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Preclinical CAR T cell target safety, biodistribution, and tumor infiltration analysis using an in situ hybridization technology

Courtney Anderson, PhD

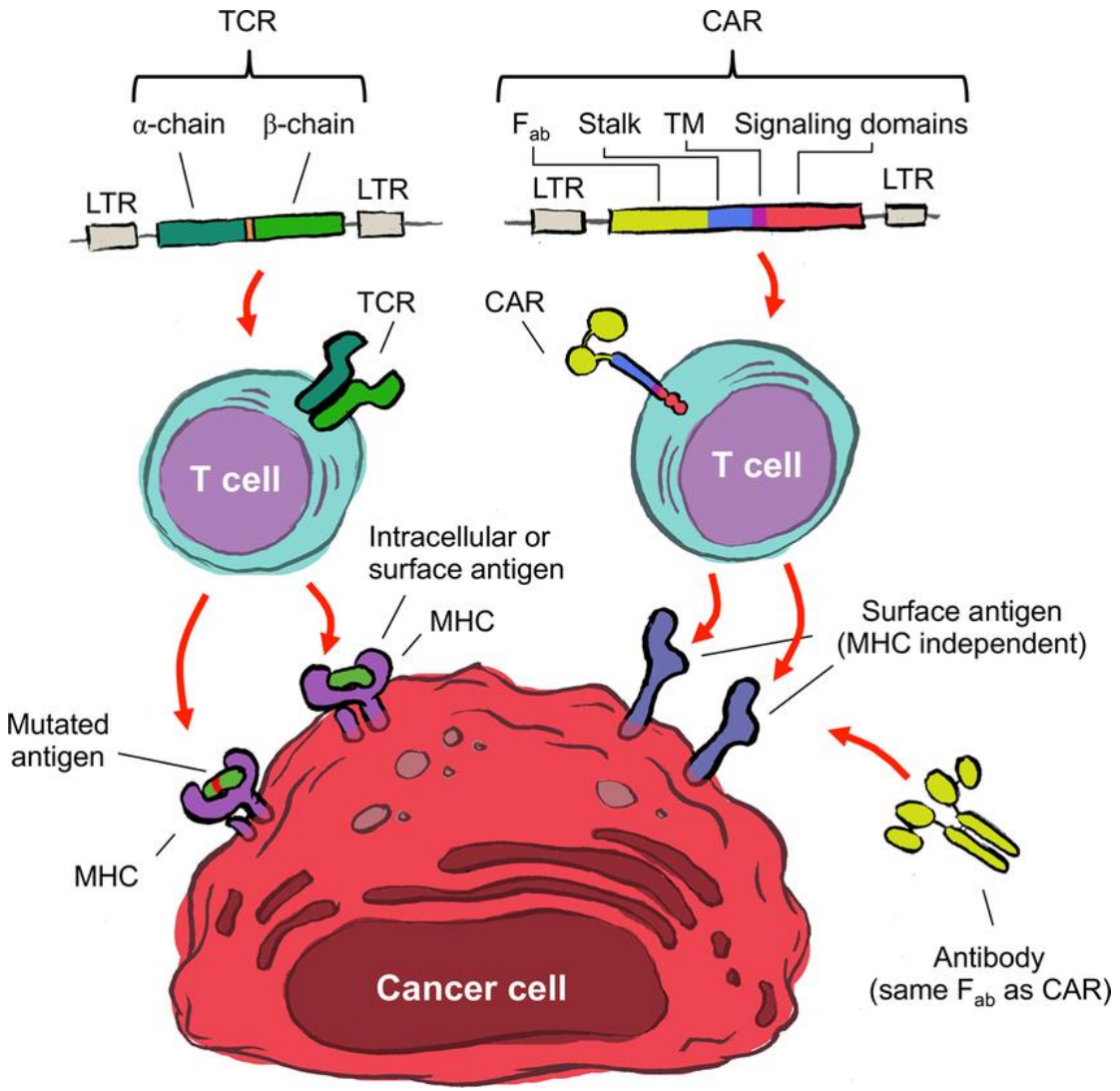
Research Manager, Applications

Advanced Cell Diagnostics, A Bio-Techne Brand

Disclosures

- Authors are employees of Bio-Techne or Bluebird Bio

The need for preclinical safety assessment of engineered T cells

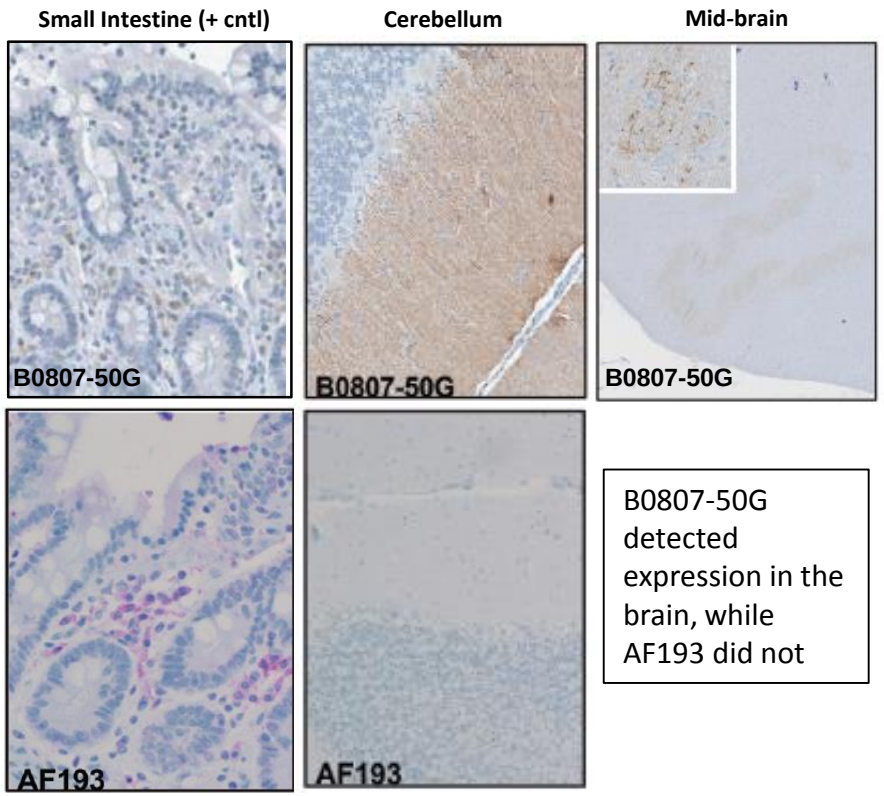


- TCR/CAR T cells are activated by target antigen engagement even at very low levels of target expression
  - Need to avoid adverse events resulting from “on-target/off-tumor” toxicity
- However, current methods are either not sensitive/specific enough to detect low levels of expression or not capable of detecting TCR/CAR T cells in the tissue context
- The highly sensitive and specific RNAscope assay can detect very low levels of target expression and track activated TCR/CAR T cells in tissue

Resolving discrepant IHC results for pre-clinical target assessment

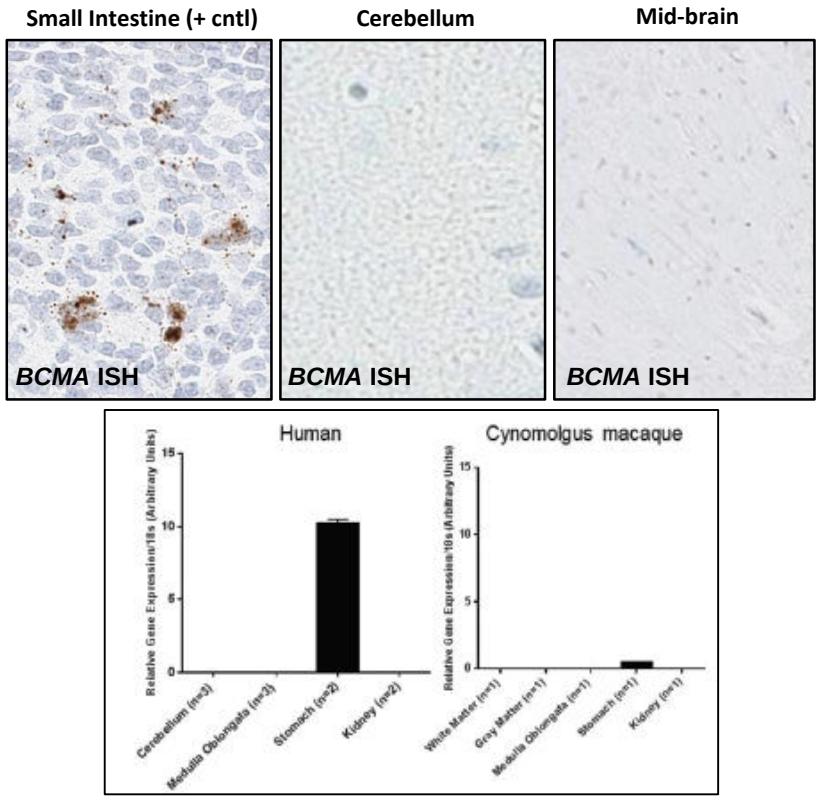
Bu et al., *Oncotarget*, 2018
Pre-clinical validation of B cell maturation antigen (BCMA) as a target for T cell immunotherapy of multiple myeloma

Disparate results between 2 different IHC antibodies for BCMA



B0807-50G detected expression in the brain, while AF193 did not

RNAscope and RT-PCR show no detection of *BCMA* in brain

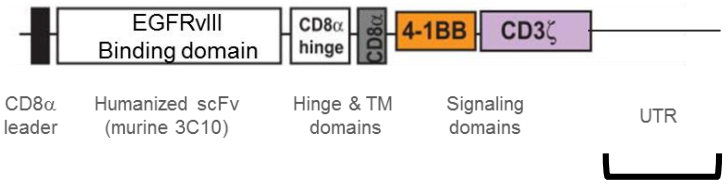


- BCMA is an attractive target for CAR T cell therapy in multiple myeloma (MM) but there have been limited reports on its expression in MM and normal tissues
- However, 2 commercially available antibodies gave discrepant results
- **RNAscope ISH** and RT-PCR were used to resolve these discordant results
  - Neither assay detected signal in the brain
- The immuno-reactivity of B0807-50G in the brain reflects cross-reactivity with an unknown epitope rather than BCMA

In situ characterization of CART-EGFRvIII in human glioblastomas

O'Rourke et al., *Sci Transl Med*, 2017
A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma

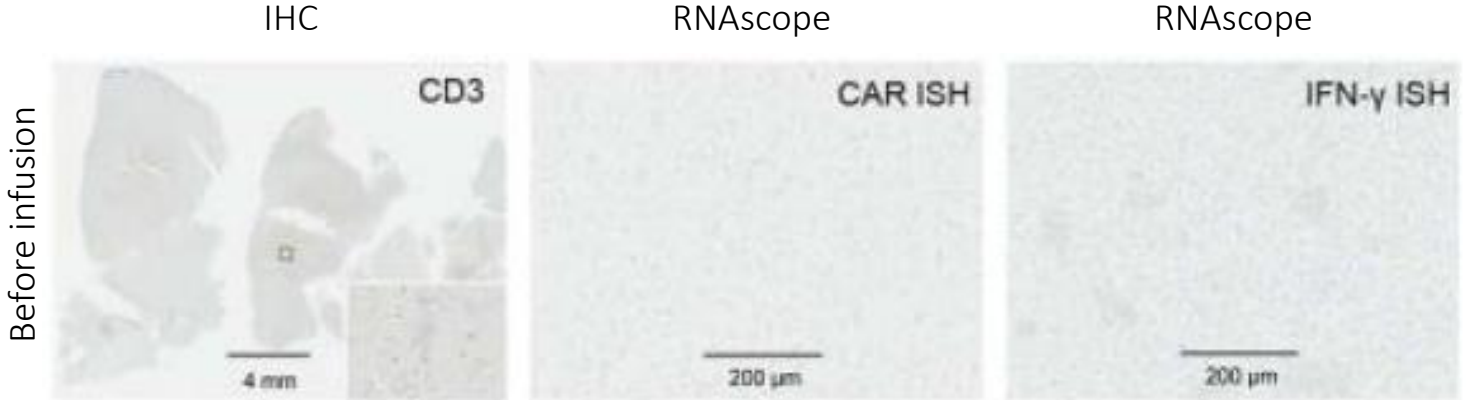
Anti-EGFRvIII CAR Design



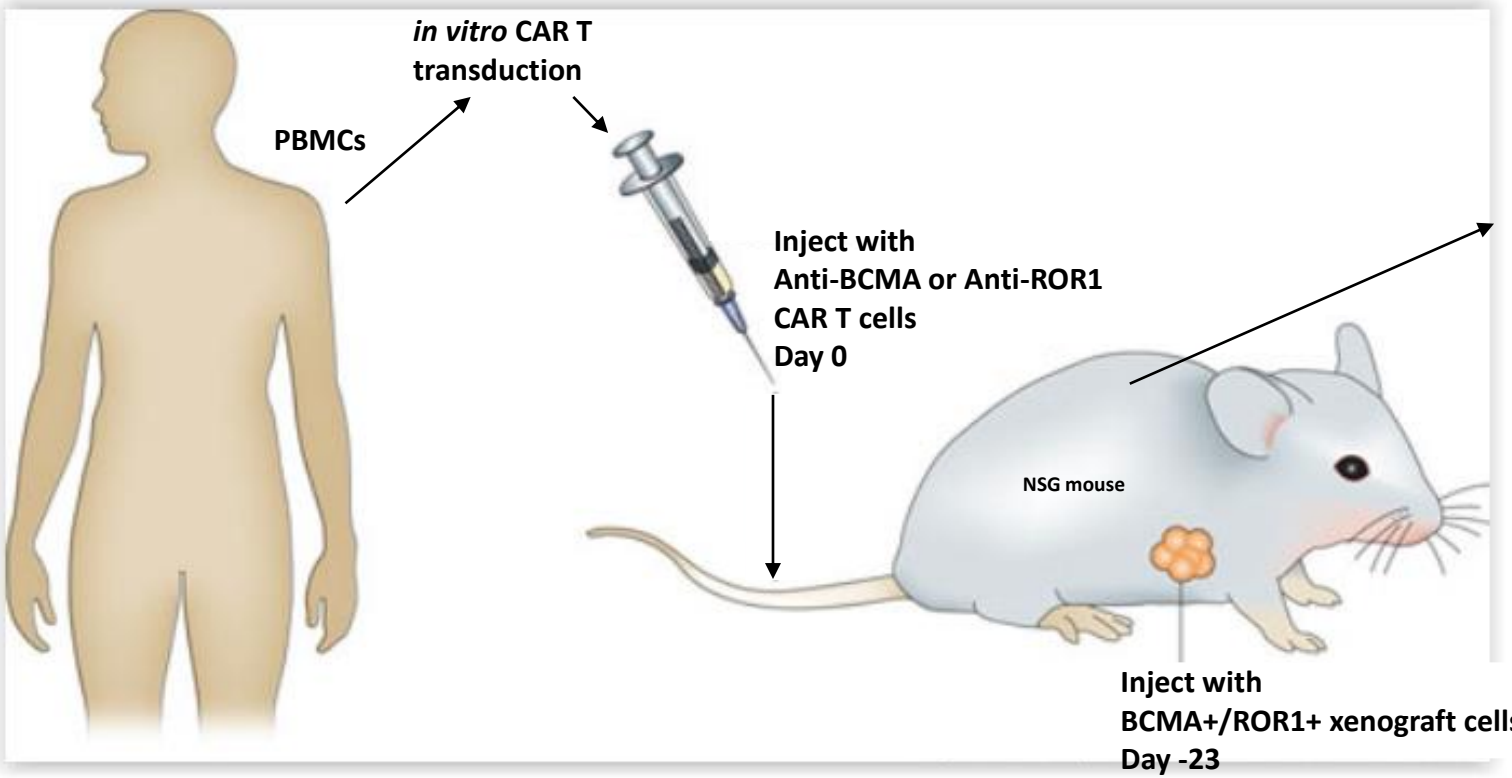
RNAscope Probe Design in 3'UTR region to target CAR+ cells

- *In situ* evaluation of tumor microenvironment after CART-EGFRvIII infusion reveals:
  - Specific detection of CAR+ cells & more IFN+ (activated) T cells (by RNAscope)
  - Increased but patchy T cell infiltration (by IHC)
- RNAscope ISH visually confirms CART-EGFRvIII cell traffic to the tumor site of glioblastomas (GBM)

In situ analysis of tumor microenvironment in GBM tissues



Study Design: Preclinical safety & activity assessment of CAR T cells



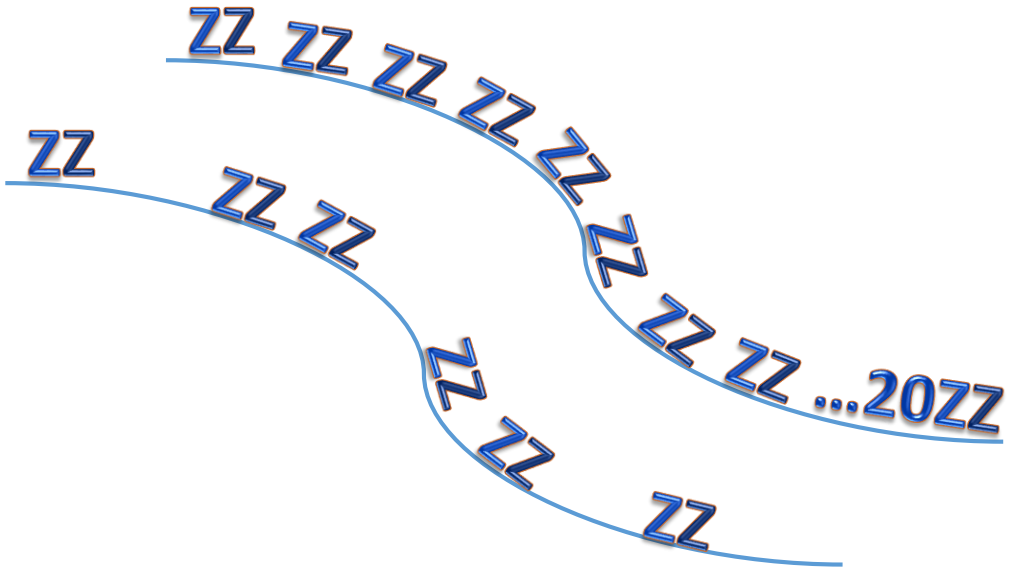
Endpoints

- Body weights and clinical symptoms
- Xenograft burden
- Histopathology
- ***In vivo* CAR T cell distribution/activation**
  - **RNAscope ISH assays for solid tissues**

Study Design: RNAscope probes

Preamplifer binding site

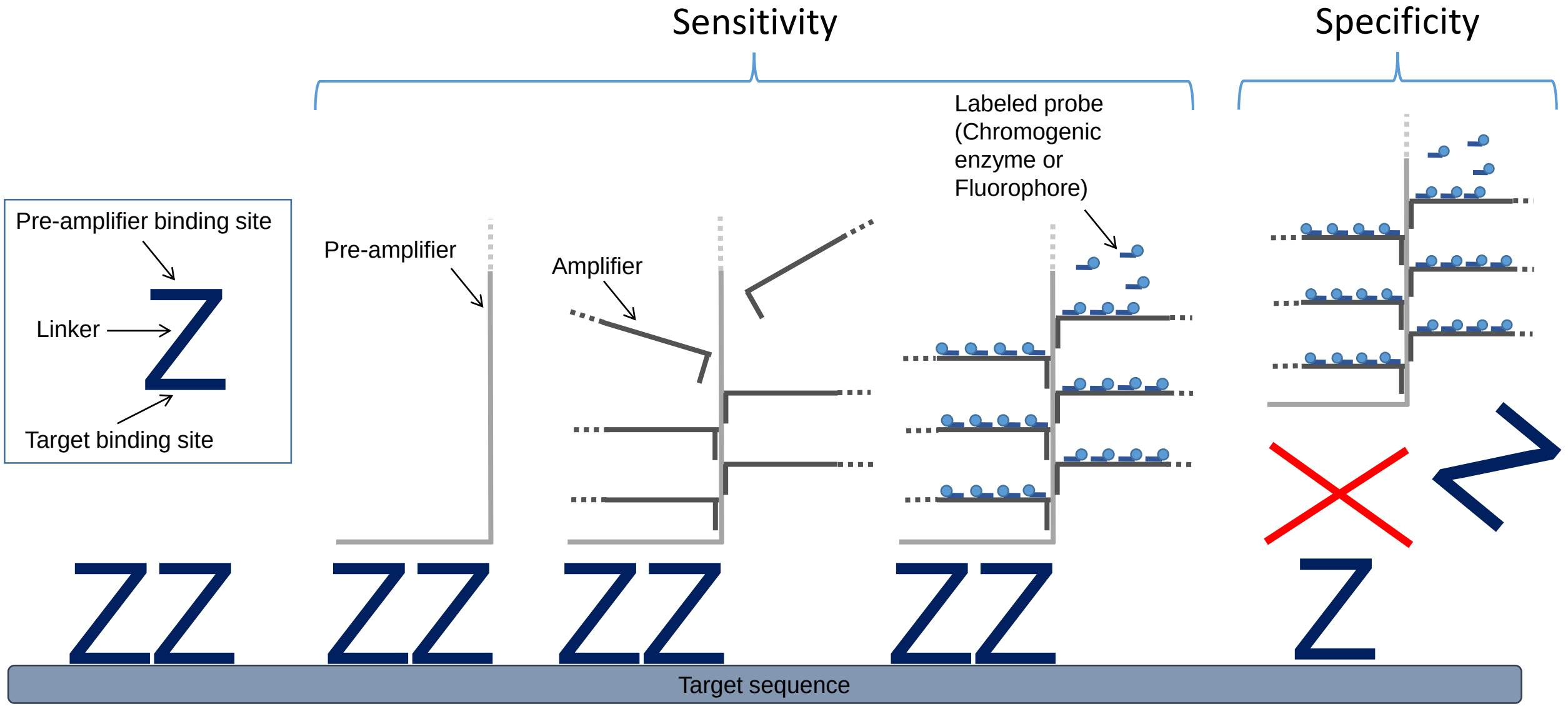
Target Probe Set:
Pool of 20 ZZ Pairs



Target-specific binding site:
~ 50-base region

RNA Target Region:
1,000 bases
(20 pairs of 50 bases)

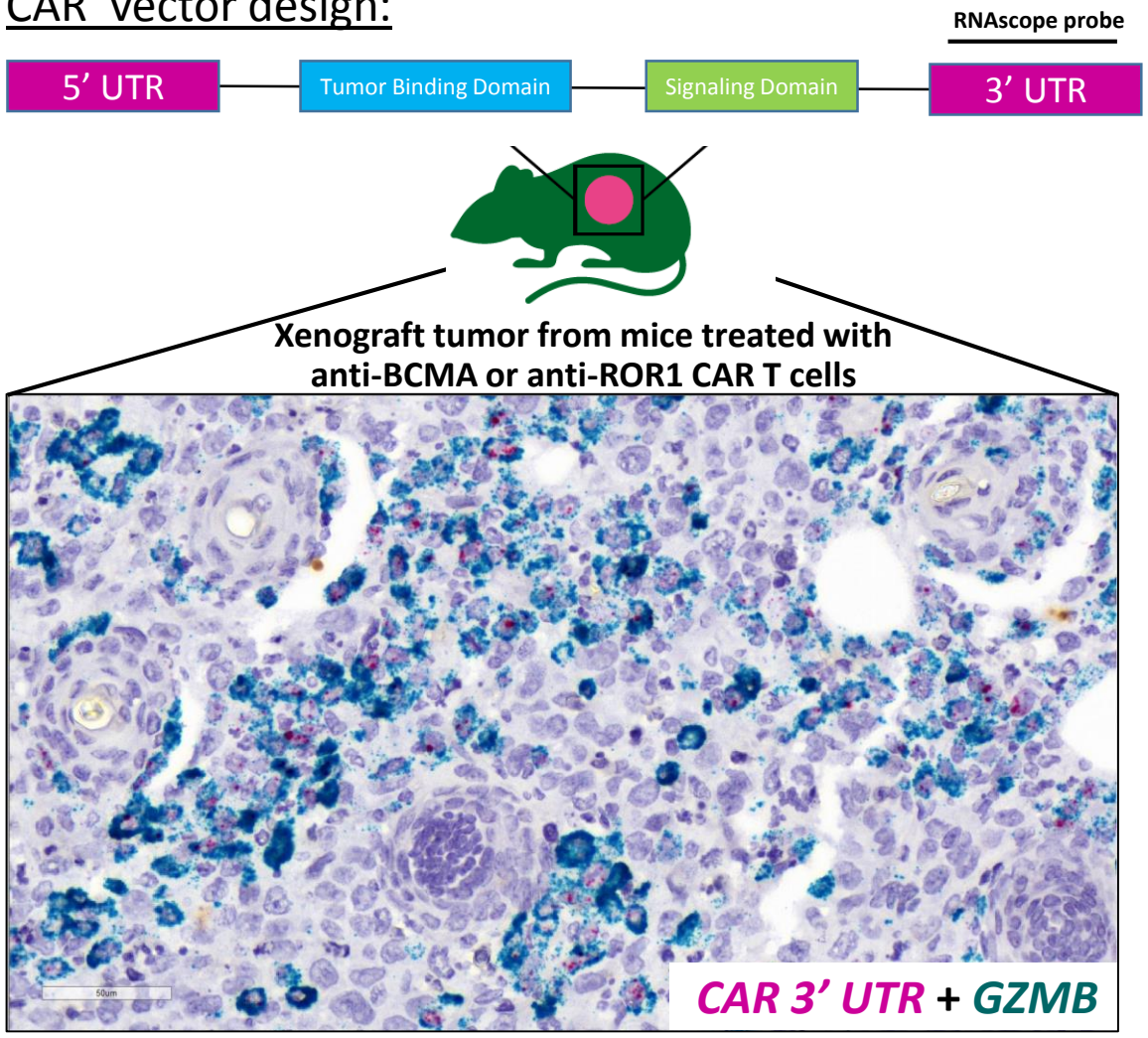
Study Design: RNAscope signal amplification



Study Design: Preclinical safety & activity assessment of CAR T cells

- Preclinical safety assessment of proposed CAR T cell targets
  - ISH Probes: *BCMA* and *ROR1*
- "On-target/on-tumor" or "On-target/off-tumor" effects
  - ISH Probes: *UTR* of CAR vector and *GZMB* or *IFNg* (markers of activation)
- Activated CAR T cells traffic to tumor
  - ISH: *UTR* of CAR vector, *GZMB*, and *IFNg*
  - IF: CD3

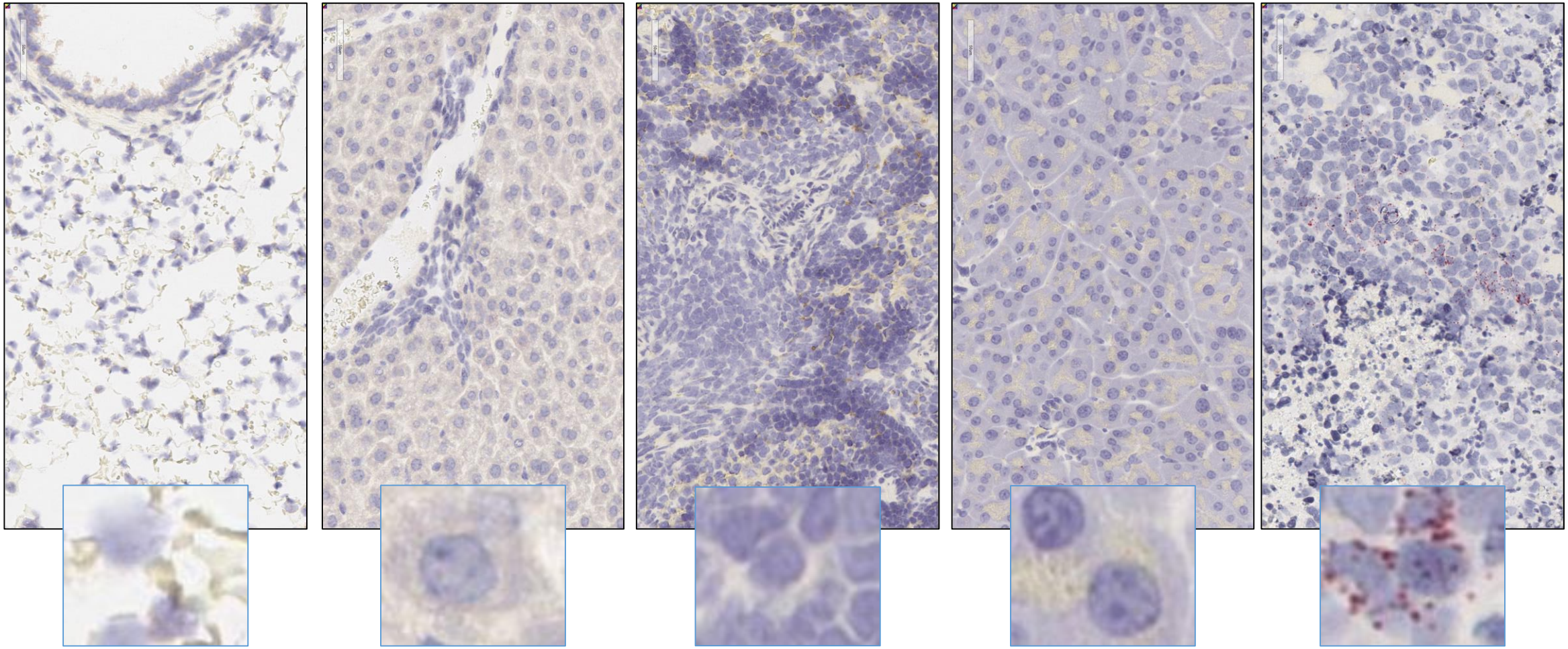
CAR vector design:



Preclinical safety assessment of proposed CAR T targets: *BCMA*

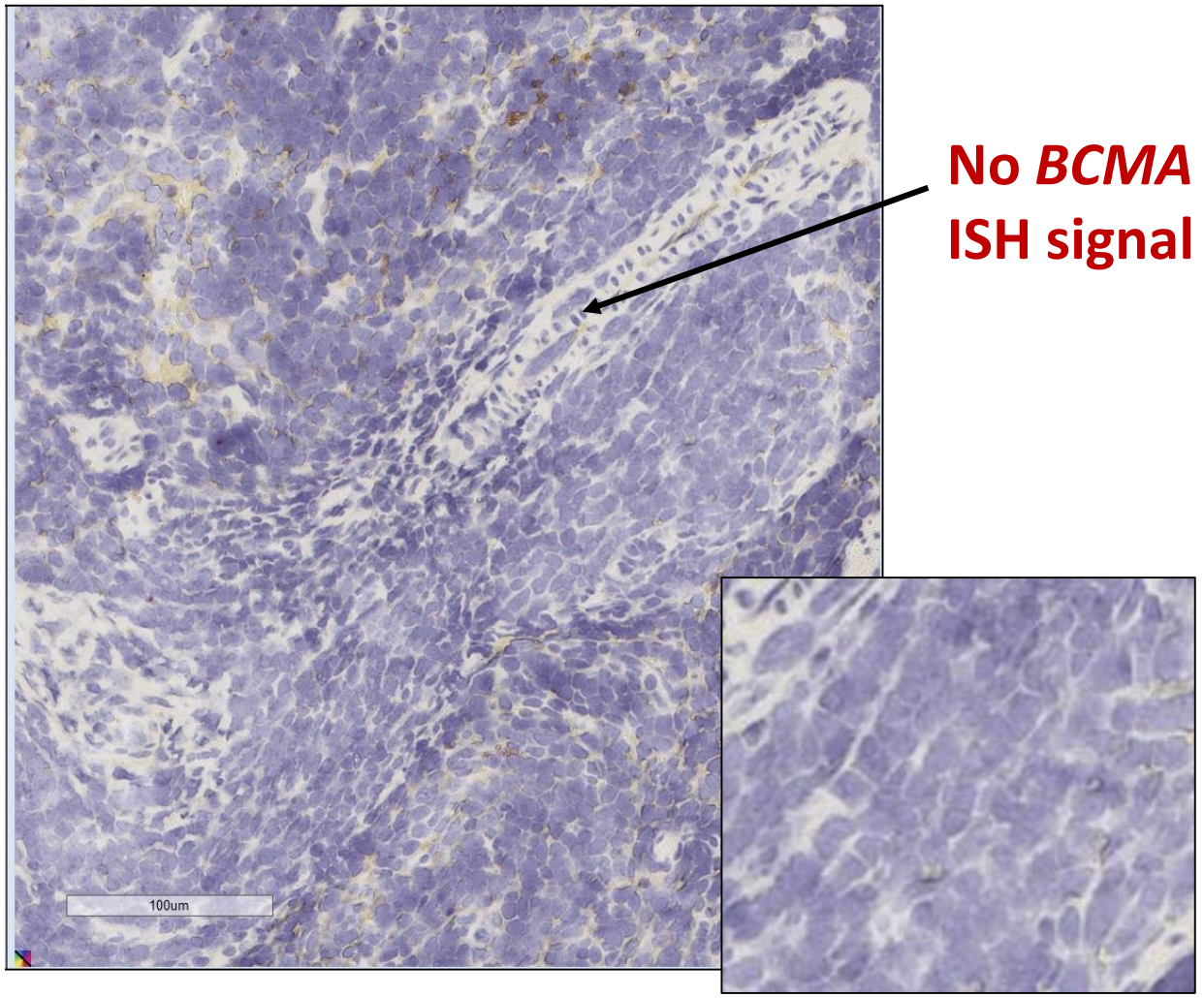
Mouse *Bcma* | **Human *BCMA***

Lung | **Liver** | **Spleen** | **Pancreas** | **Human Xenograft**



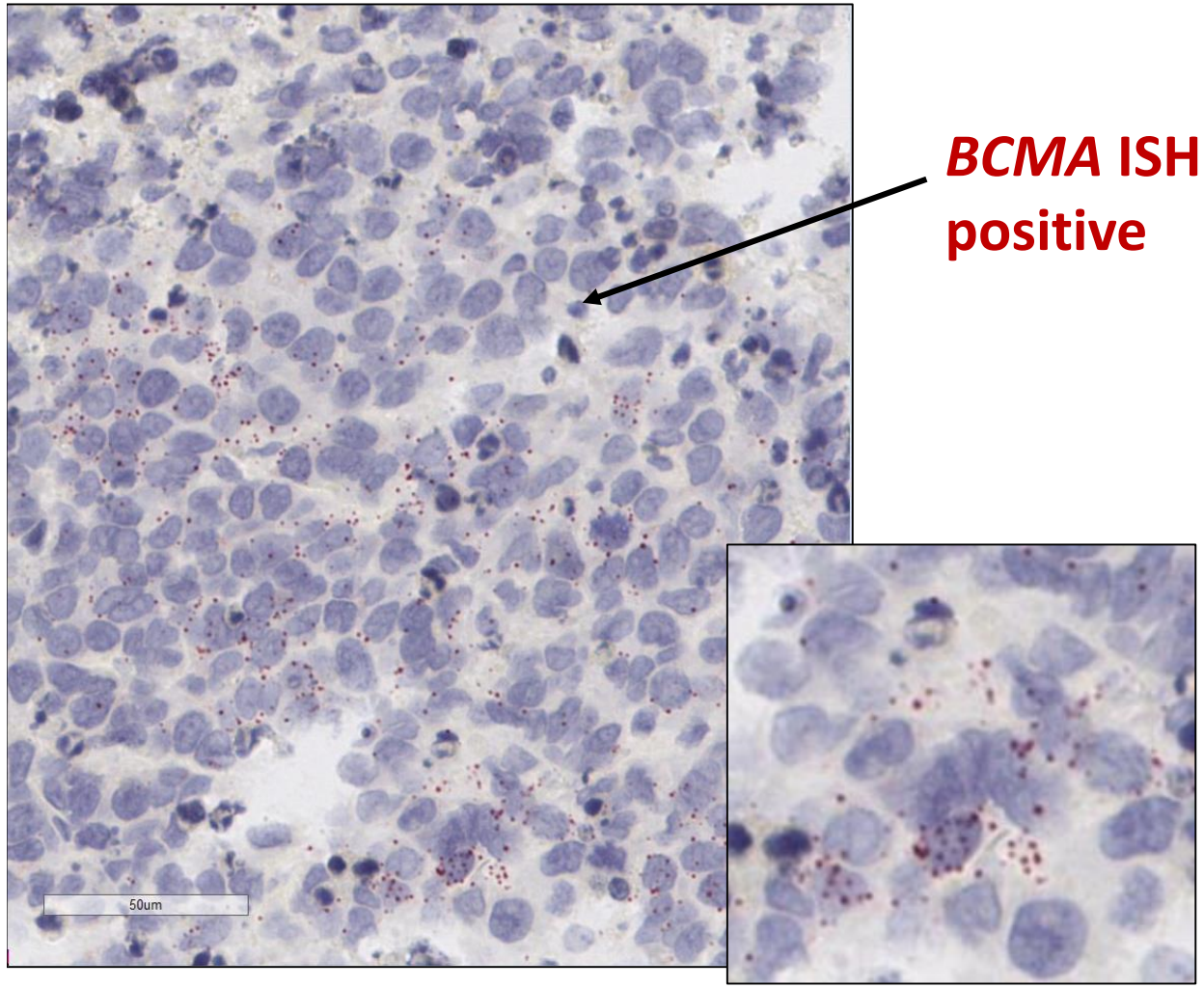
Anti-BCMA CAR T Cells in BCMA- Mouse Spleen

No *Granzyme B* expression by anti-BCMA CAR T cells in mouse spleen



Anti-BCMA CAR T Cells in BCMA+ Xenograft Tumor

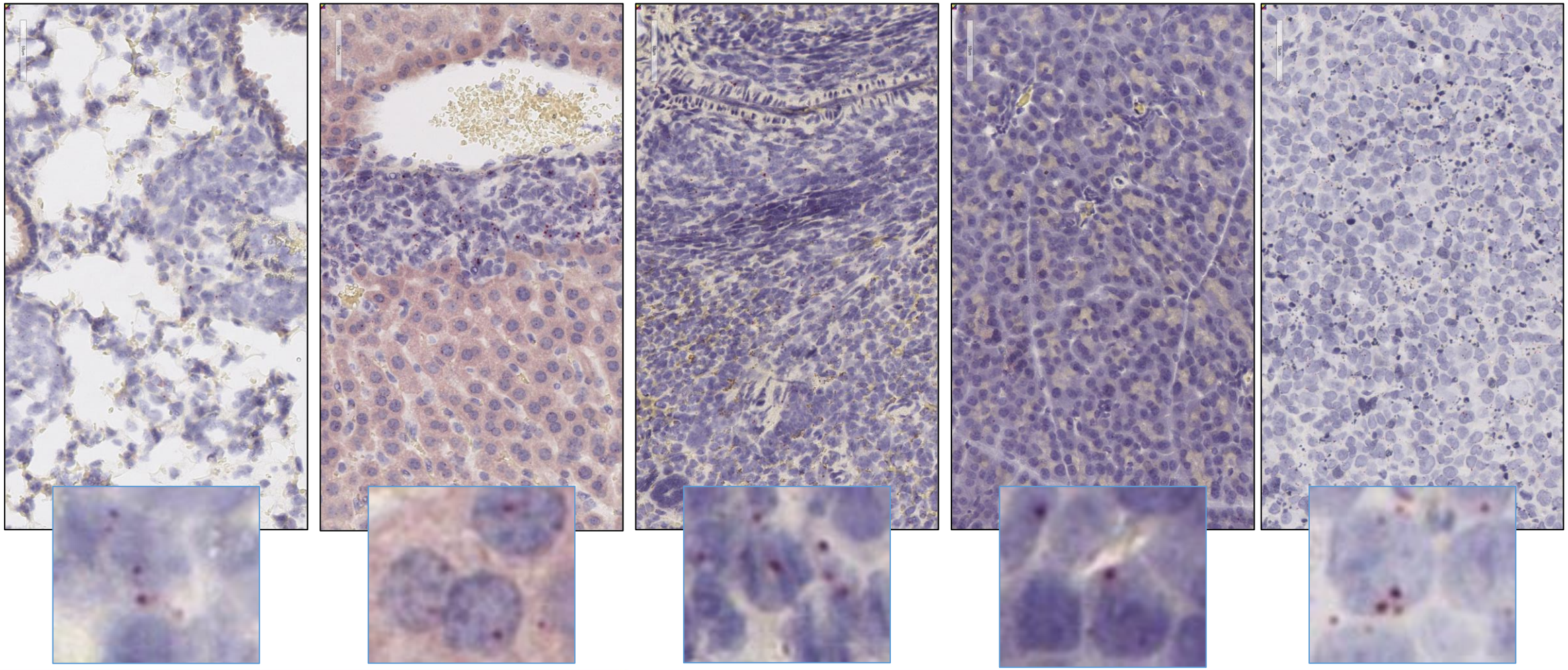
Granzyme B expression by anti-BCMA CAR T cells in *BCMA+* xenograft tumor



Preclinical safety assessment of proposed CAR T targets: *ROR1*

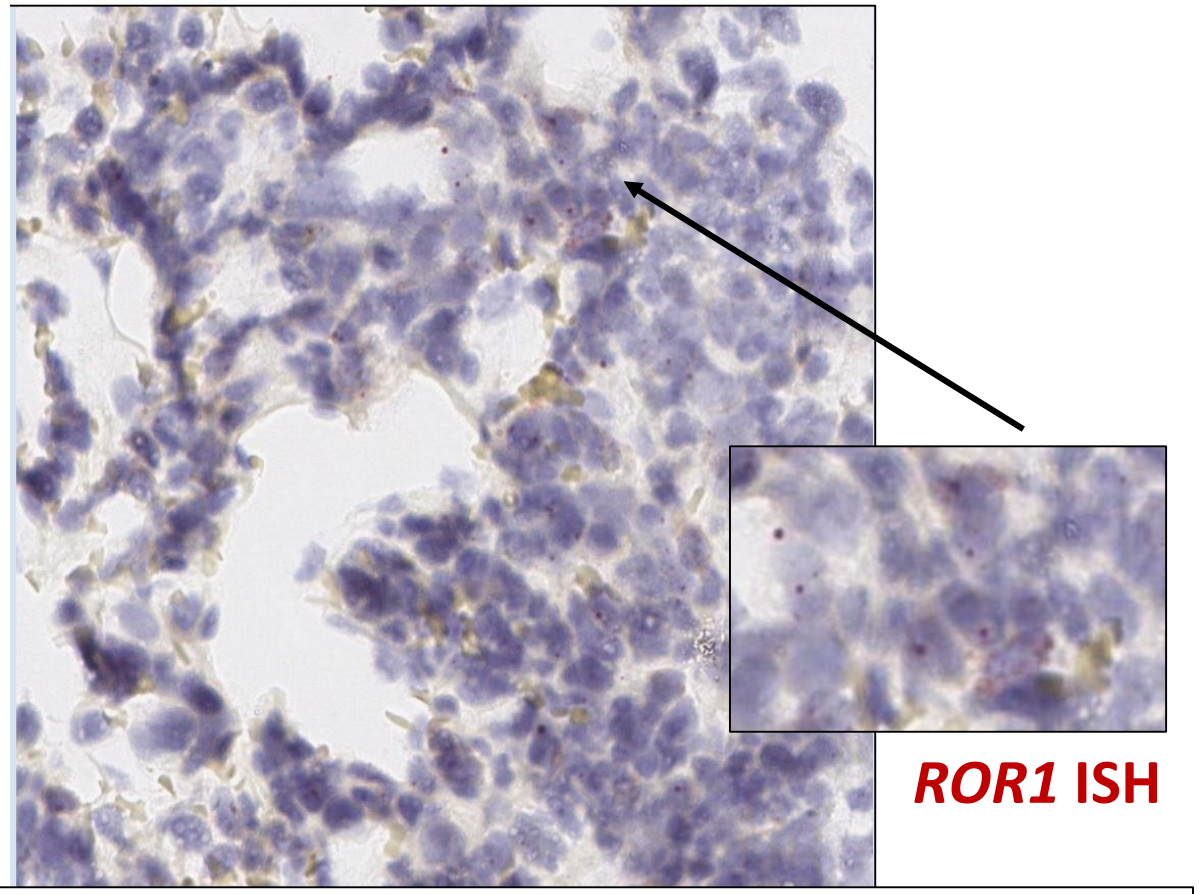
Mouse *Ror1* | **Human *ROR1***

Lung | **Liver** | **Spleen** | **Pancreas** | **Human Xenograft**



Anti-ROR1 CAR T Cell Activation in ROR1+ Mouse Lung

Granzyme B mRNA expression by anti-ROR1 CAR T cells targeting mouse lung endothelial cells expressing *ROR1*

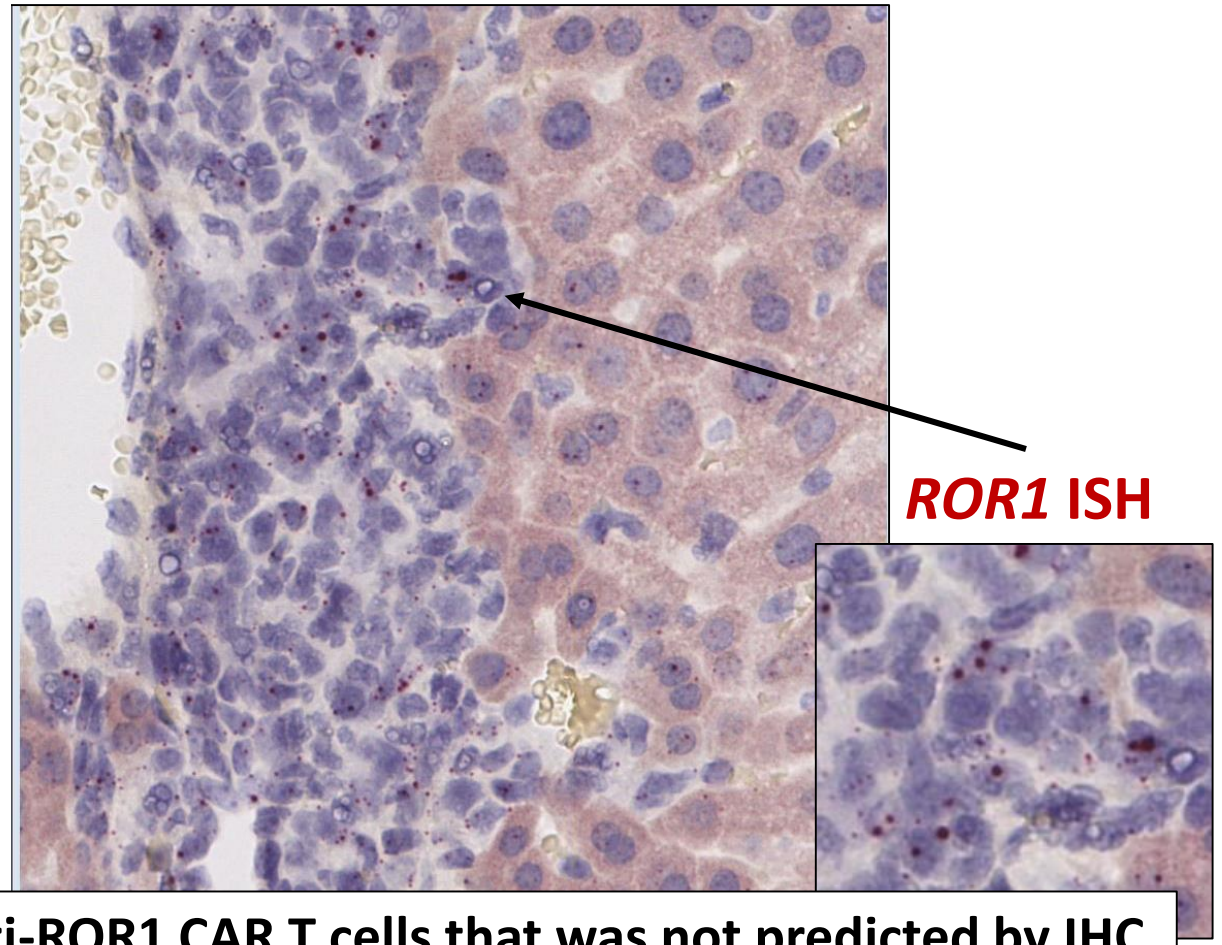


***ROR1* ISH**

Consistent with observed pulmonary toxicity of anti-ROR1 CAR T cells that was not predicted by IHC

Anti-ROR1 CAR-T Cell Activation in ROR1+ Mouse Liver

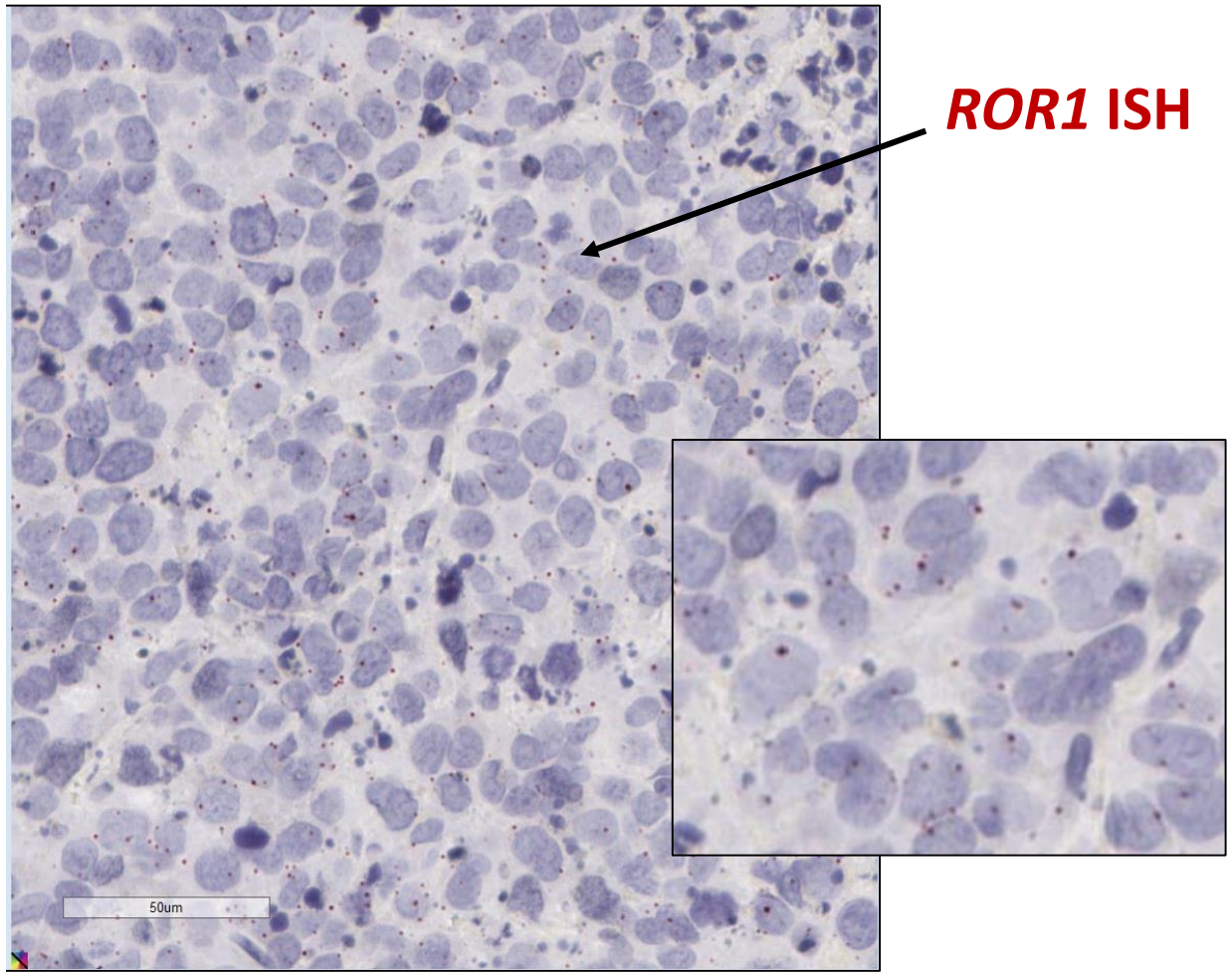
Granzyme B mRNA expression by anti-ROR1 CAR-T cells targeting mouse liver endothelial cells expressing *ROR1*



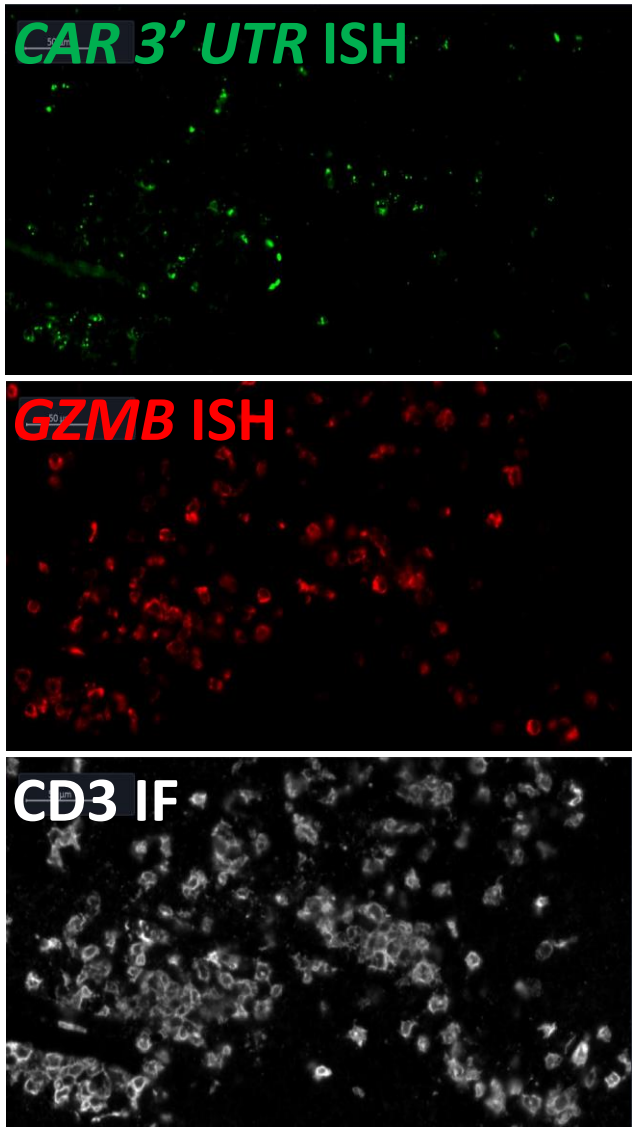
Consistent with observed hepatic toxicity of anti-ROR1 CAR T cells that was not predicted by IHC

Absence of Anti-ROR1 CAR T Cells in ROR1+ Xenograft Tumor

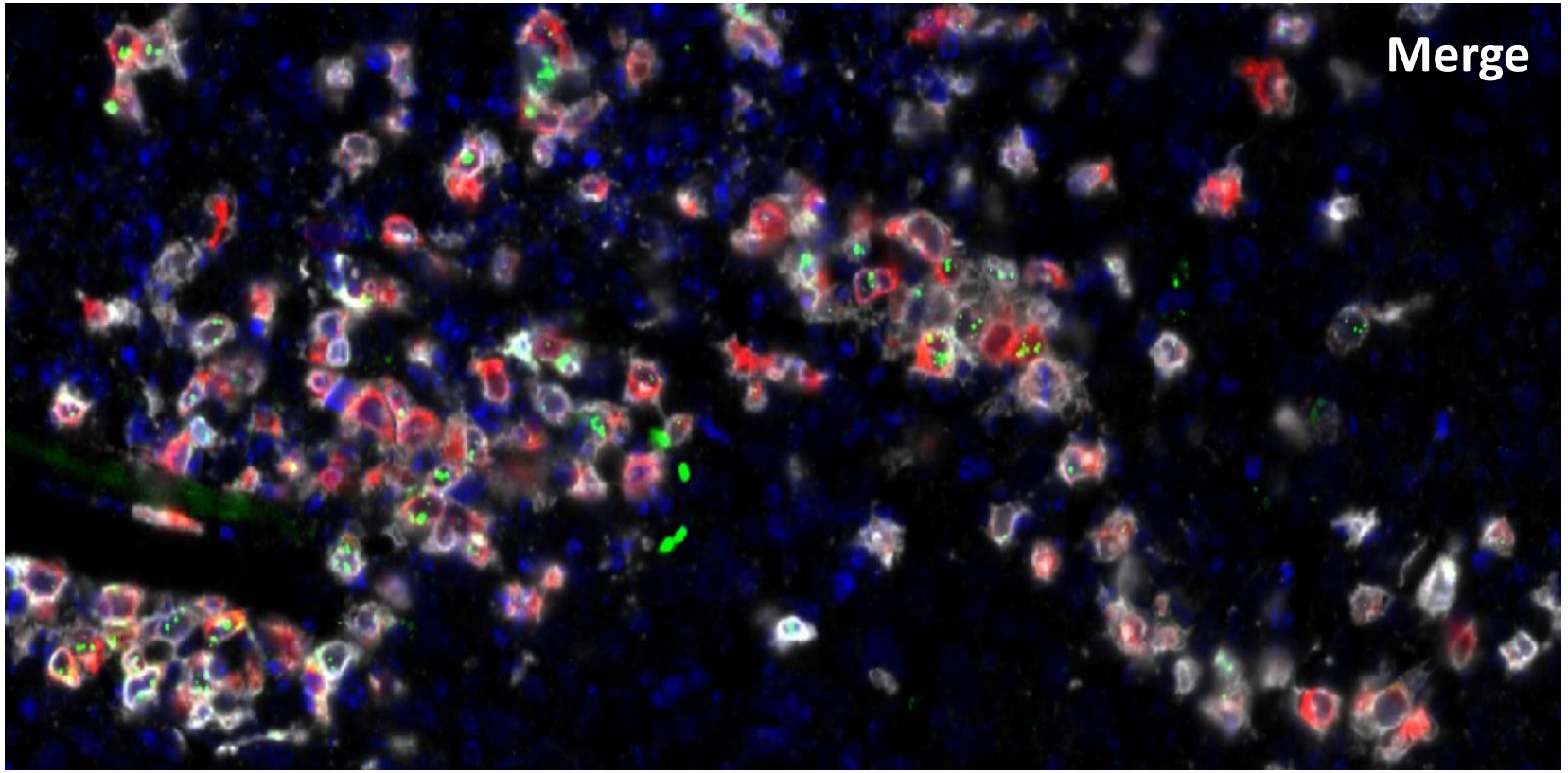
Anti-ROR1 CAR T cells do not reach or are exhausted on arrival at site of *ROR1*+ xenograft tumor



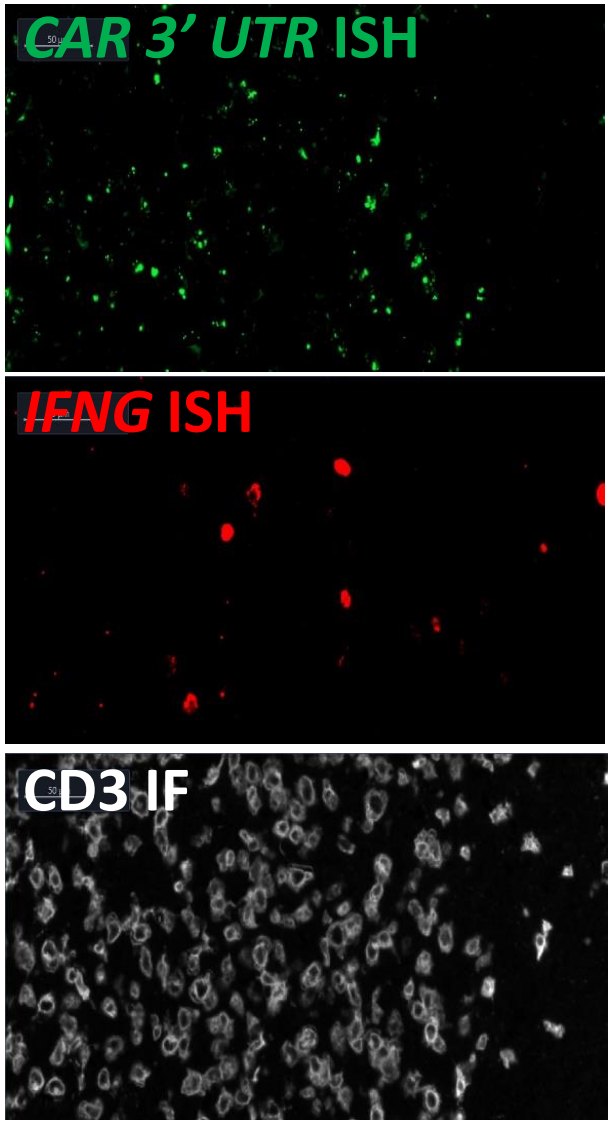
T cell recruitment and activation by anti-BCMA CAR T cells



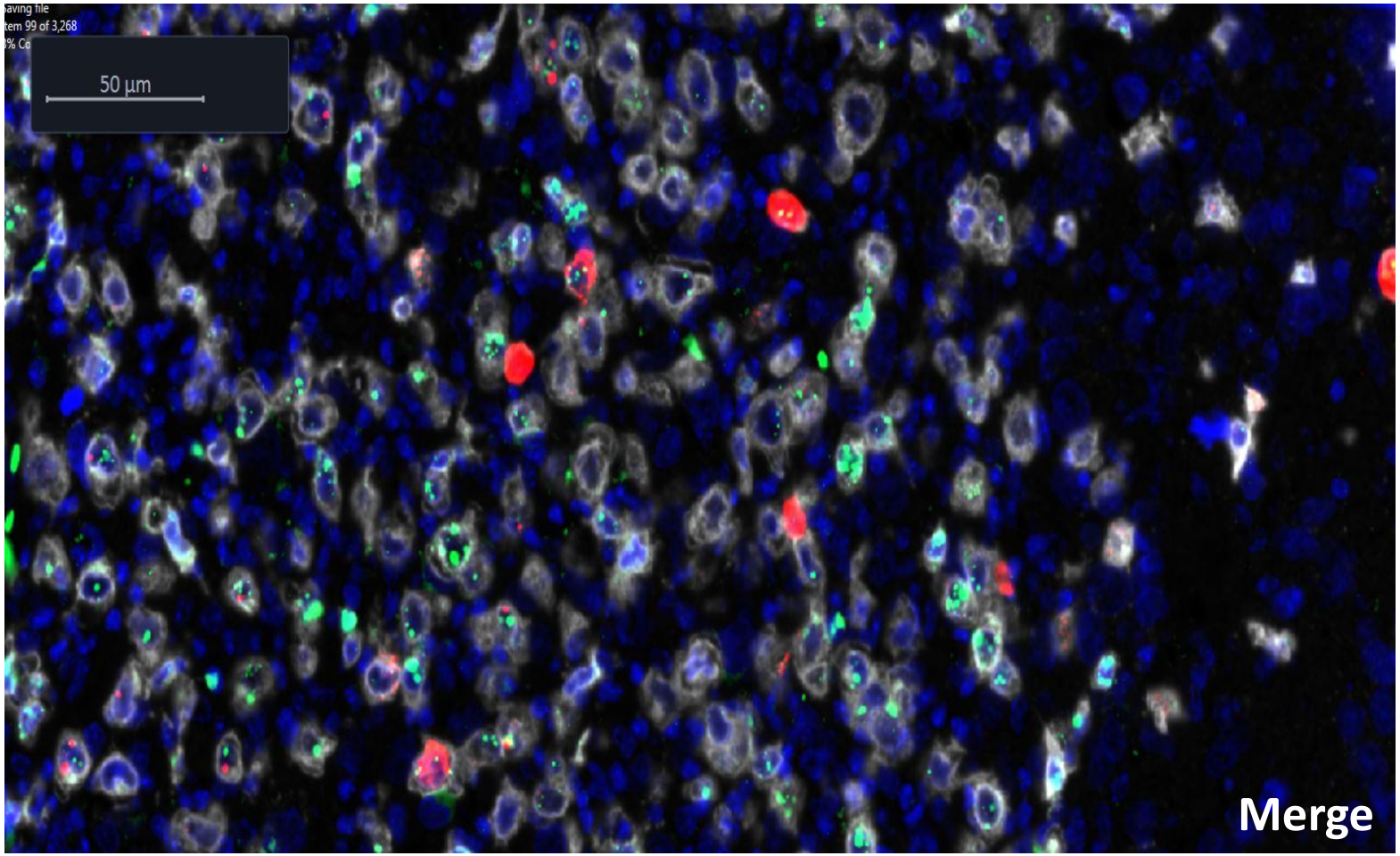
Granzyme B expression and CD3+ T cell infiltration in BCMA+ xenograft tumor treated with anti-BCMA CAR T cells



T cell recruitment and activation by anti-BCMA CAR T cells

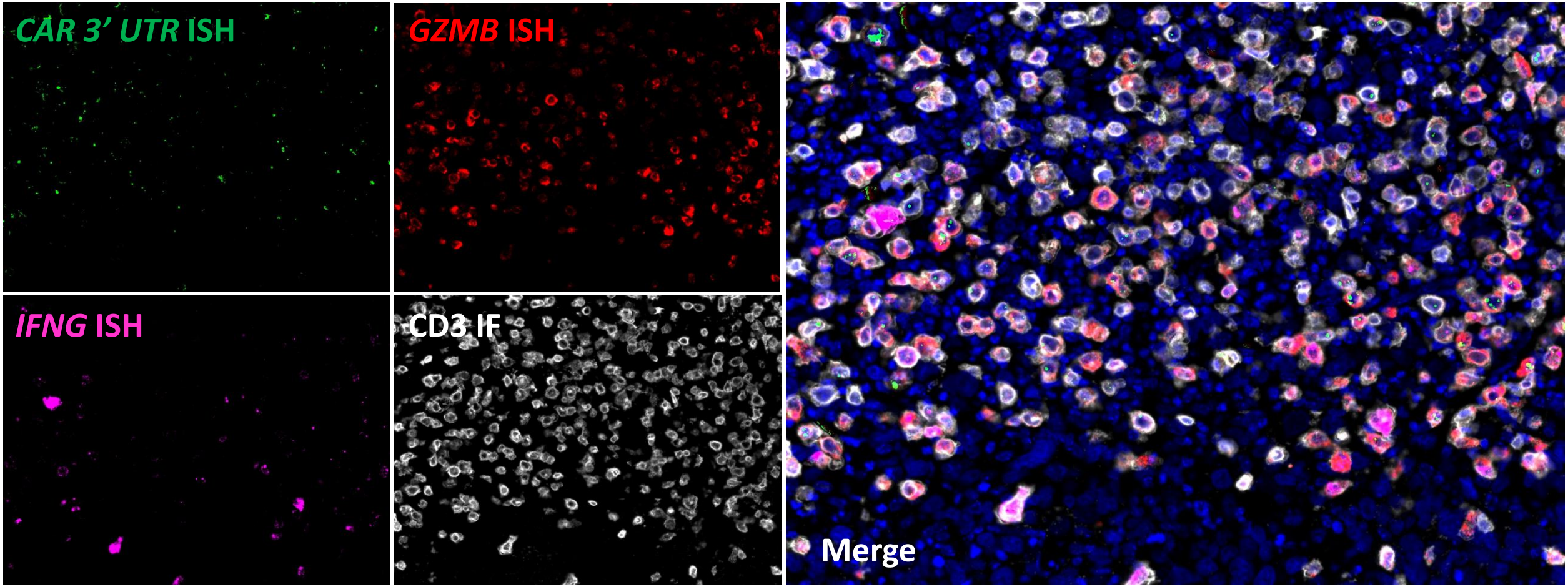


Interferon-γ expression and CD3+ T cell infiltration in BCMA+ xenograft tumor treated with anti-BCMA CAR T cells

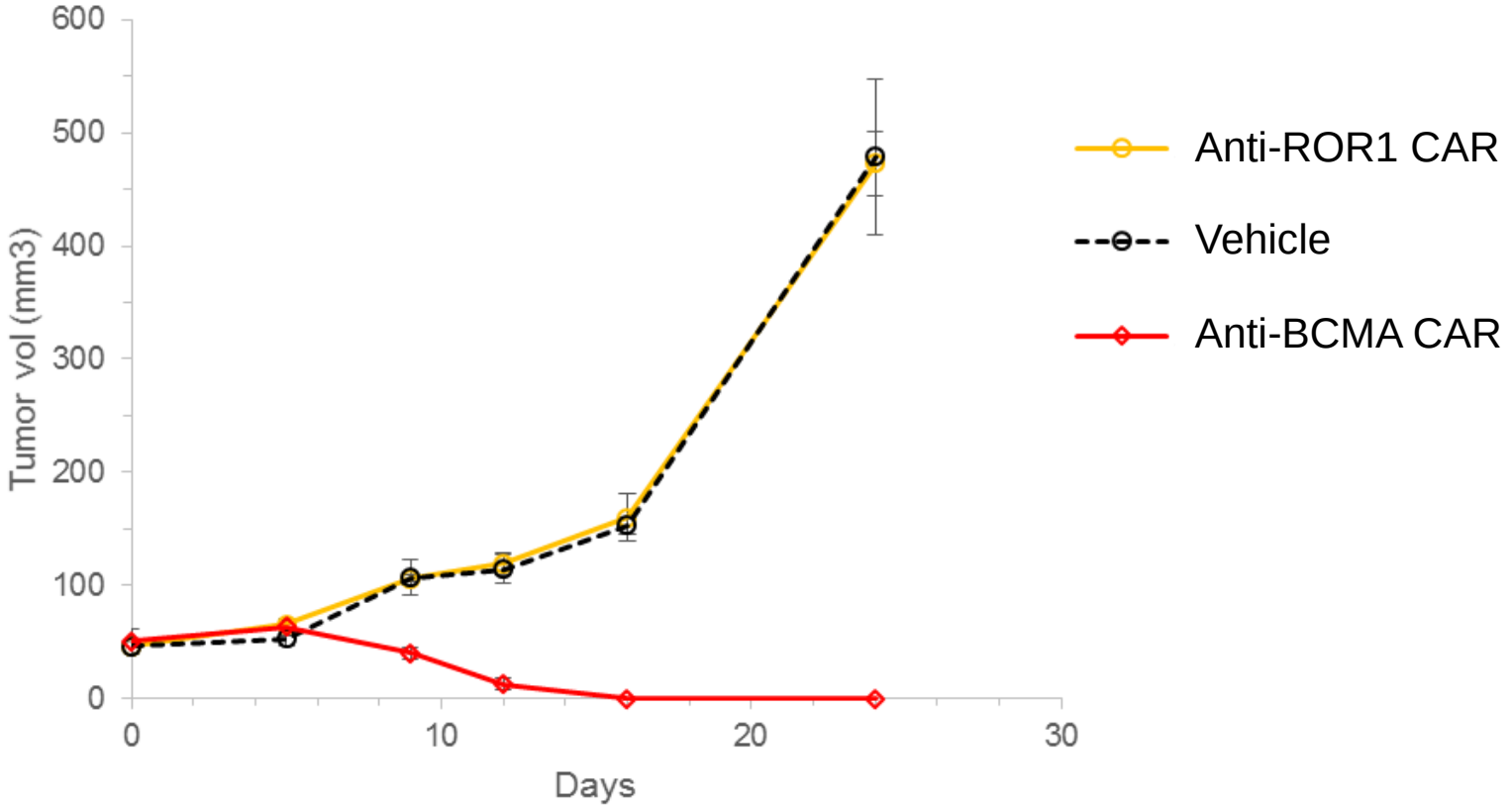


T cell recruitment and activation by anti-BCMA CAR T cells

Granzyme B and *Interferon-γ* expression and CD3+ T cell infiltration
in *BCMA+* xenograft tumor treated with anti-BCMA CAR-T cells

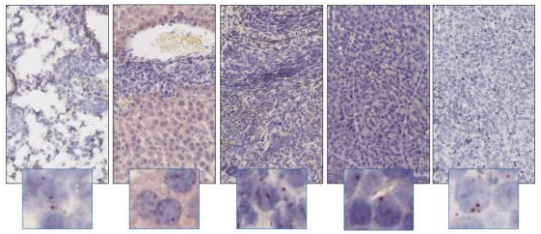


Preclinical Safety Assessment of *in vivo* Models: Tumor Volume

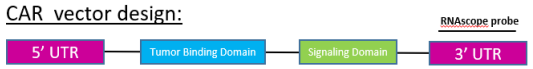


- Some anti-ROR1 CAR T cell-treated animals developed clinical signs within 24 hours
  - Lethargy, weight loss, ruffled fur, occasionally requiring euthanasia
- Affected animals usually recovered and tumor growth continued
- Anti-ROR1 CAR T cells did NOT promote xenograft rejection
- Anti-BCMA CAR T cells promoted xenograft rejection

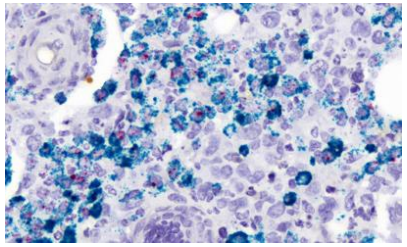
Summary



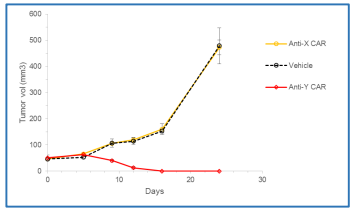
- Predict which organs to monitor for toxicity during *in vivo* studies
  - Highly sensitive RNAscope ISH assay revealed low levels of *ROR1* mRNA expression in normal lung and liver
  - Predicted anti-ROR1 CAR T cell engagement and toxicity in normal liver and lung tissues based on very low mRNA expression level



- Differentiate between engineered T cells and endogenous T cells in solid tissues
  - RNAscope probes targeting the vector transcript UTR differentiate CAR/TCR T cells from endogenous T cells



- Spatially resolve activated engineered T cells in the TME
  - Combining UTR probes with activation marker probes (i.e. *GZMB, IFNG*) enables visualization of CAR-T cell infiltration and activation in solid tissues expressing the CAR target antigen



- Preclinical efficacy studies utilizing the RNAscope technology illustrated a correlation between activated CAR T cell tumor infiltration and tumor killing

Acknowledgements

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- Fay Eng, BS
- Kevin Friedman, PhD
- Molly Perkins, PhD
- Shannon Grande, PhD

Contact Info:
Courtney Anderson, PhD
Courtney.Anderson@bio-techne.com