# Collagen anchoring of intratumorally administered IL-2 and IL-12 safely potentiates systemic immunotherapy

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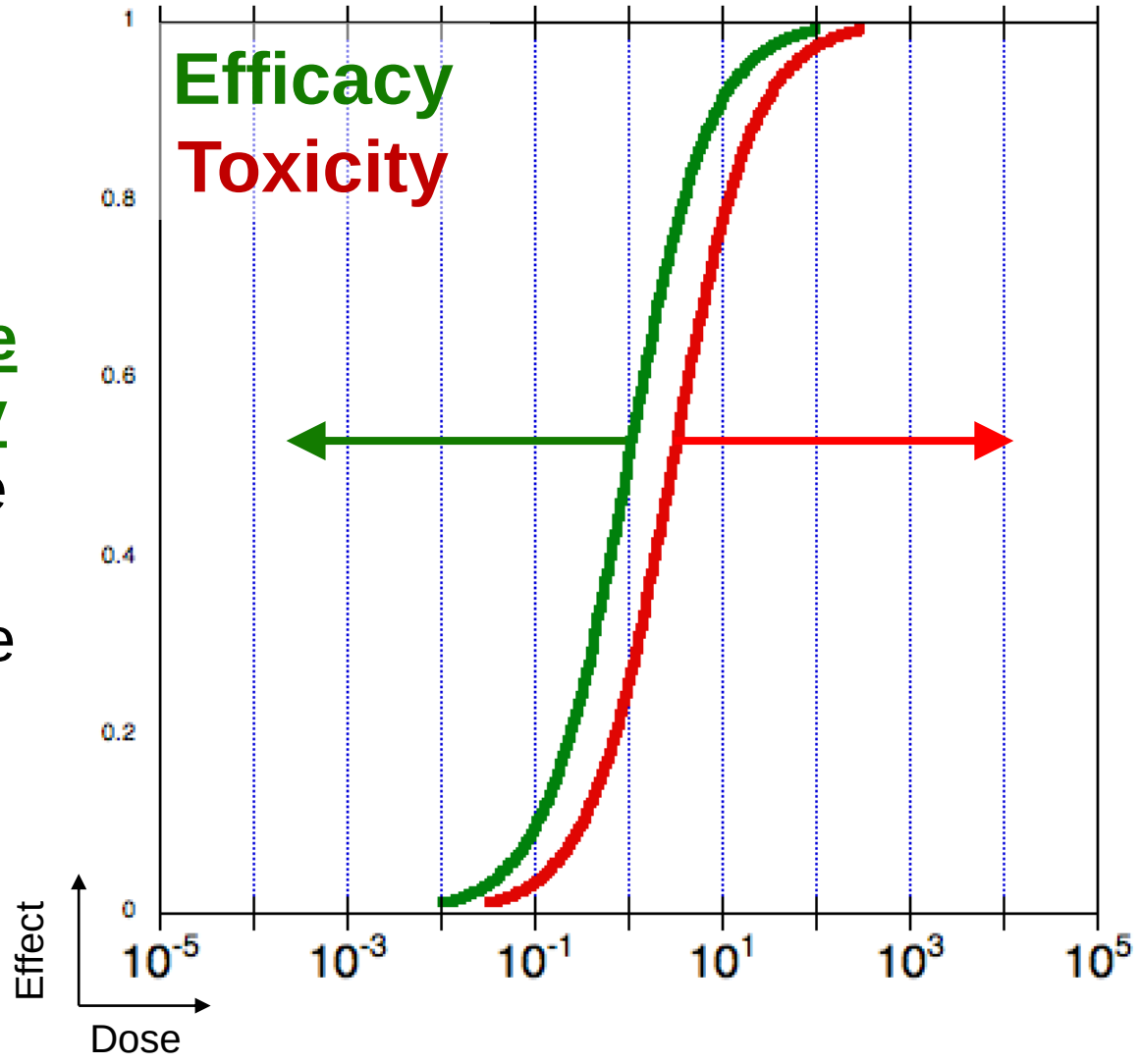
KOCH INSTITUTE for Integrative Cancer Research at MIT

# Overview

- IL-2 & IL-12 are not endocrine factors
- IV dosing to MTD does not mimic natural exposure
- Tissue localization decreases toxicity and increases potency

# Cytokine therapies have poor therapeutic windows

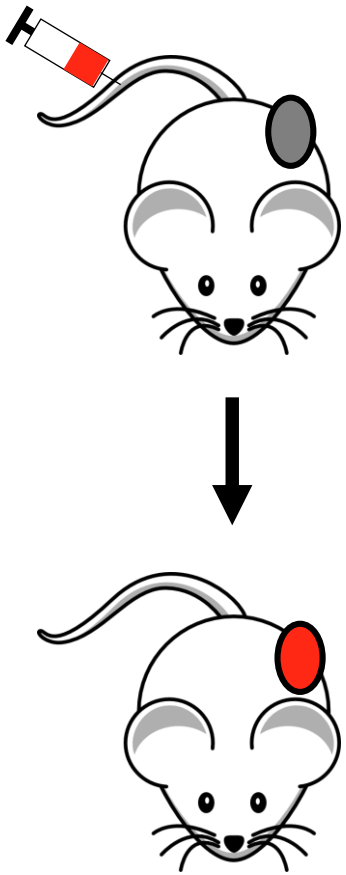
**Enhance Efficacy**
Increase tumor exposure



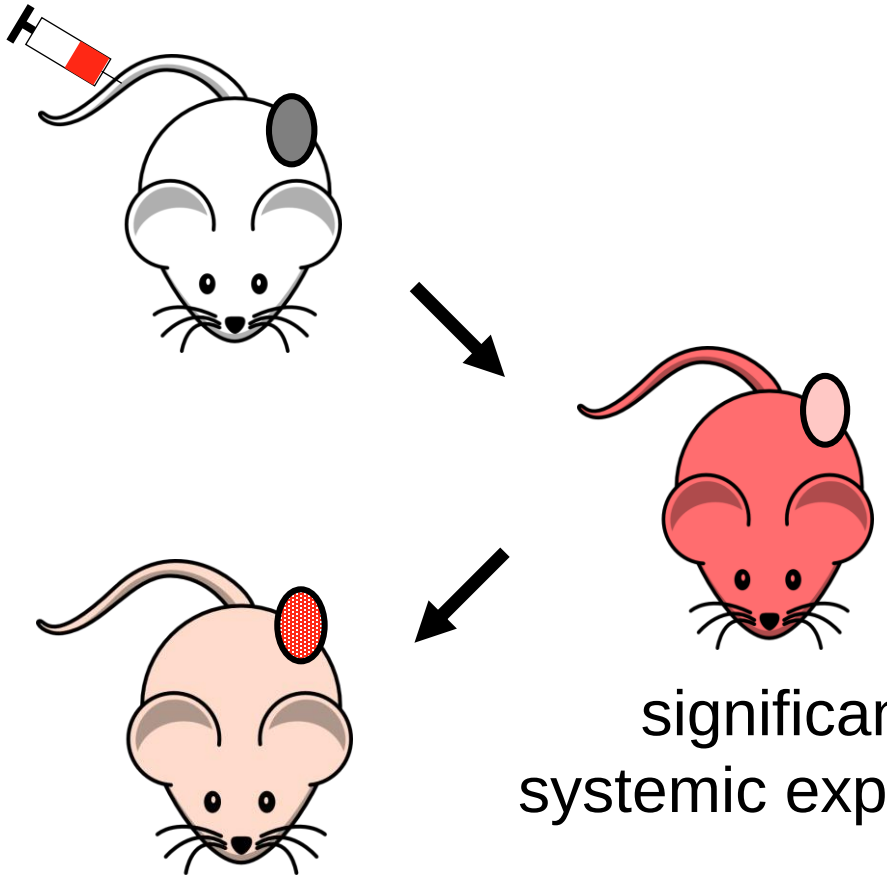

**Reduce Toxicity**
Decrease systemic exposure

# Targeted Drug (e.g. Immunocytokine) Delivery

## Expectation



## Reality

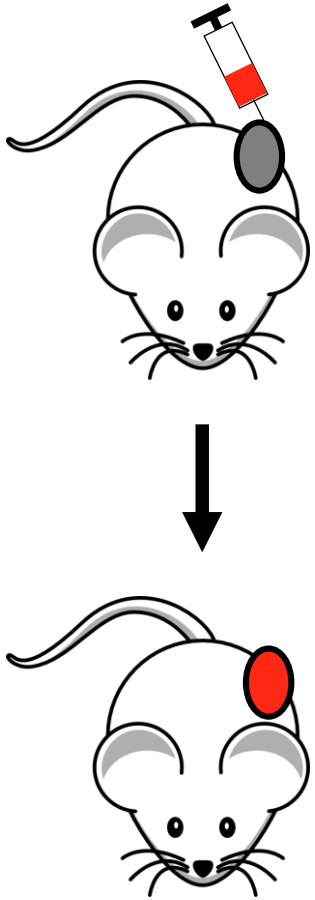


significant
systemic exposure

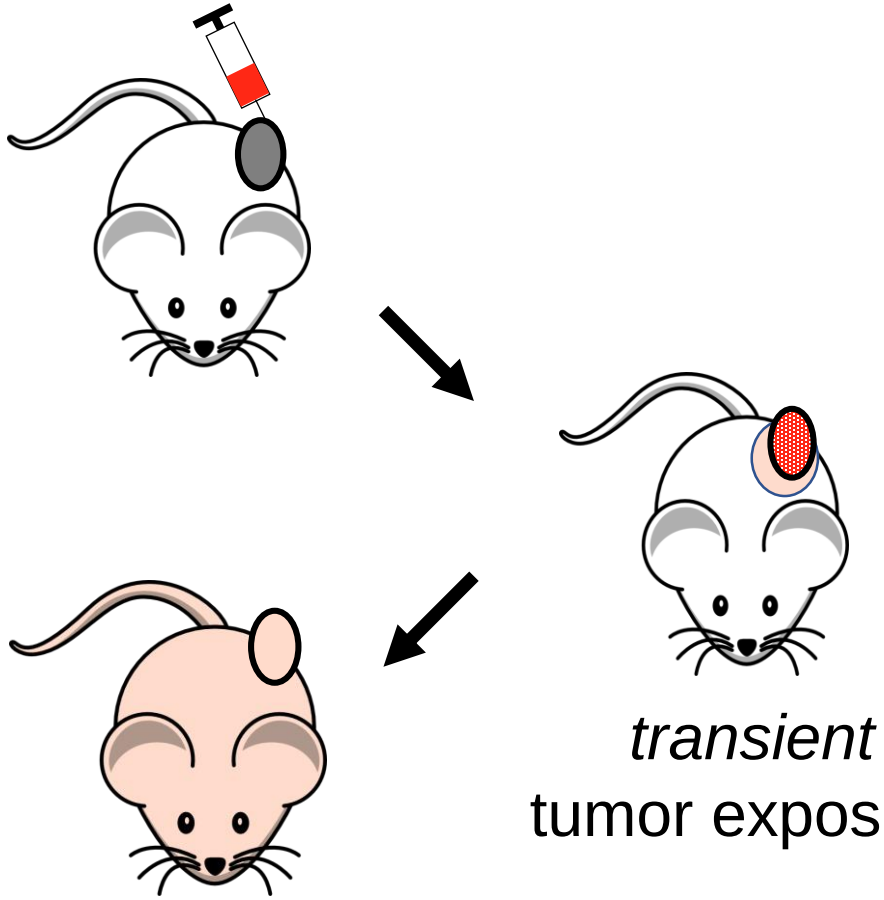
Tzeng et al. PNAS. 2015

# Intratumoral Drug Delivery

## Expectation



## Reality



*transient*
tumor exposure

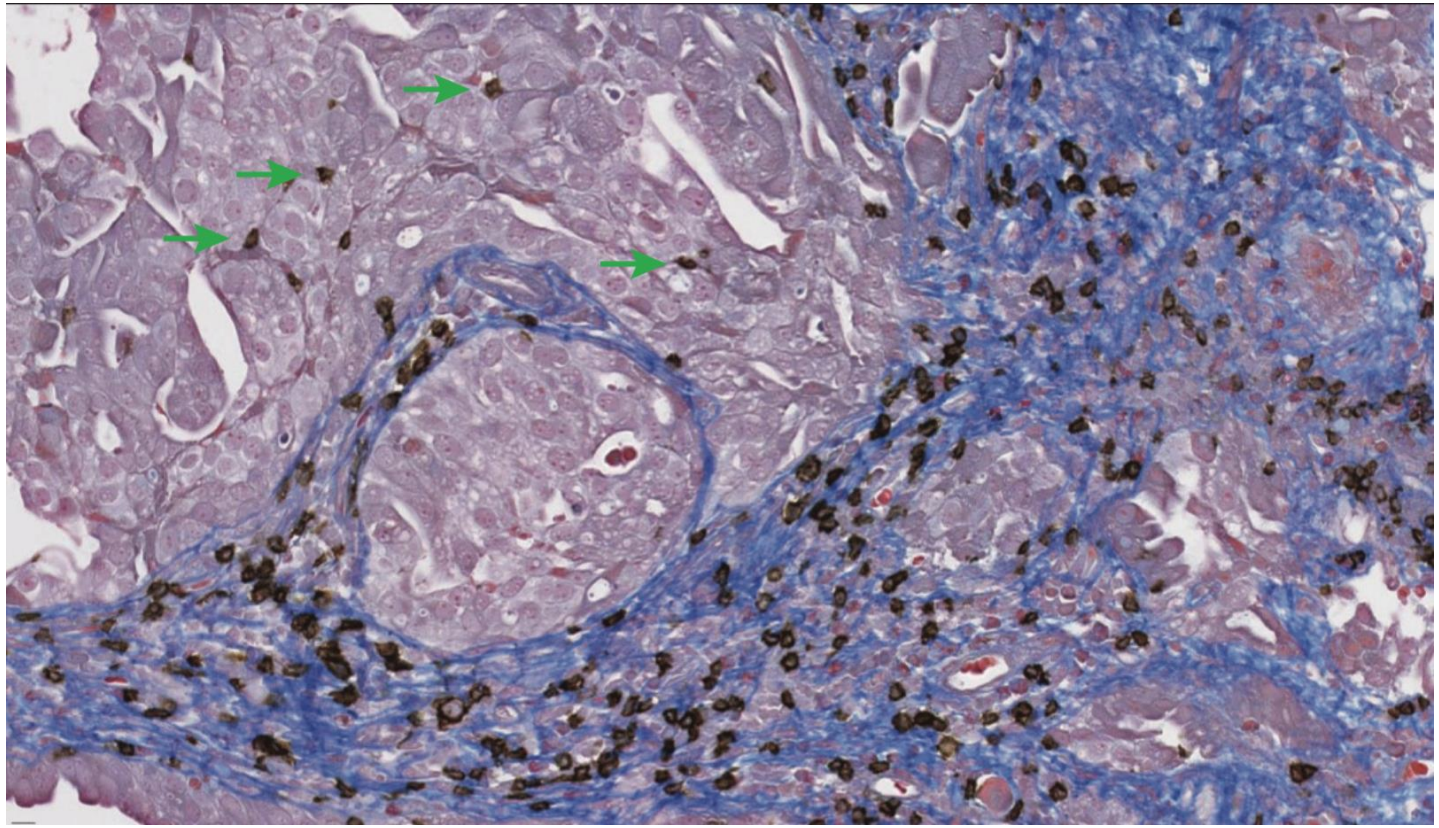
*inevitable*
systemic exposure

*Rohner & Thomas, JCR 2016*

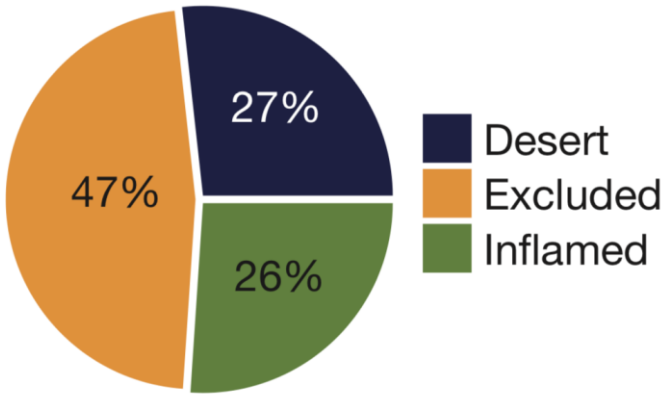
# Approach:
# Anchor intratumorally administered cytokines to collagen as fusion proteins

~30% of all protein in the body &
major component of tumor ECM
*(Naba, Mol & Cell Prot, ’12; JBC 277:4223, ‘02)*

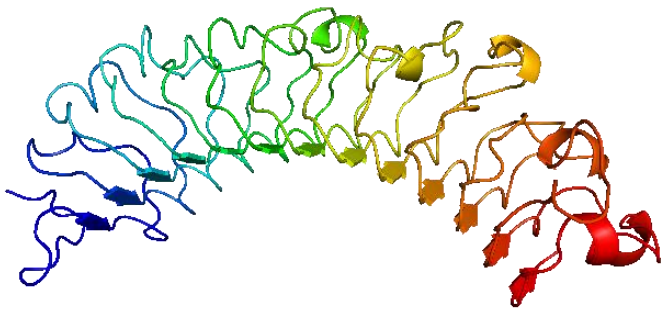
# T cells are trapped in collagen-rich stroma in immune-excluded tumors *(Mariathasan, Nature,'18)*



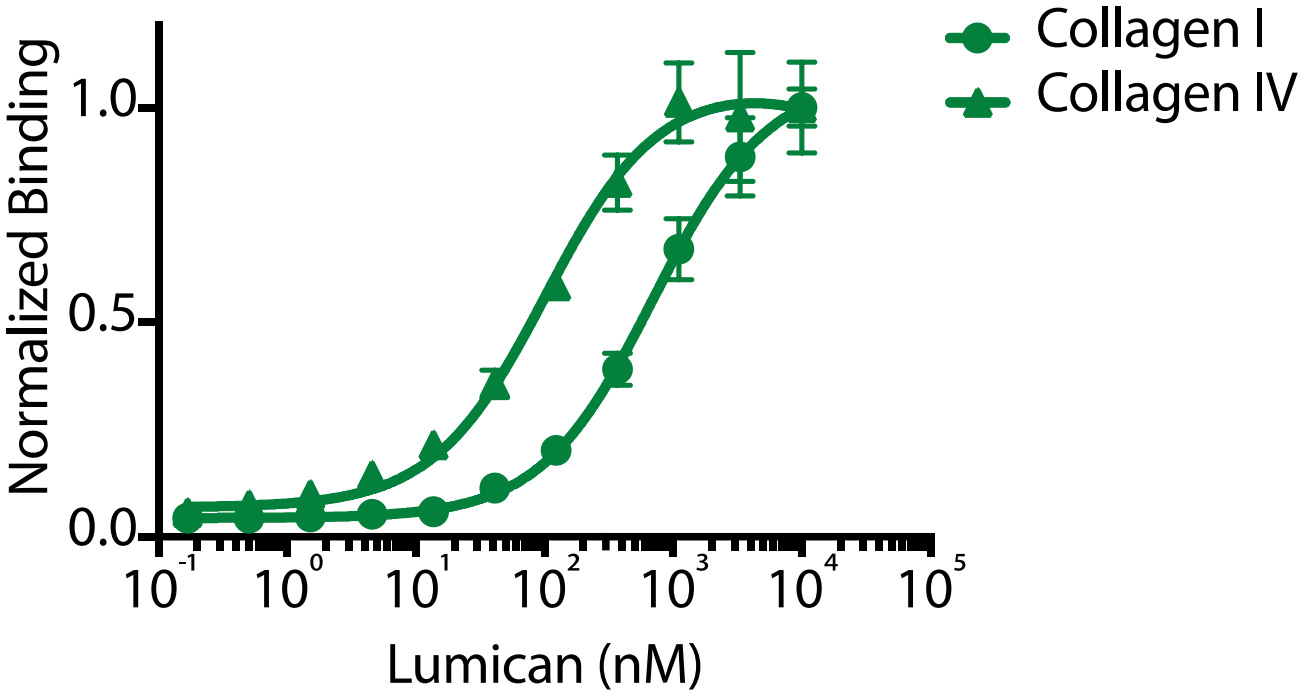
Blue: collagen
Brown: CD8+ T cells



# Lumican is a collagen anchor

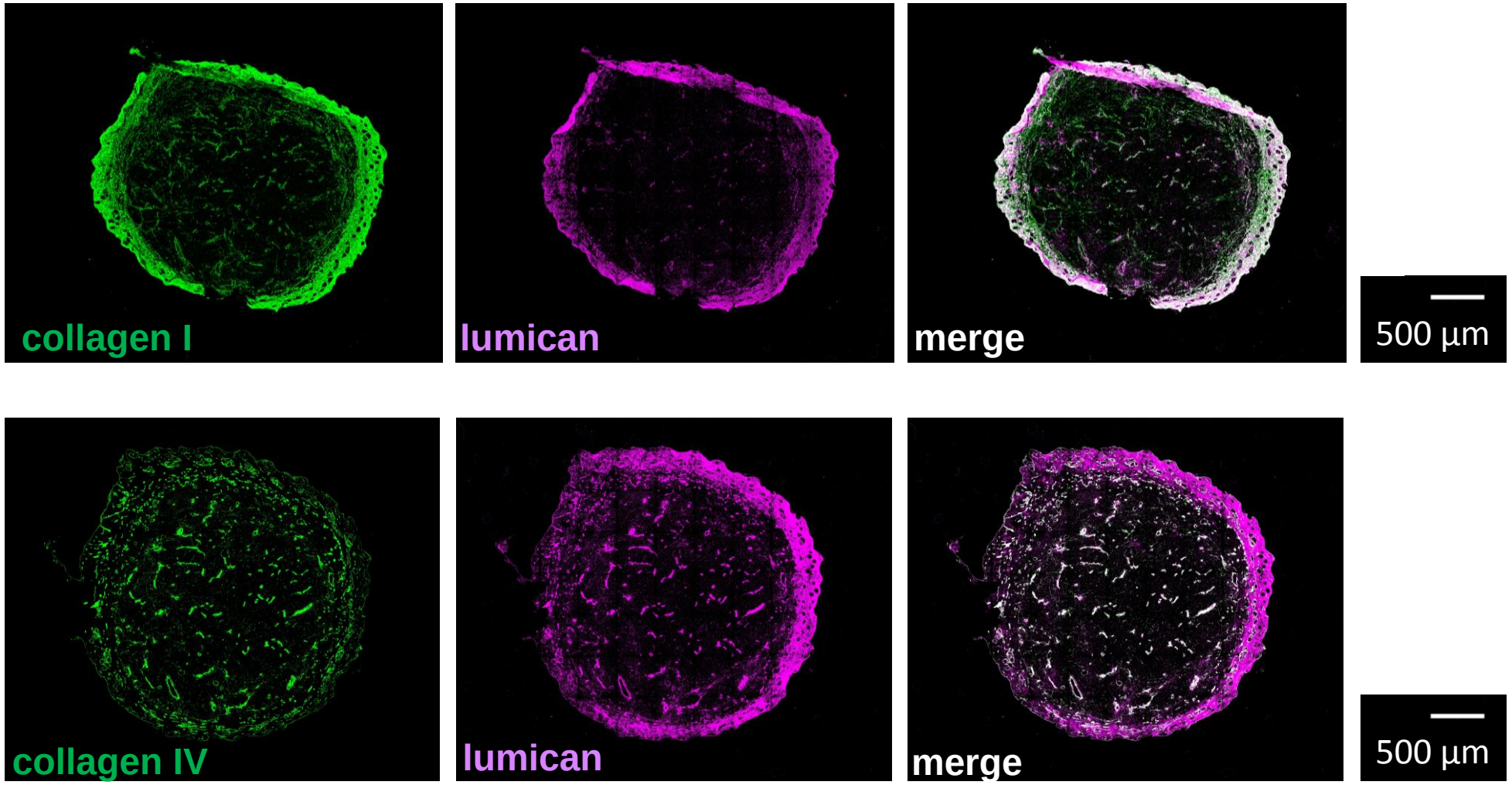


**Lumican**
*homology model*



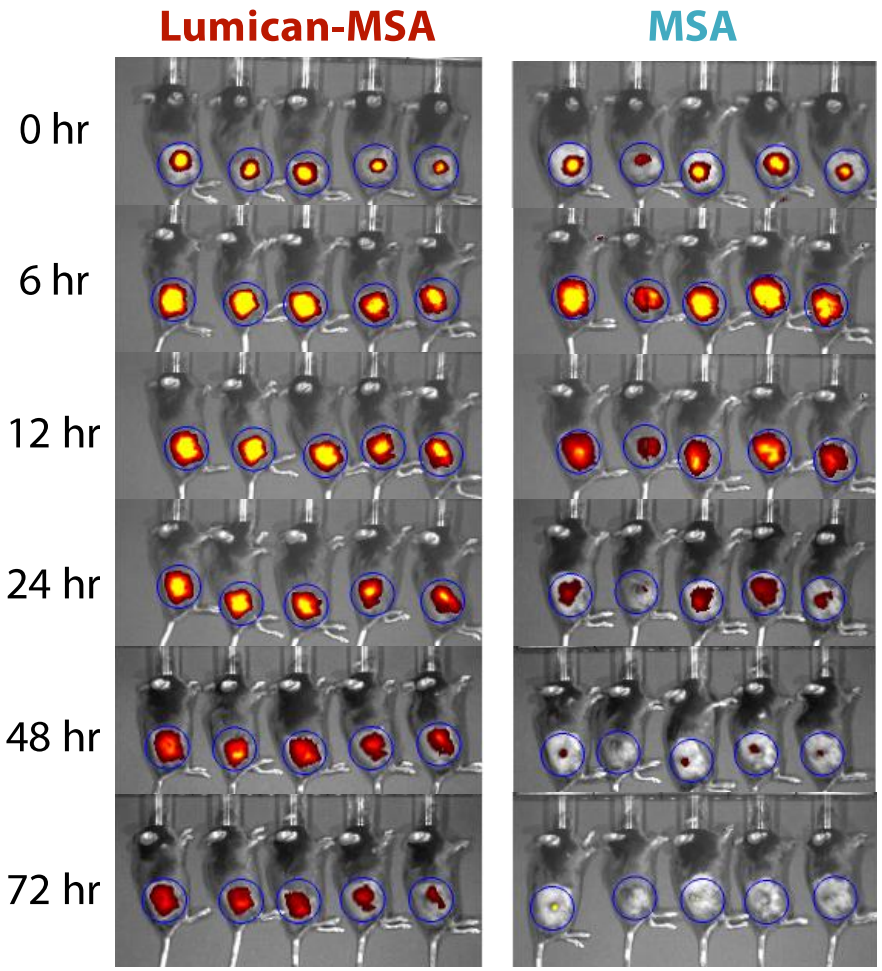

*"Lumican is a proteoglycan Class II member of the small leucine-rich proteoglycan (SLRP) family that is involved in collagen fibril organization and circumferential growth, corneal transparency, and epithelial cell migration and tissue repair."*
*https://en.wikipedia.org/wiki/Lumican*

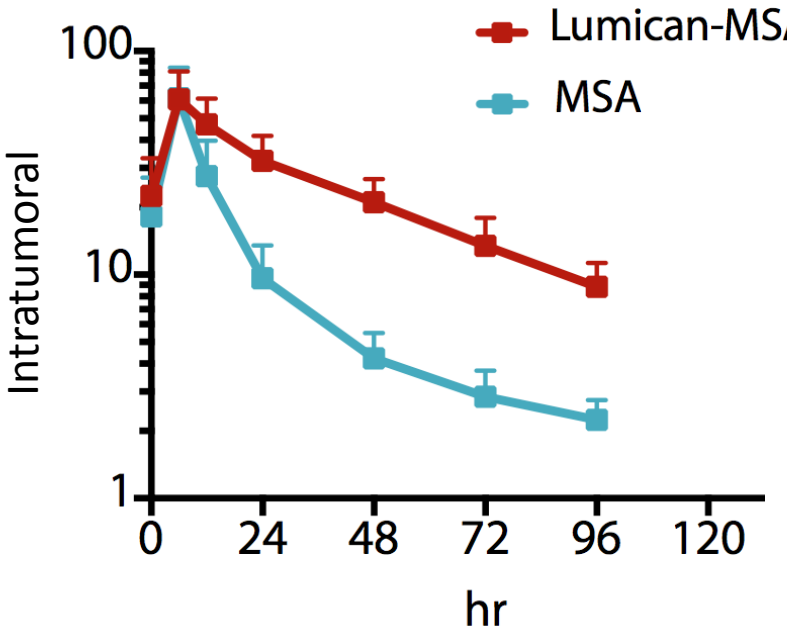
# Lumican colocalizes with collagen

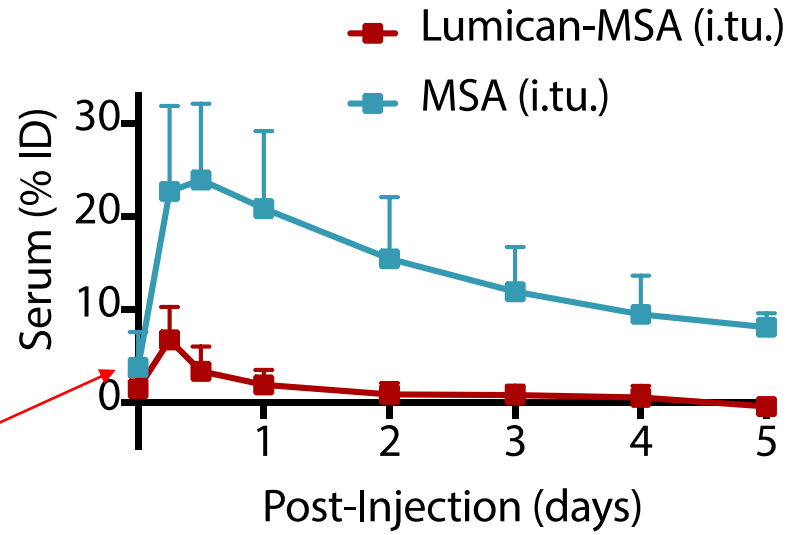



Immunofluorescence of s.c. B16F10 melanoma tumors intratumorally injected with fluorescently labeled **lumican**, excised four days later and stained for **collagen**

# Quantitative intratumoral retention for days



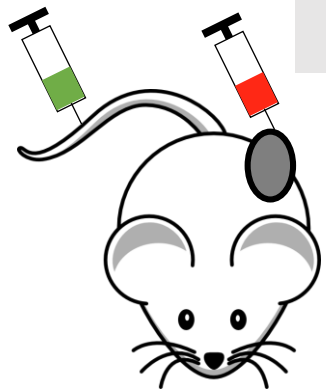




Very low escape

# Can fusion to Lumican improve the efficacy of IL-2?

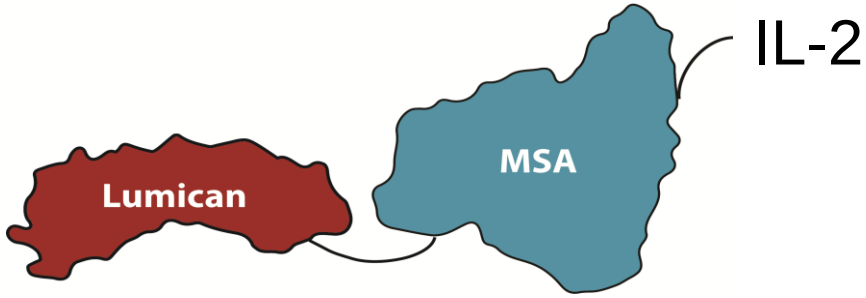
**TA99**
anti-TAA mAb



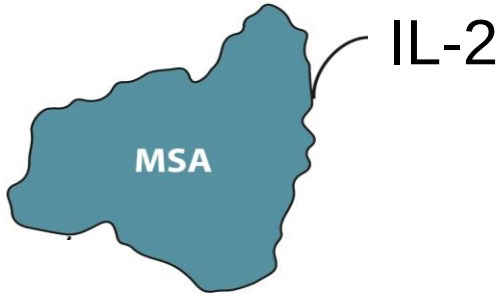
**IL-2**

**B16F10**

Zhu et al. Cancer Cell. 2015

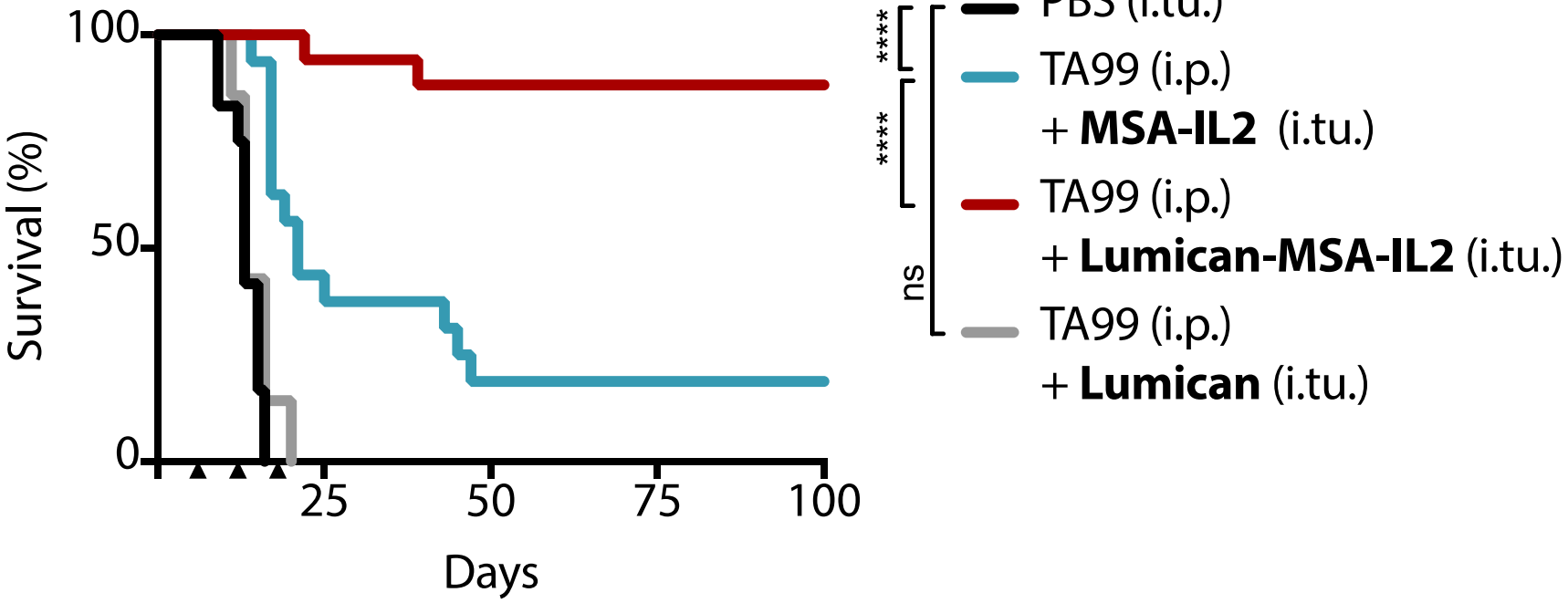


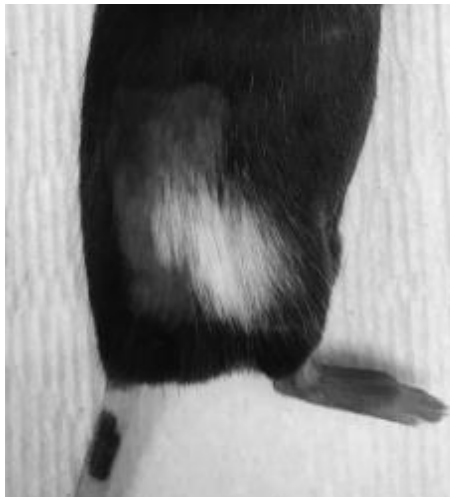

**Lumican-MSA-IL2**
collagen-anchored, locally retained

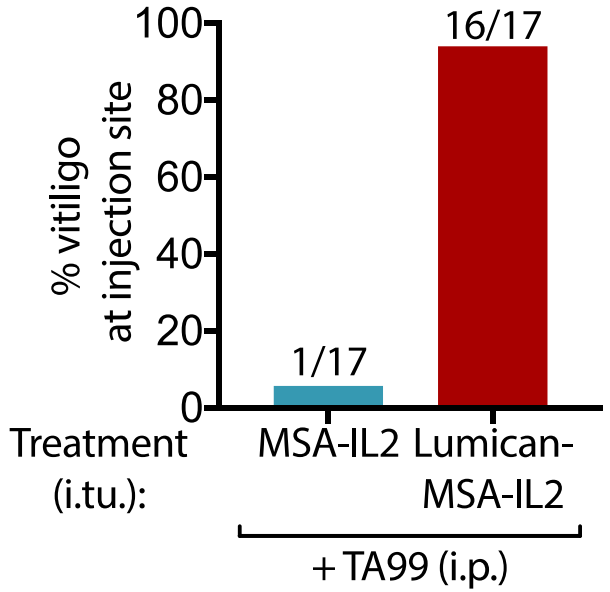



**MSA-IL2**
un-anchored, diffusible

# Collagen-anchoring IL-2 makes anti-TAA mAb therapy curative

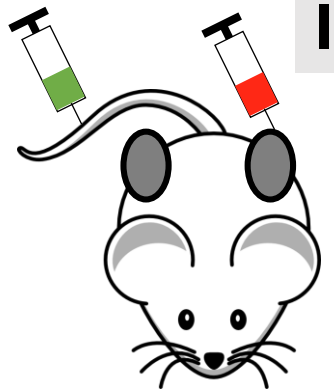




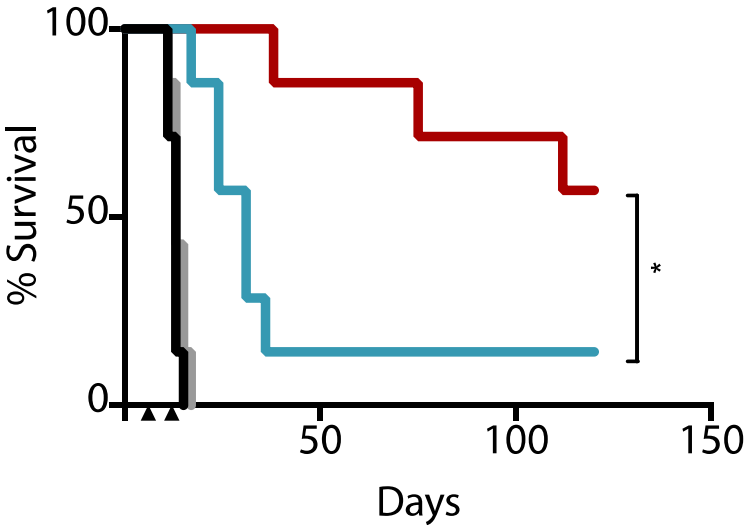
# Collagen-anchoring IL-2 + anti-TAA mAb cures distant lesions

**TA99**
anti-TAA mAb

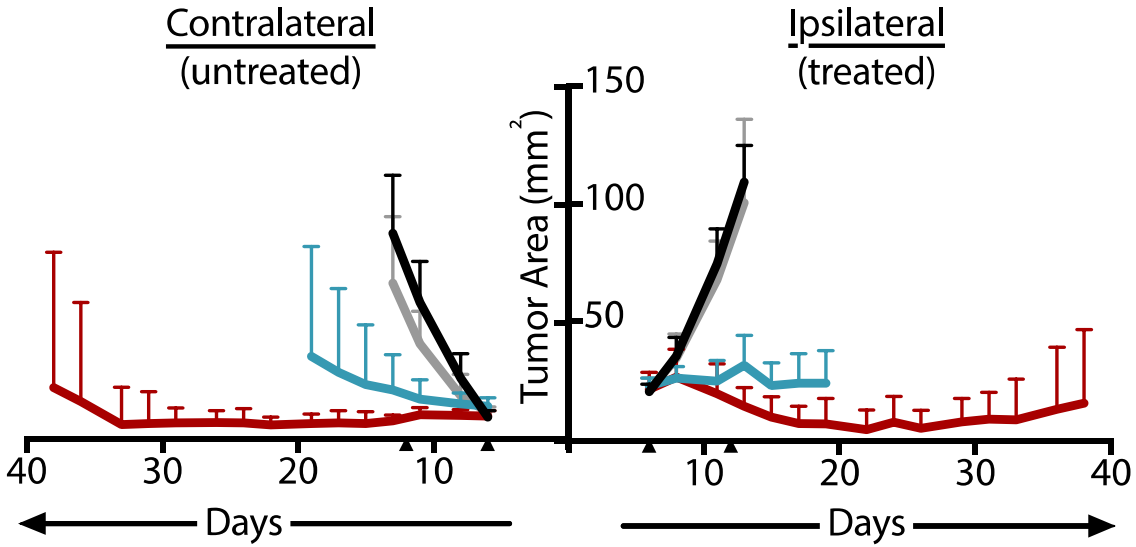


**IL-2**

**B16F10**



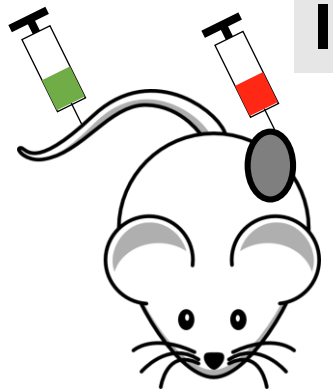

PBS (*ipsi* i.tu.)
TA99 (i.p.)
+ MSA-IL2 (*ipsi* i.tu.)
TA99 (i.p.)
+ Lumican-MSA-IL2 (*ipsi* i.tu.)
TA99 (i.p.)
+ Lumican (*ipsi* i.tu.)




**Anchored** IL-2 is superior to **un-anchored** IL-2, even at contralateral lesion control

# Can fusion to Lumican improve efficacy/reduce toxicity of IL-12?

**TA99 scFv-CAR-T**
(Leyuan Ma, Irvine lab)

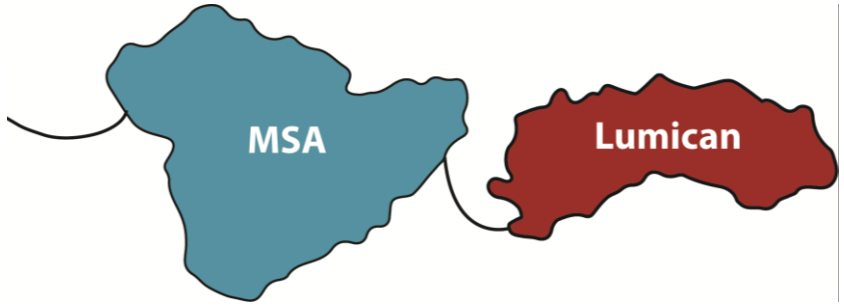


**IL-12**

**B16F10**

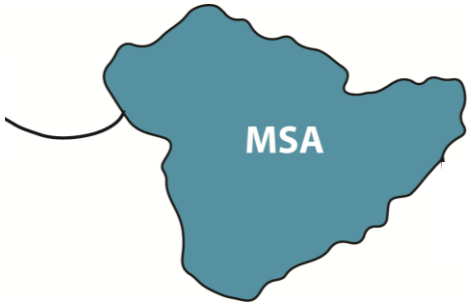
Inspired by armored CAR-T work
(Brentjens, Gilham, Koneru, etc.)

scIL-12



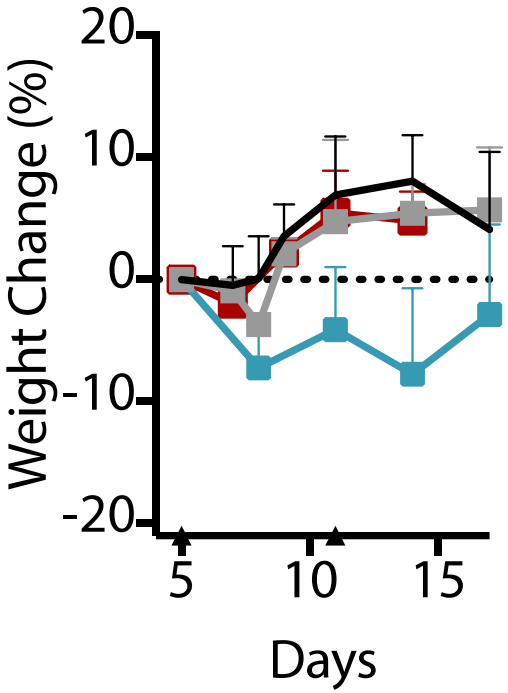

**IL12-MSA-Lumican**
collagen-anchored, locally retained

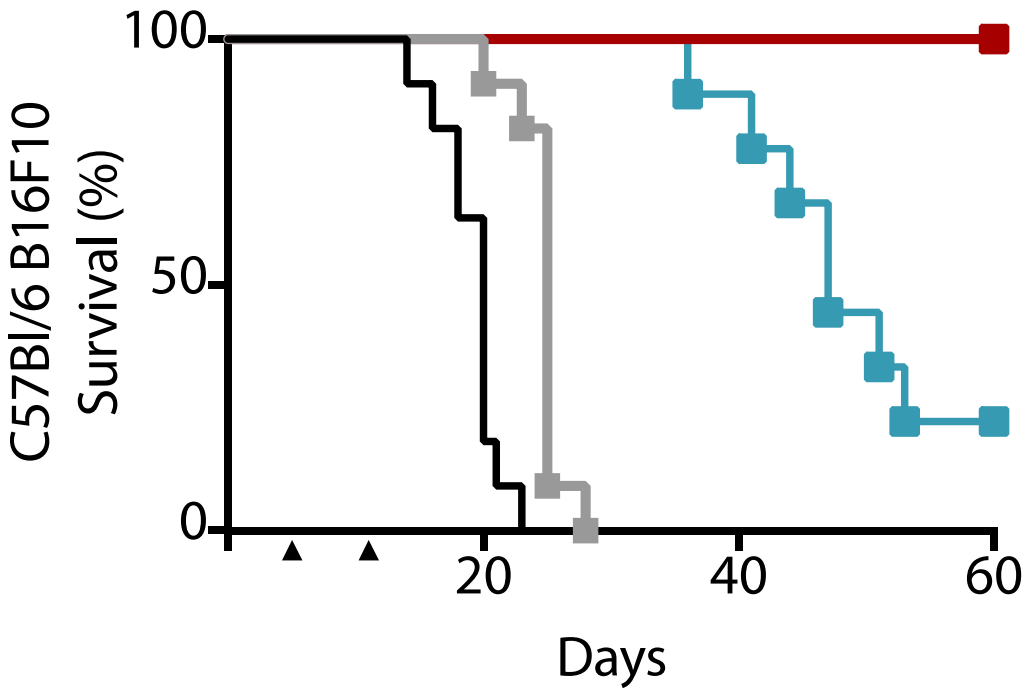
scIL-12

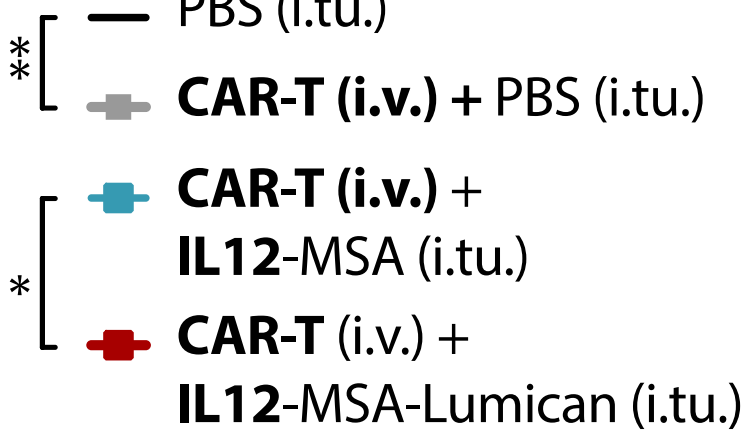



**IL12-MSA**
un-anchored, diffusible

# Collagen-anchoring IL-12 makes CAR-T therapy curative

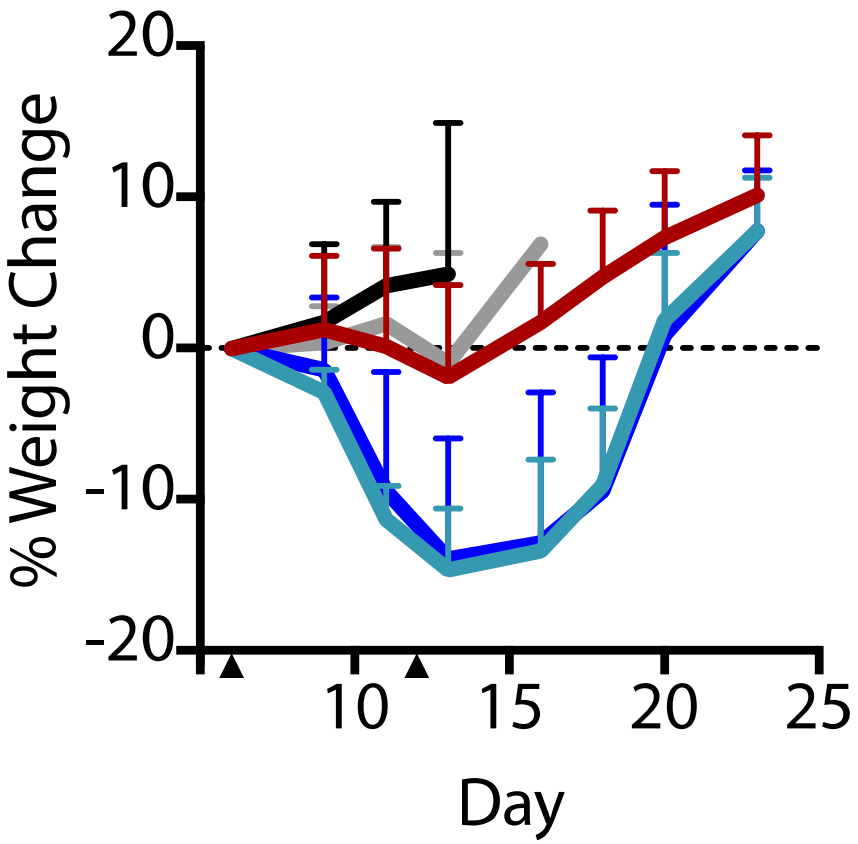


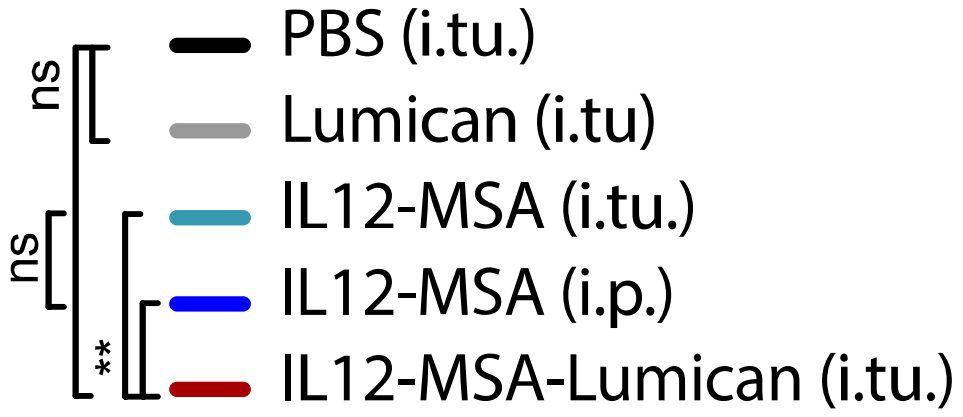




Leyuan Ma, Irvine lab
Joseph Palmeri, Wittrup lab

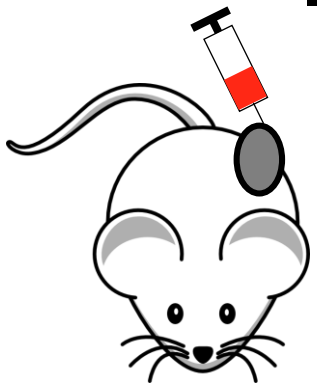
# Collagen-anchoring prevents systemic IL-12 toxicity



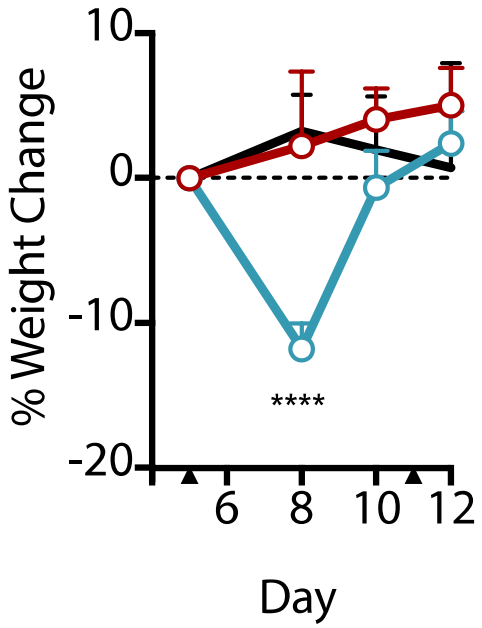

**Un-anchored intratumoral** is as **TOXIC** as **intraperitoneal administration**

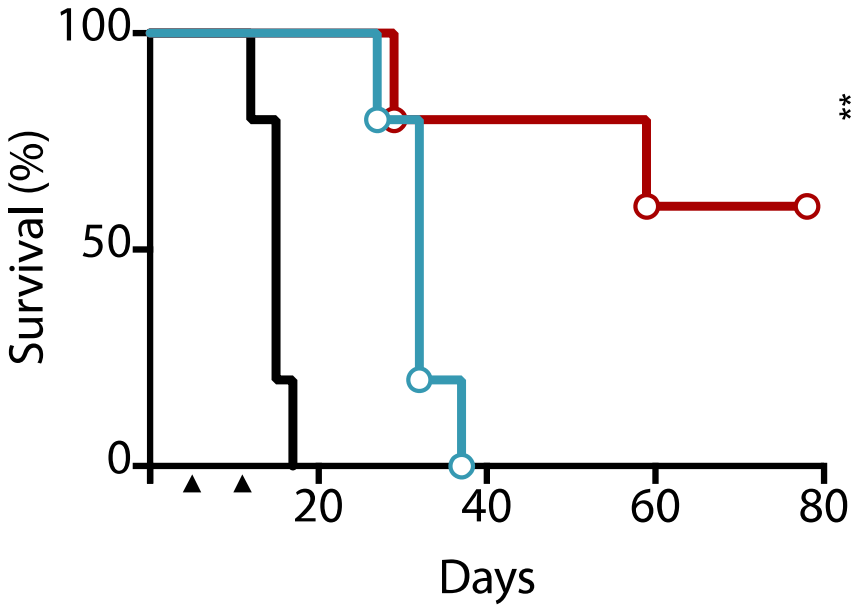
# Collagen-anchored IL-2 + IL-12, more efficacious and less toxic

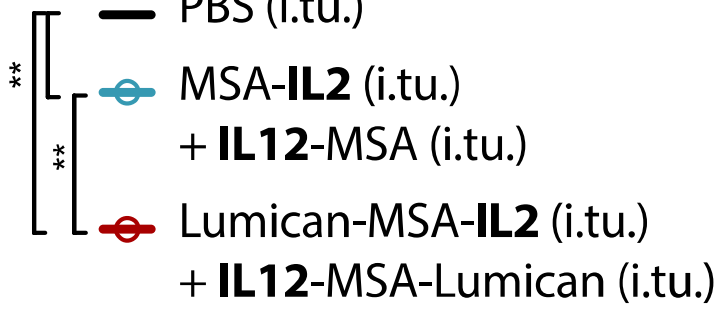


IL-2 + IL-12

B16F10

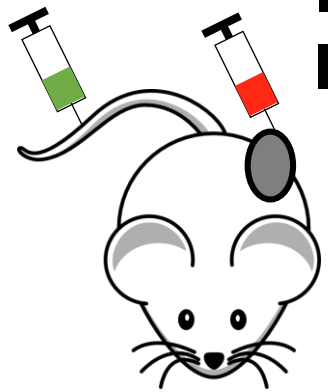


# Is treatment with collagen-anchoring cytokines tumor agnostic?

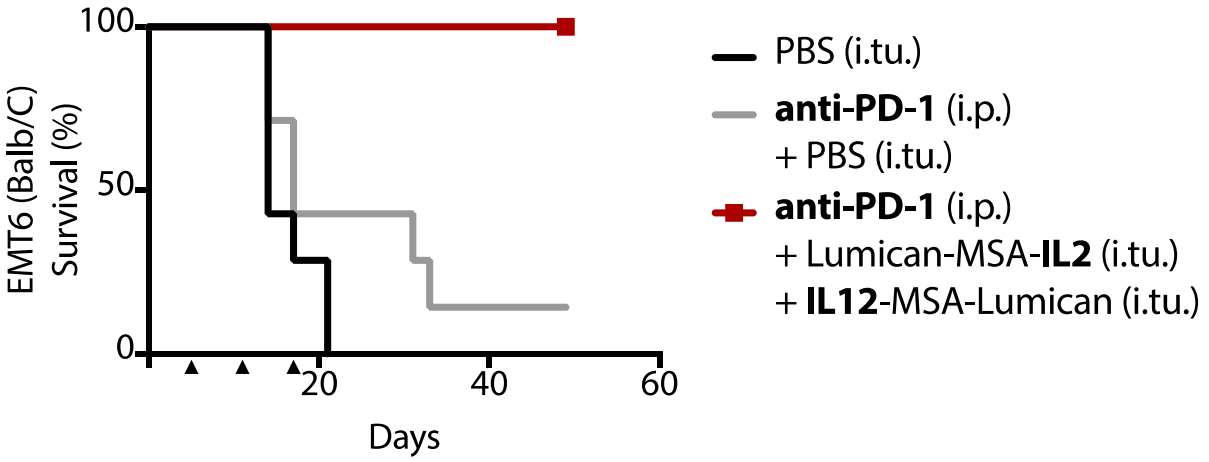
**anti-PD-1**



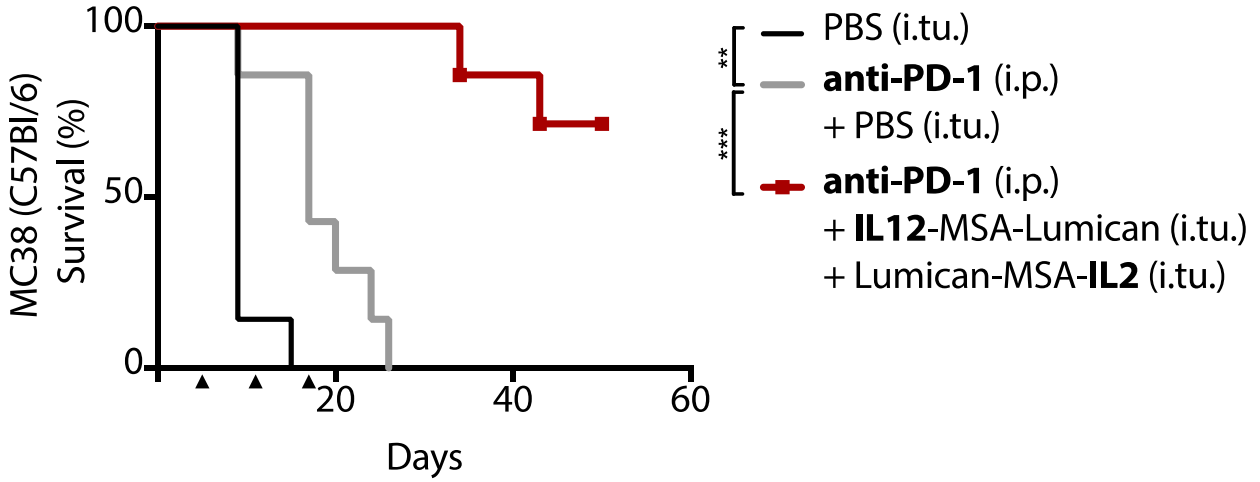
**lumican fusion**
**IL-2 + IL-12**

**tumor**

## EMT-6

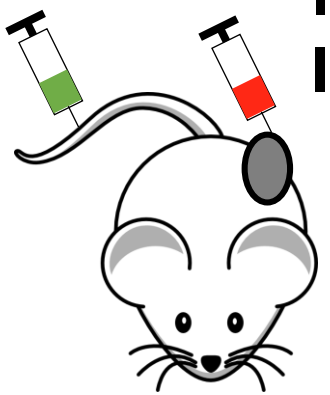



## MC38



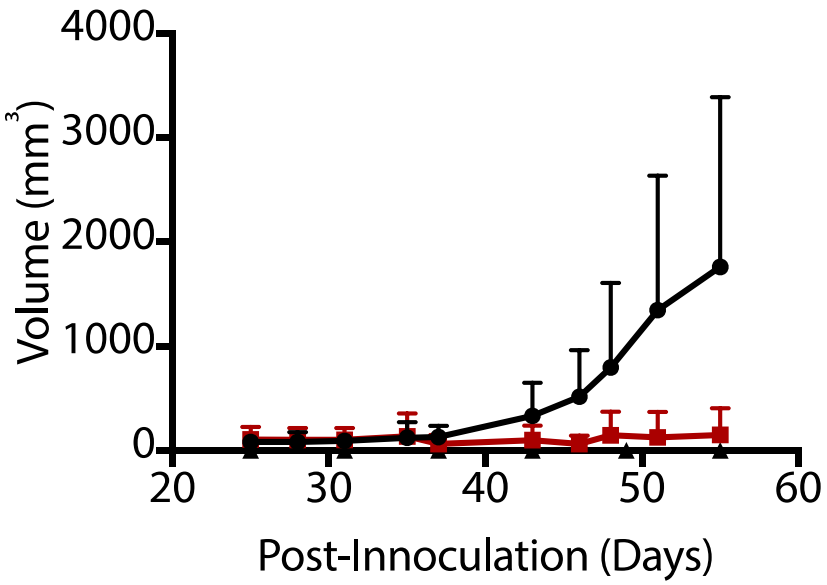
# Collagen-anchored cytokines potentiate therapy of a GEMM

**TA99**
**anti-PD-1**



**lumican fusion**
**IL-2 + IL-12**

**Braf/Pten GEMM**

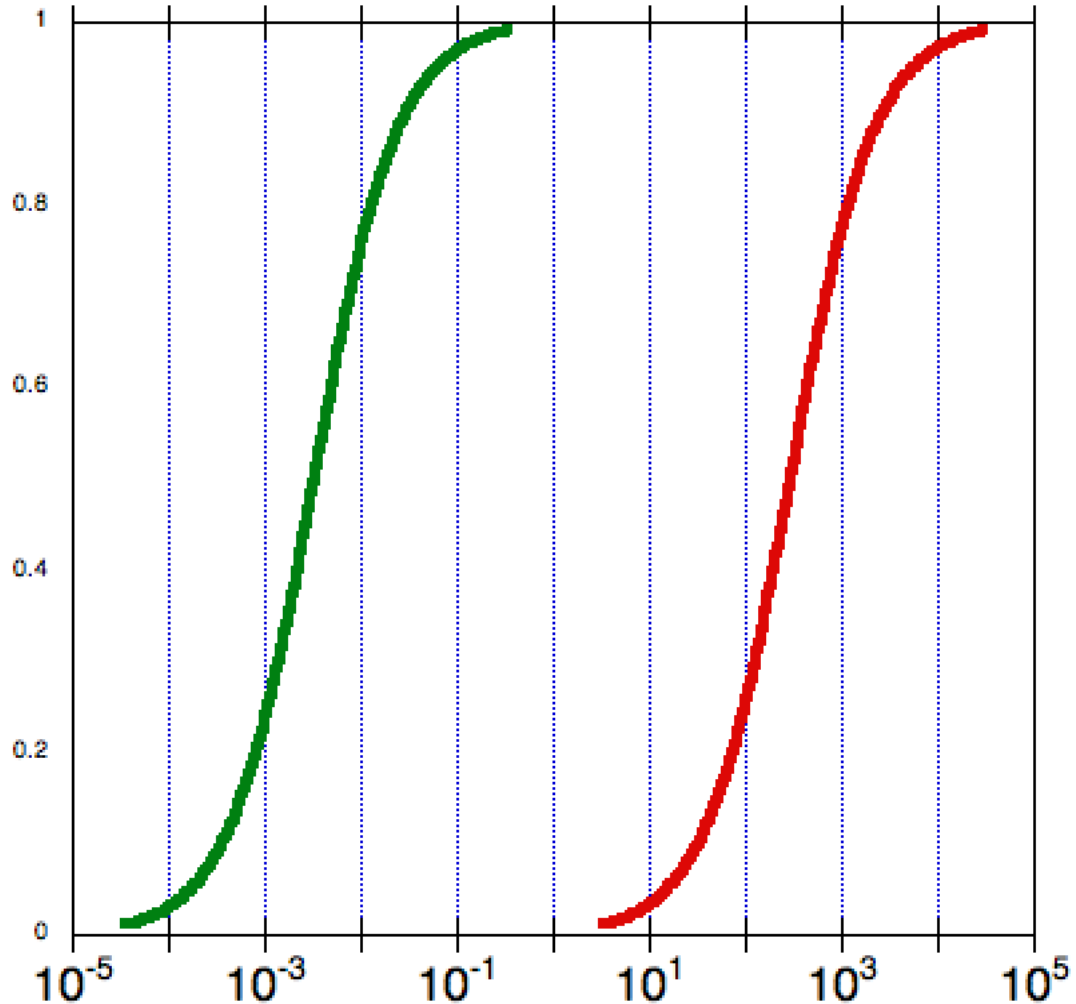



PBS (i.tu.)

Lumican-MSA-**IL2** (i.tu.)
+ **IL12**-MSA-Lumican +
**TA99** (i.p.) + **a-PD-1** (i.p.)

▲ Treatment

# Collagen-retained cytokines improve both components of therapeutic index



# Collagen-localized cytokines enhance systemic immunotherapies

- Anti-TAA mAb
- CAR-T
- Anti-PD-1 mAb
- Vaccines (not shown)

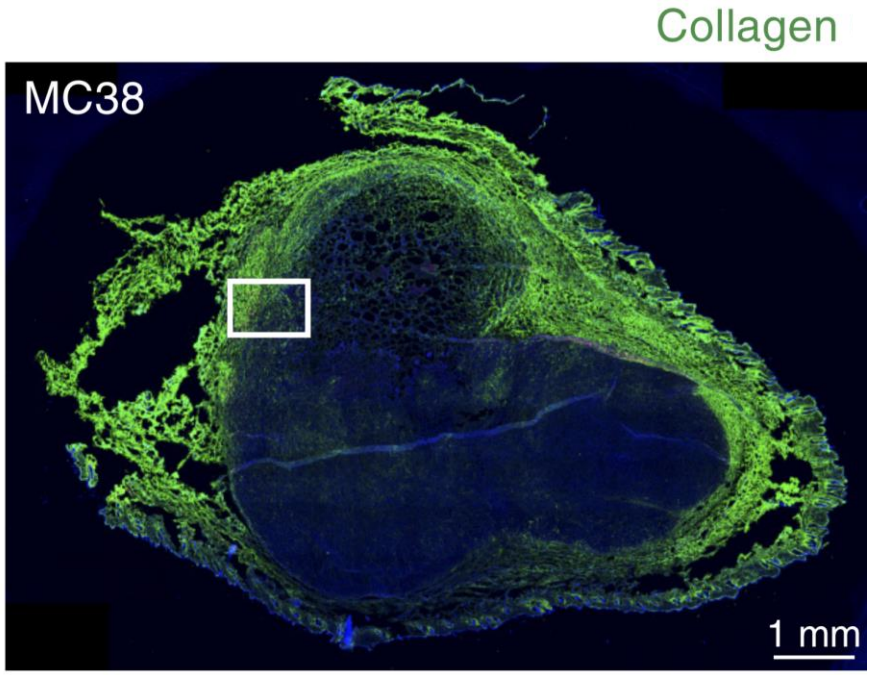
# Acknowledgments


- Naveen K. Mehta, Nitasha Bennett, Leyuan Ma, Emi A. Lutz, Magnolia M. Chinn, Joseph R. Palmeri, Byong Kang, Leyuan Ma, Nitasha Bennett, Lauren Milling
- Darrell J. Irvine, Stefani Spranger
- CA174795

# Background slides

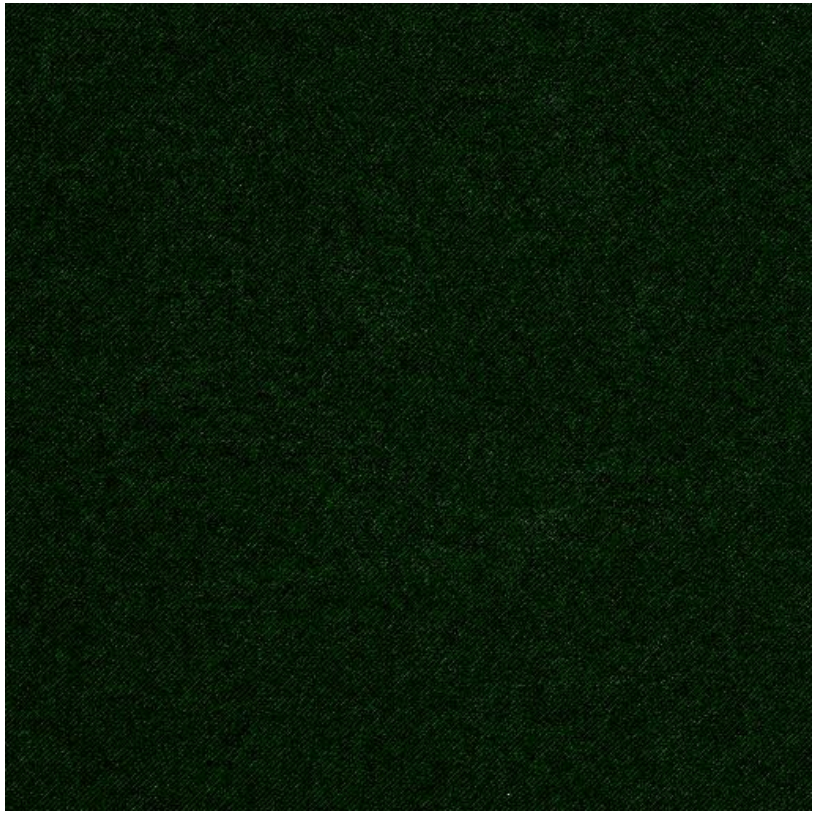
# Intratumoral collagen is an abundant anchor

~30% of all protein in the body &
major component of tumor ECM
*(Naba, Mol & Cell Prot, ’12;  JBC 277:4223, ‘02)*




*Mariathasan, Nature ‘18*

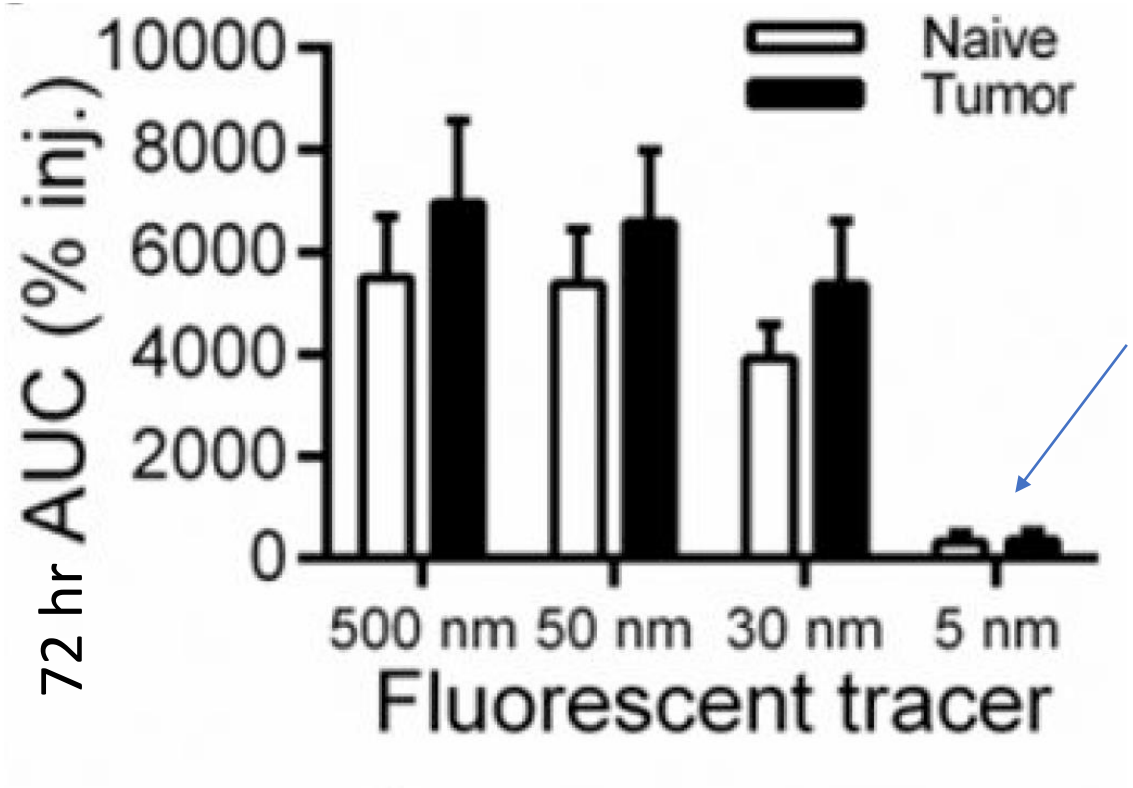
SHG image of MC38 tumor



5µm
z steps

512 µm

# Negligible intratumoral exposure of protein-sized tracers

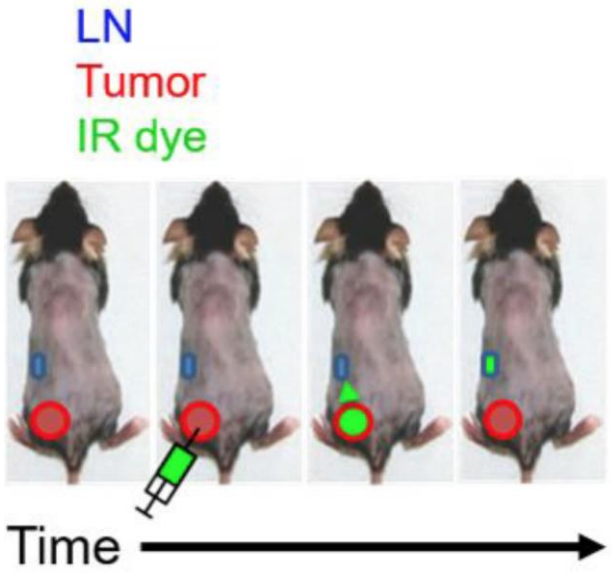



10 kDa Dextran
~ size of IL-2

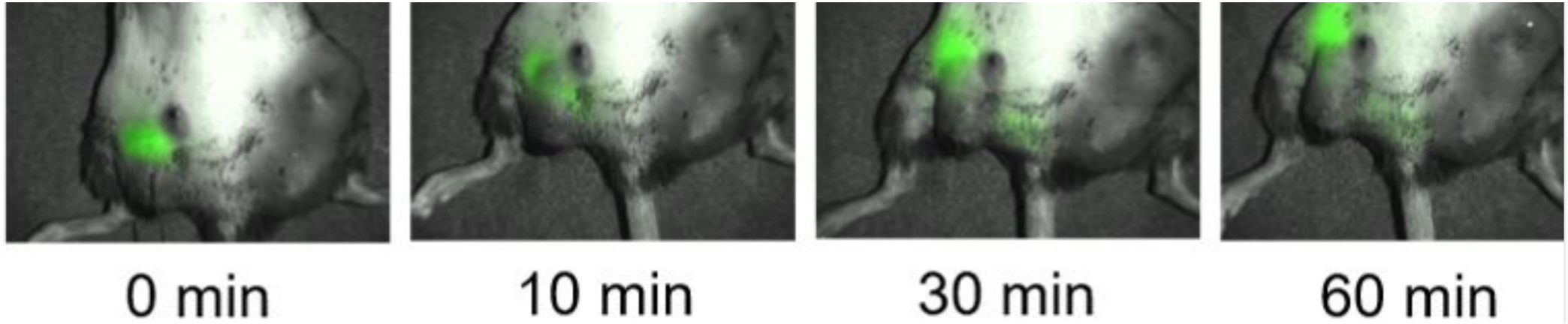
B16F10 tumors

*Rohner & Thomas, JCR 2016*

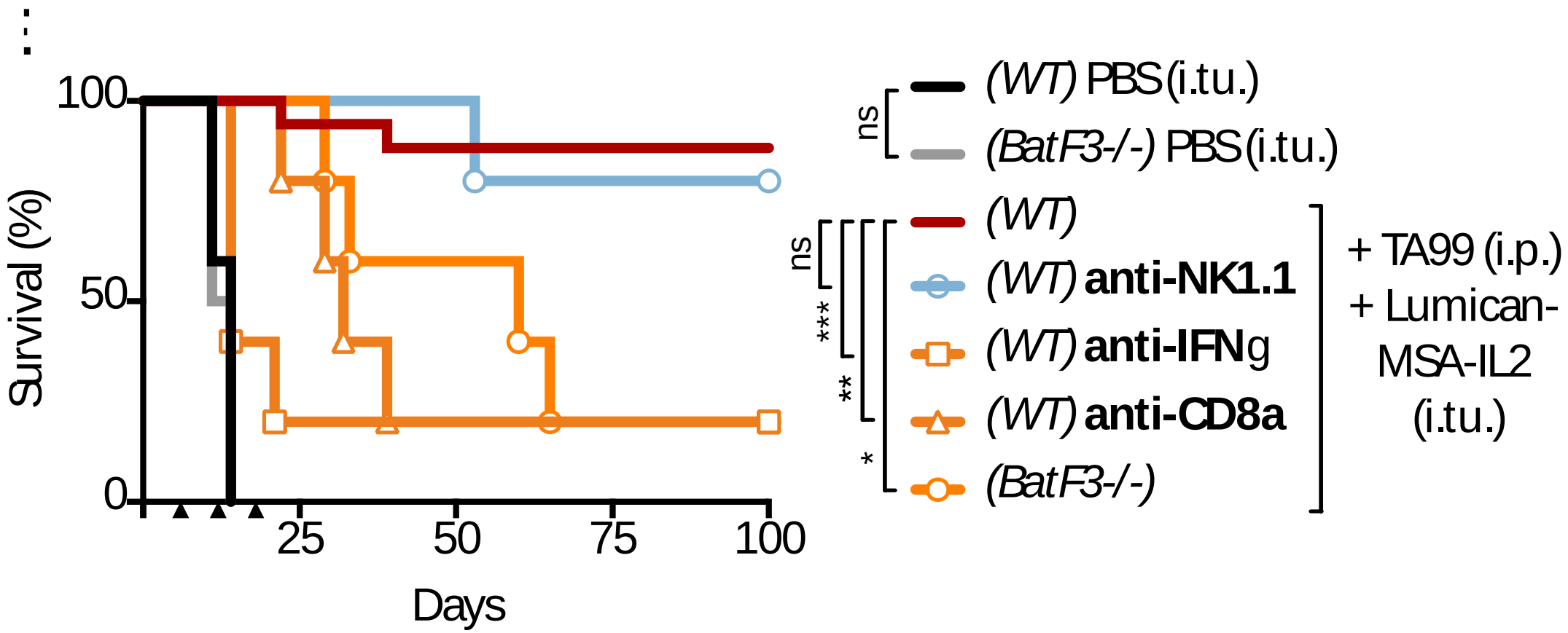
# Rapid drainage of intratumoral injections



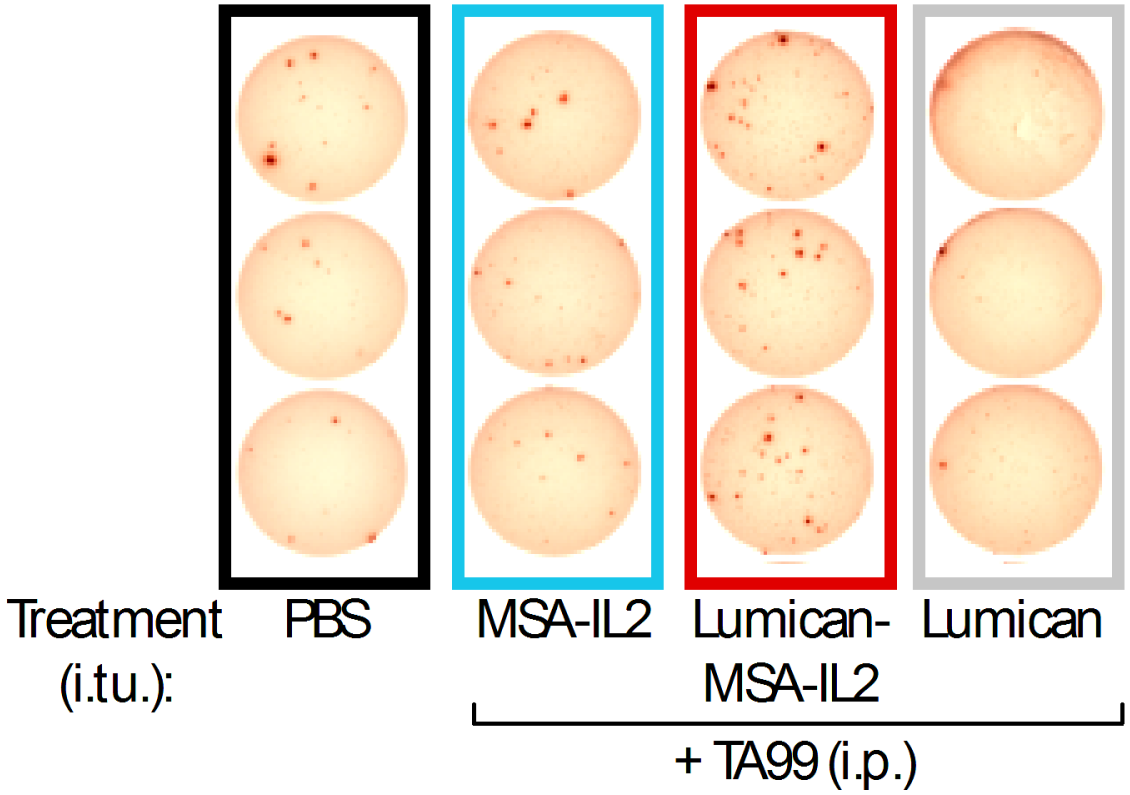

IRDye® 800CW PEG contrast agent, 25-50 kDa
day 11 C57BL/6J MC38
*Marciscano et al., Clin Canc Res. 2018*

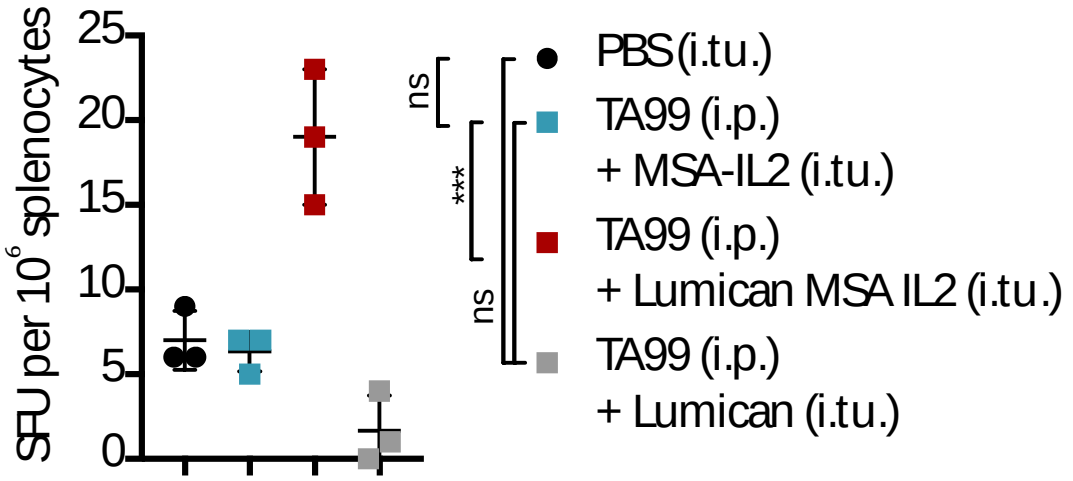


# Lumican-IL-2 + α-TAA mAb requires CD8+ DC's and T cells, IFNγ, but not NK cells



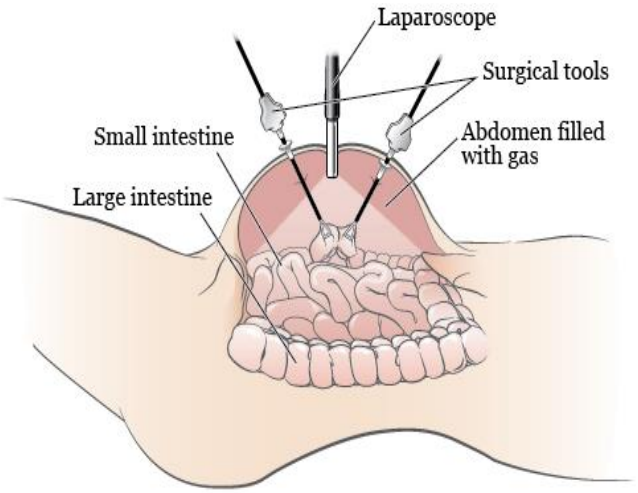
# Systemic anti-tumor CD8+ response is primed by Lumican-IL-2 + α-TAA mAb therapy



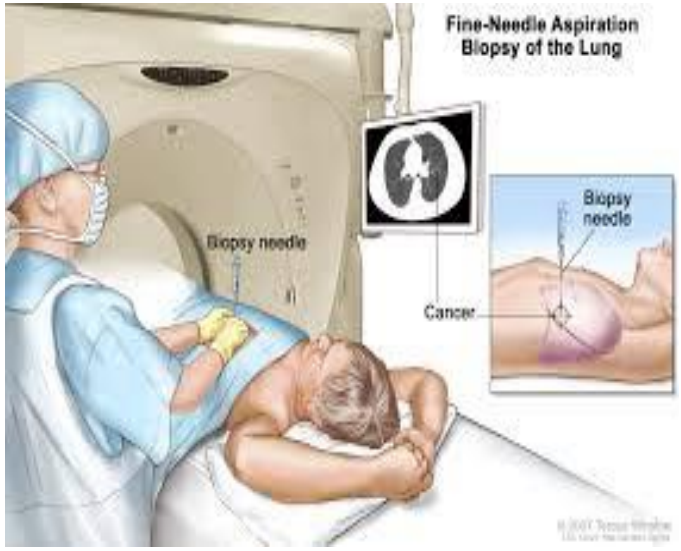
# Minimally invasive surgery is commonplace

**Diagnostic laparoscopy**



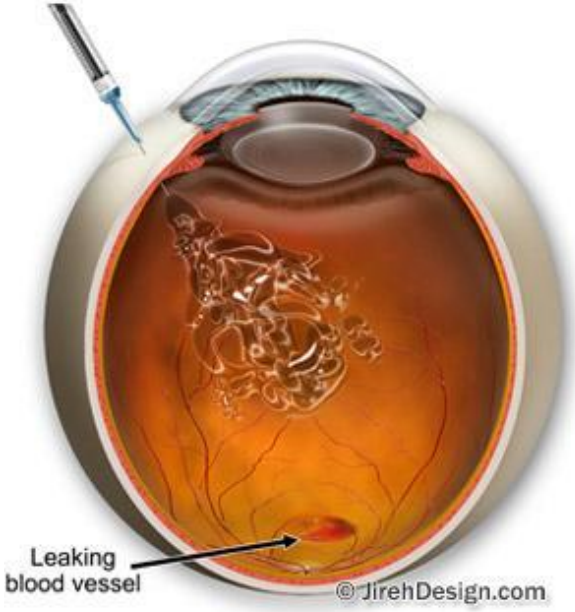

*https://www.mskcc.org/cancer-care/patient-education/laparoscopy*

**Fine needle aspirate biopsy**




*https://siteman.wustl.edu/glossary/cdr0000560745/*

**Intraocular injection**




*https://maculacenter.com/eye-procedures/eylea-treatment/*

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