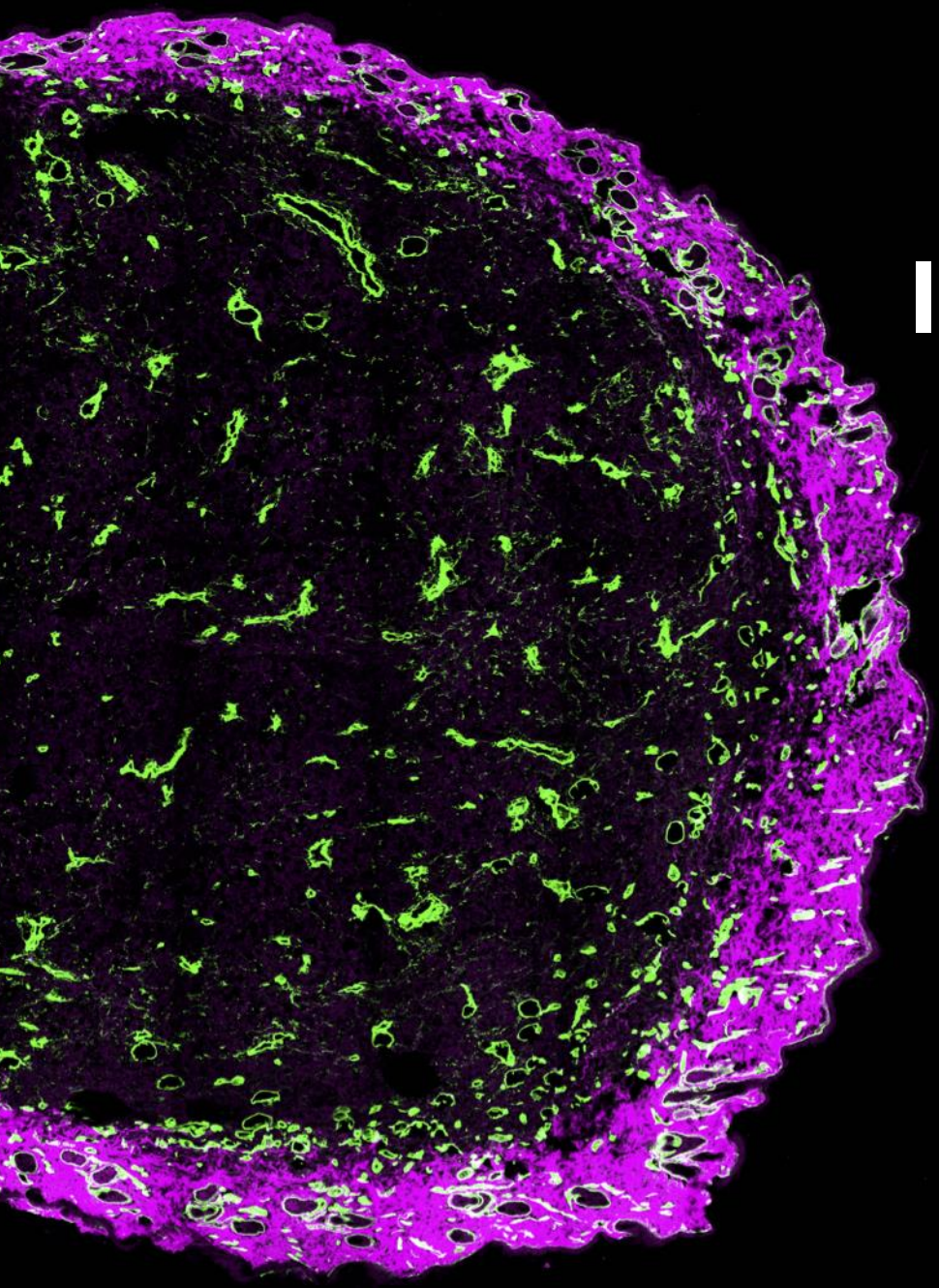


Intratumoral Cytokine Therapy: Principles and Practice

Noor Momin

(she/her/hers)

previously at MIT with Prof. Dane Wittrup

April 2022

SITC TME Workshop

Massachusetts Institute of Technology

KOCH INSTITUTE for Integrative Cancer Research at MIT

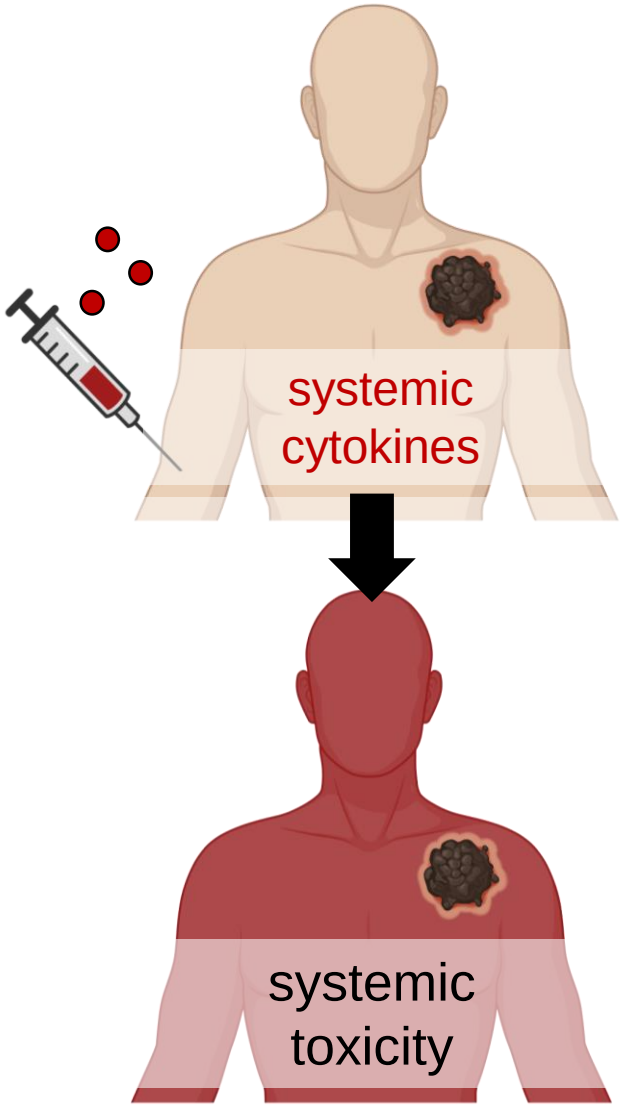
Disclosures

Noor Momin

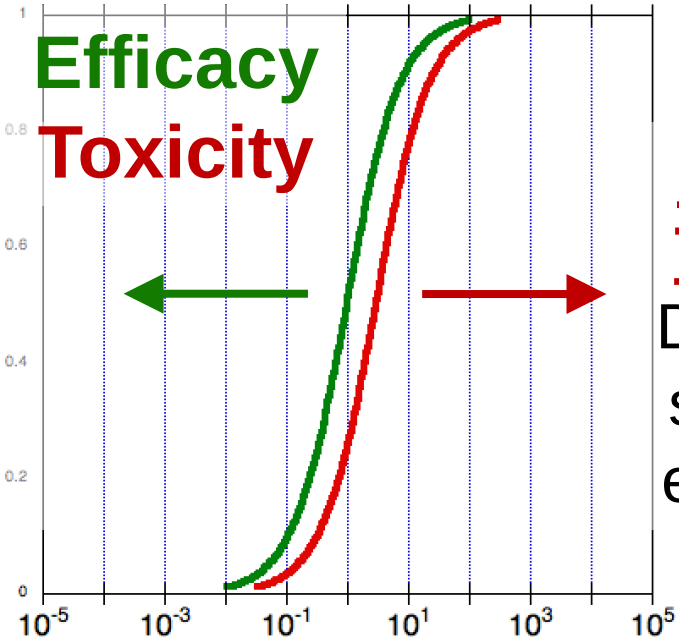
I have the following relevant financial relationships to disclose:
Consultant for: Cullinan Oncology*

*licensee of patent on collagen anchoring technology

Cytokine therapies have poor therapeutic windows

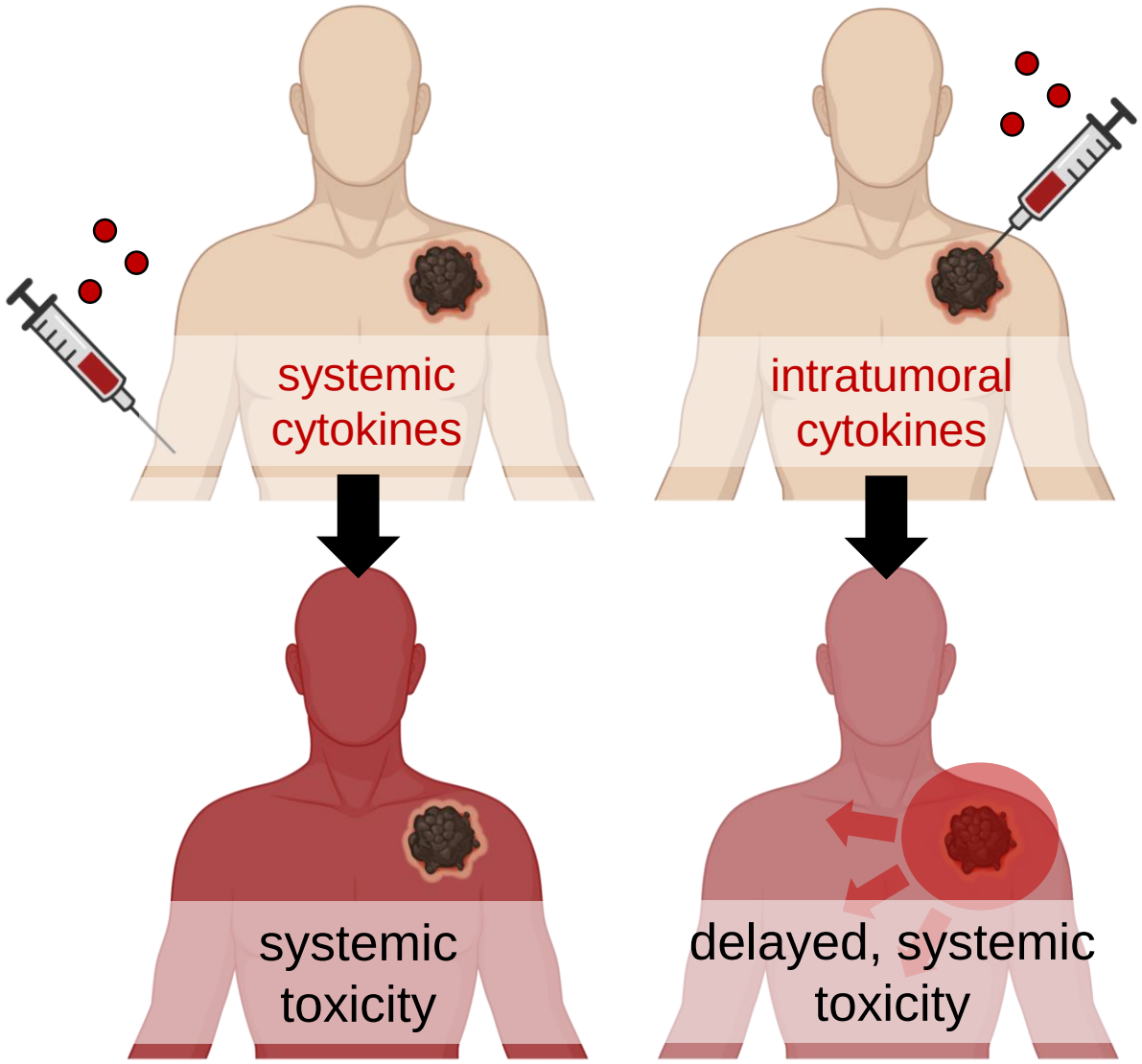


Enhance Efficacy
Increase tumor exposure

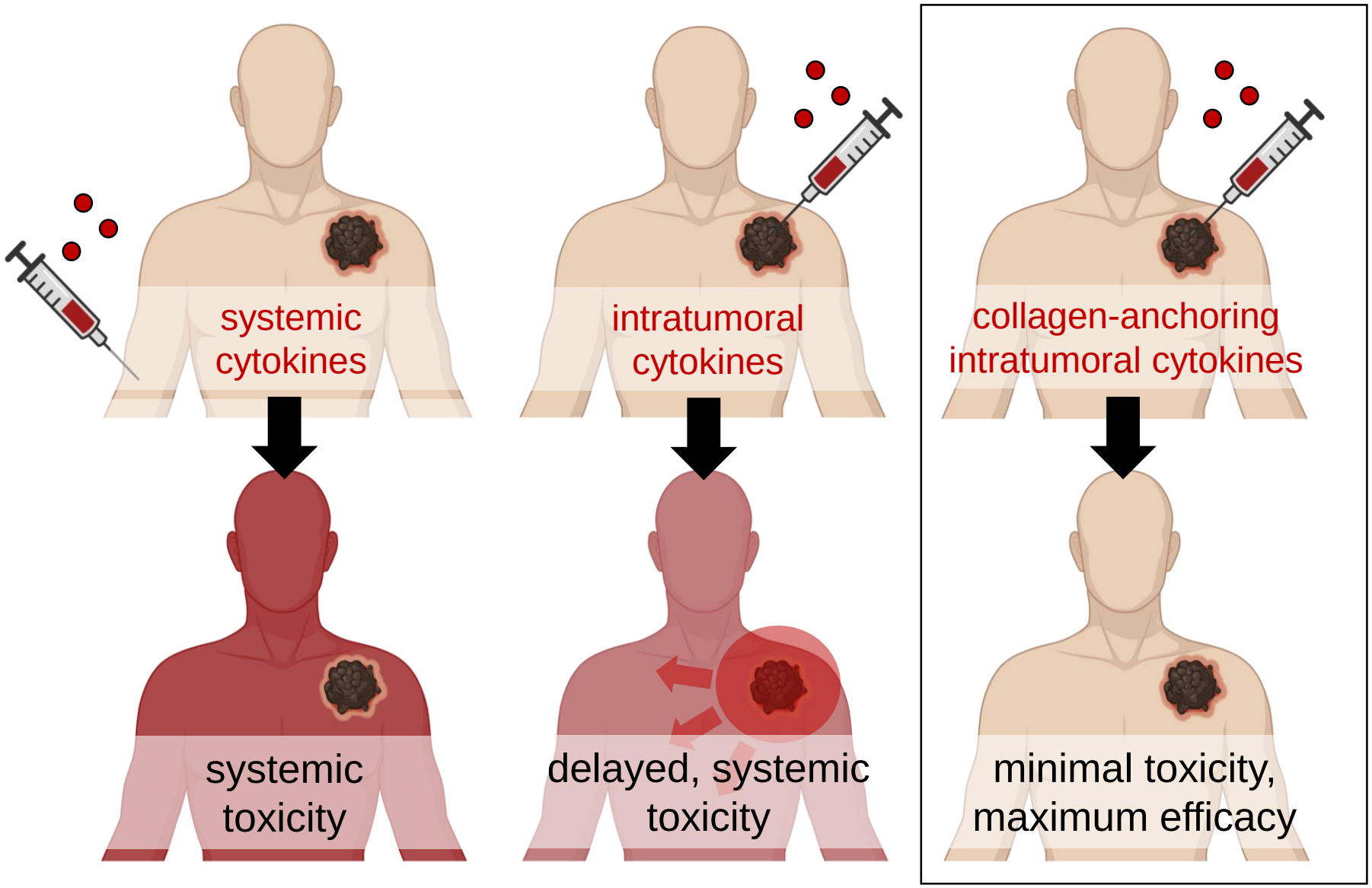


Reduce Toxicity
Decrease systemic exposure

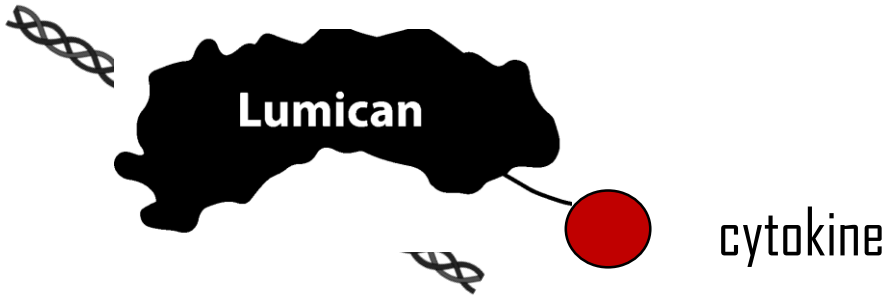
Local administration ≠ Localization



Collagen Anchoring of Intratumorally Administered Cytokines

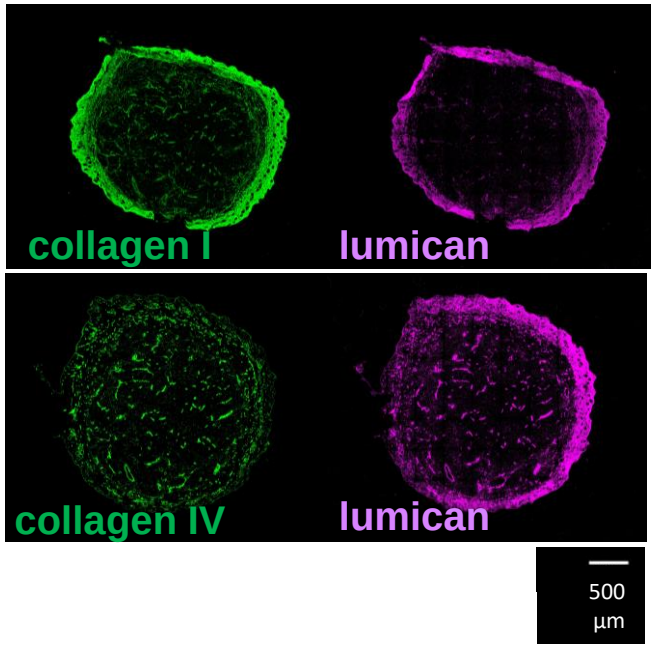


Why (and how) to anchor cytokines to collagen?



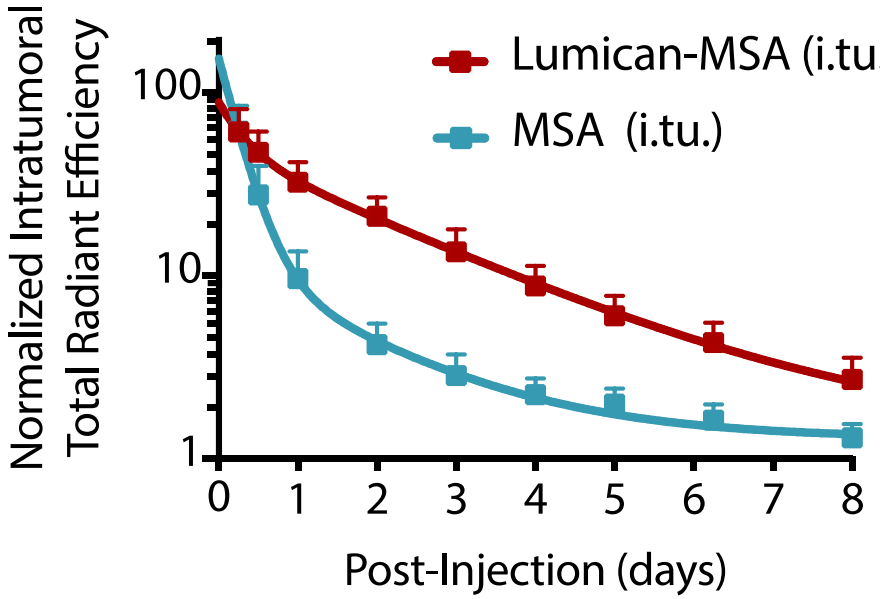
tumor-agnostic

generalizable



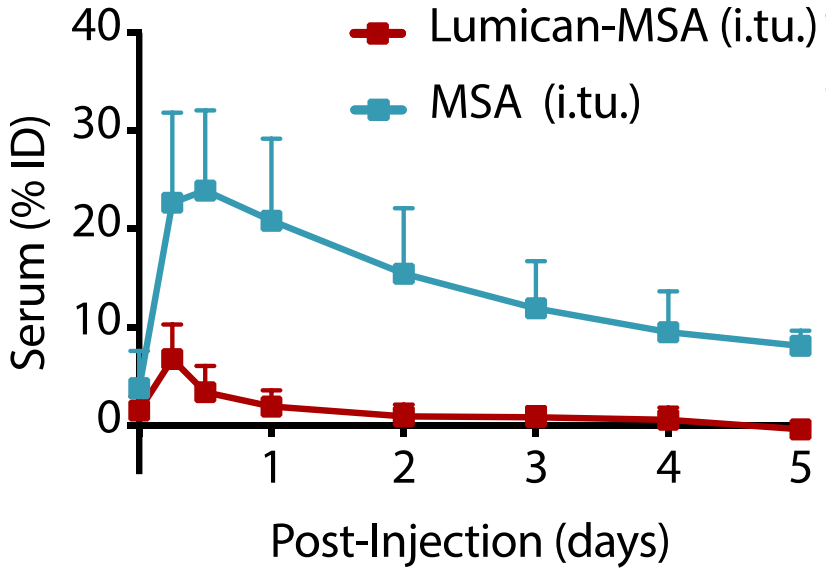
long-lived

increased **TUMOR** exposure

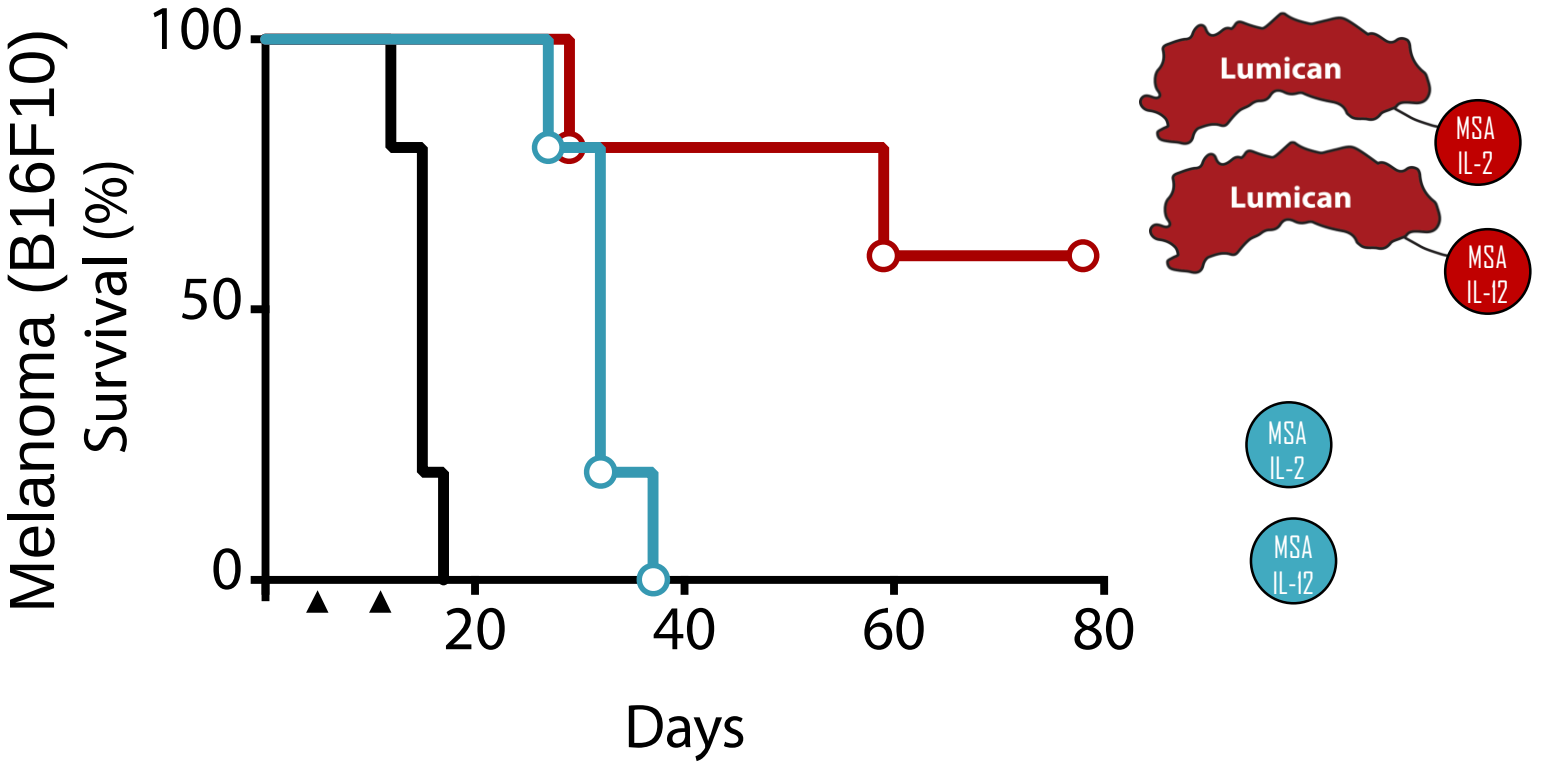


abundant

decreased **systemic** exposure



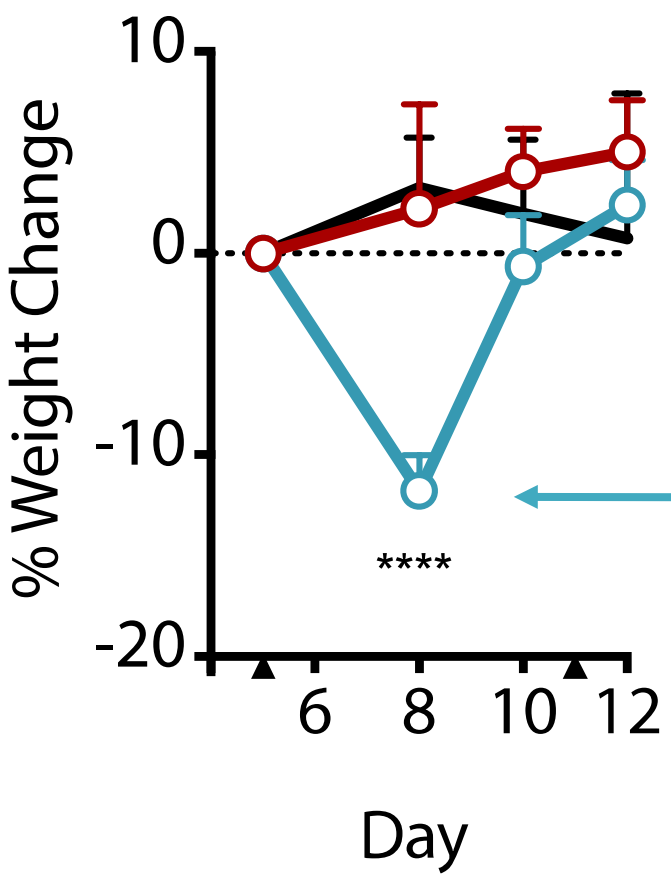
Lumican-cytokines are an effective, tumor-agnostic treatment

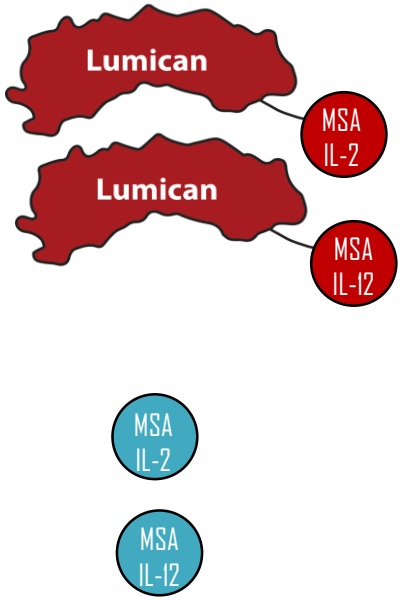


Effective against
breast (**EMT6**),
colon (**MC38**),
lung (**KP2677**),
Braf^{V600E}/Pten$^{fl/fl}$ GEMM tumor models

Adapted from Momin et al. *Sci. Trans. Med.* 2019

Lumican-cytokines are a safer, tumor-agnostic treatment



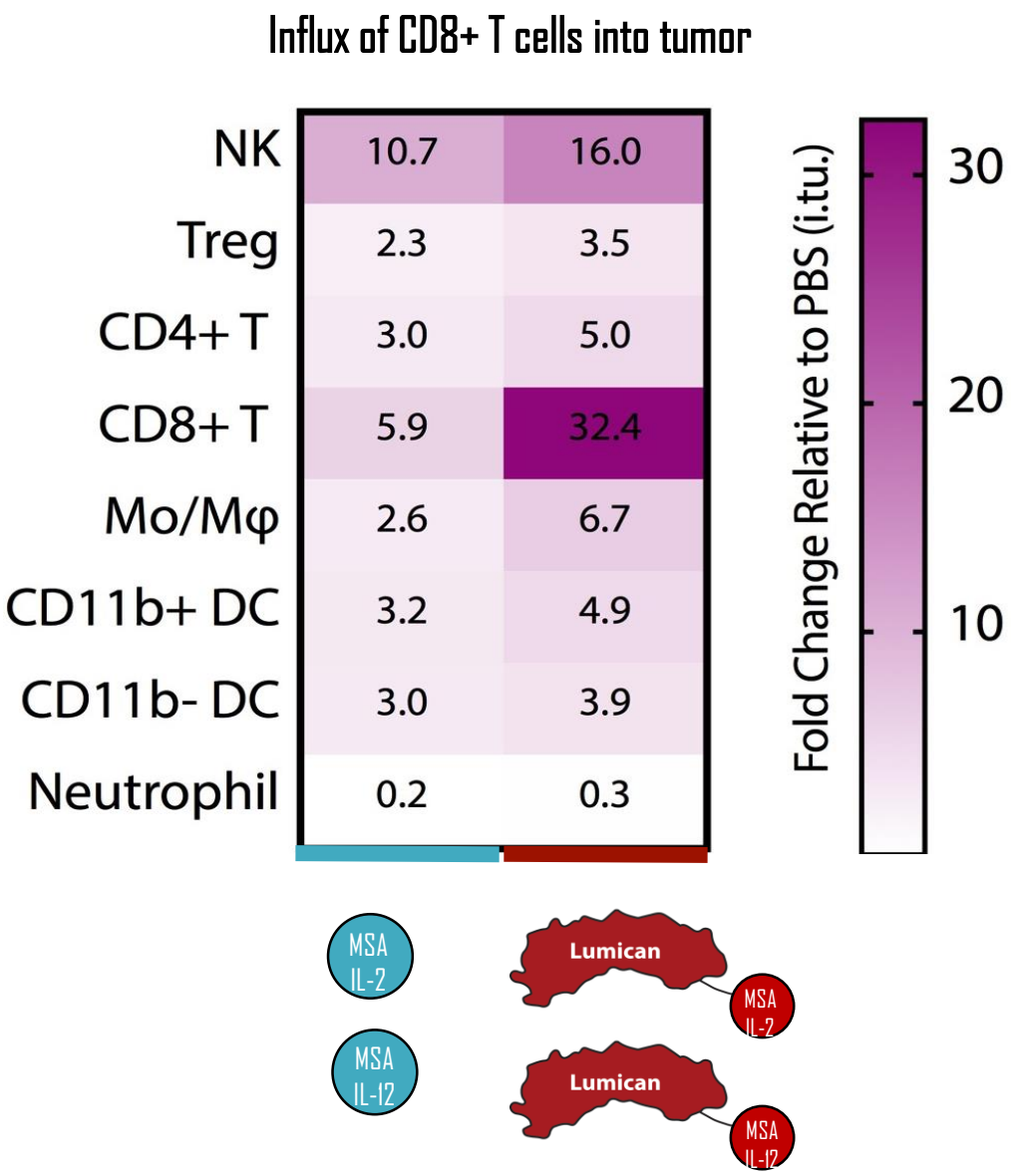


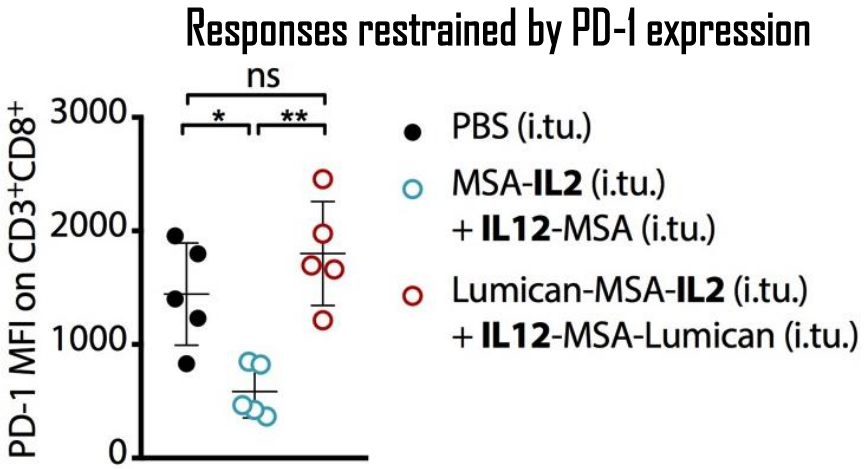
Treatment-associated toxicity (weight loss)

Effective against breast (**EMT6**), colon (**MC38**), lung (**KP2677**), **Braf**^{V600E}**/Pten**$^{fl/fl}$ **GEMM** tumor models

Adapted from Momin et al. *Sci. Trans. Med.* 2019

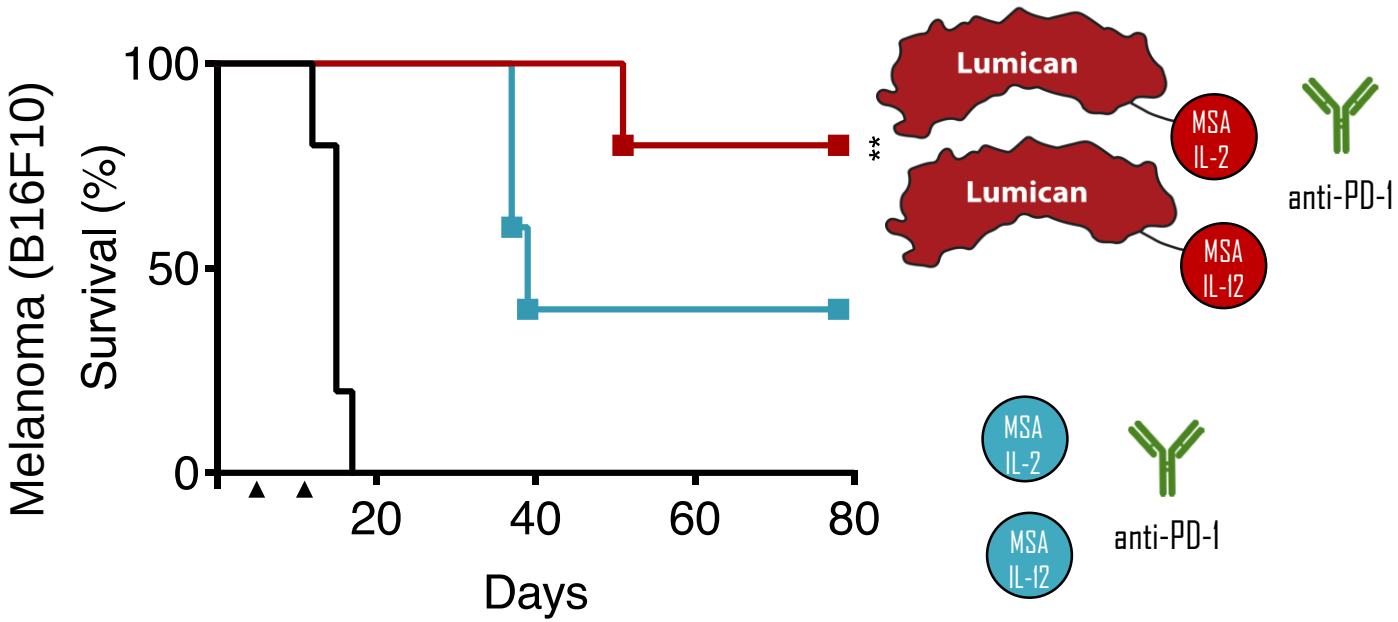
Lumican-cytokines enhances CD8 T cell infiltration

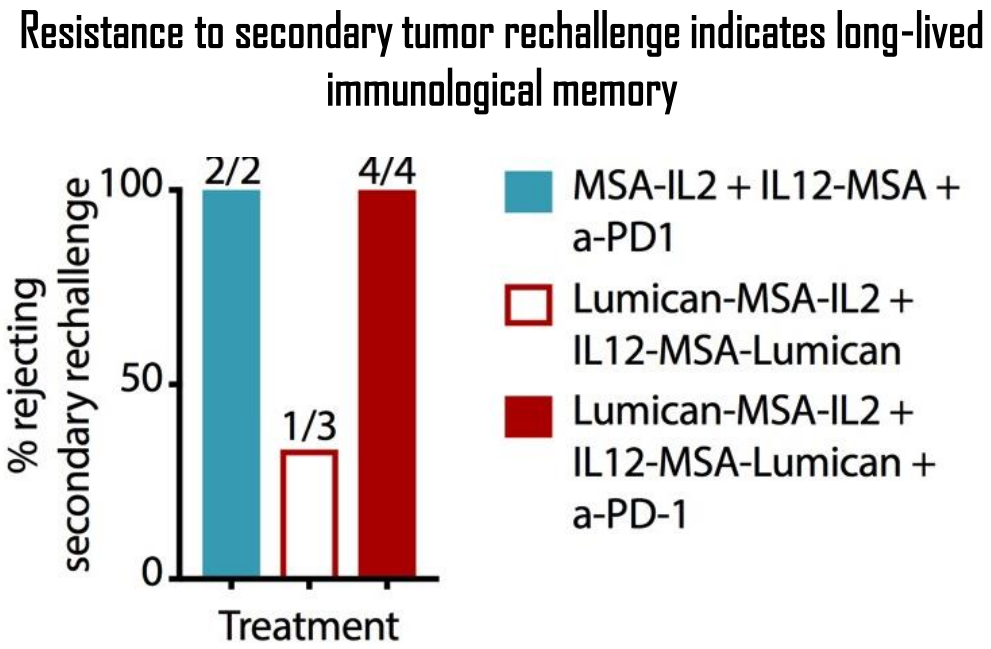




Adapted from Momin et al. *Sci. Trans. Med.* 2019

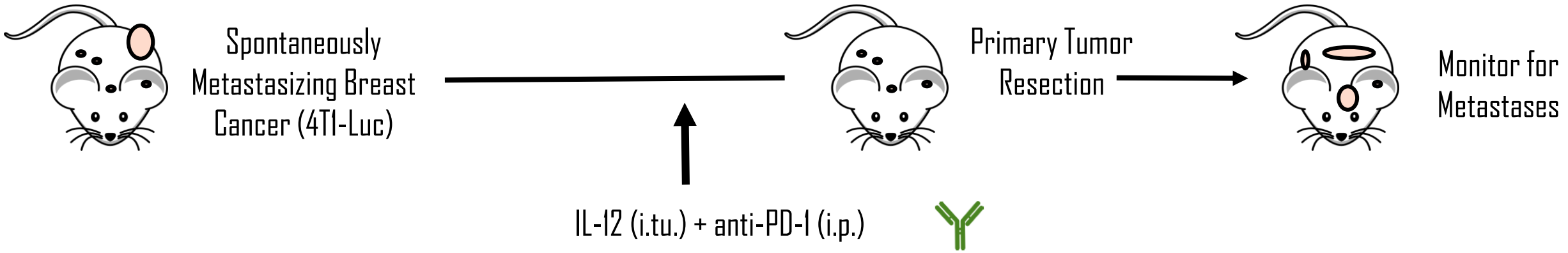
Lumican-cytokines prime durable anti-tumor immune response

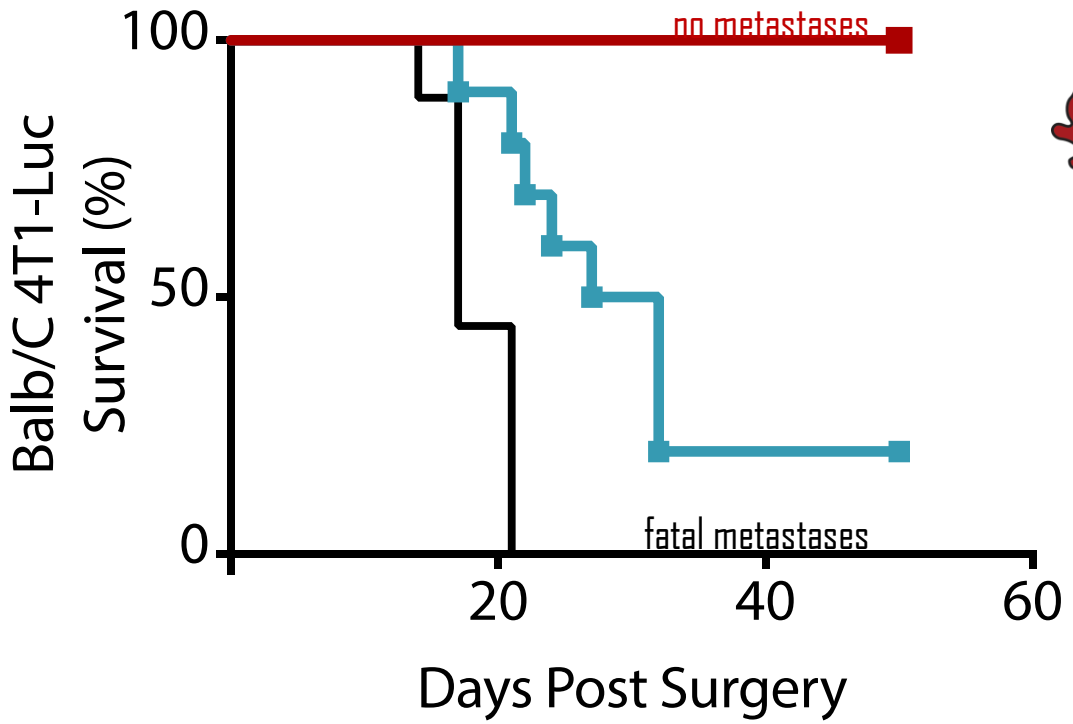


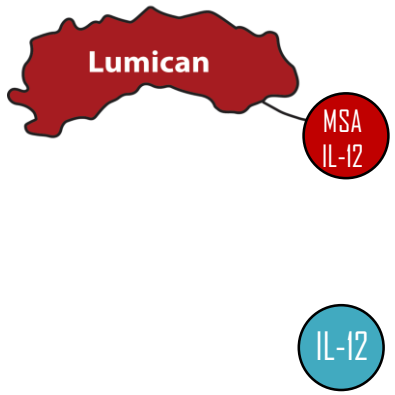


Adapted from Momin et al. *Sci. Trans. Med.* 2019

Collagen-anchoring cytokines exert superior distal tumor control







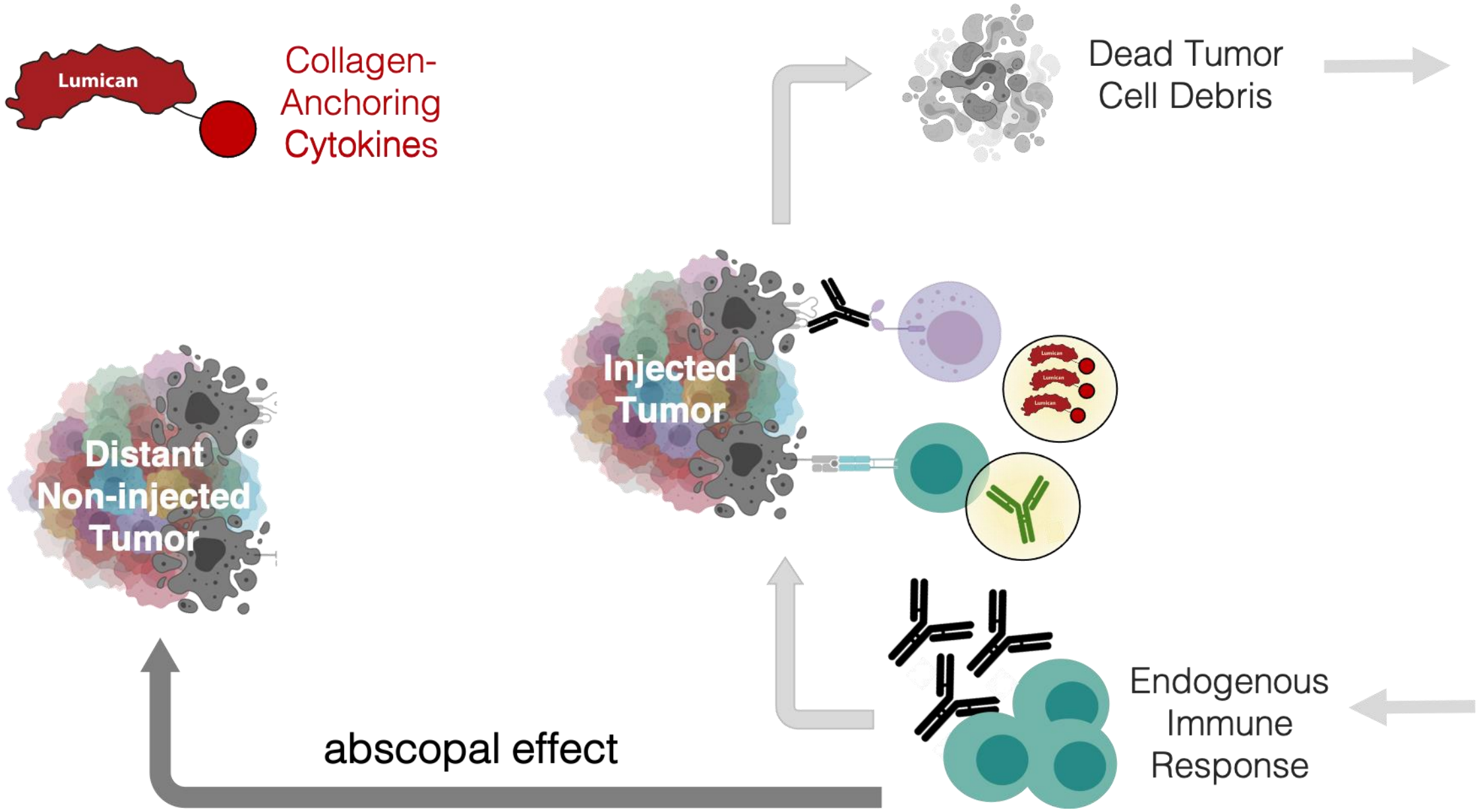
localized

systemic

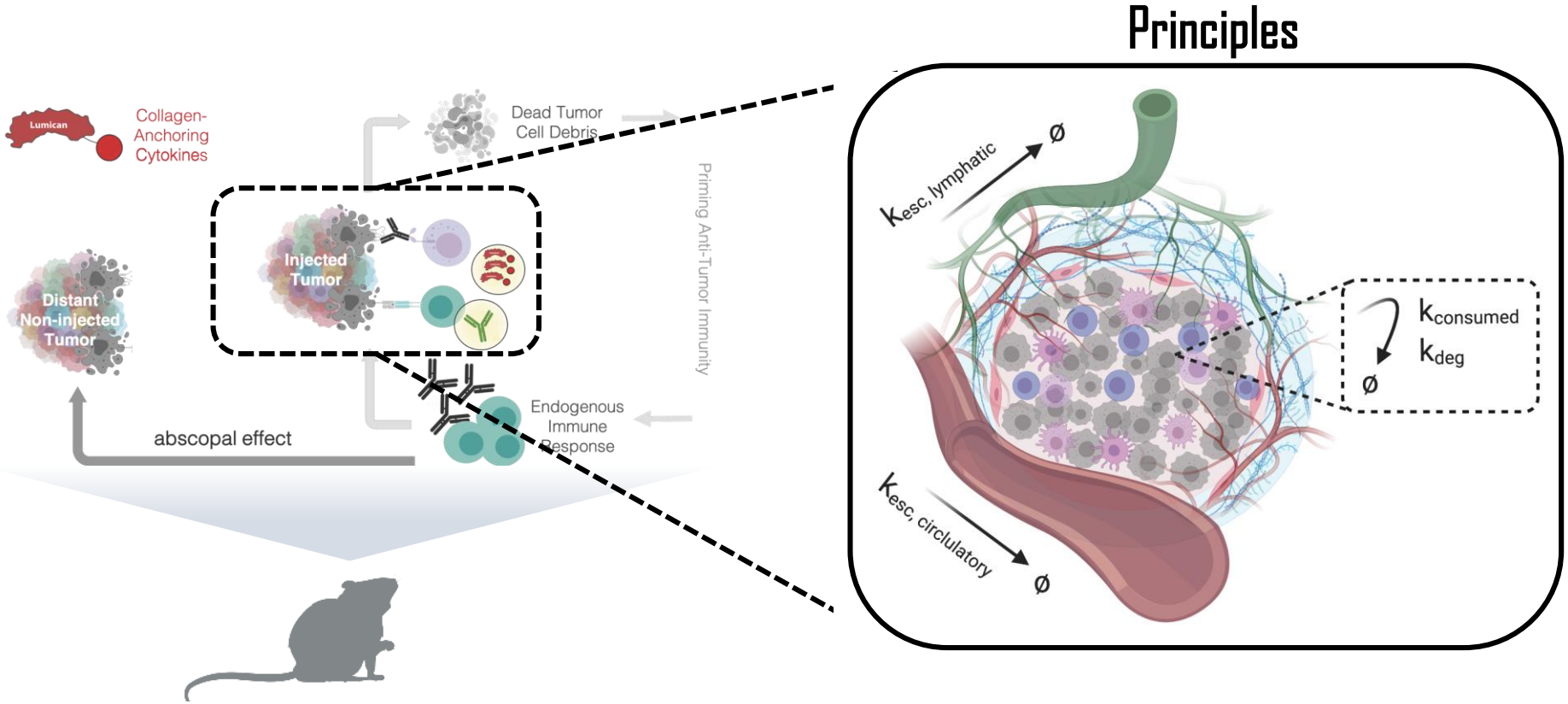
WHOLE BODY anti-tumor immunity

With Nitasha Bennett
Adapted from Momin et al. Sci. Trans. Med. 2019

Immunological snapshot collagen anchoring cytokines

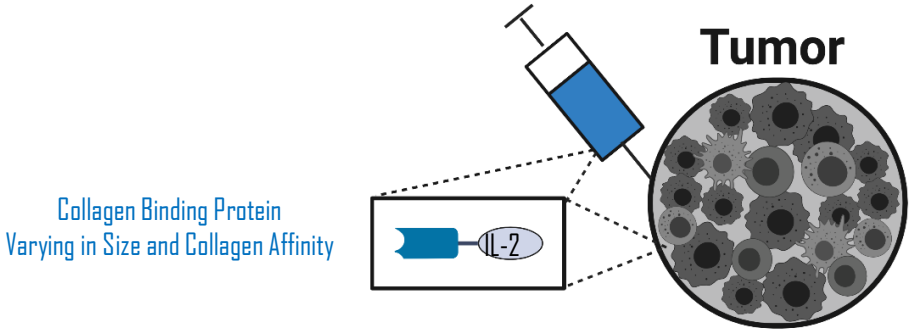


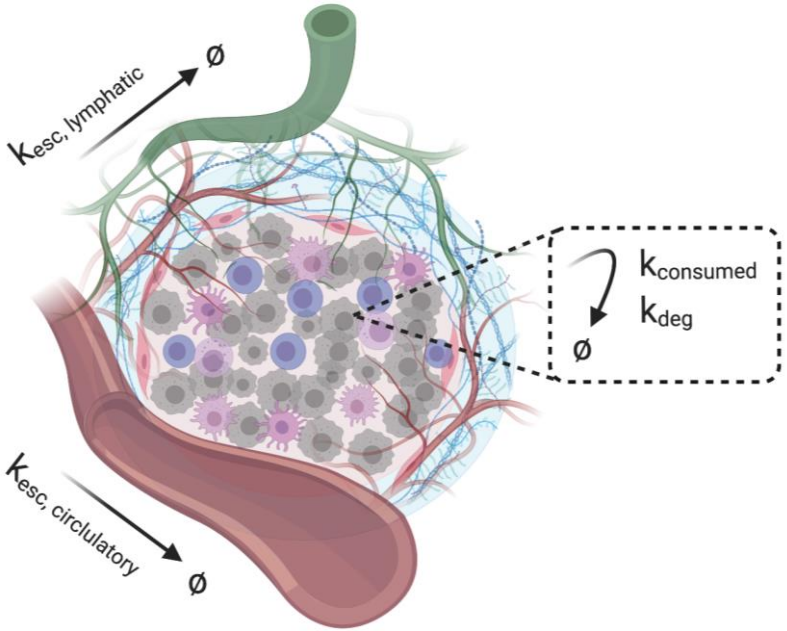
Intratumoral therapy is a new treatment paradigm

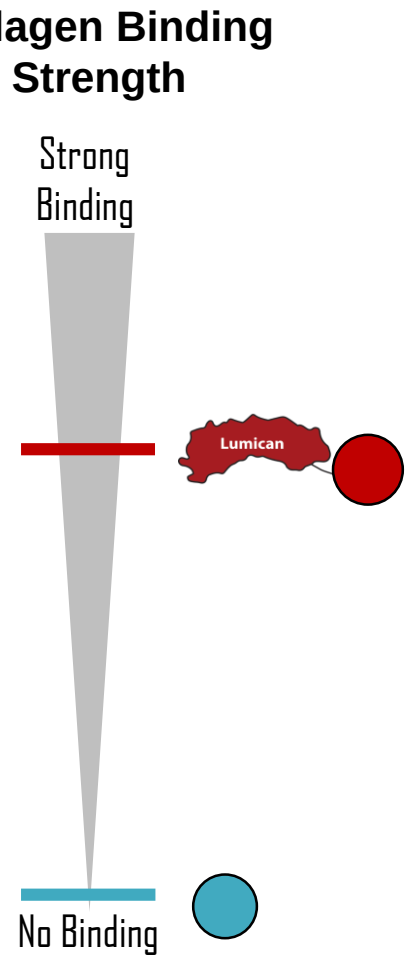


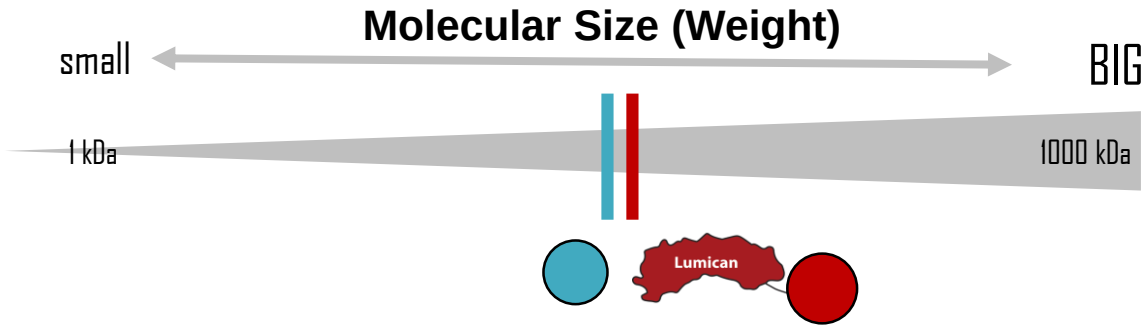
Intratumoral Therapy

How do features of our drug impact its tumor retention (and effectiveness)?

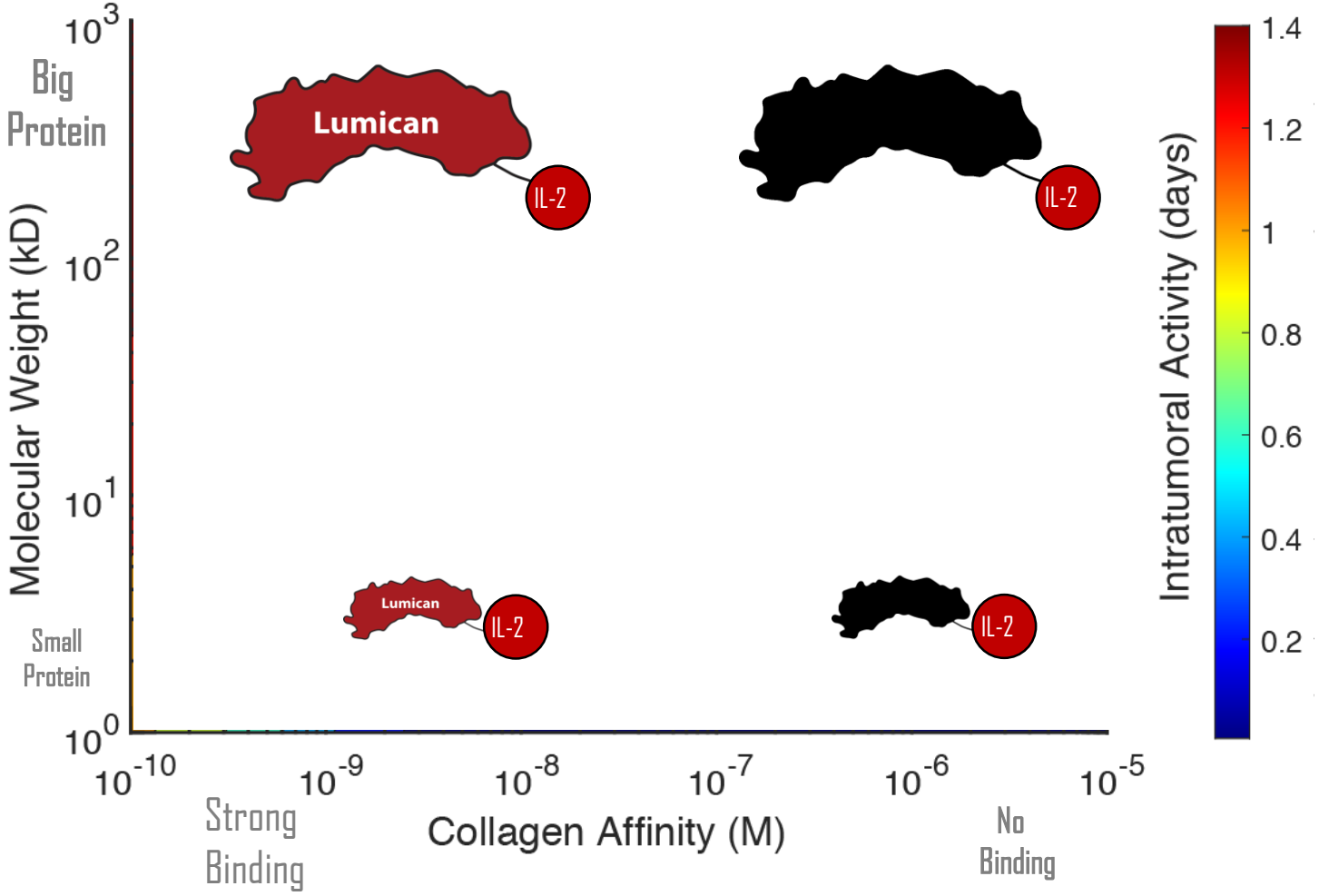






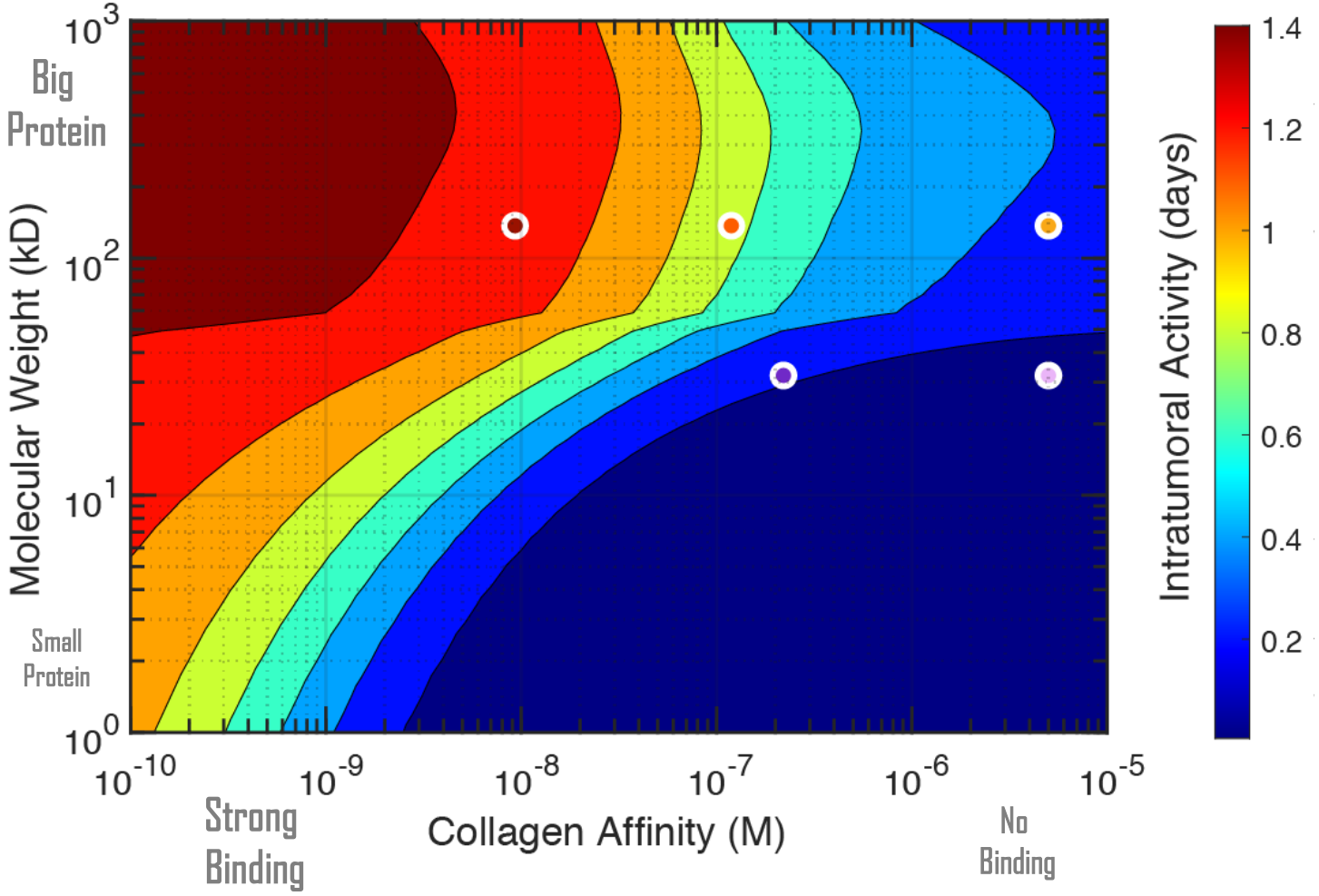


Model predicts activity of IL-2 varying in collagen affinity and size



Adapted from Momin et al. *Nat Comm.* 2022

Generating cytokines therapies to test the model's prediction



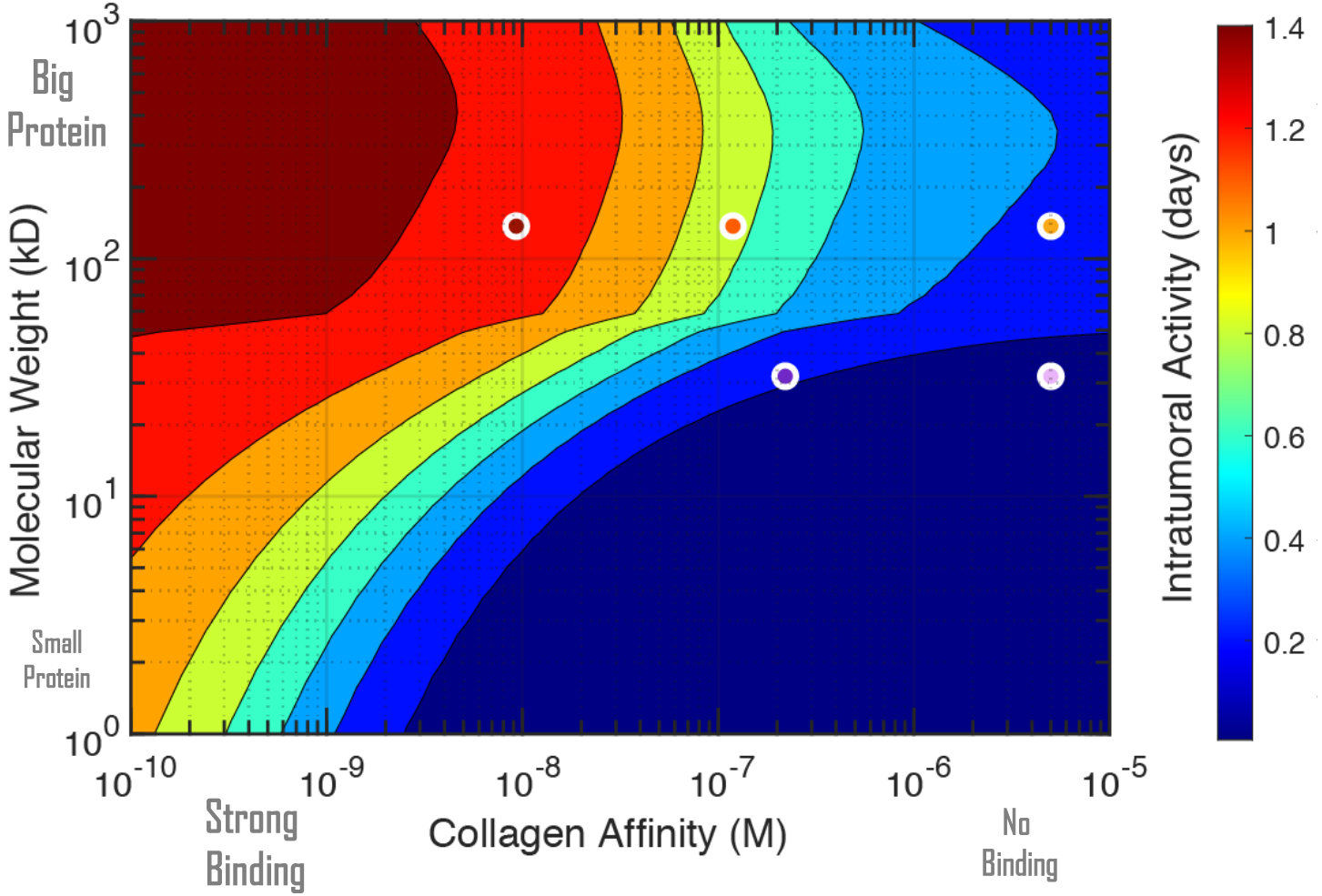
Collagen Binding
Better affinity
Smaller scaffold

LAIRx

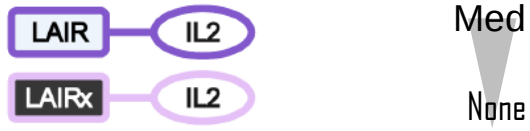
Non-Binding

Adapted from Momin et al. *Nat Comm.* 2022

Generating cytokines therapies to test the model's prediction



Collagen Binding Strength



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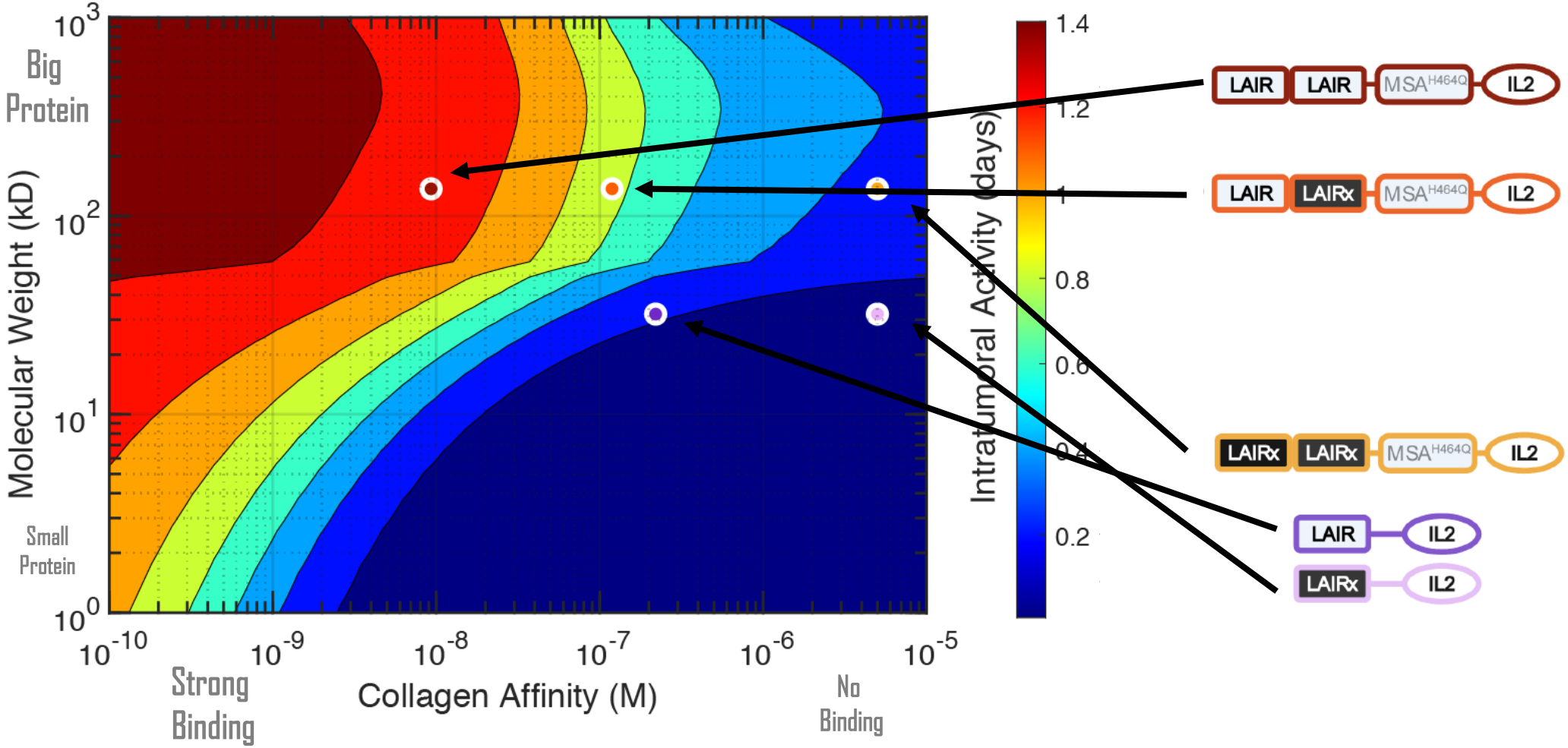
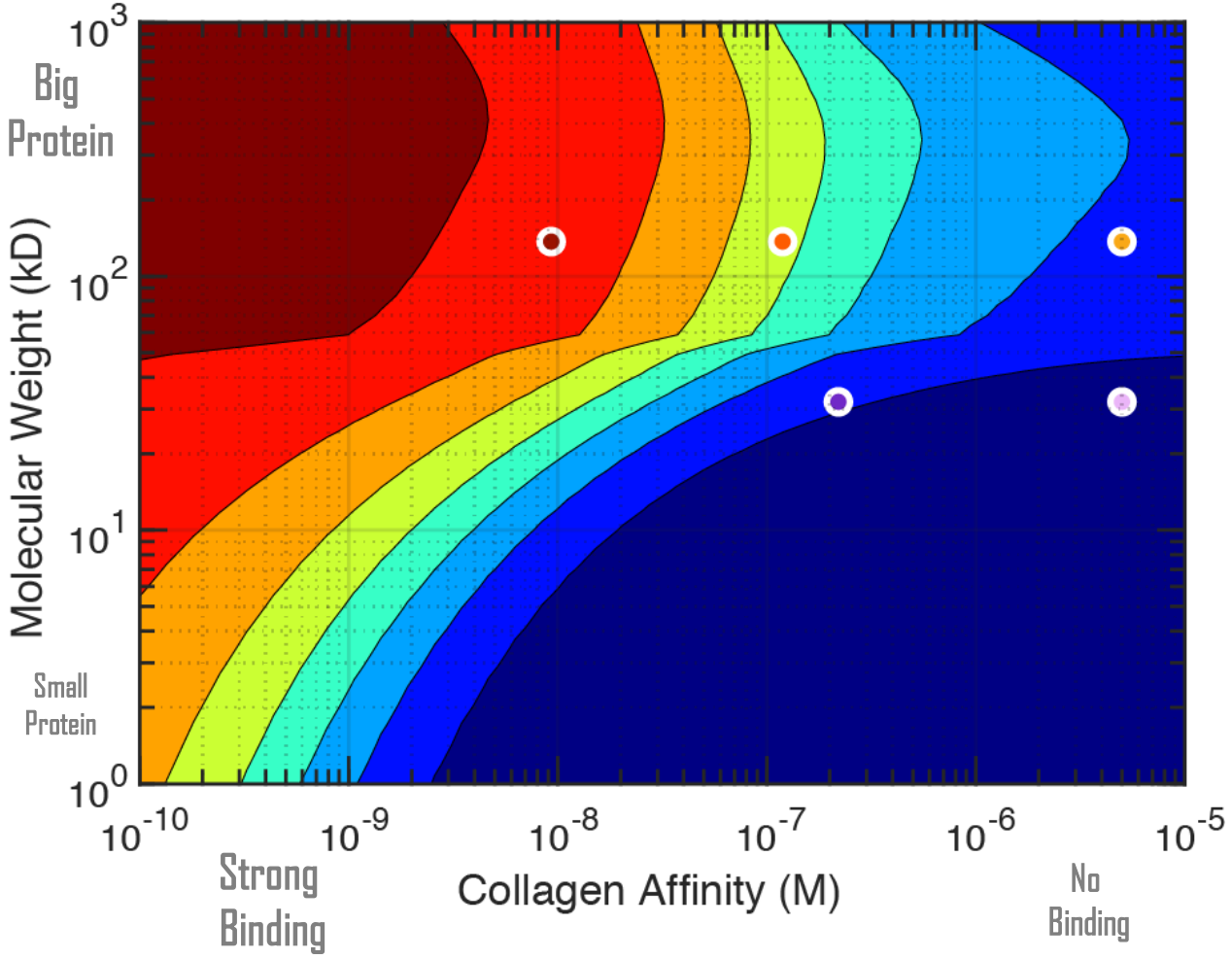
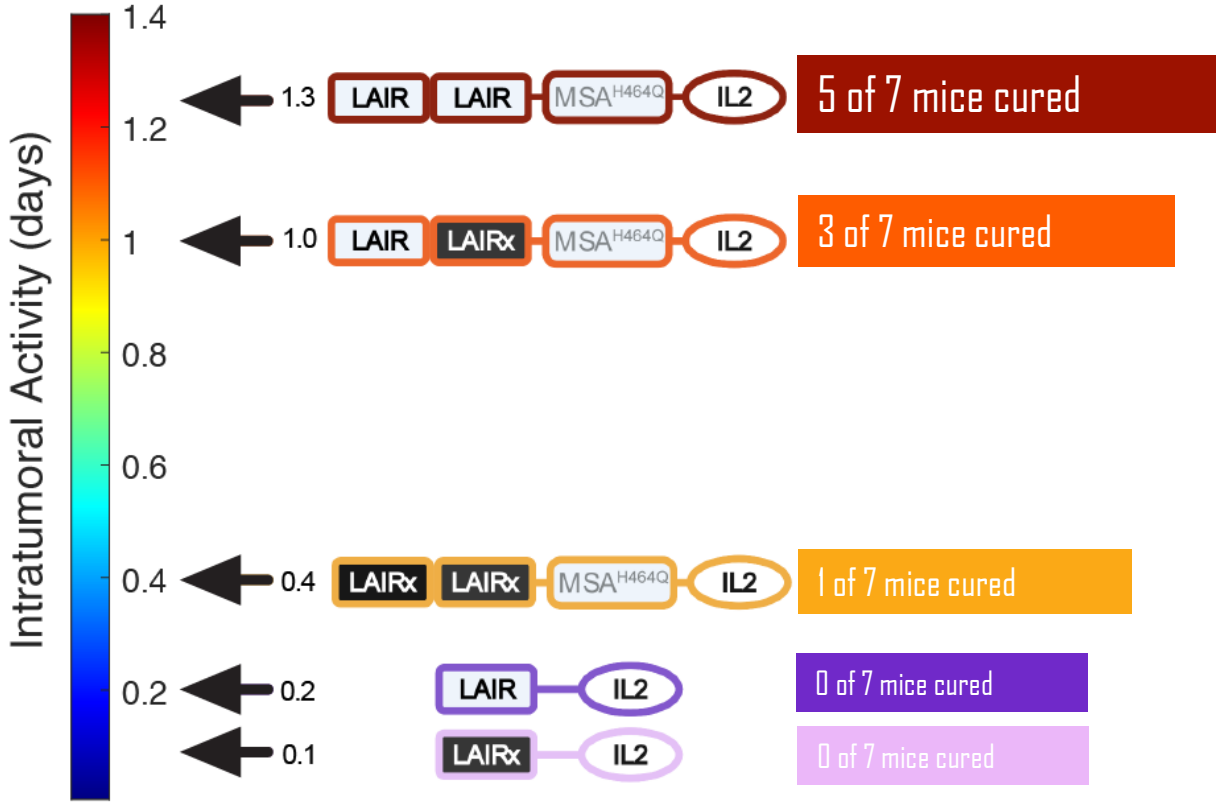
Generating cytokines therapies to test the model's prediction



Adapted from Momin et al. *Nat Comm.* 2022

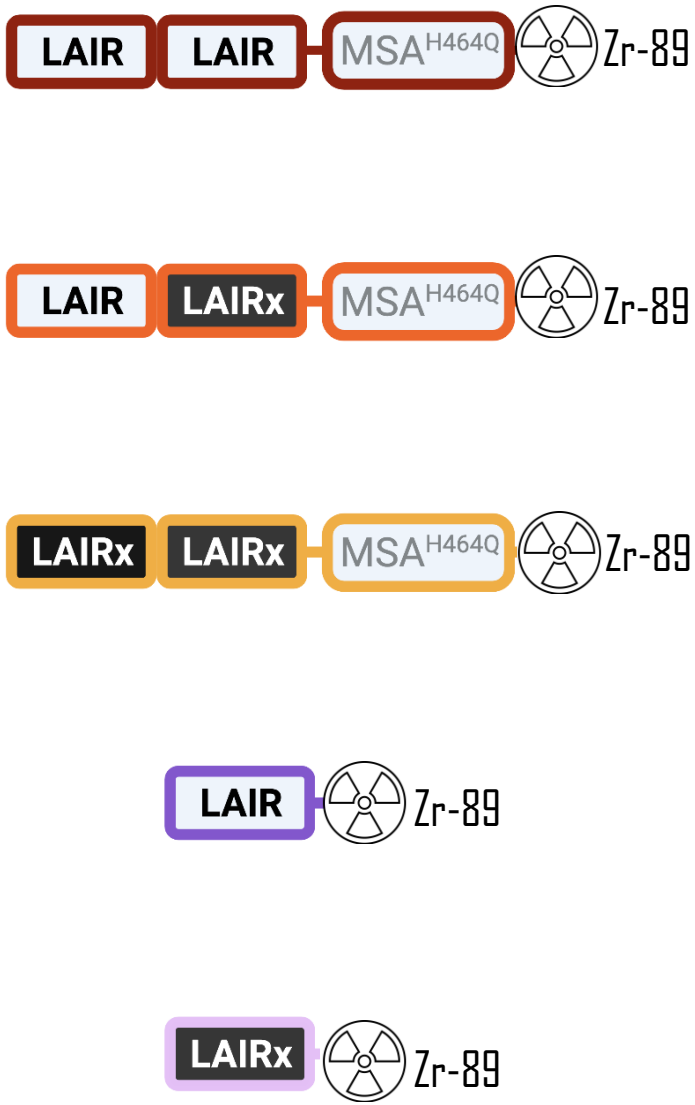
Empirically validating the model in B16F10 mouse model

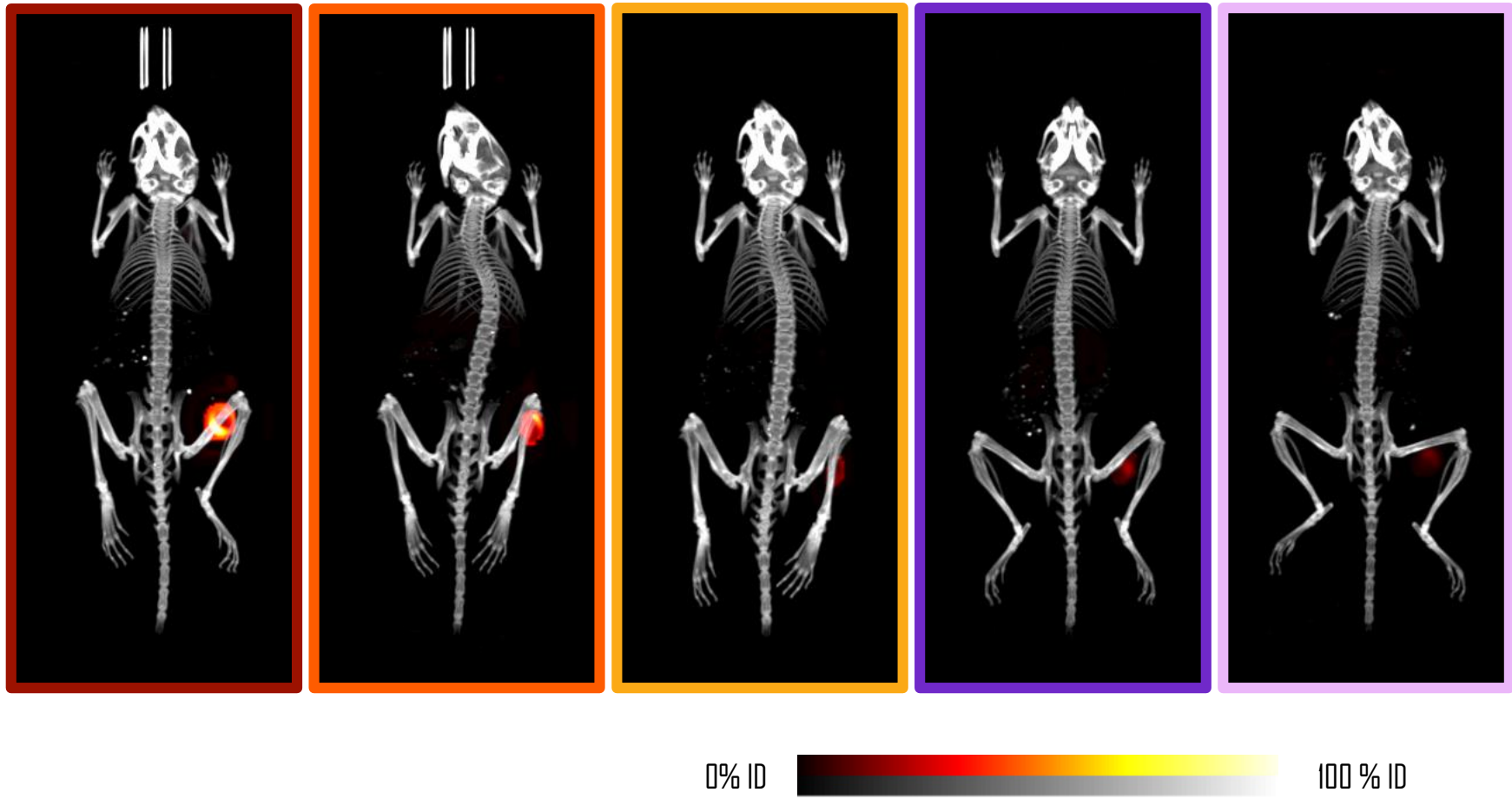




Adapted from Momin et al. *Nat Comm.* 2022

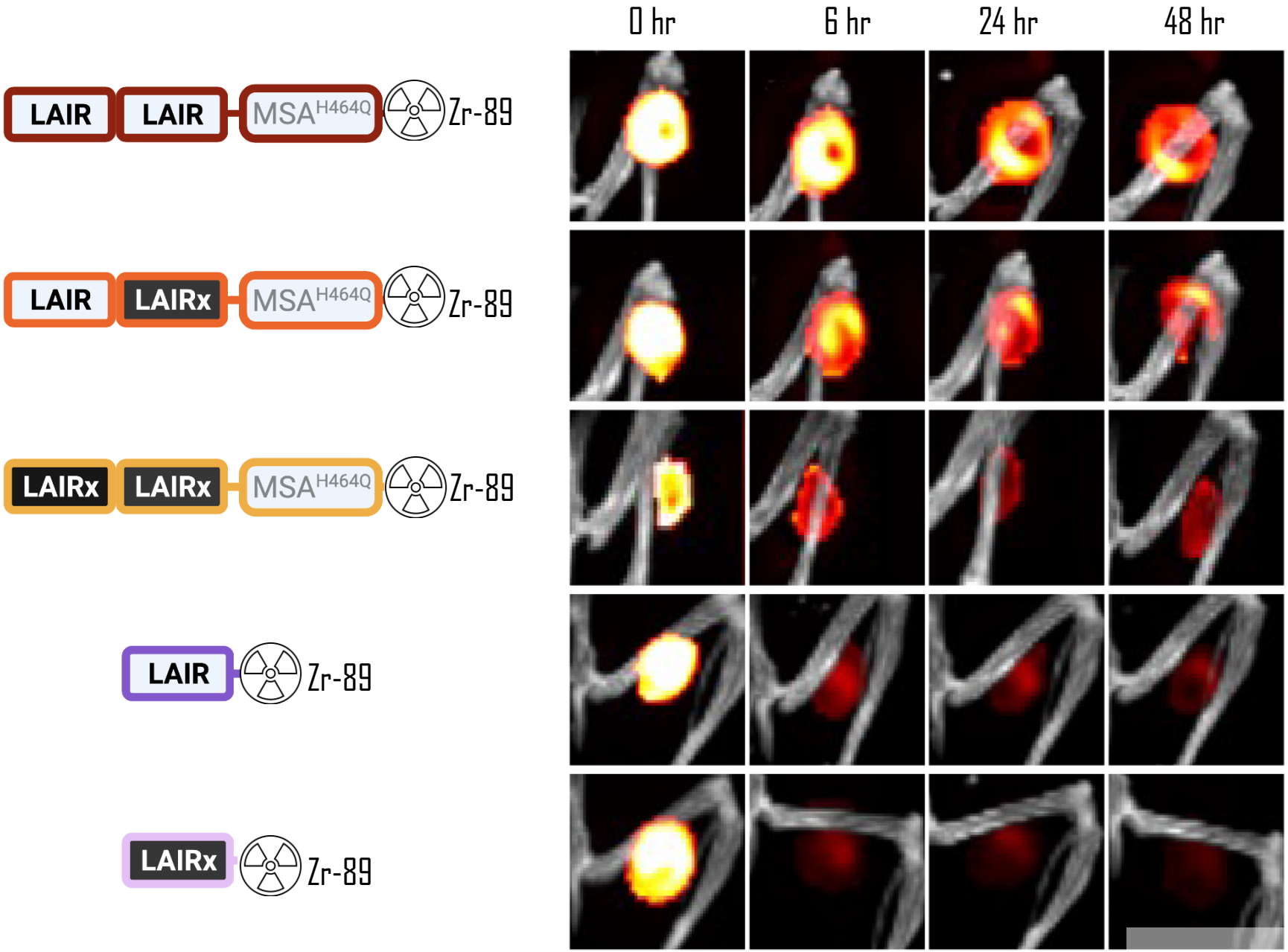
Quantitatively validating the model using PET imaging





Adapted from Momin et al. *Nat Comm.* 2022

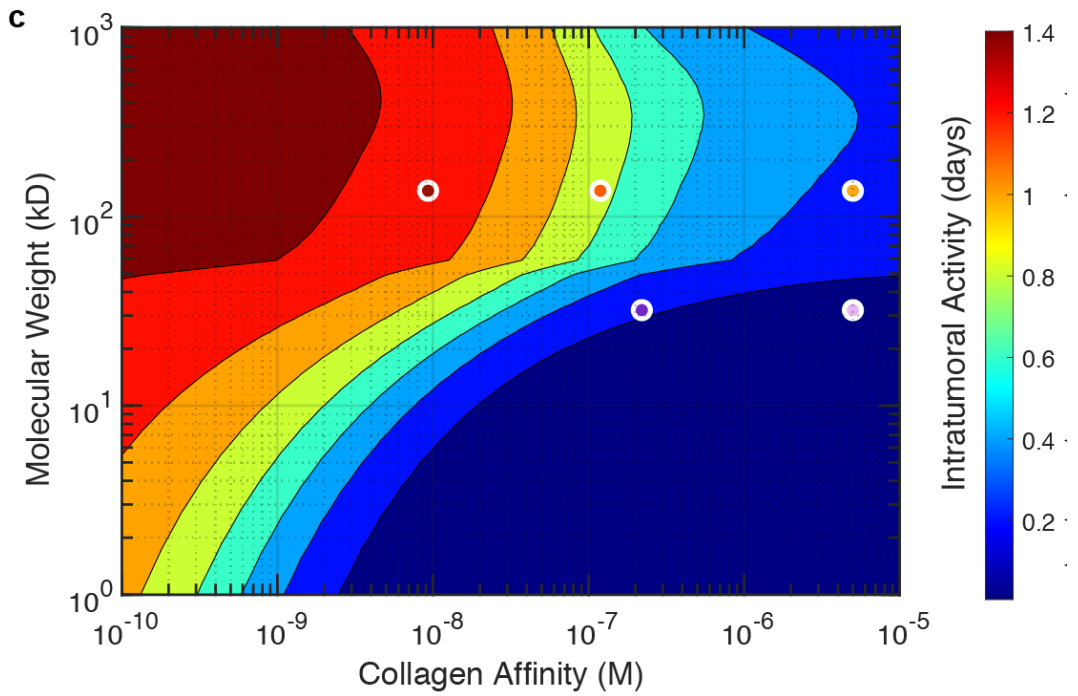
Contribution of size to retention outweighs affinity to collagen

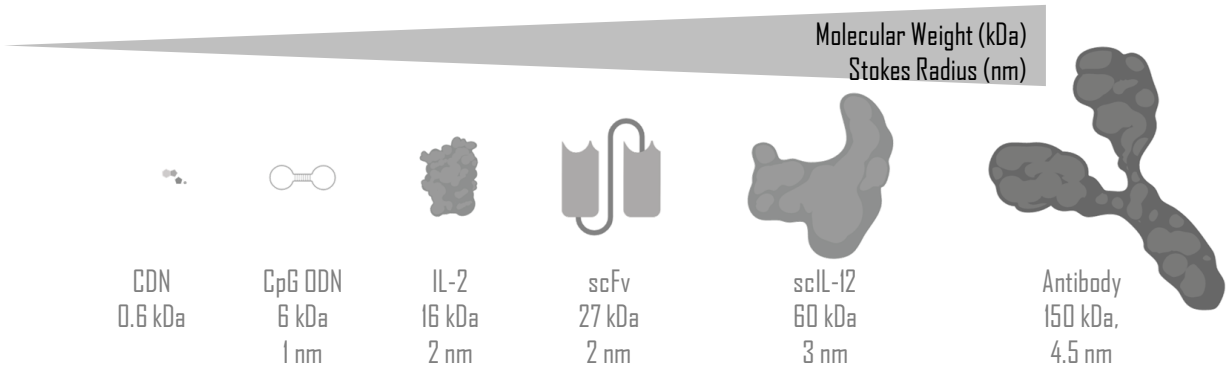


Adapted from Momin et al. *Nat Comm.* 2022

Framework to increase tumor retention of local therapy

Molecular Modifications

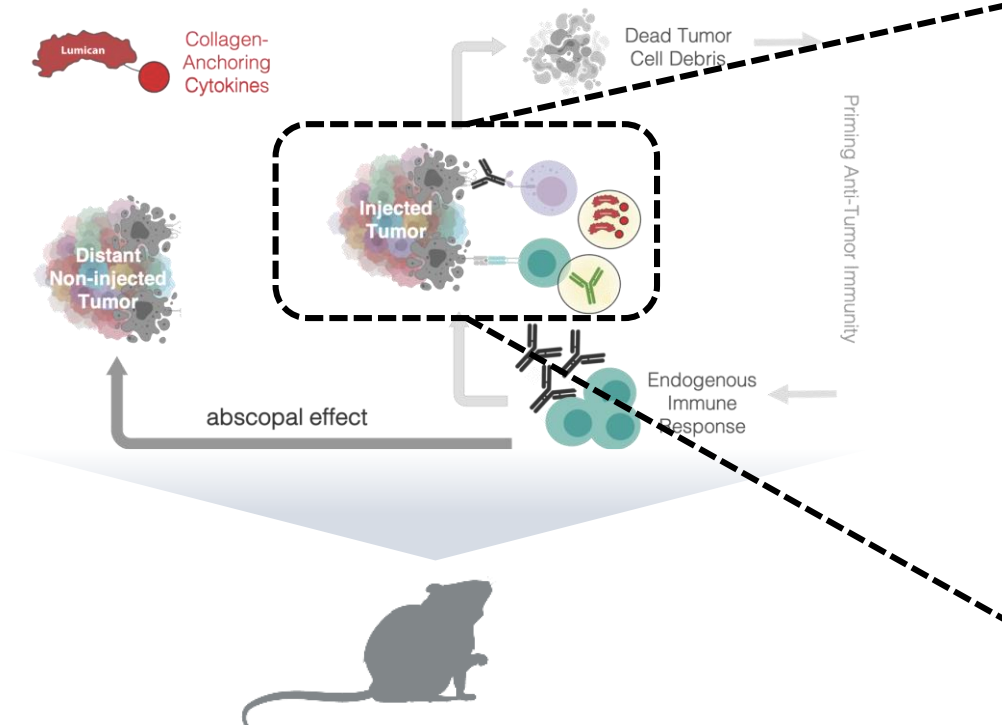




Code deposited on Github!

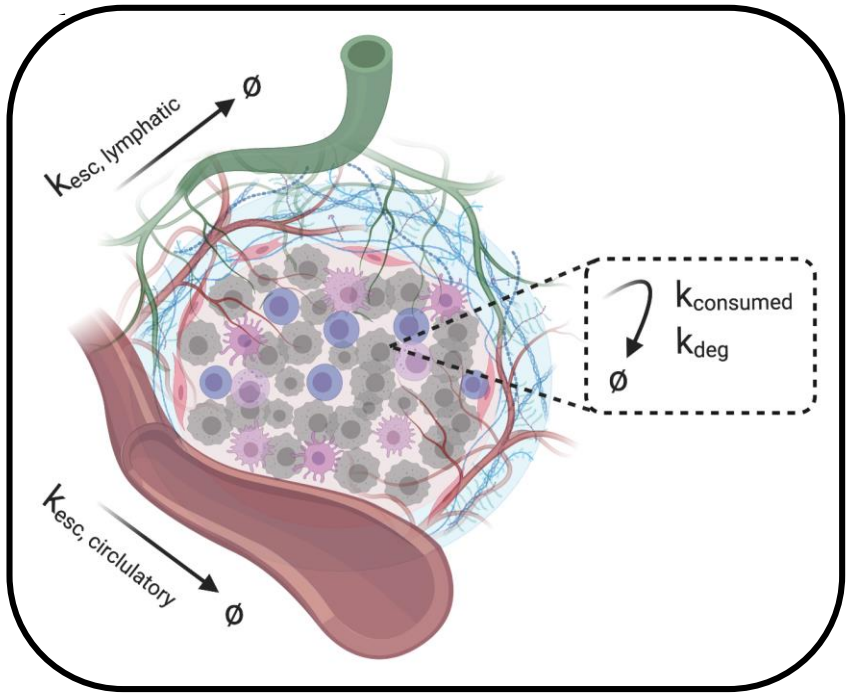
Adapted from Momin et al. *Nat Comm.* 2022

Translating intratumoral collagen cytokines therapy to the clinic

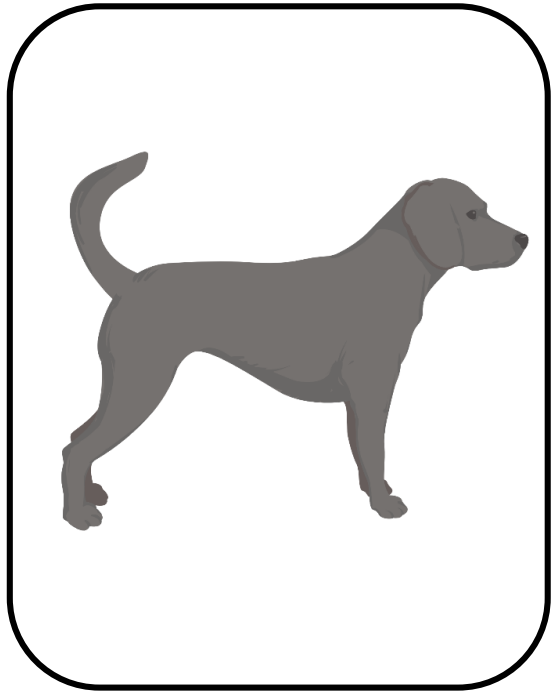


Momin et al. *Sci Trans Med.* 2019

Principles



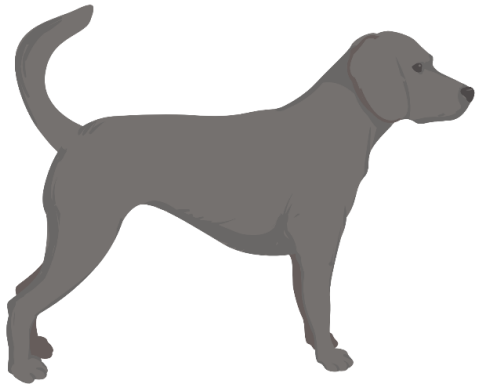
Practice

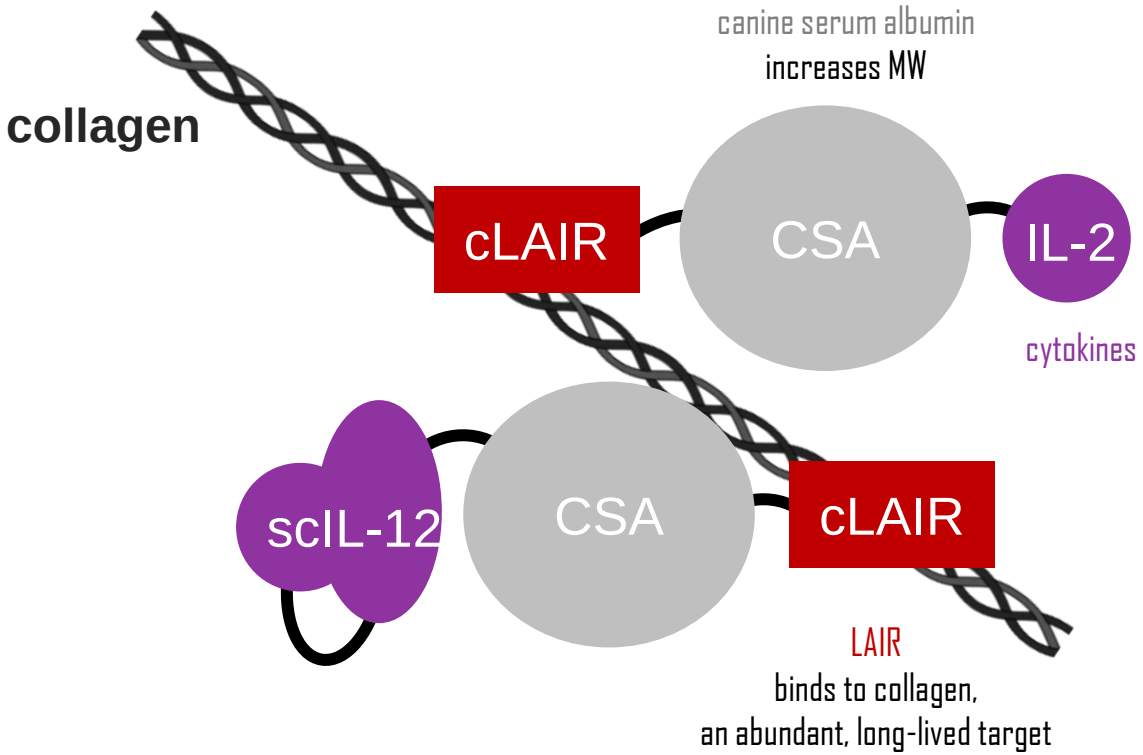


Momin et al. *Nat Comm.* 2022

Intratumoral Therapy

Pet dogs with naturally-occurring cancer can bridge translation





Prof. Tim Fan, UIUC

Dr. Rebecca Bernstein, DVM

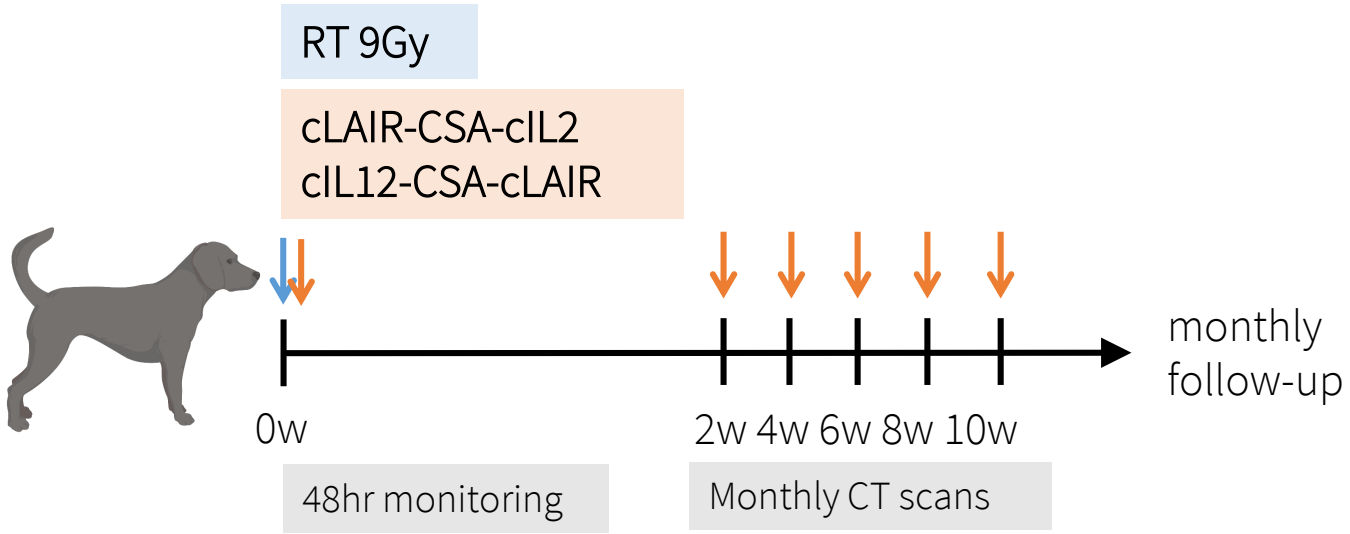
Dr. Jordan Hampel, DVM

Prof. Dane Wittrup, MIT

Jordan Stinson | Dr. Noor Momin | Allison Sheen

Cancer Center at Illinois

Phase I/II – Oral Malignant Melanoma

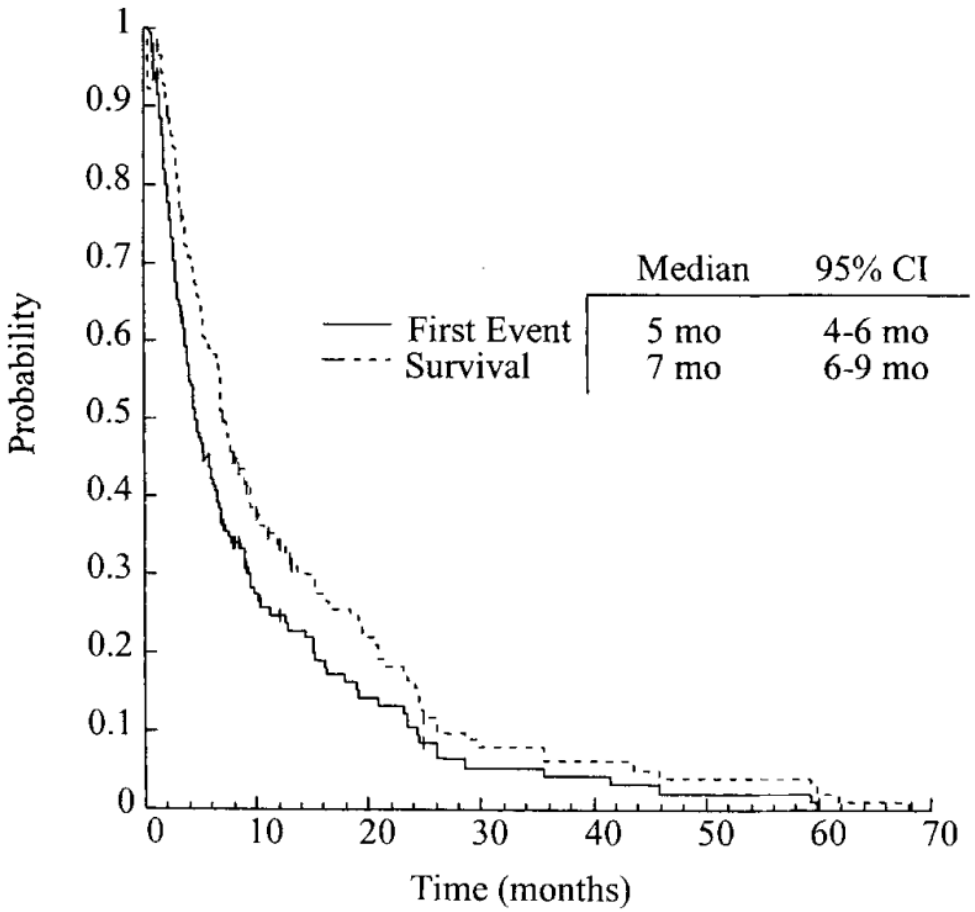


Starting dose (1X):

cLAIR-CSA-cIL-2: 17.4 µg/kg

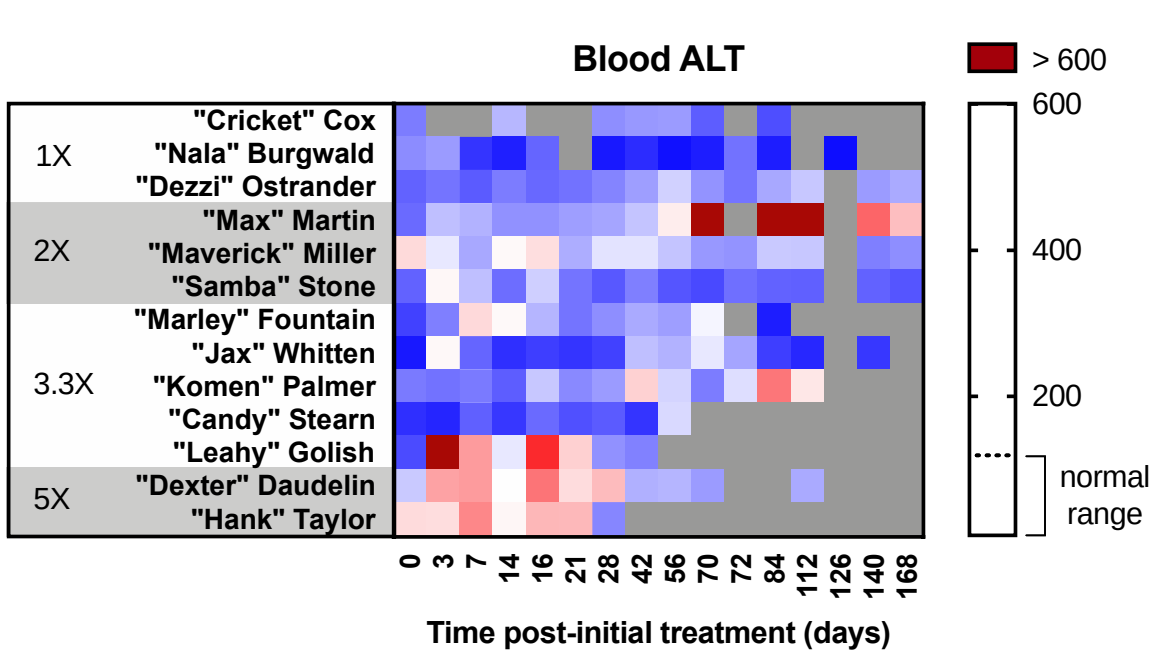
cIL-12-CSA-cLAIR: 0.041 mg/m^2

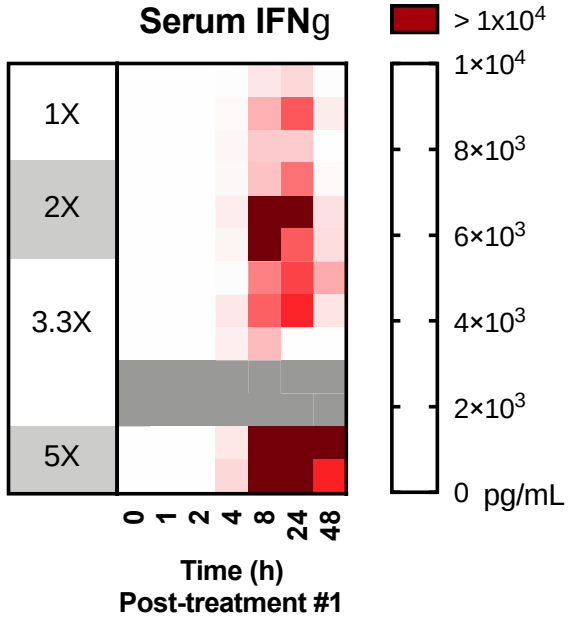
Escalated to 2X, 3.3X, 5X dose

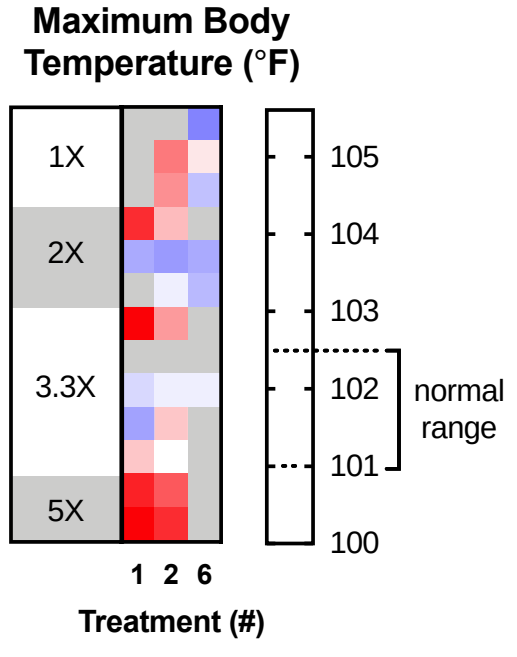


Proulx, D. R., Veterinary Radiology Ultrasound 2003

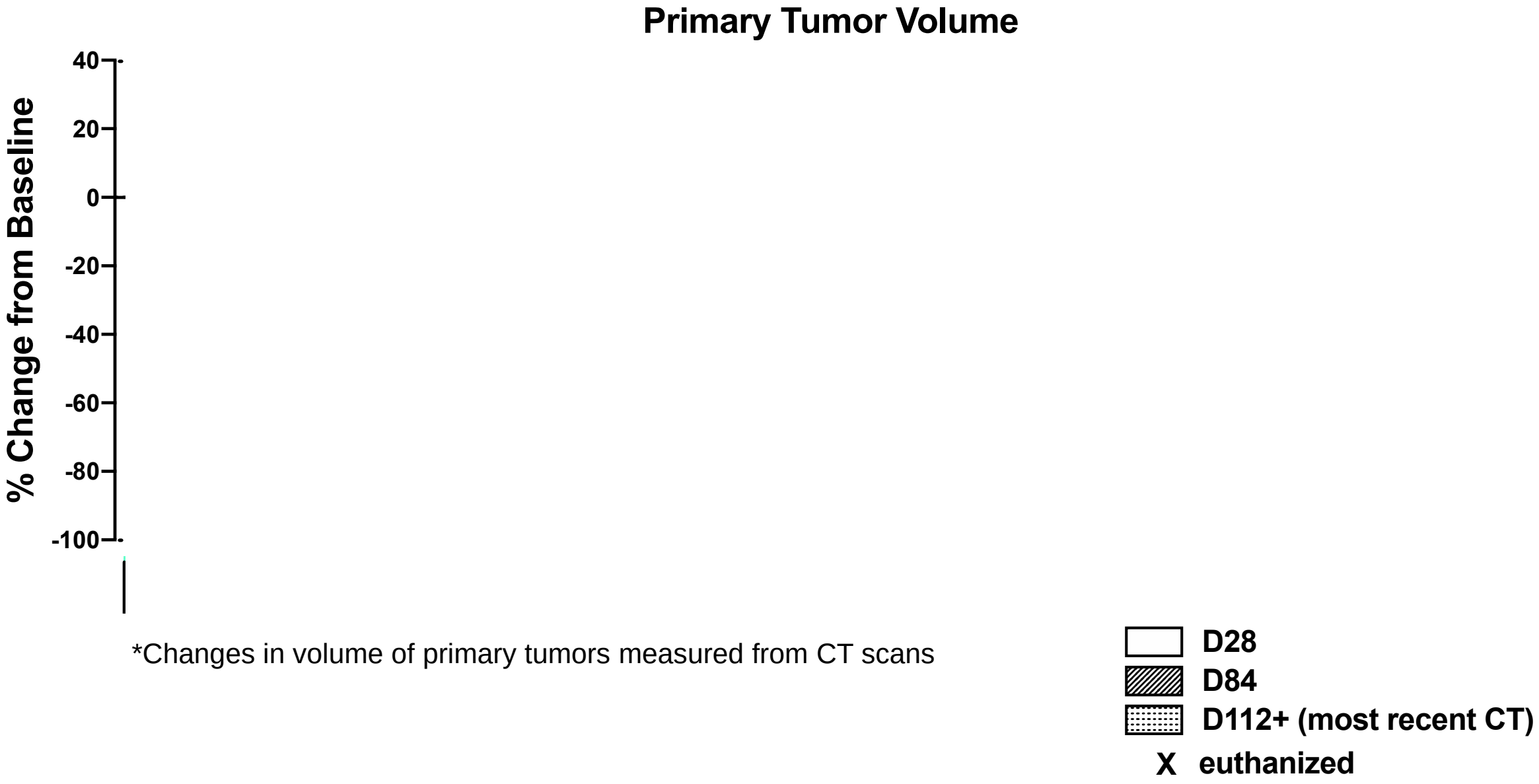
Safety – intratumoral cytokine treatment is tolerable ≤ 3.3X dose





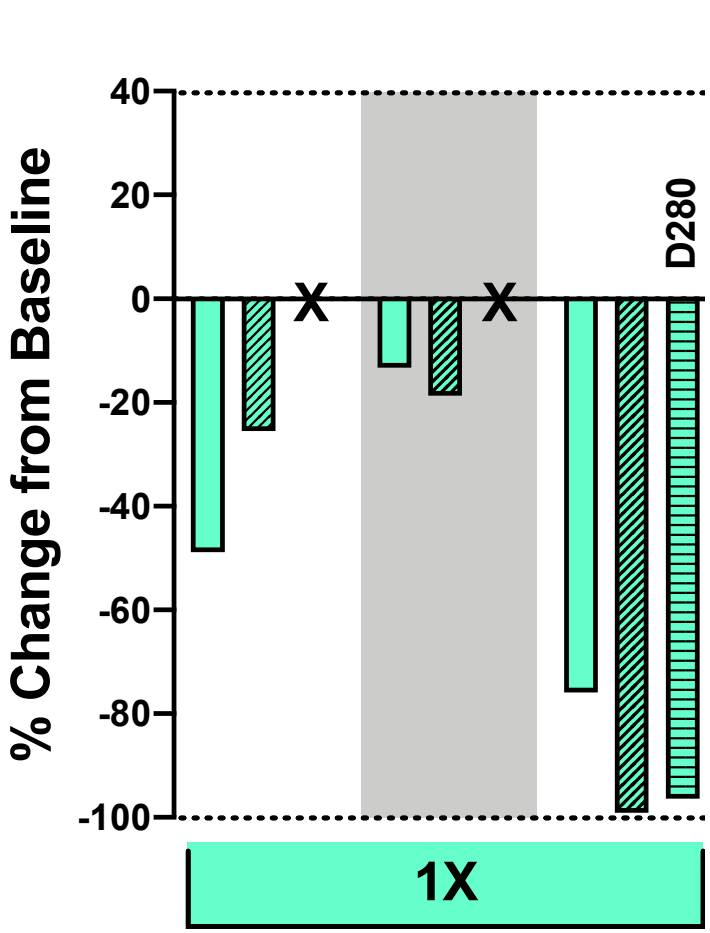


Efficacy – intratumoral cytokines results in regression of melanoma



*Changes in volume of primary tumors measured from CT scans

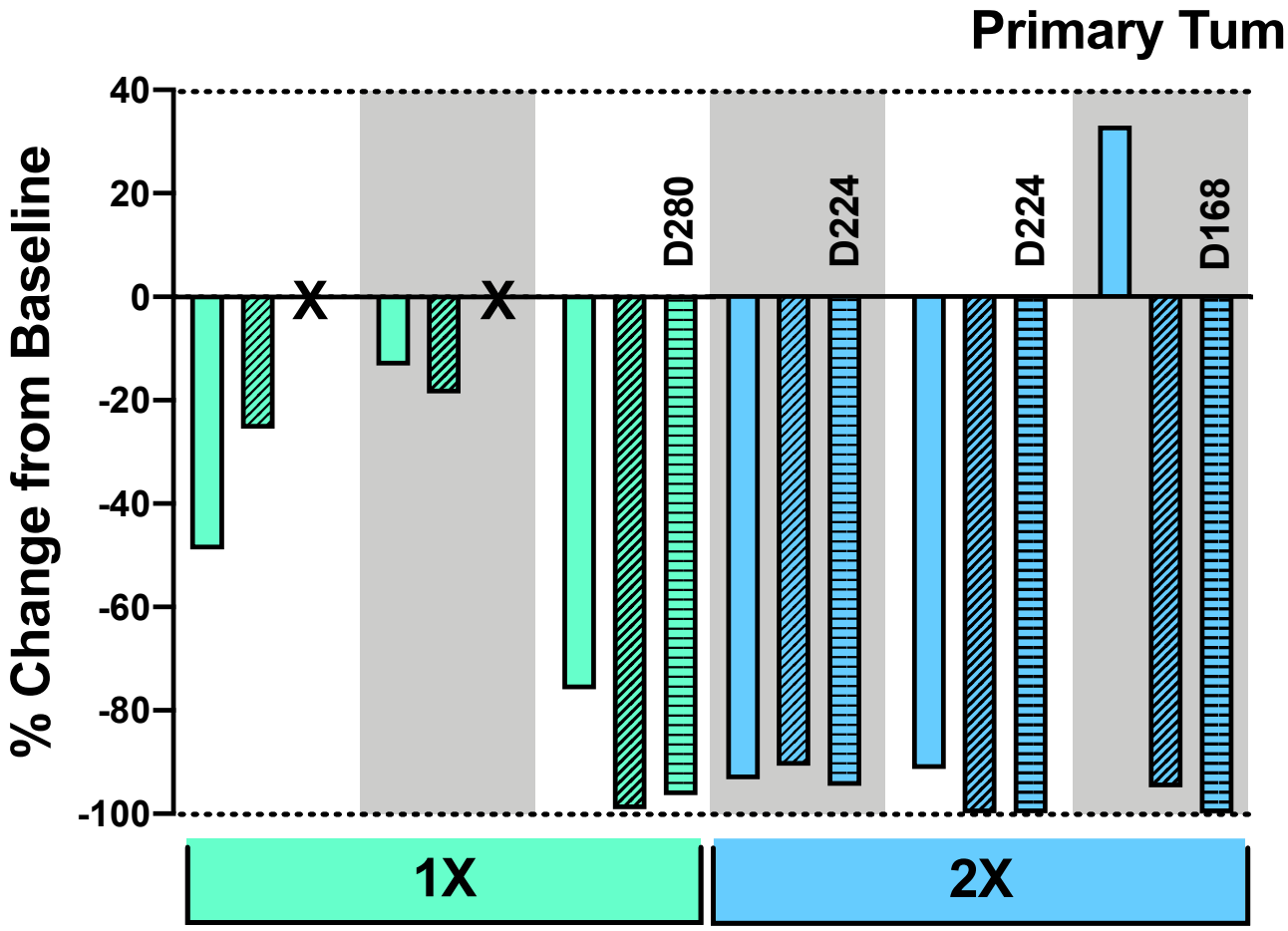
Efficacy – intratumoral cytokines results in regression of melanoma



*Changes in volume of primary tumors measured from CT scans

D28
D84
D112+ (most recent CT)
X euthanized

Efficacy – intratumoral cytokines results in regression of melanoma



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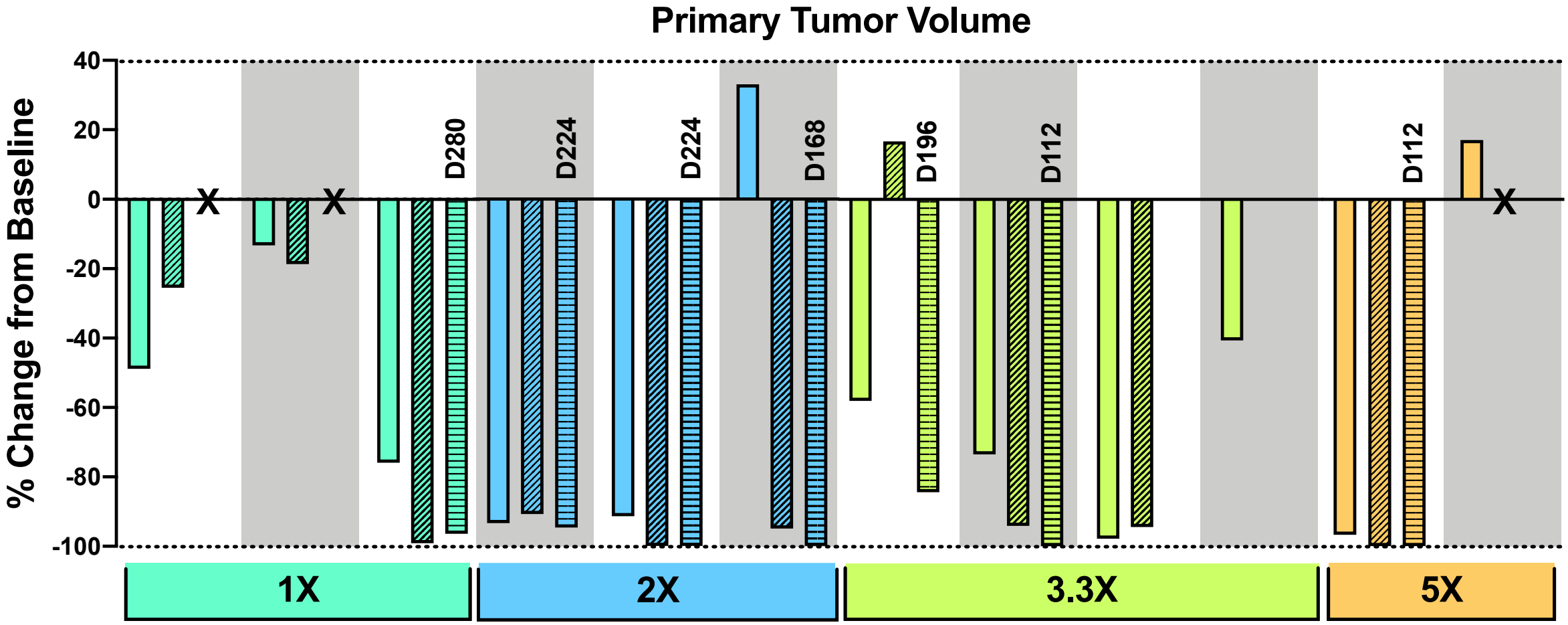


*Changes in volume of primary tumors measured from CT scans

Efficacy – evidence of long-lived anti-tumor immune response

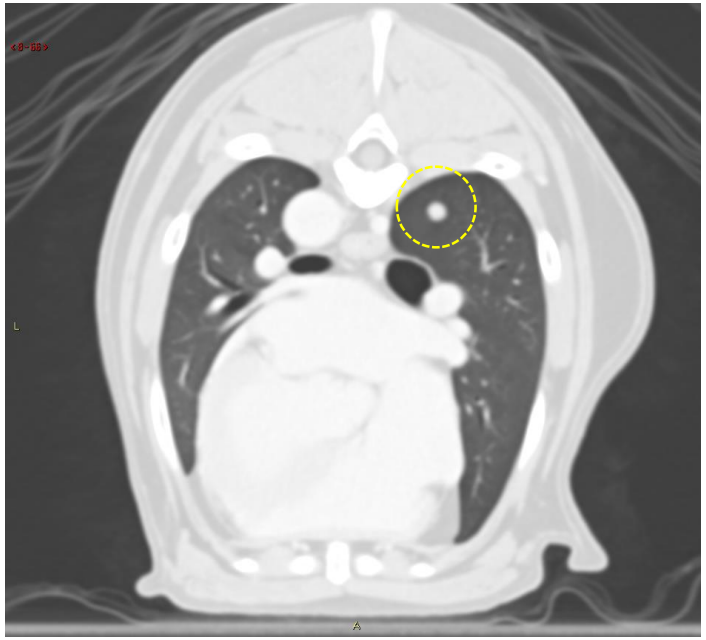
“Max” Martin (2X dose)

D1 D112 D140 D224

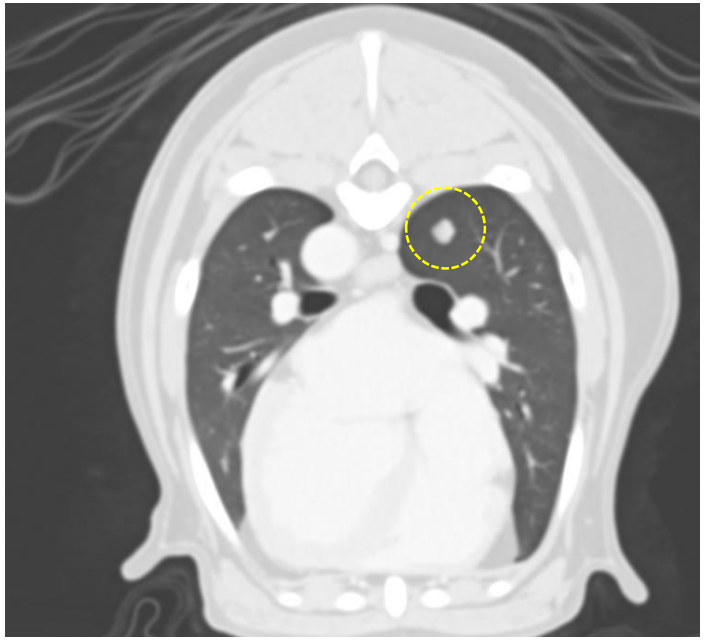
Tumor regrowth followed by regression after last cytokine dose (d84) suggests long-lived anti-tumor immune response

Efficacy – evidence of abscopal responses

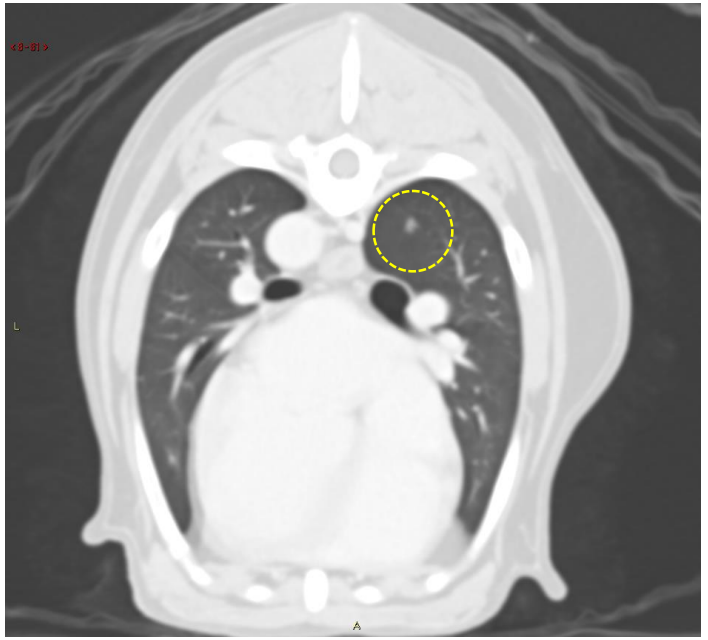
“Candy” Stern (3.3X dose)



Pre-treatment



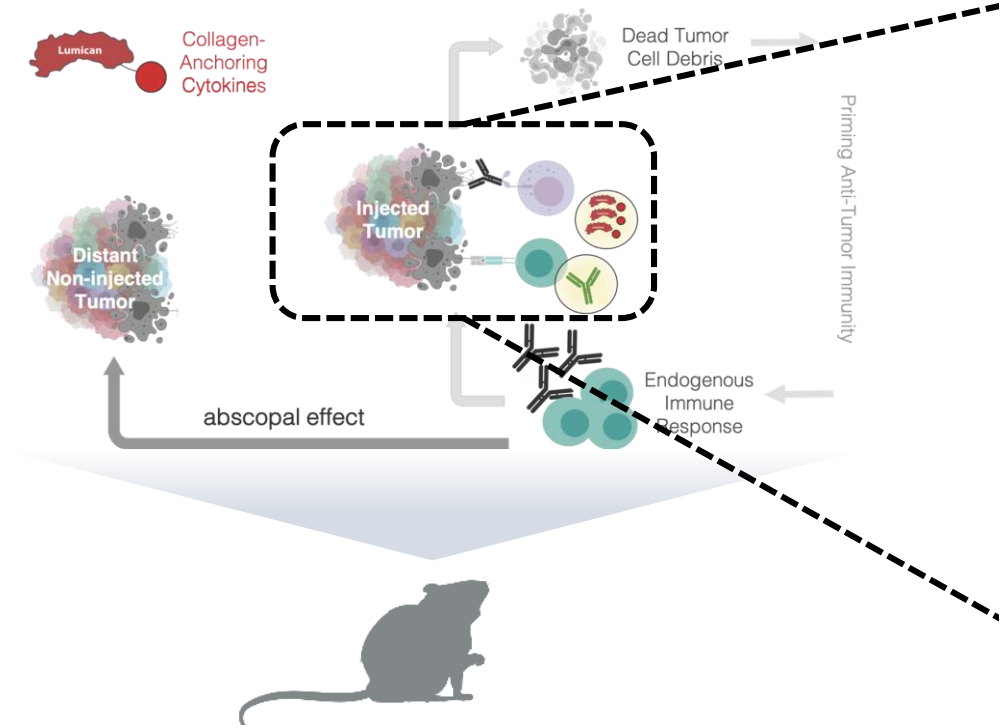
D28 (2 doses)



D70 (5 doses)

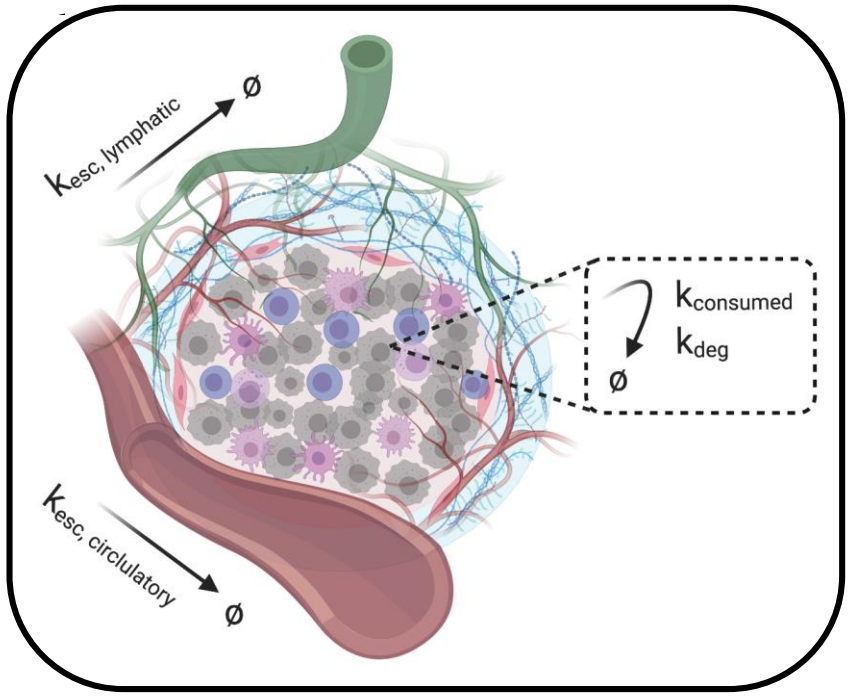
Evidence of distant response at lung metastatic site (CT)

Intratumoral Cytokine Therapy: Principles and Practice



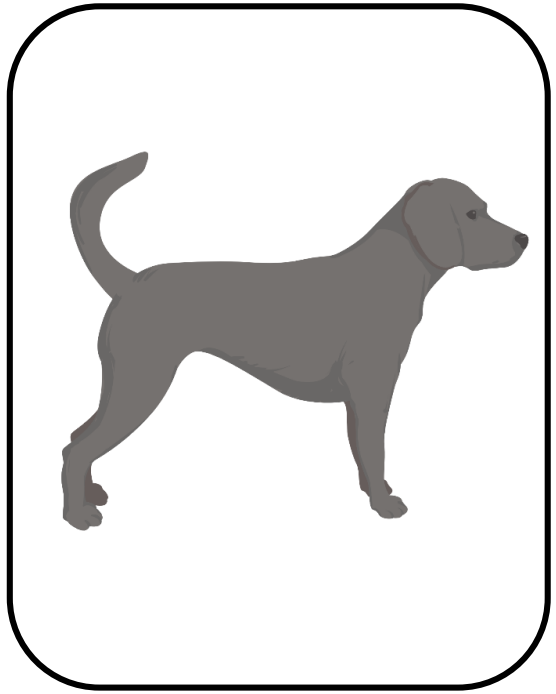
Tumor localization of collagen binding IL-2 and IL-12 can safely drive anti-tumor efficacy in several mouse tumor models.

Principles



Molecular weight, in addition to matrix affinity, contributes substantially to the retention of injected agents.

Practice



Treatment of canine soft tissue sarcoma and malignant melanoma with collagen binding IL-2 and IL-12 is safe and effective

Momin Lab

Cardio-Immunoengineering

Join us!

Penn Engineering
University of Pennsylvania

Prof. K. Dane Wittrup
Jordan Stinson
Allison Sheen
Emi Lutz
Dr. Naveen Mehta
Dr. Adrienne Rothschilds
Joseph Palmeri
Anthony Tabet
Magnolia Chinn
Izumi de los Rios Kobara
Yash Agarwal
Luciano Santollani
Sarah Cowles
Megan Hoffman
Keith Cheah

Prof. Timothy Fan (UIUC)
Dr. Rebecca Bernstein, DVM
Dr. Jordan Hampel, DVM
Rebecca Kamerer
Dr. Jonathan Samuelson
Prof. Kimberly Selting

Prof. Darrell Irvine (MIT)
Dr. Nitasha Bennet
Dr. Leyuan Ma (Penn)

Prof. Stefani Spranger (MIT)

Canine Patients and their Families

Cancer Center at Illinois

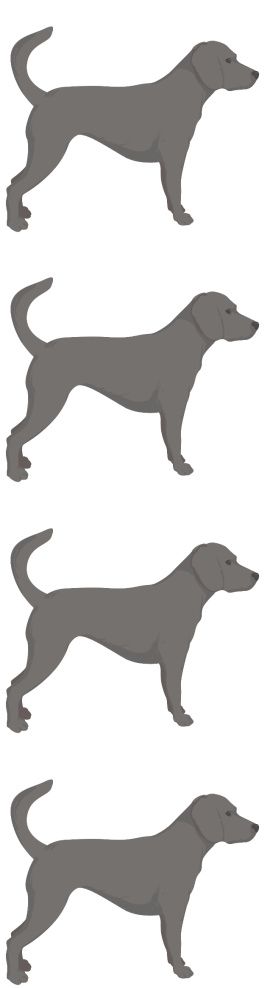
KOCH INSTITUTE for Integrative Cancer Research at MIT

Siebel Scholars

GRFP NSF GRADUATE RESEARCH FELLOWSHIP PROGRAM

NIH

Immunological Measurements - Soft Tissue Sarcoma Trial



Cohort 1
D1 Tx/D3 Resect
(n = 3)

Cohort 2
D1 Tx/D9 Resect
(n = 3)

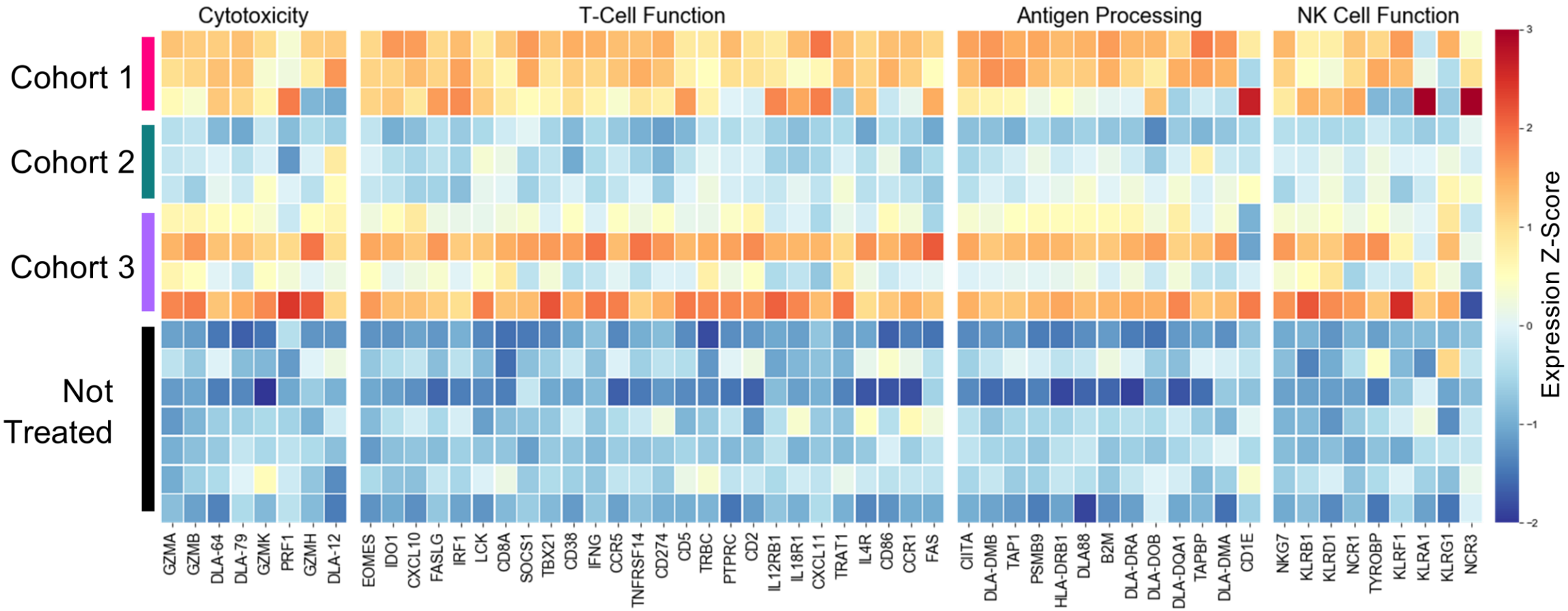
Cohort 3
D1, D7 Tx/D9 Resect
(n = 4)

Untreated
No Tx/Resect
(n = 7)

Treatment: Intratumoral dosing of cytokines

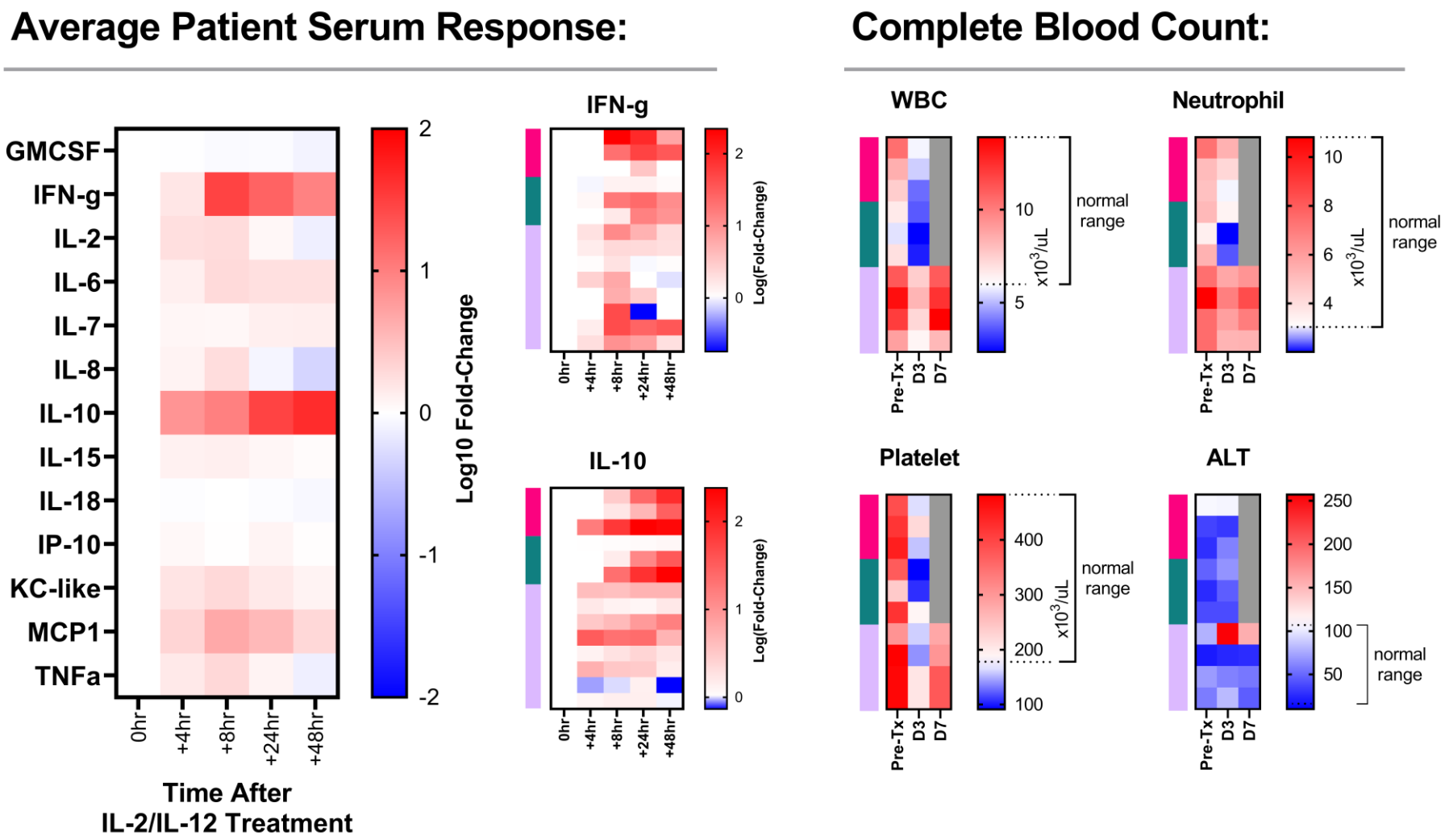
- cLAIR-CSA-cIL-2: 17.4 µg/kg
- cIL-12-CSA-cLAIR: 0.041 mg/m^2

Treated, resected sarcomas display enhanced immune infiltration



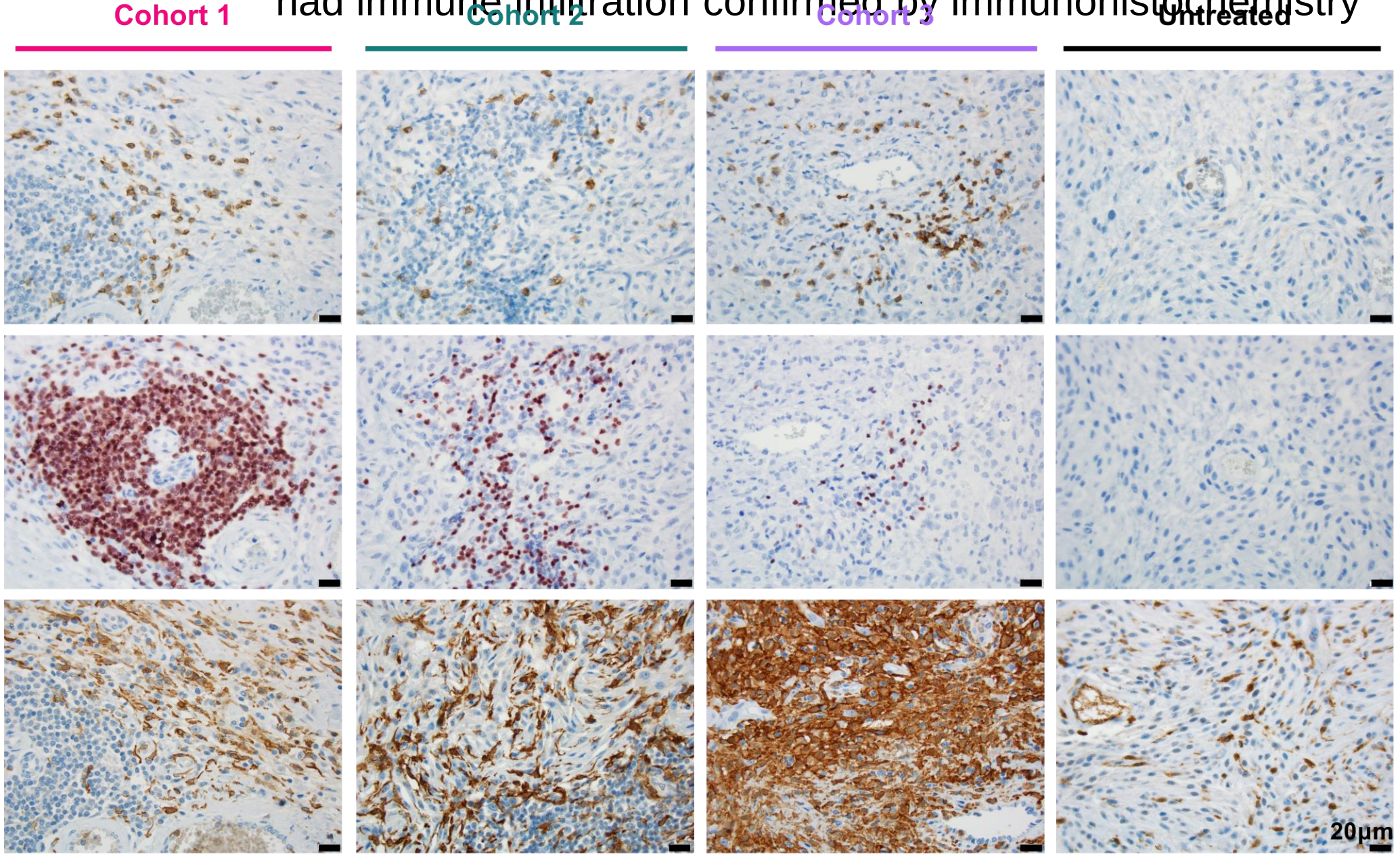
FFPE-derived tumor RNA profiled by Nanostring confirmed infiltration of CD45+ immune populations

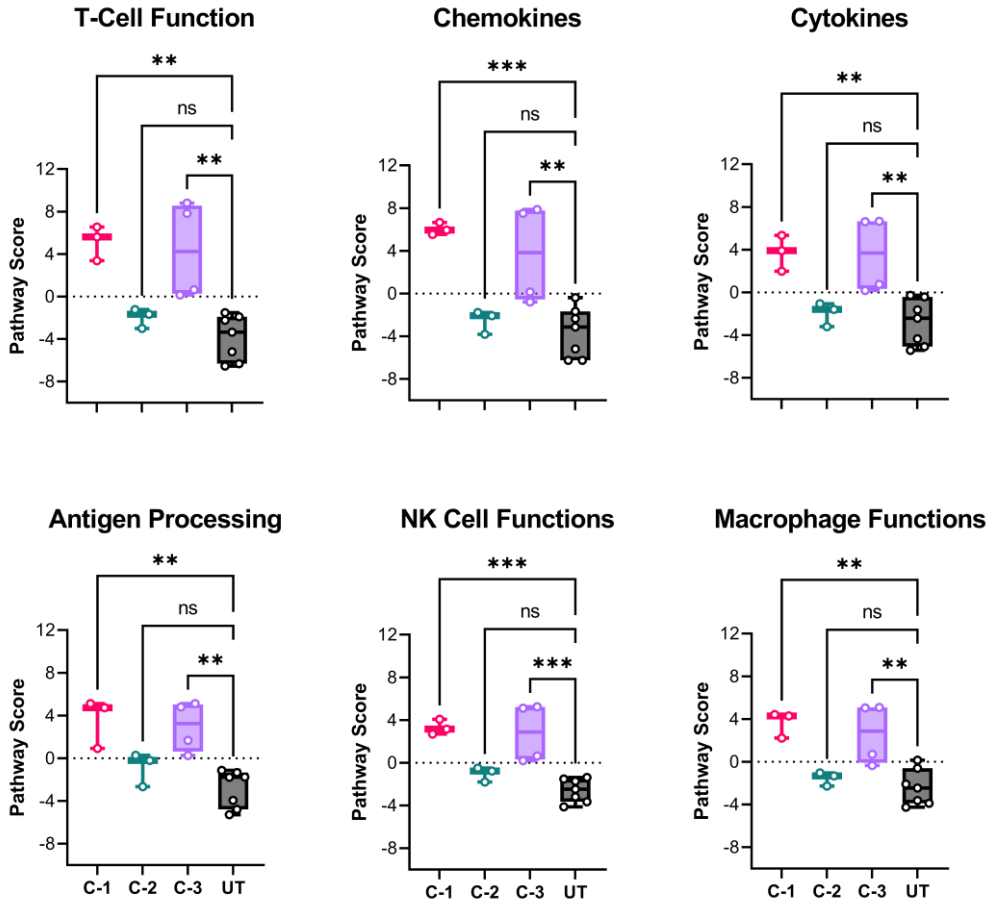
Counter-regulatory responses limit TIL effector functions after intratumoral cytokine therapy



Multiplex analysis of serum analytes revealed a common IFN-g and IL-10 response pattern to treatment, which led to minor neutropenia and thrombocytopenia

Immunologically “cold” soft-tissue sarcomas treated with IL-2 and IL-12 had immune infiltration confirmed by immunohistochemistry



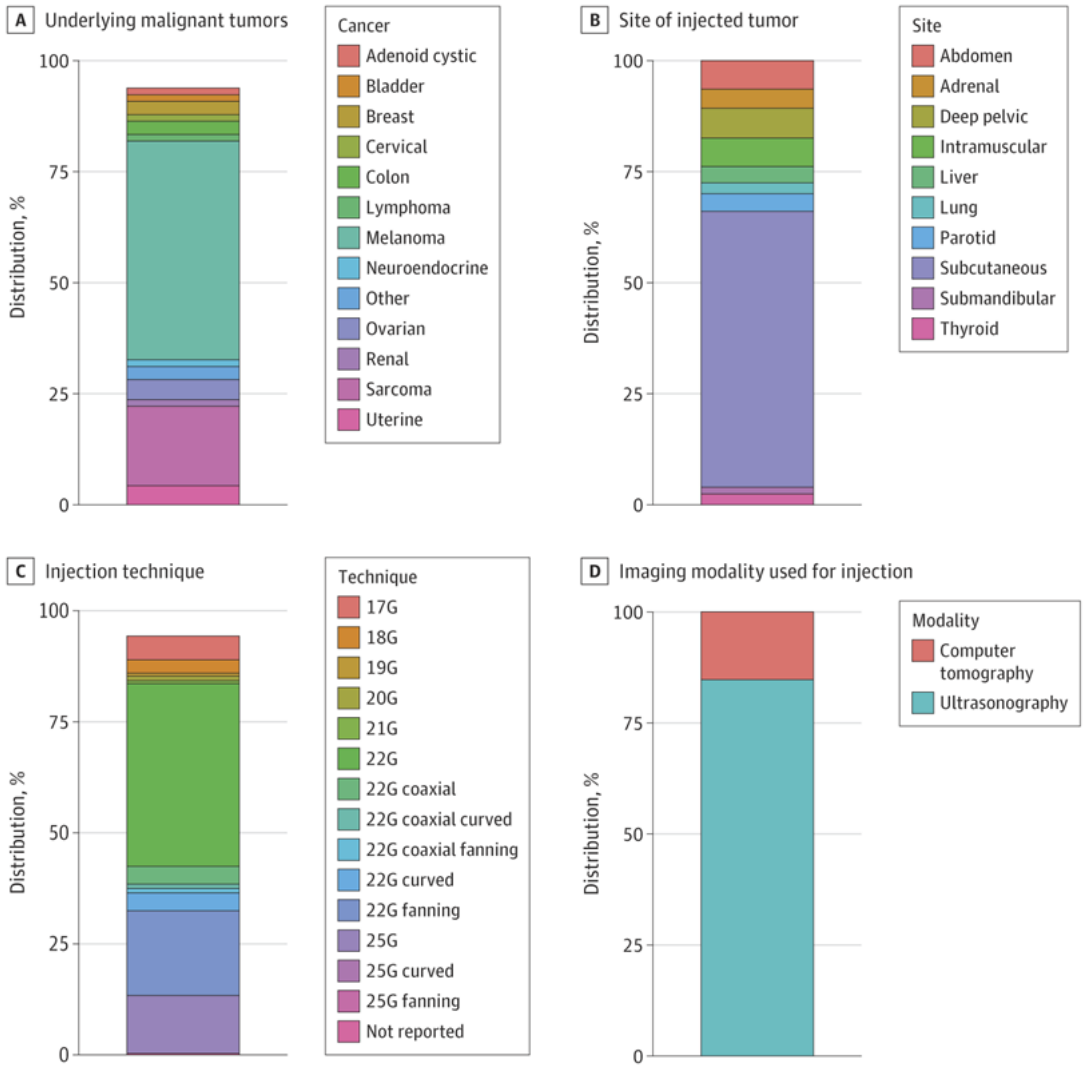


RNA profiling of treated sarcomas suggest immune infiltrates display hallmarks of activation, antigen presentation, and anti-tumor effector functions

FFPE-derived tumor RNA profiled by Nanostring confirmed infiltration of CD45+ immune populations

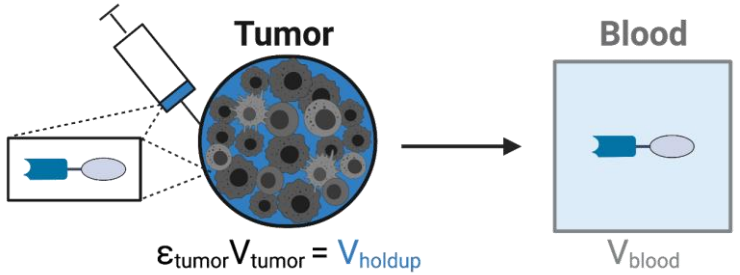
Outlook on the Field

Intratumoral injections are the future



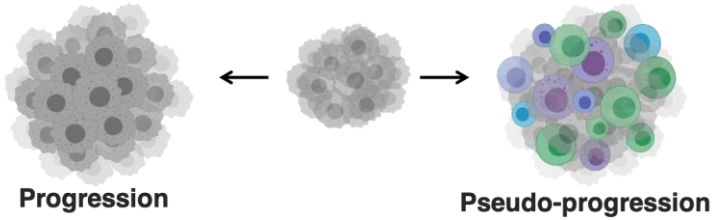
Sheth et al, *Jama Nework Open* 2020

How to inject intratumoral therapies?

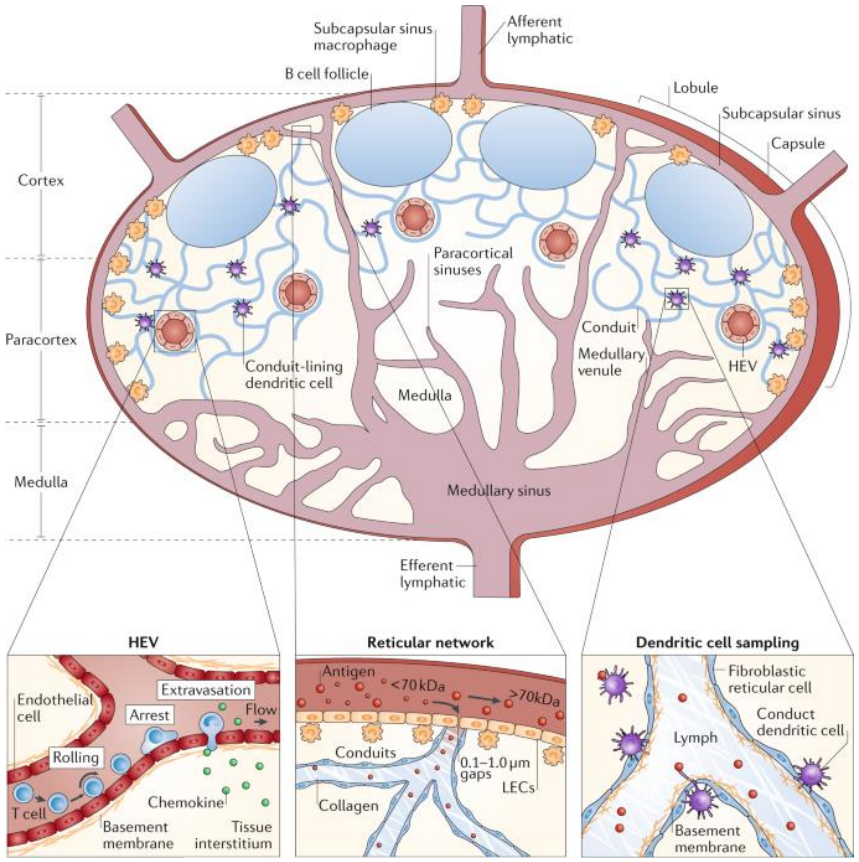


How to sequence local therapies with standard of care?

How to measure clinical responses?

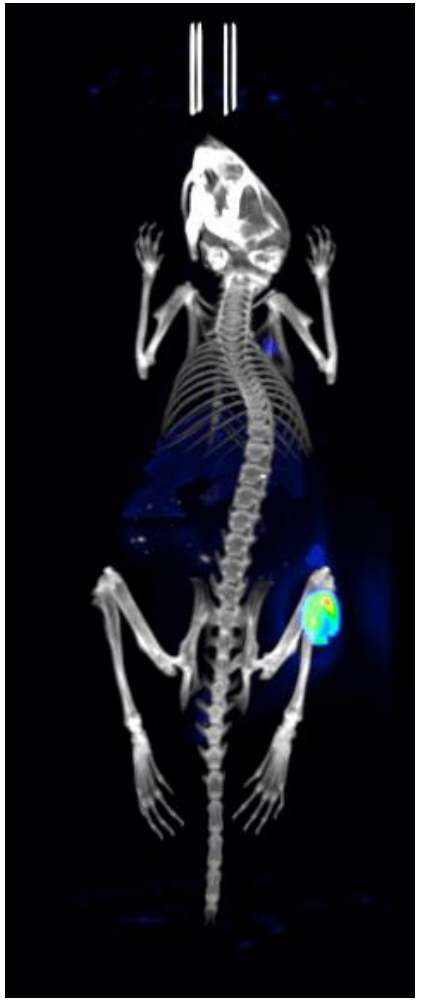


Why are there major changes in the lymph node?



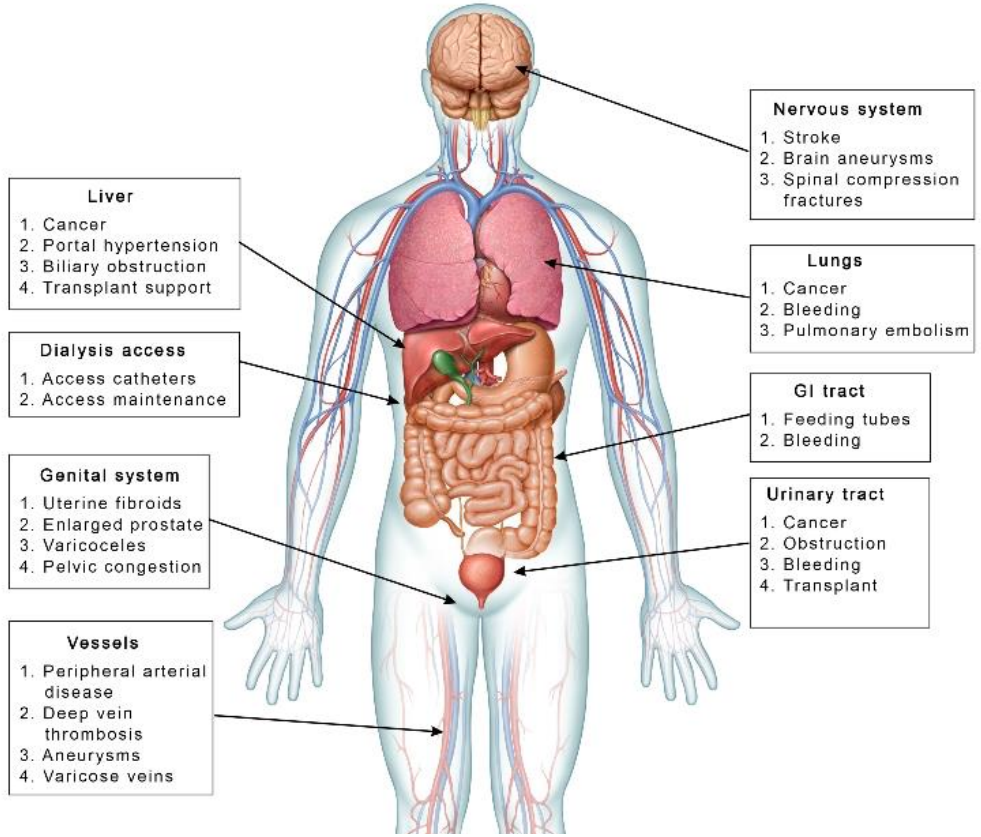
Schudel et al Nature Review Materials, 2019

LAIR LAIRx MSA^{H464Q} Zr-89



LAIRx LAIRx MSA^{H464Q} Zr-89

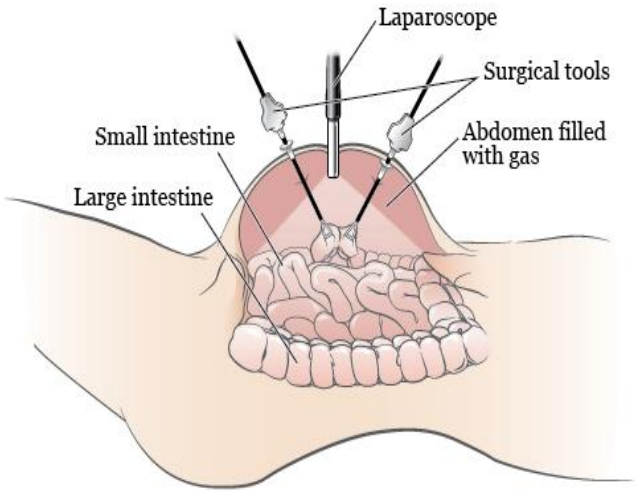
What about non-superficial tumors?



Very few sites are inaccessible to interventional radiology, guided by ultrasound, CT, MRI

https://www.sirweb.org/patients/what-is-interventional-radiology/

Diagnostic laparoscopy



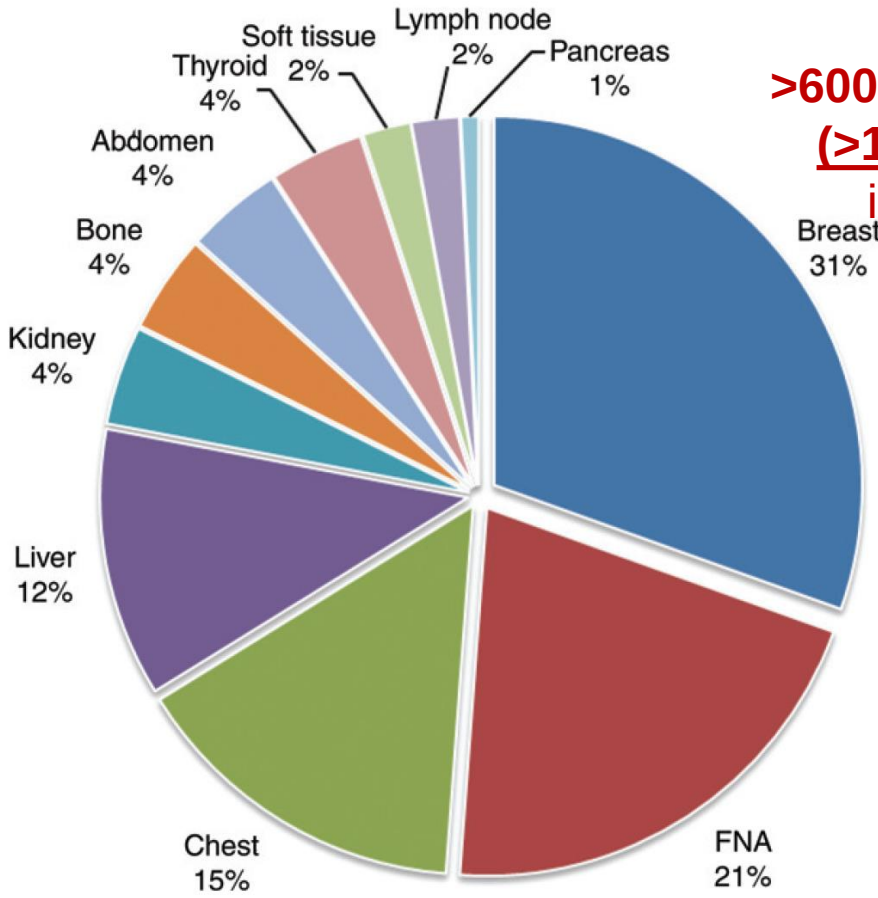
"During laparoscopic procedures, various types of needles can be used, including aspiration, injection, irrigation, puncture needles, and Veress needles with stopcocks." Millennium Surgical Instruments *(www.surgicalinstruments.com)*

Minimally-invasive surgery is commonplace

https://www.mskcc.org/cancer-care/patient-education/laparoscopy

Needle biopsies are standard of care

- Breast
  - *Image-guided breast biopsy: state-of-the-art. O'Flynn E et al., Clin Radiol. 65: 259, '10.*
- Lung
  - *Feasibility of image-guided transthoracic core-needle biopsy in the BATTLE lung trial. Tam et al., J Thorac Oncol. 2013 8:436.*
- Prostate
  - *Image-Guided Prostate Biopsy Using Magnetic Resonance Imaging–Derived Targets: A Systematic Review. Moore et al., Eur Urol. 63:125, '13.*
- Liver
  - *Complications following percutaneous liver biopsy.A multicentre retrospective studyon 68,276 biopsies. Piccinino et al., J. Hepatol 2:165, '86*



>600K biopsies (>1/minute) in 2008

Radiology 256:751, '10

∴ ***Curative intratumoral treatments will find a straightforward path to clinical practice***

How is TVEC injected?

Table 1. TVEC and theoretical maximum injectable volumes			
Legion size (Longest dimension, cm)	TVEC injection volume (mL)	Predicted V_{max}(mL)	TVEC/V_{max}
>5	4	16	0.25
2.5-5	2	2.0-16	0.125-1
1.5-2.5	1	0.42-2.0	0.5 – 2.4
0.5-1.5	0.5	0.016-0.42	1.2 – 31
<0.5	0.1	0.016	6.3

Canine Back Up Slides

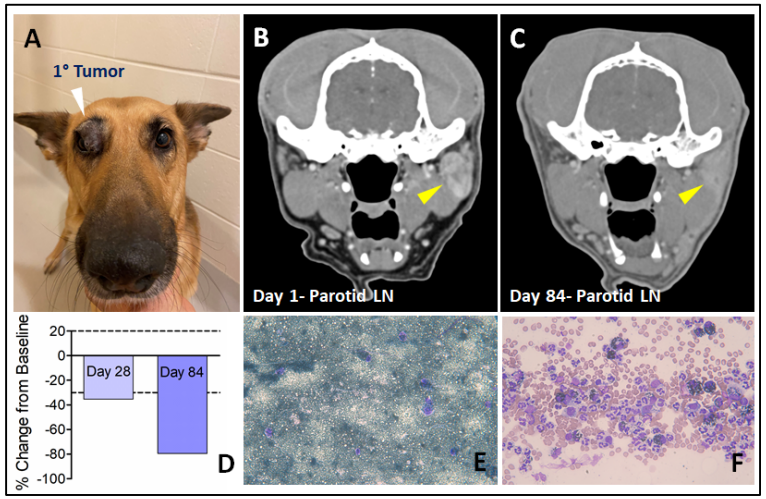


Fig 9. Periocular melanoma (**A**) treated with localized radiation and intratumoral cytokine therapy. Regional metastatic parotid lymph node pretreatment identified on computed tomography and cytology (**B** and **E**, respectively), with reassessment following treatment with radiation and intratumoral cytokines (**C** and **F**, respectively). Strong partial abscopal response (**D**) achieved at days 28 & 84 of therapy.

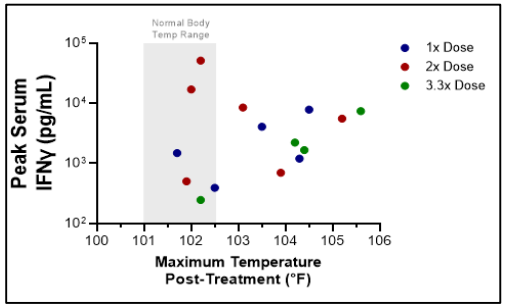
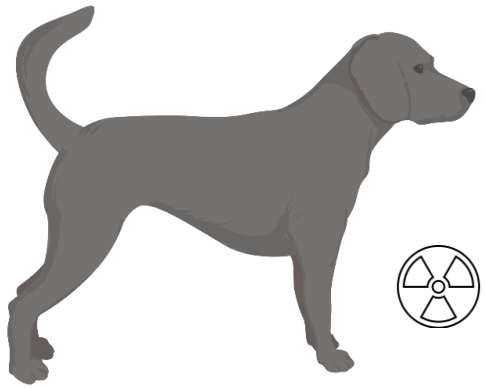


Fig 6. Peak temperature & plasma IFNγ in dogs treated with intratumoral cytokines.

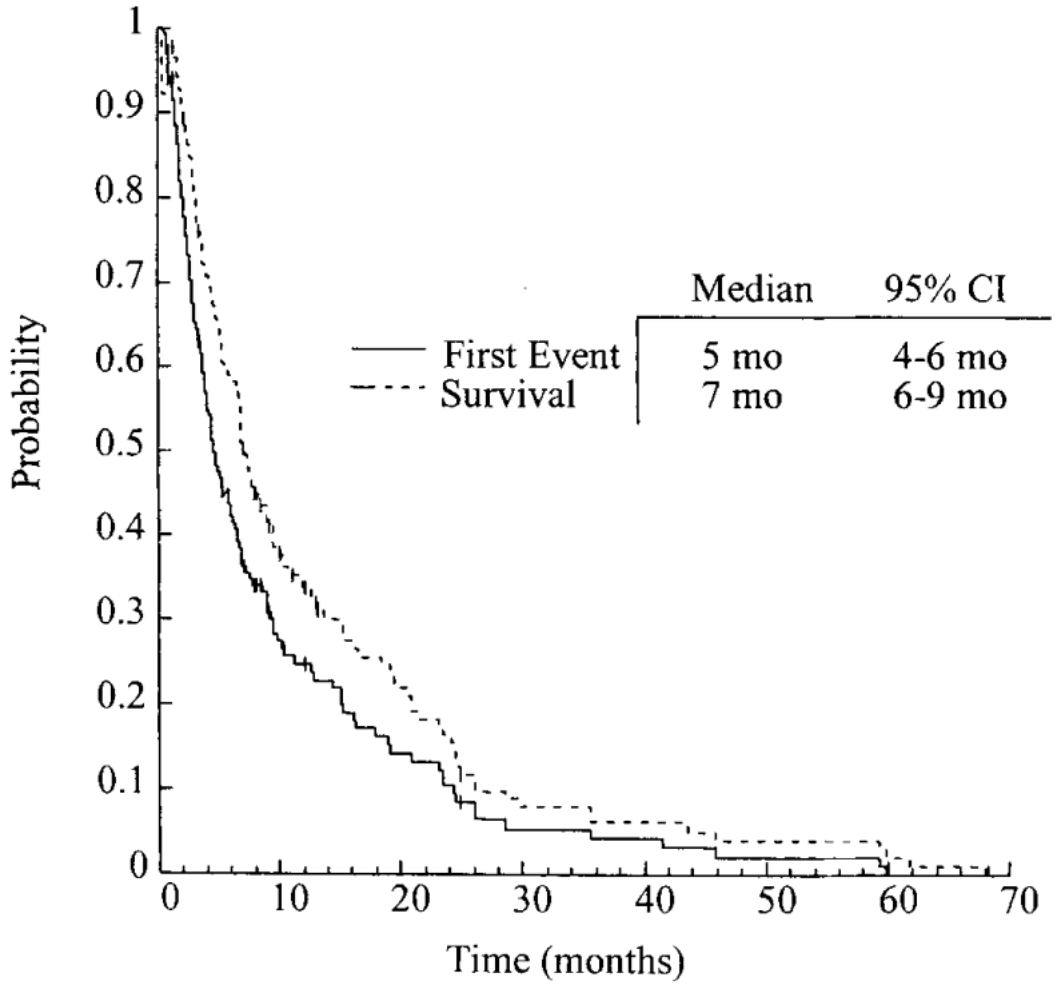
Phase II: Treating pet dogs with spontaneous melanoma

MTD



10 Gy Local Radiation

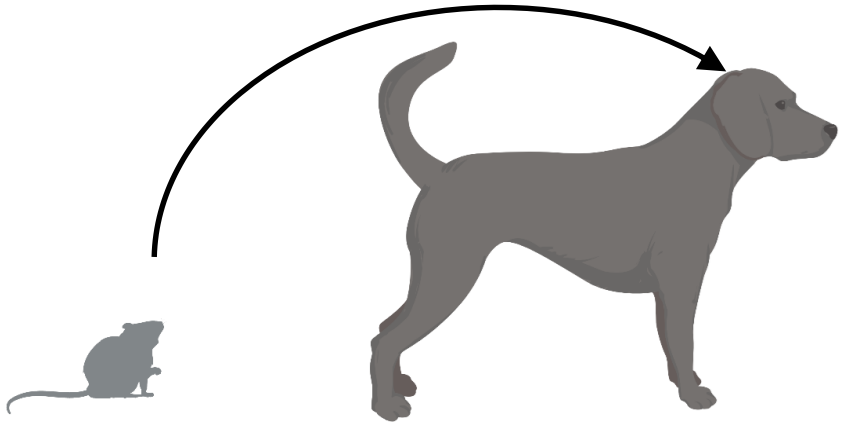
Melanoma bearing dogs

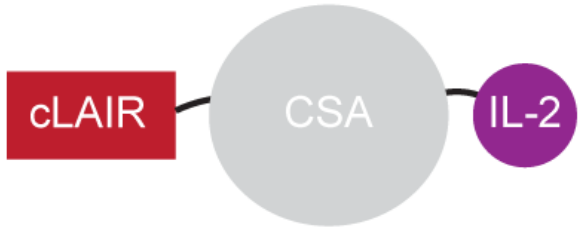


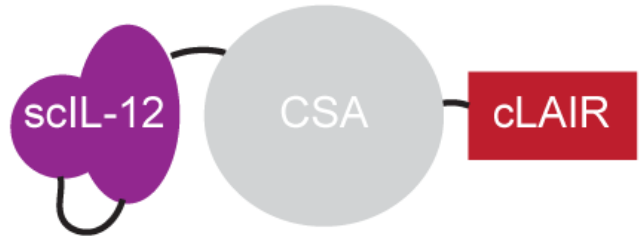
Proulx, D. R., Veterinary Radiology Ultrasound 2003

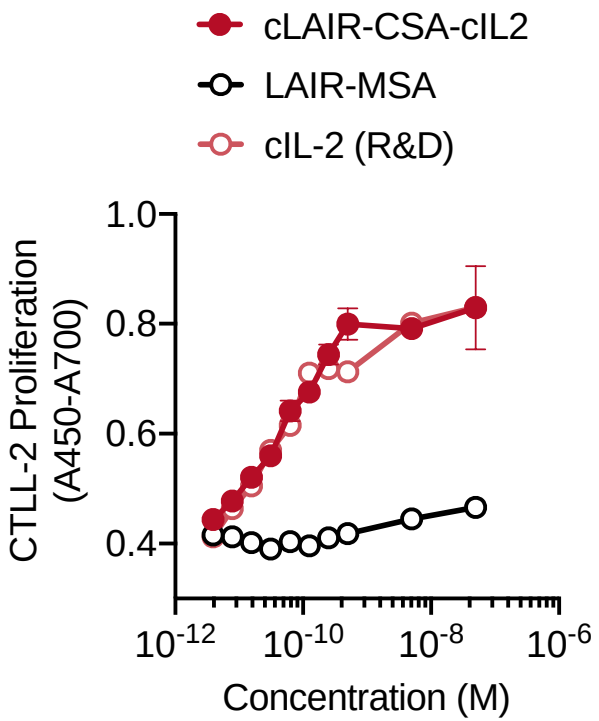
Phase I | Phase II

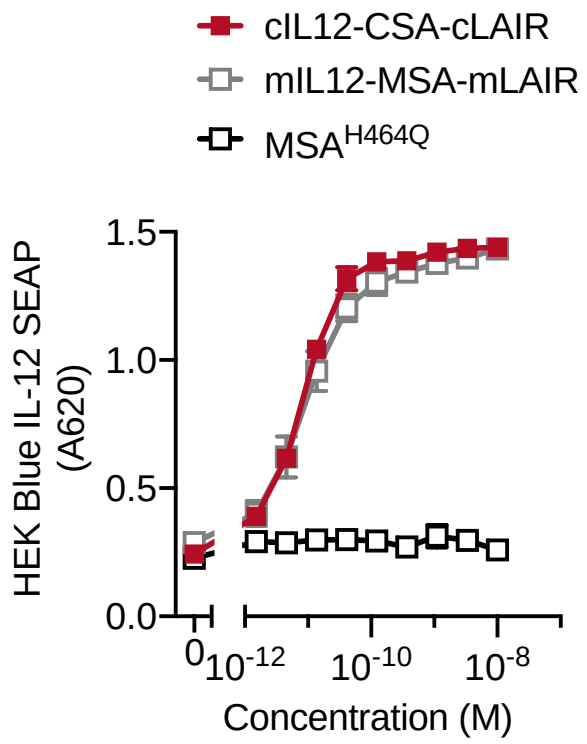
Canine collagen anchoring cytokines are functional





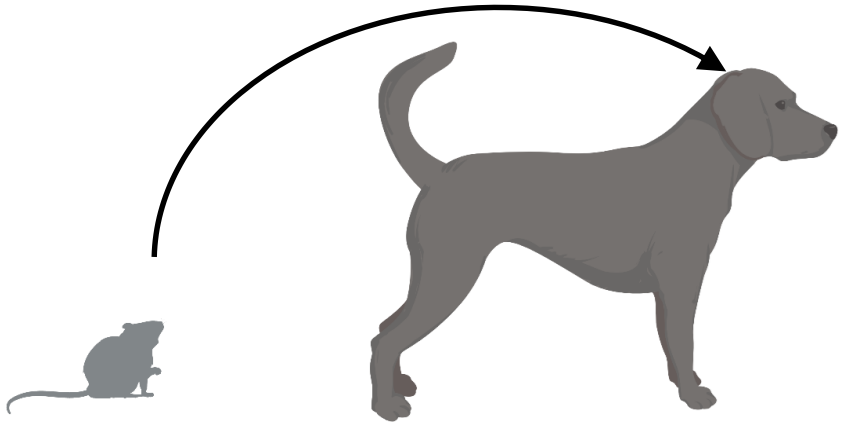


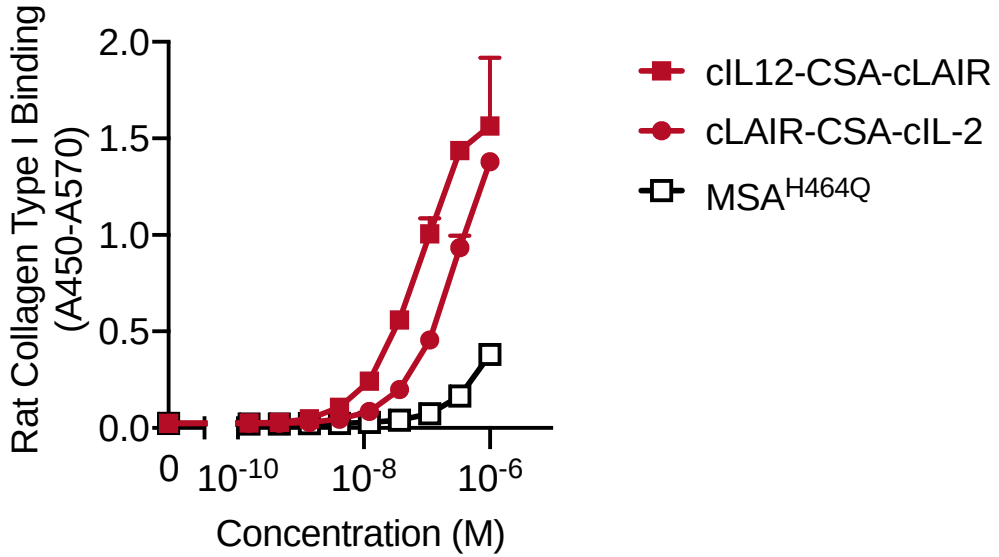




Preclinical | Phase I | Phase II

Canine collagen anchoring cytokines are functional





Preclinical | Phase I | Phase II

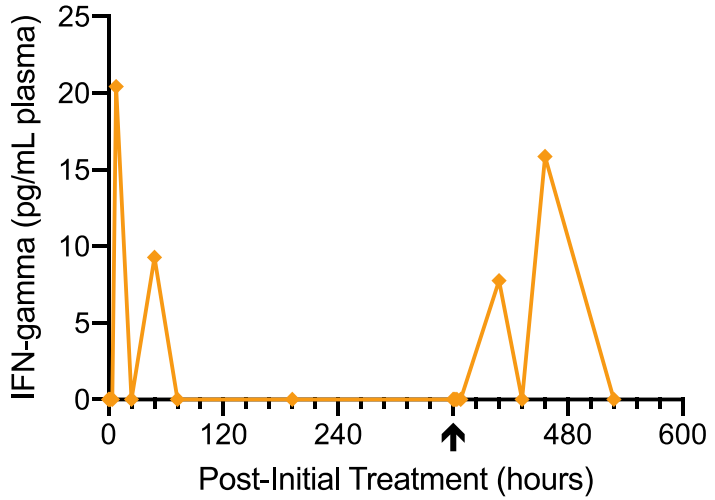
Phase I: Defining a maximum tolerated dose in healthy beagles

Treatment
cLAIR-CSA-cIL2
+ cIL12-CSA-cLAIR
in 600 uL, with 25G needle
into shaved shoulder

High Dose

Med Dose

Low Dose



Preclinical | Phase I | Phase II

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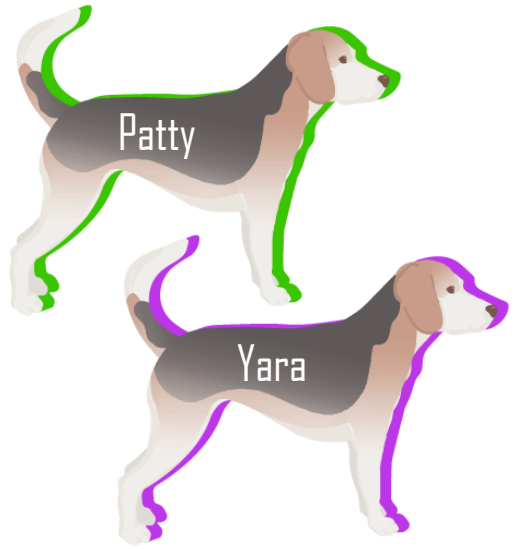
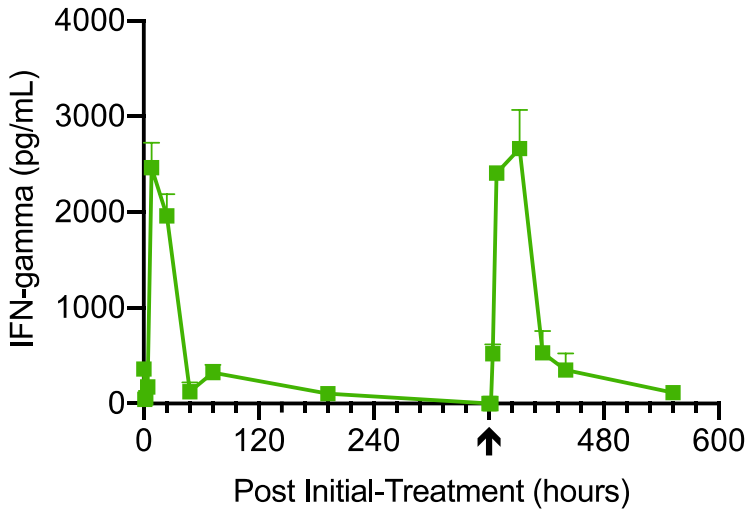
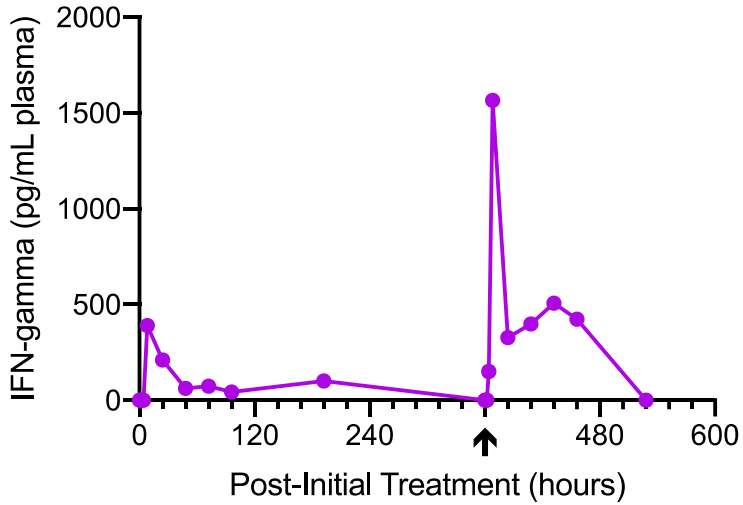
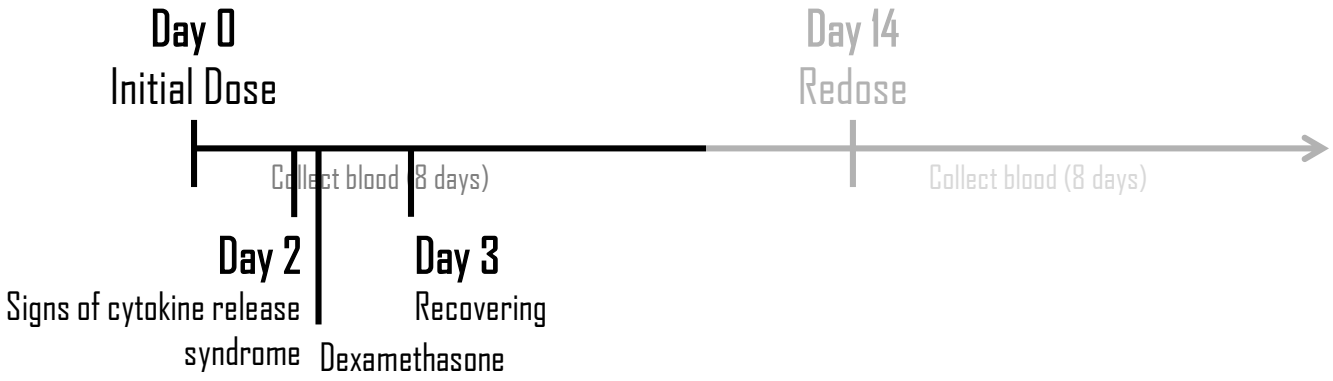






Preclinical | Phase I | Phase II

Phase I: Defining a maximum tolerated dose in healthy beagles

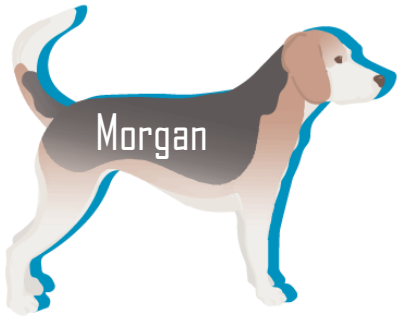


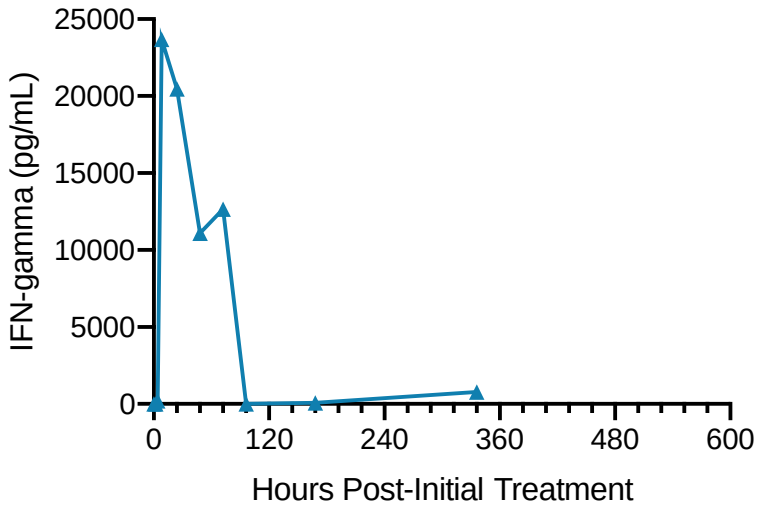
Treatment
cLAIR-CSA-cIL2
+ cIL12-CSA-cLAIR
in 600 uL, with 25G needle
into shaved shoulder

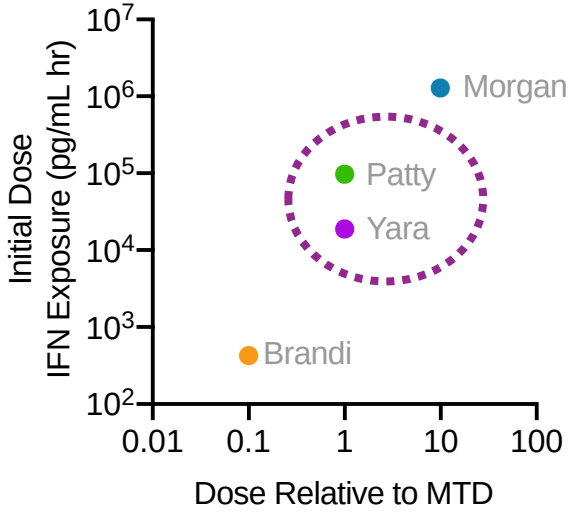
High Dose

Med Dose

Low Dose

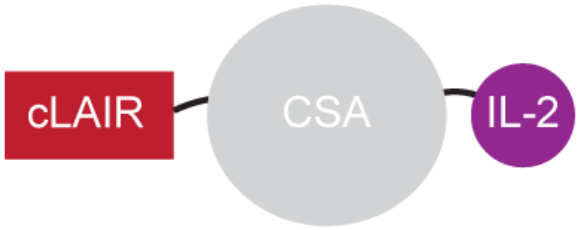


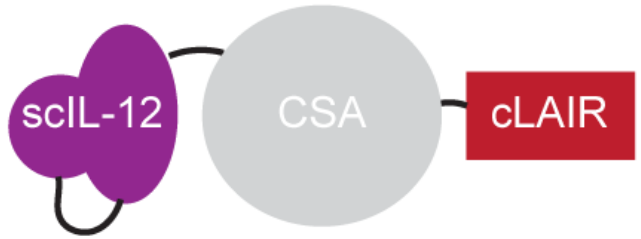




Preclinical | Phase I | Phase II

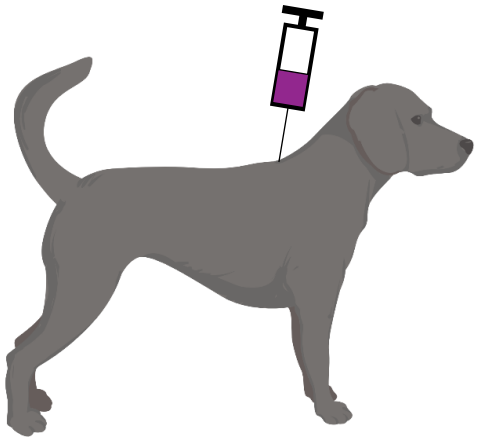
Phase I: Defining a maximum tolerated dose in healthy beagles





High Dose

Low Dose



Healthy Dogs

High Dose	=	173.6 µg/kg alloscaled Proleukin MTD	+	0.4 mg/m^2 NHS-IL12 canine MTD
Med Dose	=	17.36 µg/kg cLAIR-CSA-cIL2	+	0.04 mg/m^2 cIL12-CSA-cLAIR
Low Dose	=	1.736 µg/kg	+	0.004 mg/m^2

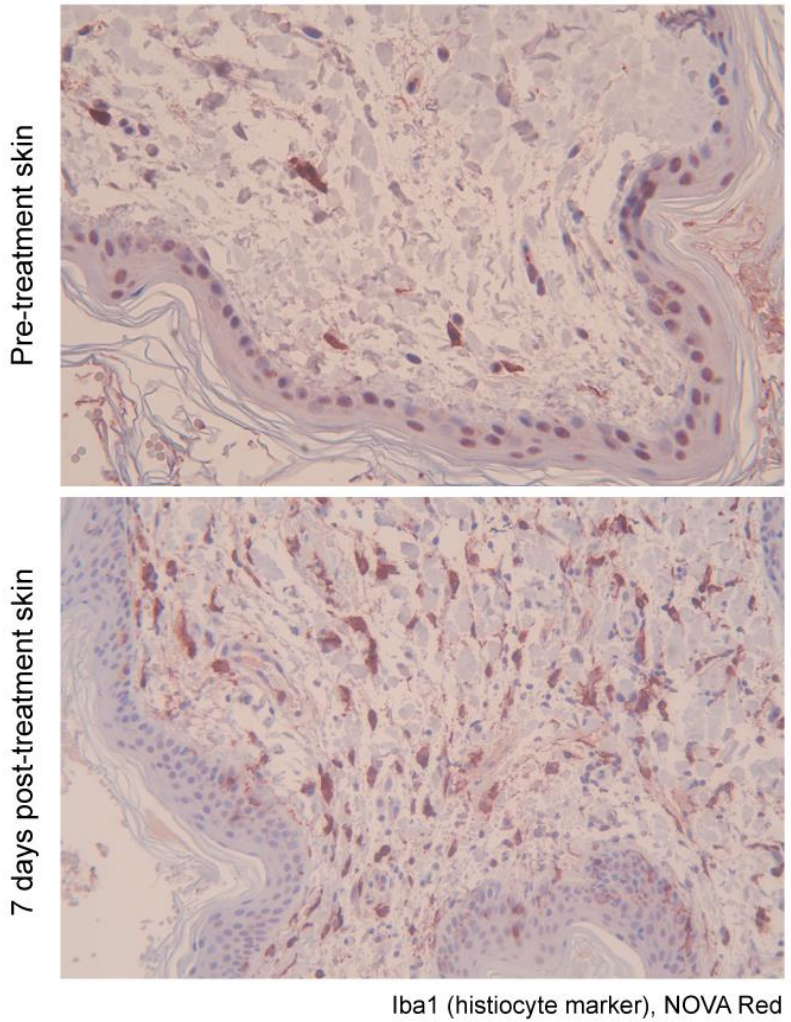
Phase I | Phase II

Myeloid cell infiltration into dermis

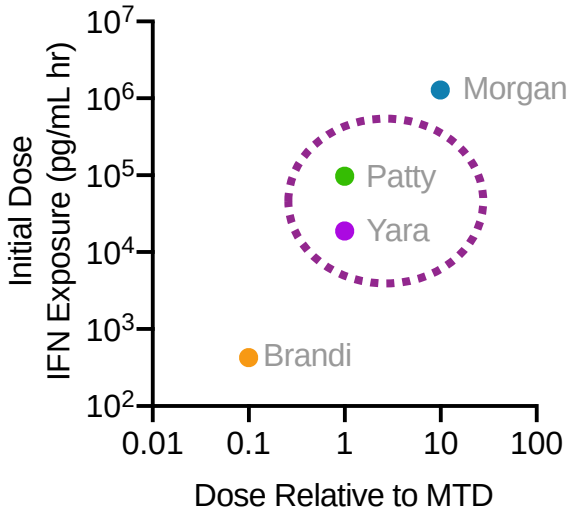
High Dose

Med Dose

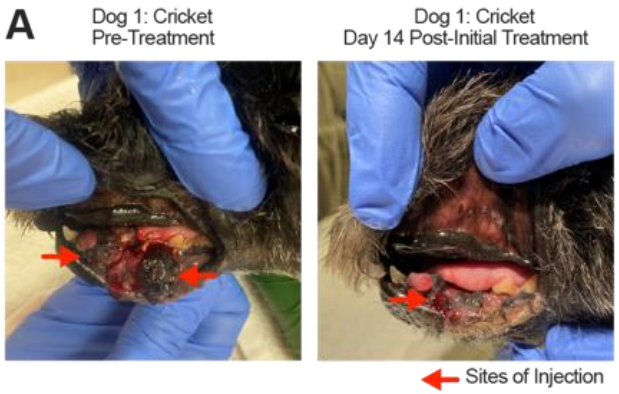
Low Dose

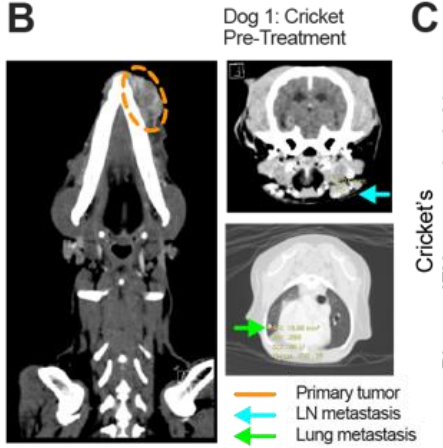


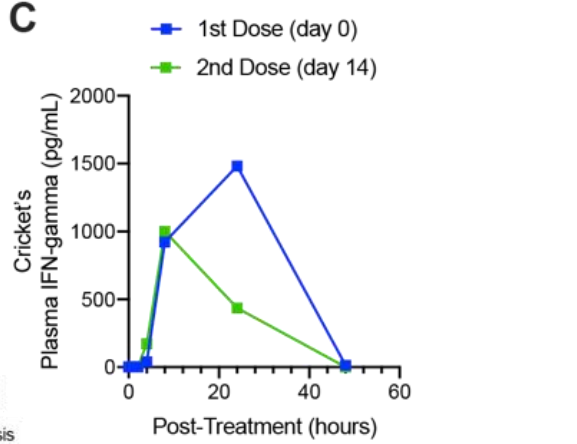
Iba1 (histiocyte marker), NOVA Red

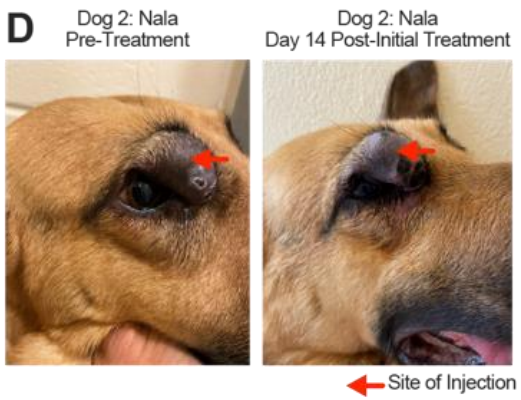


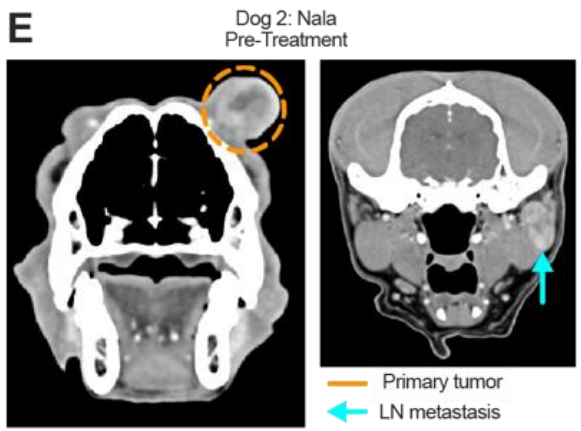
Preclinical | Phase I | Phase II

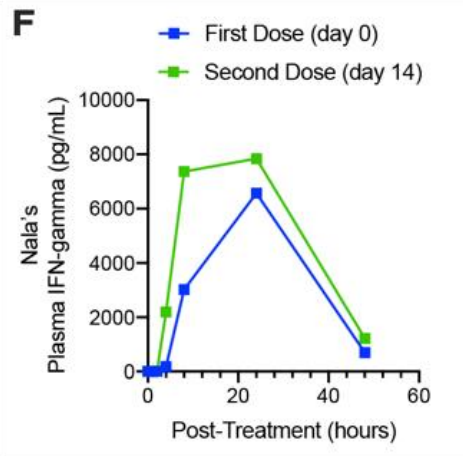

A
Dog 1: Cricket
Pre-Treatment
Dog 1: Cricket
Day 14 Post-Initial Treatment
Sites of Injection


B
Dog 1: Cricket
Pre-Treatment
Primary tumor
LN metastasis
Lung metastasis


C
1st Dose (day 0)
2nd Dose (day 14)
Cricket's
Plasma IFN-gamma (pg/mL)
2000
1500
1000
500
0
0
20
40
60
Post-Treatment (hours)

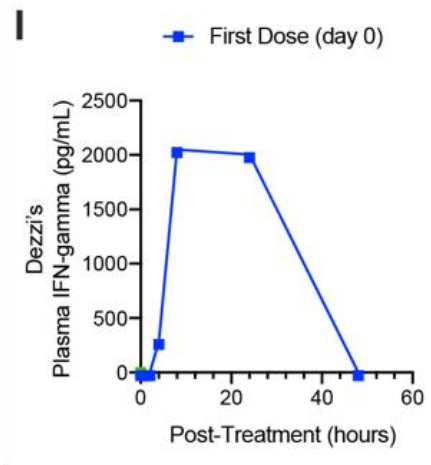

D
Dog 2: Nala
Pre-Treatment
Dog 2: Nala
Day 14 Post-Initial Treatment
Site of Injection

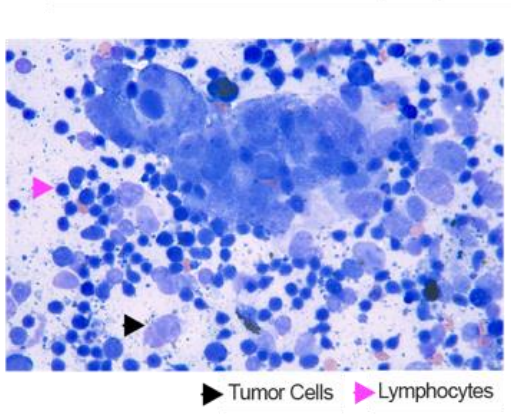

E
Dog 2: Nala
Pre-Treatment
Primary tumor
LN metastasis


F
First Dose (day 0)
Second Dose (day 14)
Nala's
Plasma IFN-gamma (pg/mL)
10000
8000
6000
4000
2000
0
0
20
40
60
Post-Treatment (hours)

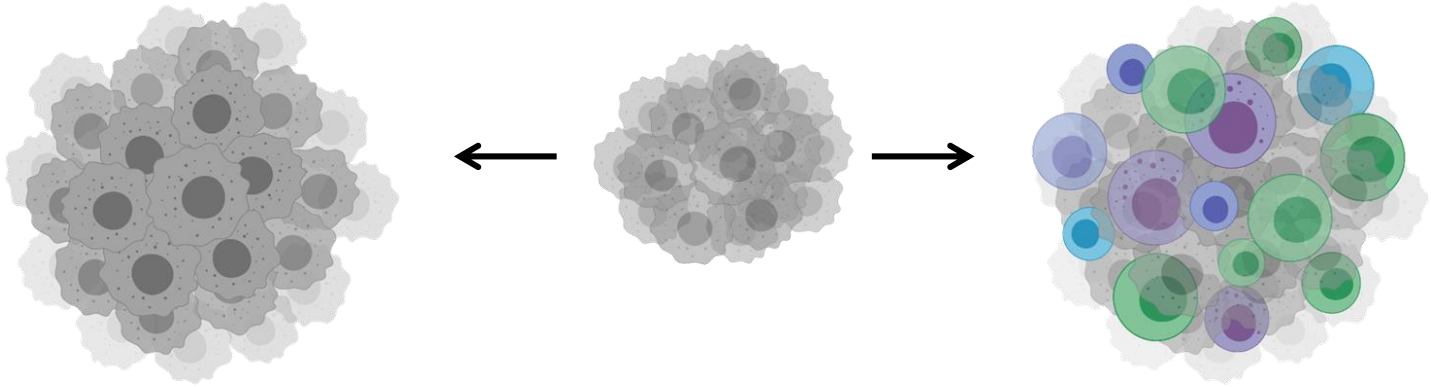

G
Dog 3: Dezzi
Pre-Treatment
Site of Injection


H
Dog 3: Dezzi
Pre-Treatment
Primary tumor


I
First Dose (day 0)
Dezzi's
Plasma IFN-gamma (pg/mL)
2500
2000
1500
1000
500
0
0
20
40
60
Post-Treatment (hours)


J
Tumor Cells
Lymphocytes

Immunotherapy trials require patients and patience



Progression

Pseudo-progression

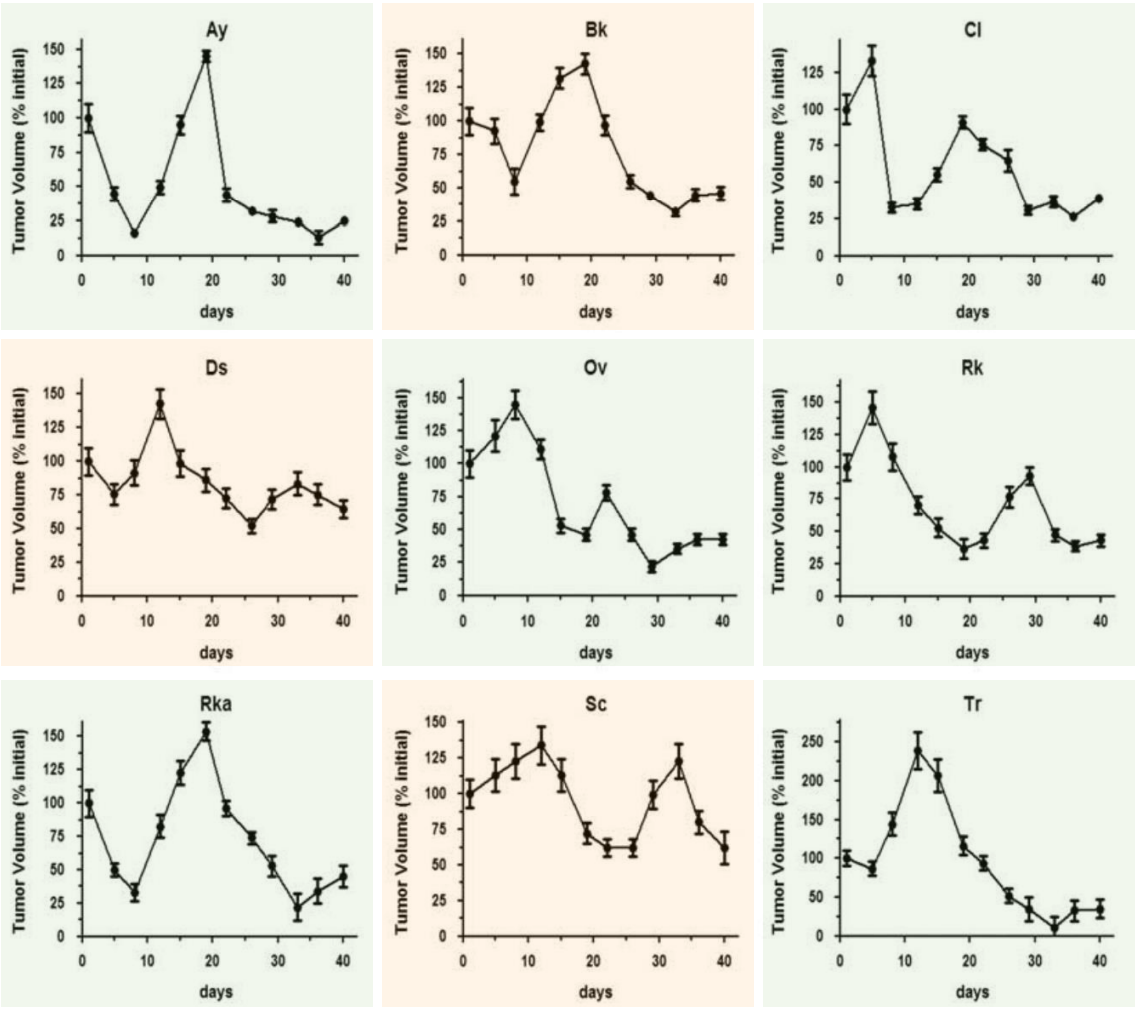
Inflammation/ immune cell infiltration appear to increase tumor size

Liver metastases of malignant melanoma human patient treated with **nivolumab**

Start Treatment 31 mm | 3-month 63 mm | 5 month 31 mm | 8-month 20 mm | 12-month 13 mm

Ozaki et al, BMC Cancer 2017

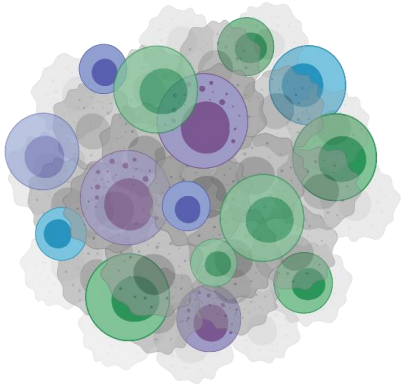
Immunotherapy trials require patients and patience



Stable Disease

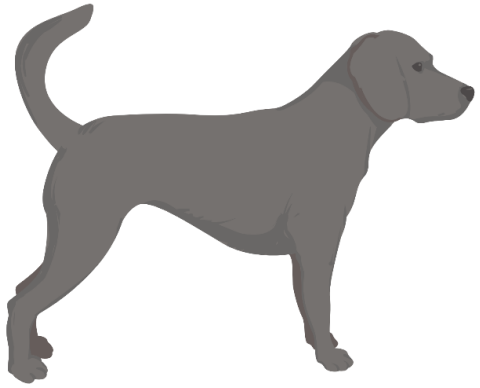
Partial Response

Fondello et al, Melanoma Research 2018

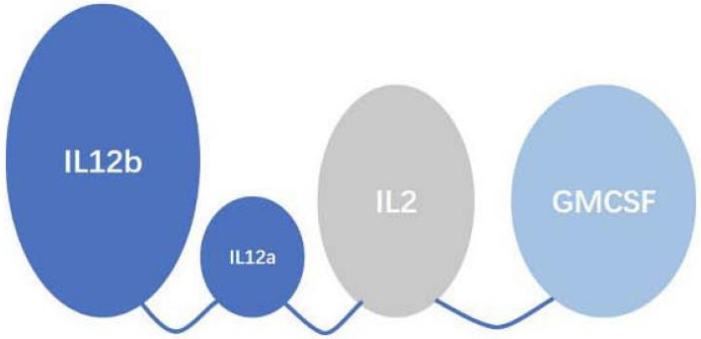


Pseudo-progression

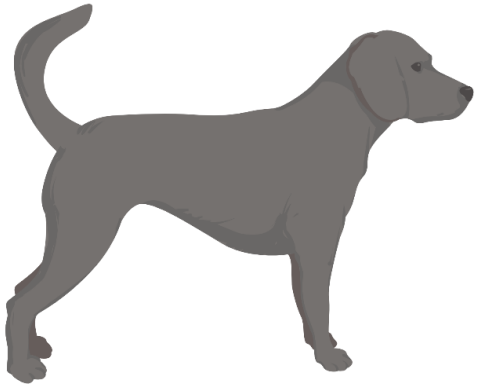
Inflammation/ immune cell infiltration appear to increase tumor size



Immunotherapy trials require patients and patience



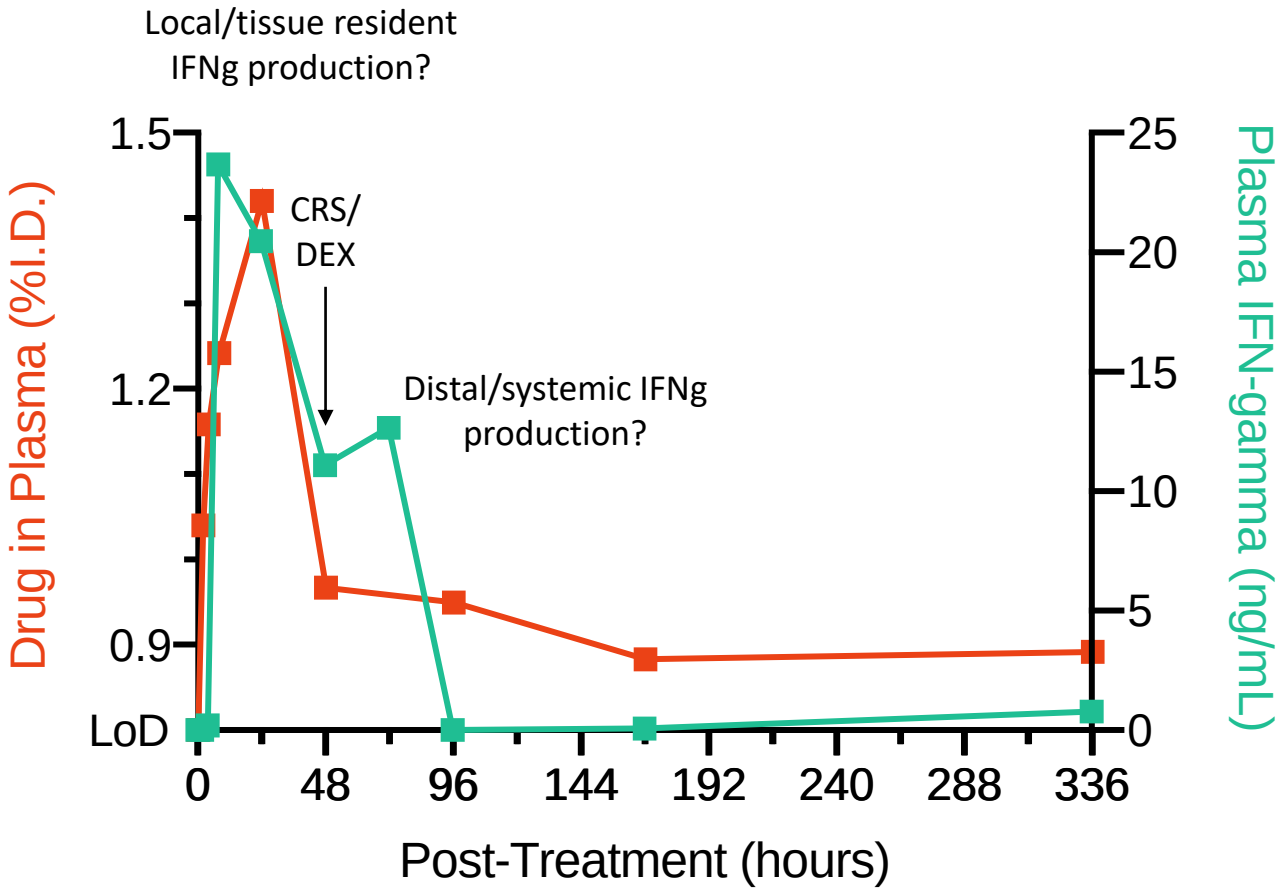
16.7 ug/kg of IL12-IL2-GMCSF
every other week for 4 total treatments



Du et al, bioRxiv 2020

Morgan (1x dose)
2 mg cLAIR-CSA-cIL2
0.22 mg cIL12-CSA-cLAIR

Interesting temporal dynamics though…



n = 1 dog and k = 1 technical replicate due to scant sample

Major and minor peaks in IFN-gamma levels

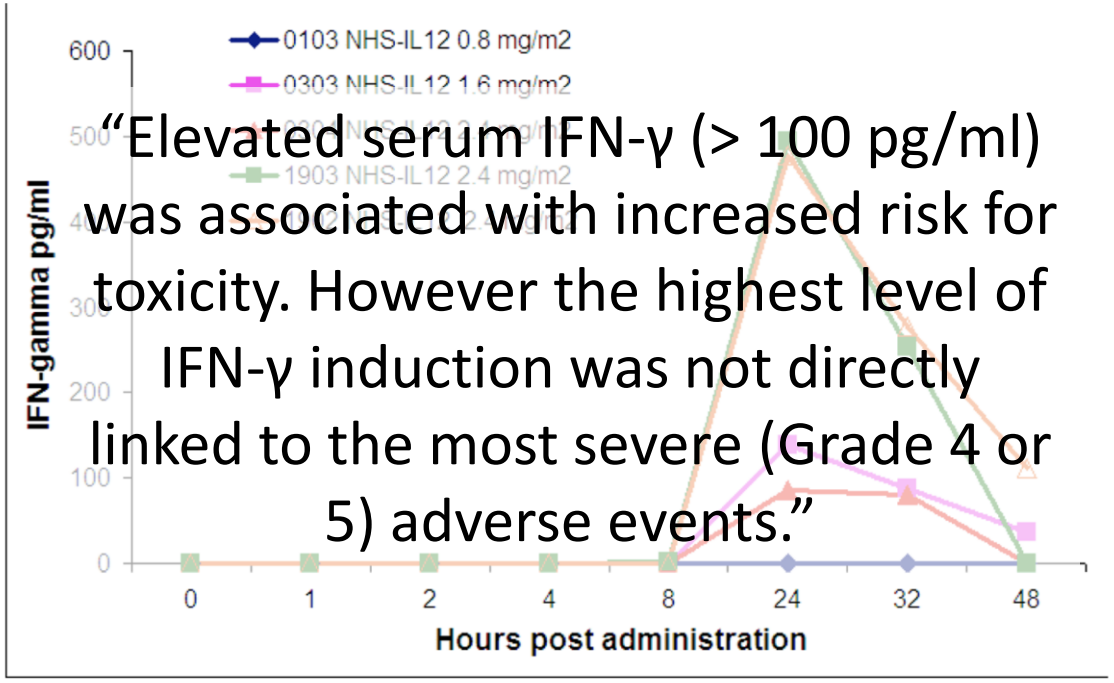
$C_{max,IFNg}$ precedes $C_{max,drug}$

Elevated plasma IFN-gamma

Morgan (1x dose)
2 mg cLAIR-CSA-cIL2
0.22 mg cIL12-CSA-cLAIR

Paoloni et al, 2015

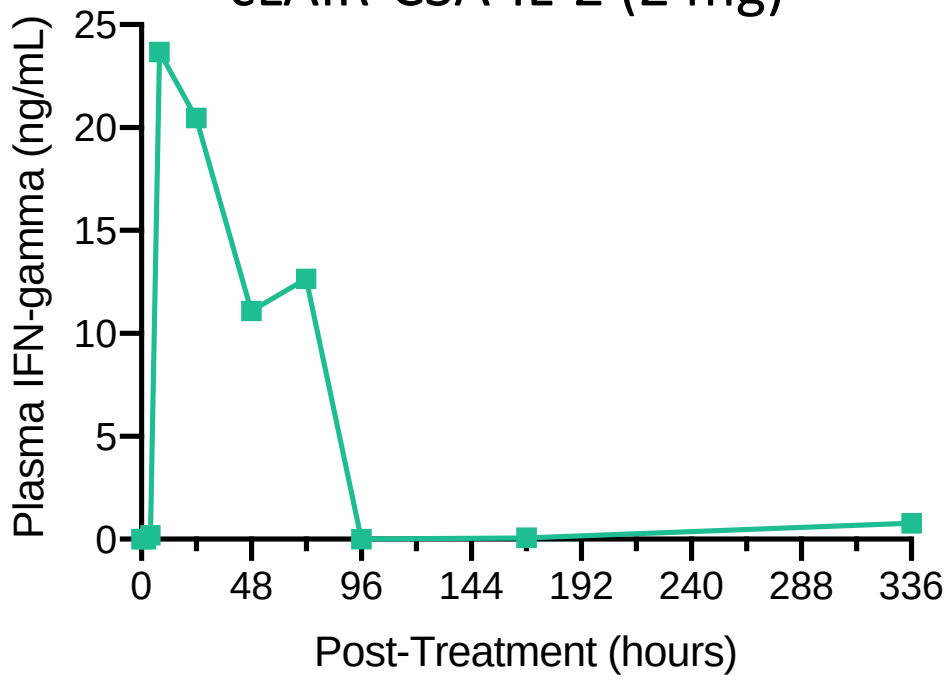
Subcutaneous administration
NHS-IL12 in dogs (dose 0.8 mg/m^2)



"Elevated serum IFN-γ (> 100 pg/ml) was associated with increased risk for toxicity. However the highest level of IFN-γ induction was not directly linked to the most severe (Grade 4 or 5) adverse events."

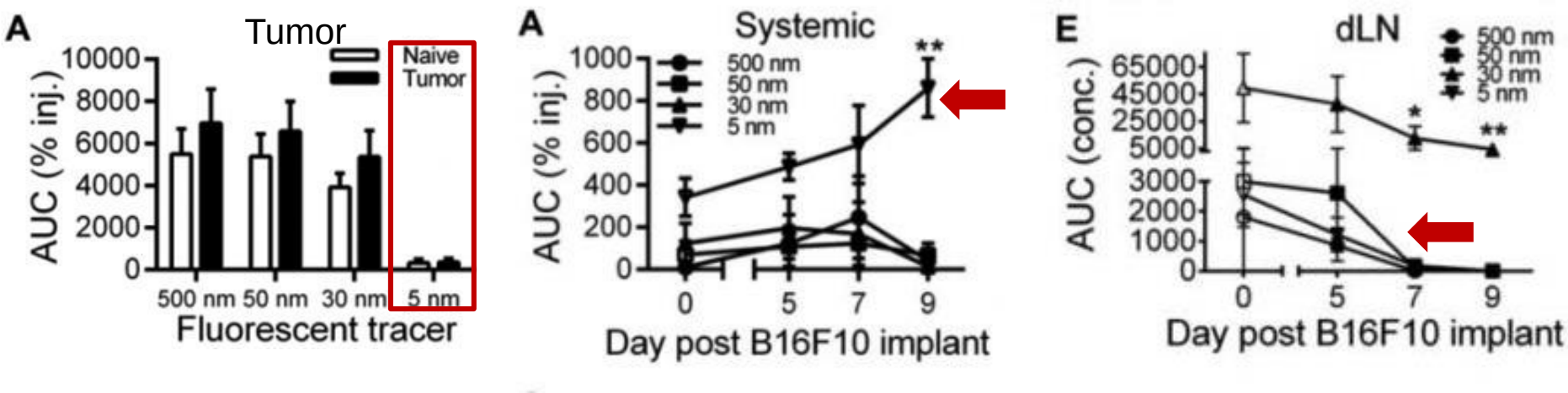
Our Study

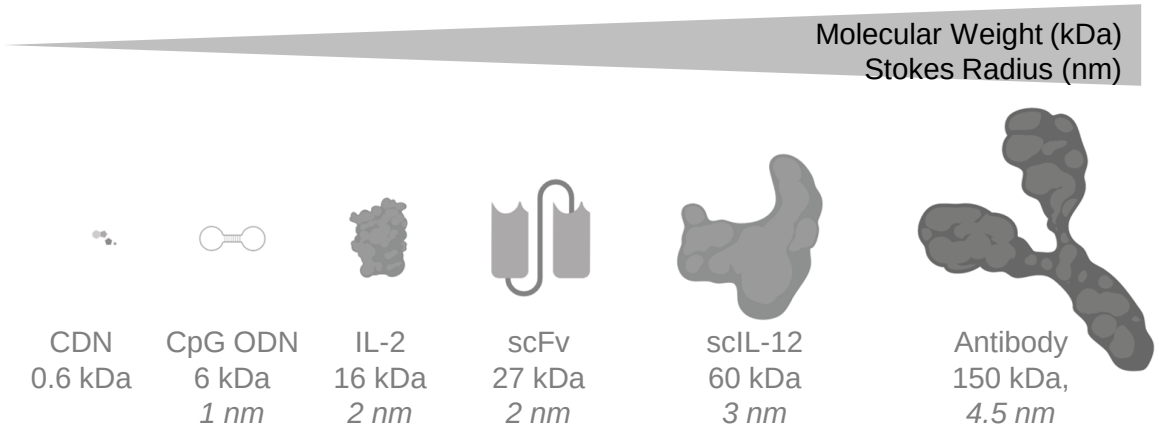
Intradermal administration
cIL12-CSA-cLAIR (0.4 mg/m^2 -> 0.2 mg)
cLAIR-CSA-IL-2 (2 mg)



Need to characterize pharmacokinetics of <5 nm radius agents

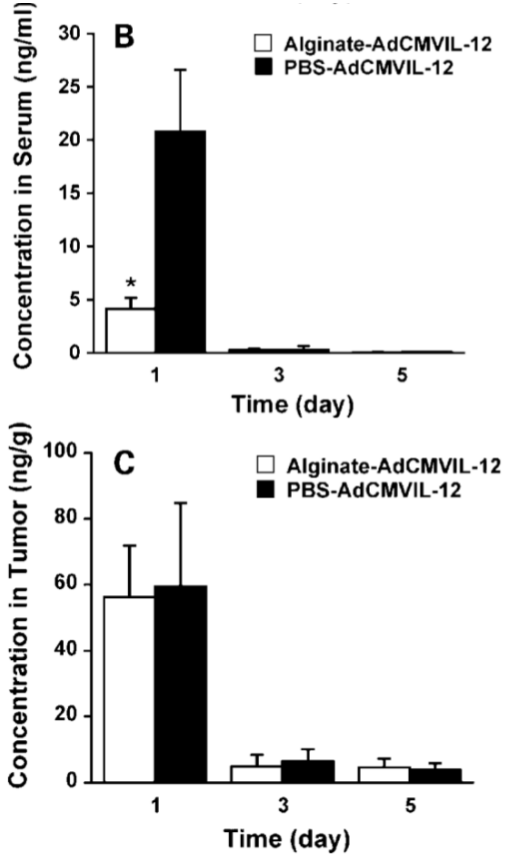
Rohner and Thomas *J Control Release*, 2016





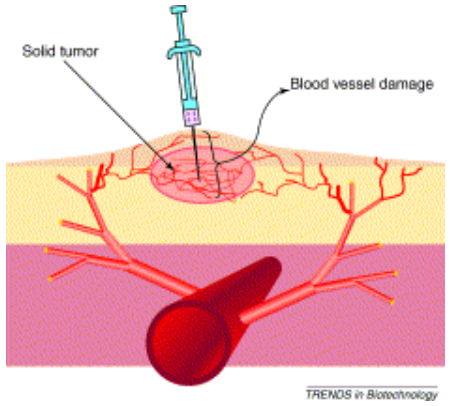
Drug dissemination observed <u>DURING</u> i.tu. injection

Posited Systemic Dissemination Occurred
During IT Injection of Viral Vector

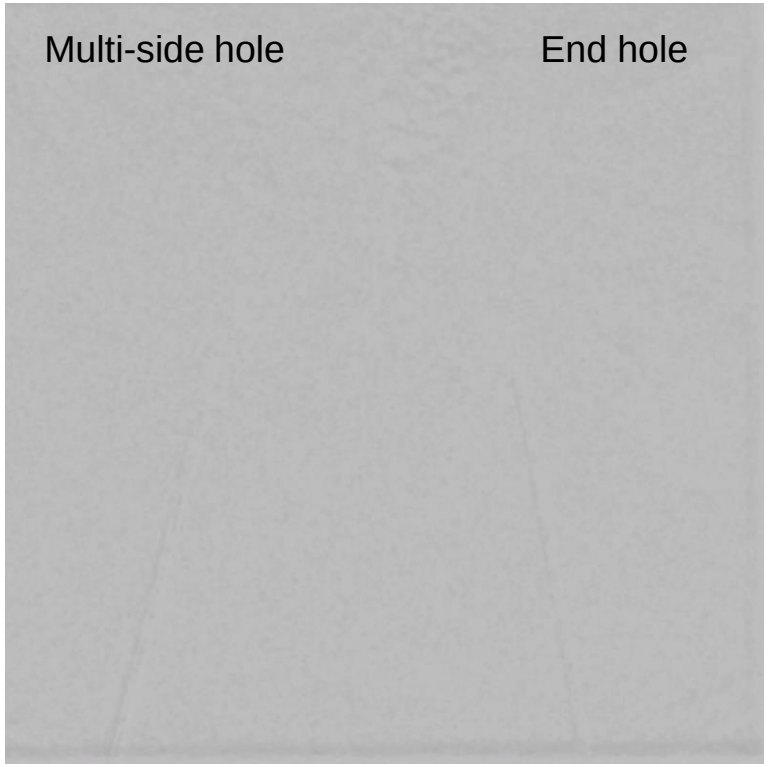


Wang et al. Molecular Cancer Therapeutics. 2003

Exit Route: Damaged Blood Vessels



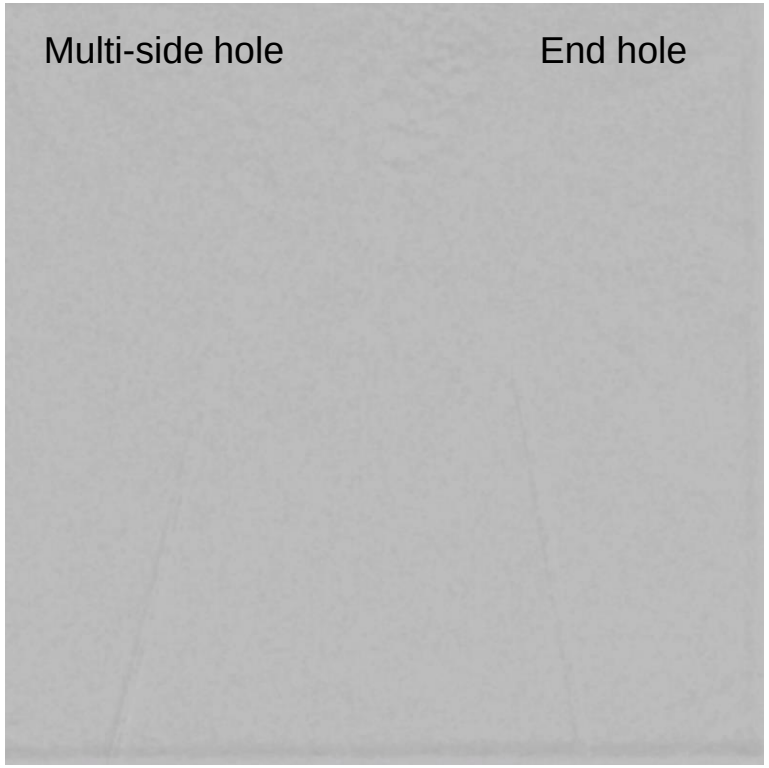
Luo. Trends in Biotechnology, 2004



Munoz et al. JITC. 2021

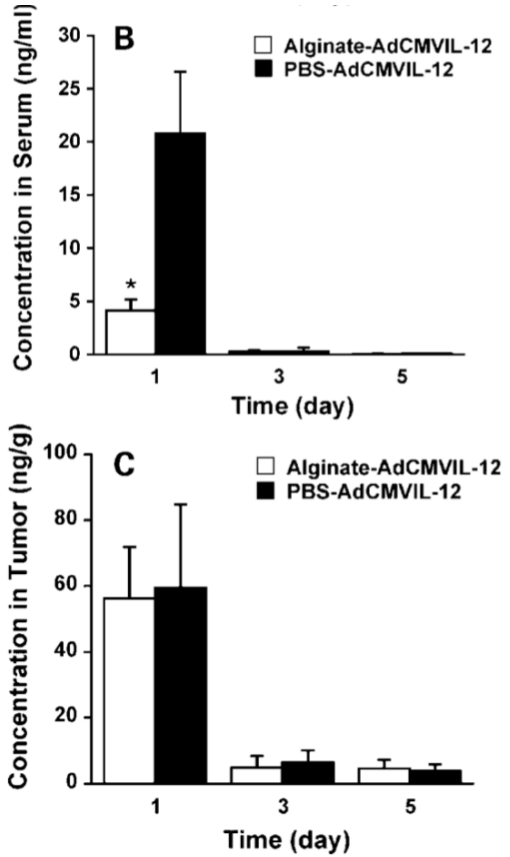
Potential avenues to increase V_{holdup}

1) Slow injection rate (with pump)
2) Multi-side hole needle (laser etch?)



Munoz et al. JITC. 2021

3) Formulation with alum, alginate, matrigel, hydrogel etc.

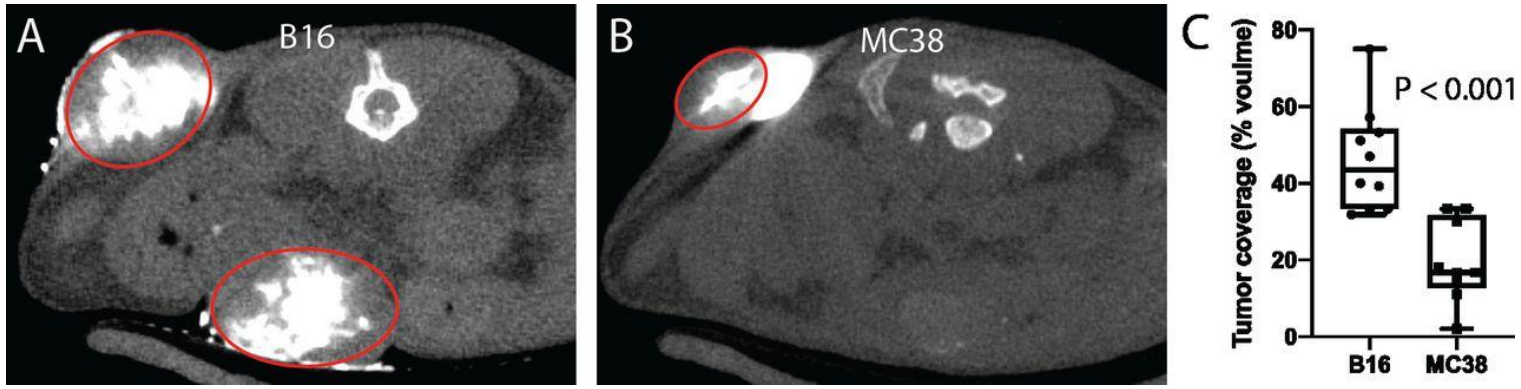


Wang et al. Molecular Cancer Therapeutics. 2003

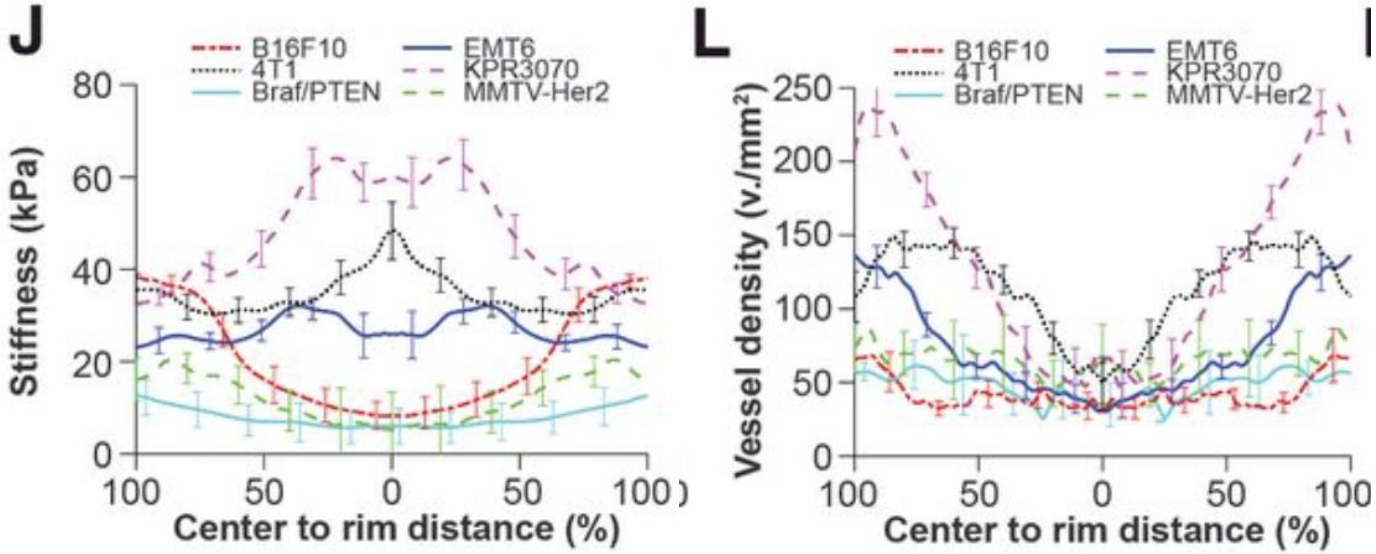
But also V_{holdup} will vary by tumor type!

Stiffer tumors have lowering V_{holdup}

Munoz et al. JITC. 2021



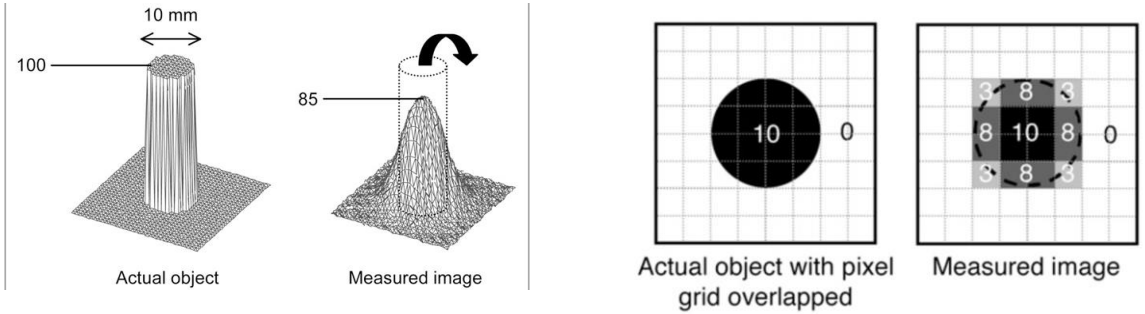
Different tumor types have different **stiffnesses** …that changes with **tumor staging** (d6 v. d12 v. d18) …that changes with respect to **spatial dimension**



Riegler et al. Clinical Cancer Research. 2018

PET without additional correction is qualitative

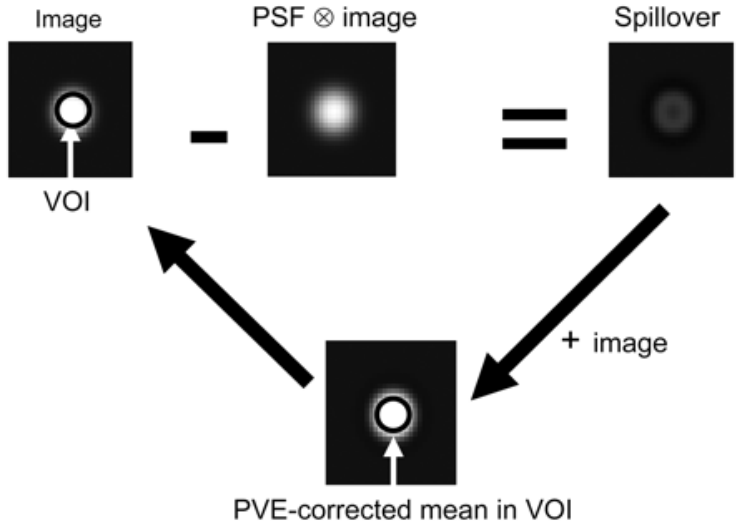
Partial Volume Effect (PVE)
PVE is a **loss in apparent activity** when an object partially occupies the volume of an imaging instruction in both **space and time**.



Soret, Bacharach, and Buvat. J. Nuclear Medicine. 2007

Interregional correction for tumor-draining lymph node and tumor spillover**: Labbe and Region-Based Voxel-Wise Correction** *(withstands errors in machine's point spread function, used for spherical structure in the brain, fast-performing)*

Intraregional correction for the same compartment: **Van-Cittret** (*assumes a Gaussian noise distribution and additive correction step leading to optimal signal to noise*)

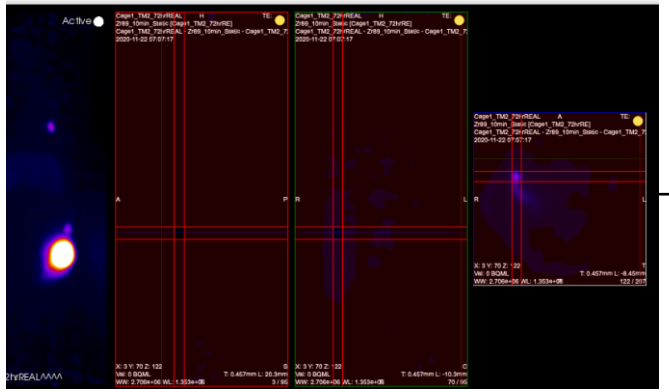


Teo et al Journal of Nuclear Medicine 2007

PET without additional correction is qualitative

Manually Convert DICOM Files to NIFTI format using **MRI-Convert Software**

Define Compartments in **Amide Software** Tumor and Tumor-Draining Lymph Node for the Labbe + RVC correction

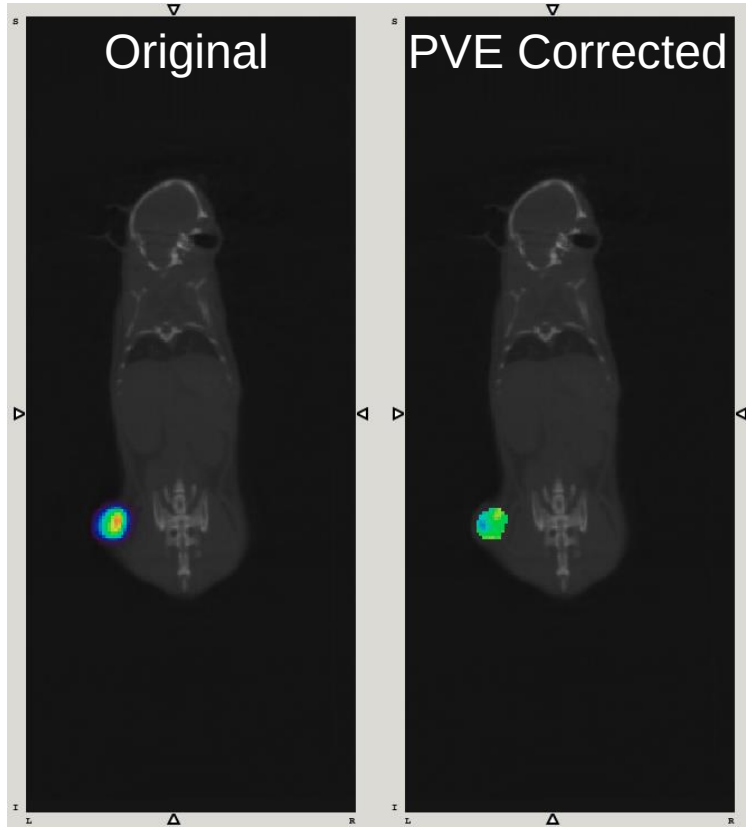


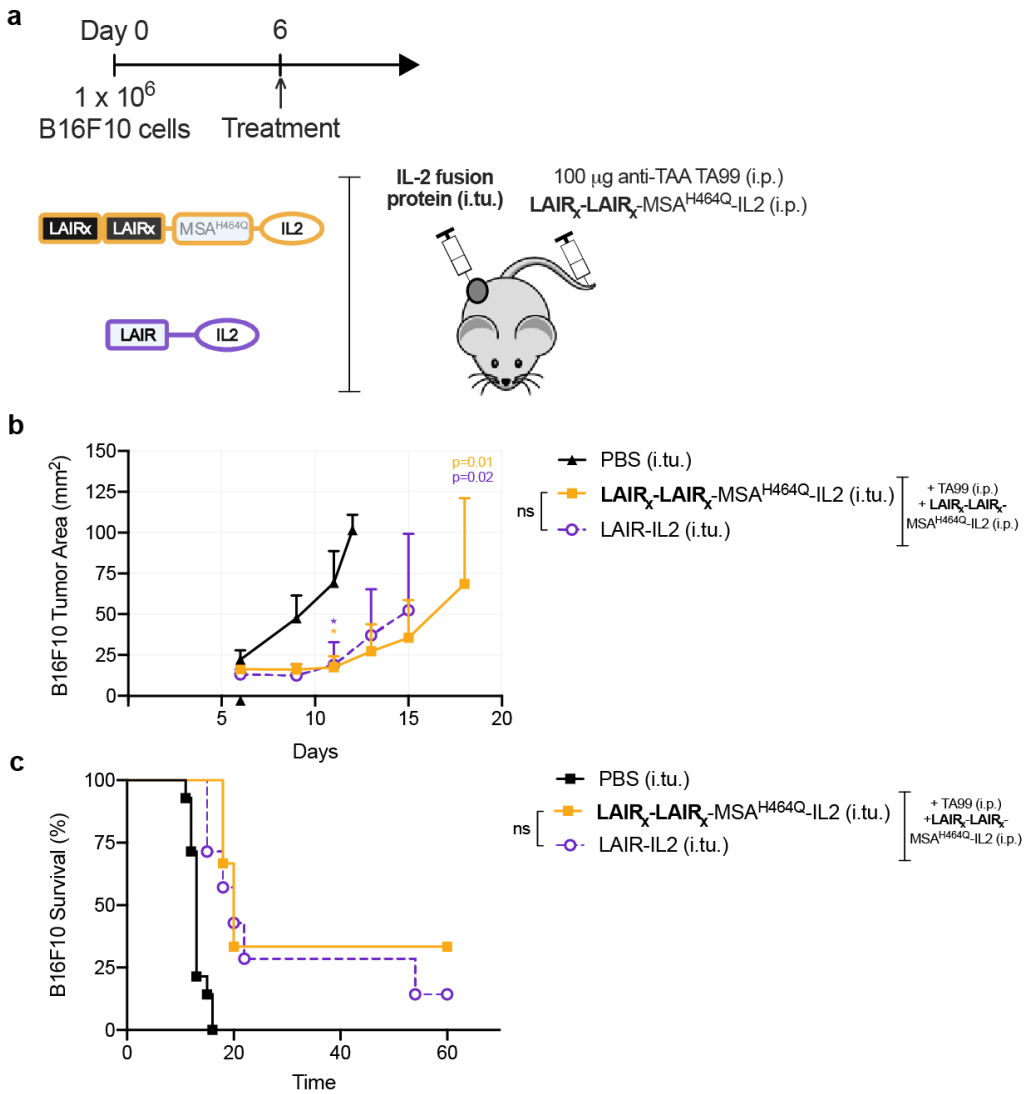
Create 3D Mask in **Fiji** Using MorphoLibJ Connected Components Plugin

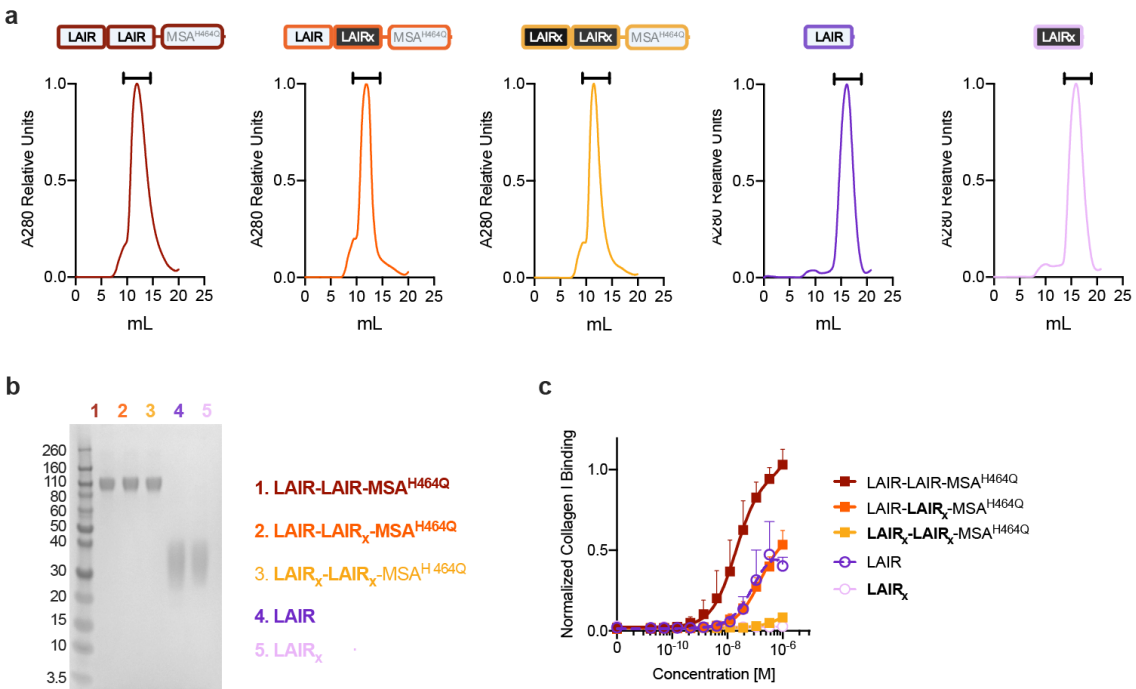
Create 4D Mask using UCL PETPVC **pvc_make4D function** (Github published code)

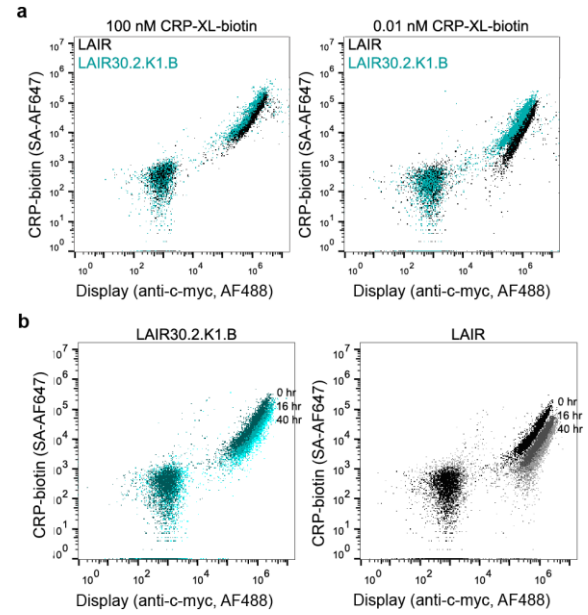
Input 4D Mask and PET Data into UCL PETPVC **petpvc function** (Github published code)

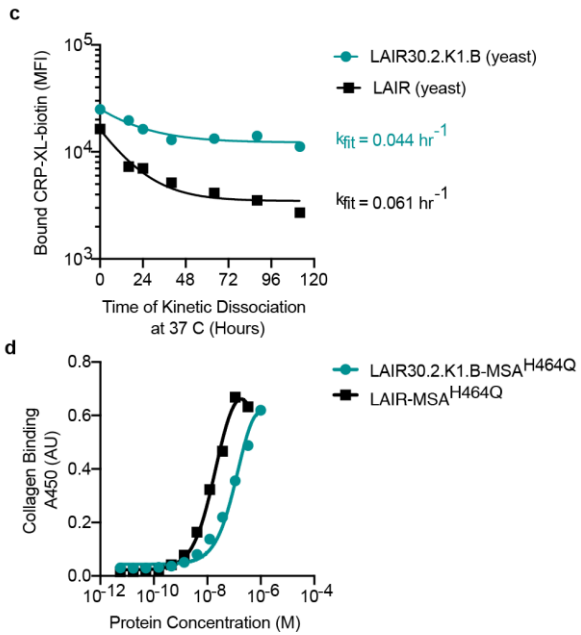
Corrected PET Image

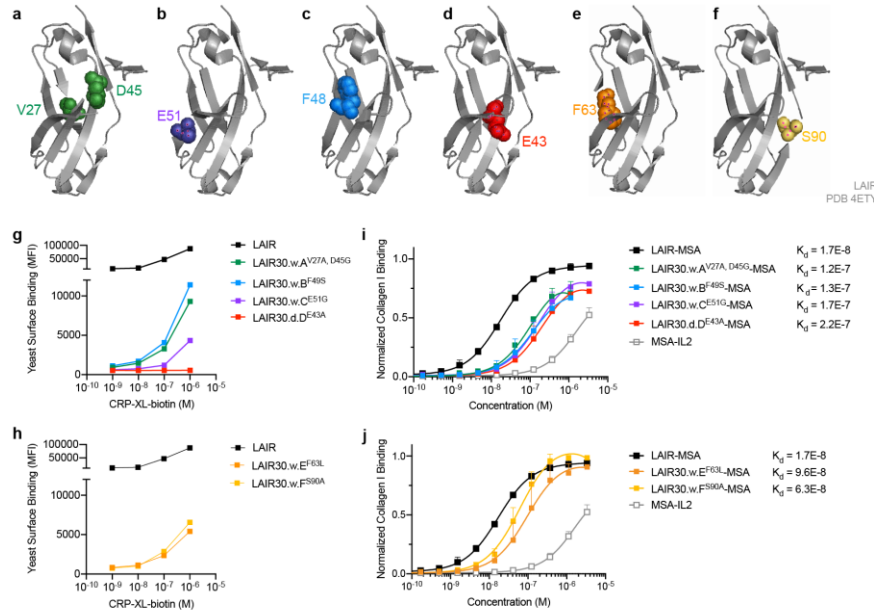


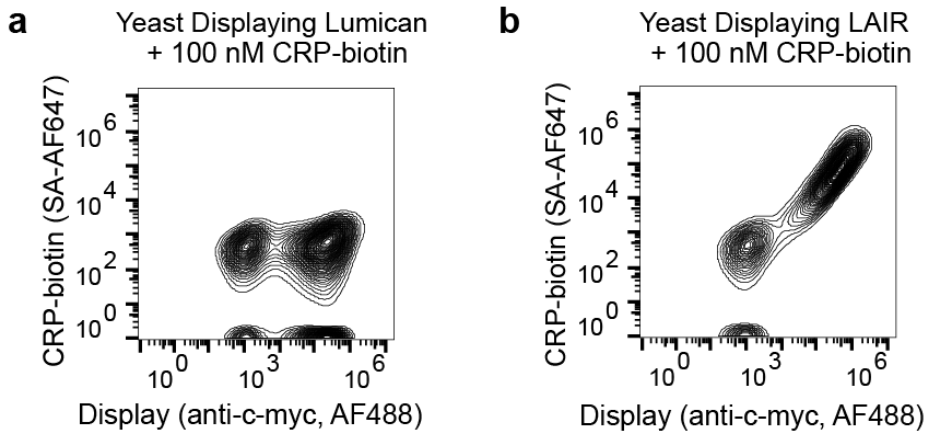

a
Day 0
6
1 x 10^6
B16F10 cells
Treatment
LAIRx
LAIRx
MSA^H464Q
IL2
LAIR
IL2
IL-2 fusion protein (i.tu.)
100 μg anti-TAA TA99 (i.p.)
LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.p.)
b
B16F10 Tumor Area (mm^2)
Days
p=0.01
p=0.02
PBS (i.tu.)
LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.tu.)
LAIR-IL2 (i.tu.)
ns
+ TA99 (i.p.)
+ LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.p.)
c
B16F10 Survival (%)
Time
PBS (i.tu.)
LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.tu.)
LAIR-IL2 (i.tu.)
ns
+ TA99 (i.p.)
+LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.p.)

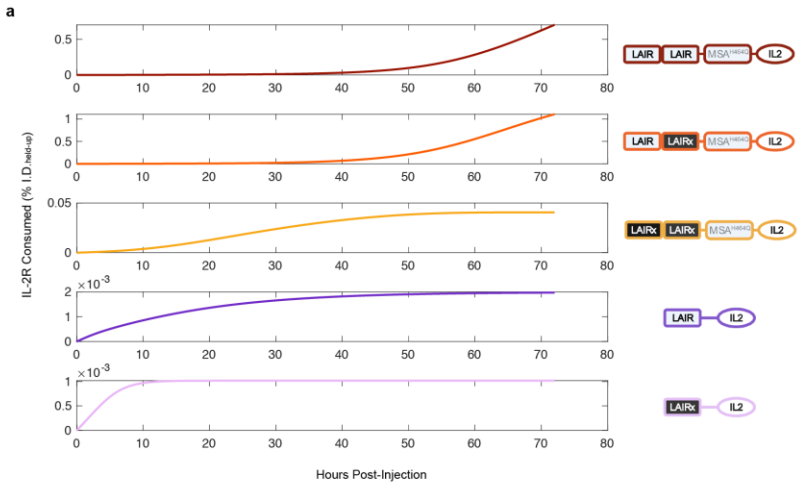

a
LAIR
LAIR
MSA^H464Q
LAIR
LAIRx
MSA^H464Q
LAIRx
LAIRx
MSA^H464Q
LAIR
LAIRx
A280 Relative Units
mL
b
1 2 3 4 5
1. LAIR-LAIR-MSA^H464Q
2. LAIR-LAIR_x-MSA^H464Q
3. LAIR_x-LAIR_x-MSA^H464Q
4. LAIR
5. LAIR_x
c
Normalized Collagen I Binding
Concentration [M]
LAIR-LAIR-MSA^H464Q
LAIR-LAIR_x-MSA^H464Q
LAIR_x-LAIR_x-MSA^H464Q
LAIR
LAIR_x

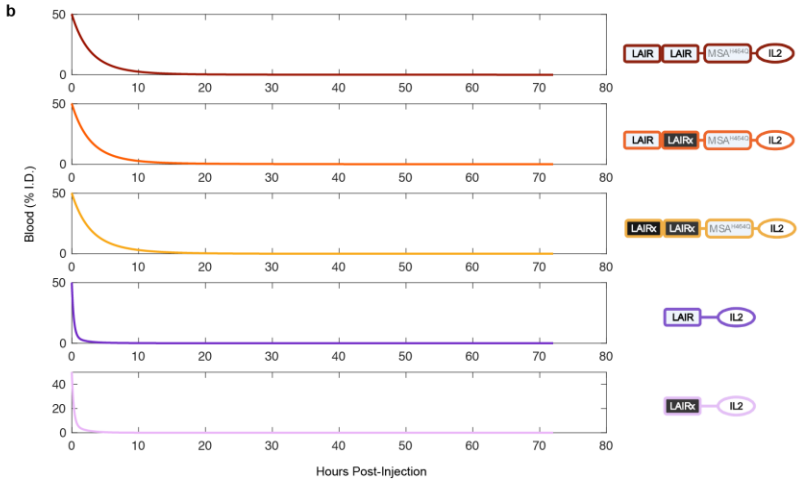

a
100 nM CRP-XL-biotin
0.01 nM CRP-XL-biotin
LAIR
LAIR30.2.K1.B
CRP-biotin (SA-AF647)
Display (anti-c-myc, AF488)
b
LAIR30.2.K1.B
LAIR
0 hr
16 hr
40 hr
CRP-biotin (SA-AF647)
Display (anti-c-myc, AF488)

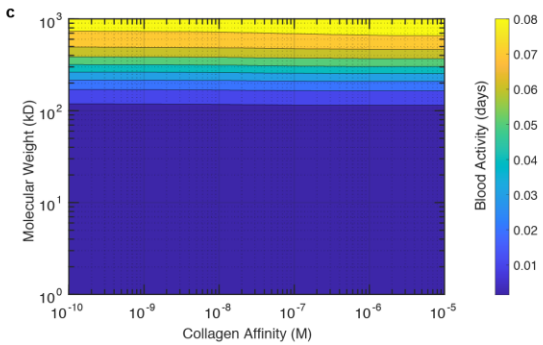

c
Bound CRP-XL-biotin (MFI)
LAIR30.2.K1.B (yeast)
LAIR (yeast)
kfit = 0.044 hr-1
kfit = 0.061 hr-1
Time of Kinetic Dissociation at 37 C (Hours)
d
Collagen Binding A450 (AU)
LAIR30.2.K1.B-MSAH464Q
LAIR-MSAH464Q
Protein Concentration (M)

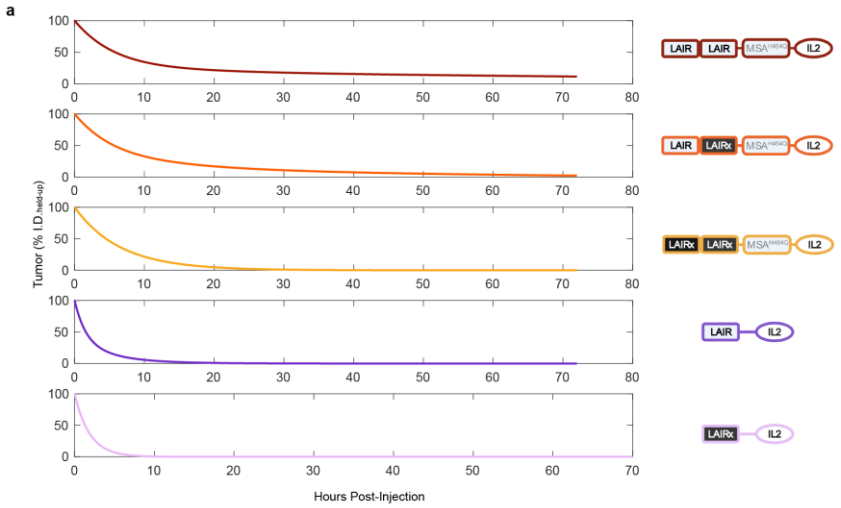

a
b
c
d
e
f
V27
D45
E51
F48
E43
F63
S90
LAIR
PDB 4ETY
g
Yeast Surface Binding (MFI)
LAIR
LAIR30.w.AV27A, D45G
LAIR30.w.BF49S
LAIR30.w.CE51G
LAIR30.d.DE43A
CRP-XL-biotin (M)
i
Normalized Collagen I Binding
LAIR-MSA
LAIR30.w.AV27A, D45G-MSA
LAIR30.w.BF49S-MSA
LAIR30.w.CE51G-MSA
LAIR30.d.DE43A-MSA
MSA-IL2
Kd = 1.7E-8
Kd = 1.2E-7
Kd = 1.3E-7
Kd = 1.7E-7
Kd = 2.2E-7
Concentration (M)
h
LAIR
LAIR30.w.EF63L
LAIR30.w.FS90A
j
LAIR-MSA
LAIR30.w.EF63L-MSA
LAIR30.w.FS90A-MSA
MSA-IL2
Kd = 1.7E-8
Kd = 9.6E-8
Kd = 6.3E-8

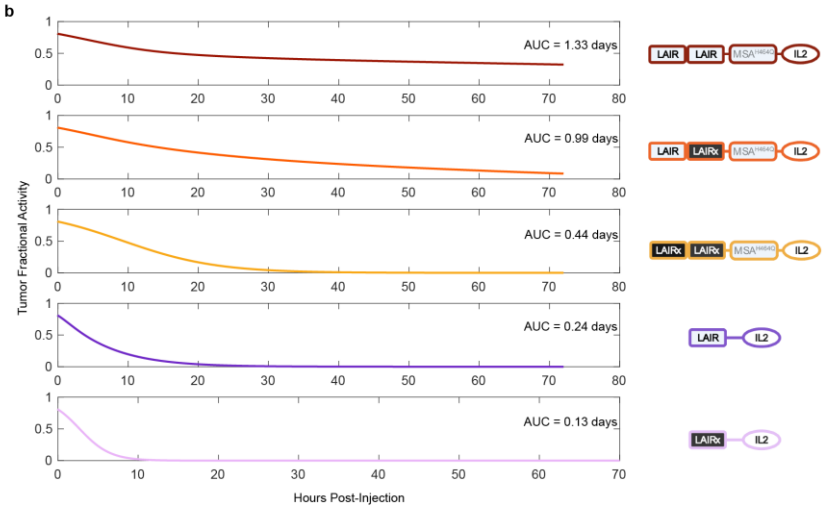

a
Yeast Displaying Lumican
+ 100 nM CRP-biotin
b
Yeast Displaying LAIR
+ 100 nM CRP-biotin
CRP-biotin (SA-AF647)
Display (anti-c-myc, AF488)





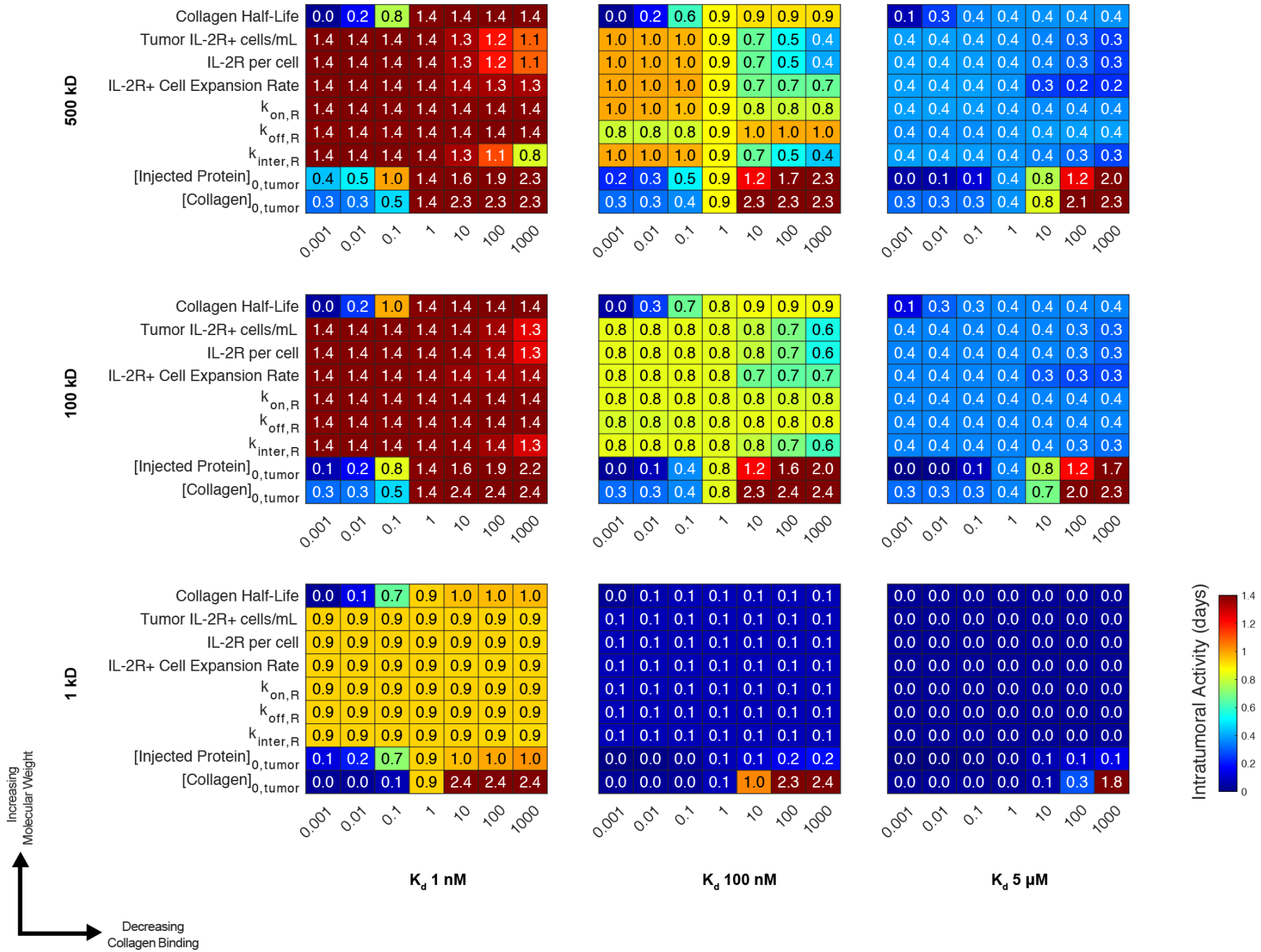


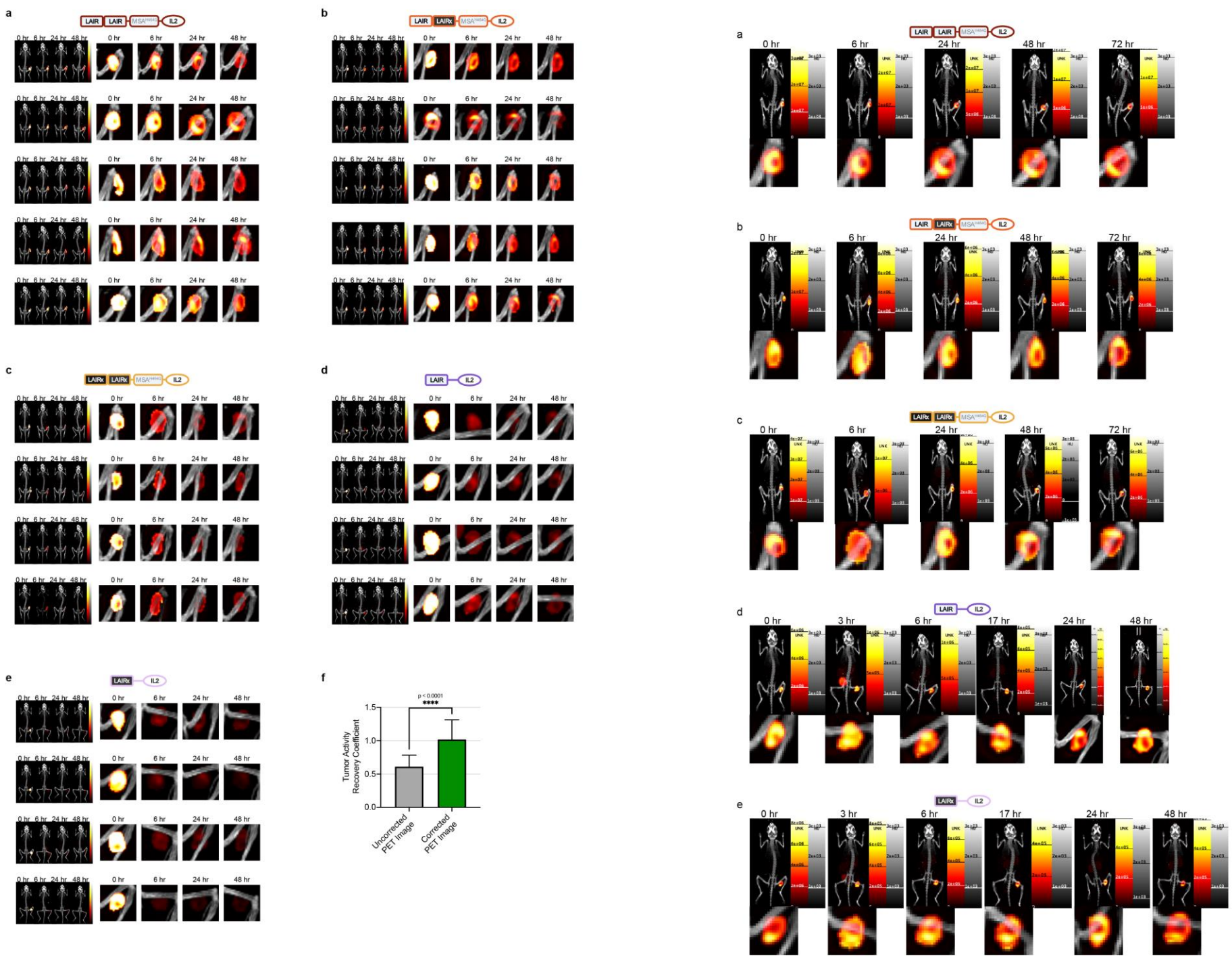




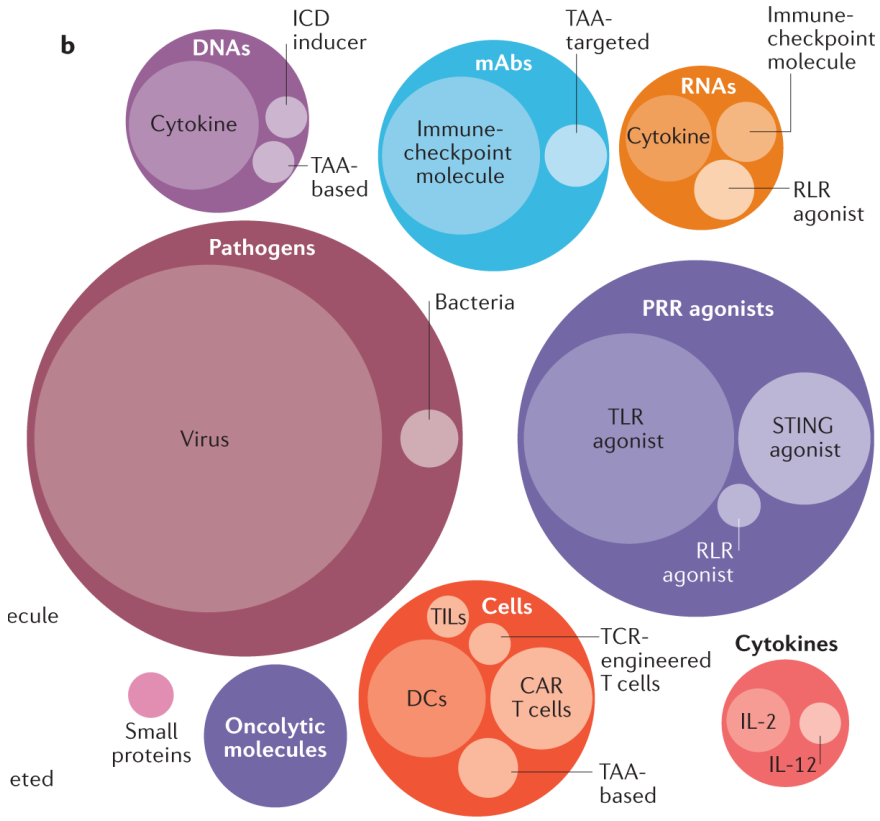
$$Fractional\,Activity = \frac{1}{1 + \frac{EC_{50,IL2}}{[Intratumoral\,Fusion\,Protein]}}$$

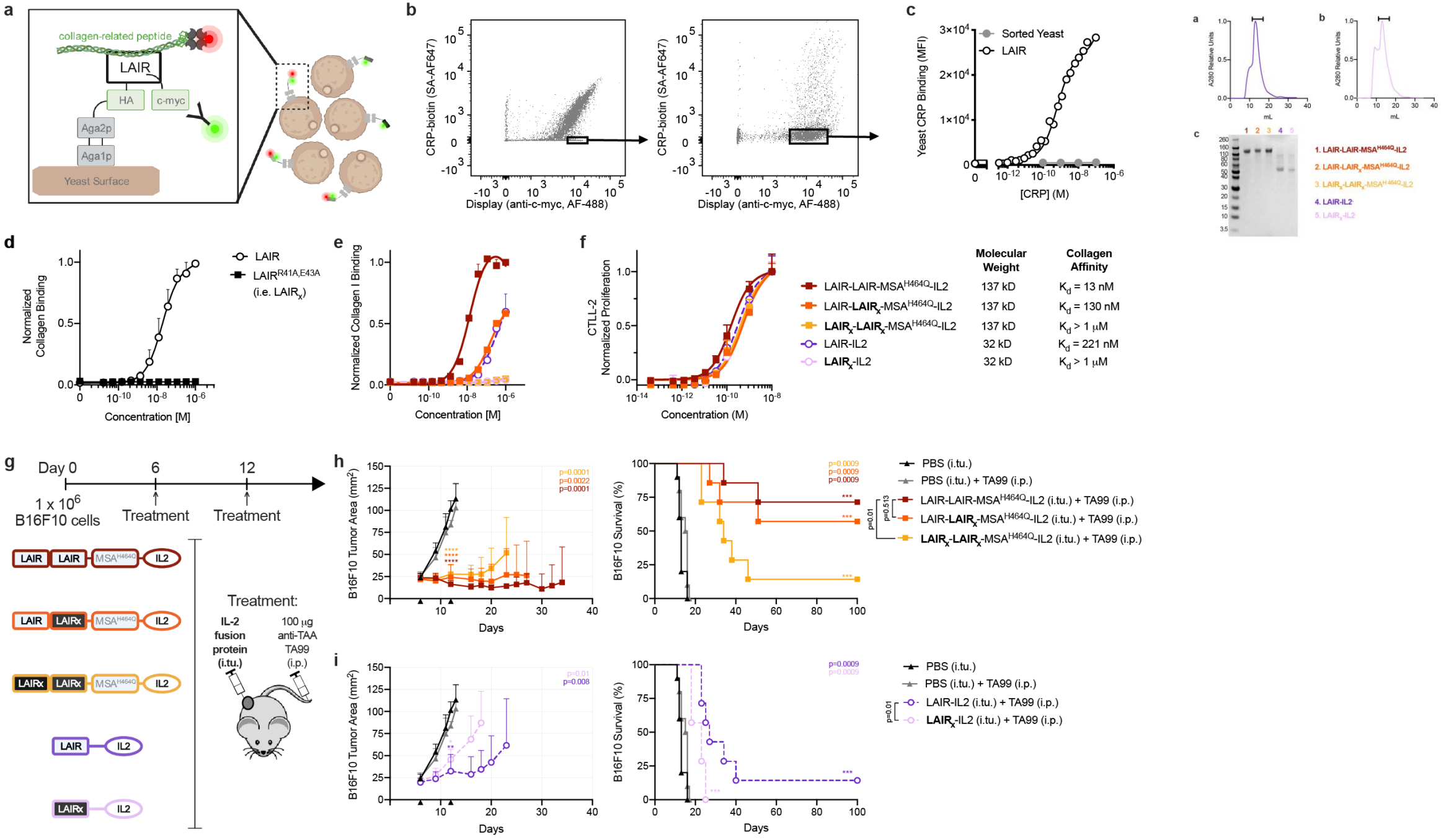
a


500 kD
100 kD
1 kD
Collagen Half-Life
Tumor IL-2R+ cells/mL
IL-2R per cell
IL-2R+ Cell Expansion Rate
$k_{on,R}$
$k_{off,R}$
$k_{inter,R}$
$[Injected\ Protein]_{0,tumor}$
$[Collagen]_{0,tumor}$
0.001
0.01
0.1
1
10
100
1000
K_d 1 nM
K_d 100 nM
K_d 5 μM
Increasing Molecular Weight
Decreasing Collagen Binding
Intratumoral Activity (days)
1.4
1.2
1
0.8
0.6
0.4
0.2
0

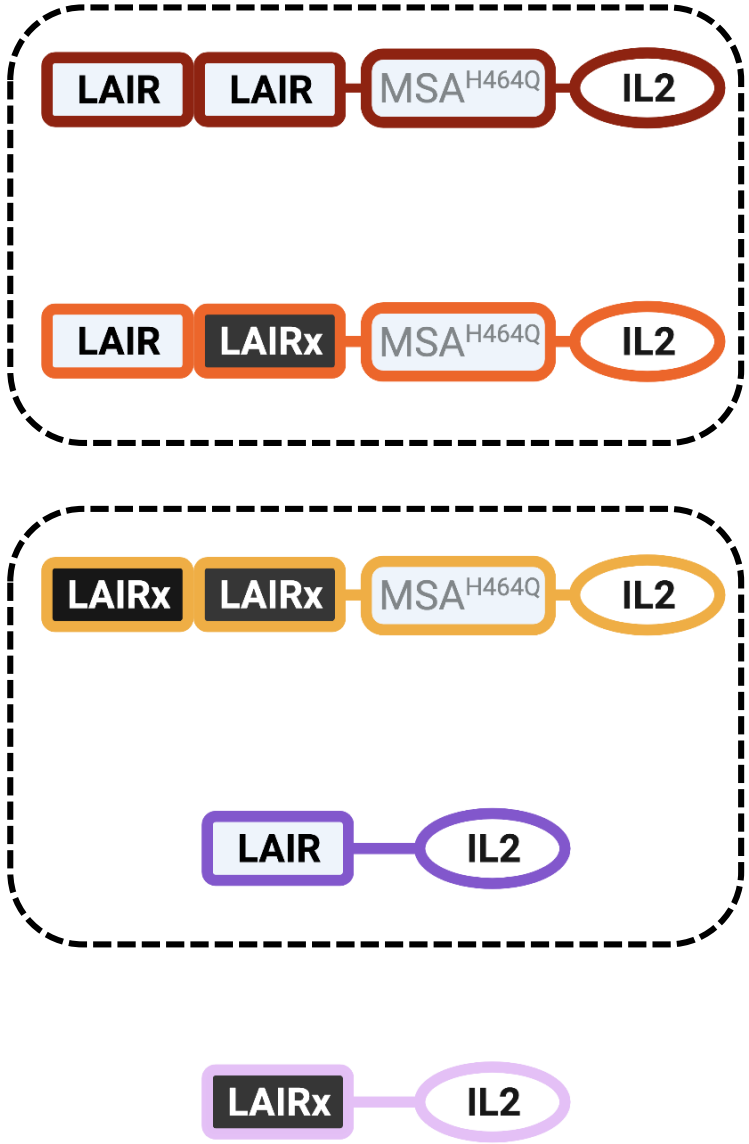

a
LAIR LAIR MSA IL2
0 hr 6 hr 24 hr 48 hr
b
LAIR LAIRx MSA IL2
c
LAIRx LAIRx MSA IL2
d
LAIR IL2
e
LAIRx IL2
f
p < 0.0001
****
Tumor Activity Recovery Coefficient
1.5
1.0
0.5
0.0
Uncorrected PET Image
Corrected PET Image
72 hr
3 hr
17 hr

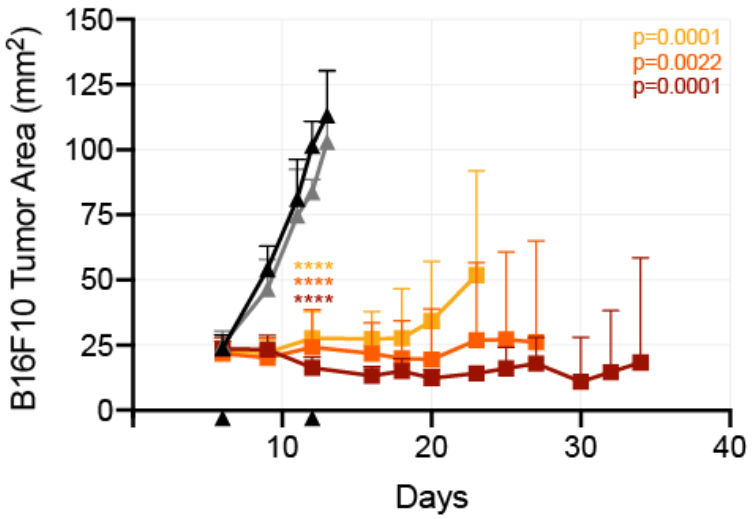
Different Immunotherapies Injected Locally

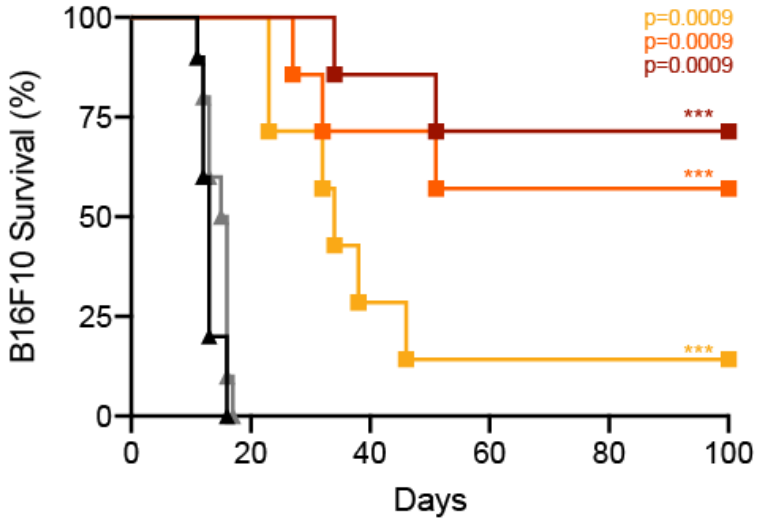


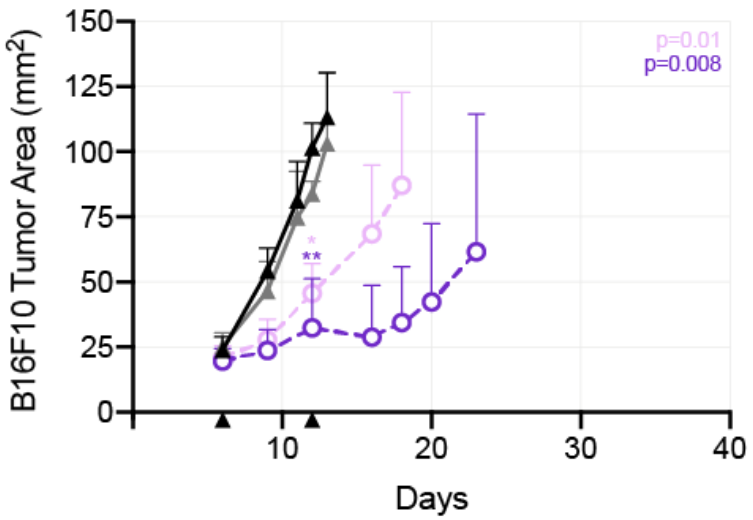

a
collagen-related peptide
LAIR
HA
c-myc
Aga2p
Aga1p
Yeast Surface
b
CRP-biotin (SA-AF647)
Display (anti-c-myc, AF-488)
c
Sorted Yeast
LAIR
Yeast CRP Binding (MFI)
[CRP] (M)
d
LAIR
LAIR^R41A,E43A (i.e. LAIR_x)
Normalized Collagen Binding
Concentration [M]
e
Normalized Collagen I Binding
Concentration [M]
f
CTLL-2 Normalized Proliferation
Concentration (M)
LAIR-LAIR-MSA^H464Q-IL2 137 kD Kd = 13 nM
LAIR-LAIR_x-MSA^H464Q-IL2 137 kD Kd = 130 nM
LAIR_x-LAIR_x-MSA^H464Q-IL2 137 kD Kd > 1 μM
LAIR-IL2 32 kD Kd = 221 nM
LAIR_x-IL2 32 kD Kd > 1 μM
Molecular Weight
Collagen Affinity
g
Day 0
6
12
1 x 10^6 B16F10 cells
Treatment
Treatment
LAIR LAIR MSA^H464Q IL2
LAIR LAIRx MSA^H464Q IL2
LAIRx LAIRx MSA^H464Q IL2
LAIR IL2
LAIRx IL2
Treatment:
IL-2 fusion protein (i.tu.)
100 μg anti-TAA TA99 (i.p.)
h
B16F10 Tumor Area (mm²)
Days
B16F10 Survival (%)
PBS (i.tu.)
PBS (i.tu.) + TA99 (i.p.)
LAIR-LAIR-MSA^H464Q-IL2 (i.tu.) + TA99 (i.p.)
LAIR-LAIR_x-MSA^H464Q-IL2 (i.tu.) + TA99 (i.p.)
LAIR_x-LAIR_x-MSA^H464Q-IL2 (i.tu.) + TA99 (i.p.)
i
B16F10 Tumor Area (mm²)
Days
B16F10 Survival (%)
PBS (i.tu.)
PBS (i.tu.) + TA99 (i.p.)
LAIR-IL2 (i.tu.) + TA99 (i.p.)
LAIR_x-IL2 (i.tu.) + TA99 (i.p.)

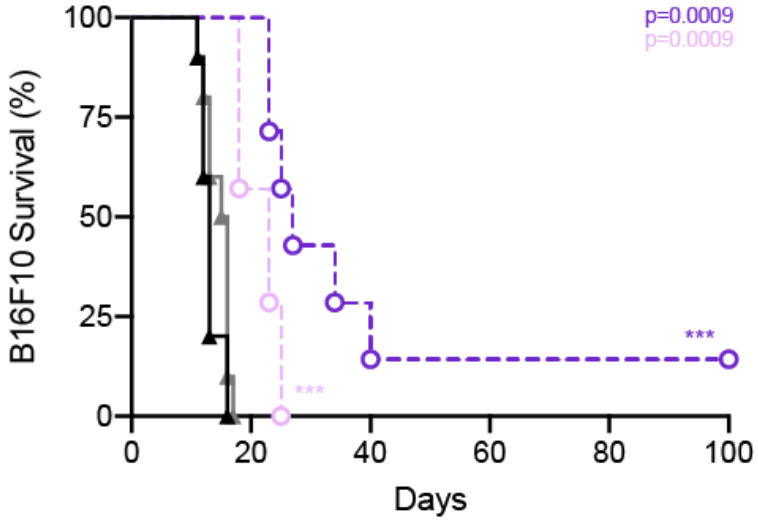
a
A280 Relative Units
mL
b
A280 Relative Units
mL
c
1 2 3 4 5
1. LAIR-LAIR-MSA^H464Q-IL2
2. LAIR-LAIR_x-MSA^H464Q-IL2
3. LAIR_x-LAIR_x-MSA^H464Q-IL2
4. LAIR-IL2
5. LAIR_x-IL2





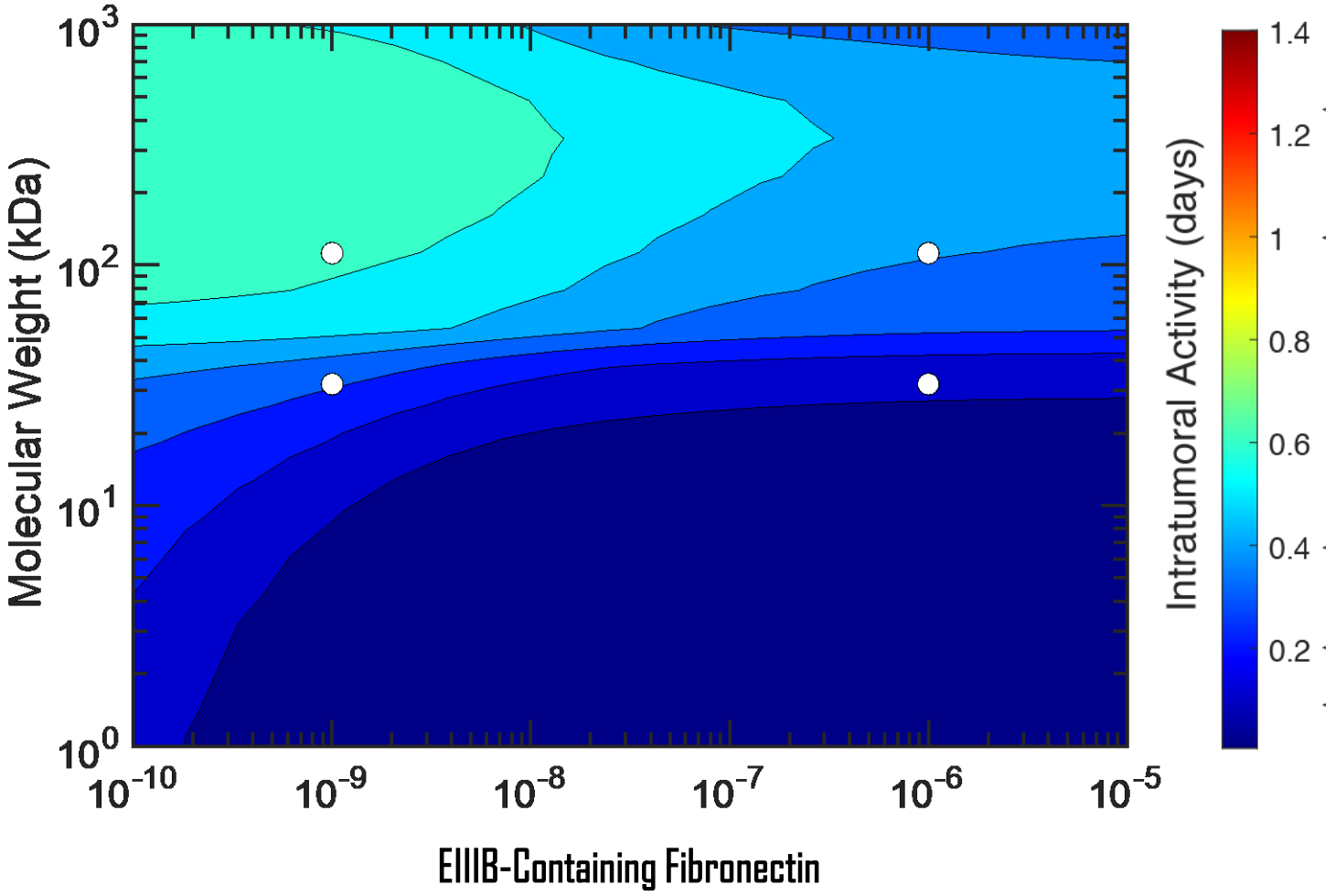


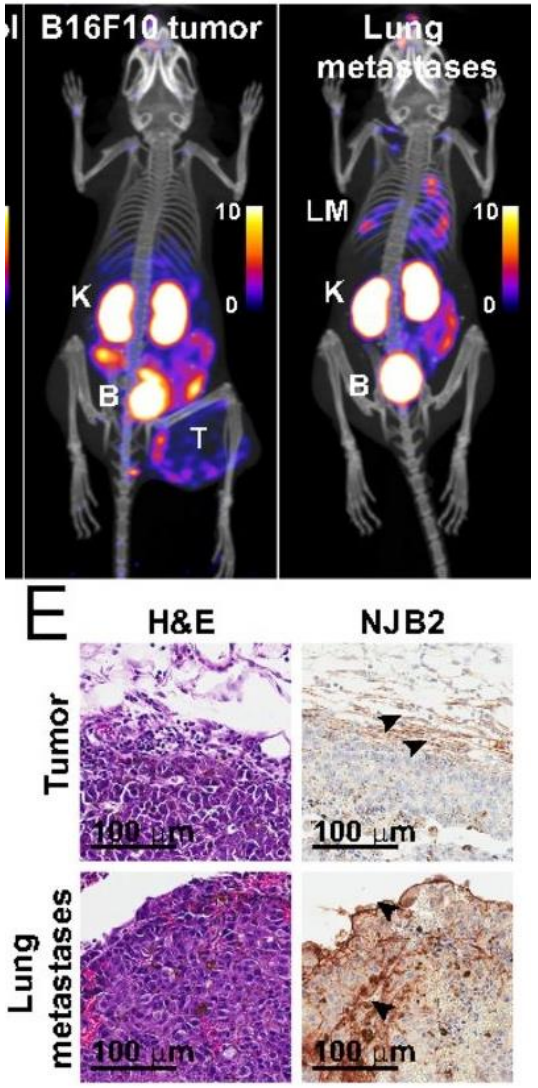




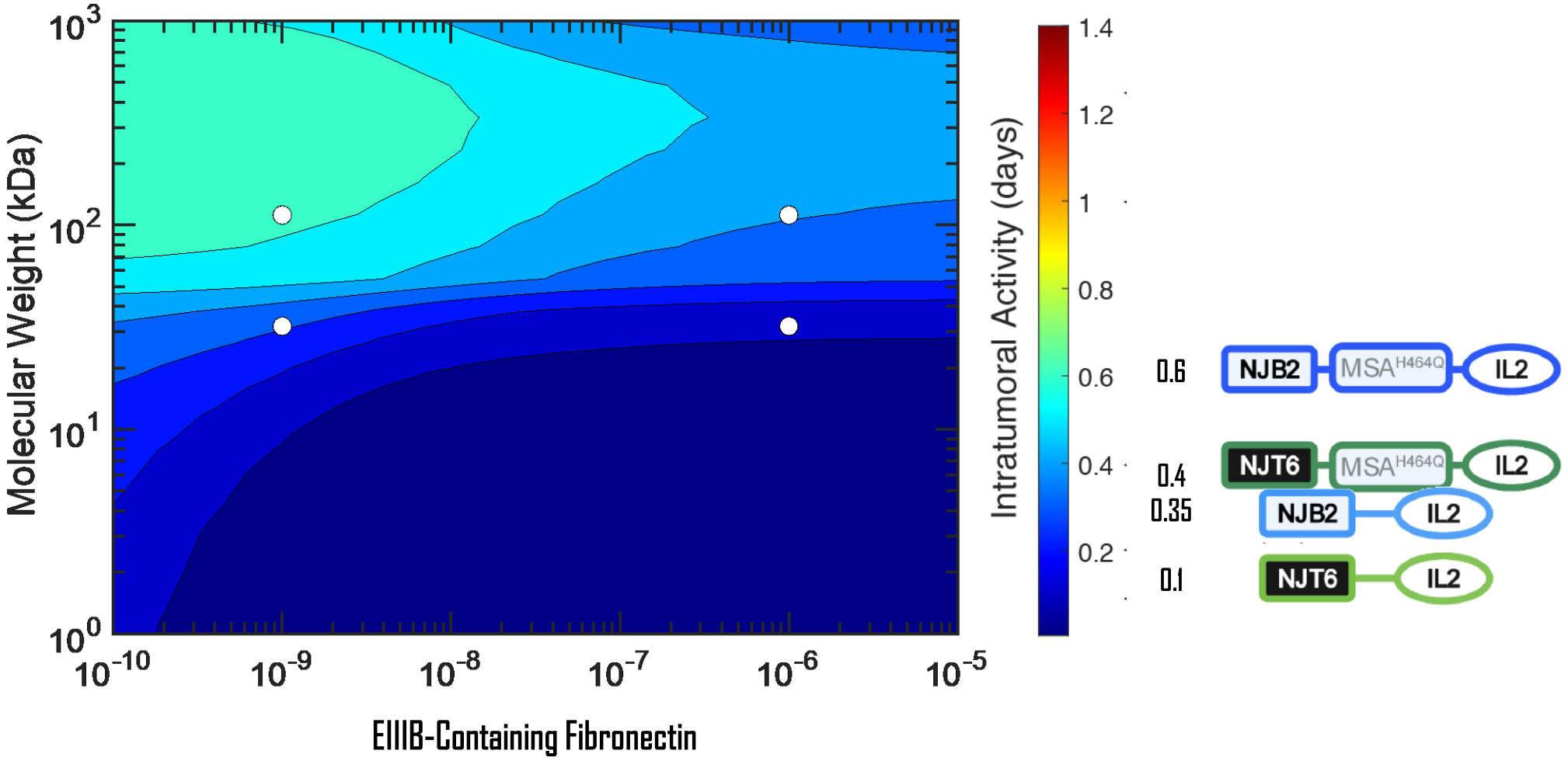
Momin et al. *2021 (submitted)*

EIIIB Simulation

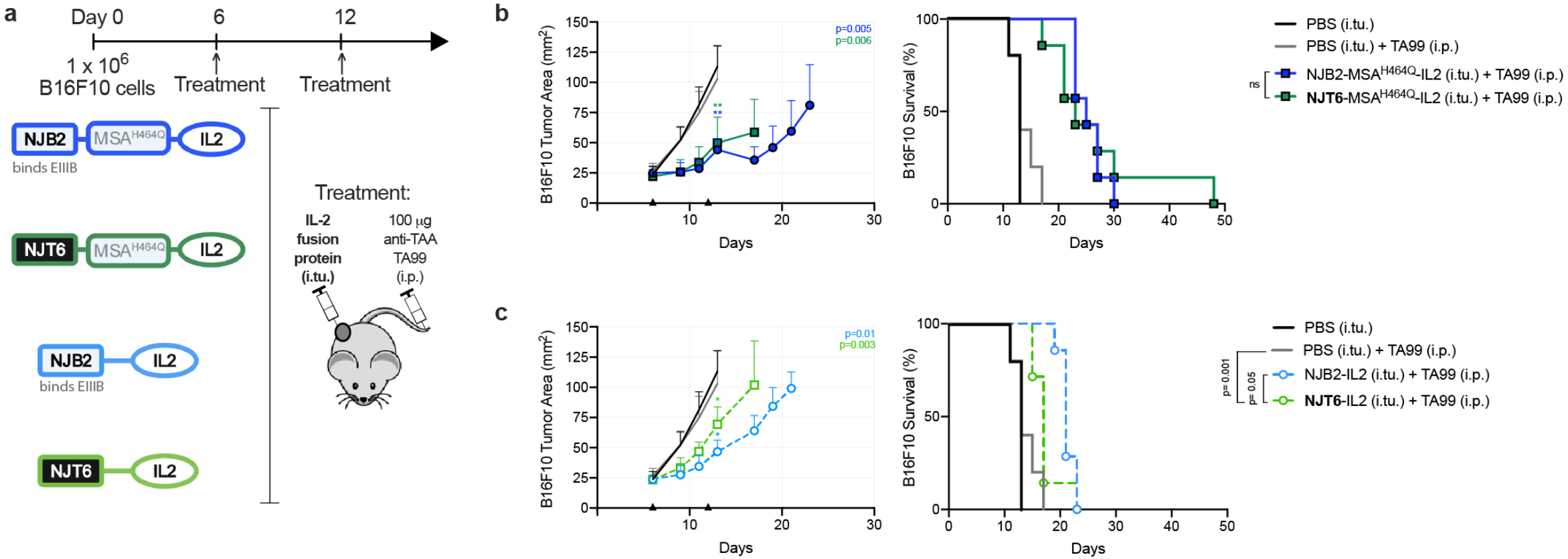




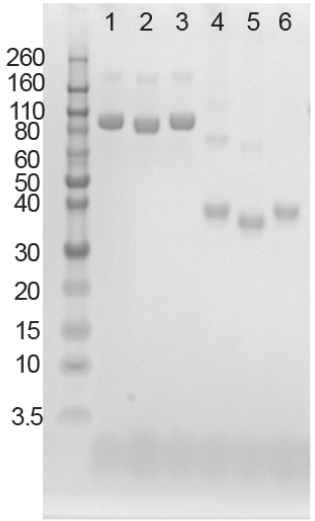
Jailkhani et al. PNAS, 2019


Molecular Weight (kDa)
10^3
10^2
10^1
10^0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
EIIIB-Containing Fibronectin
Intratumoral Activity (days)
1.4
1.2
1
0.8
0.6
0.4
0.2
0.6
NJB2
MSA^{H464Q}
IL2
0.4
NJT6
MSA^{H464Q}
IL2
0.35
NJB2
IL2
0.1
NJT6
IL2

Supplementary

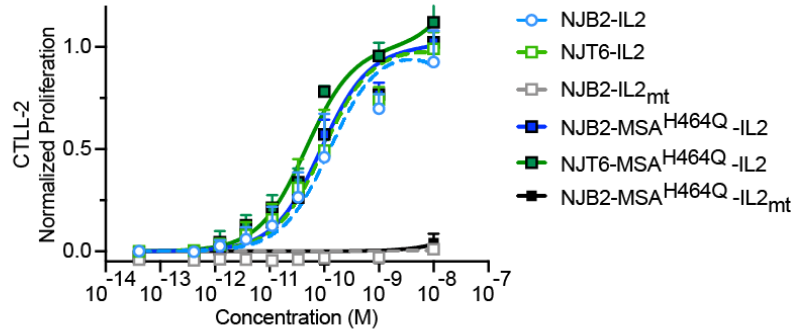


a



1. NJB2-MSA^{H464Q}-IL2	97.7 kDa
2. NJT6-MSA^{H464Q}-IL2	98.1 kDa
3. NJB2-MSA^{H464Q}-IL2mt	97.7 kDa
4. NJB2-IL2	31.8 kDa
5. NJT6-IL2	32.2 kDa
6. NJB2-IL2mt	31.8 kDa

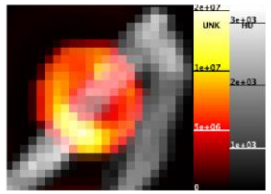
b



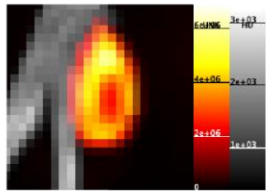
Need to characterize the pharmacokinetics of local therapies

48 hr

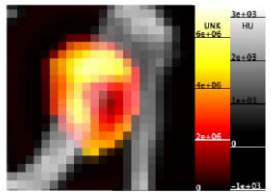
LAIR LAIR MSA^{H464Q} Zr-89



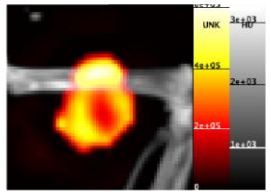
LAIR LAIRx MSA^{H464Q} Zr-89



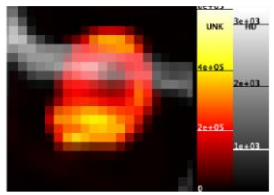
LAIRx LAIRx MSA^{H464Q} Zr-89

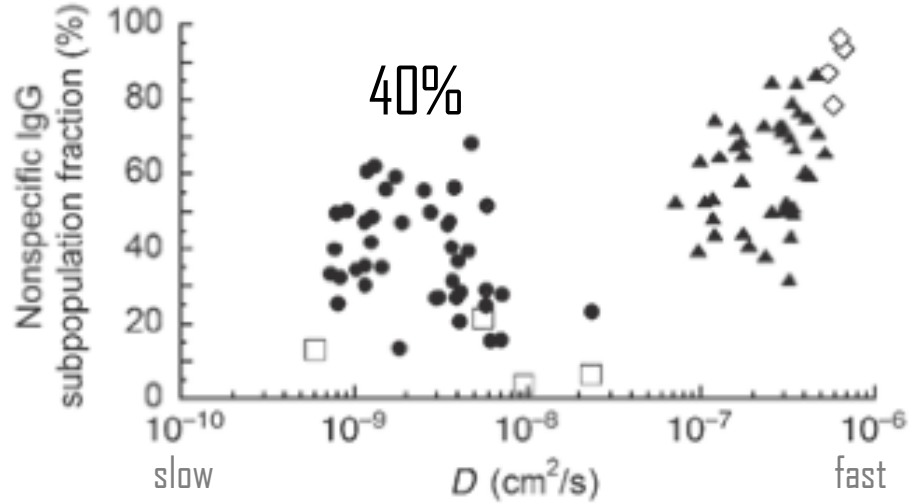


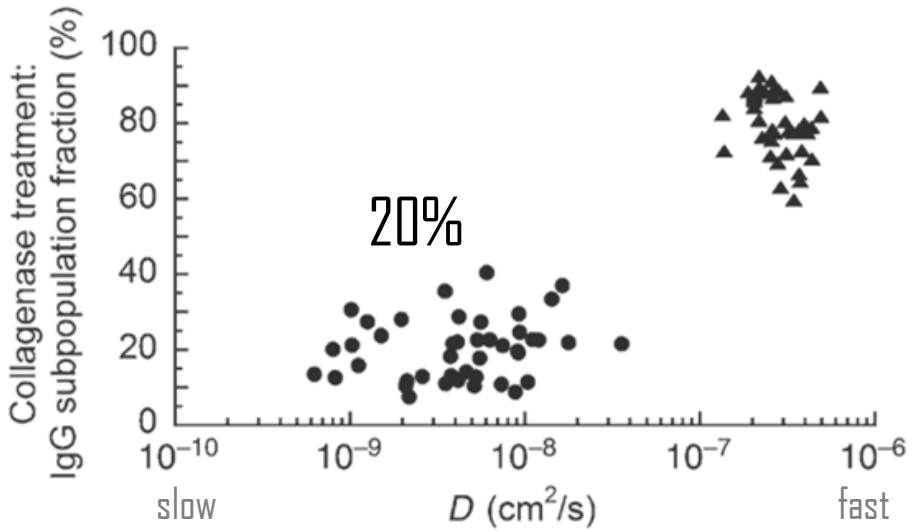
LAIR Zr-89



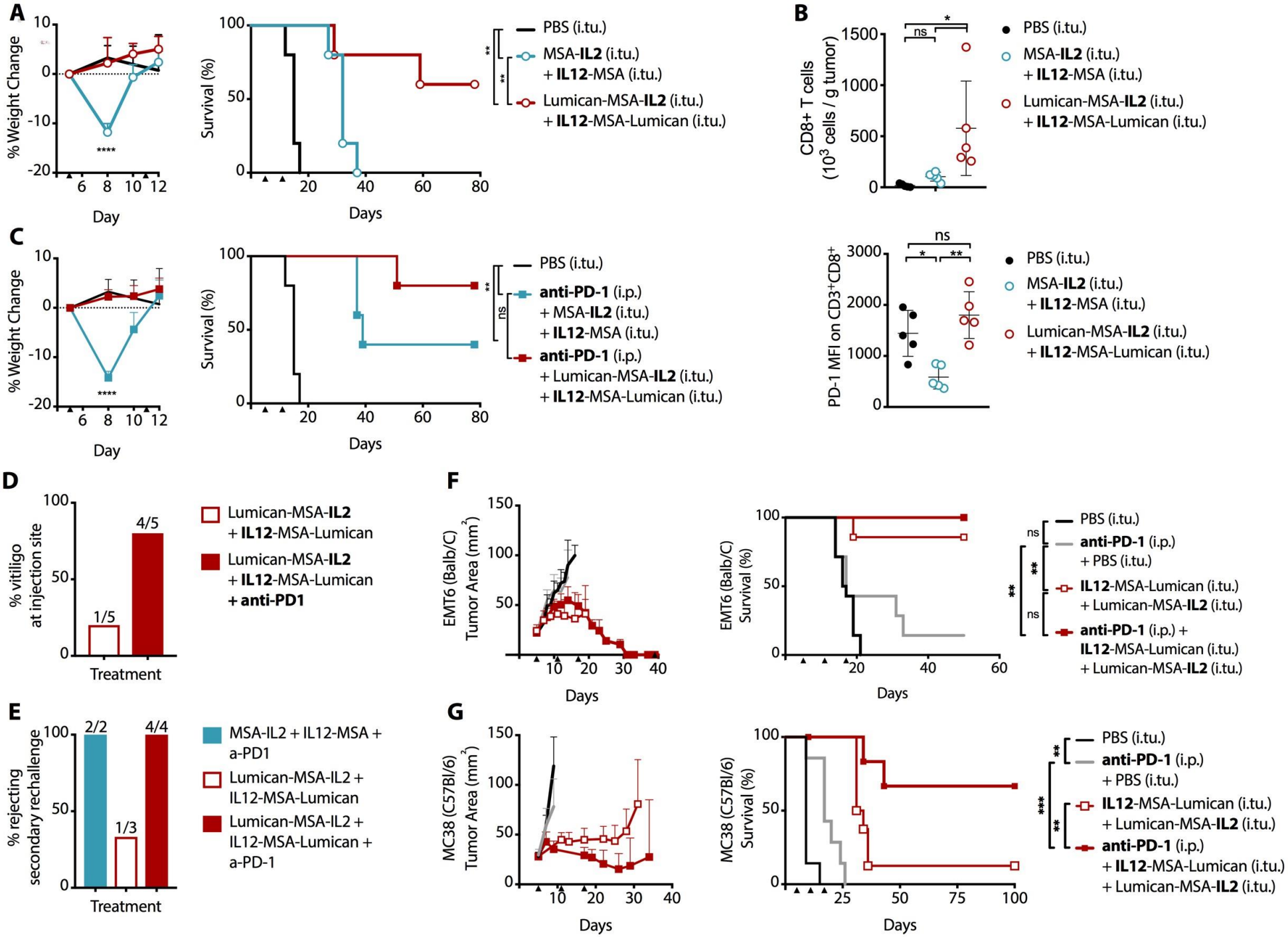
LAIRx Zr-89

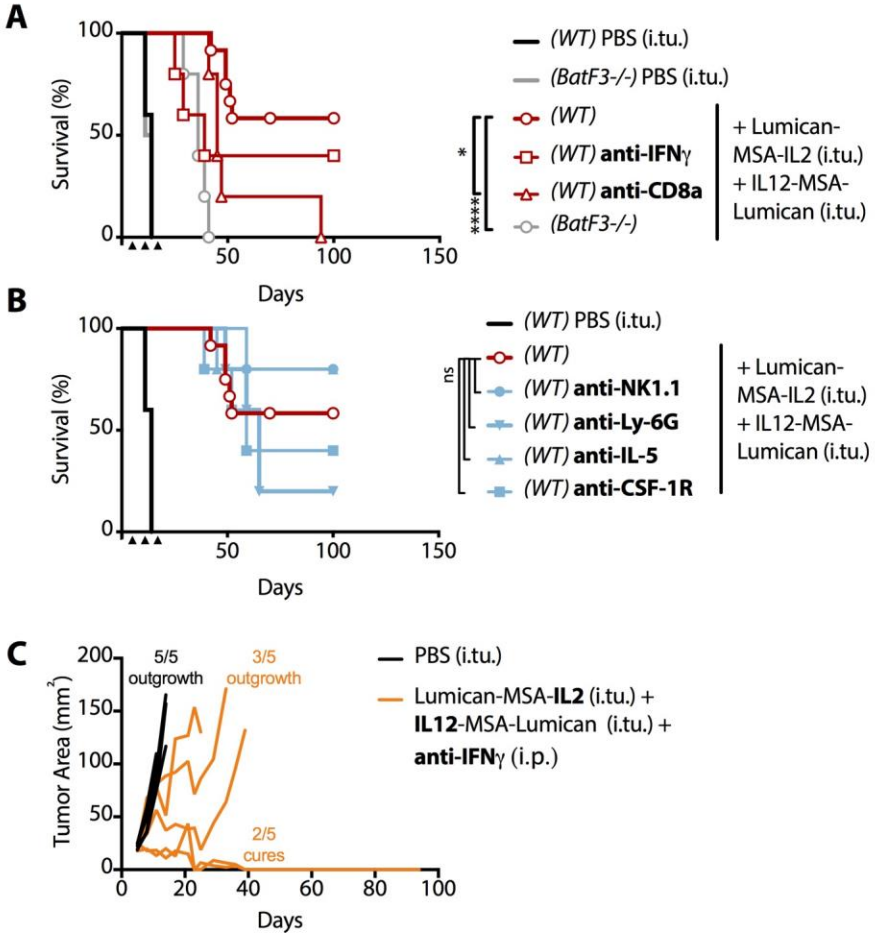






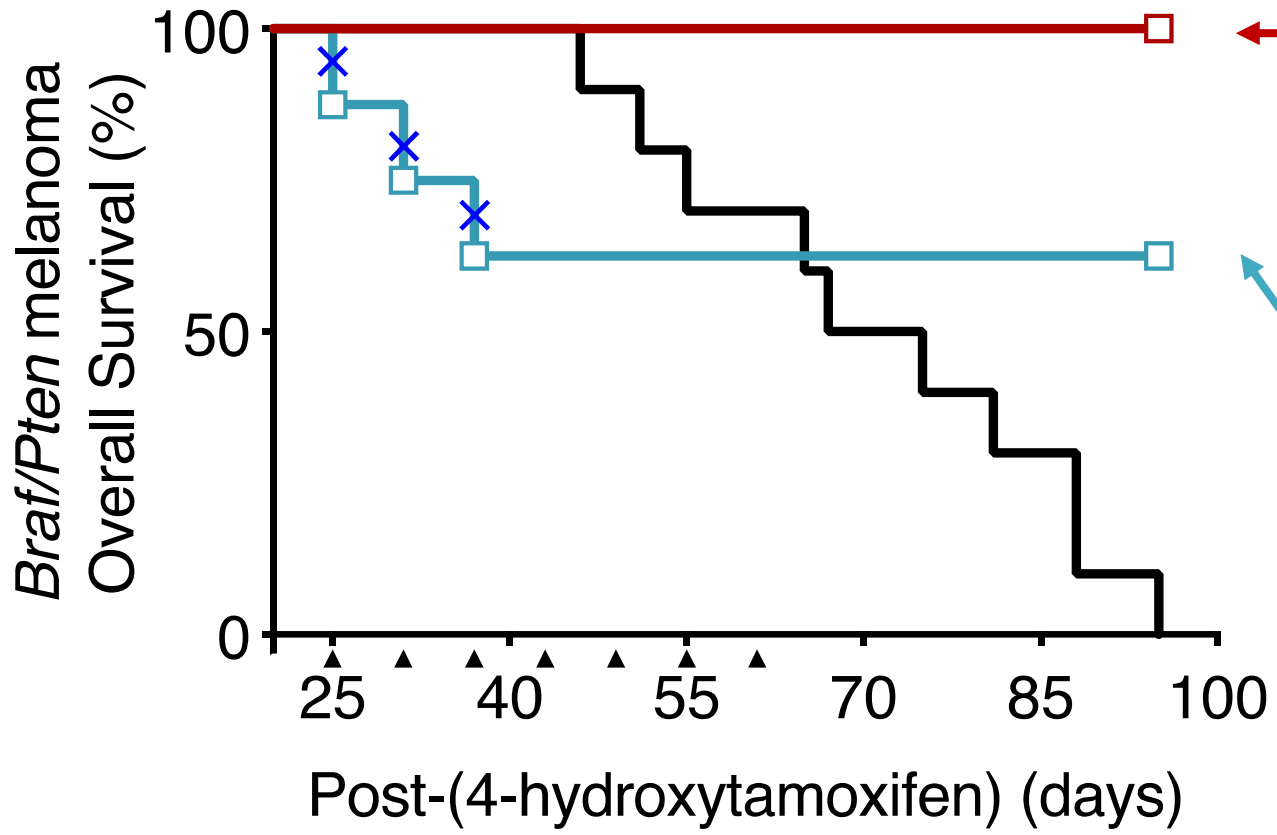
Alexandrakis et al. *Nat Med.* 2004

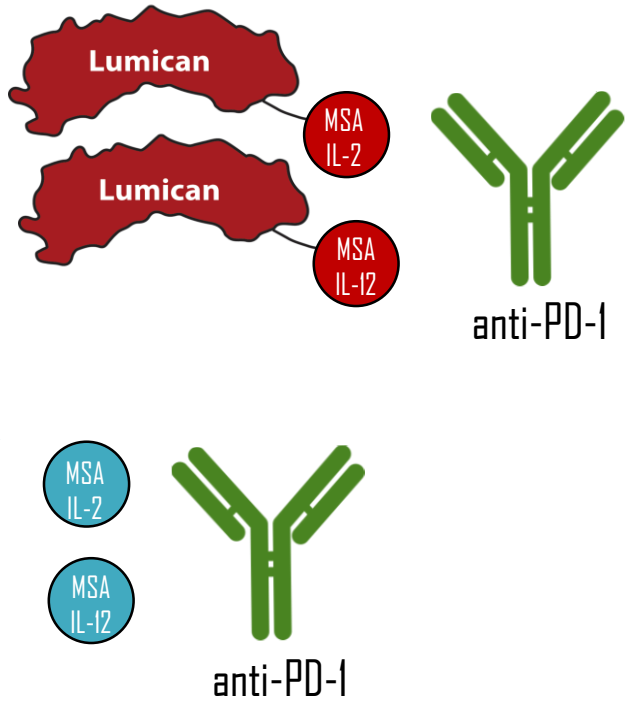

A
% Weight Change
Day
****
Survival (%)
Days
PBS (i.tu.)
MSA-IL2 (i.tu.)
+ IL12-MSA (i.tu.)
Lumican-MSA-IL2 (i.tu.)
+ IL12-MSA-Lumican (i.tu.)
B
CD8+ T cells
(10^3 cells / g tumor)
ns
*
C
anti-PD-1 (i.p.)
+ MSA-IL2 (i.tu.)
+ IL12-MSA (i.tu.)
anti-PD-1 (i.p.)
+ Lumican-MSA-IL2 (i.tu.)
+ IL12-MSA-Lumican (i.tu.)
PD-1 MFI on CD3+CD8+
D
% vitiligo at injection site
Treatment
1/5
4/5
Lumican-MSA-IL2
+ IL12-MSA-Lumican
Lumican-MSA-IL2
+ IL12-MSA-Lumican
+ anti-PD1
E
% rejecting secondary rechallenge
2/2
1/3
4/4
MSA-IL2 + IL12-MSA + a-PD1
Lumican-MSA-IL2 + IL12-MSA-Lumican
Lumican-MSA-IL2 + IL12-MSA-Lumican + a-PD-1
F
EMT6 (Balb/C) Tumor Area (mm²)
EMT6 (Balb/C) Survival (%)
PBS (i.tu.)
anti-PD-1 (i.p.)
+ PBS (i.tu.)
IL12-MSA-Lumican (i.tu.)
+ Lumican-MSA-IL2 (i.tu.)
anti-PD-1 (i.p.) +
IL12-MSA-Lumican (i.tu.)
+ Lumican-MSA-IL2 (i.tu.)
G
MC38 (C57Bl/6) Tumor Area (mm²)
MC38 (C57Bl/6) Survival (%)


A
100
50
0
Survival (%)
50
100
150
Days
(WT) PBS (i.tu.)
(BatF3-/-) PBS (i.tu.)
(WT)
(WT) anti-IFNγ
(WT) anti-CD8a
(BatF3-/-)
*
****
+ Lumican-MSA-IL2 (i.tu.) + IL12-MSA-Lumican (i.tu.)
B
100
50
0
Survival (%)
50
100
150
Days
(WT) PBS (i.tu.)
(WT)
(WT) anti-NK1.1
(WT) anti-Ly-6G
(WT) anti-IL-5
(WT) anti-CSF-1R
ns
+ Lumican-MSA-IL2 (i.tu.) + IL12-MSA-Lumican (i.tu.)
C
200
150
100
50
0
Tumor Area (mm²)
5/5 outgrowth
3/5 outgrowth
2/5 cures
0
20
40
60
80
100
Days
PBS (i.tu.)
Lumican-MSA-IL2 (i.tu.) + IL12-MSA-Lumican (i.tu.) + anti-IFNγ (i.p.)

Lumican-cytokines are a safer, tumor-agnostic treatment

$Braf^{V600E}/Pten^{fl/fl}$ Genetically Engineering Mouse Model



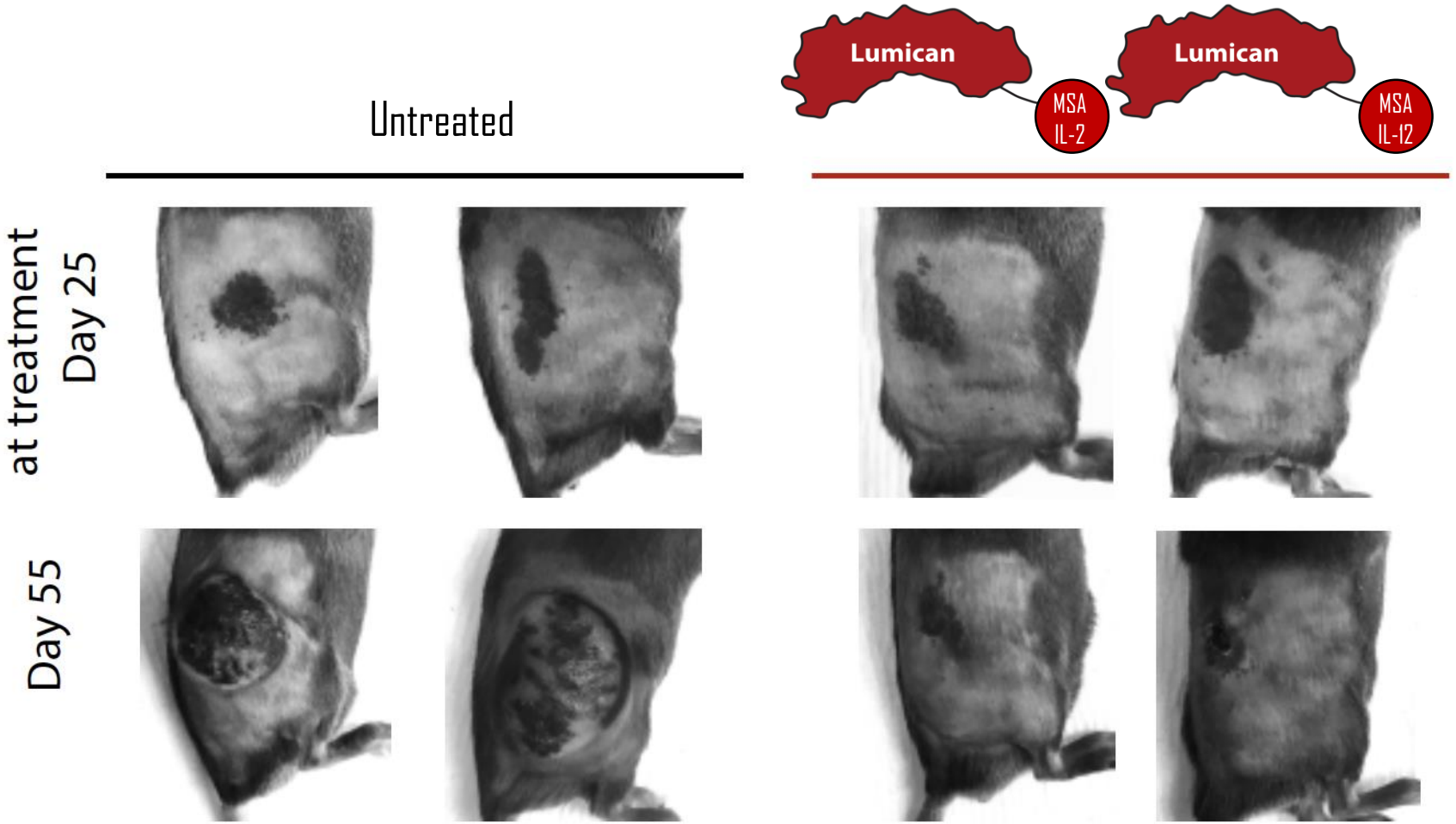


x: Euthanized due to >20% weight loss

Adapted from Momin et al. *Sci. Trans. Med.* 2019

Lumican-cytokines are a safer, tumor-agnostic treatment

$Braf^{V600E}/Pten^{fl/fl}$ Genetically Engineering Mouse Model



Adapted from Momin et al. *Sci. Trans. Med.* 2019