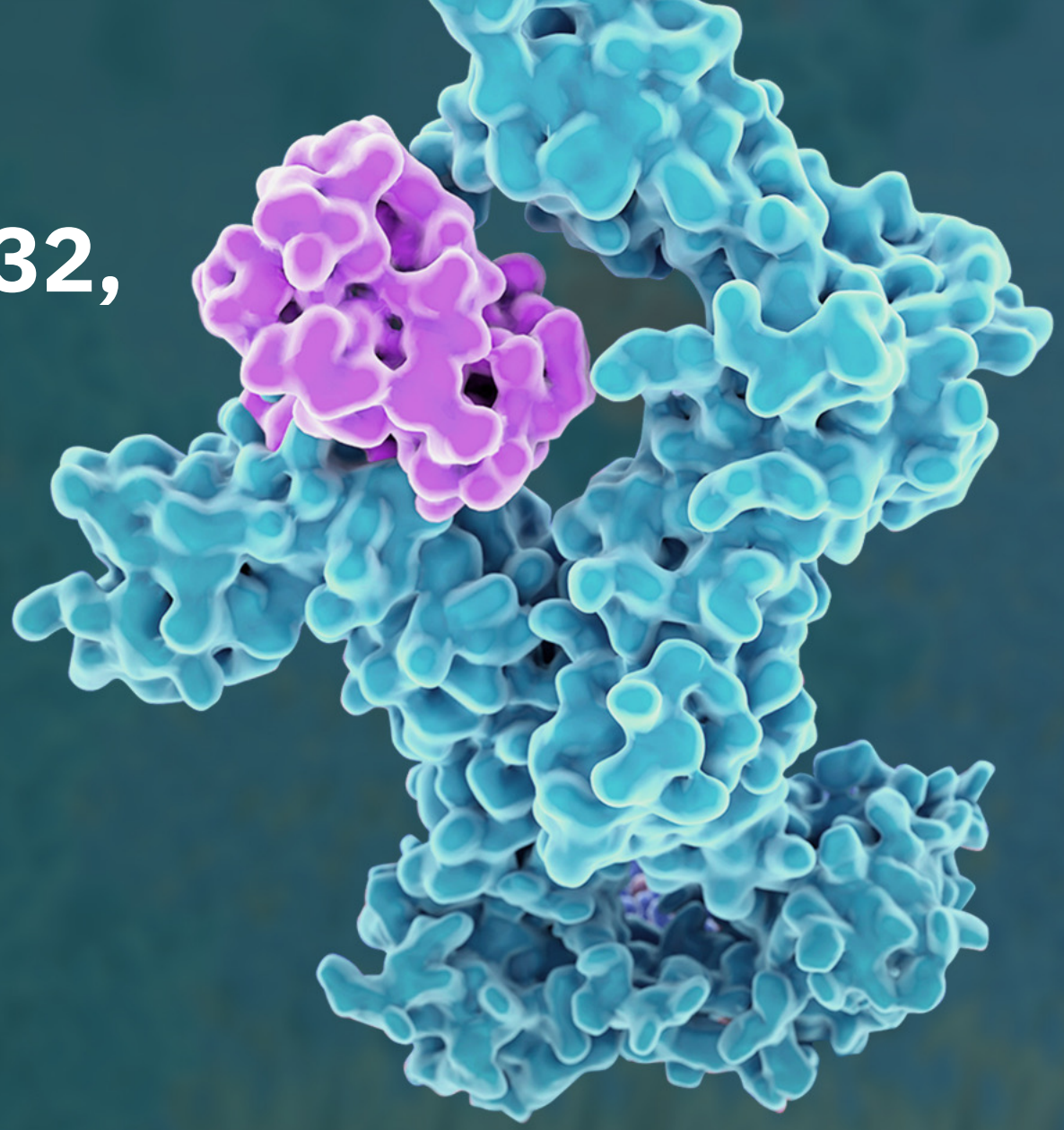
AACR 2023

Characterization of MDNA132, an IL-13 Decoy Receptor Selective Superkine for Targeted Delivery of Immunotherapies to the Tumor Microenvironment



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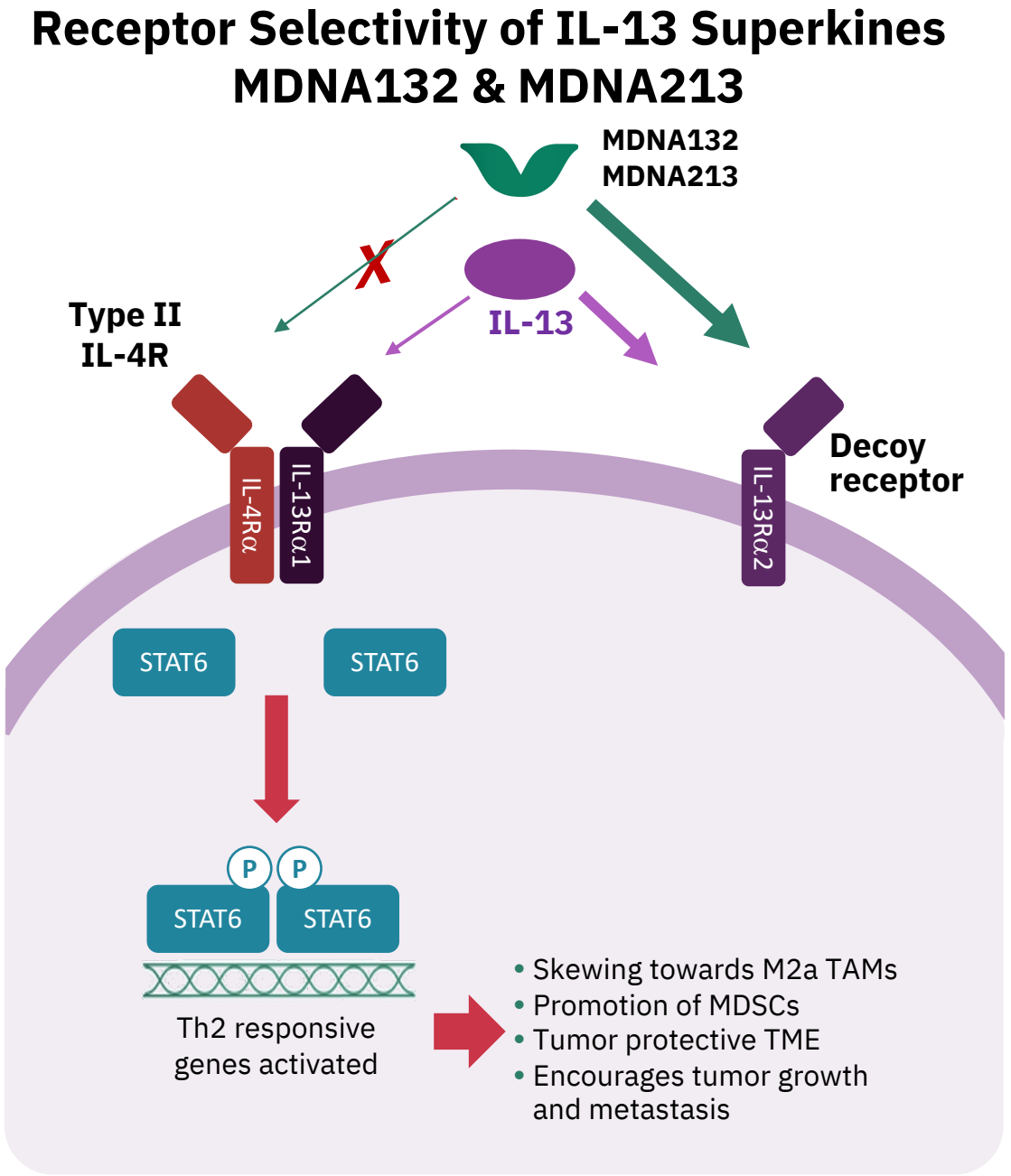
Overview of IL-13 Pathway in Cancer

- IL-13 signals through Type II (IL-4Rα/ IL-13Rα1) receptors to promote Th2 responsive genes and an immune-suppressive TME.
- IL-13 also binds with high affinity to IL-13α2 decoy receptor, but exact function of this receptor remains unclear.



Targeting IL-13Rα2: A Highly Selective Tumor Associated Antigen

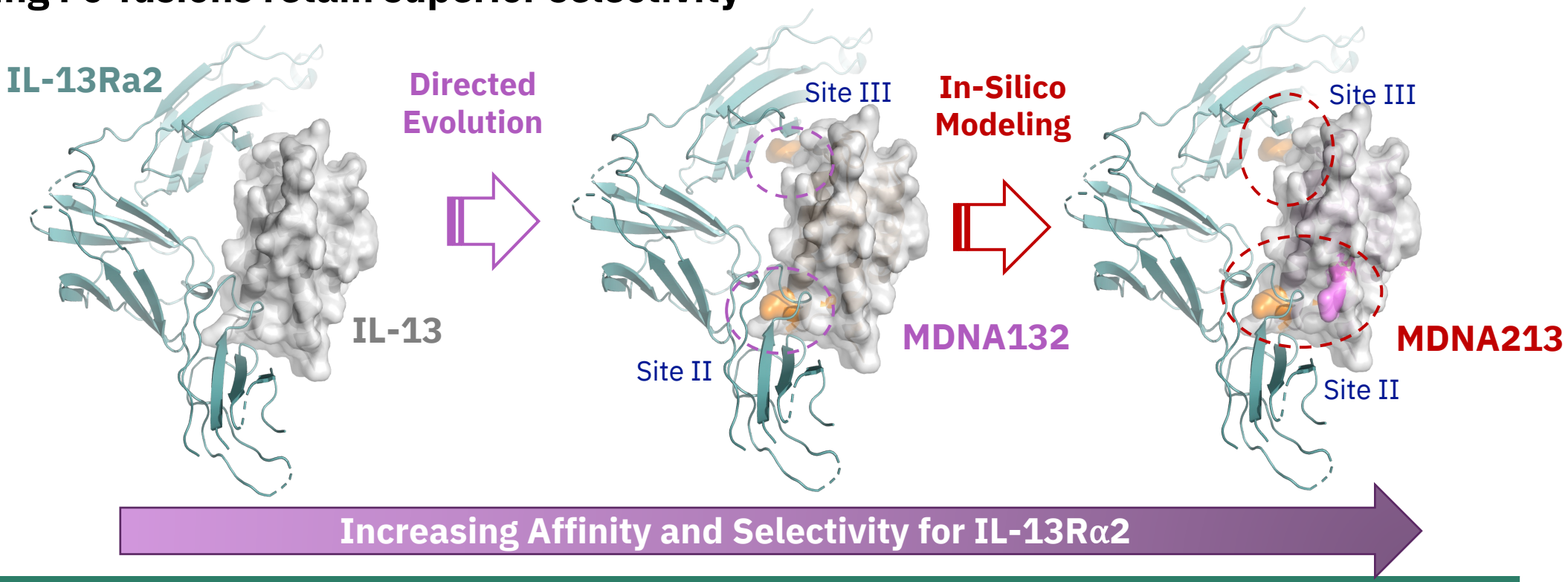
- IL-13Rα2 is overexpressed in a wide range of solid tumors with no or minimal expression in normal tissues
- High IL-13Rα2 correlates with cancer invasion, metastasis and poor survival
- IL-13Rα2 has gained momentum as an attractive target given its tumor specificity, pro-metastatic properties and high expression in immune suppressed “cold tumors”

Targeting IL-13Rα2 enables highly selective delivery of therapeutic payloads or immunotherapies to tumors

Tumors over-expressing IL-13Rα2 (>25%)
Pancreatic Cancer
Prostate Cancer
Colorectal Cancer
Triple Negative Breast Cancer
Bladder Cancer
Lung cancer
Mesothelioma
Head & Neck Cancer
Ovarian Cancer
Glioblastoma

IL-13 Superkines Have Better Selectivity Than IL-13

Long-acting Fc-fusions retain superior selectivity



K_D (nM)	Fc-IL13	Fc-MDNA132	Fc-MDNA213
IL-13Rα1	202	No binding*	No binding*
IL-13Rα2	0.7	2.5	0.3

SPR performed on immobilized ligands with receptors as flow analytes using MCK

*Tested at >1000 nM

MDNA213 in complex with IL-13Ra2: Model built based on PDB ID 3LB6

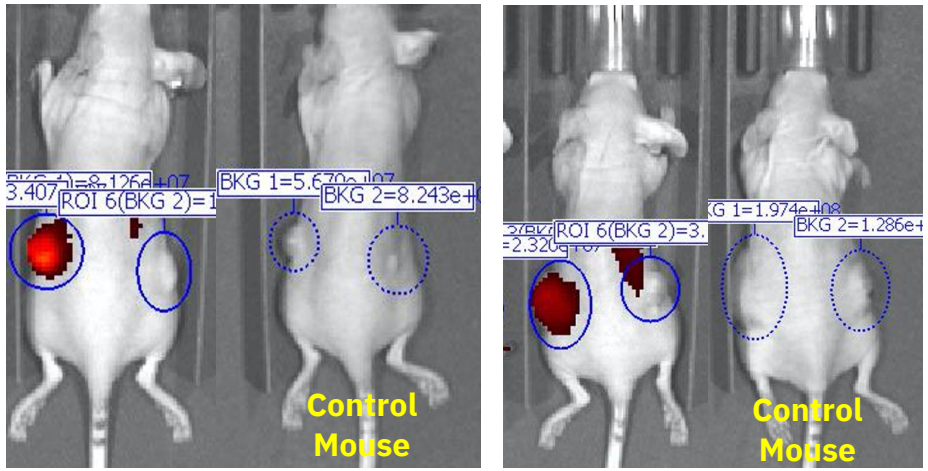
Precise and Durable Localization of IL-13 Superkines in IL-13Rα2 Tumors

Selective accumulation of labelled Fc-MDNA132 and Fc-MDNA213 in IL-13Rα2 expressing tumors

Fc-MDNA132

Human Tumor Xenografts

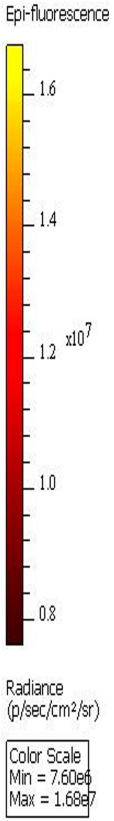
120 h Post Dose



Left Tumor: A375 (Melanoma)
Expresses IL-13Rα2

Left Tumor: U87 (Glioblastoma),
Expresses IL-13Rα2

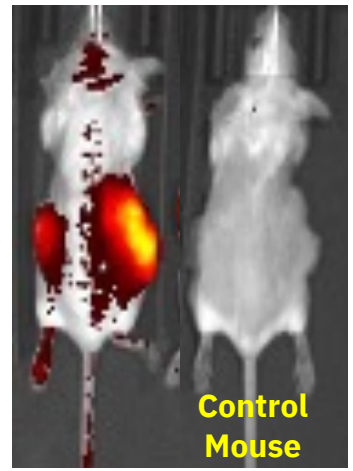
Right Tumors: A549, Low Expression of IL-13Rα2



Fc-MDNA213

Mouse Syngeneic Breast Tumors

144 h Post Dose

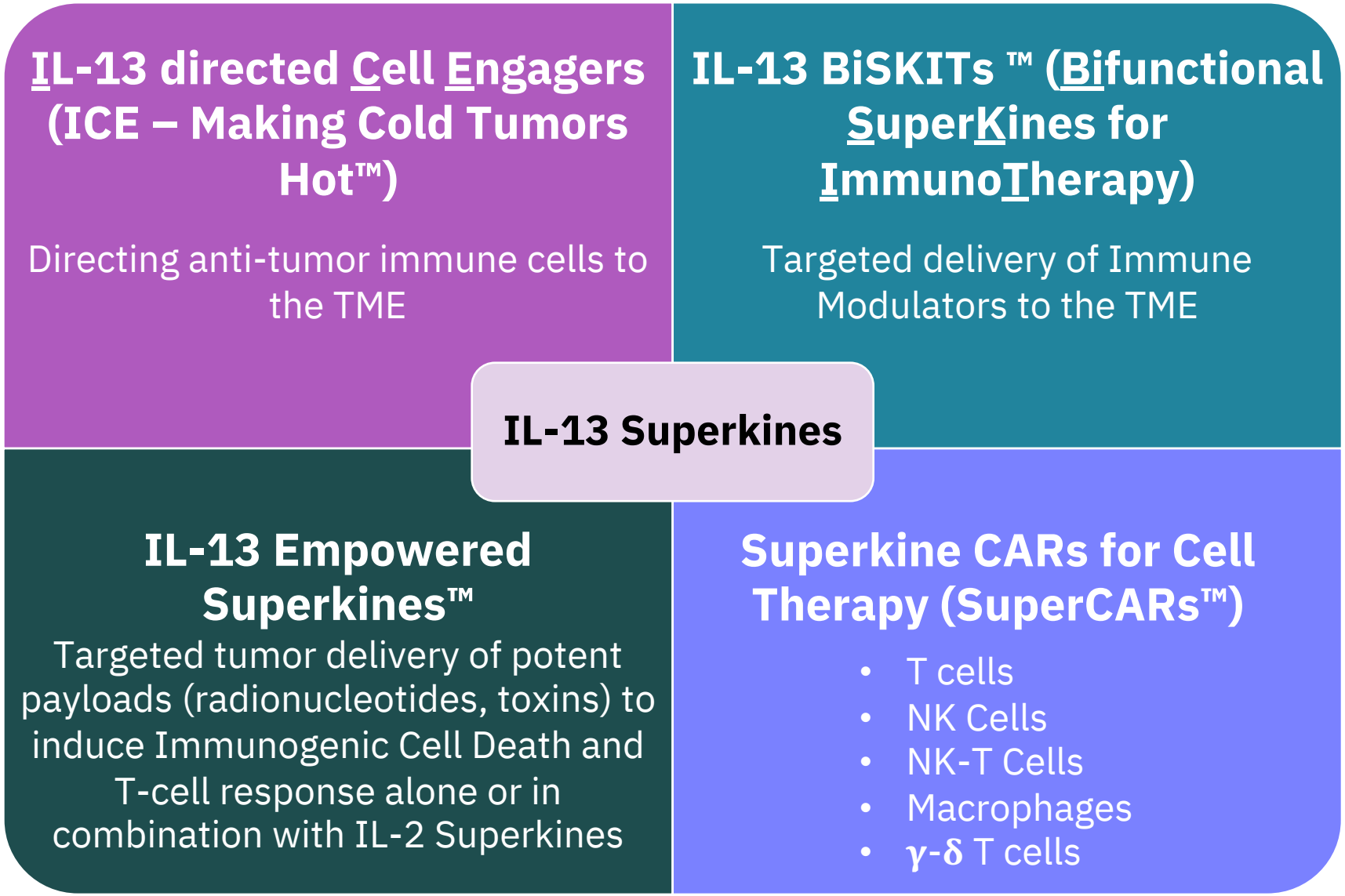


Right Tumor: EMT6-IL13Rα2,
Stable ectopic IL-13Rα2 expression

Left Tumor: EMT6 WT, Low IL-13Rα2

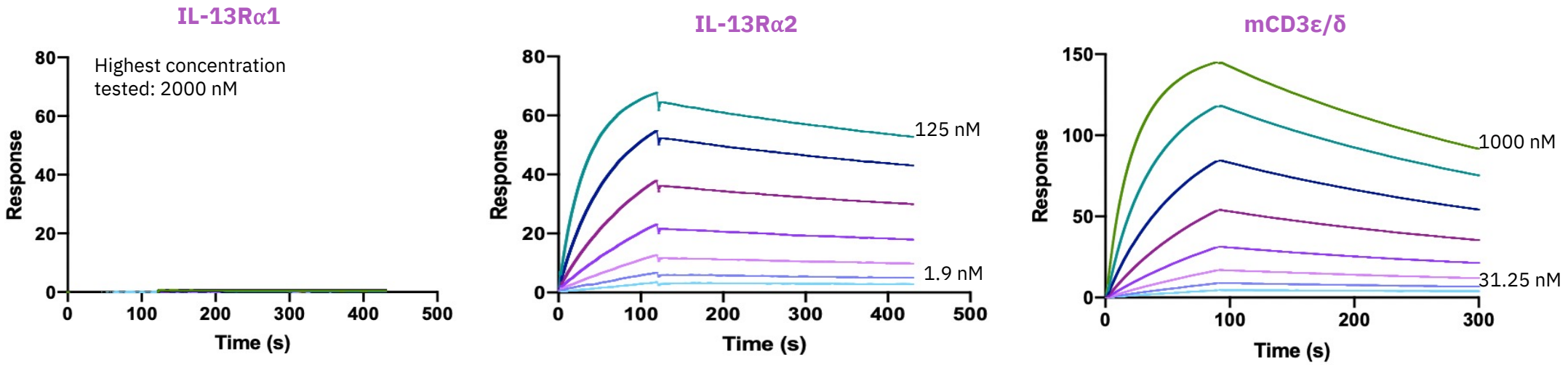
IVIS Images of tumors treated with a single IV dose of VivoTag800 labelled Fc-MDNA132 or Fc-MDNA213.
Control mice were not treated with labeled probe.

Designing Next Generation IL-13 Superkine Therapies



Fusion of MDNA132 with Anti-CD3: An **I**L-13 Directed **C**ell **E**ngager (ICE – Making Cold Tumors Hot™)

Designed to localize CD8⁺ T cell activation within IL-13Rα2 expressing tumors



SPR data K_D (nM)	Anti-mCD3-MDNA132
IL-13Rα1	No binding
IL-13Rα2	3.7
mCD3ε/δ	50.9

SPR performed on immobilized ligands with receptors as flow analytes using MCK

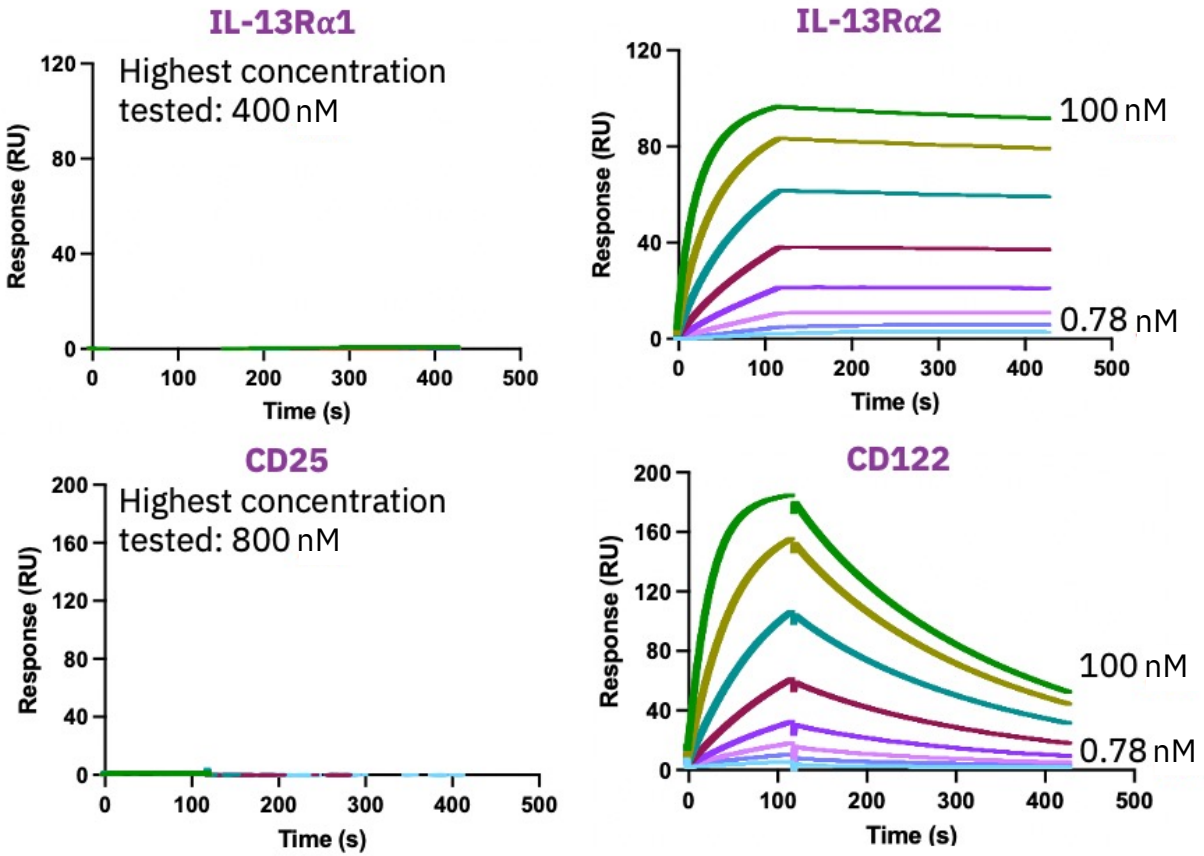
Fusion of MDNA213 with an IL-2 Superkine: Targeted Delivery of Immune Modulators to the Tumor Micro-environment

MDNA19-MDNA213 retains binding properties of both moieties

MDNA19 is an engineered IL-2 superkine with extended half life (via Fc fusion) and enhanced receptor selectivity by:

- Increased affinity to CD122 (enhanced beta)
- No binding to CD25 (non-alpha)

Stimulates expansion and function of $CD8^{+}$T & NK cells in TME

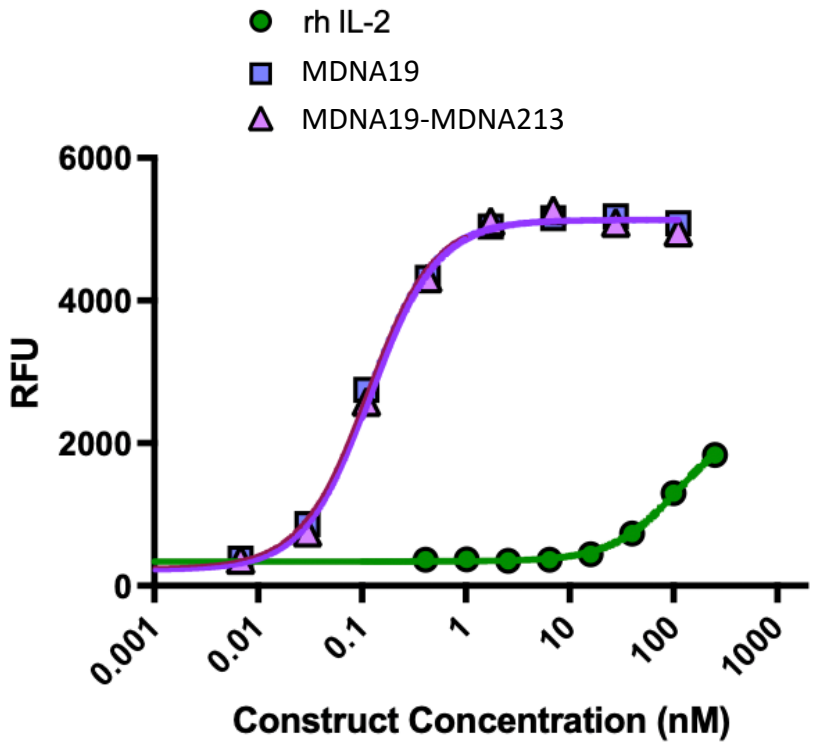


K_D (nM)	IL-13Rα1	IL13Rα2	CD25	CD122
MDNA19-MDNA213	No binding	0.6	No binding	13

SPR performed on immobilized ligands with human receptors as flow analytes using MCK

Fusion of MDNA213 with an IL-2 Superkine: Targeted Delivery of Immune Modulators to the Tumor Micro-environment

MDNA19-MDNA213 potentiates IL-2R signaling and pSTAT5 activity



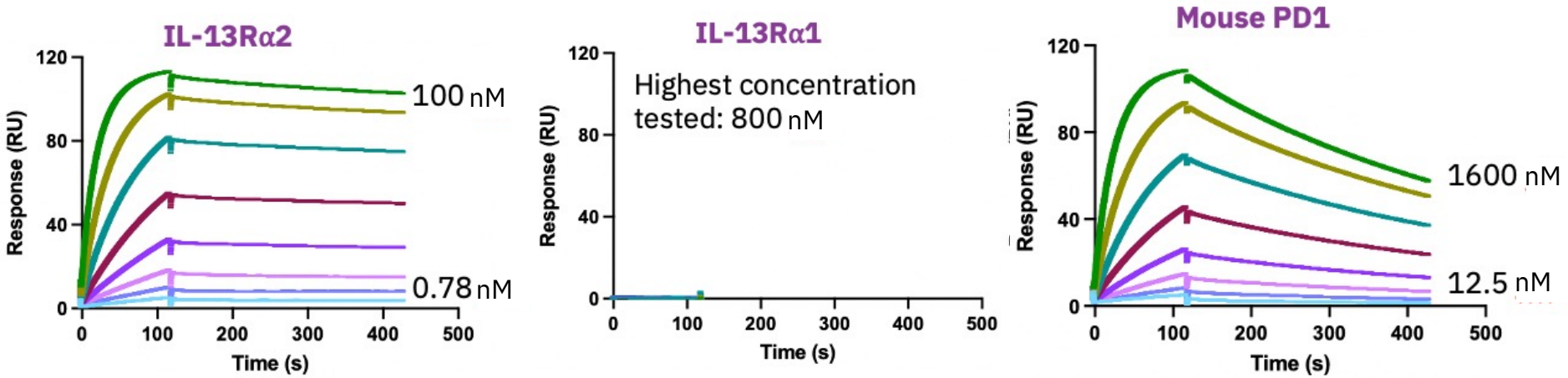
IL-2Rβγ Bioassay	EC_{50} (pM)
MDNA19	110
MDNA19-MDNA213	120

IL-2 Signaling in Promega IL-2Rβγ Bioassay In Jurkat Cells lacking CD25 expression

Fusion of MDNA213 with an Anti-PD1 Antibody: Targeted Delivery of Immune Modulators to the Tumor Micro-environment

Blockade of immune cell checkpoint in the TME to invigorate anti-tumor response

Receptor Binding:

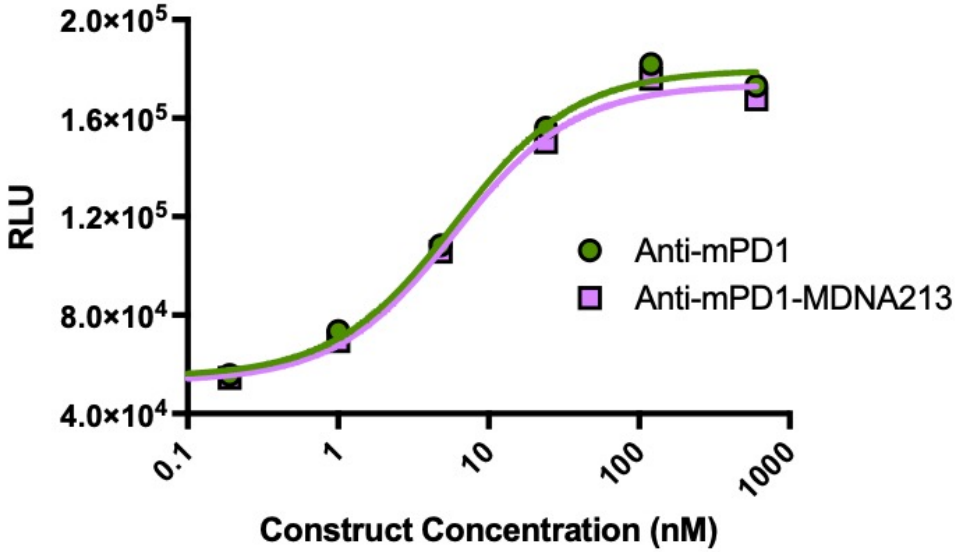


SPR data K_D (nM)	Anti-mPD1-MDNA213
Human IL-13Rα1	No binding
Human IL-13Rα2	0.6
Mouse PD1	87.7

SPR performed on immobilized ligands with receptors as flow analytes using MCK

Fusion of MDNA213 with an Anti-PD1 Antibody: Targeted Delivery of Immune Modulators to the Tumor Micro-environment

Immune checkpoint blockade retained

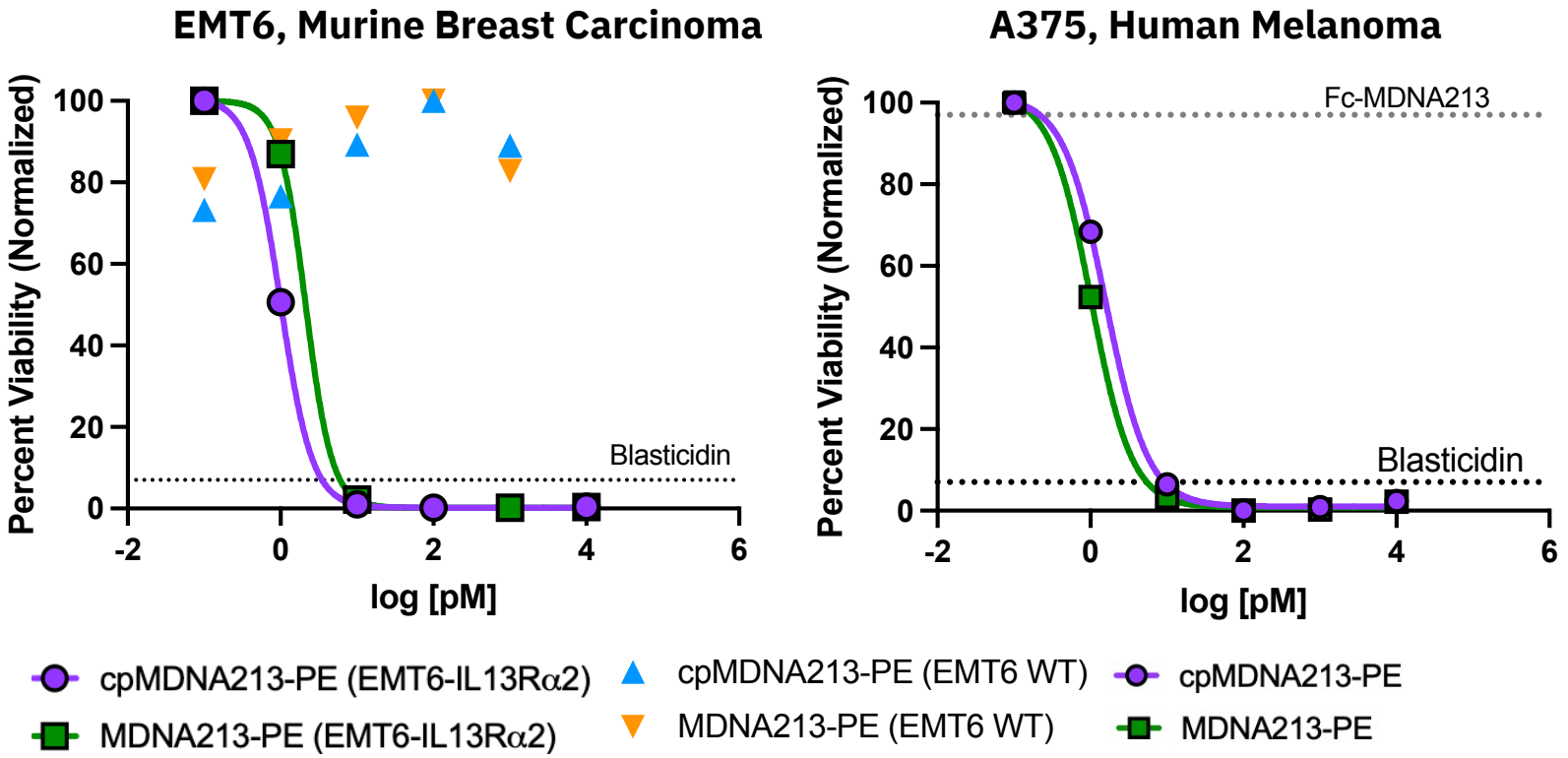


PD1 Reporter Assay	EC_{50} (nM)
Anti-mPD1	5.9
Anti-mPD1-MDNA213	6

PD-1 Reporter Assay in Jurkat cells expressing PD-1 and PD-L1 expressing aAPC/CHO-K1 cells

IL-13 Empowered Superkines ™: Targeted Tumor Delivery of Potent Payloads to Induce Immunogenic Cell Death

MDNA213-PE & circularly permuted (cp) MDNA213-PE induce cytotoxicity of IL-13Rα2 expressing cancer cells

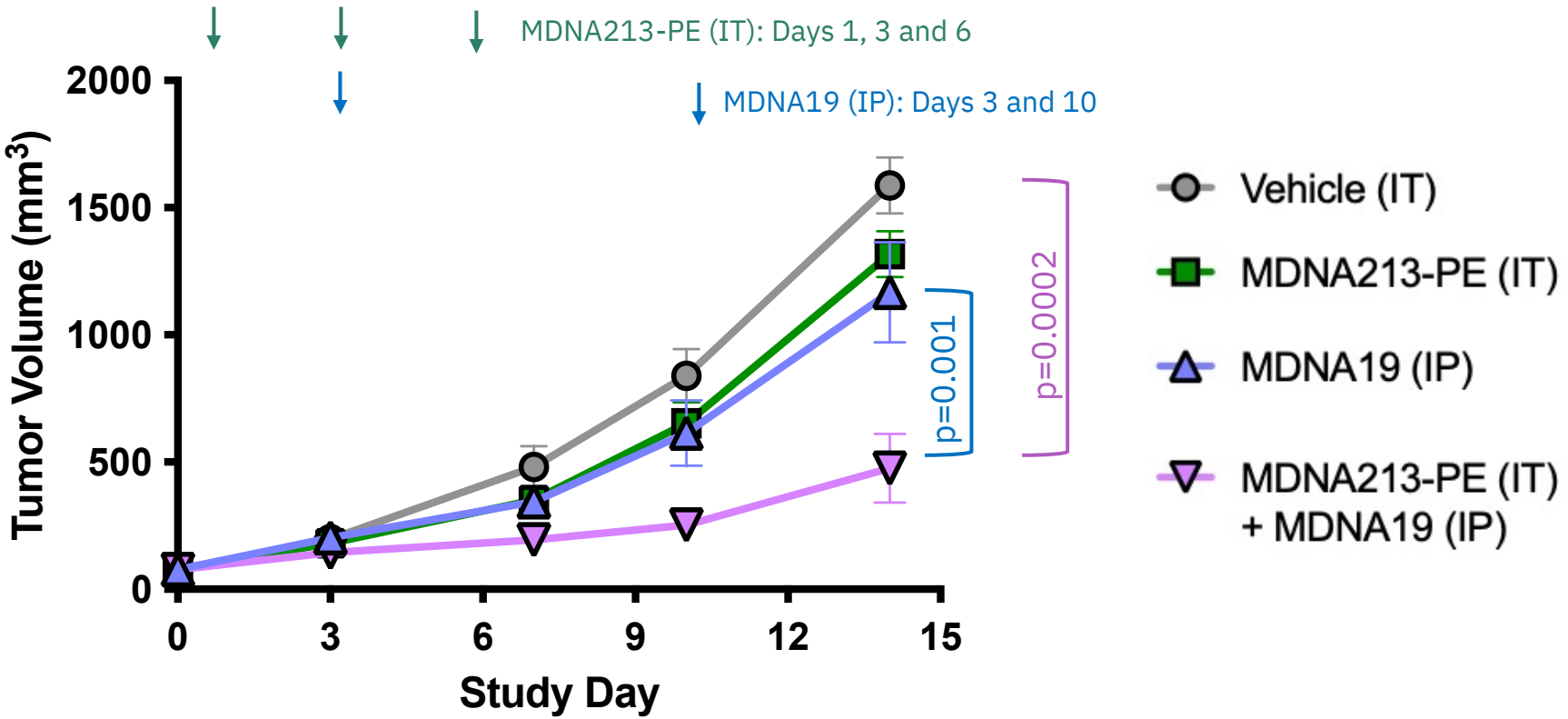


IC_{50} (pM)	MDNA213 -PE	cpMDNA213 -PE
EMT6 WT	No cell death	No cell death
EMT6-IL13Ra2	2.1	1.0
A375	1.02	1.5

- MDNA213-PE and cpMDNA213-PE exhibited selective and potent cytotoxic activity towards human and murine cancer cells that express IL-13Rα2 but show no activity in non-expressing tumors

Synergy of IL-13 Empowered Superkine (MDNA213-PE) in Combination with IL-2 Superkine in Immunologically Cold Tumors

EMT6 is a triple negative breast cancer model that is refractory to immune-checkpoint inhibition



- Intra-tumoral (IT) treatment with MDNA213-PE (2 µg/tumor, 3 doses) inhibited tumor growth
- MDNA213-PE (IT) synergizes with MDNA19 (5 mg/kg IP) to significantly enhance therapeutic efficacy
- MDNA213-PE treated animals showed steady weight gain during the study

Tumor bearing mice were treated with either MDNA213-PE 2 µg/tumor on days as indicated or MDNA19 5 mg/kg once weekly X 2 IP on days 3 and 10 or their combination. Avg tumor size at initiation of dosing was 50 mm^3.P-values generated using t-test based on day 14 data.

Summary of Key Findings

- IL-13 Superkines (MDNA132 and MDNA213) are designed for high IL-13Rα2 selectivity
- These Superkines preferentially accumulate in TME of IL-13Rα2 expressing tumors
- MDNA19-MDNA213 and anti-mPD1-MDNA213 BiSKITs retain binding and functional properties of respective moieties, namely IL-2 agonism and PD-1 blockade.
- MDNA213 fusion to Pseudomonas Exotoxin (PE) induces cell cytotoxicity only in IL-13Rα2 expressing tumors
- MDNA213-PE and MDNA19 (an IL-2 Superkine) act in synergy against tumors expressing IL-13Rα2
- MDNA213 is a versatile platform for engineering next generation of precision immunotherapies for many immune-resistant IL-13Rα2 expressing tumors

Thank you

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