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

Helly Pimentel[1], Helen Jarnagin[1], Hailing Zong[1], Courtney Todorov[1], Kenneth Ganley[2], Fay Eng[2], Kevin Friedman[2], Molly Perkins[2], Shannon Grande[2], Courtney Anderson[1], Bingqing Zhang[1], Christopher Bunker[1], James B. Rottman[2], Xiao-Jun Ma[1]

[1]Advanced Cell Diagnostics, 7707 Gateway Blvd, Newark, CA 94560; [2]Bluebird Bio, 60 Binney St, Cambridge, MA 02142

Background

Chimeric antigen receptor (CAR) T cell therapy has proven to be highly effective in treating hematologic malignancies, and major efforts are being made to achieve similar efficacy in solid tumors. These efforts face multiple challenges, including off-tumor target expression and checkpoint inhibition of CAR T cell activity in the tumor microenvironment. CAR T cells are much more potent compared to antibody therapeutics, therefore there is a need for more stringent CAR T target safety assessment to avoid adverse events resulting from "on-target/off-tumor" activity. Furthermore, it is critical to track and monitor CAR+ T cells within the context of intact tissue and tumor to understand the mechanisms underlying off-tumor toxicity and efficacy in tumor killing.

Design

We employed the RNAscope in situ hybridization (ISH) technology to assess target expression specificity and to track CAR T cell distribution and activation in xenograft and host tissues using the RPMI-8226 xenograft mouse model treated with or without anti-BCMA or anti-ROR1 CAR T cells.

Samples: 5μm sections from formalin-fixed paraffin embedded (FFPE) mouse multi-tissue arrays and xenograft tumor were used.

RNAscope ISH assays: The RNAscope 2.5 LS Red or 2.5 LS Duplex chromogenic assays or the RNAscope 2.5 LS Multiplex Fluorescent assay combined with immunofluorescence were used and performed on the Leica BondRX automated stainer (Figure 1). RNAscope probes were designed to target the 3' UTR of the CAR vector–derived transcript (Figure 2), *IFNG*, *GZMB*, *BCMA*, and *ROR1*.

Imaging: Brightfield images were acquired using a Leica Biosystems Aperio AT2 Digital Pathology Slide Scanner. Fluorescent images were acquired using the Akoya Biosciences Vectra Polaris Automated Quantitative Pathology Imaging System with a 40x objective.

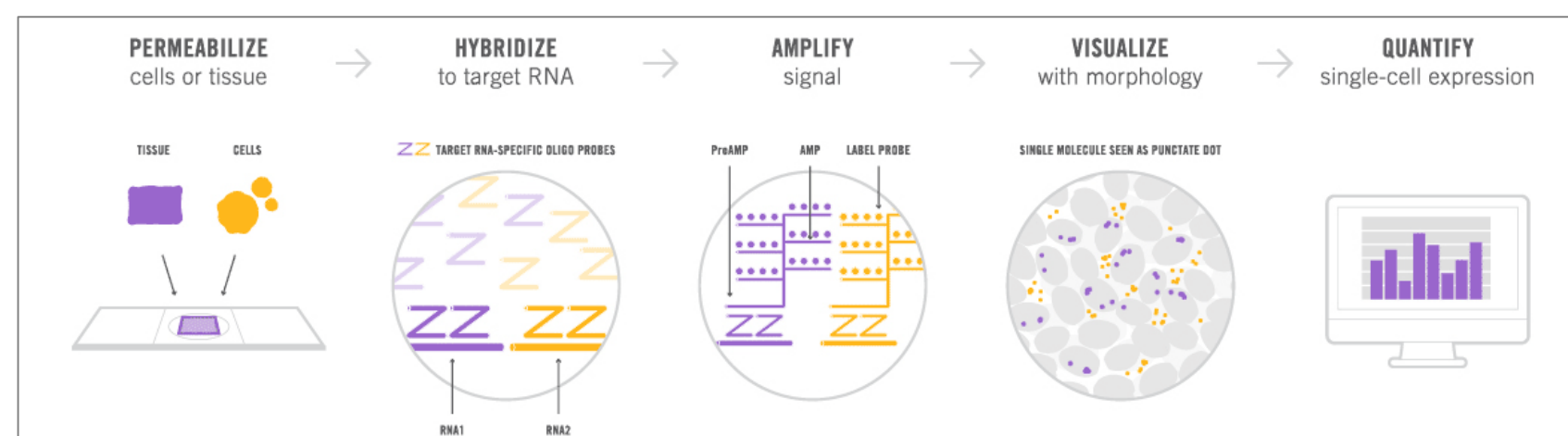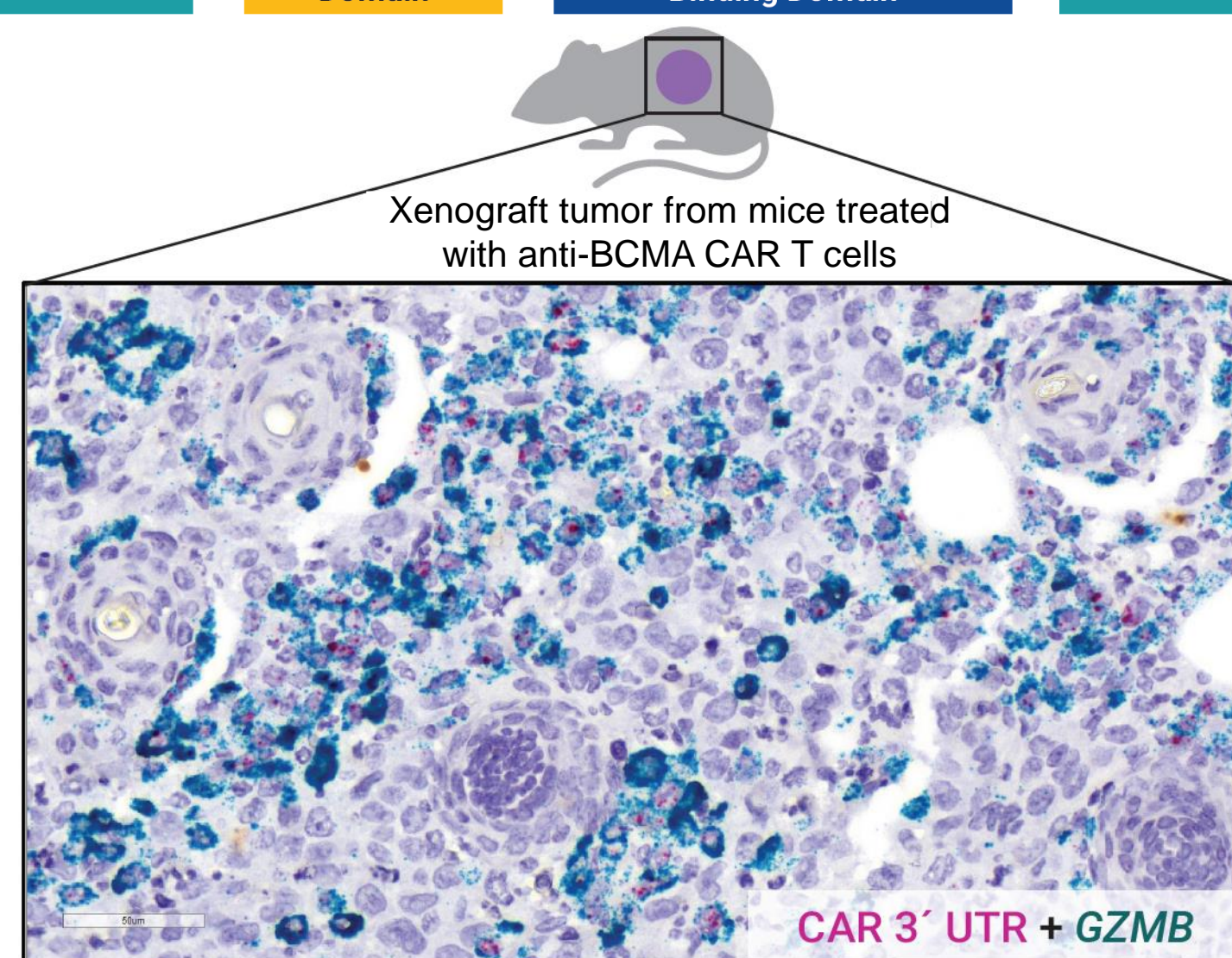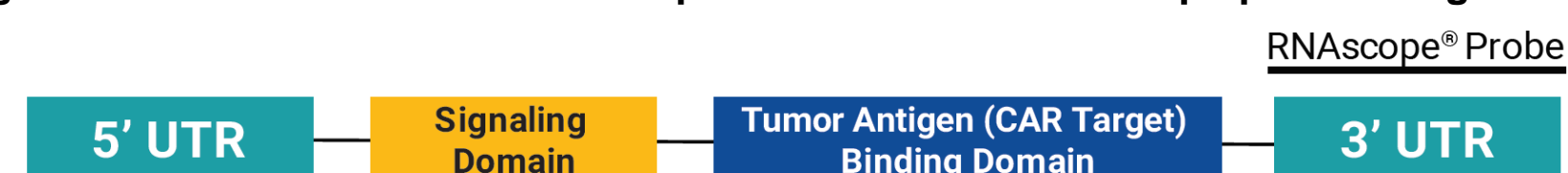### Figure 1. The RNAscope technology and workflow

Figure 2. CAR vector-derived transcript schematic and RNAscope probe design

Results

Figure 3. Target expression pattern for safety assessment
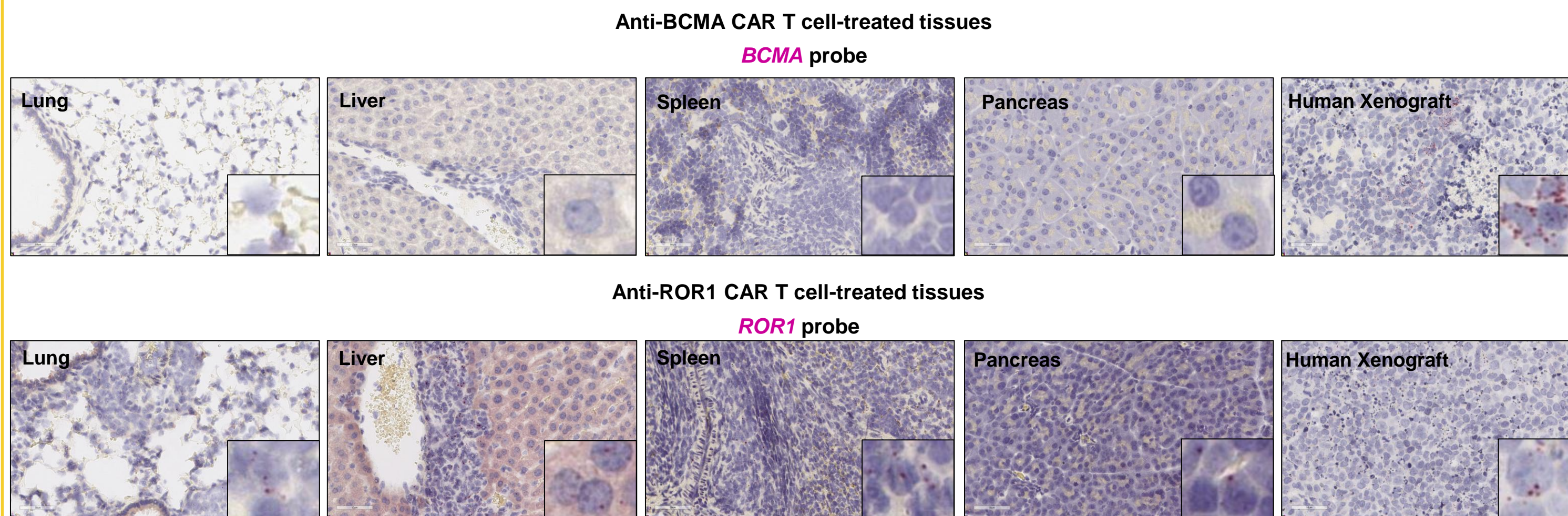

Figure 4. Activated anti-BCMA CAR T cells detected only in xenograft tumor, demonstrating on-target/on-tumor activity
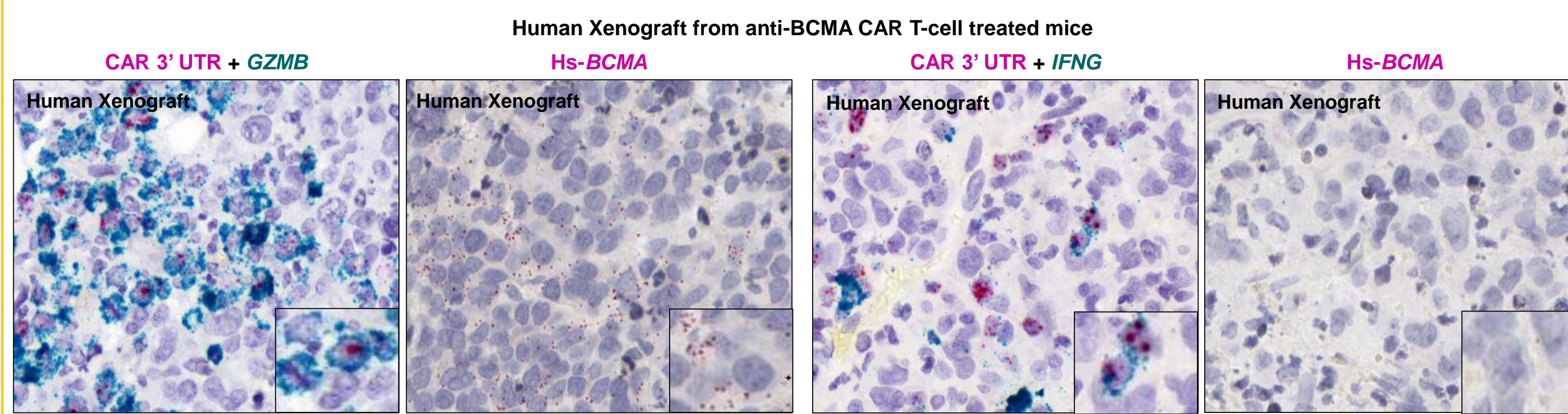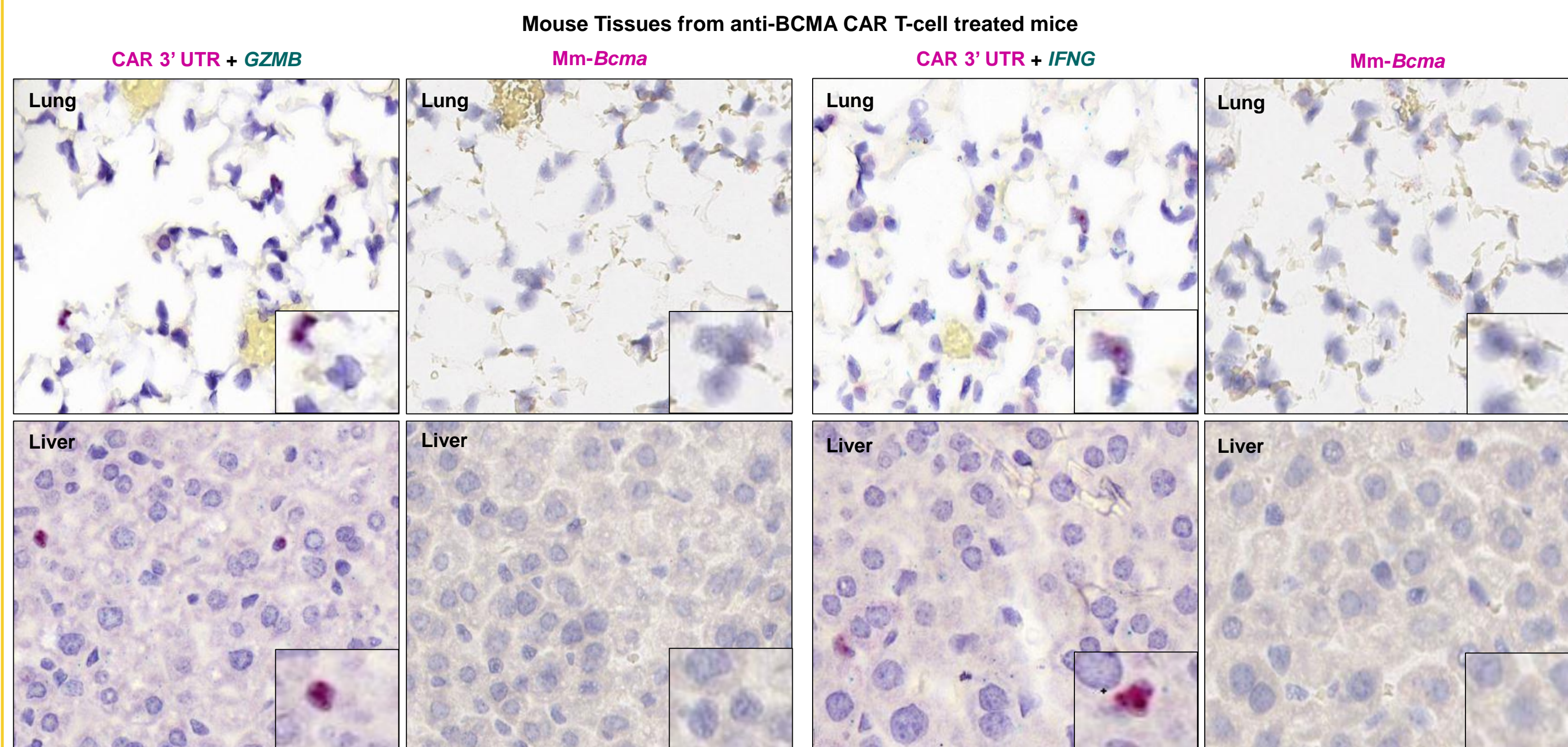

Figure 5. Activated anti-ROR1 CAR T cells detected in lung, liver, and xenograft tumor, revealing on-target/off-tumor activity
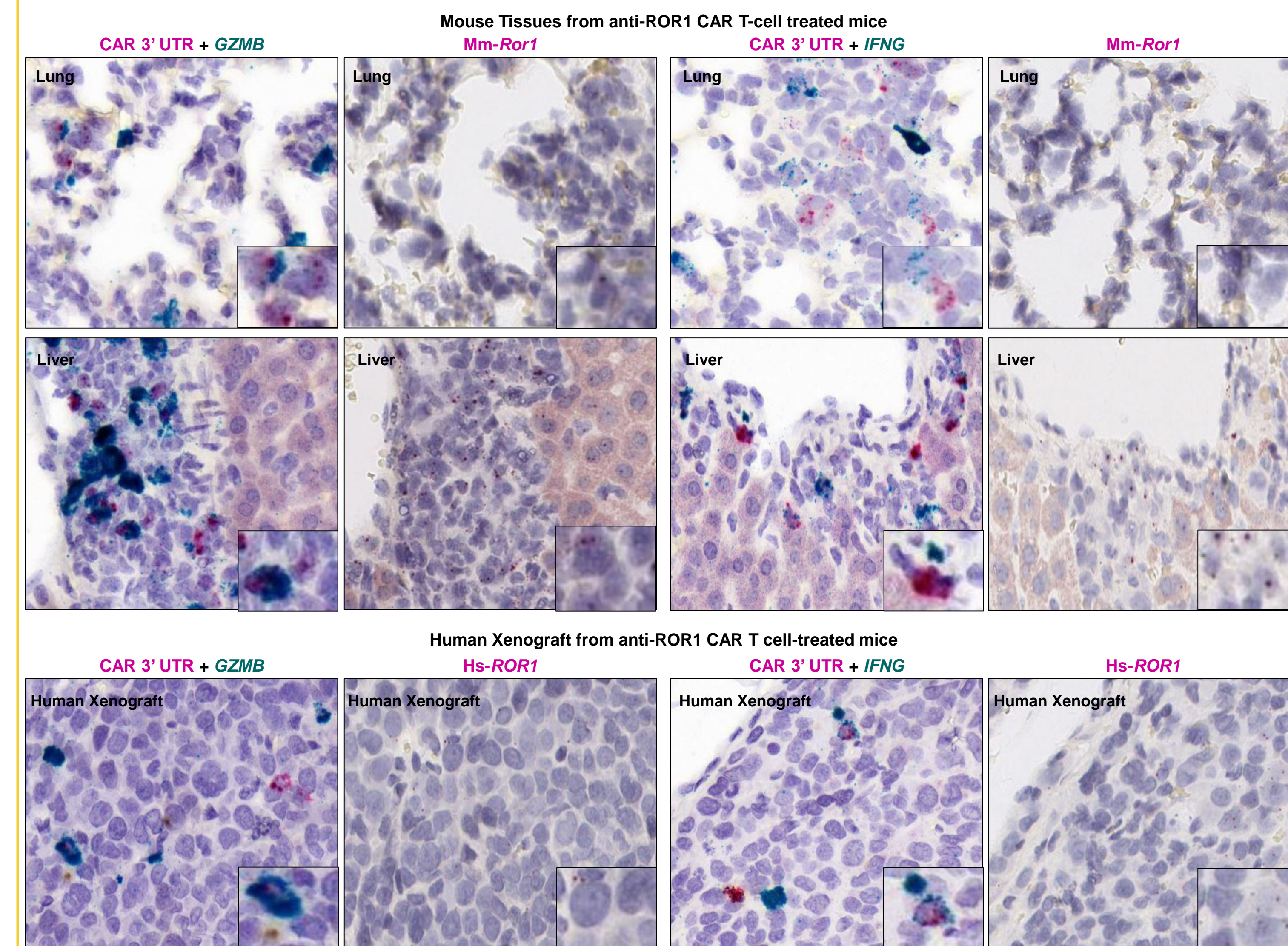

Figure 6. Trafficking of activated CAR T cells to the tumor site. Dual RNAscope ISH-IF workflow (below). Detection of activated anti-BCMA CAR+ T cells in the xenograft tumor using RNAscope ISH to detect the 3' UTR, *IFNG*, and *GZMB* (right).

Conclusions

These data thus demonstrate how the RNAscope ISH assay can be utilized for CAR T efficacy and safety/toxicity assessment in preclinical models by detecting very low levels of target antigen expression in off-tumor tissues and monitoring CAR T cell pharmacodynamics and activation in tumor models. This technology has equal utility in understanding both CAR T and TCR T cell activity in patient tumors.