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

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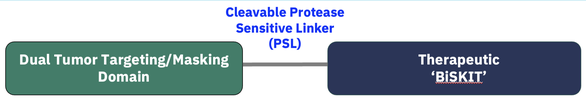
Distinctive Features of the T-MASK Platform

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

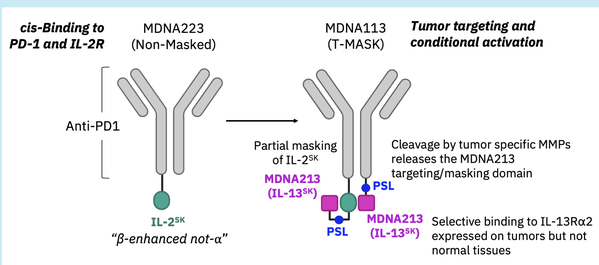
- **Minimize risk of systemic toxicity**
- **Maximize therapeutic activity at the tumor site**



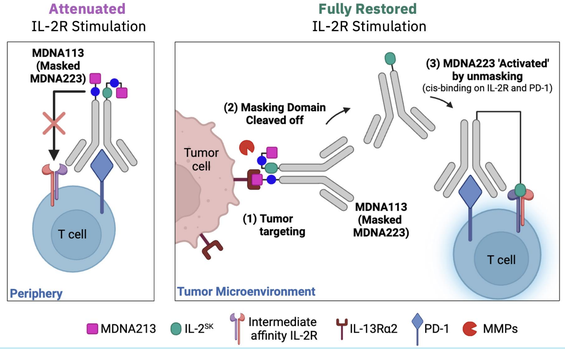
- IL-13 Superkine with high selectivity for IL-13Rα2, a tumor associated antigen
- Sterically hinder activity of the therapeutic domain ('MASK')
- Retains T-MASK within the TME to maximize PSL cleavage ('Un-MASK')

- Bifunctional SuperKine for ImmunoTherapy
- MDNA223 (Anti-PD1-IL-2^{SK}), a potent activator of immune effector function
- c-binding to IL-2R and PD1 on the same effector cell maximizes synergy between immune activation and checkpoint blockade

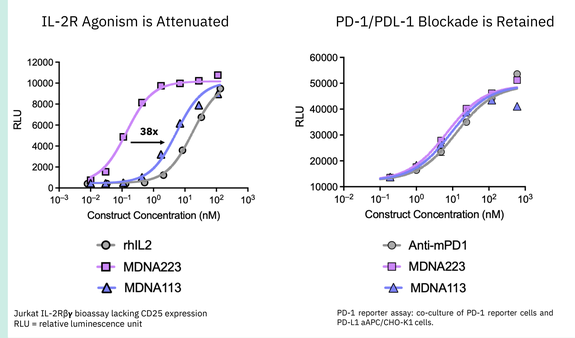
MDNA113 is a Masked IL-13^{SK} Tumor Targeting Anti-PD1-IL-2^{SK}



Mechanism of Action



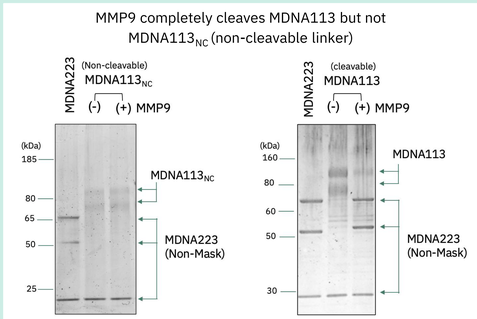
MDNA113: Attenuated IL-2R Signaling with Intact PD-1/PDL-1 Immune Blockade



Jurkat IL-2Rβγ bioassay lacking CD25 expression
RLU = relative luminescence unit

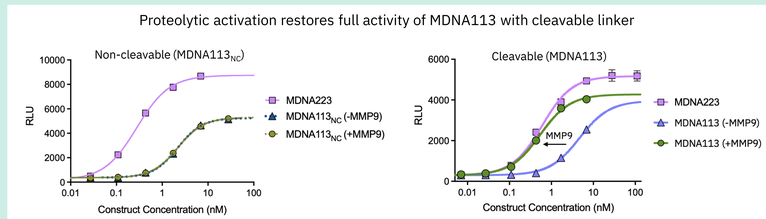
PD-1 reporter assay: co-culture of PD-1 reporter cells and PD-L1 aAPC/CHO-K1 cells.

MMP9 Cleavage of MDNA113 Releases the MDNA213 MASK Domain



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

MMP9 Cleavage Fully Restores IL-2R Agonism to MDNA113

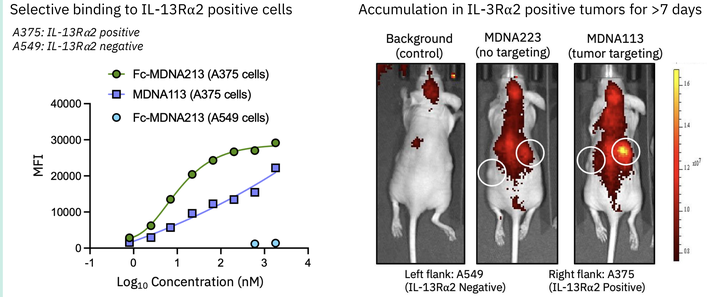


	EC_{50} (pM)
MDNA223	279
$MDNA113_{NC}$ (-) MMP9	2176
$MDNA113_{NC}$ (+) MMP9	2206

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

Jurkat IL-2Rβγ bioassay lacking CD25 expression

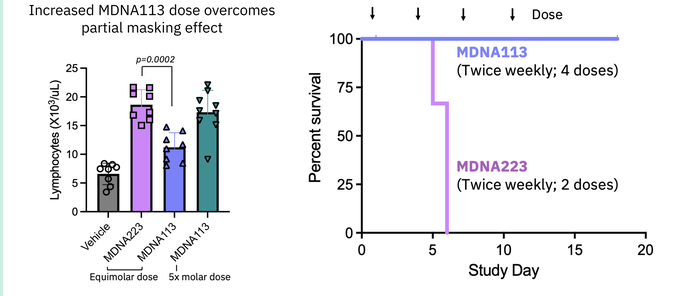
Selective and Durable Accumulation of MDNA113 in IL-13Rα2 Positive Tumors



Cell binding studies by by flow cytometry
MFI: mean fluorescence intensity

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

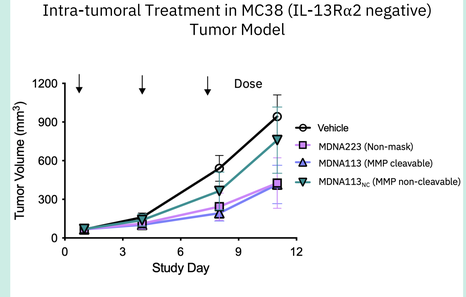
Masking with MDNA213 Attenuates Peripheral Lymphocyte Expansion and Demonstrates Greater In Vivo Tolerability



Blood samples were subjected to hematology analysis 3 days post treatment

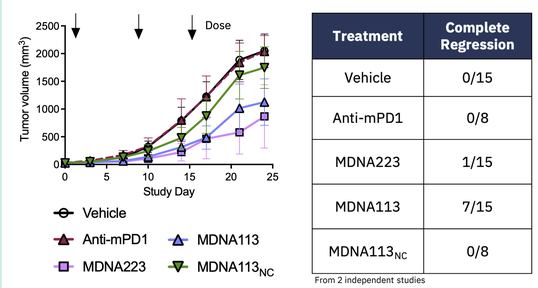
C57Bl/6 mice were treated with equimolar doses of MDNA223 and MDNA113 on a twice weekly schedule

Proteolytic Activation of MDNA113 within Tumors Potentiates In Vivo Efficacy



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

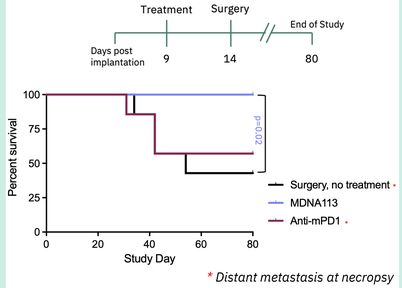


Treatment	Complete Regression
Vehicle	0/15
Anti-mPD1	0/8
MDNA223	1/15
MDNA113	7/15
$MDNA113_{NC}$	0/8

From 2 independent studies

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

Single Neo-adjuvant Treatment with MDNA113 Provides Survival Benefit



* *Distant metastasis at necropsy*

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-13Rα2 positive tumor cells *in vitro* and durably accumulates (>7 days) in IL-13Rα2 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

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