

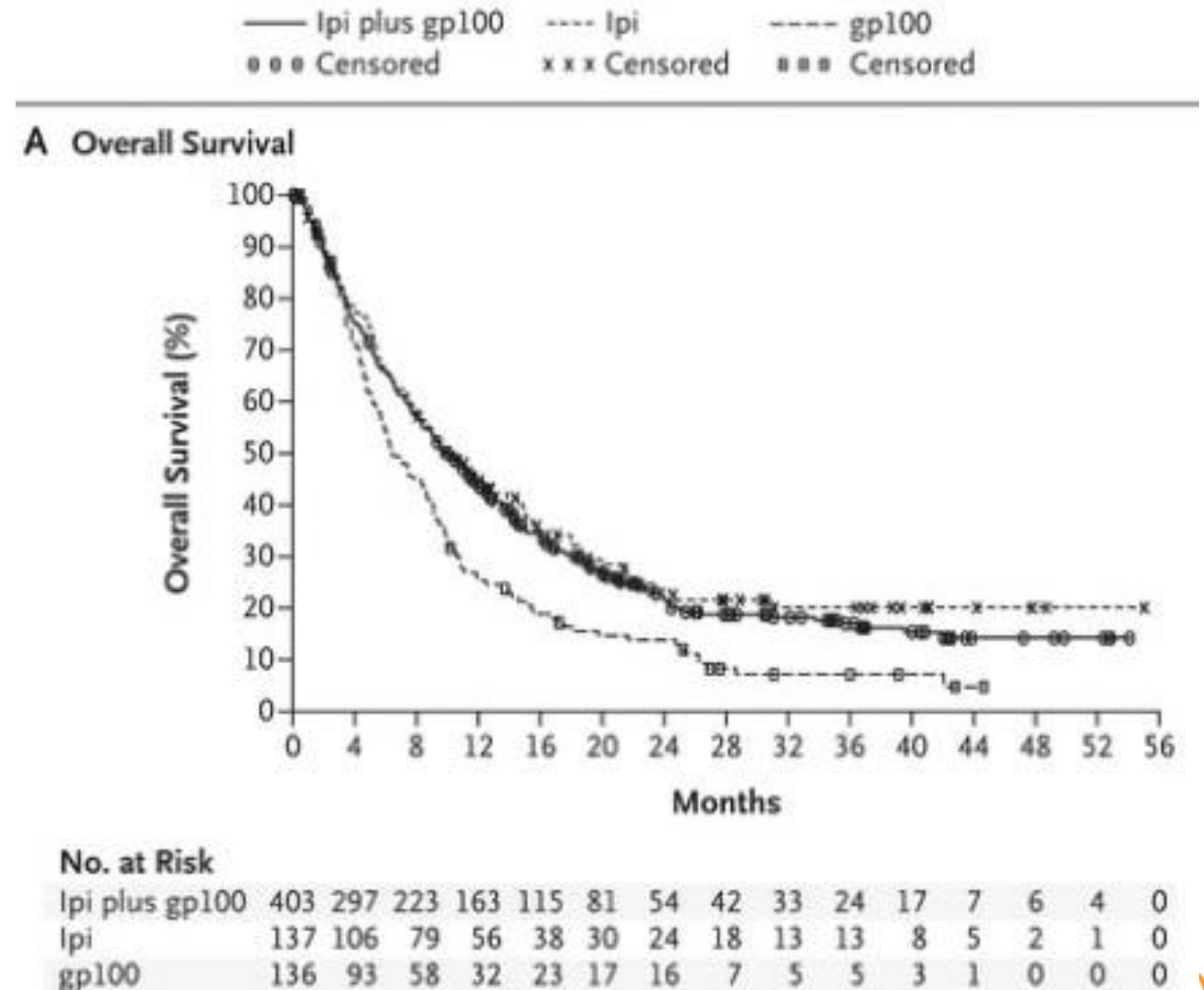


**Granzyme B PET Imaging: A Novel Clinical Biomarker
Transforming Oncology and Immunology Drug
Development**

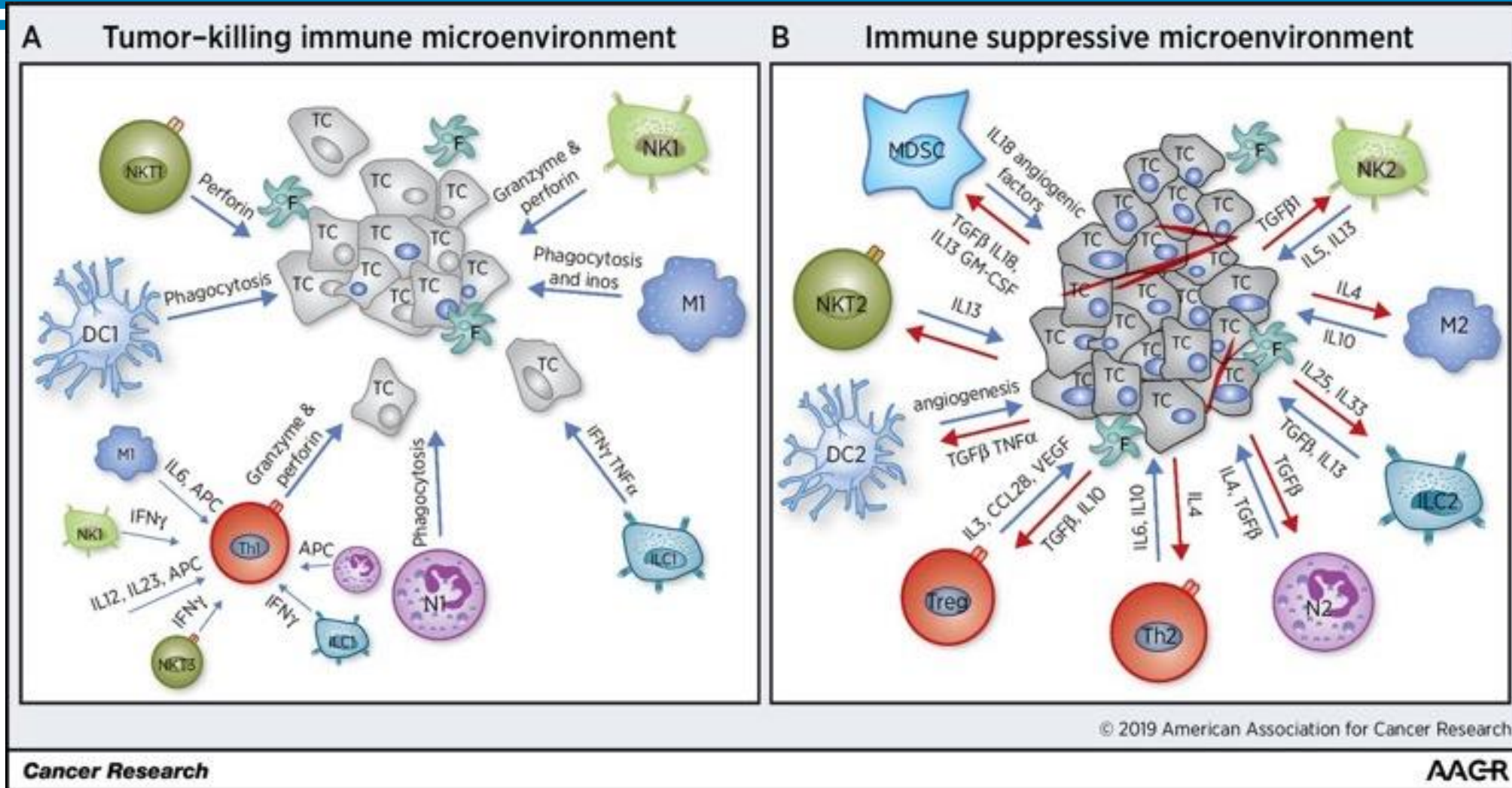
**Ben Larimer, PhD
CEO, CytoSite Bio
PINTaD January 29, 2025**

Determining Response in Immuno-Oncology

- Remarkable Responses
 - ▶ Durable remissions of >10 years in Stage 4 melanoma, NSCLC
- Response rate limited
 - ▶ Expensive, side effects
- Monitoring early response difficult
- New Biomarkers



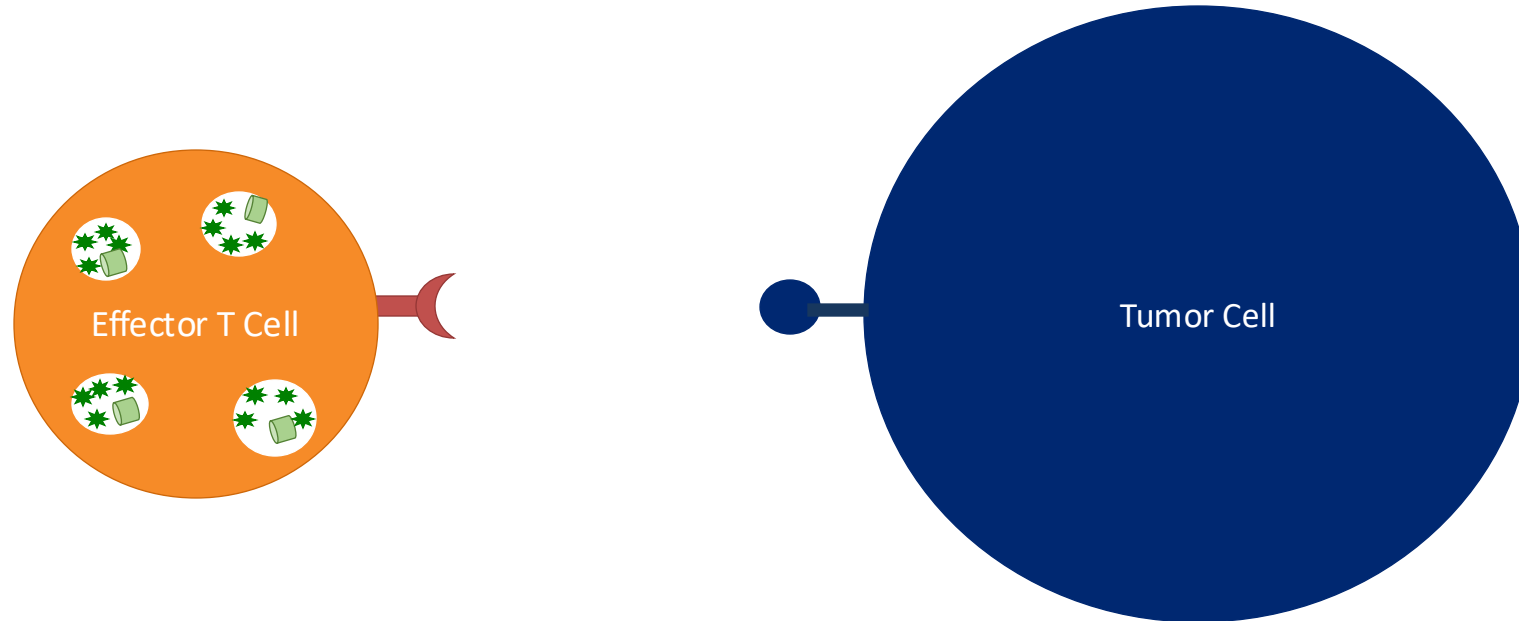
Imaging the Tumor Immune Microenvironment



Dominique C. Hinshaw, and Lalita A. Shevde *Cancer Res* 2019;79:4557-4566

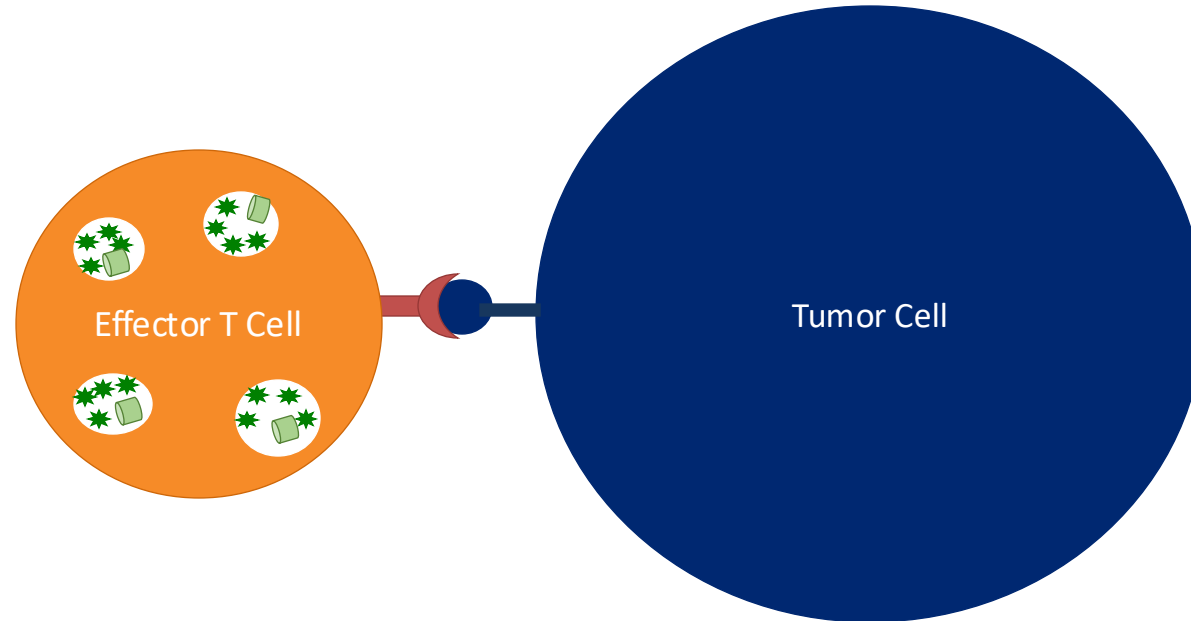
Granzyme B Biology

Effector T Cell Infiltrates Tumor



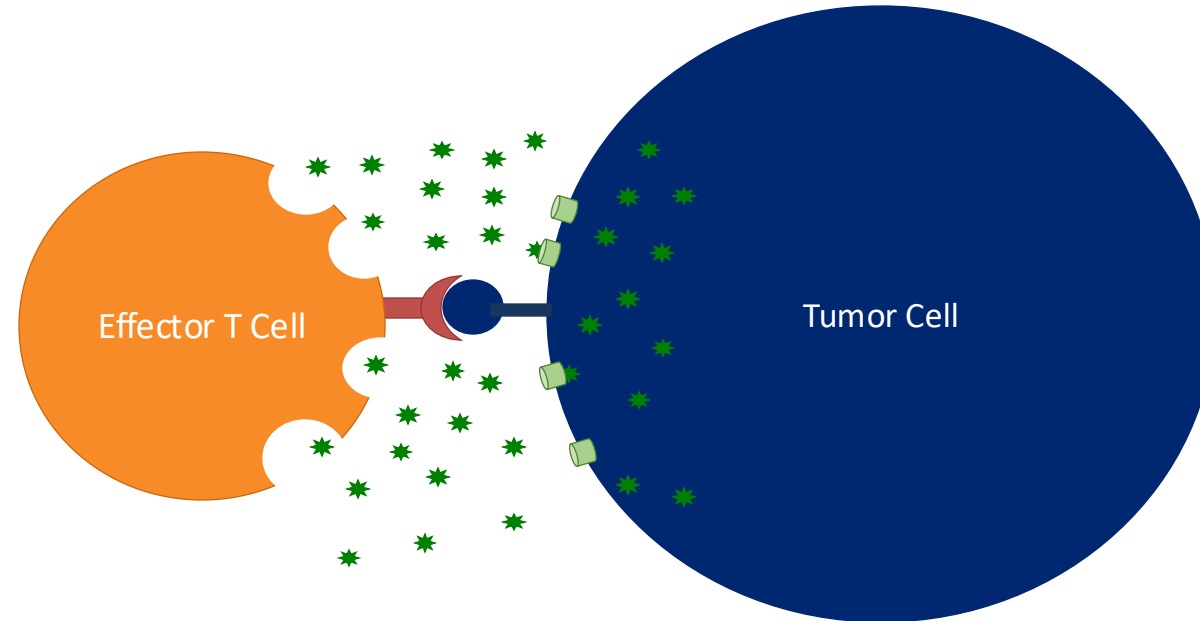
Granzyme B Biology

Effector T Cell Recognizes Tumor Antigen and is Activated

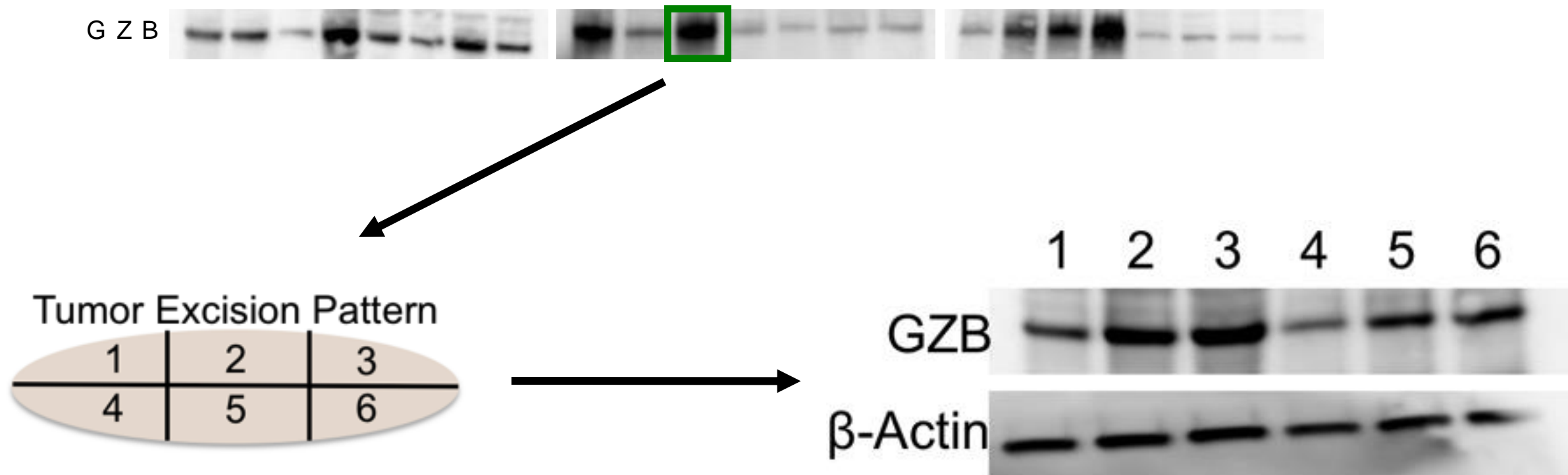


Granzyme B Biology

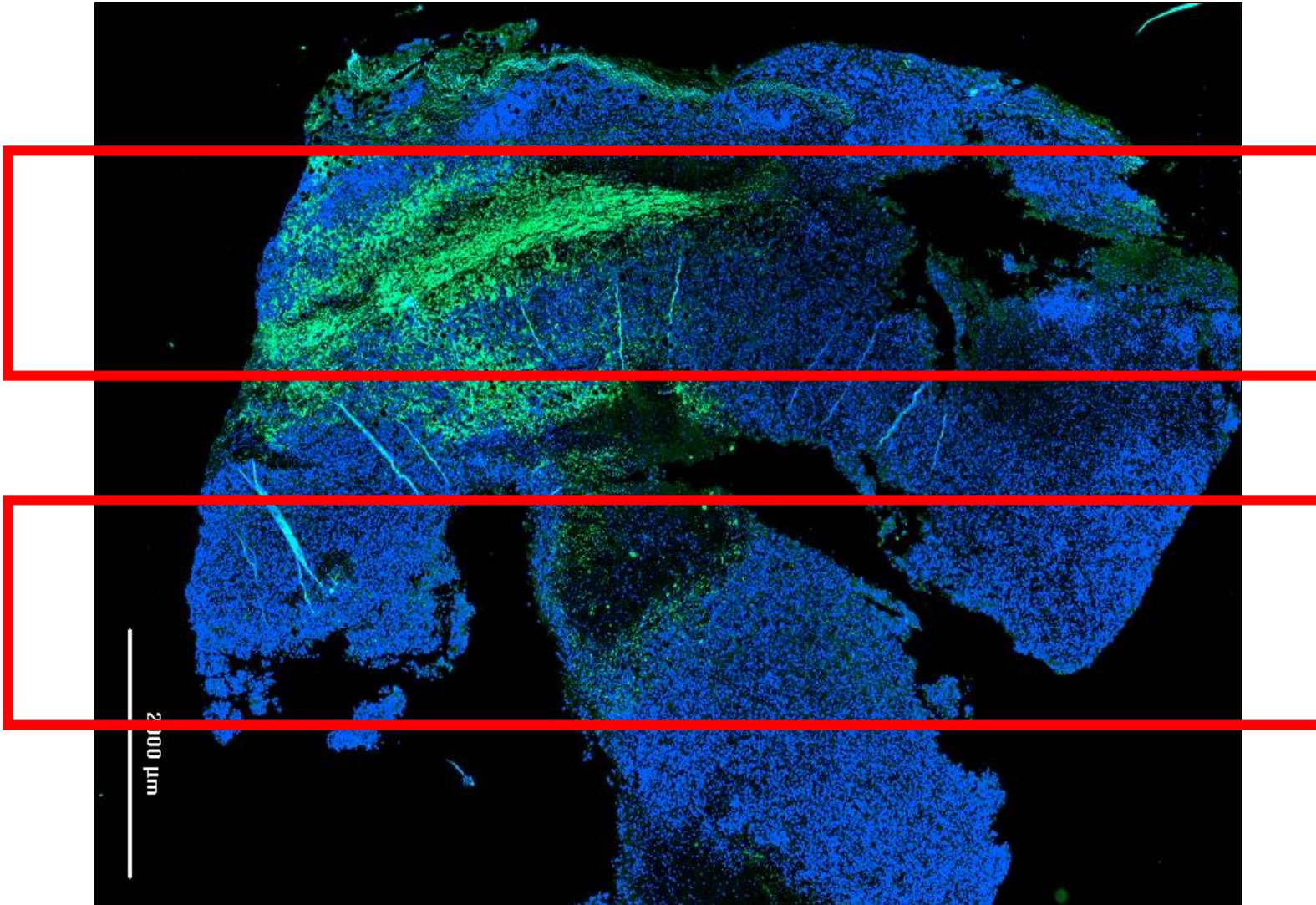
Granzyme B and Perforin are Released, Killing Nearby Tumor Cells



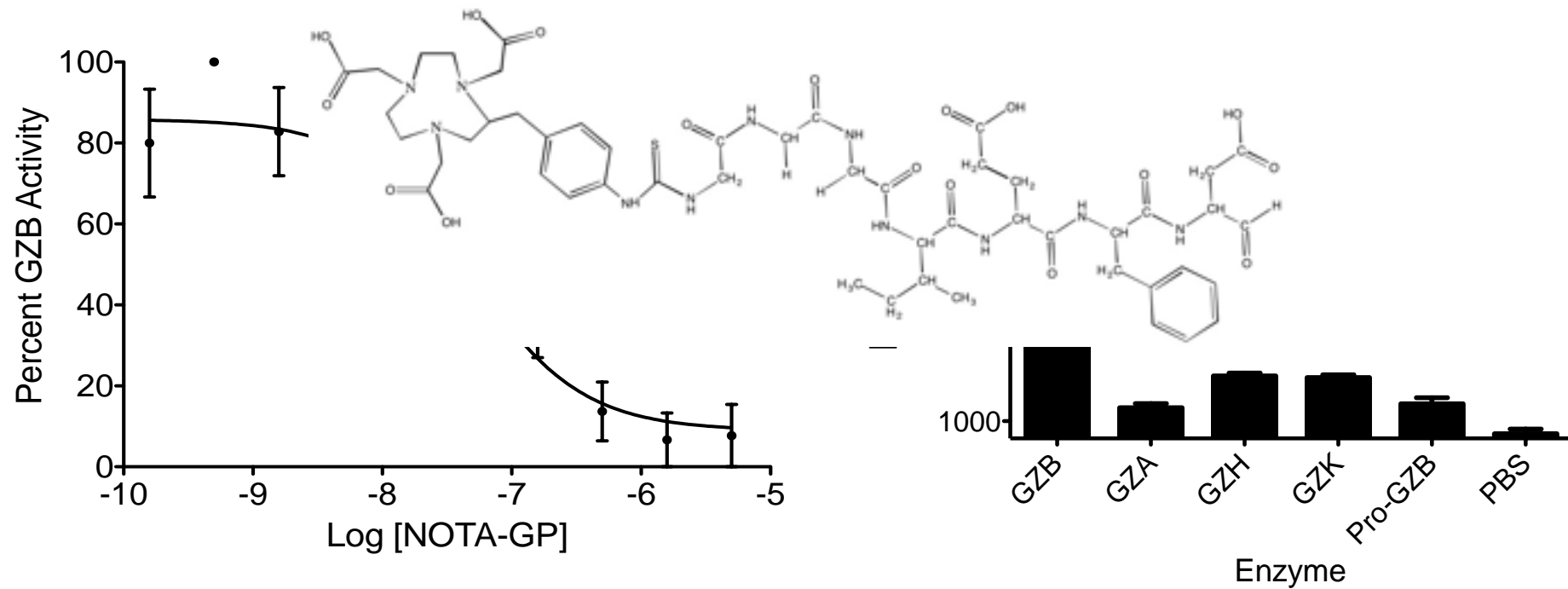
Immune Cells are Highly Heterogeneous in a Tumor



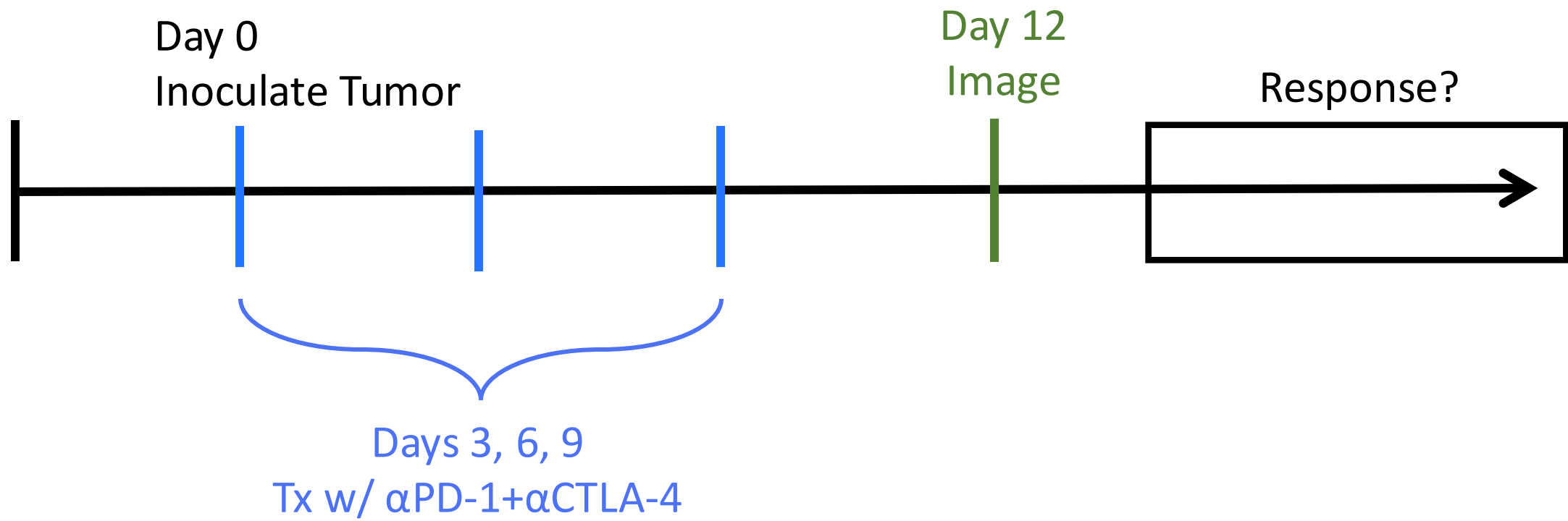
The Limitations of Needle Biopsies for Target Quantification



Design and Characterization of Granzyme B Peptide



Murine Model of Immunotherapy



Granzyme B PET of Treated Mice Differentiates into High and Low Uptake Groups

Tx High
Granzyme B



Tx Low
Granzyme B



Granzyme B PET of Treated Mice Differentiates into High and Low Uptake Groups

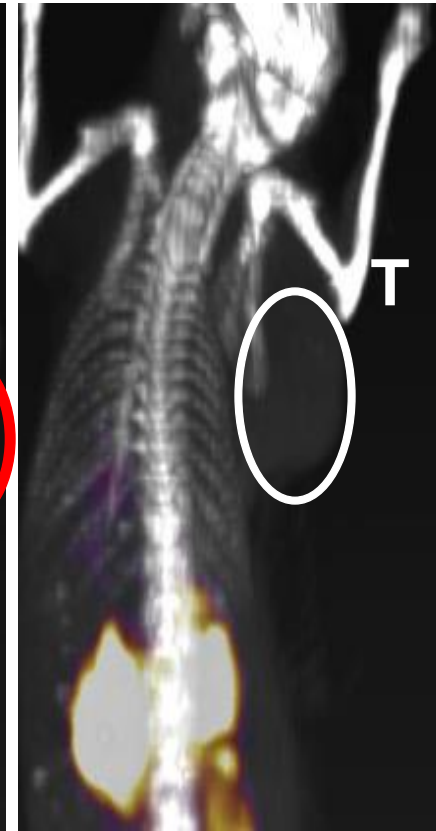
Tx High
Granzyme B



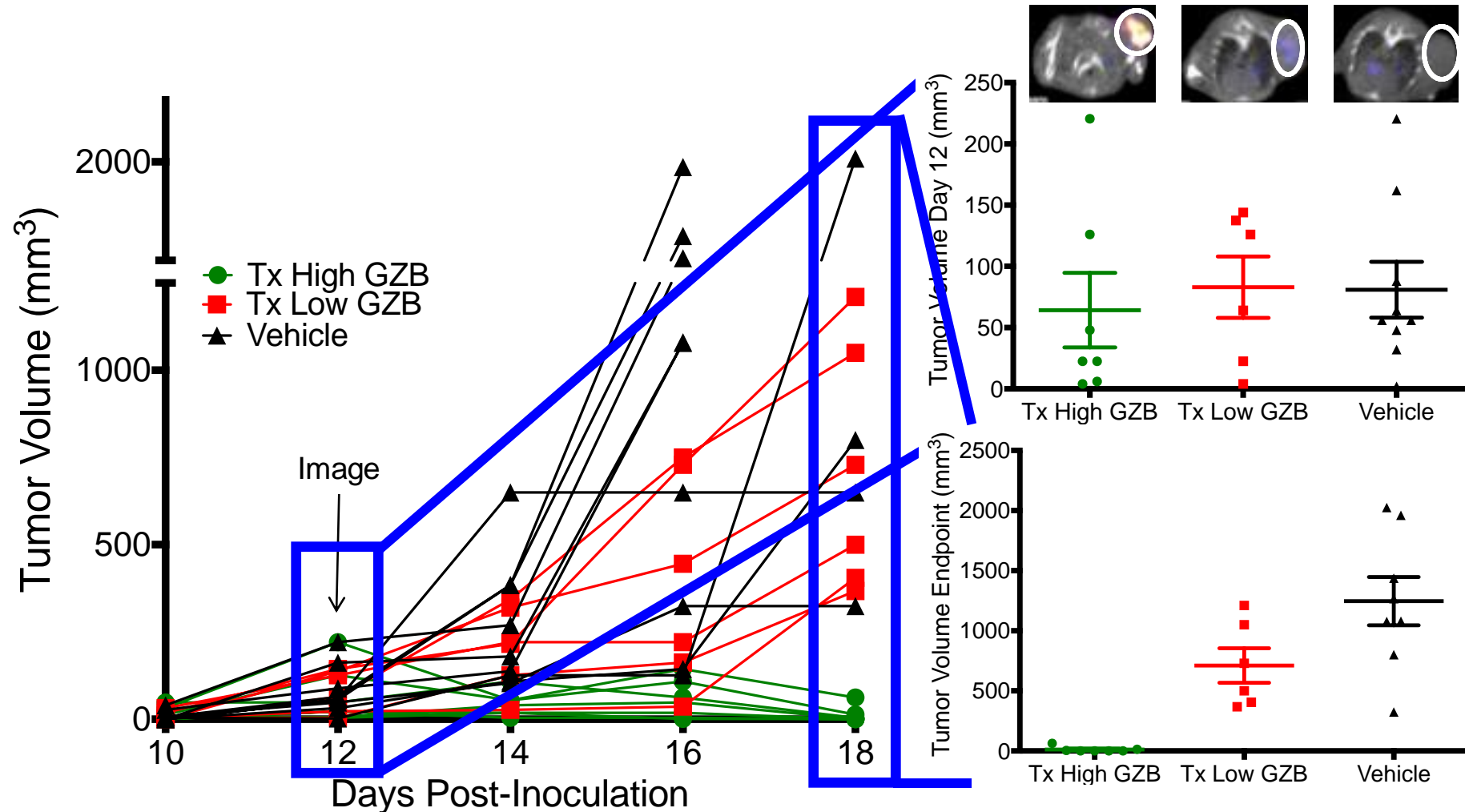
Tx Low
Granzyme B



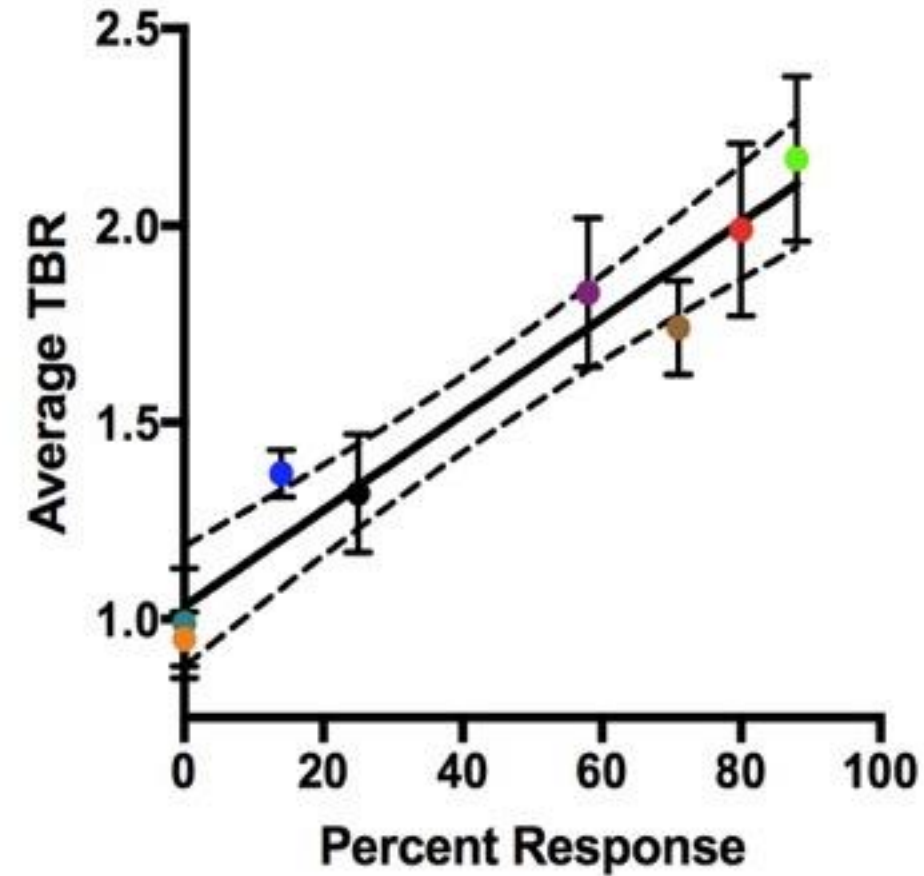
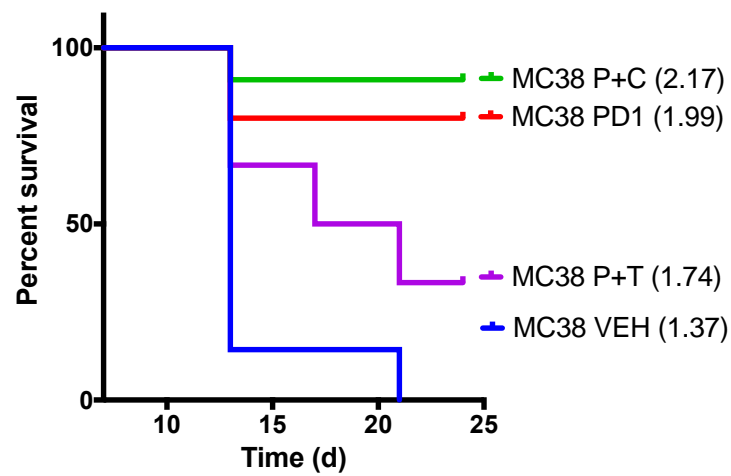
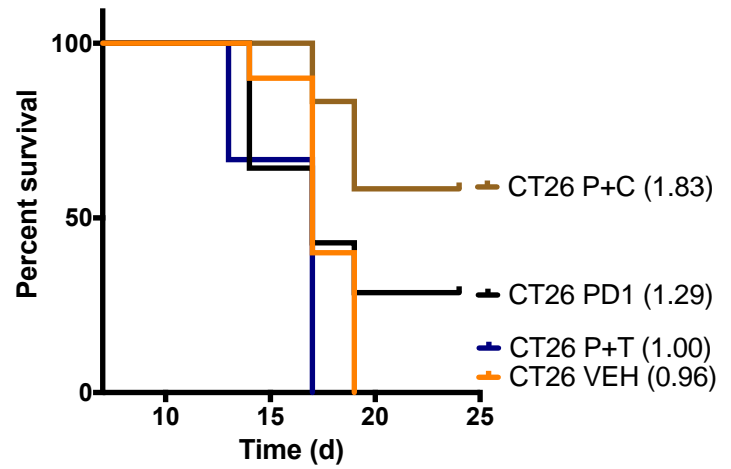
Vehicle



Granzyme B PET of Treated Mice Differentiates into High and Low Uptake Groups



Testing GZB PET Imaging Prediction of Combination Efficacy

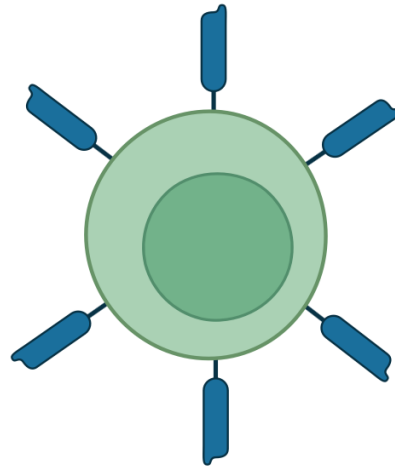


Non-Checkpoint Inhibitor Assessment

**Checkpoint
Inhibitors**



**Adoptive Cell
Transfer**

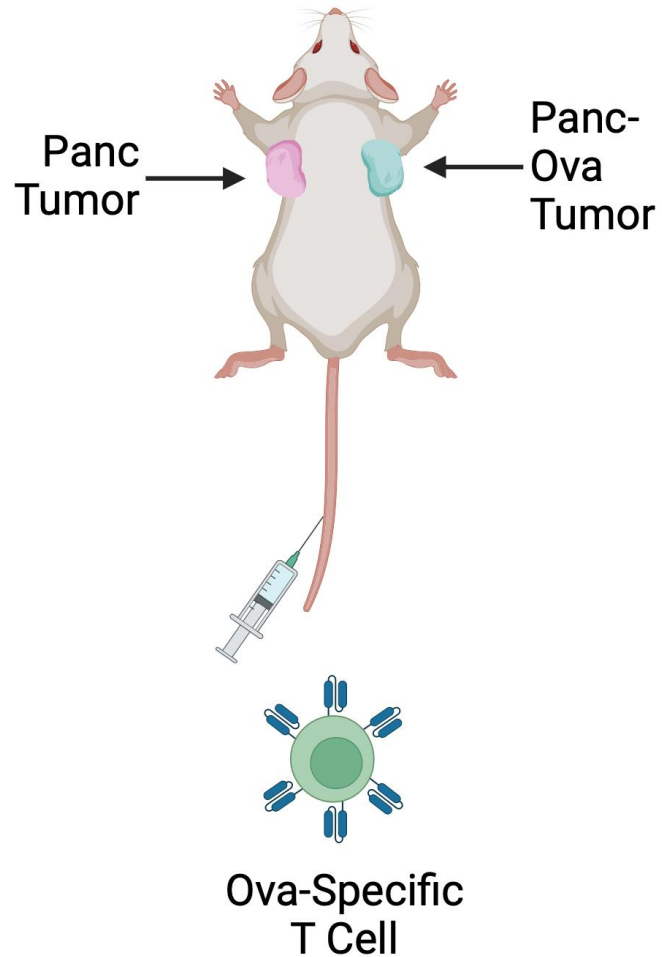


Radiation

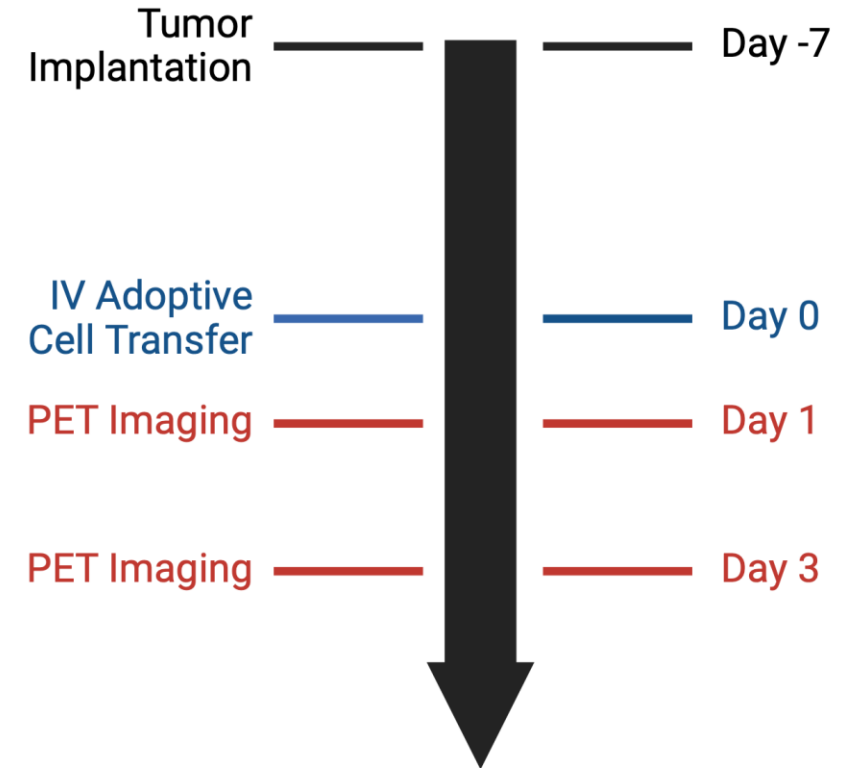
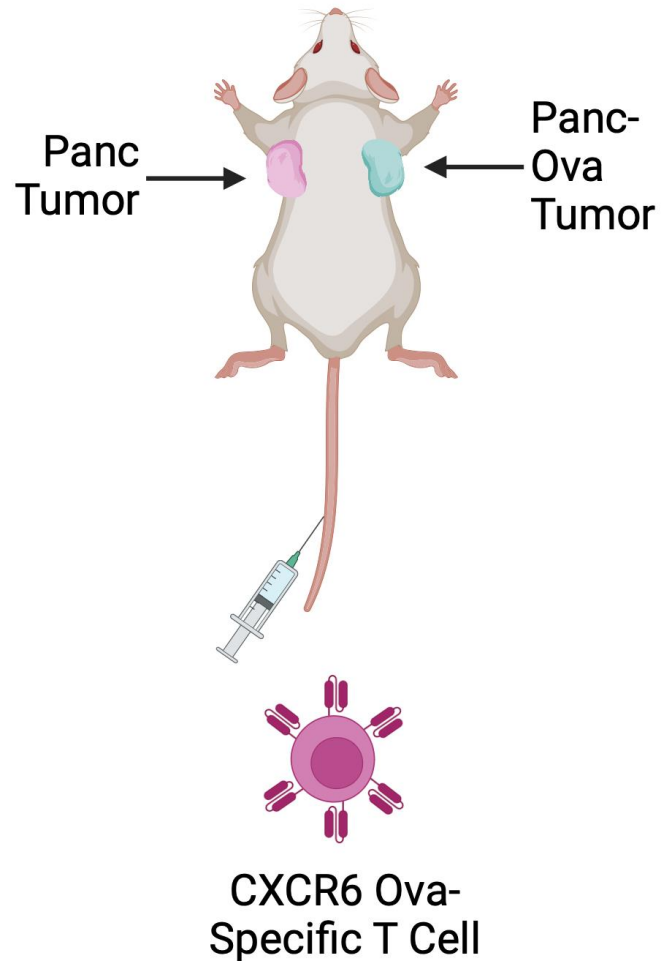


Adoptive Cell Transfer Model

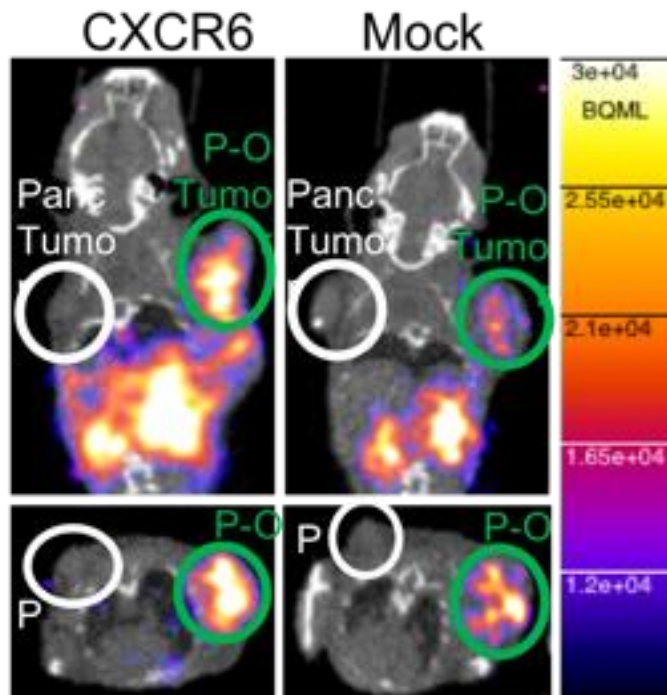
Standard Adoptive Cell Therapy



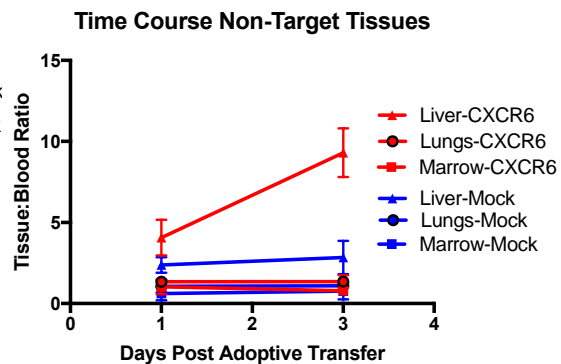
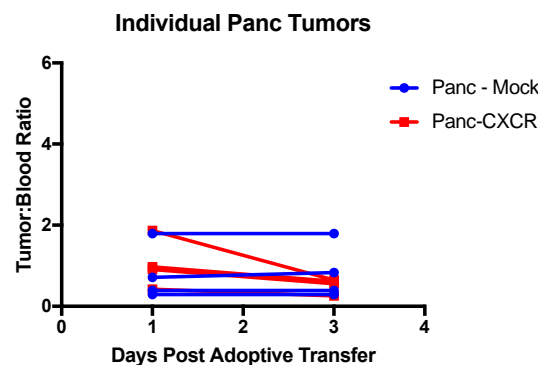
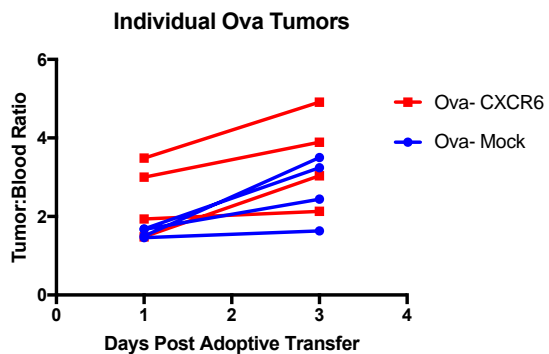
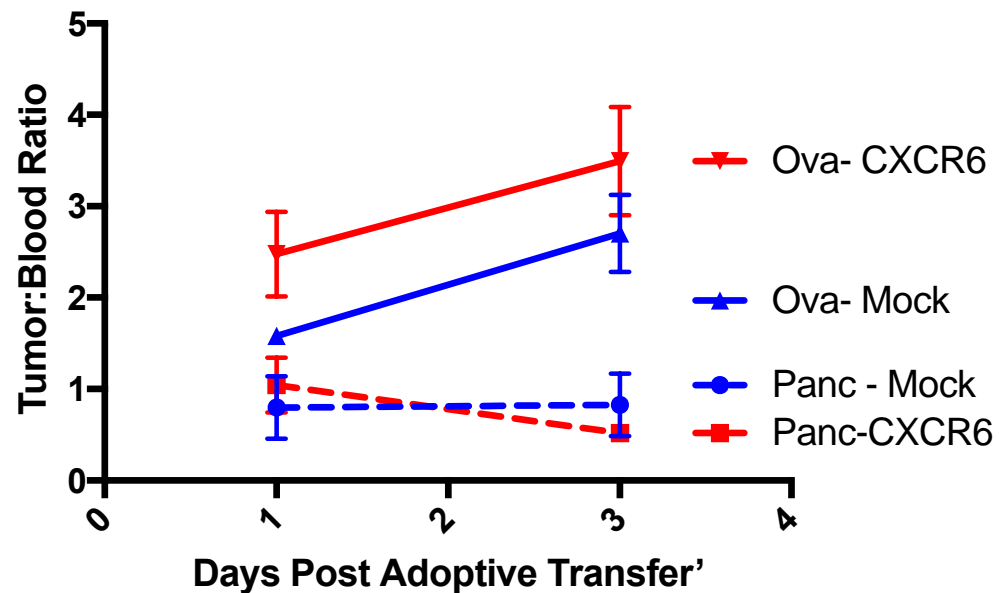
Modified Adoptive Cell Therapy



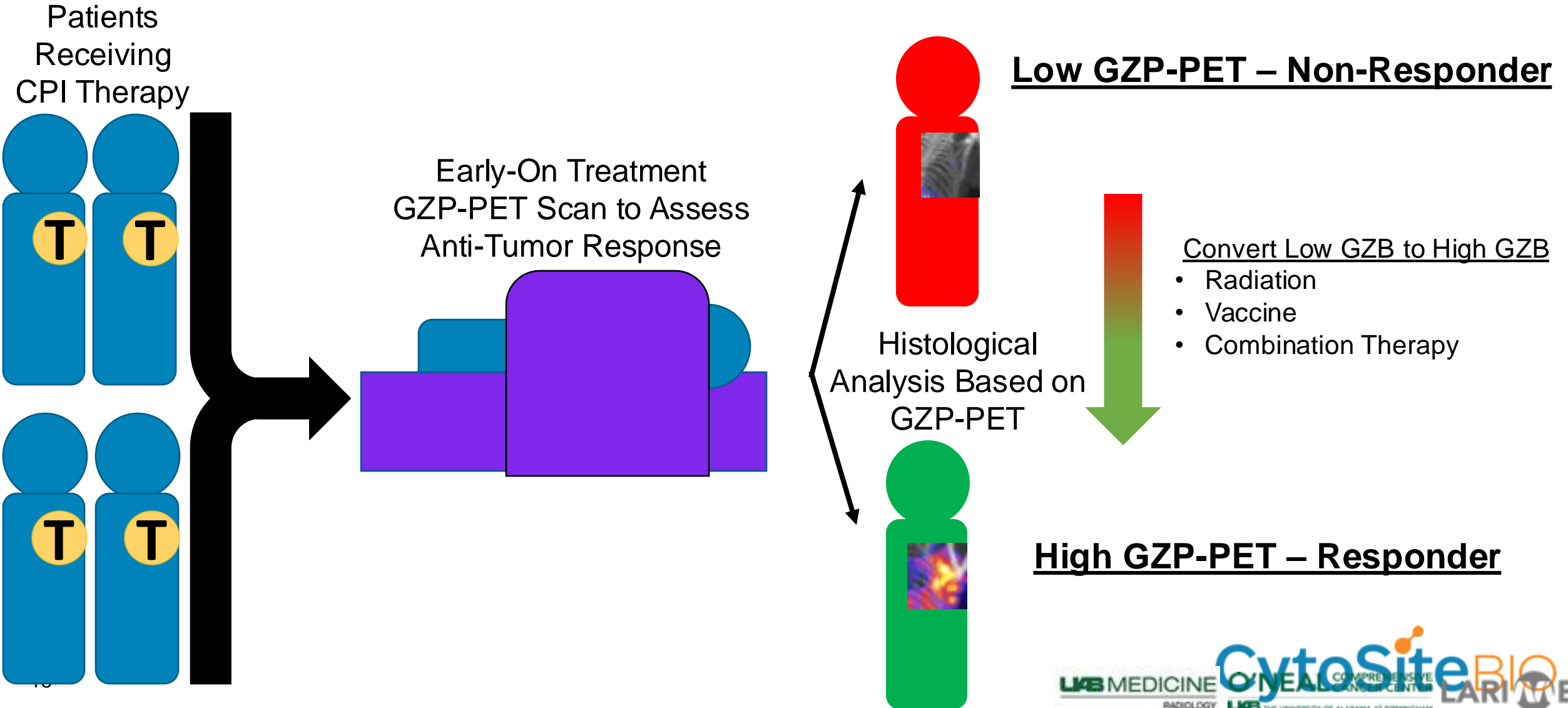
GZP PET Assessment of Longitudinal ACT



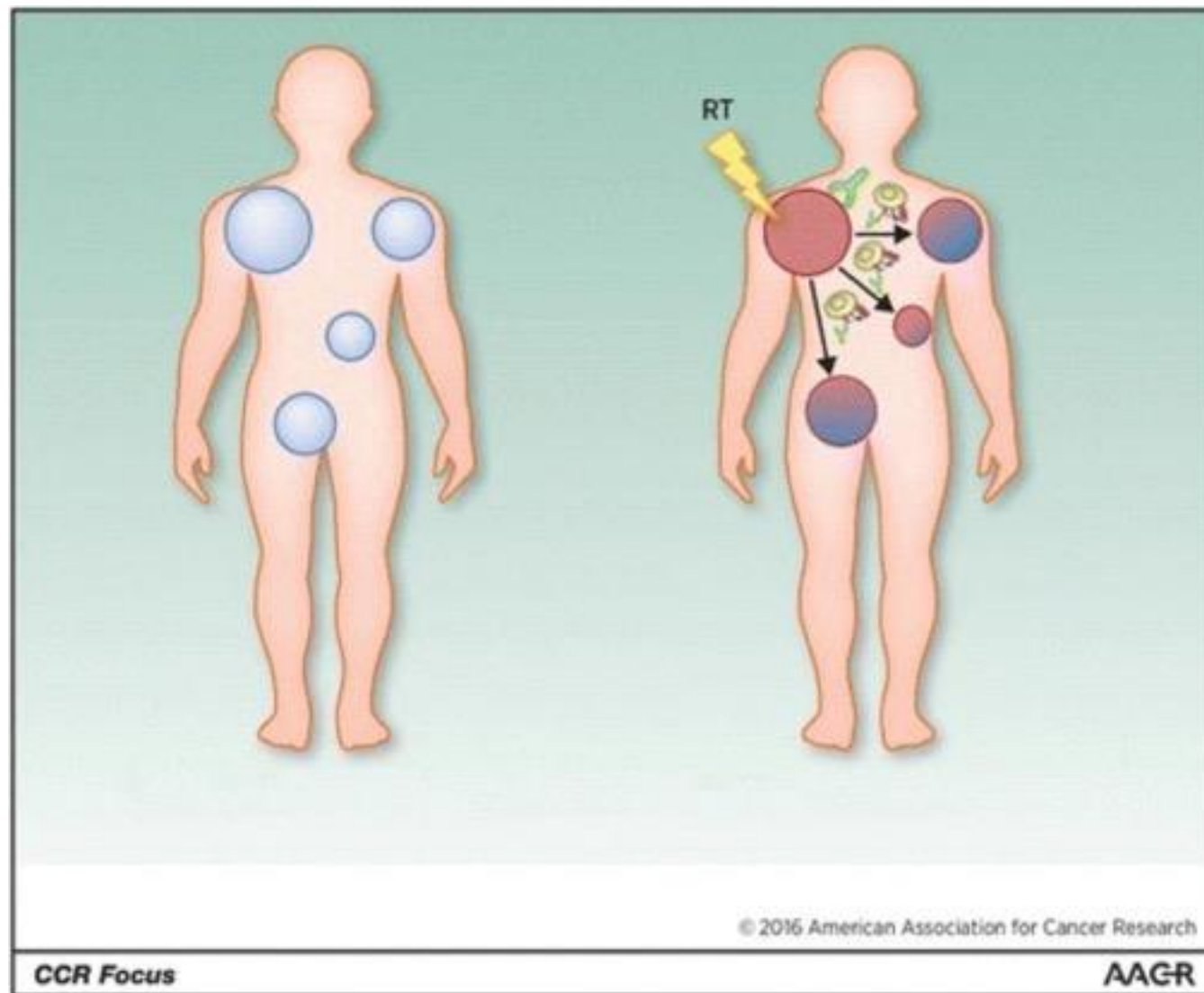
Mean Tumor Granzyme B PET Signal



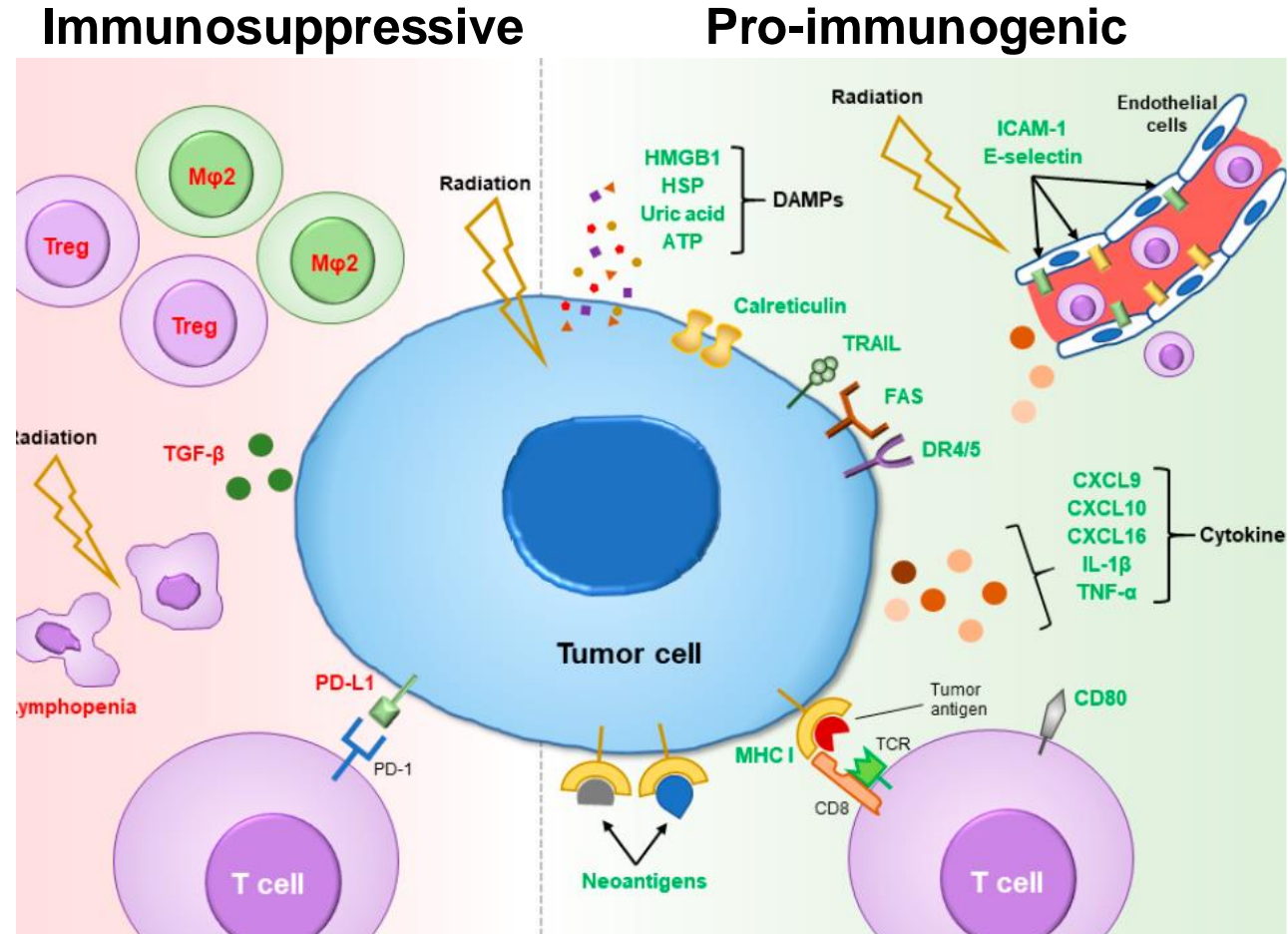
Correlations between GZP-PET and Cytokine Phenotypes



Quantifying the Abscopal Effect

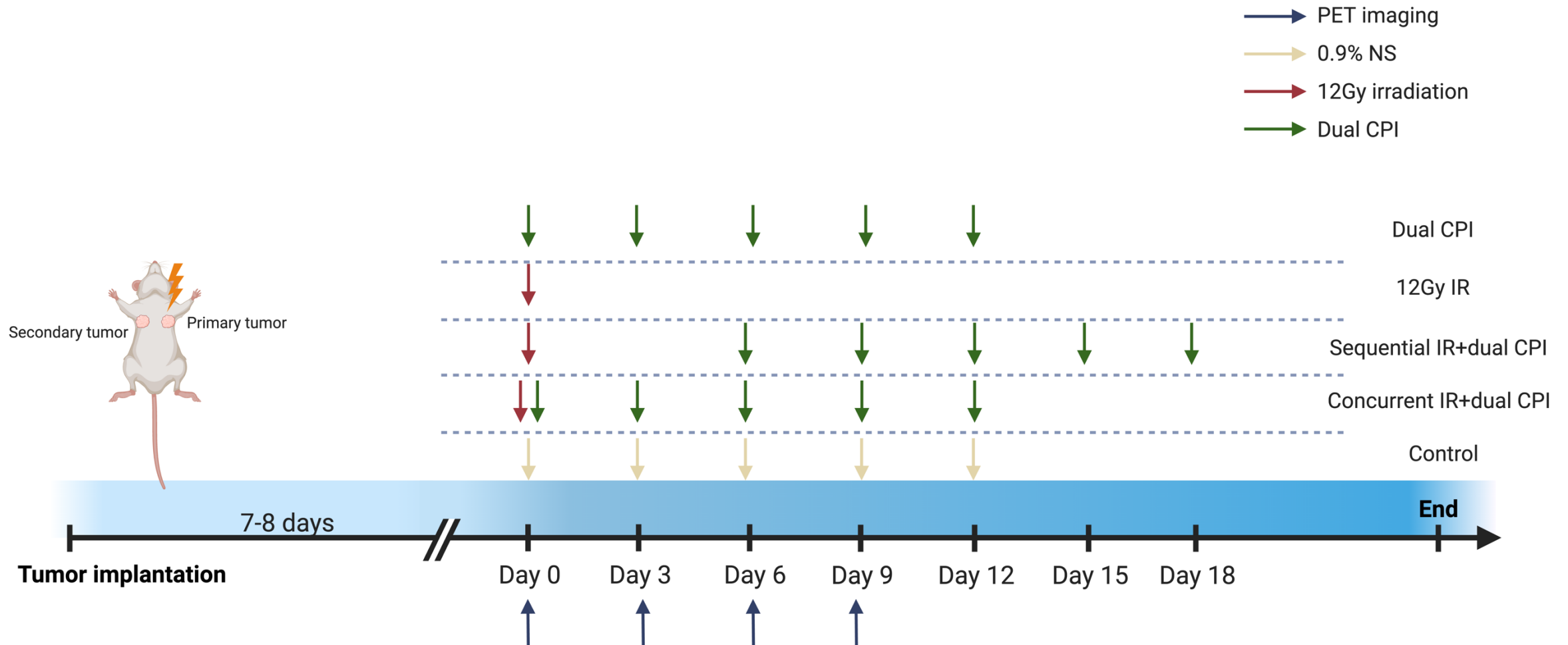


Pleiotropic Effects of Radiation on the Tumor Microenvironment

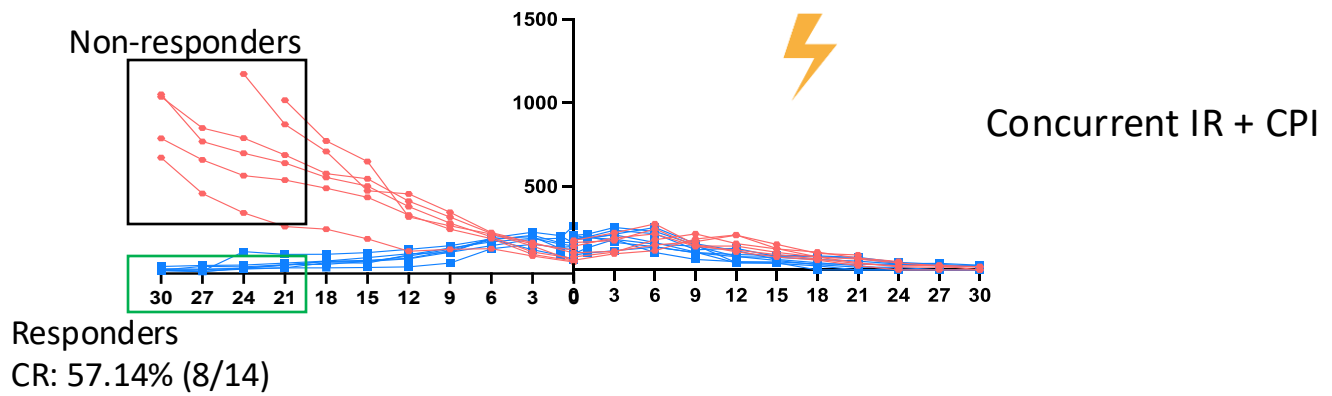
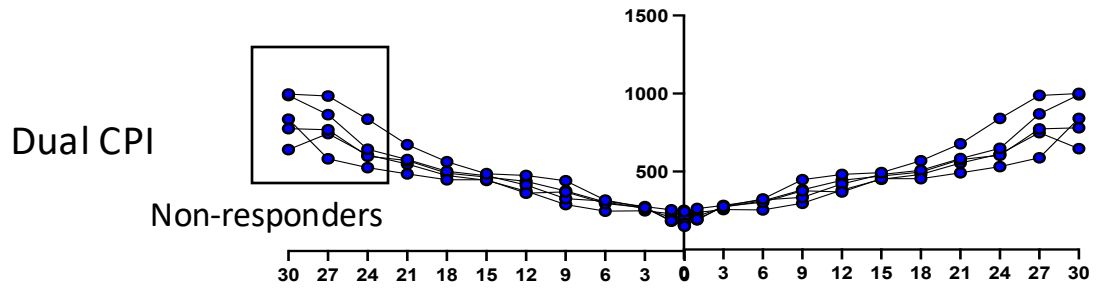
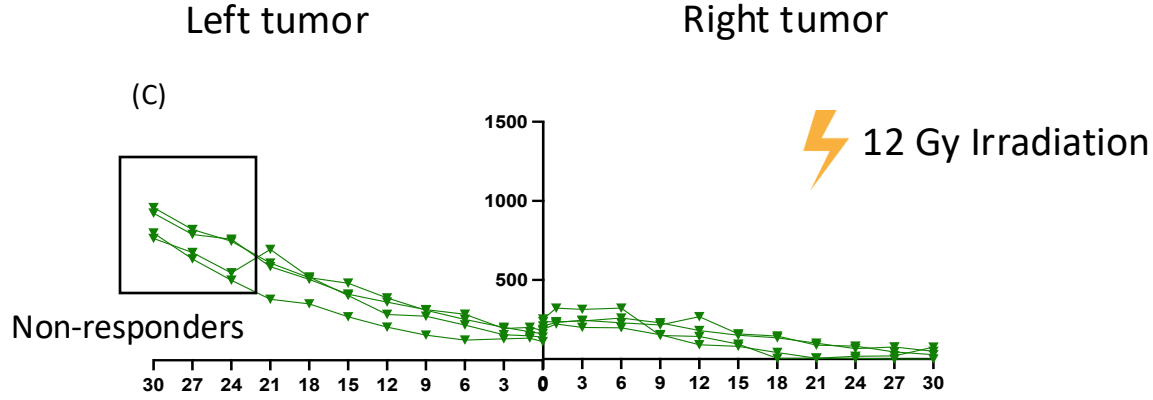
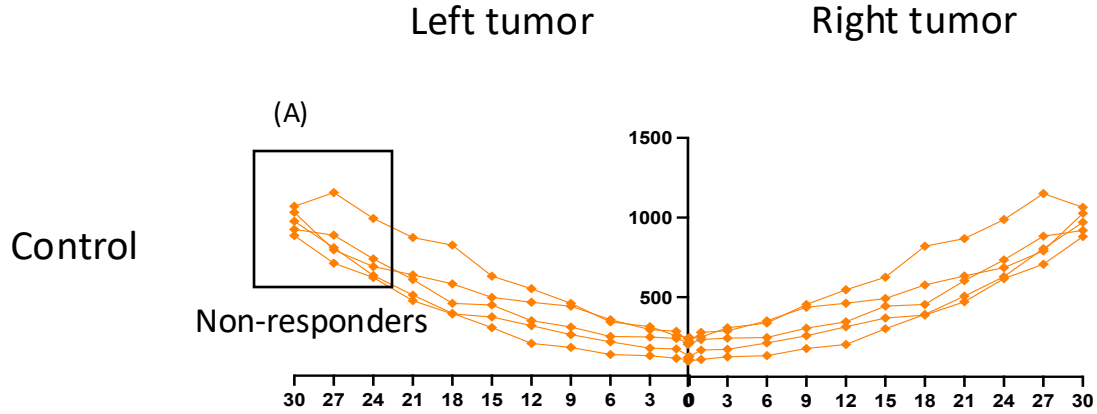


Abscopal Effect Tumor Growth Curves

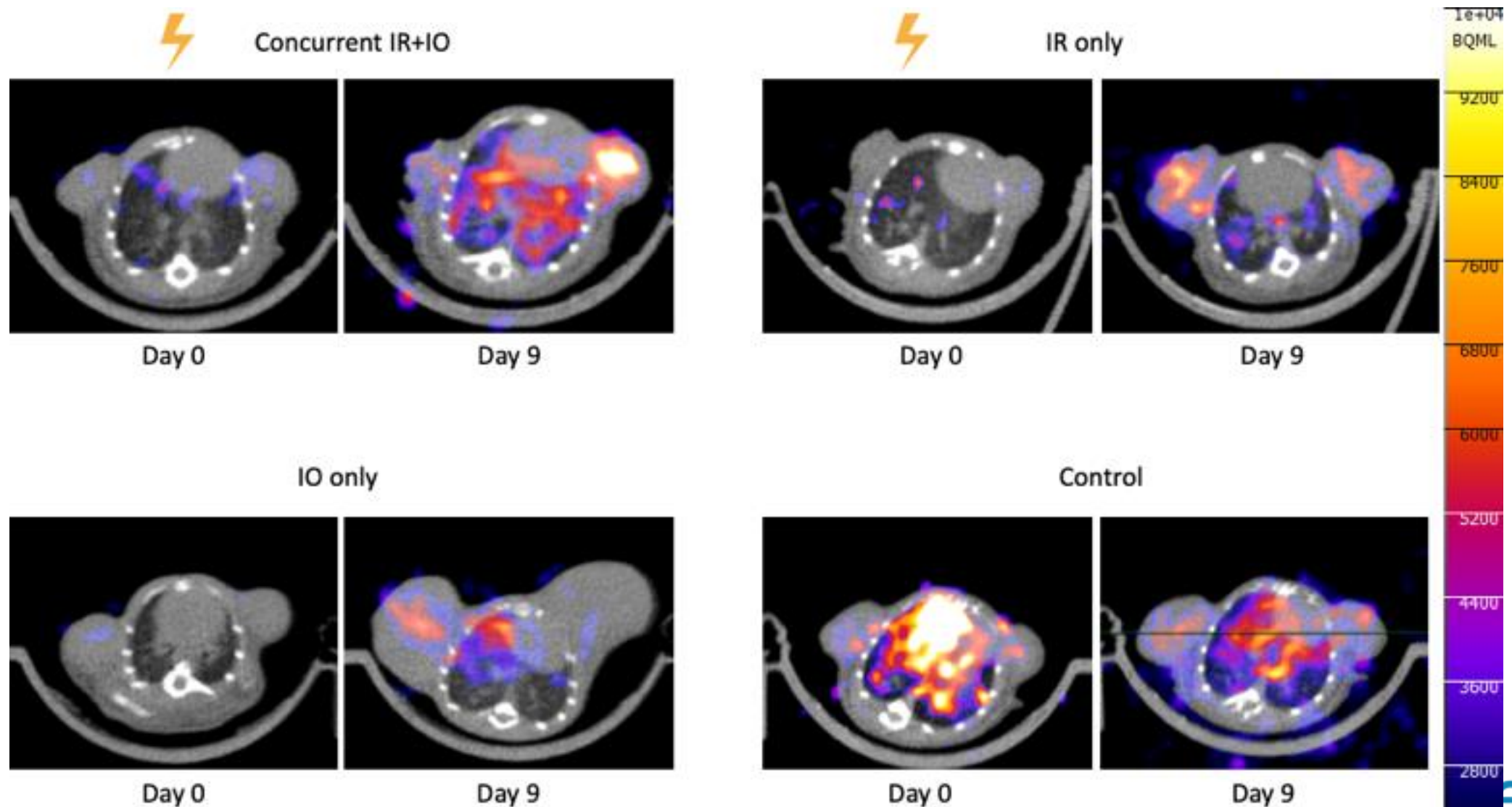
Tumor Volume (mm³)



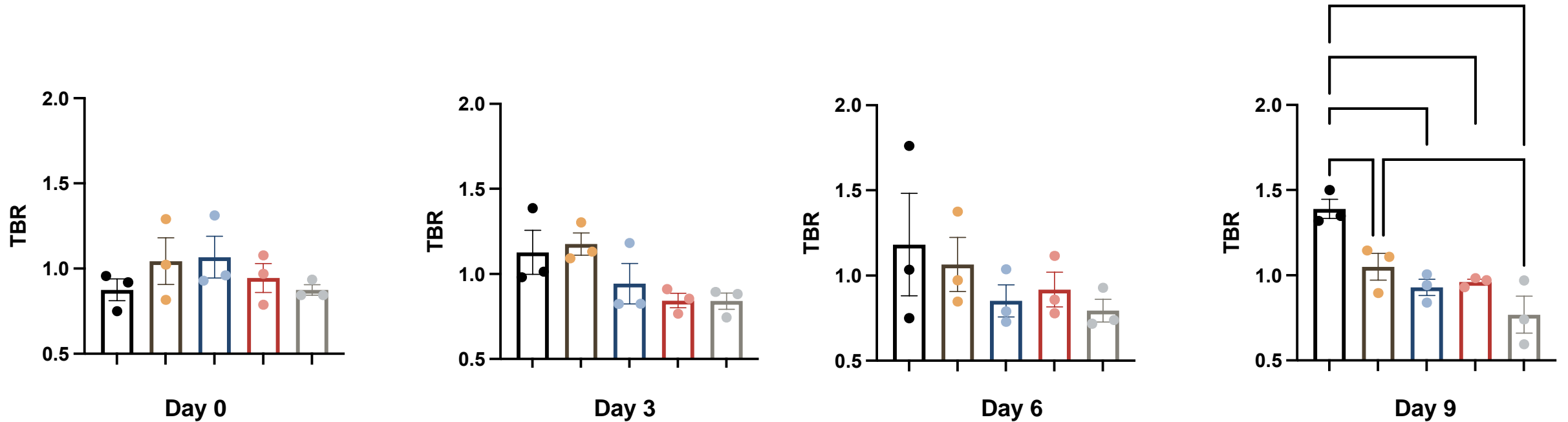
Abscopal Effect Tumor Growth Curves



Granzyme B PET Imaging Identifies the Abscopal Effect



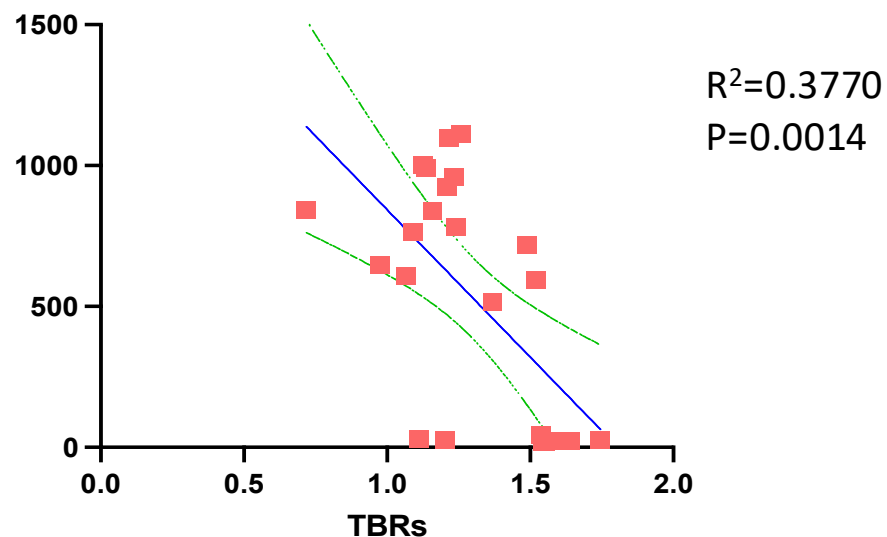
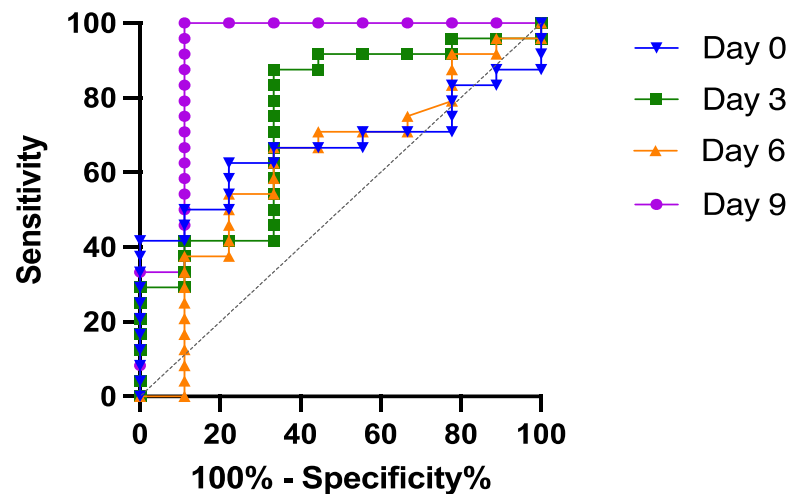
Longitudinal Granzyme B PET Imaging



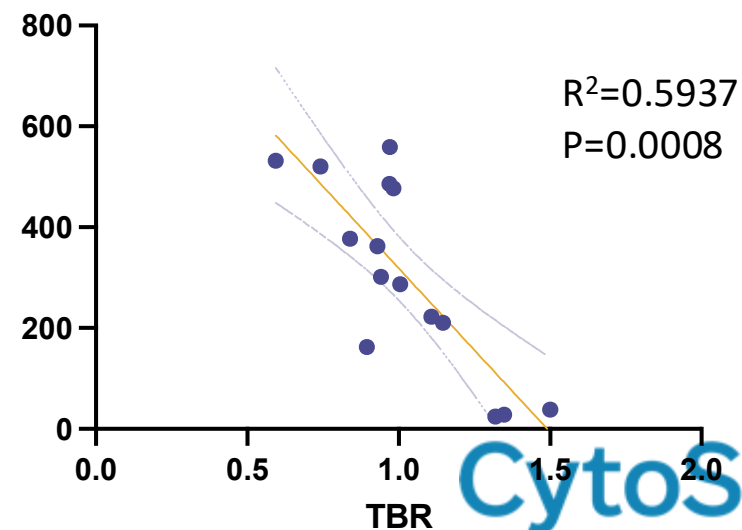
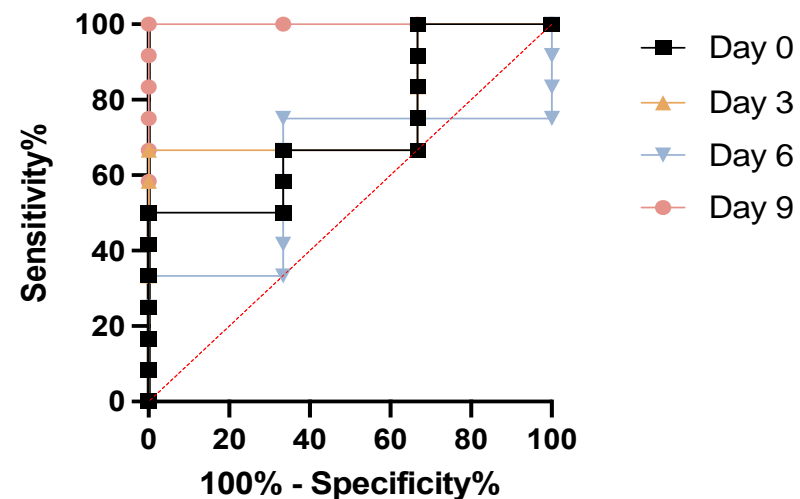
- Control
- 12 Gy irradiation
- Dual CPI
- Concurrent non-responding
- Concurrent responding

Granzyme B PET Imaging Identifies the Abscopal Effect

Triple Negative Breast Cancer



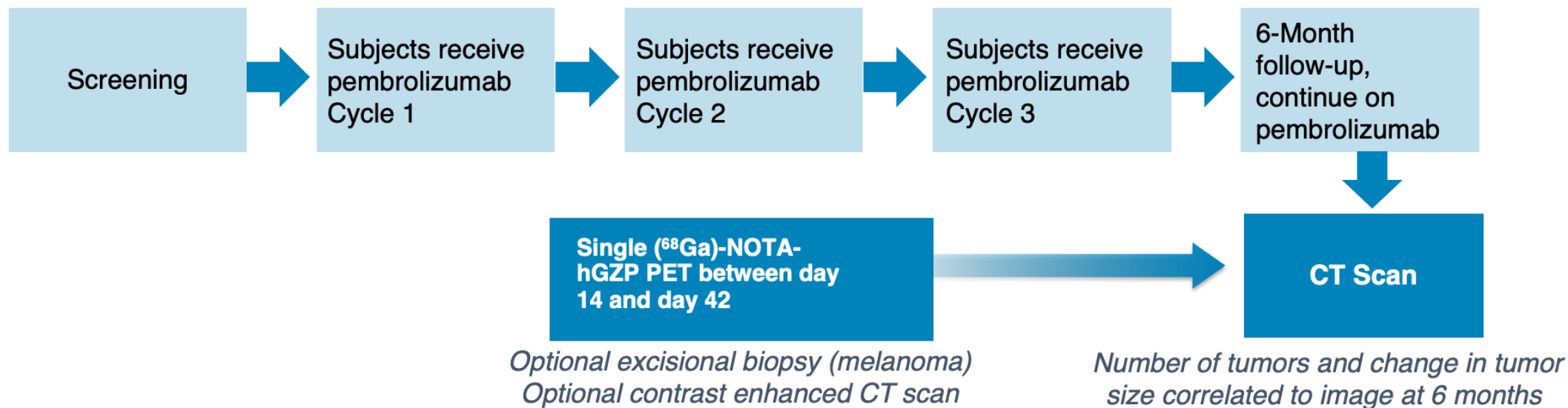
Lewis Lung Carcinoma



First-in-Human Granzyme B Clinical Trial

⁶⁸Gallium First-in-Human Trial: Design

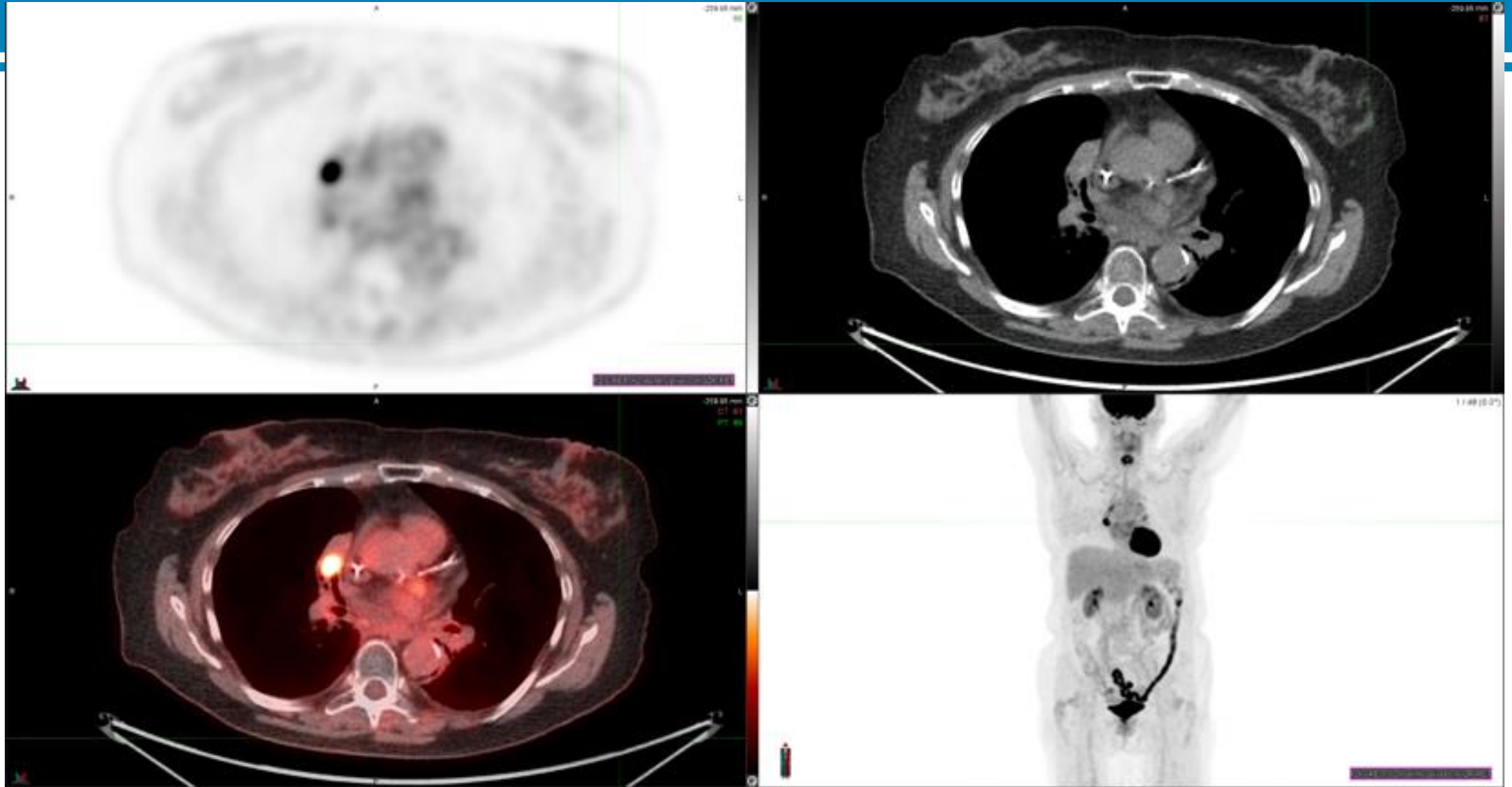
20 patients with metastatic melanoma or NSCLC, treated with 1L pembrolizumab (Keytruda)



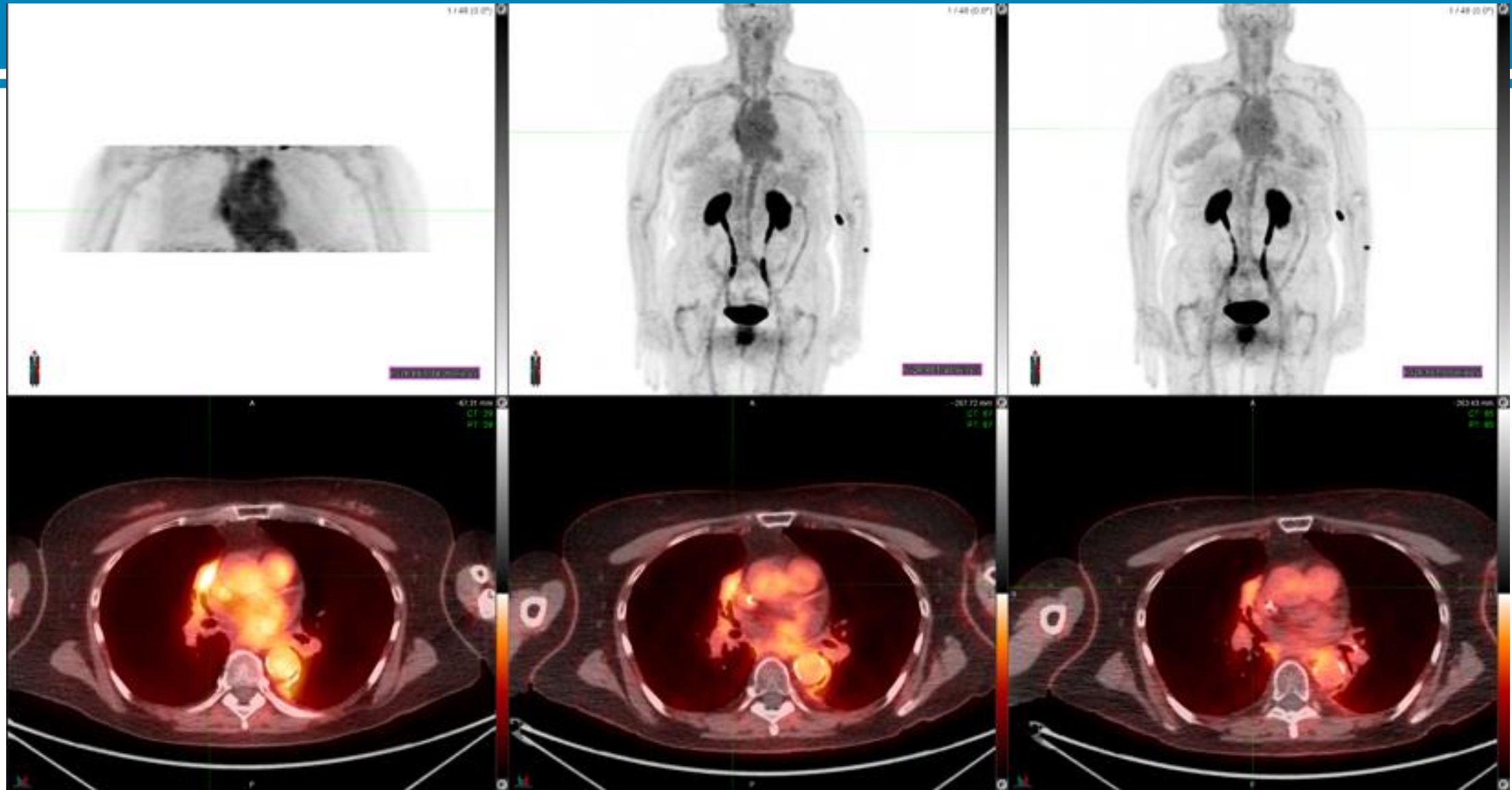
Trial Sites:

- Massachusetts General Hospital, in Boston (MGH)
- University of Alabama Birmingham (UAB)
- Chang Gung Memorial Hospital, in covid-free Taiwan (CGMH)

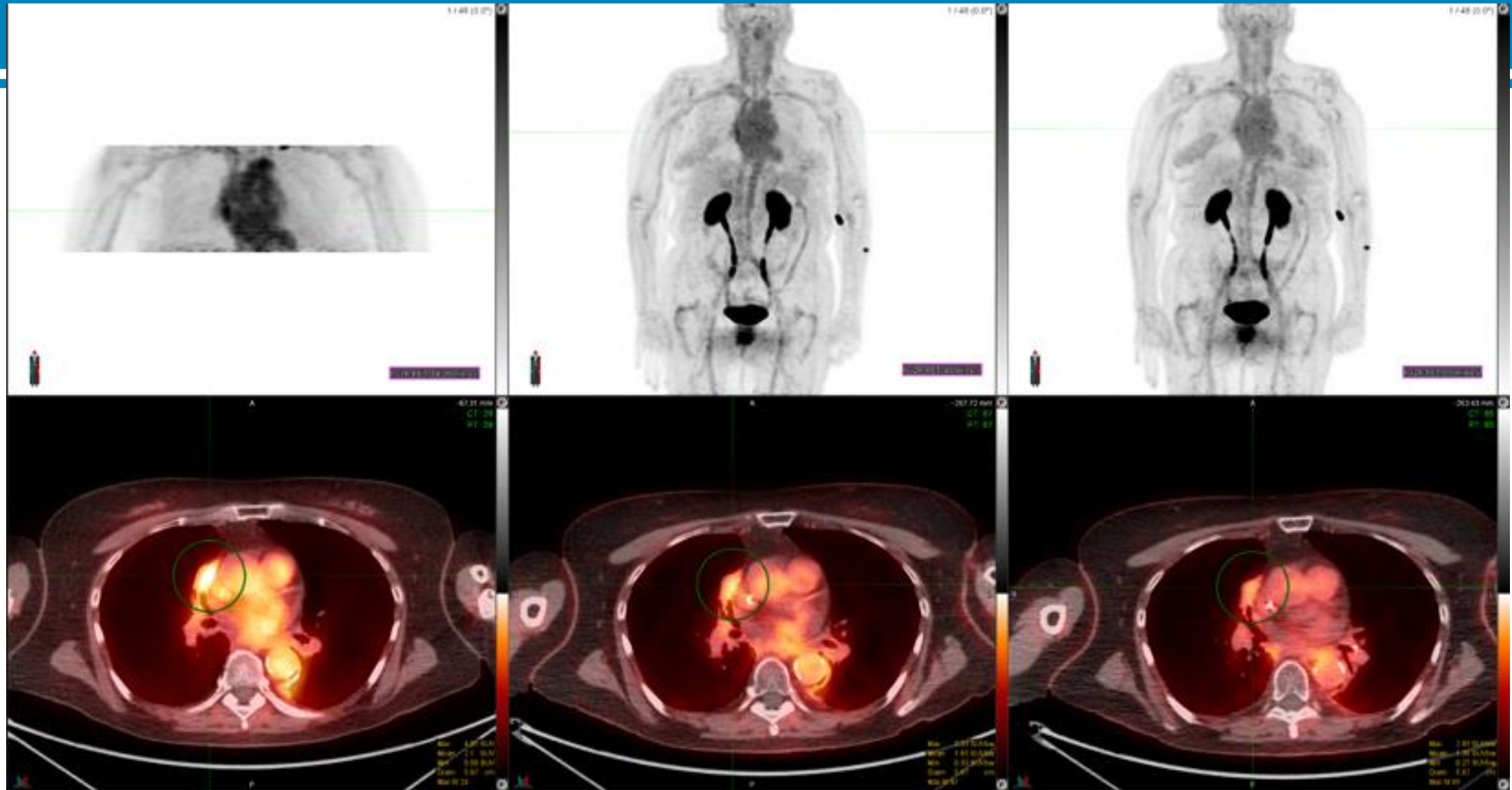
FDG-PET/CT 2 months prior to hGZP study



hGZP-PET/CT images



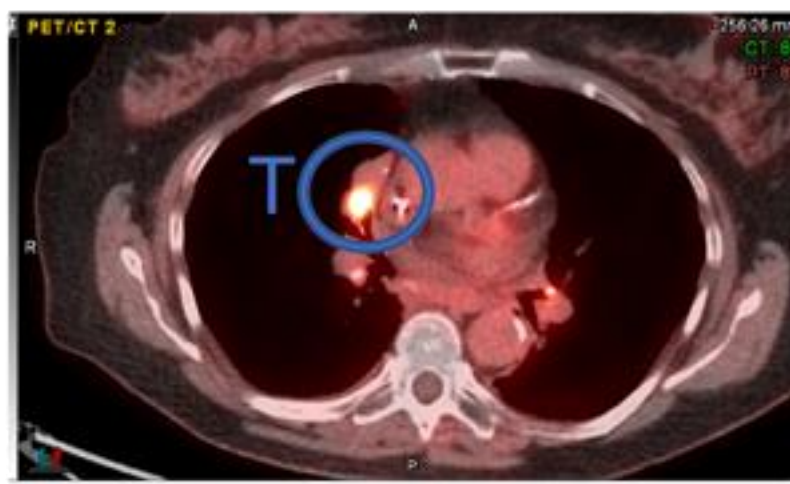
hGZP-PET/CT images



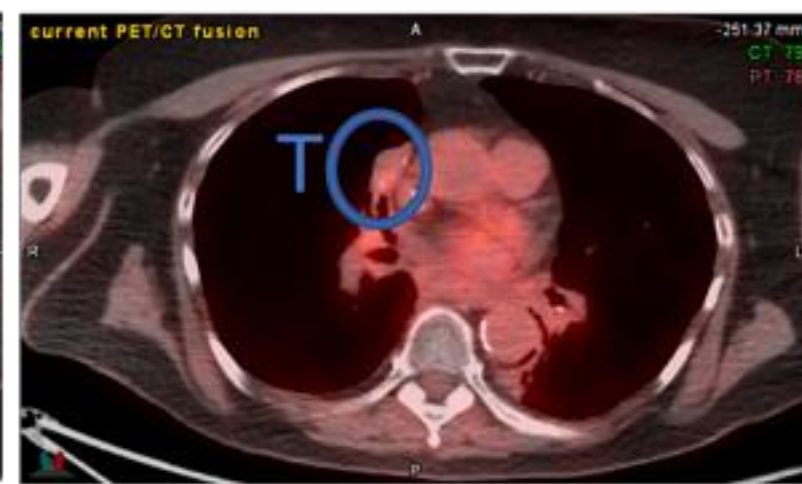
Follow-Up



GZP PET



FDG Pre-Tx



FDG Post-Tx

Selective Granzyme PET Tracer Uptake in Bone Metastasis Responding to Checkpoint Inhibitor Rx

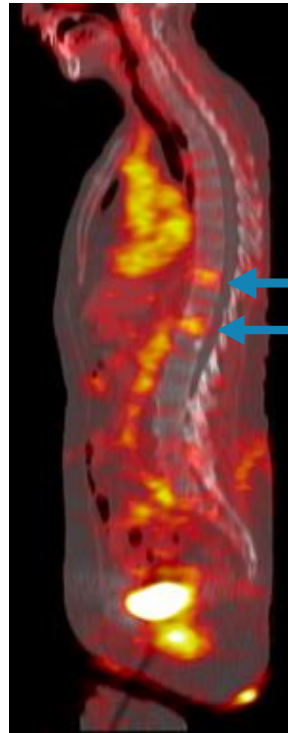
^{68}Ga Probe Uptake in Bone Metastases

- Selective visualization of GzmB tracer in the tumor
- Clear differentiation in uptake from tumor & surrounding normal tissue

Lateral View



CT Scan

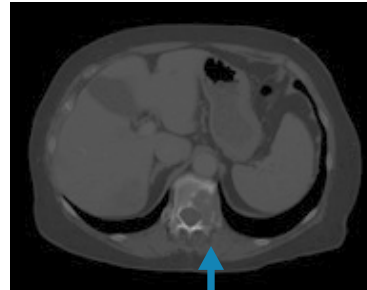


CT/PET Fused Image

T10
T12

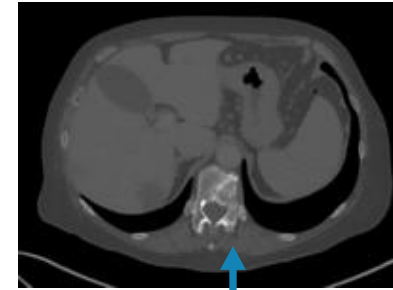
Axial View

Pembrolizumab



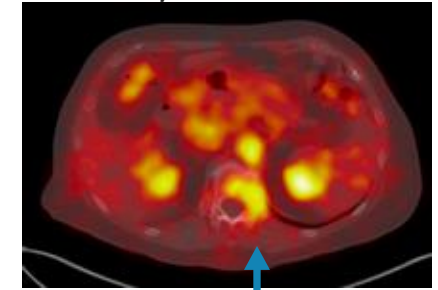
T12 metastatic lesion

Pembrolizumab



Tumor clinical response with bone regrowth

CT/PET Fused



GzmB tracer illuminating patient response

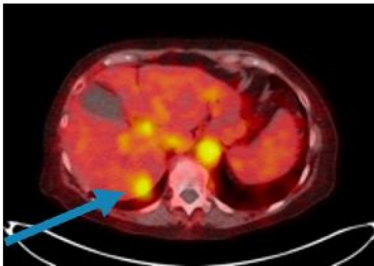
Note: Uptake (red/yellow) in lesions at T10 & T12 vertebrae

➔ Denotes metastatic lesion

Hepatic Metastasis Responding to Checkpoint Inhibitor Rx

Hepatic Metastasis Responding to I-O Therapy

GzmB PET

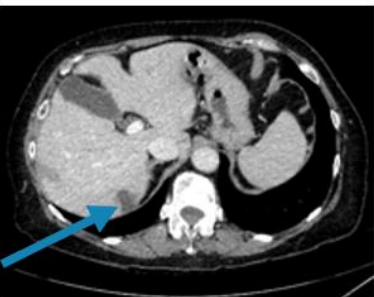


Nov. 2020

CT

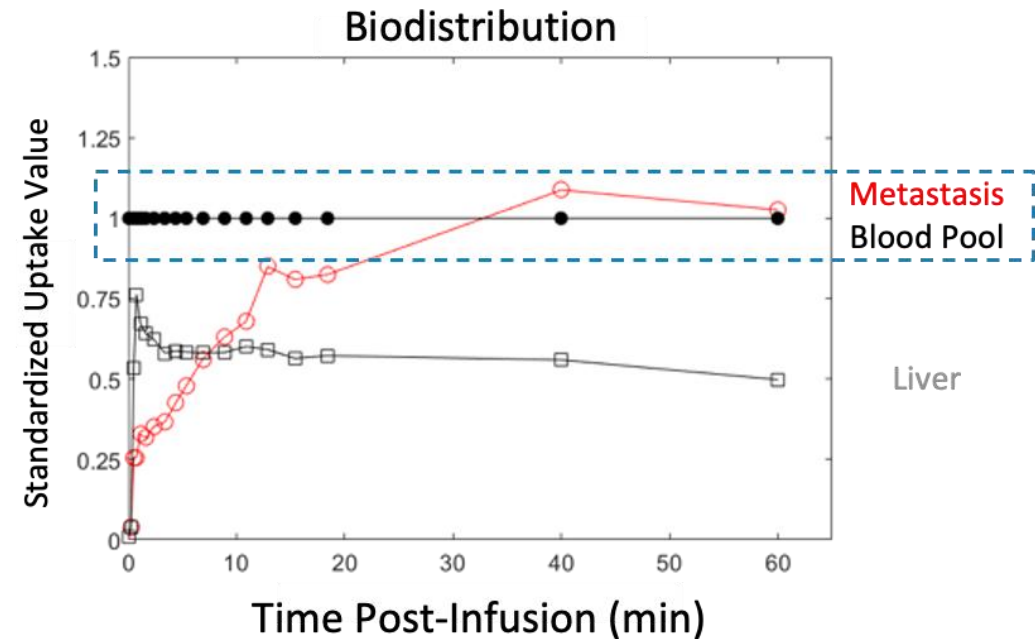


CT



Jun. 2021

Determination of Response Status

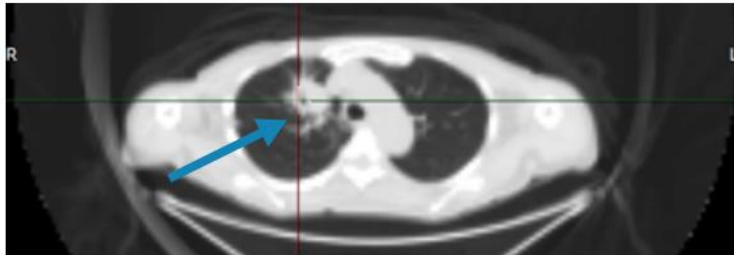


- Tracer uptake levels are determined in tumor & compared to blood pool
- Uptake levels higher in tumor than blood pool indicates a positive response

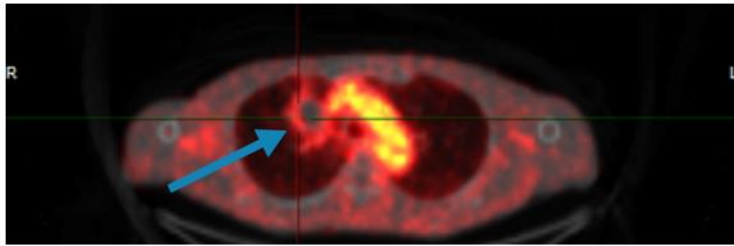
Metastatic Lung Lesion Not Responding to Checkpoint Inhibitor Rx

Metastatic Lesion Not Responding to I-O Therapy

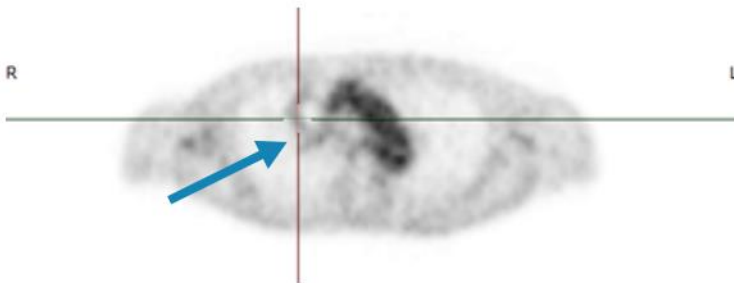
CT



GzmB
PET

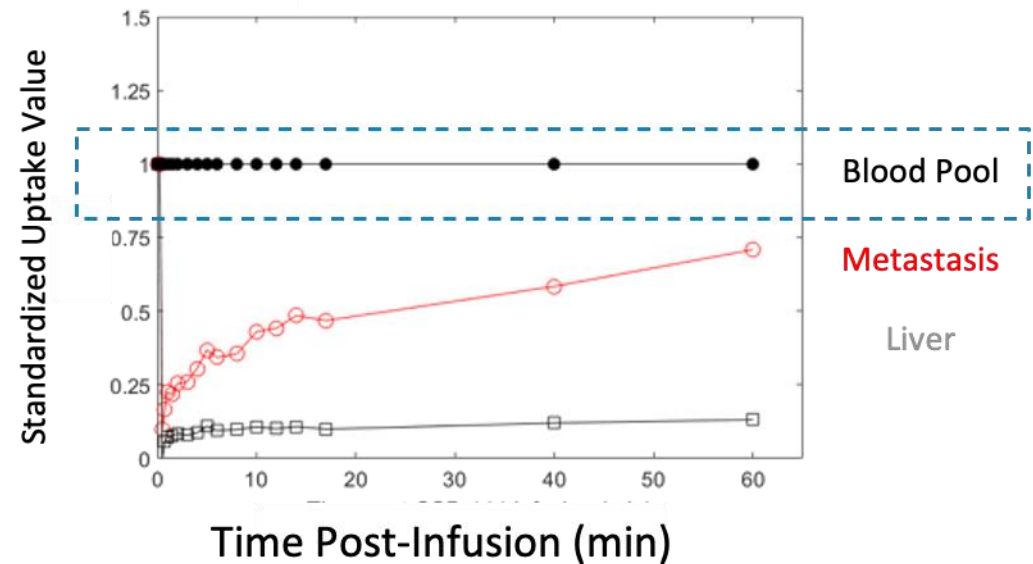


PET



Determination of Response Status

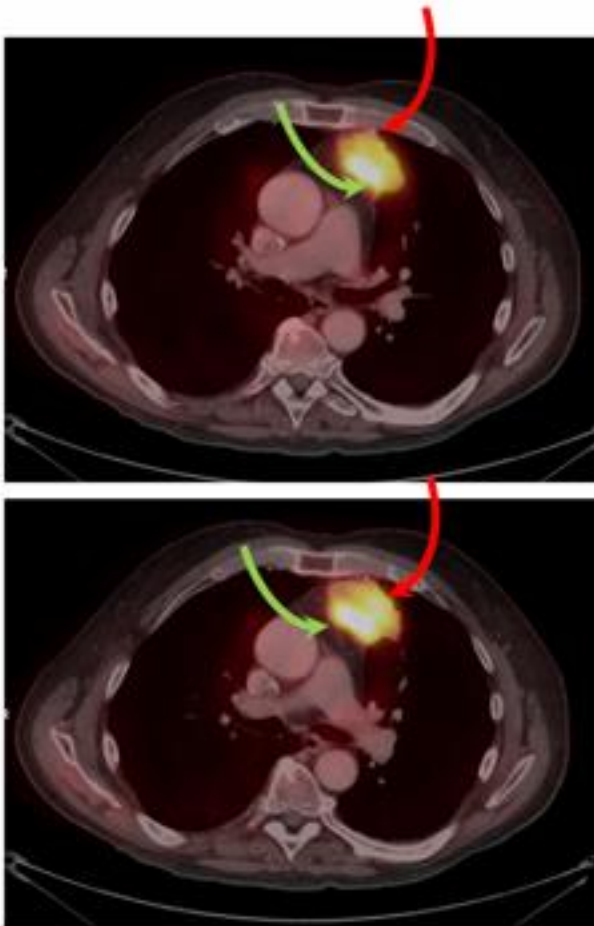
Biodistribution



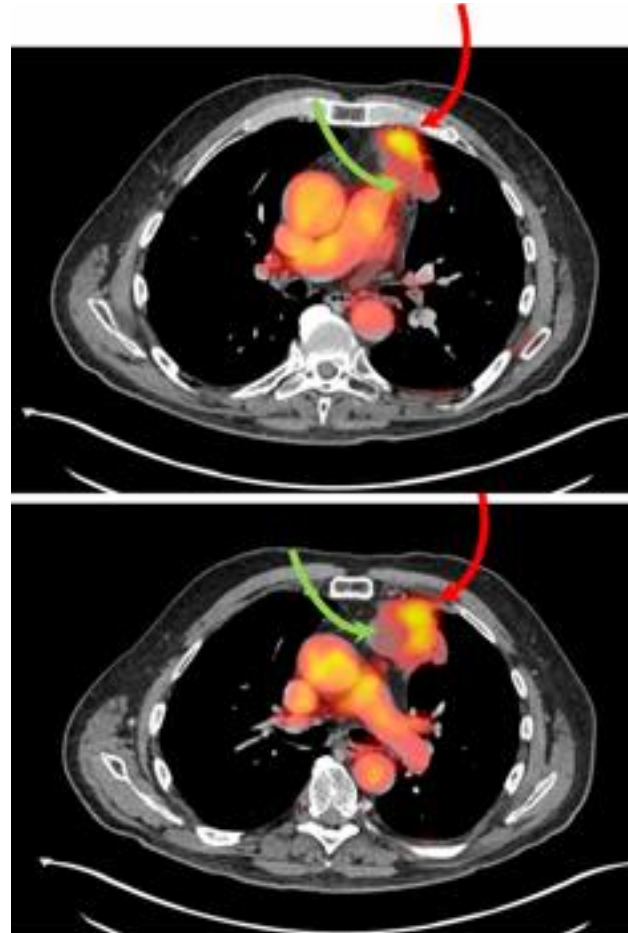
- Tracer uptake levels are determined in tumor & compared to blood pool
- Uptake levels lower in tumor than blood pool indicates non-response

Lesion showing Bimodal response: ^{18}F -FDG PET v GZB PET

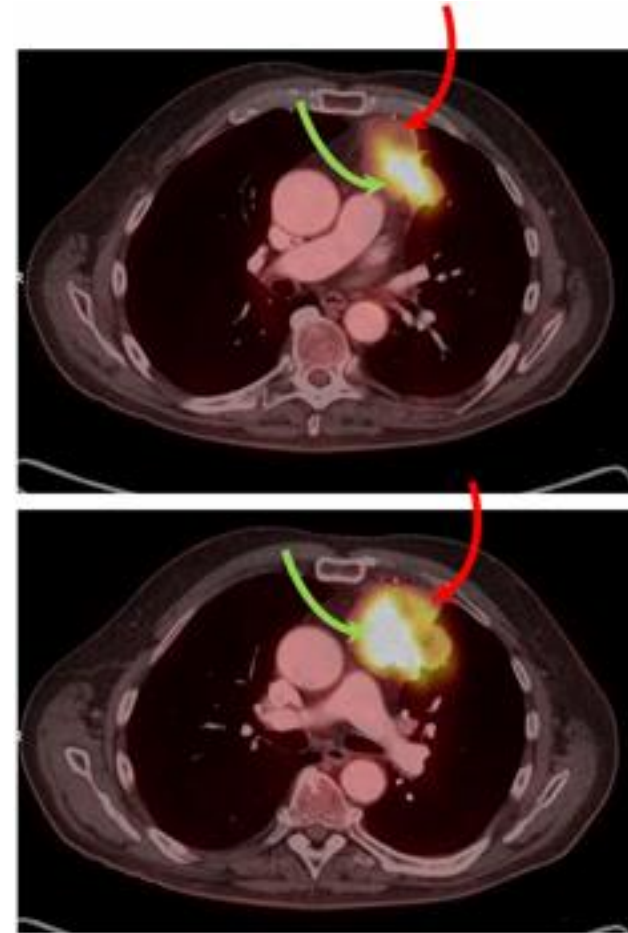
^{18}F -FDG PET scan acquired 4/2/21



GZB PET scan acquired 6/2/21

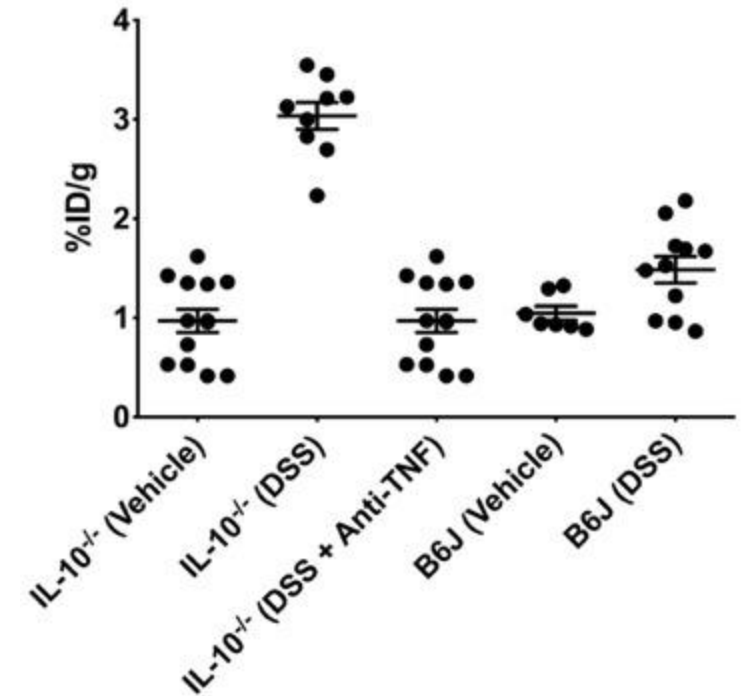
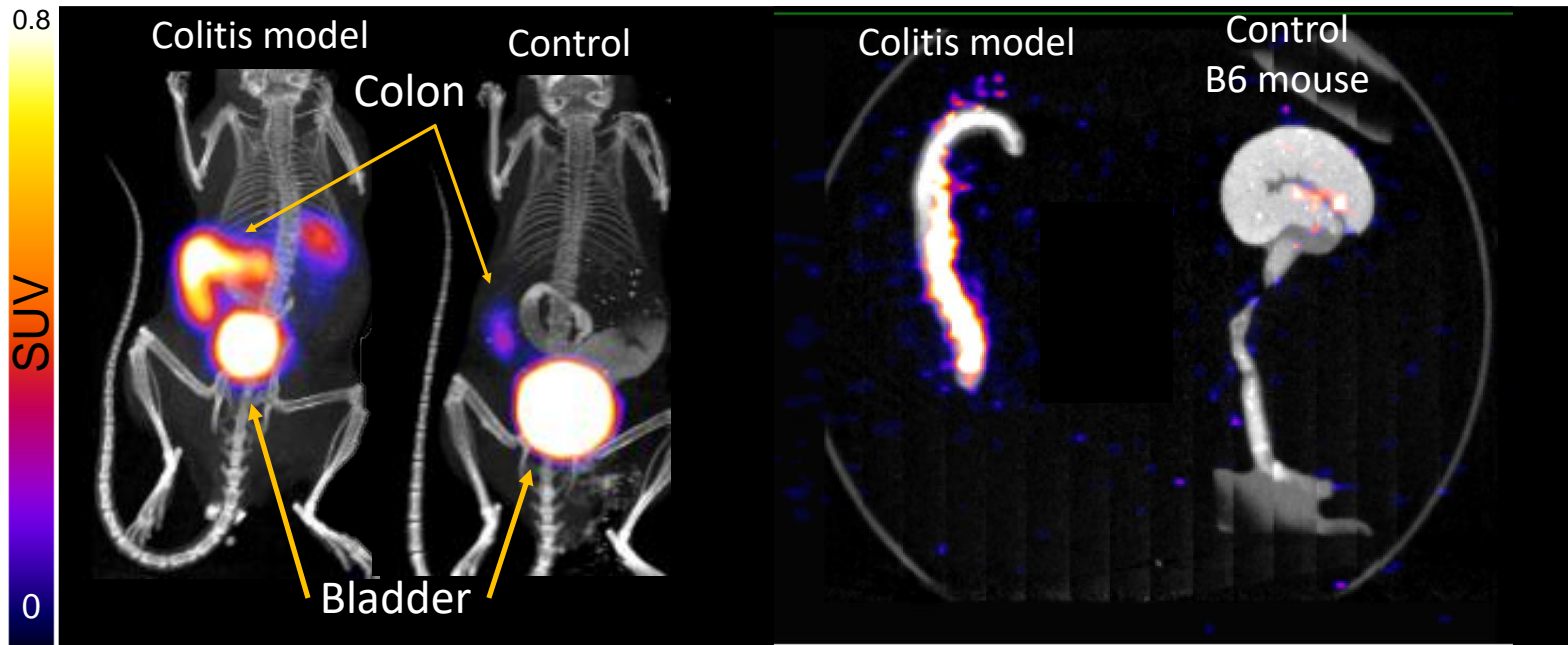


^{18}F -FDG PET scan acquired 7/26/21



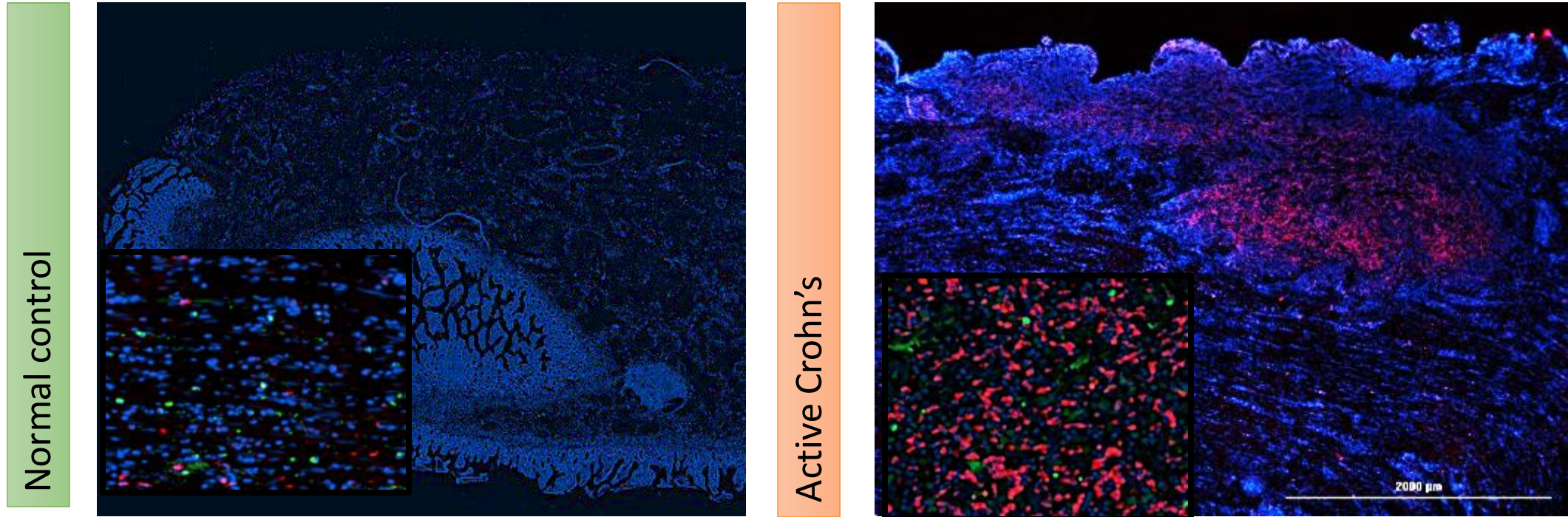
Non-Oncology Indications

Granzyme B Imaging of IL10^{-/-} Colitis Model



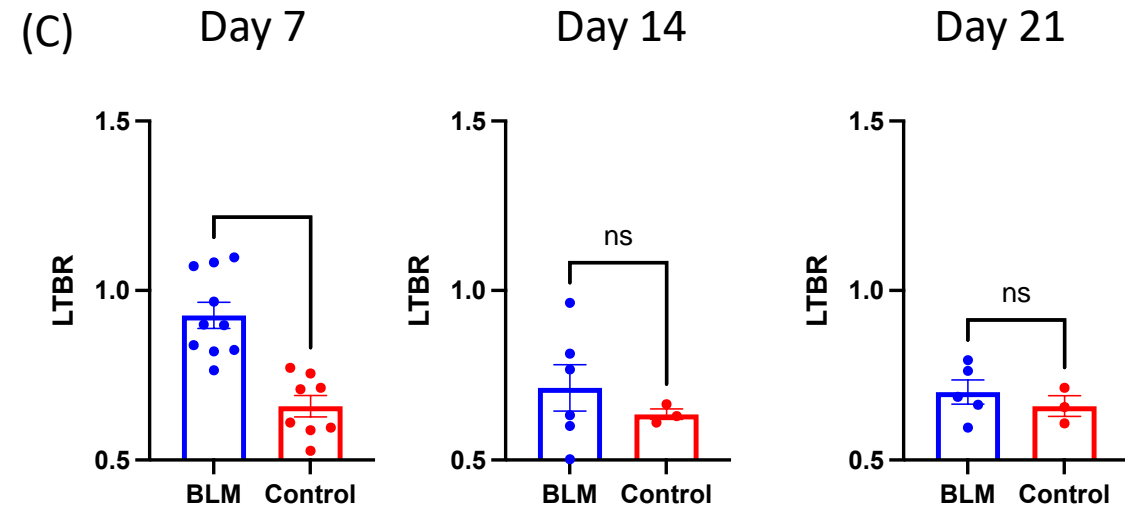
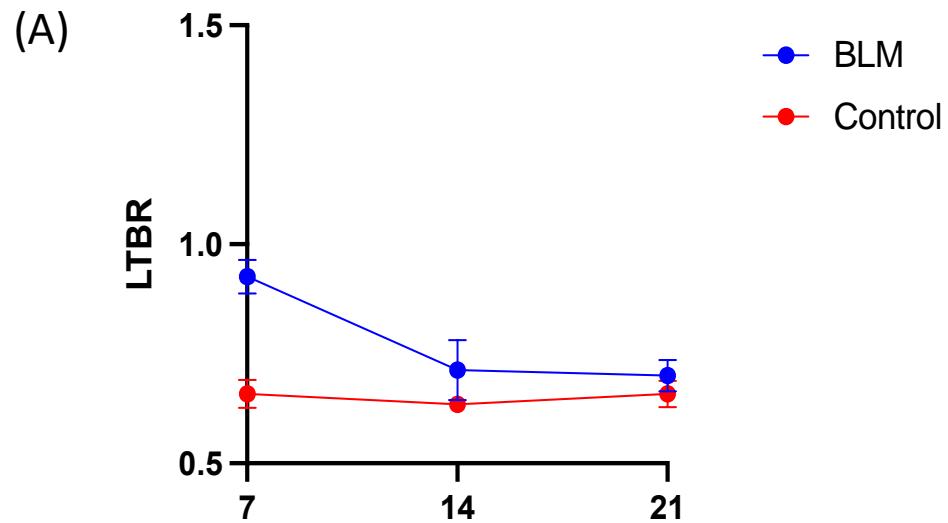
Mice with induced colitis have significantly higher granzyme B PET signal than control. TNF-blockade in this model reduces granzyme B PET signal to baseline.

Granzyme B IHC of Human IBD Confirms Expression in Active Disease

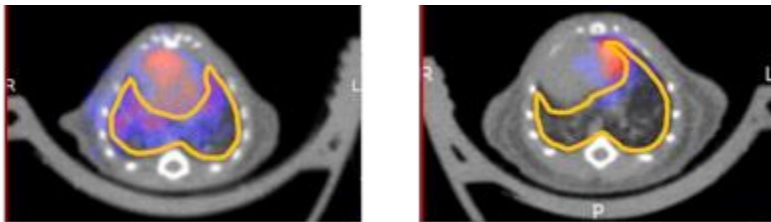


Tissue samples from normal control patients (left) and patients with active Crohn's disease right were stained for cellularity (blue) and granzyme B (red). Patients with active Crohn's have significantly higher granzyme B than control patients, suggesting utility of granzyme B imaging in these patients.

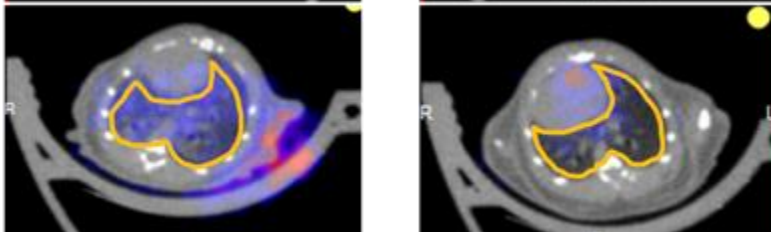
Granzyme B PET signal is elevated early during inflammatory phase of IPF



Day 7



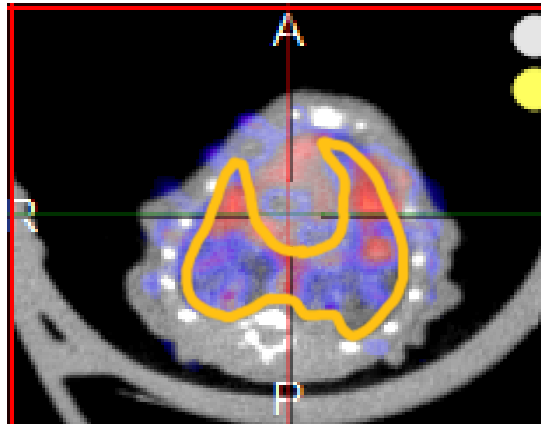
Day 14



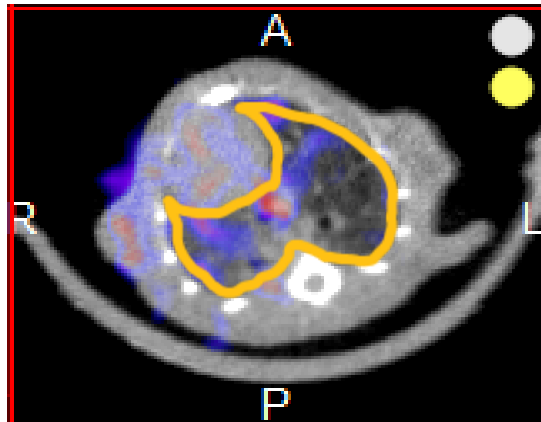
Longitudinal Imaging of granzyme B (as measured by total lung to blood ratio - LTBR) in mice reveals high levels of granzyme B 7 days after bleomycin instillation. As fibrosis develops (days 14-21) the granzyme B signal returns to baseline.

GZP PET imaging quantifies pirfenidone treatment efficacy

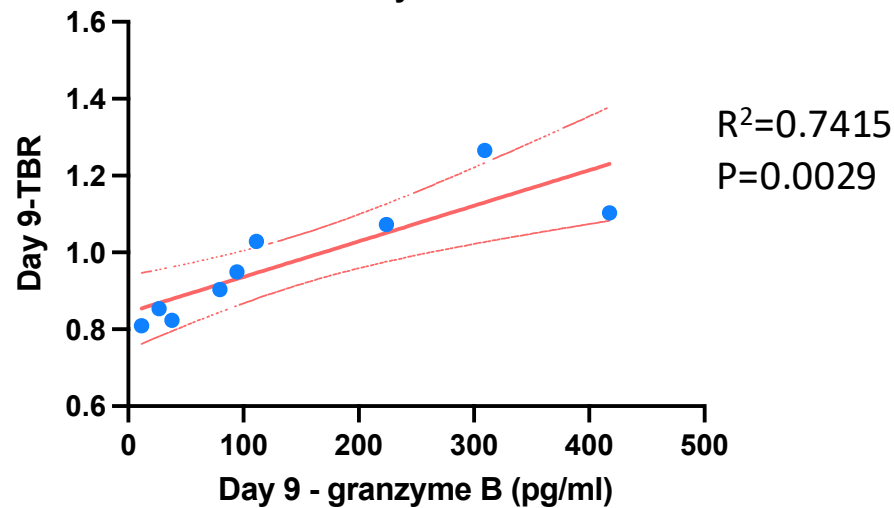
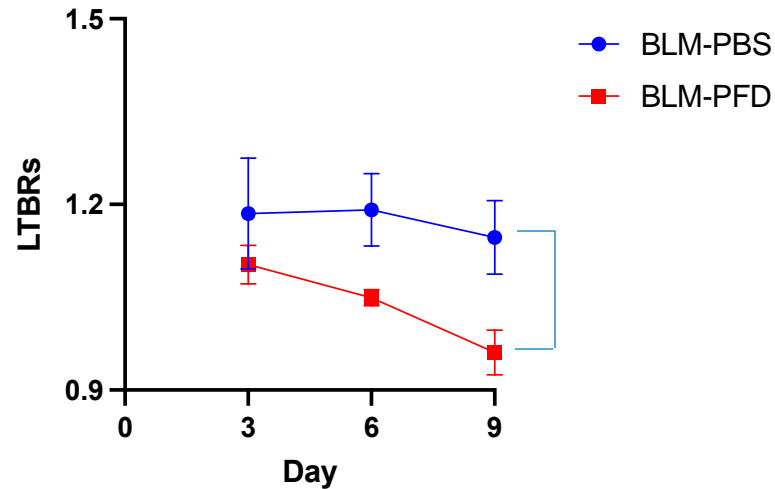
BLM-PBS



BLM-PFD



Day 9



GZP PET imaging in bleomycin-instilled mice with and without pirfenidone treatment reveals decreases in GZP-PET signal in the pirfenidone group consistent with decreased evidence of subsequent fibrosis by CT.

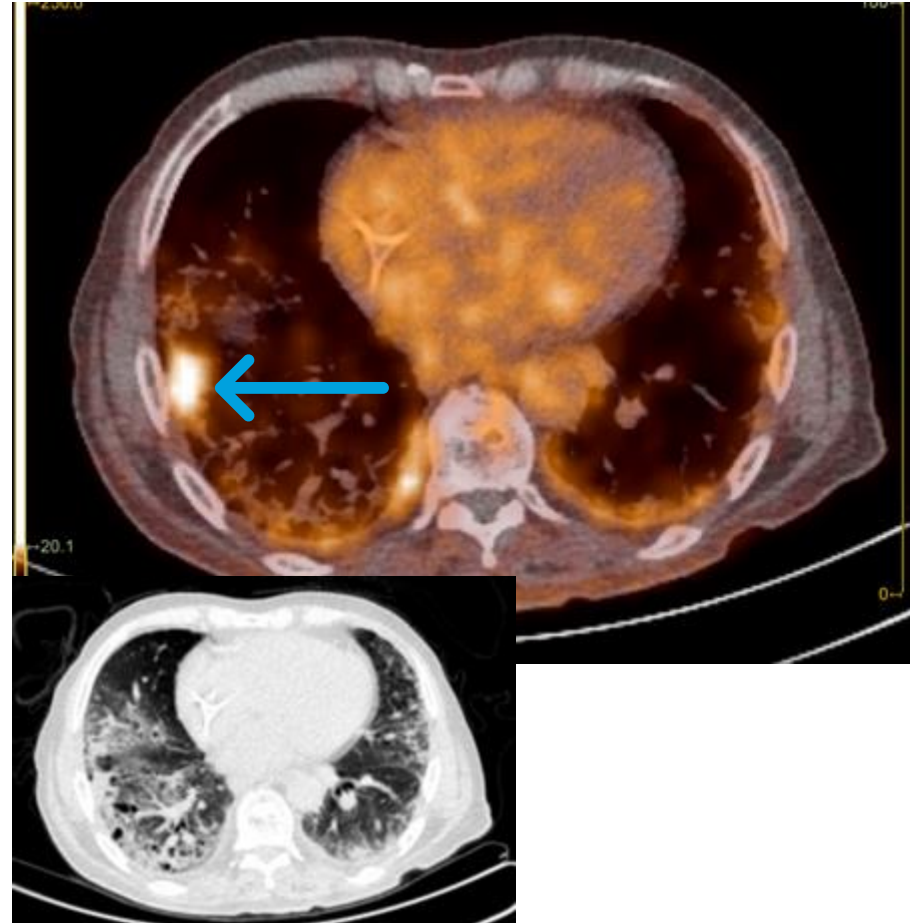
GZP PET signal is correlated with the amount of granzyme B detected by ELISA in the bronchial alveolar lavage fluid of mice.

Clinical Granzyme B PET Imaging Visualizes PET Uptake in Potential Sites of Inflammation

Colorectal Cancer Patient Lung Disease Imaging

Both right and left lung have areas of focal GZB tracer uptake in areas of consolidation

CT images consistent with either inflammation or metastasis – awaiting clinical confirmation



Cytosite Pharmaceutical Services

- Several clinical trials completed
- Multiple existing pharmaceutical partners
- >95% Sensitivity and Specificity
- Experience at 5 sites across 3 continents

Experienced Clinical and Regulatory Team

- Three INDs approved
- Clinical trials across three continents

Modular IND

- Designed to accommodate new cohorts
- Can be appended to existing INDs

Real-Time Trial Updates and Data Sharing

- Web-based portal provides notification of enrollment and instant image access

The Value of PET Imaging for Drug Development

- Complimentary to biopsy
 - ▶ Whole body
 - ▶ Functional information
 - ▶ Longitudinal
- Non-invasive
 - ▶ Access sites not amenable to biopsy, not specific to blood biomarkers
- Nuclear medicine is expanding
 - ▶ Better technology
 - ▶ More access
 - ▶ Lower cost



CytoSite^{BIO}

Contact:

Ben Larimer, CEO

blarimer@cytositebio.com