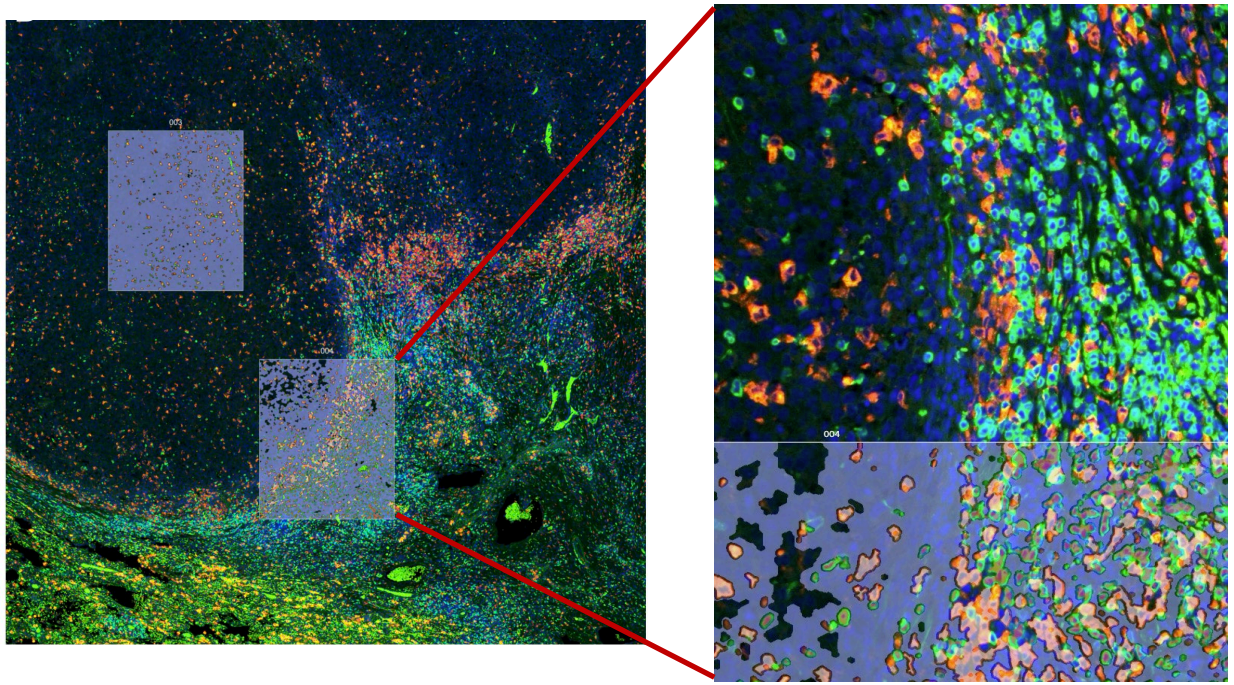
Canine to Human: Translating Novel Immunotherapy Treatments for Osteosarcoma



Dan Regan DVM, PhD
Associate Professor
Flint Animal Cancer Center
Dept. Microbiology, Immunology, and Pathology
Colorado State University

CCTSI CU-CSU Summit
August 13, 2025

COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

FLINT ANIMAL CANCER CENTER
COLORADO STATE UNIVERSITY

Tumor-host interactions govern metastatic site selection

Figure 1 | **Stephen Paget.**

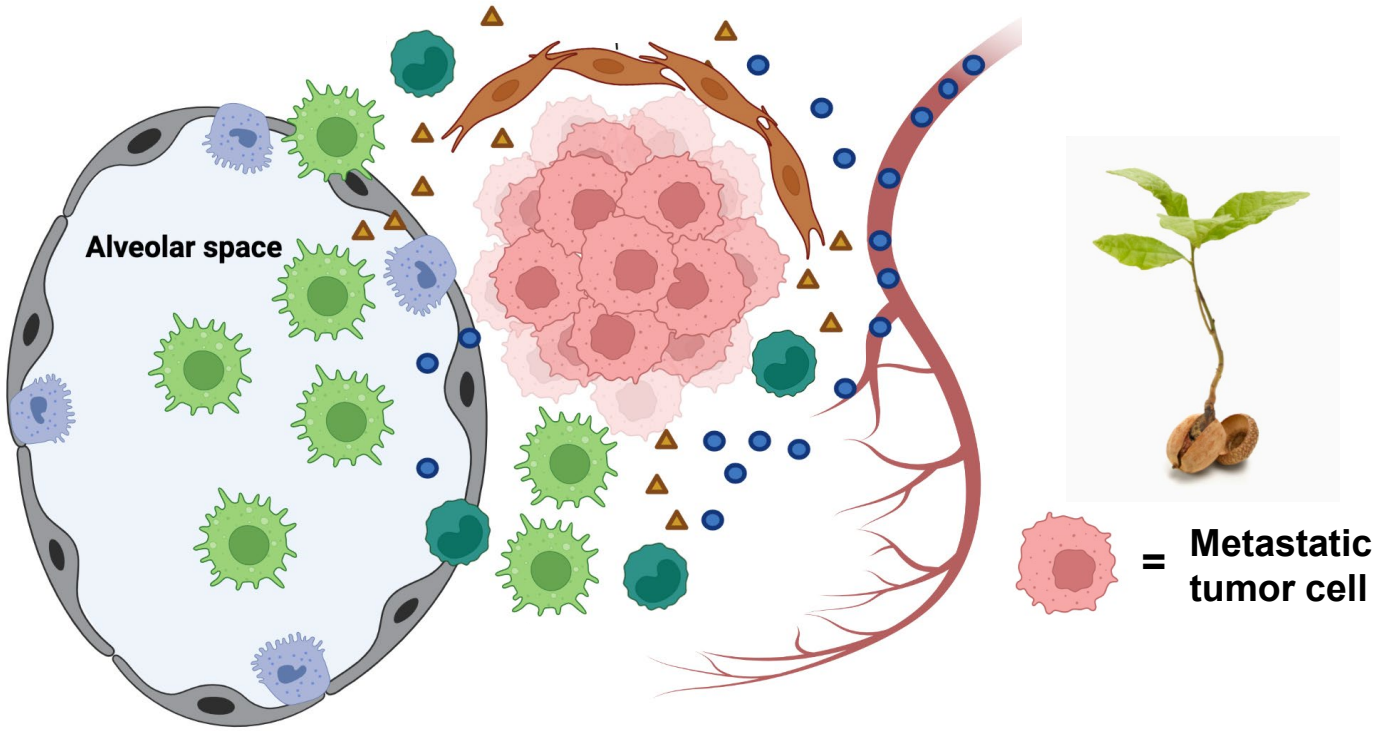
THE

DISTRIBUTION OF SECONDARY GROWTHS IN CANCER OF THE BREAST.

By STEPHEN PAGET, F.R.C.S.,

ASSISTANT SURGEON TO THE WEST LONDON HOSPITAL AND THE METROPOLITAN HOSPITAL.

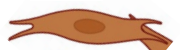
An attempt is made in this paper to consider "metastasis" in malignant disease, and to show that the distribution of the secondary growths is not a matter of chance. It



= **Metastatic tumor cell**

Cytokines & chemokines

Endothelial cells



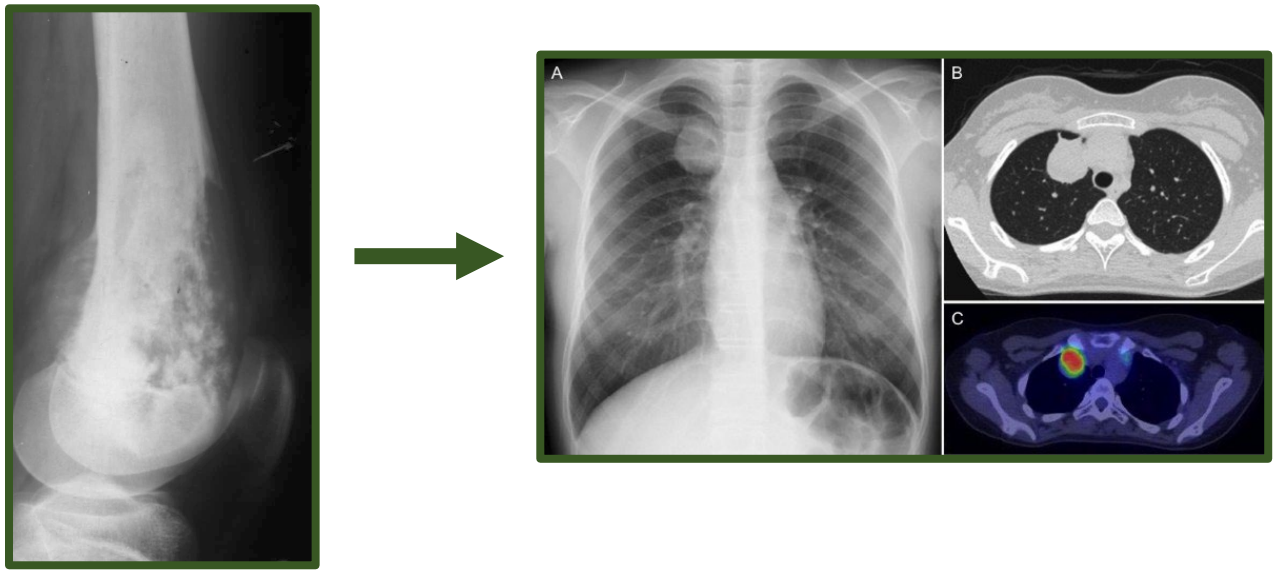
fibroblasts

macrophages

monocytes

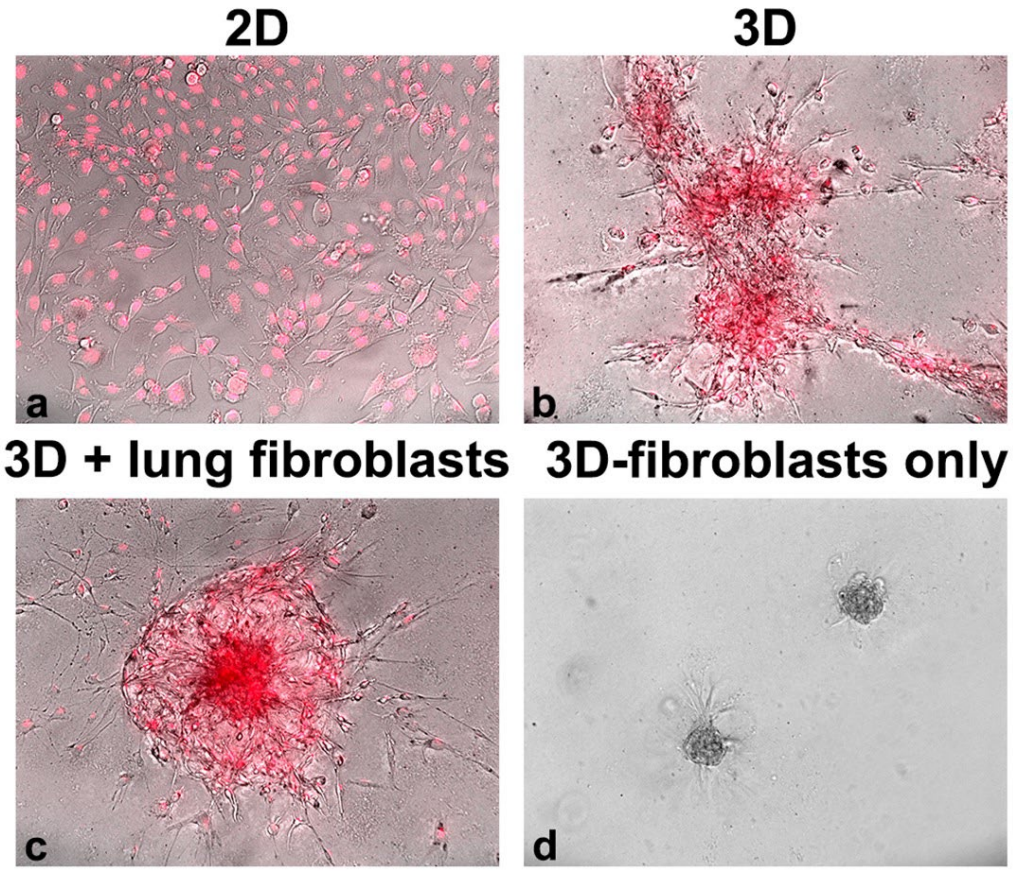
Osteosarcoma (OS), the most common primary malignant bone tumor, exhibits an almost exclusive tropism for lung metastasis

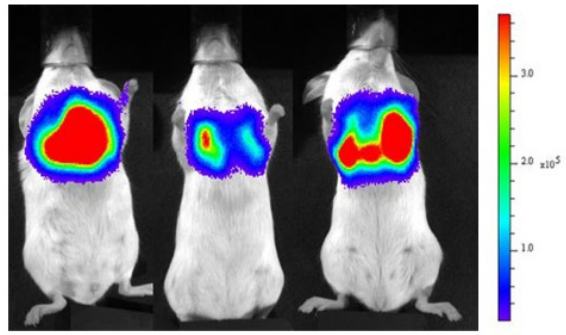
- 30-40% of patients experience relapse
  - Median time to relapse of 1.6 years
  - **Pulmonary metastasis** accounts for 75-90% of relapses
  - 5 yr overall survival (OS) of 23%
  - 4-month progression free survival (PFS) of 12%
- Difficulty in identifying effective targeted therapy, due to:
  - Significant heterogeneity of the disease
  - Absence of pathognomonic, “druggable” mutations

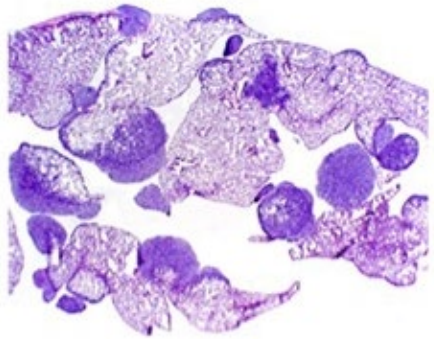


Kempf-Bielack B et al, JCO, 2005; Hawkins DS & Arndt CA, Cancer, 2003; Bacci G et al, Cancer, 2006

Approaches for modeling the OS tumor microenvironment (TME)

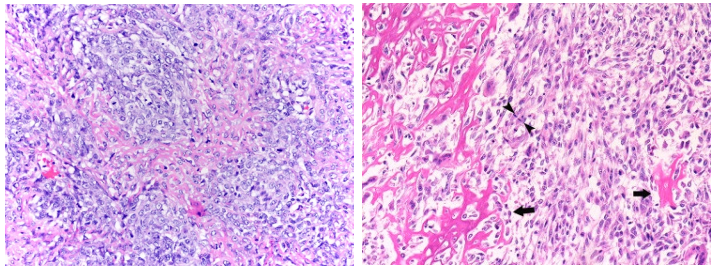






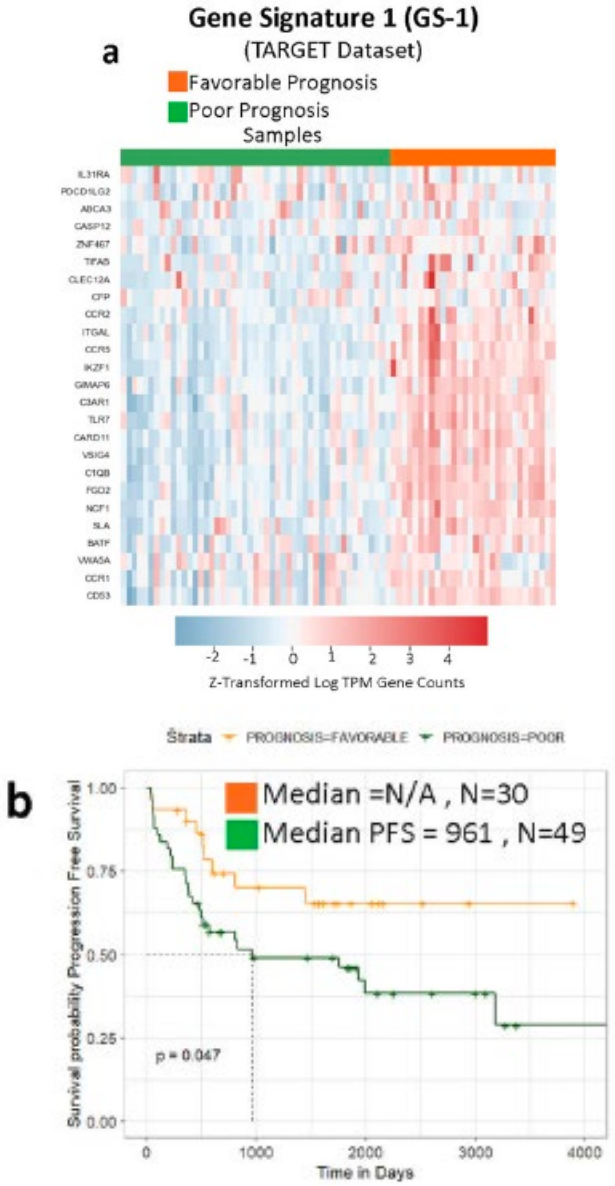
Dogs with spontaneous osteosarcoma are a valuable surrogate for the human disease

- Species similarities in:
  1) Primary tumor location
  2) Histology
  3) Response to therapy
  4) Genomics
  5) High tropism for lung metastasis



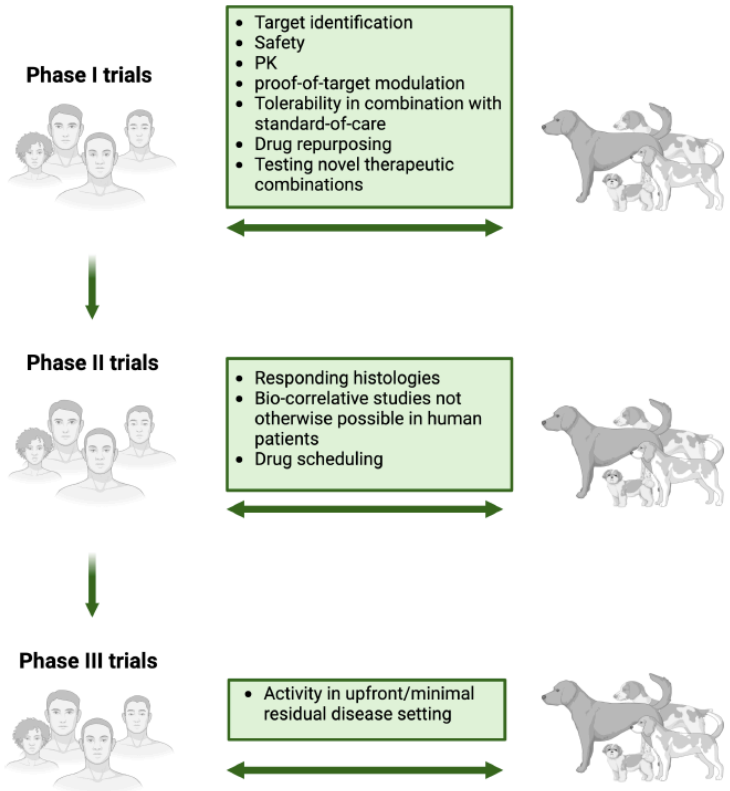
Canine Human

KM Rogers. 2019



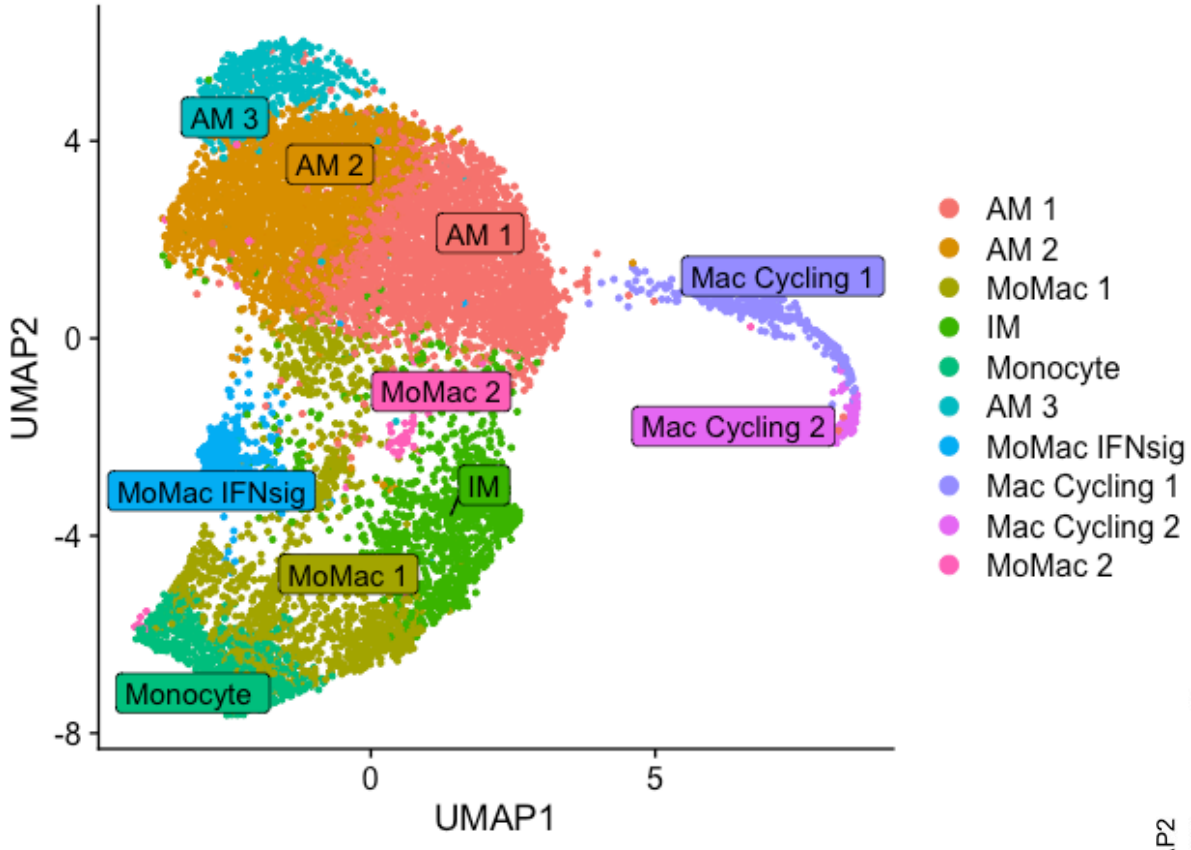
Mannheimer et al. 2023

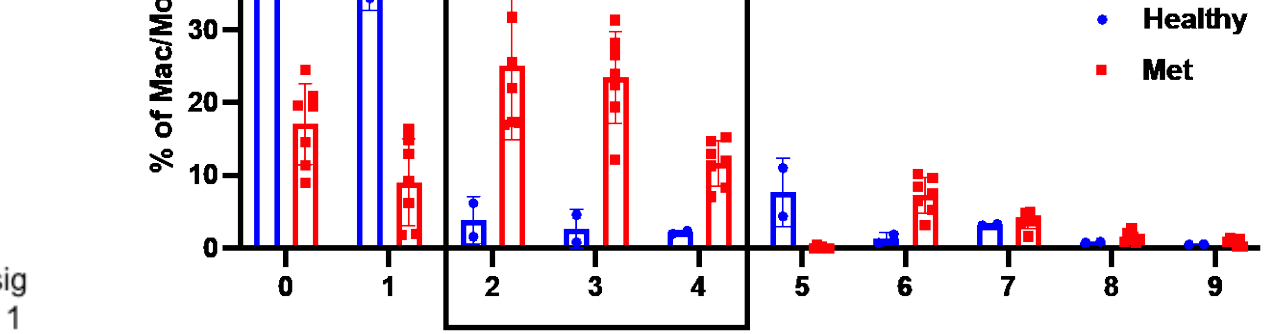
- Advantages of canine model
  - ➢ Accelerated pace of clinical trials
  - ➢ Greater feasibility for ”high-risk” studies

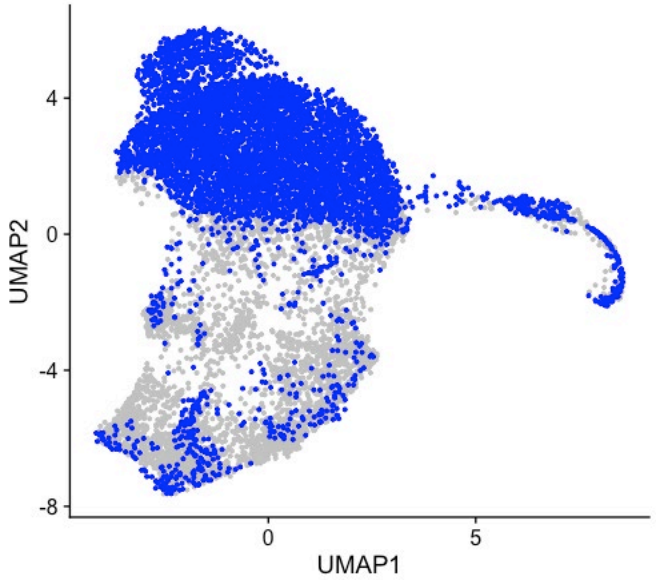


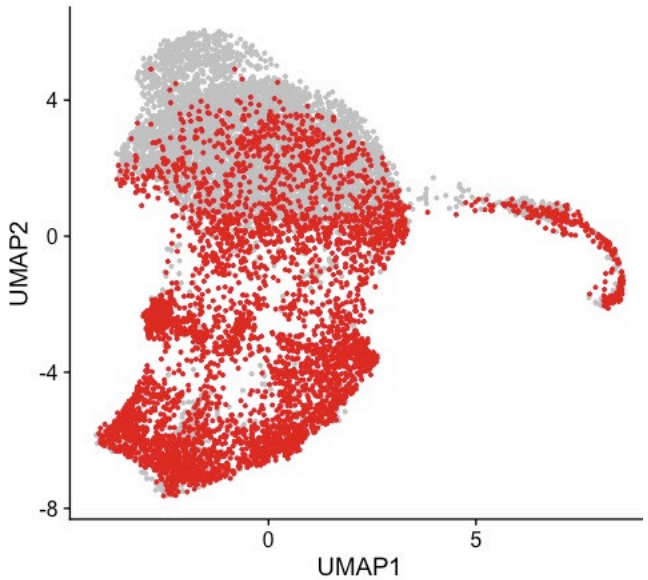
Klosowski and Regan. 2023

Canine osteosarcoma lung metastases overproduce CCL2 and are enriched for myeloid cells

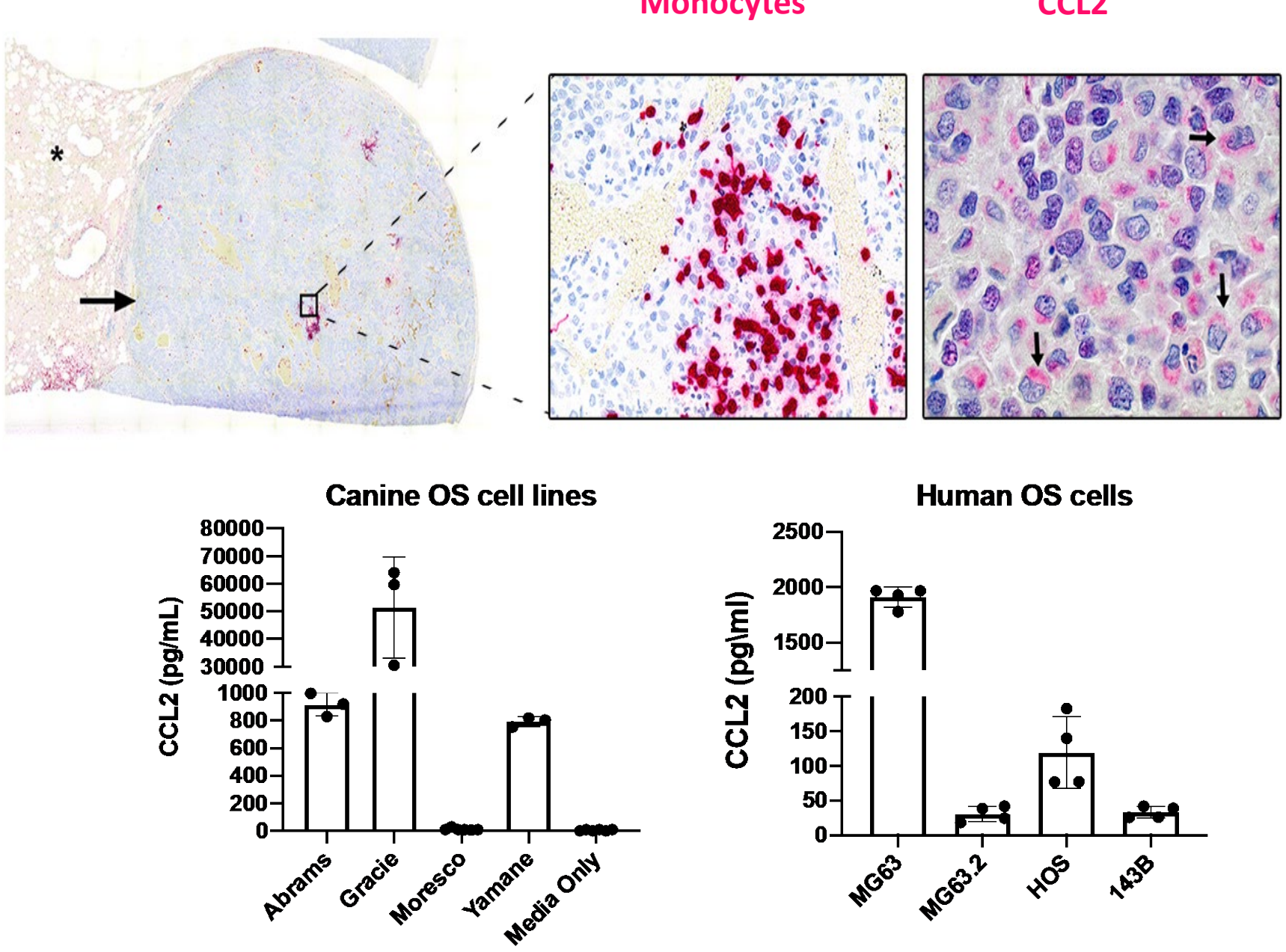






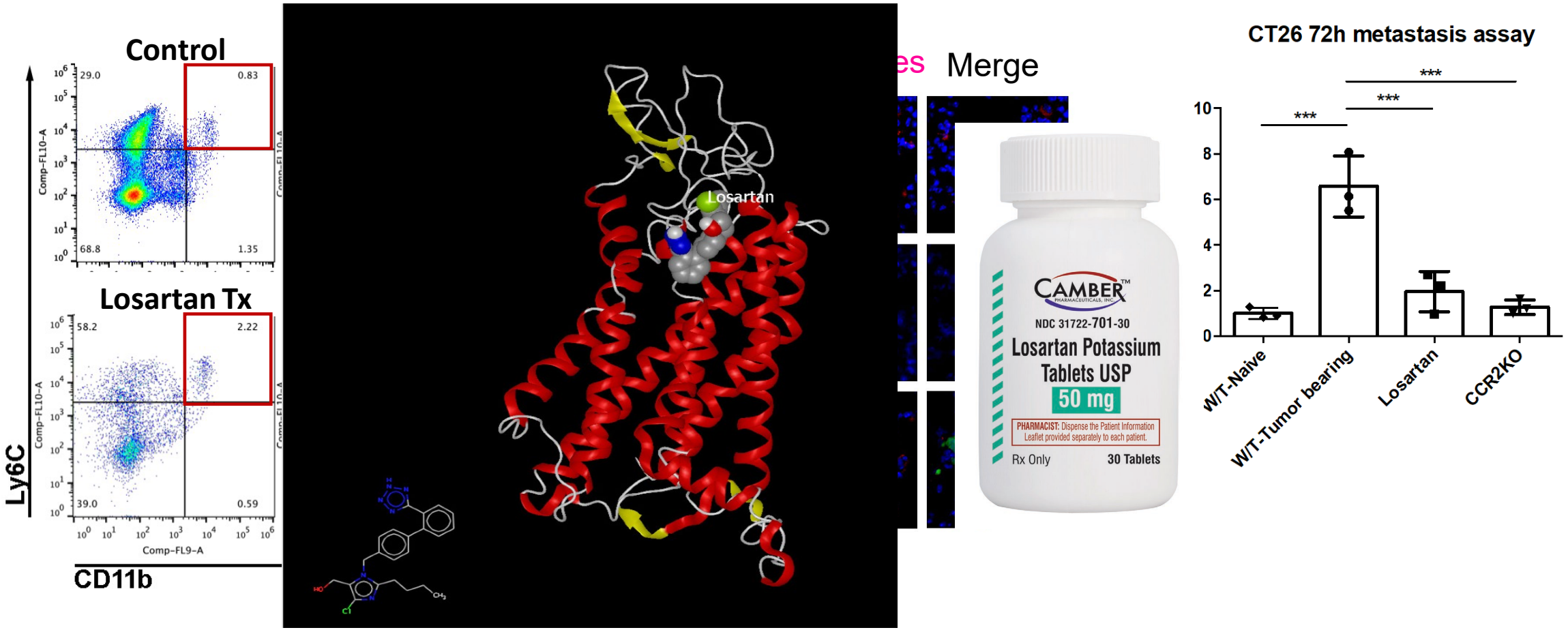


Katie Cronise, unpublished



Regan et al. *Clin Cancer Res, 2021*

The angiotensin receptor blocker losartan suppresses monocyte recruitment to lung metastases in mice



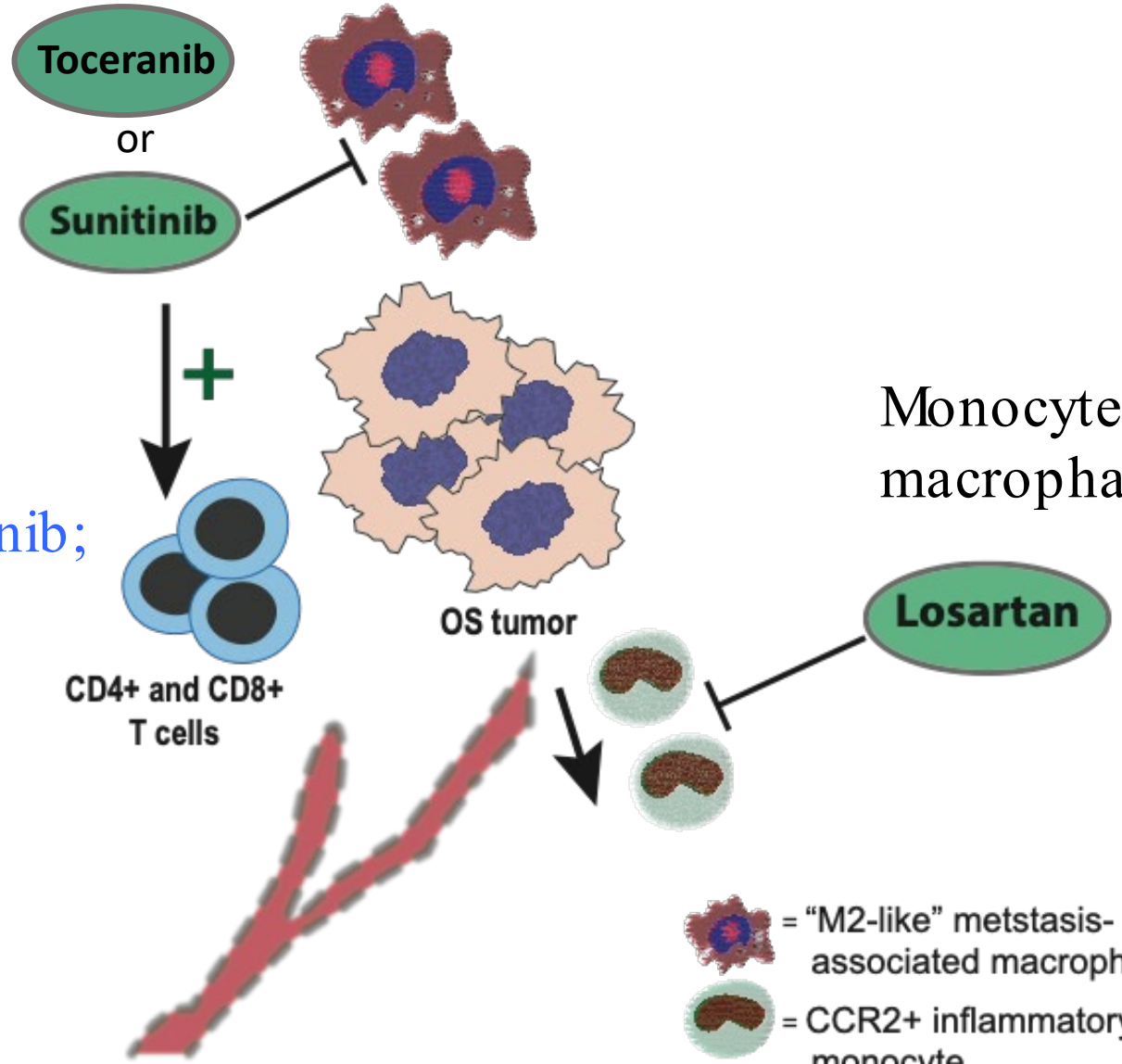
Regan et al. *J Immunol*, 2019

Complementary strategies for targeting immune suppressive myeloid cells to treat canine metastatic osteosarcoma

Myeloid-derived suppressor cells

Target: STAT3
Agents: Toceranib/sunitinib;

FLINT ANIMAL CANCER CENTER
COLORADO STATE UNIVERSITY



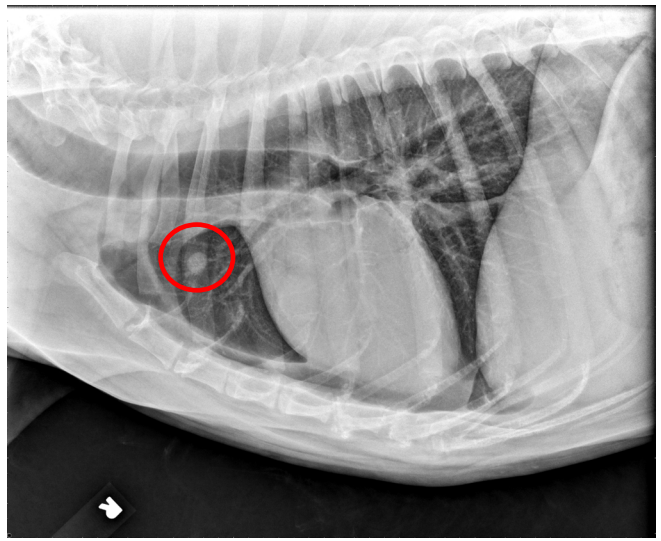
Monocytes/Tumor-associated macrophages

Target: CCR2
Agent: Losartan

High-dose losartan combined with toceranib exerts significant clinical benefit in dogs with metastatic osteosarcoma

Best Response	Toceranib (n=22)	Losartan 1mg/kg + toceranib (n=8)	Losartan 10mg/kg + toceranib (n=20)
CR	0	0	0
PR	0	0	**4/16* (25%)**
SD (min 8 wks)	3/17 (17.6%)	3/8 (37.5%)	4/16 (25%)
PD	14/17 (82.4%)	5/8 (62.5%)	8/16 (50%)
ORR (PR, CR)	0	0	25%
CB (SD, PR, CR)	17.6 %	37.5 %	**50 %**
PFS (days)	57	61	**110.5**
OS (days)	89	109	**148**

**16 /20 dogs evaluable; 3 removed due to owner request, 1 removed for AE*



Week 0

8 weeks post-treatment

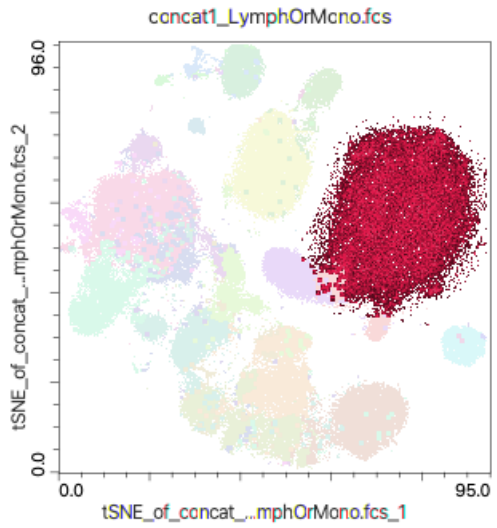
Regan et al. ***Clin Cancer Res, 2021***

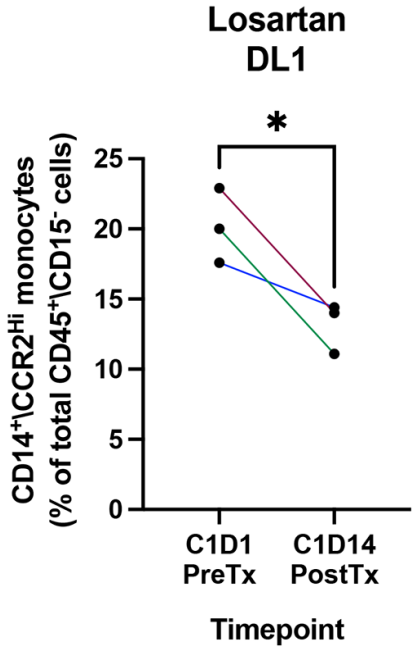
Phase I trial of losartan + sunitinib in Pediatric/Young Adult Patients with Metastatic Osteosarcoma

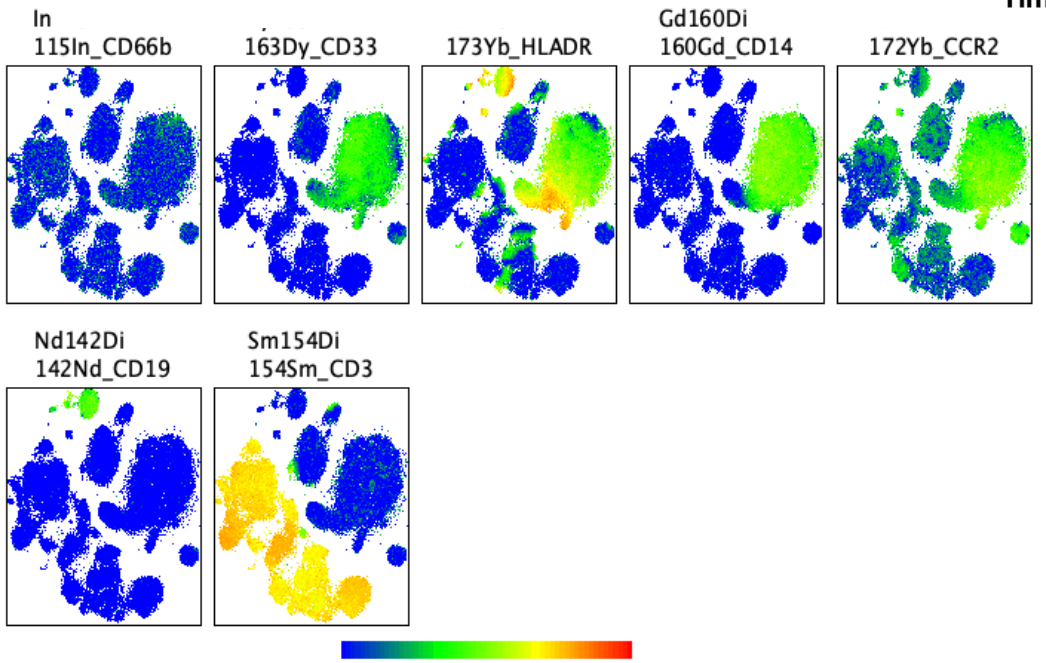
Dose Level	Losartan	Sunitinib
-1	0.4 mg/kg	BSA < 1.6= 8 mg [c] BSA ≥ 1.6= 16 mg [c]
*1	0.7 mg/kg [a]	BSA < 1.6= 12.5 mg BSA ≥ 1.6= 25 mg
2	1.4 mg/kg [b]	
3	2.8 mg/kg [b]	
4		BSA < 1.6= 25 mg BSA ≥ 1.6= 37.5 mg

*Starting Dose
[a] Max 50 mg
[b] Max 100 mg
[c] Must utilize liquid formulation

ClinicalTrials.gov Identifier: NCT03900793







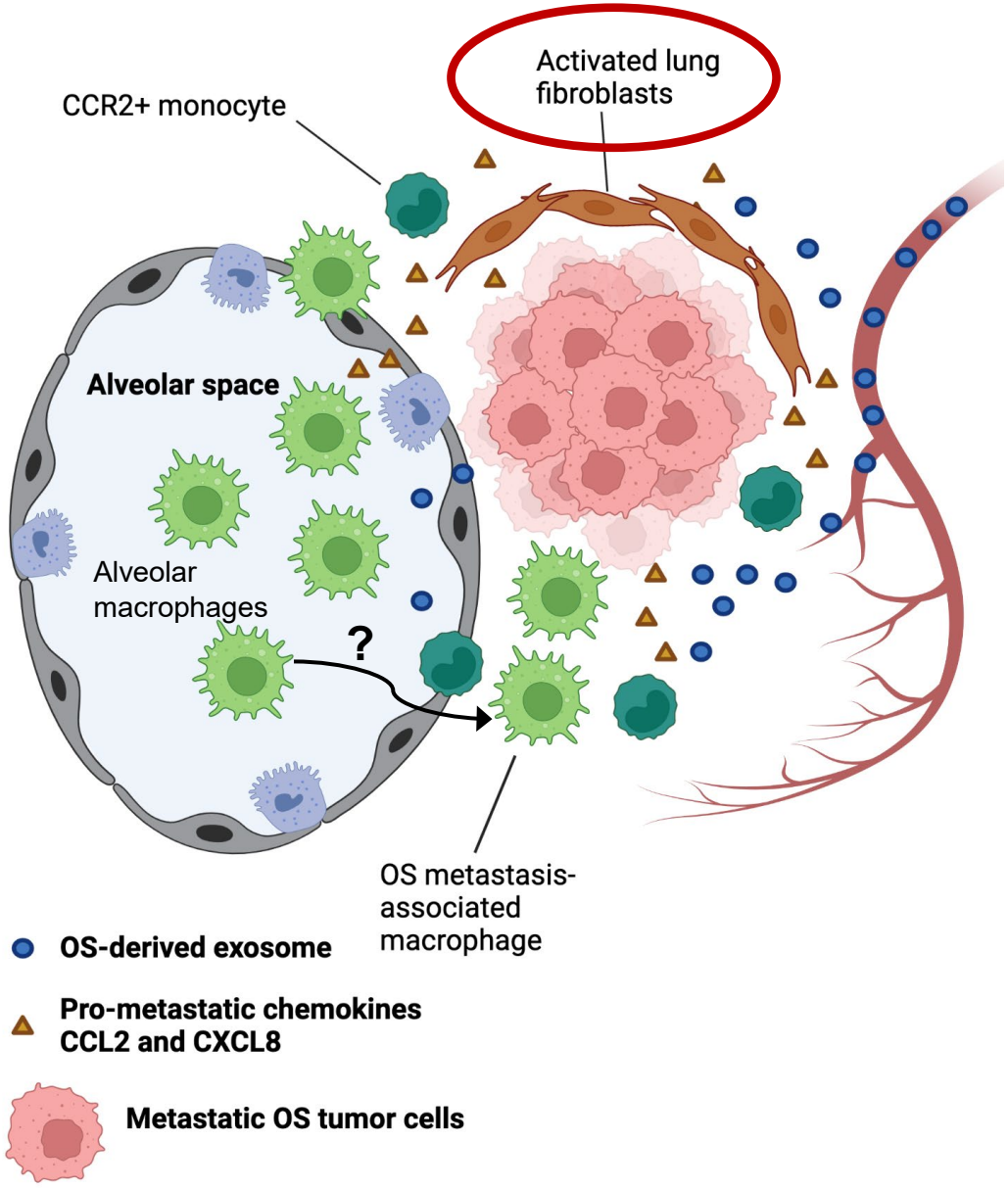
CSU | one cure FLINT ANIMAL CANCER CENTER

Children's Healthcare of Atlanta

Cancer Center
NCI-DESIGNATED COMPREHENSIVE CANCER CENTER

Children's Hospital Colorado

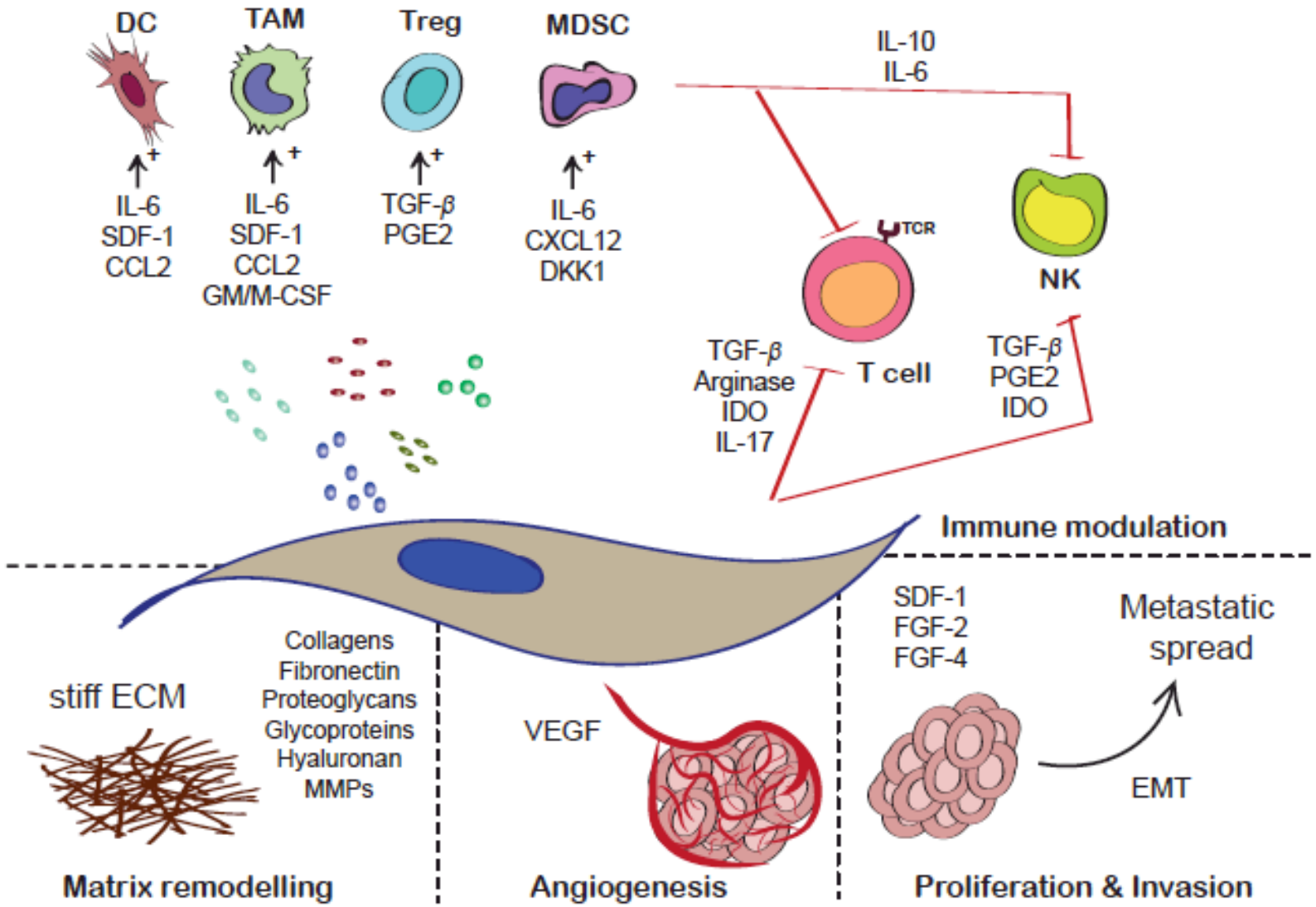
Fibroblasts as a linchpin of the metastatic OS TME and key driver of myeloid cell recruitment ?



Katie Cronise, PhD

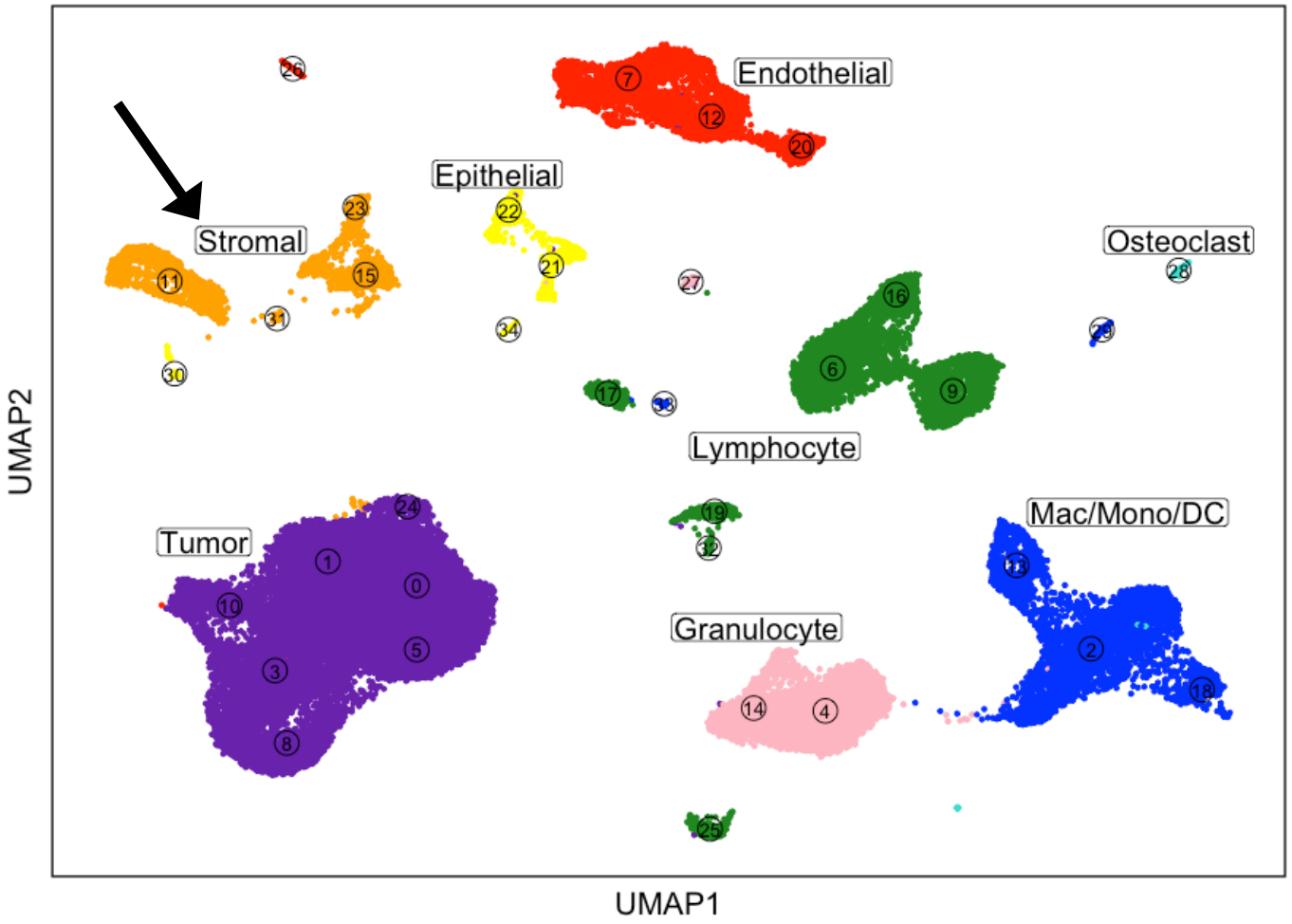
Eric Palmer, PhD

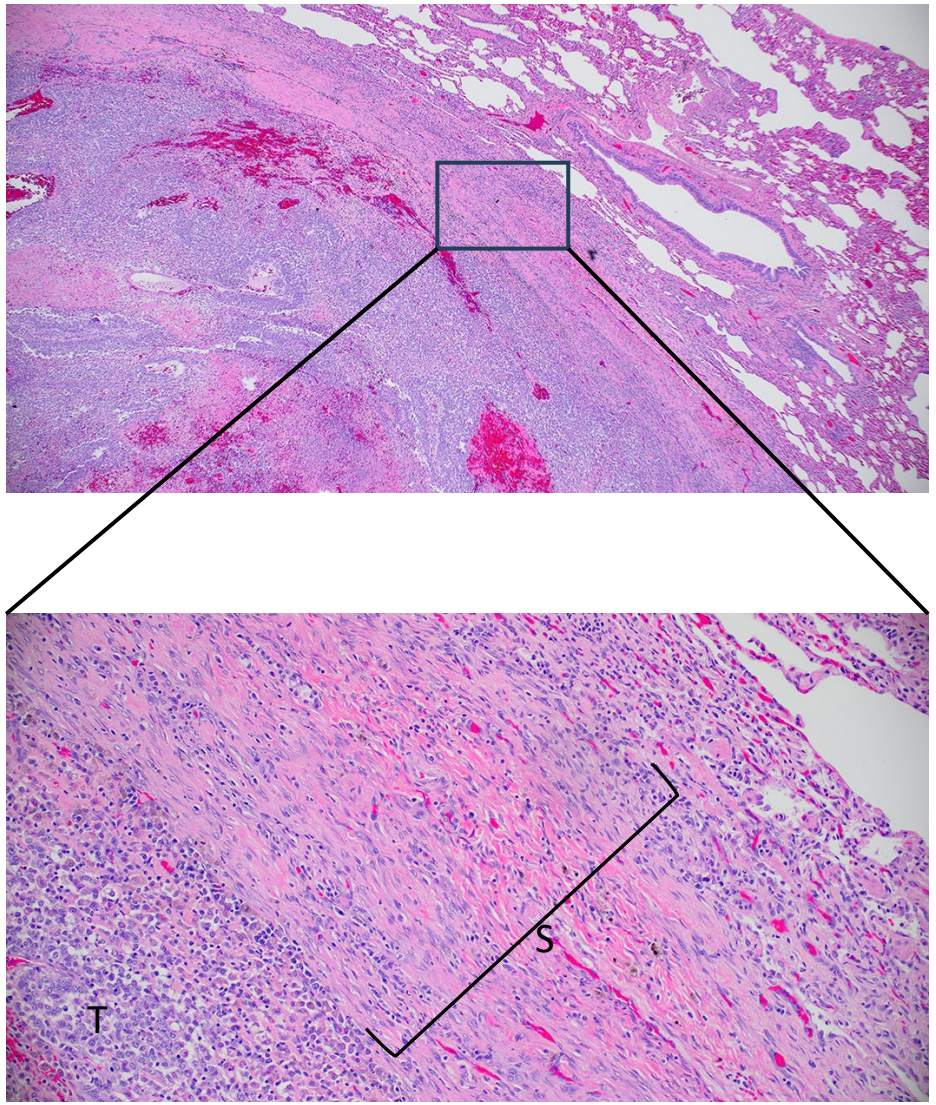
Functions of cancer-associated fibroblasts (CAFs) in the TME



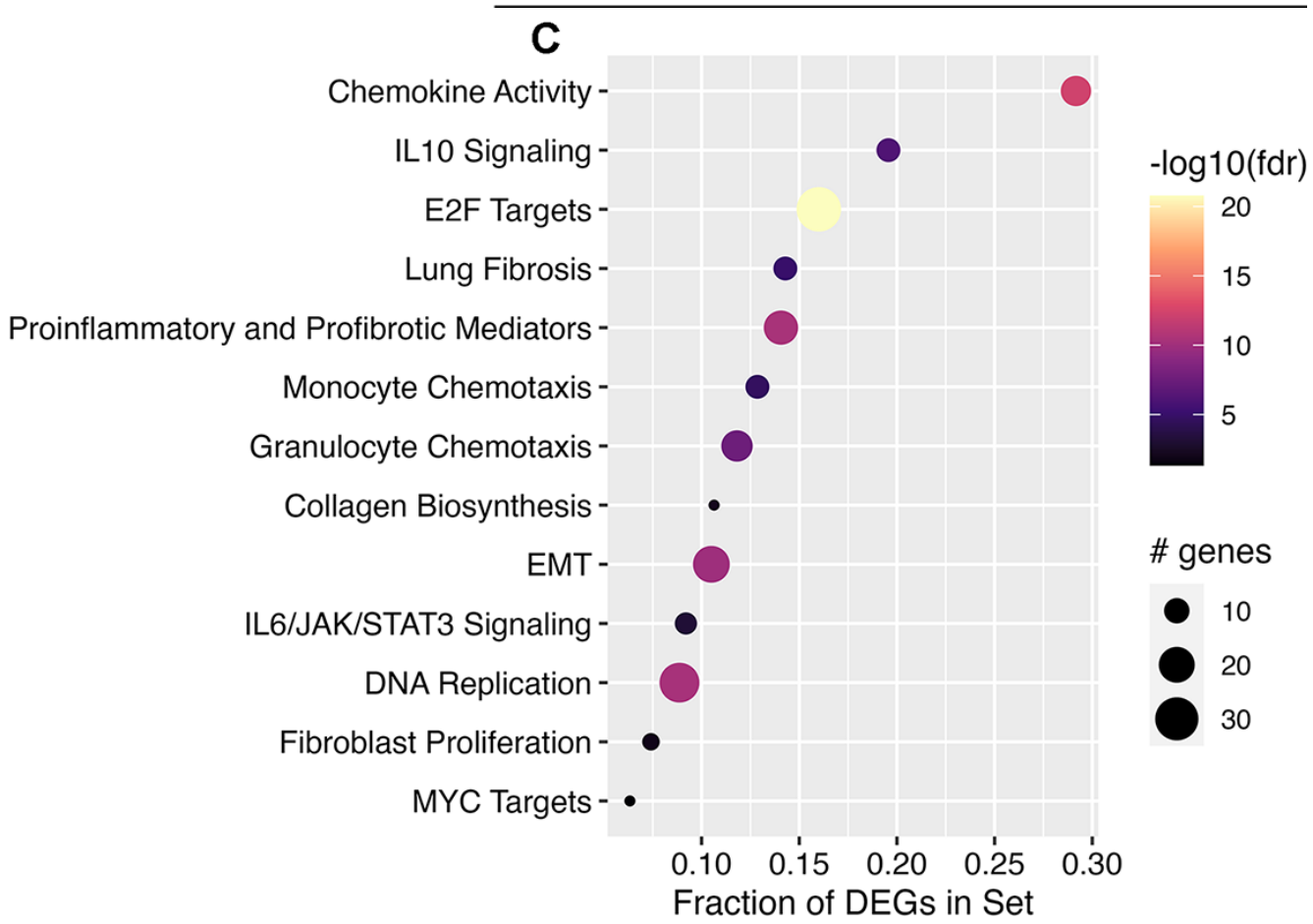
Caligiuri and Tuveson, *Cancer Cell*. 2023

Fibroblasts are a prominent cell type in the lung metastatic OS TME

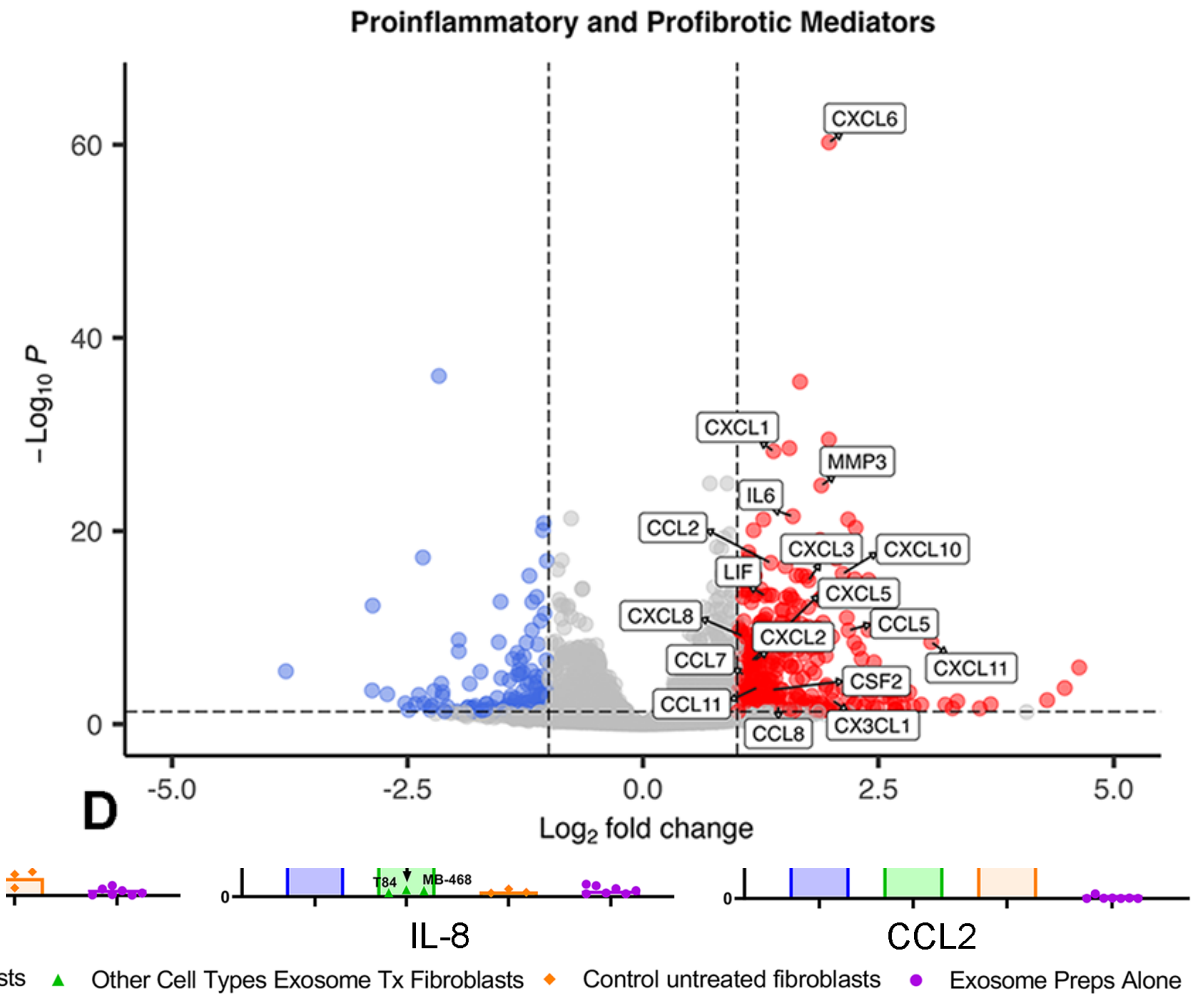




OS cells drive lung fibroblasts towards an inflammatory CAF (iCAF) phenotype in vitro

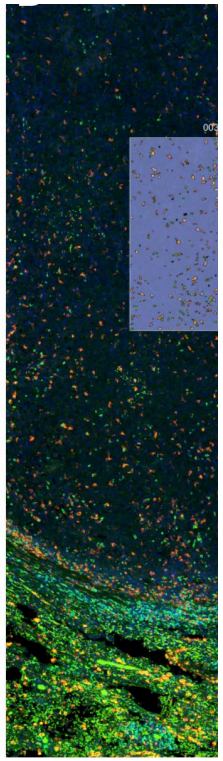


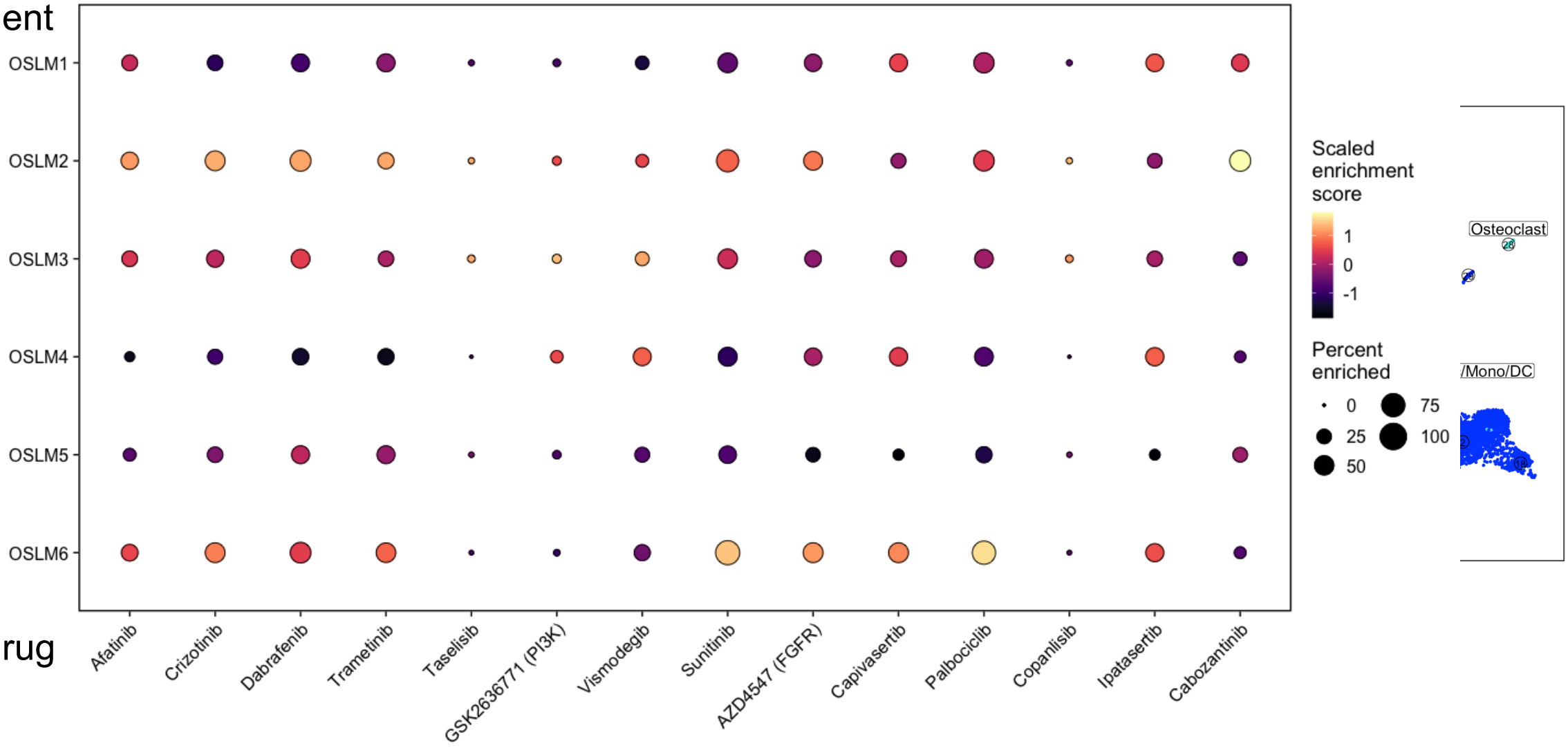
IL-8	33.51	6111	6077	0.001338

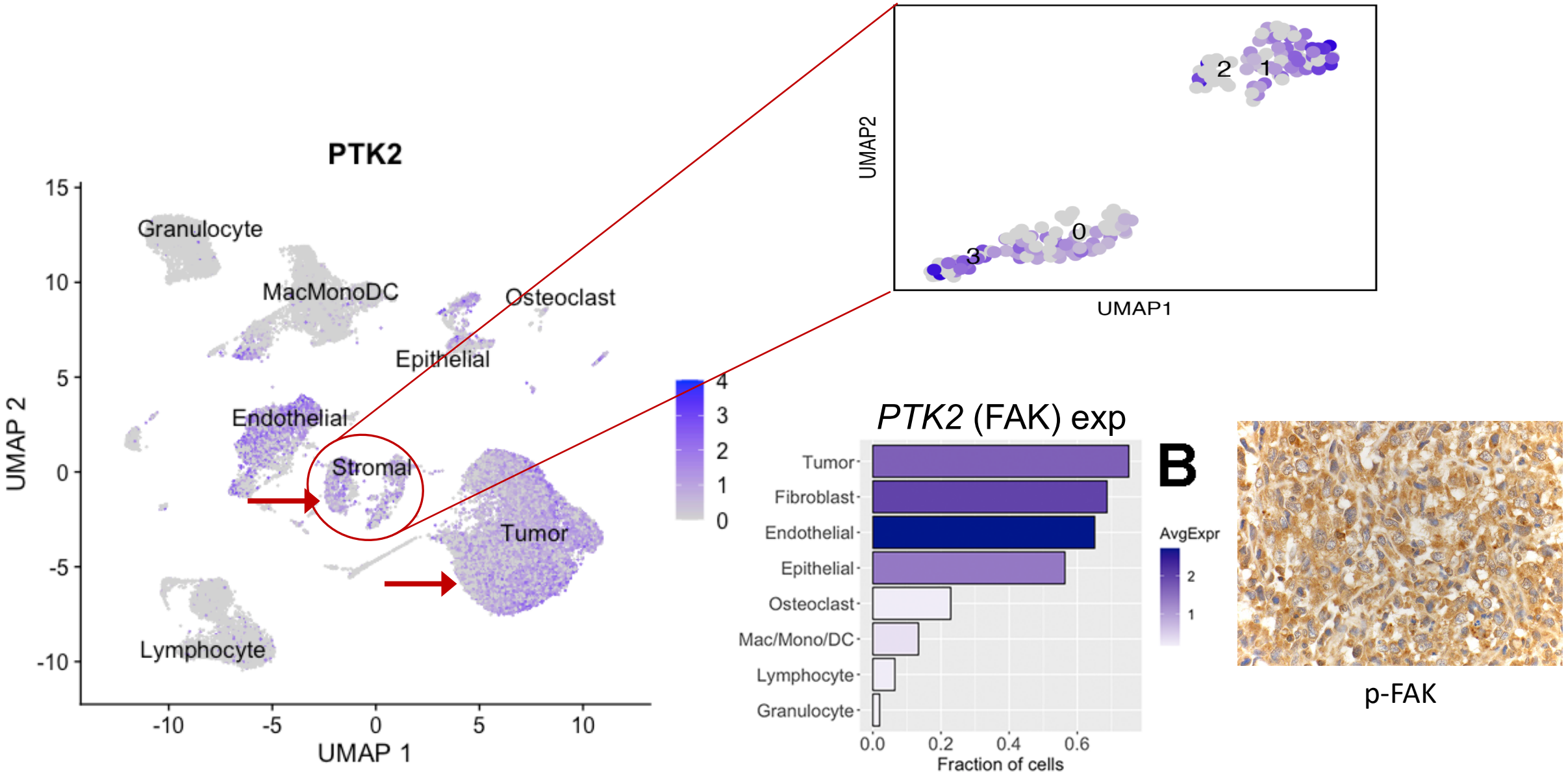


Palmer et al. *Cancer Res Commun*. 2025

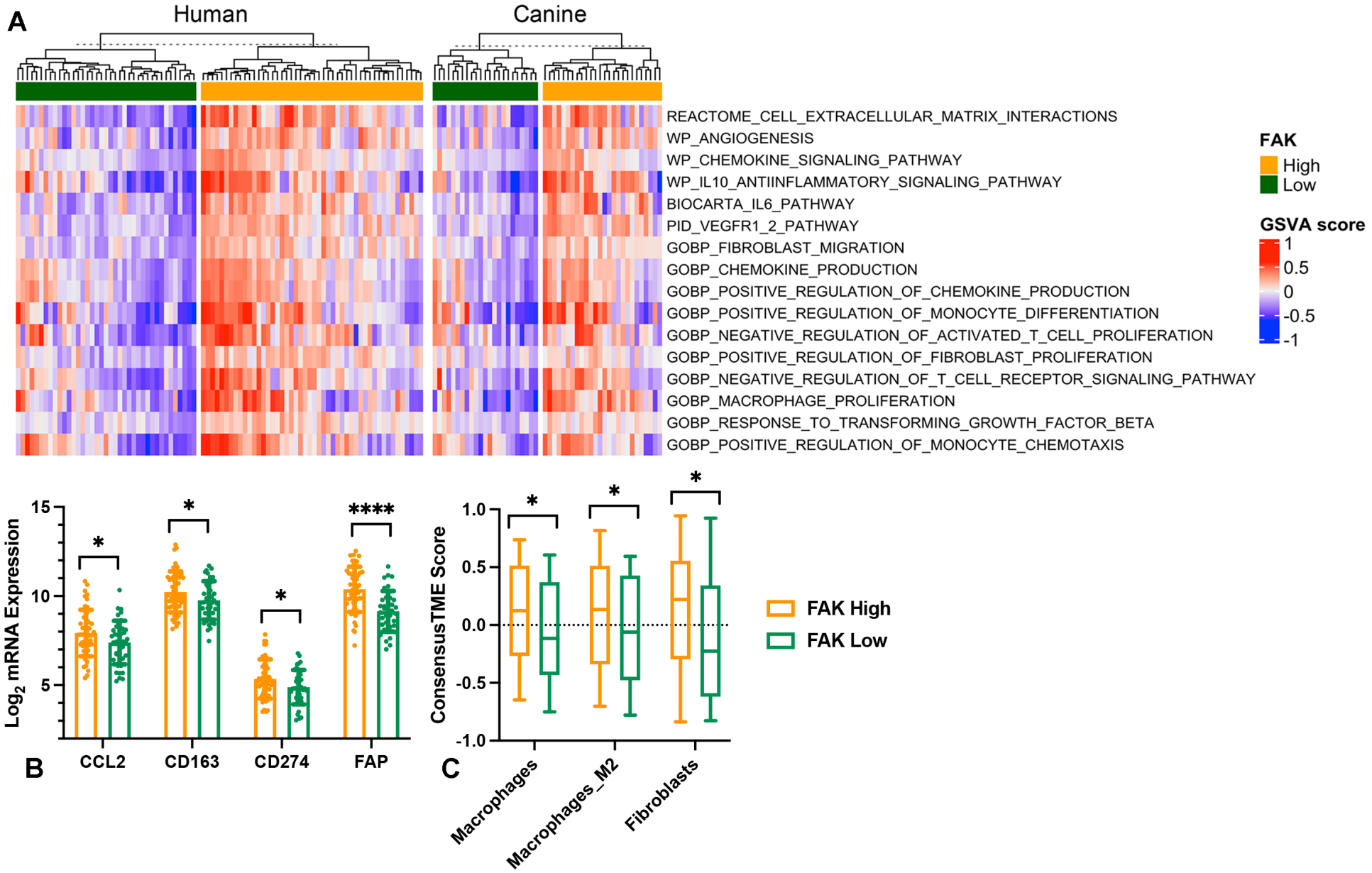
Single cell profiling to identify therapeutic targets shared between tumor cell and TME compartments of metastatic OS



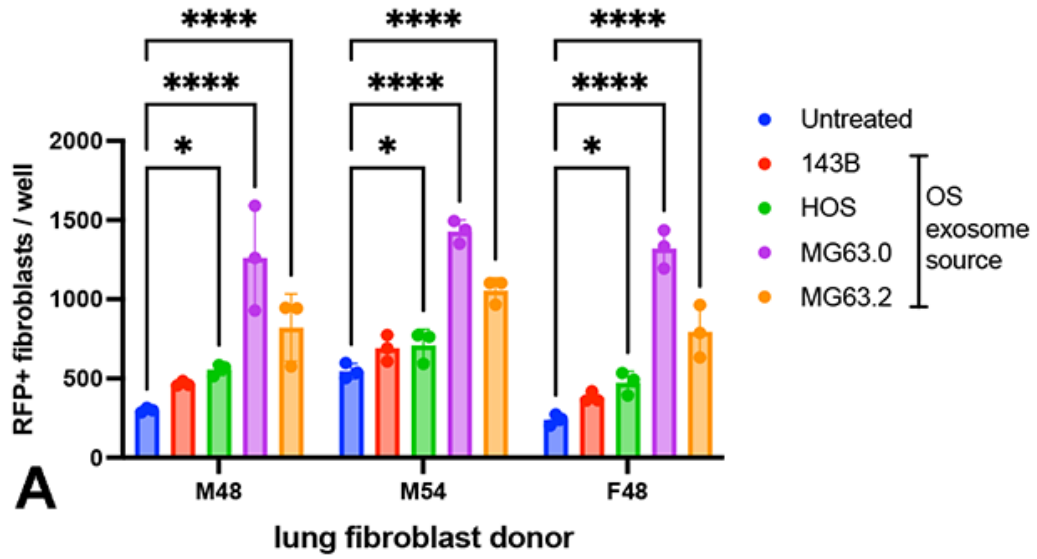


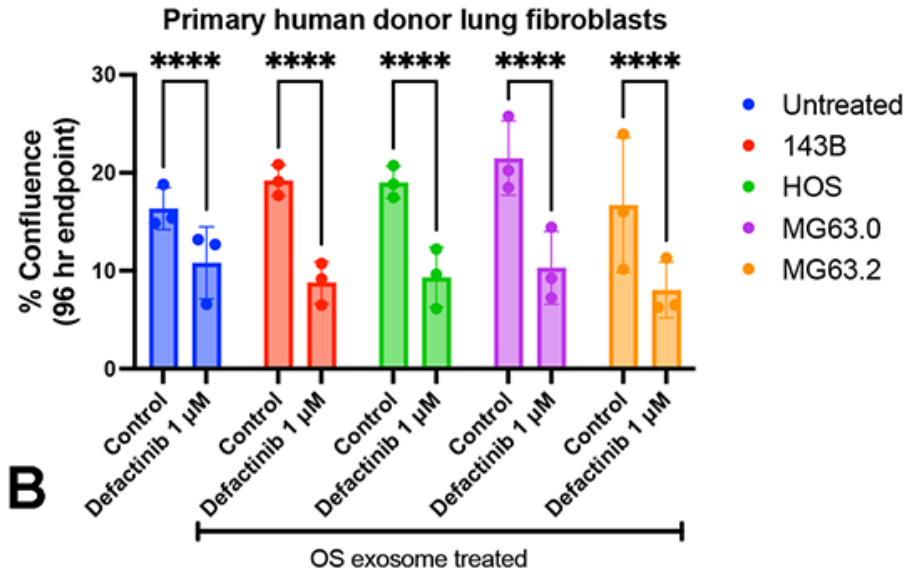

PTK2
Granulocyte
MacMonoDC
Osteoclast
Epithelial
Endothelial
Stromal
Tumor
Lymphocyte
UMAP 1
UMAP 2
15
10
5
0
-5
-10
-10
-5
0
5
10
4
3
2
1
0
UMAP2
UMAP1
2
1
0
3
PTK2 (FAK) exp
Tumor
Fibroblast
Endothelial
Epithelial
Osteoclast
Mac/Mono/DC
Lymphocyte
Granulocyte
0.0
0.2
0.4
0.6
Fraction of cells
B
AvgExpr
2
1
p-FAK

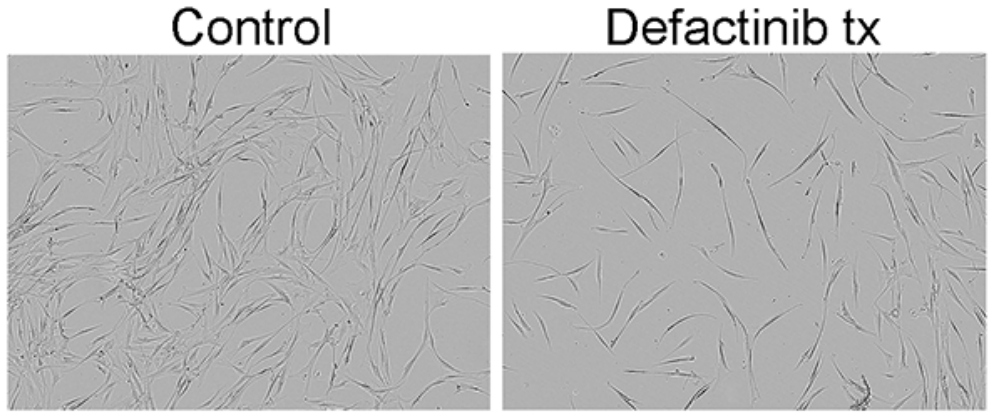
FAK signaling is enriched in OS and mediates an immune suppressive TME associated with CAF and Mϕ enrichment and T cell suppression

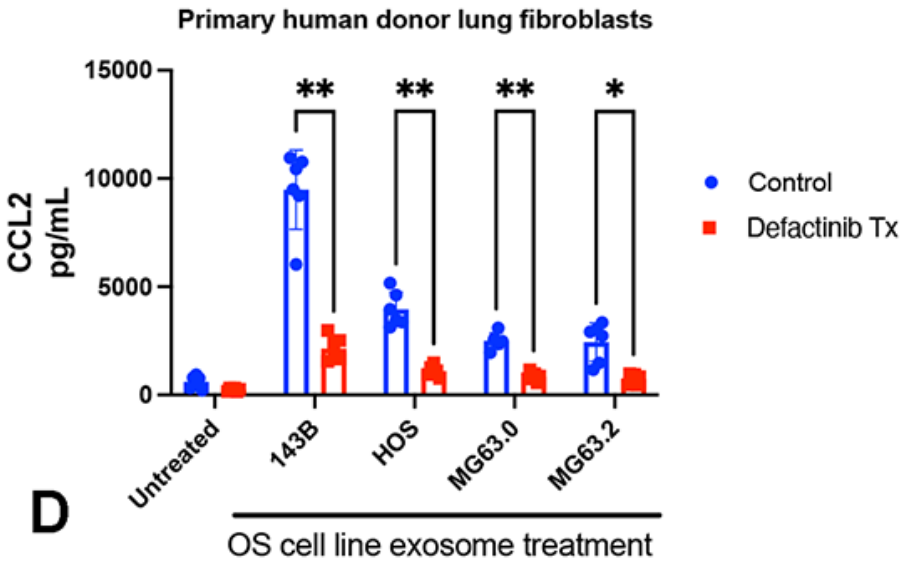


FAKi therapy blocks OS tumor cell-driven lung fibroblast proliferation and CCL2 secretion

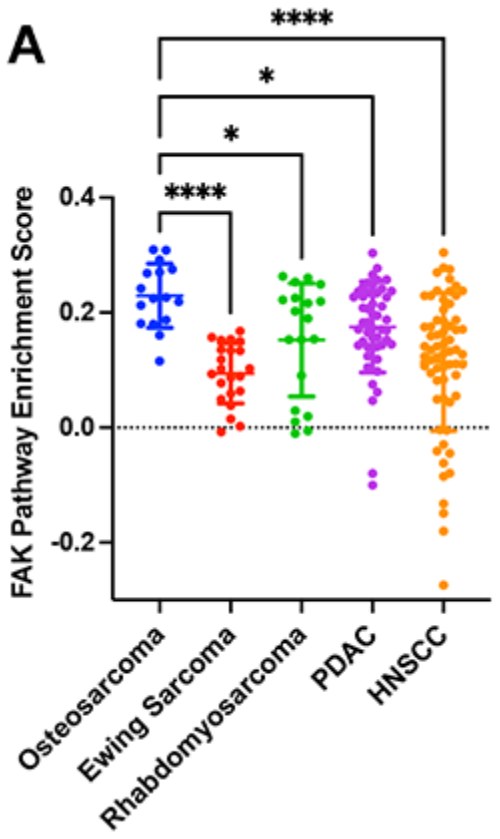


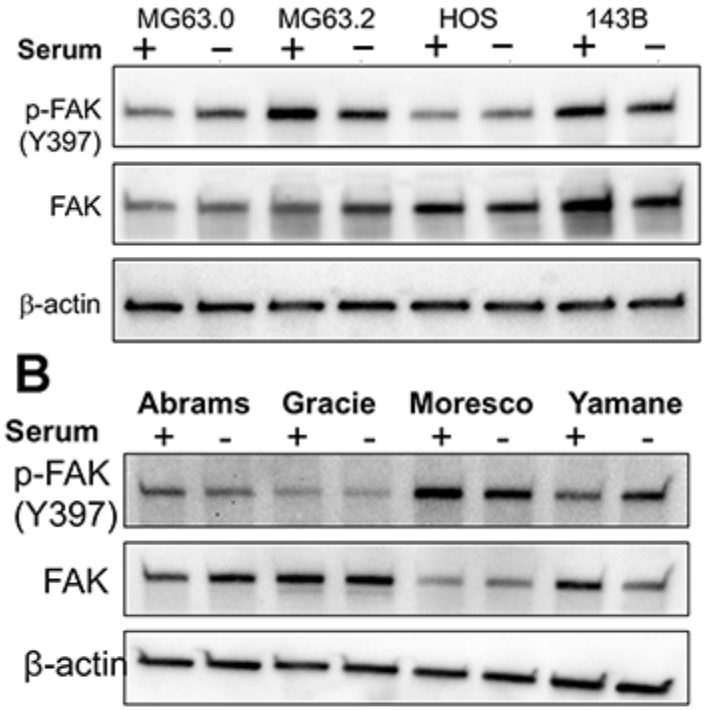


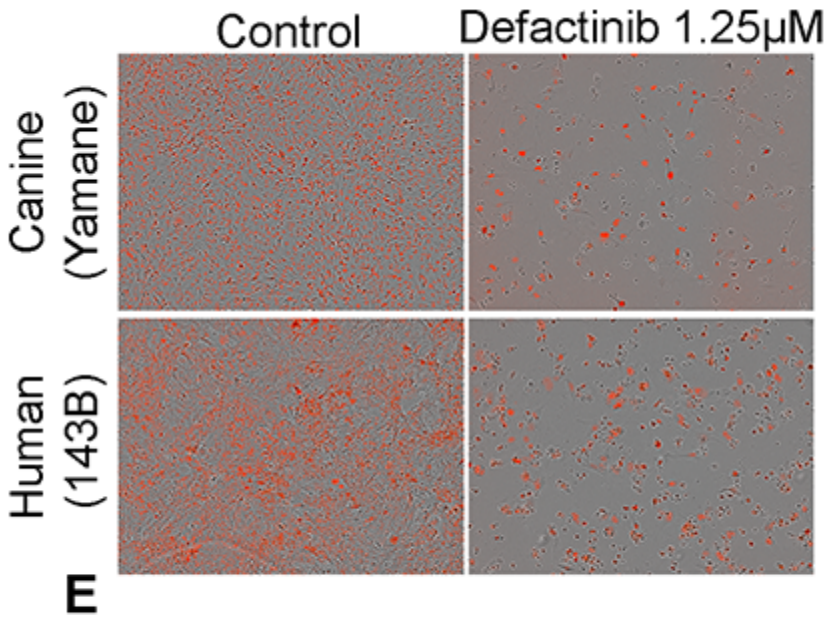


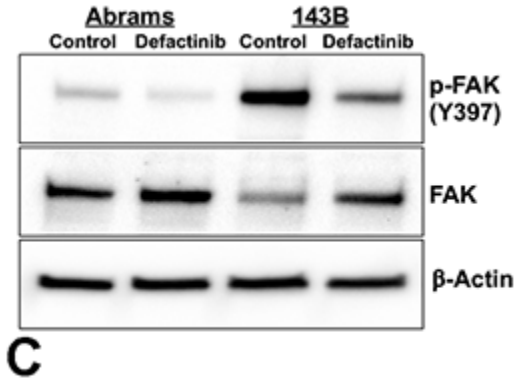


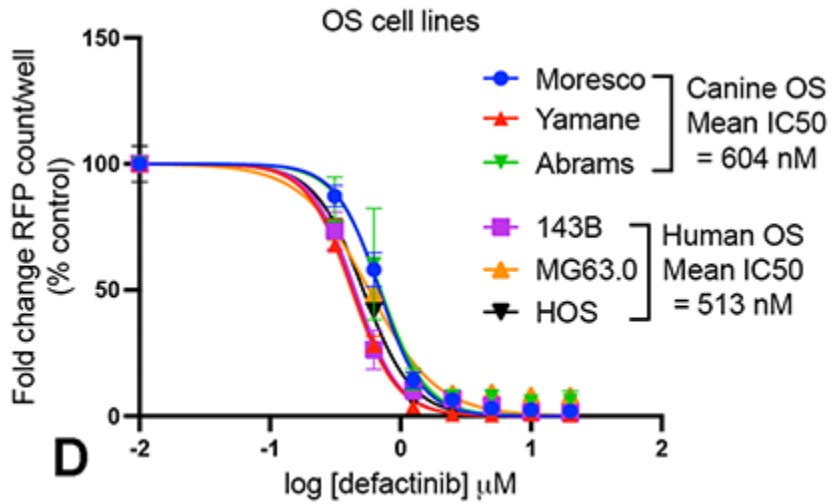
FAK is also a cell intrinsic therapeutic vulnerability in OS

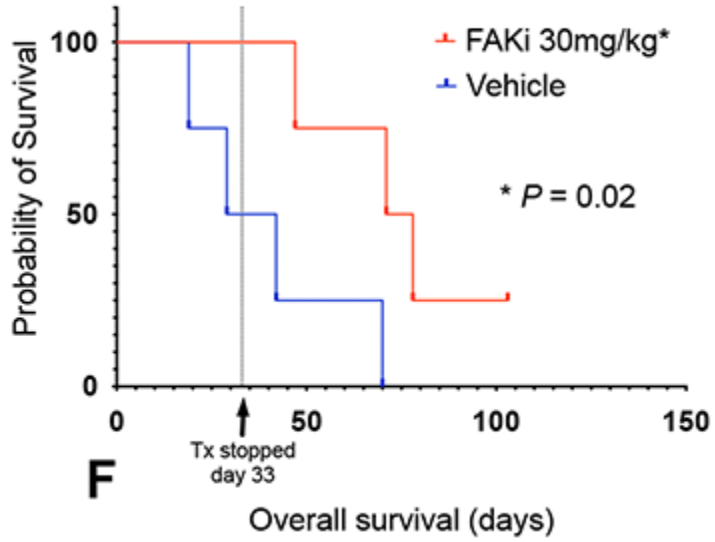




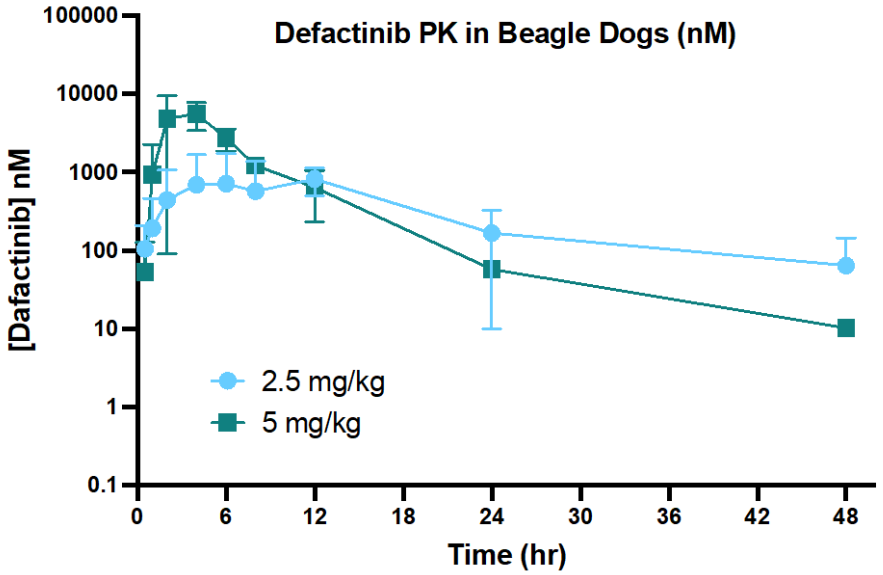


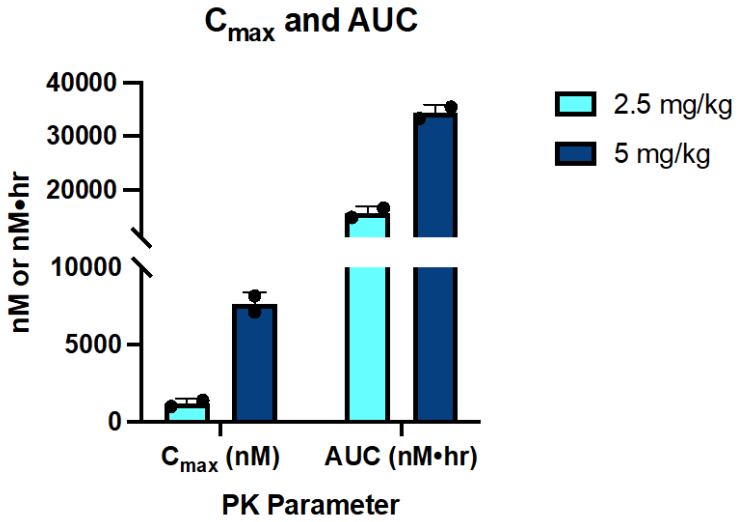


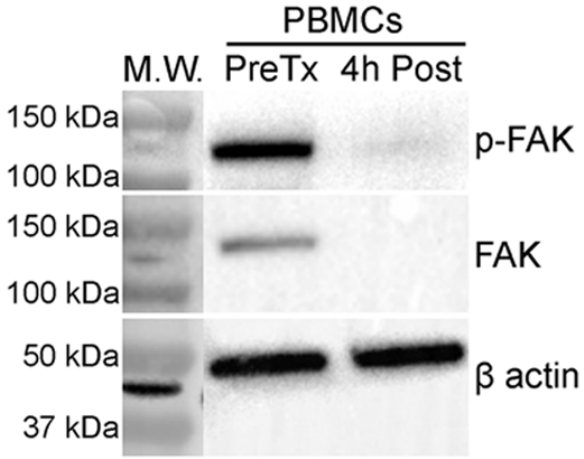


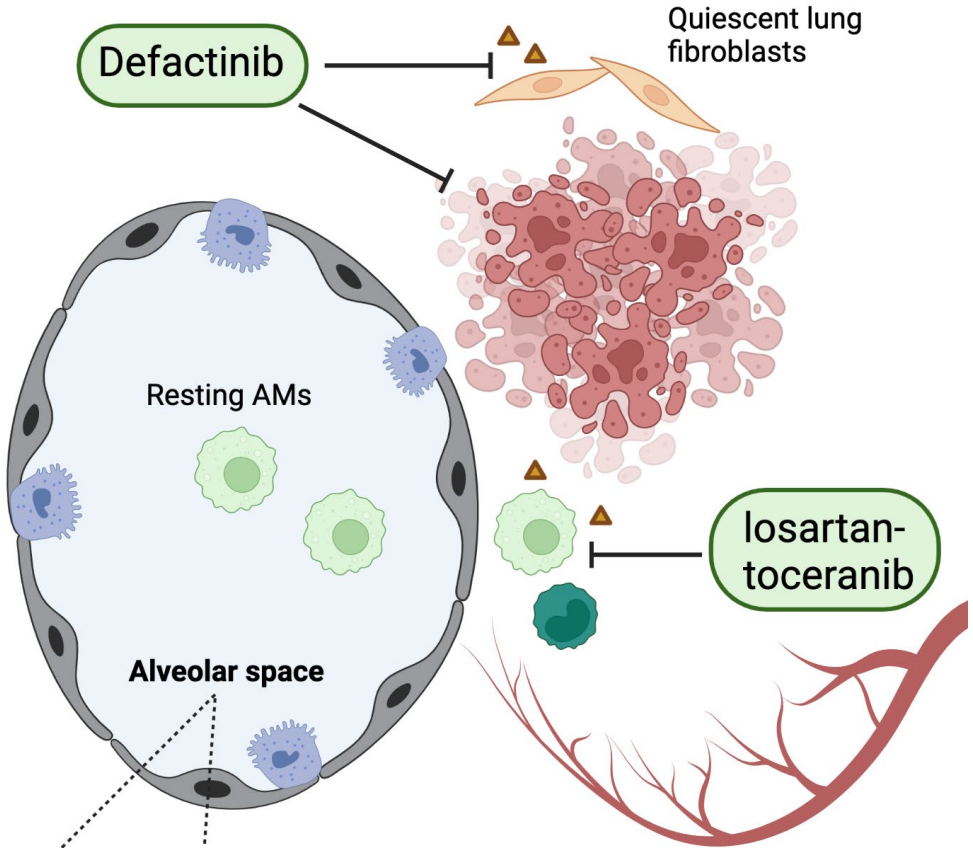


FAKi therapy for dual targeting of tumor and stromal compartments in canine metastatic OS

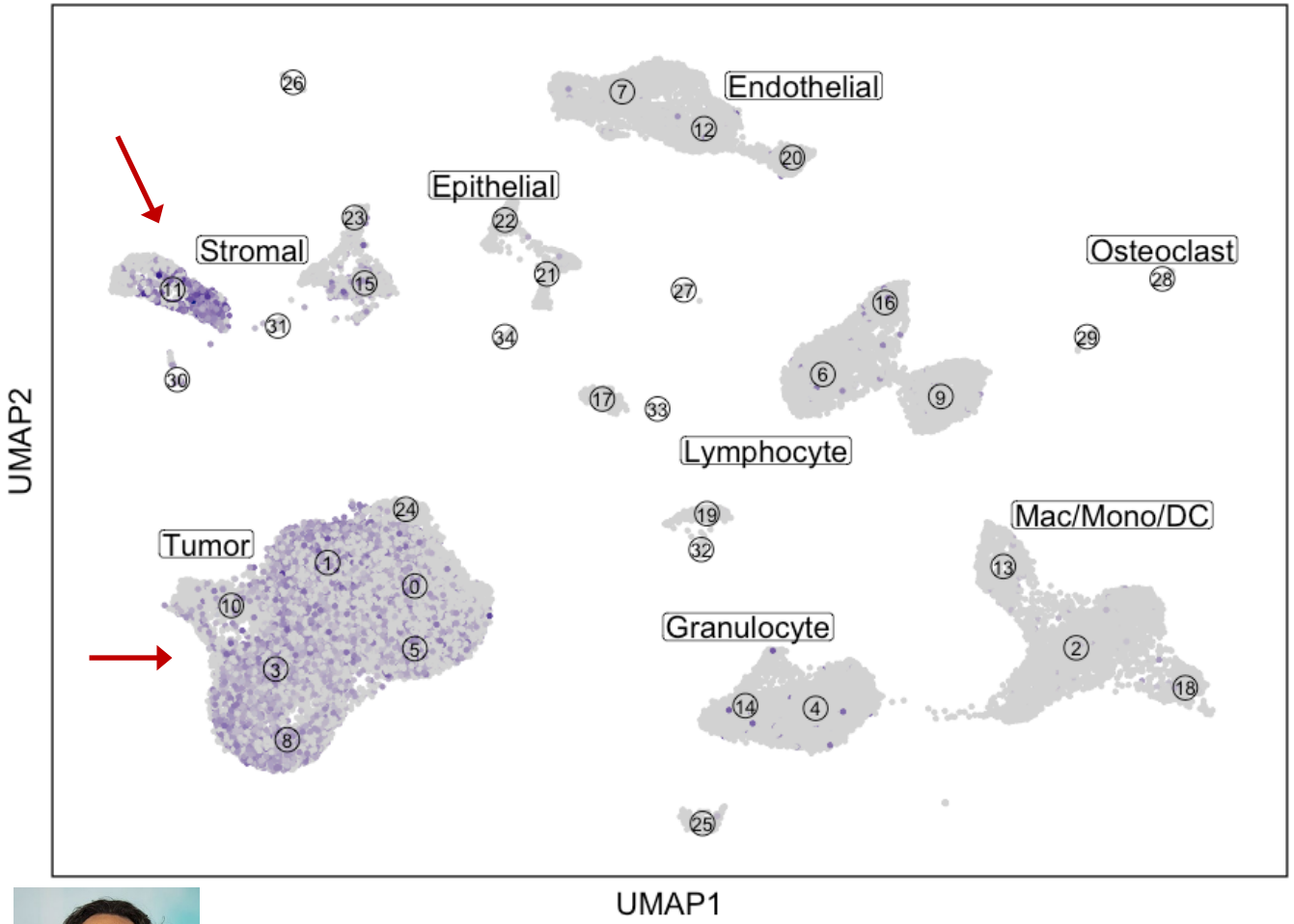




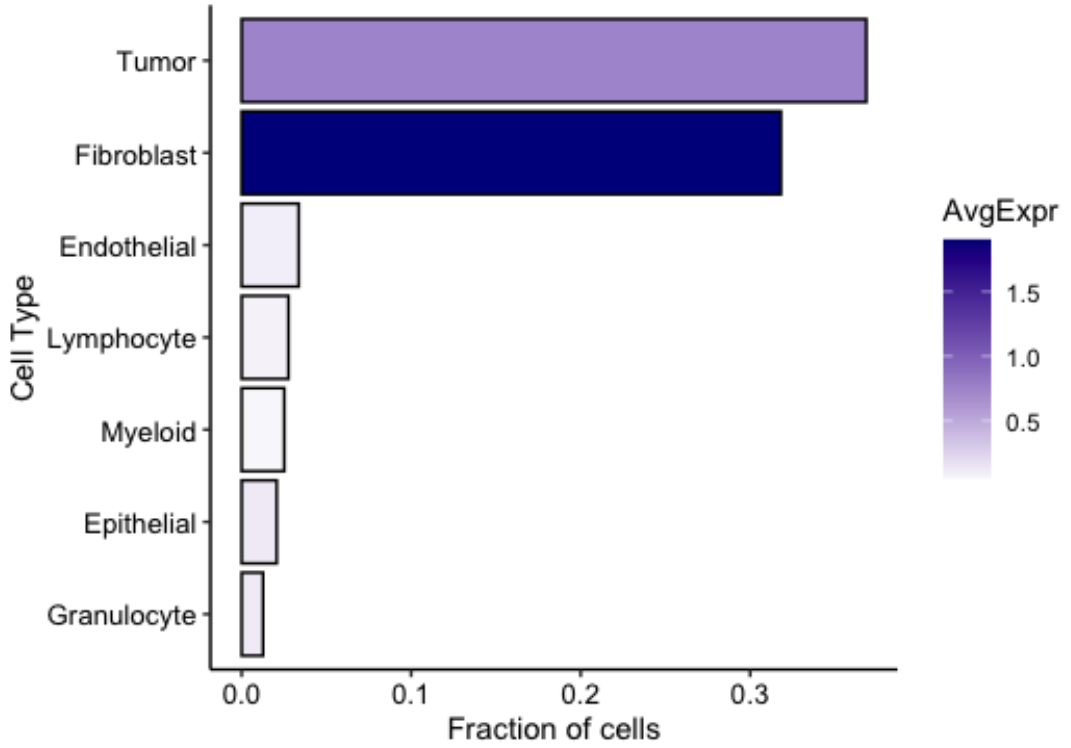




Fibroblast Activation Protein (FAP) – another approach for dual targeting of lung fibroblasts and OS tumor cells



FAP expression

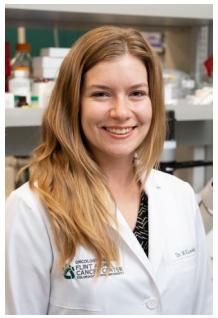


FAP expression

Mike Leibowitz
MD, PHD
Children's Hospital Colorado

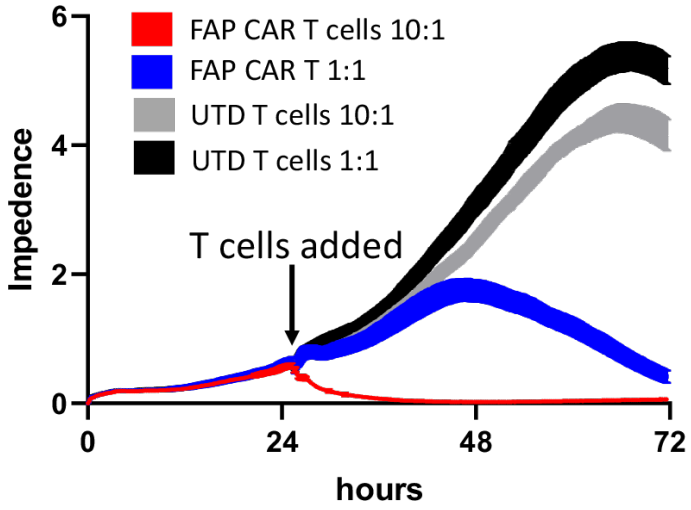
V FOUNDATION®
Victory Over Cancer

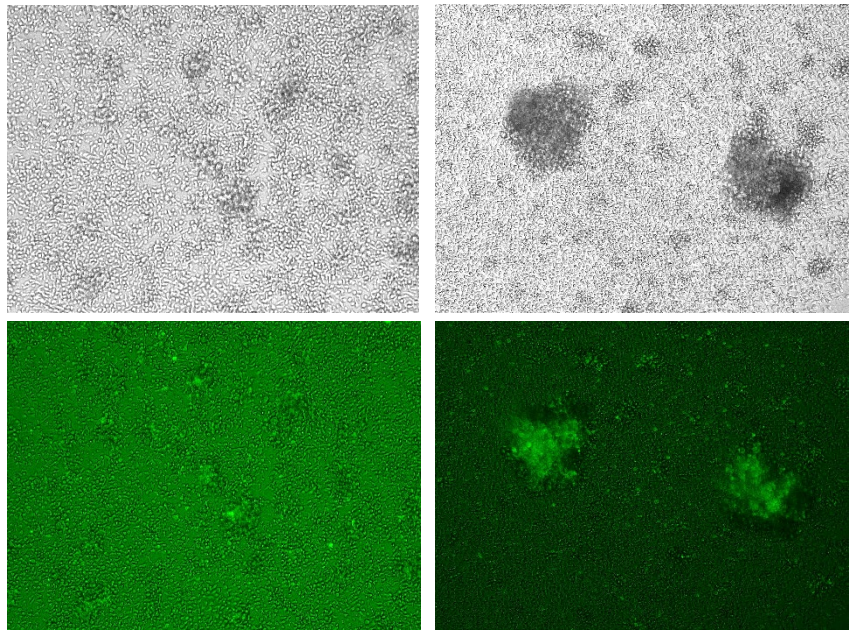
Canine FAP CAR T cells kill FAP+ OS target cells

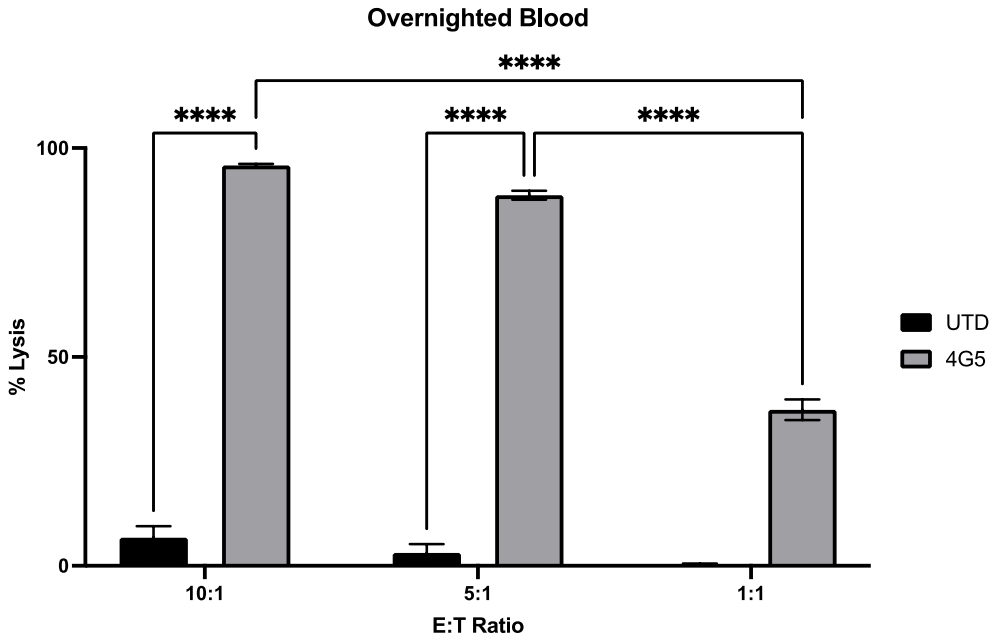


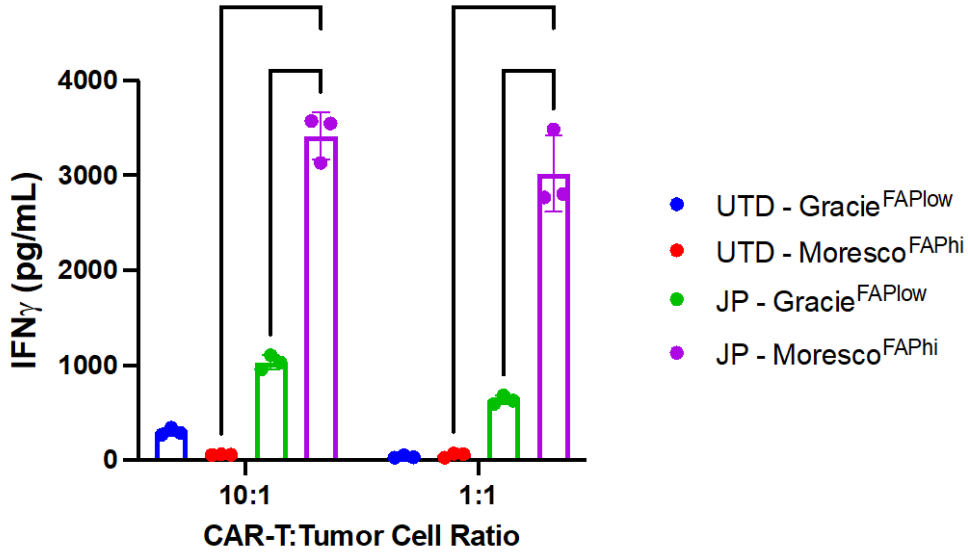
Marika Klosowski
CSU FACC

Mike Leibowitz Allie McMellen
Children's Hospital Colorado



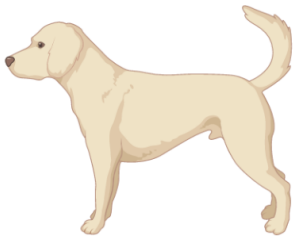


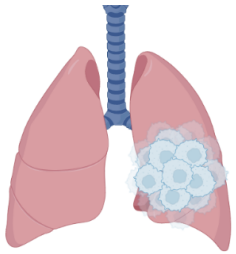




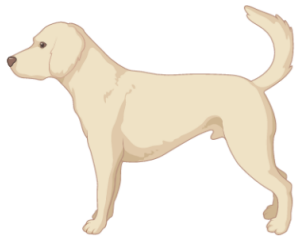
Evaluation of FAP CAR T cells and macrophage-targeted immunotherapy in canine metastatic OS

Arm 1:
FAP CAR T cells alone





Arm 2:
FAP CAR T cells + losartan/toceranib



- Initially enroll n=9/arm
- CAR T cell dose identified in safety run-in trial in dogs with primary OS
- < 3 objective responses in either/both arms:
  - Both arms - trial halted
  - otherwise "pick the winner" - Arm with highest ORR chosen for continued enrollment --> enroll additional 15 patients

Day -14

- Screening
- Peripheral blood
- Leukapheresis for CAR generation
- +/- begin losartan-toceranib tx

Day -4

- Cyclophosphamide (500mg/m2 PO

Day 0

- CAR T cell infusion
- in hospital monitoring for day
- Peripheral blood
  - CBC/chem
  - multiplex cytokine analysis
  - flow cytometry

Day 3

- Peripheral blood
  - CBC/chem
  - flow cytometry
  - multiplex cytokine analysis

Day 7, 14, 28, 56

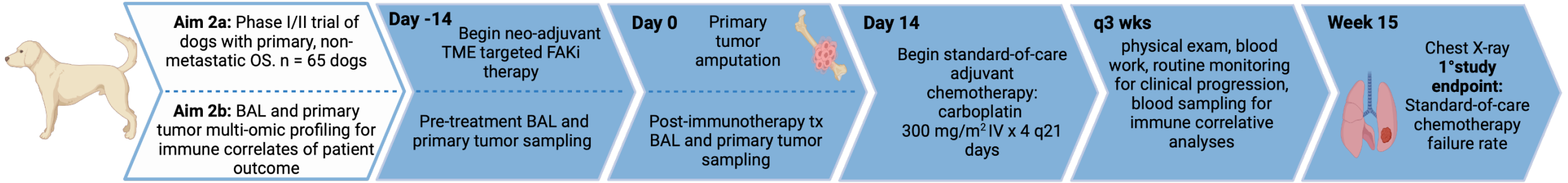
- Peripheral blood
  - CBC/chem
  - flow cytometry
  - multiplex cytokine analysis
- Re-check CXRs @8wks
  - Repeat again @16wks to confirm progression

Conclusions, potential impact and next steps

- Osteosarcoma TME dominated by myeloid cell responses
  - Fibroblasts as primary mediators of their recruitment ?
- Drug repurposing is an effective strategy for developing myeloid targeted immunotherapies
  - Targeting myeloid cells exerts significant antitumor activity against canine metastatic OS in gross disease setting
- Canine immunotherapy trials can inform human translation
  - Losartan-sunitinib Phase I study in human metastatic OS (NCT03900793)

Next steps:

- losartan/sunitinib as platform for other combination therapies
  - Cytotoxic/molecular targeted therapy?
  - T cell targeted immunotherapy ?
- Clinical trial of FAKi therapy + standard-of-care chemotherapy in adjuvant setting of canine OS



Acknowledgements

Regan Lab

Katie Cronise
Laurel Haines
Eric Palmer
Kendall Malmstrom
Marika Klosowski
Becca Makii
Kelly McAuliffe

Past

Carina Easton
Aaron Offermann
Alex Baeckler
Lauren Alfino
Tea Ned

Dow lab (FACC)

Steve Dow
Lyndah Chow
Dylan Ammons

Kelly Faulk
Mike Leibowitz
Allie McMellen
Kim Jordan and HIMSR
Bree Wilky

Thomas Cash

CSU FACC and Clinical Trials Team

Kristen Weishaar
Jenna Burton
Sarah Shropshire (Medicine)

TL1 postdoctoral fellowships

Questions?