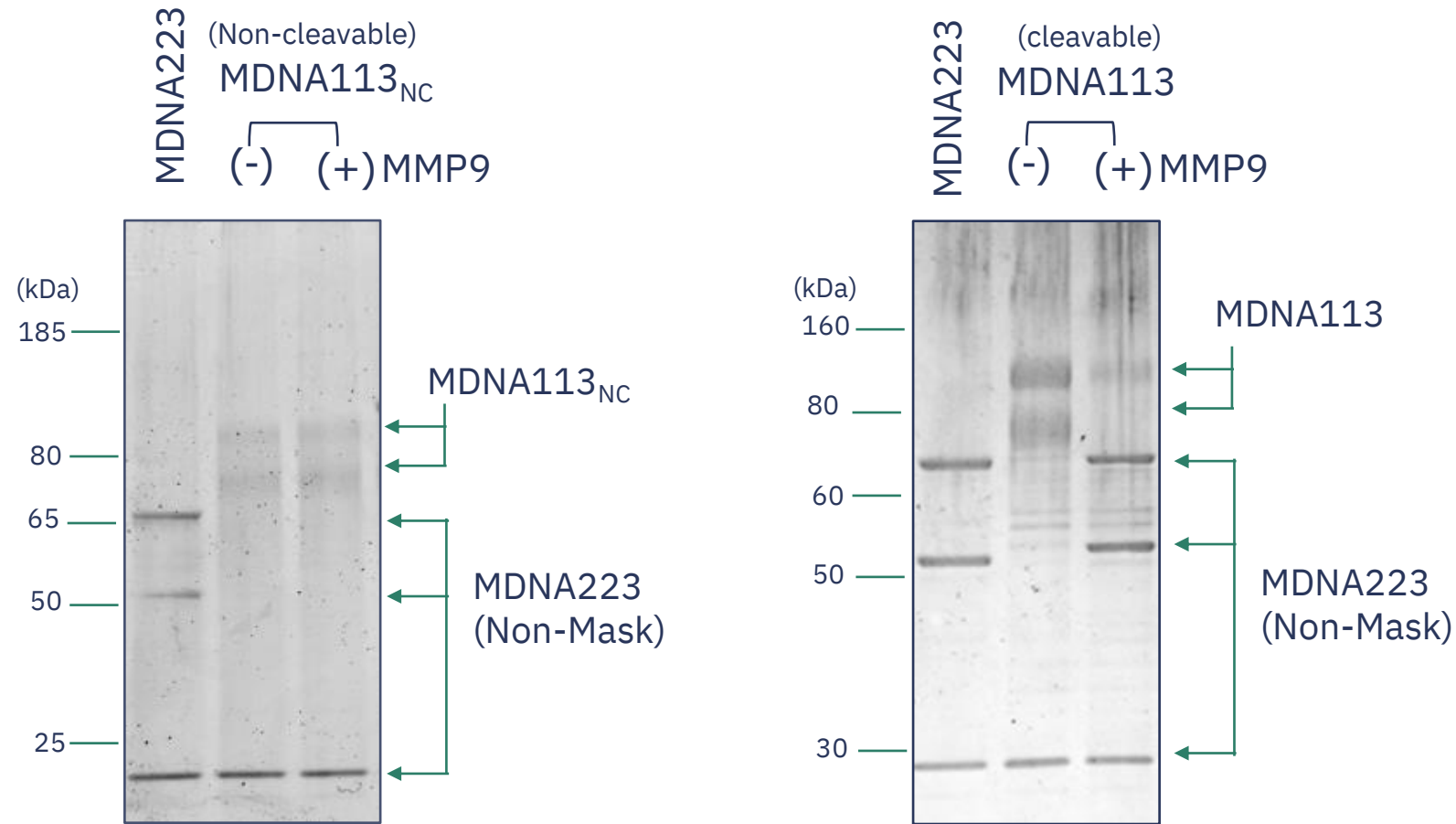
Jurkat IL-2R $\beta\gamma$ bioassay lacking CD25 expression
RLU = relative luminescence unit

PD-1/PDL-1 Blockade is Retained



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

MMP9 Cleavage of MDNA113 Releases the MDNA213 MASK Domain

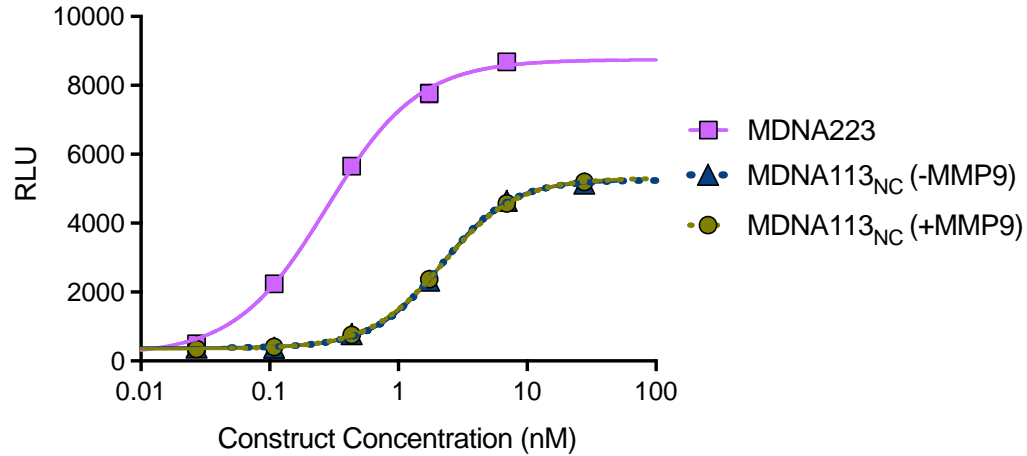


rMMP9 incubation at 5 μ g/mL at 37°C for 1 h

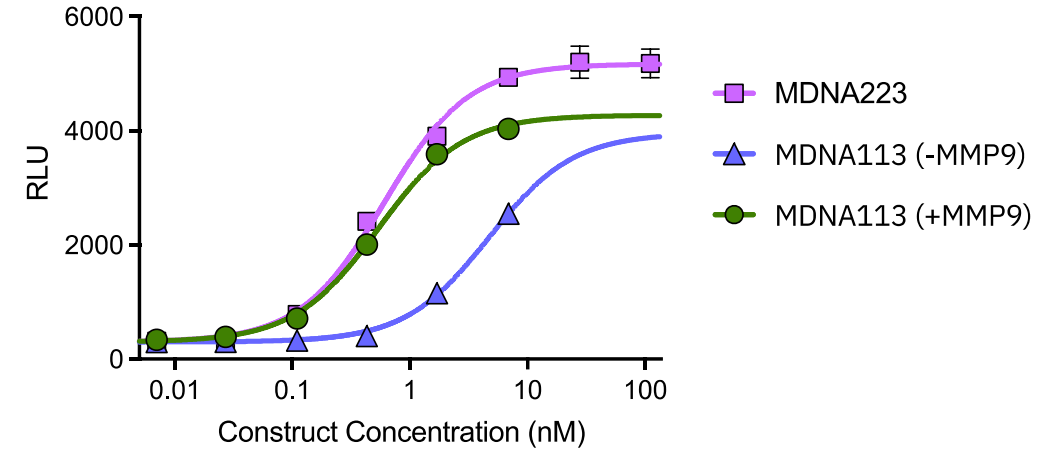
MMP9 completely cleaves MDNA113 but not MDNA113_{NC} (non-cleavable linker)

MMP9 Cleavage Fully Restores IL-2R Agonism to MDNA113

Non-cleavable (MDNA113_{NC})



Cleavable (MDNA113)



	EC ₅₀ (pM)
MDNA223	279
MDNA113 _{NC} (-) MMP9	2176
MDNA113 _{NC} (+) MMP9	2206

	EC ₅₀ (pM)
MDNA223	597
MDNA113 (-) MMP9	4477
MDNA113 (+) MMP9	532

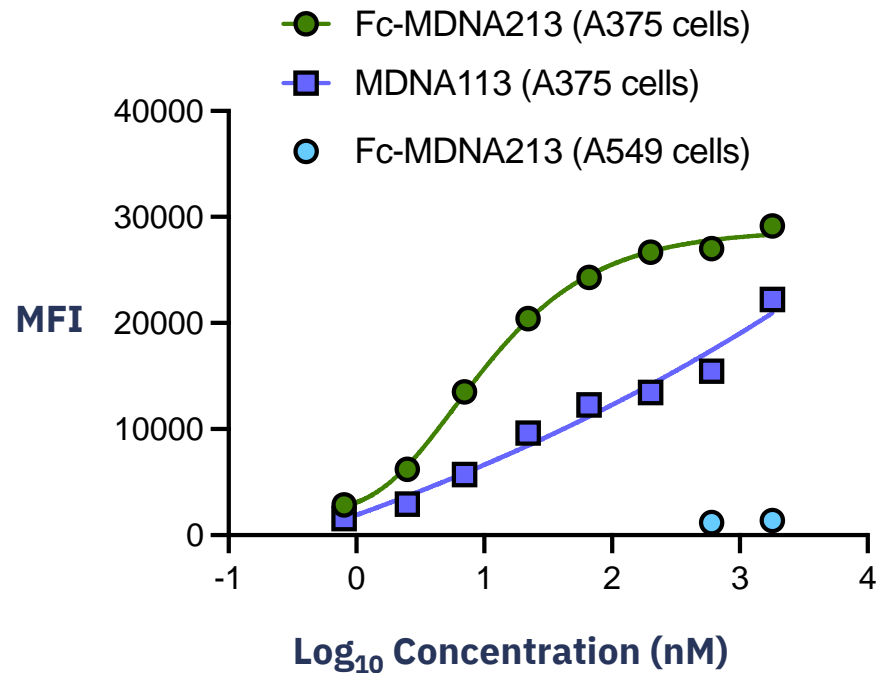
Jurkat IL-2Rβγ bioassay lacking CD25 expression

Proteolytic activation restores full activity of MDNA113 with cleavable linker

Selective and Durable Accumulation in IL-13R α 2 Positive Tumors

Selective binding to IL-13R α 2 positive cells

A375: IL-13R α 2 positive
A549: IL-13R α 2 negative



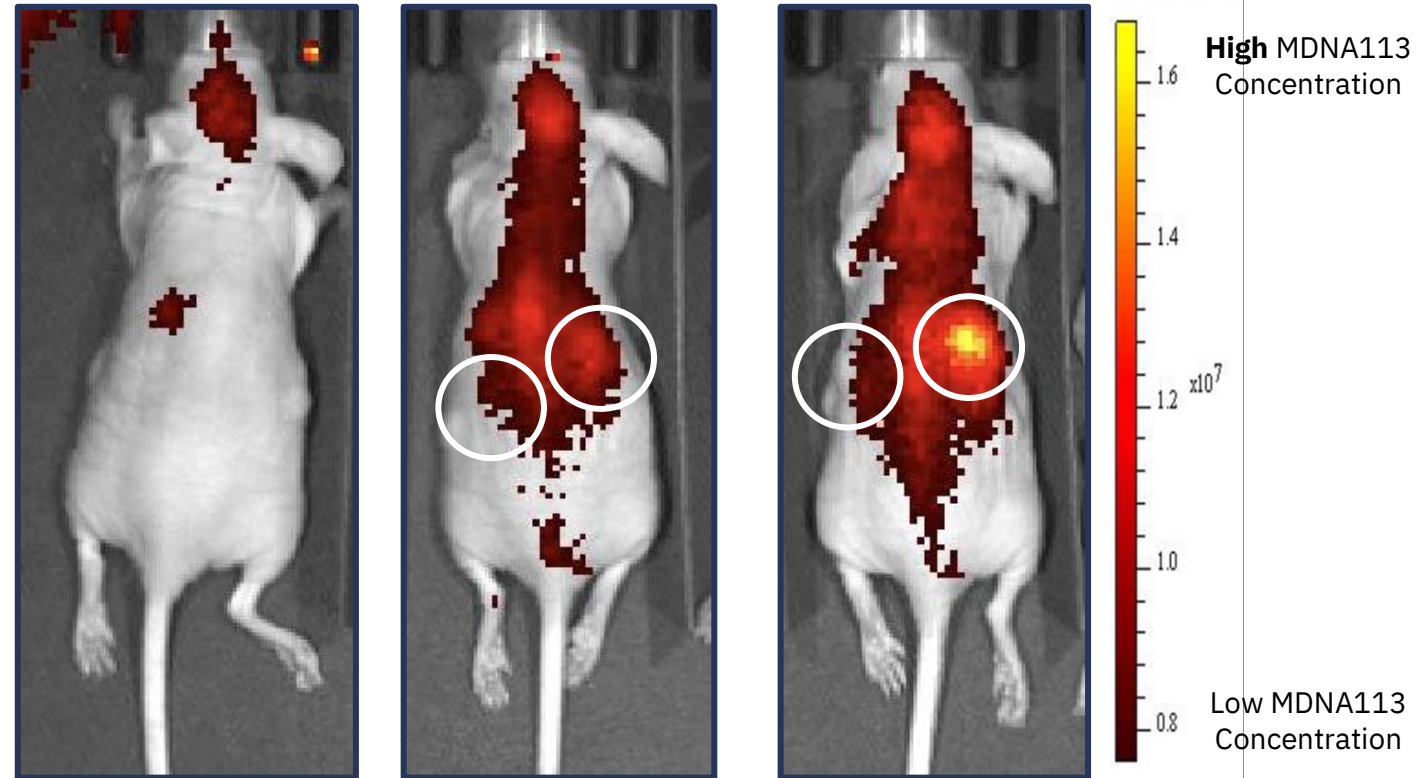
Cell binding studies by flow cytometry
MFI: mean fluorescence intensity

Accumulation in IL-3R α 2 positive tumors for >7 days

Background
(control)

MDNA223
(no targeting)

MDNA113
(tumor targeting)



Left flank: A549
(IL-13R α 2 Negative)

Right flank: A375
(IL-13R α 2 Positive)

Tumor bearing athymic mice were IV injected with a single dose of VivoTag800 labelled MDNA223 or MDNA113 (2 mg/kg)

Masking with MDNA213 Attenuates Peripheral Lymphocyte Expansion in Mice

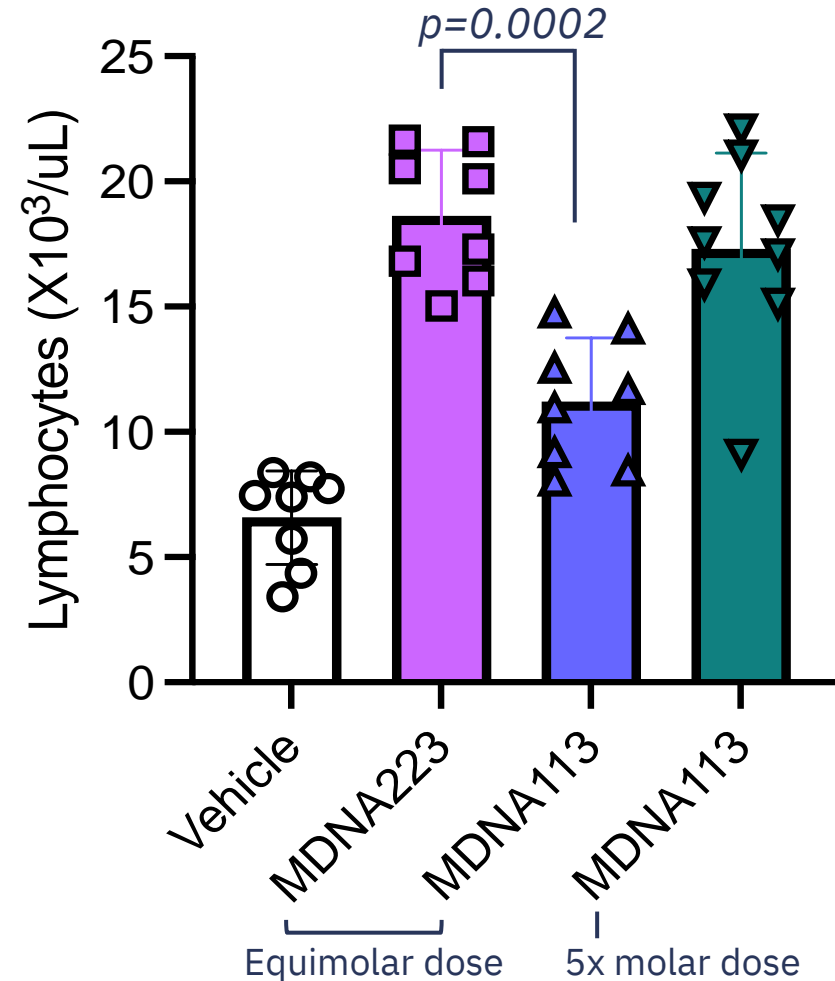
Increased MDNA113 dose overcomes partial masking effect

Single IP administration
(Day 1)

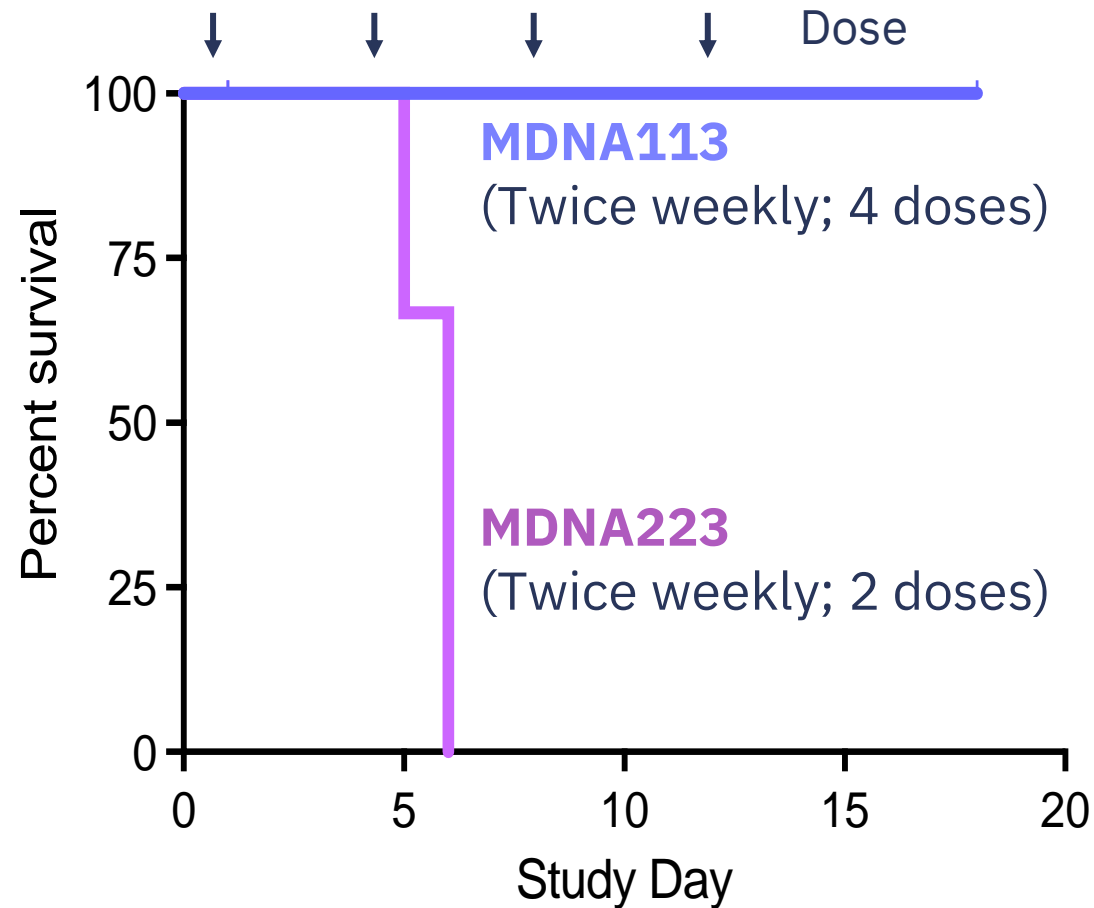


Balb/c Mice

Hematology
Analysis of Whole
Blood
(Day 3)



MDNA113 Demonstrates Greater In Vivo Tolerability



C57Bl/6 mice were treated with equimolar doses of MDNA223 and MDNA113 on a twice weekly schedule

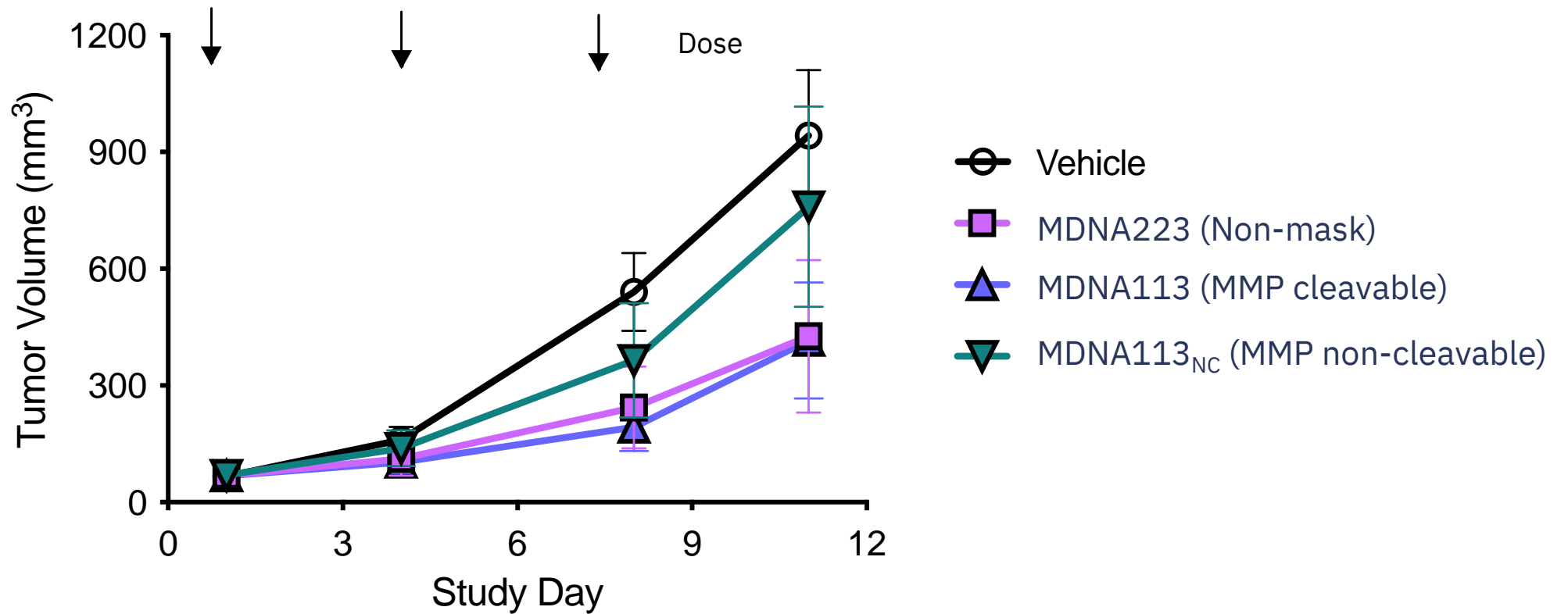
Proteolytic Activation of MDNA113 within Tumors Potentiates In Vivo Efficacy

Intra-tumoral Treatment in MC38 (IL-13R α 2 negative) Tumor Model

IT administration



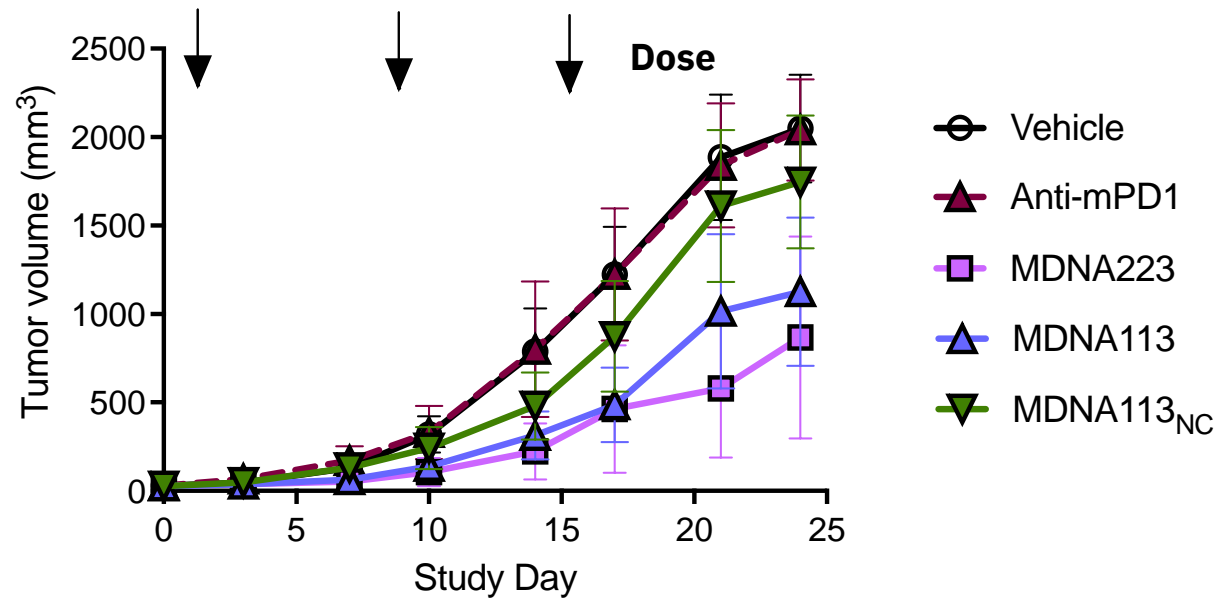
C57Bl/6 Mice



Avg tumor volume of 40 mm³ at initiation of dosing; Dose of 15 ug/tumor by IT injection

Systemic MDNA113 Treatment Shows Potent Tumor Inhibition

Intra-peritoneal Treatment in MC38 (IL-13R α 2 negative) Tumor Model



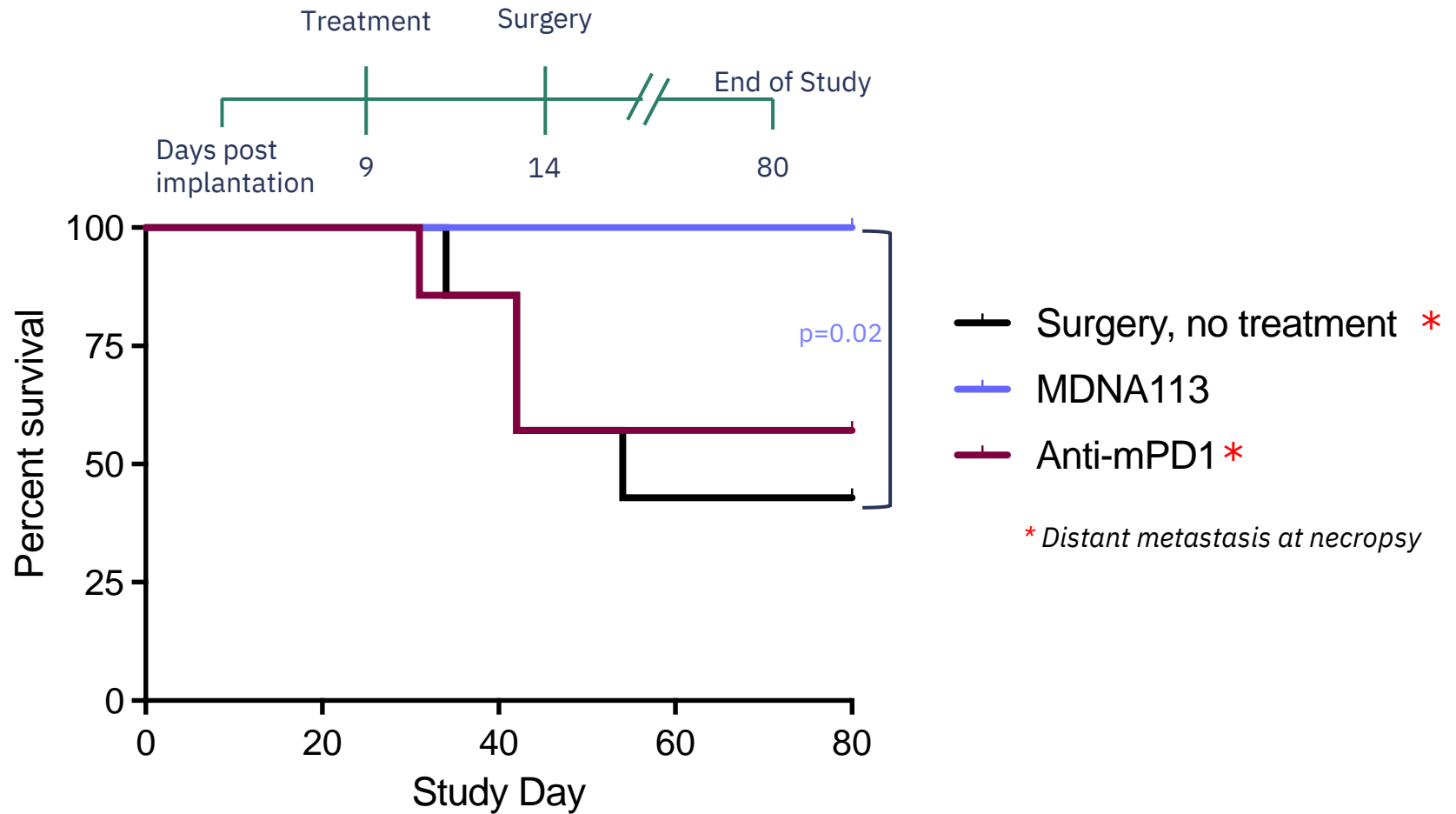
Treatment	Complete Regression
Vehicle	0/15
Anti-mPD1	0/8
MDNA223	1/15
MDNA113	7/15
MDNA113 _{NC}	0/8

Avg tumor volume of 30 mm³ at initiation of dosing; All dosed once weekly at molar equivalent doses

From 2 independent studies

Single Neo-adjuvant Treatment with MDNA113 Provides Survival Benefit

4T1.2 (IL-13R α 2 negative) Orthotopic Breast Cancer Model



Equimolar doses of MDNA113 and Anti-mPD1 were administered, IP.

Summary

- MDNA113 exhibits attenuated IL-2R stimulation without altering PD1/PDL-1 blockade activity *in vitro*.
- MMP cleavage of MDNA113 releases the MASK domain (MDNA213), restoring IL-2R signaling *in vitro*.
- MDNA113 selectively binds IL-13R α 2 positive tumor cells *in vitro* and durably accumulates (>7 days) in IL-13R α 2 positive tumors in mice.
- MDNA113 is better tolerated than non-masked counterpart (MDNA223), supporting higher dose and more frequent dosing schedule.
- Cleavable MDNA113 shows similar efficacy as non-masked MDNA223, consistent with proteolytic activation within TME.
- Single neoadjuvant treatment with MDNA113 in a highly invasive orthotopic 4T1.2 breast cancer model significantly increases survival by preventing metastasis.
- T-MASK is a highly versatile platform with unique tumor targeting and conditionally activatable features to mitigate risk of systemic toxicity and maximize therapeutic activity at tumor site