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IN VIVO BIODISTRIBUTION OF CAR-T CELLS BASED ON RNA IN SITU HYBRIDIZAITON TECHNOLOGY JOINT TECHNOLOGY PLATFORM OF JOINN LABORATORIES-R&D SYSTEMS

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Background: Chimeric antigen receptor (CAR)-T cell therapy has shown good efficacy in the treatment of hematological malignancies. At the same time, more and more research is devoted to the application of CAR-T cell therapy to solid tumors, with the hope to achieve similar effects. There are many challenges in CAR-T therapy on solid tumors, including whether the target genes corresponding to the CAR-T cells are intended to be expressed and distributed only in tumor cells, and whether the monitoring points in the tumor microenvironment will inhibit the activity of CAR-T cells, etc. Therefore, for the safety assessment of target genes corresponding to CAR-T cells, it is very important to prevent CAR-T cells from attacking normal tissues. Based on this, tracking and detecting the distribution of CAR-T cells in tumors and other tissues will also help to understand the in vivo toxicity and tumor-killing effects of the CAR-T cells.

Design: In this study, the RNAscope in situ hybridization (ISH) technology was used to inject CAR-T cells targeting BCMA or inject CAR-T cells targeting ROR1 (anti-ROR1 CAR-T) in a mouse model inoculated with human myeloma cell line (RPMI-8226) tumor. Analyze the results in reference to the tumor mouse model not inoculated with CAR-T cells, evaluate the expression level of CAR-T target genes in tumor and non-tumor tissues, and track the distribution and activity of CAR-T cells in tumor tissues and normal tissues.

Sampling: A variety of tissue samples and tumor samples of experimental mice were prepared and paraffin sectioned (FFPE). The thickness of the section was 5μm. RNAscope in situ hybridization (ISH) analysis: Use Leica automated instruments to perform single staining (red), RNAscope 2.5 LS double staining or RNAscope LS multiple fluorescent staining combined with immunofluorescence detection and data analysis (Figure 1). The RNAscope probes of CAR-T cells are designed to target the 3'UTR area of CAR vector transcript, and the other RNAscope detection probes target the mRNA sequences of IFNG, GZMB, BCMA and ROR1, respectively.

Imaging: Use the Leica Biosystem Aperio AT2 digital pathology slice scanner to acquire brightfield images, and use Akoya Biosciences Vectra Polaris automatic quantitative pathology imaging system to acquire fluorescent images (40X).

Figure 1 Basic Technical Process of RNAscope

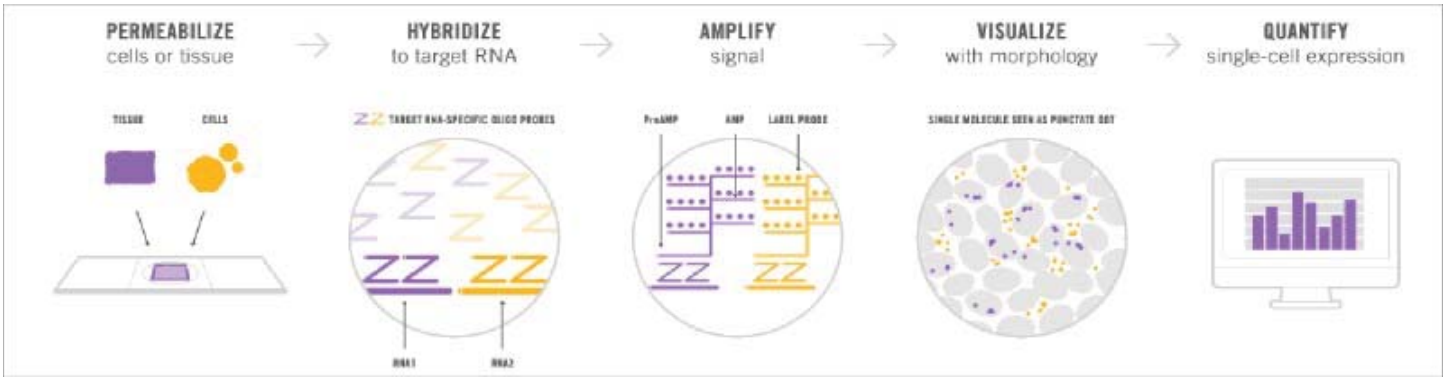
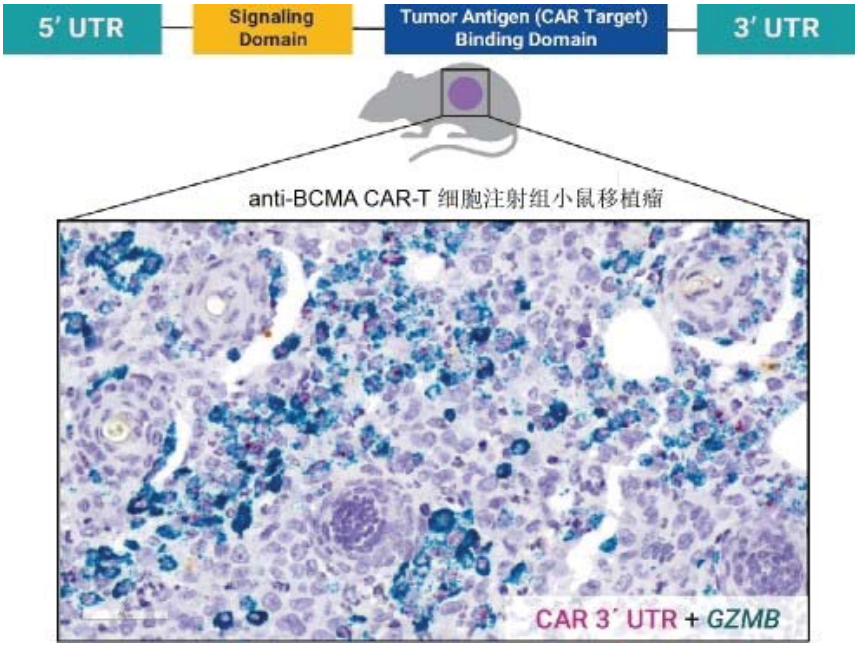


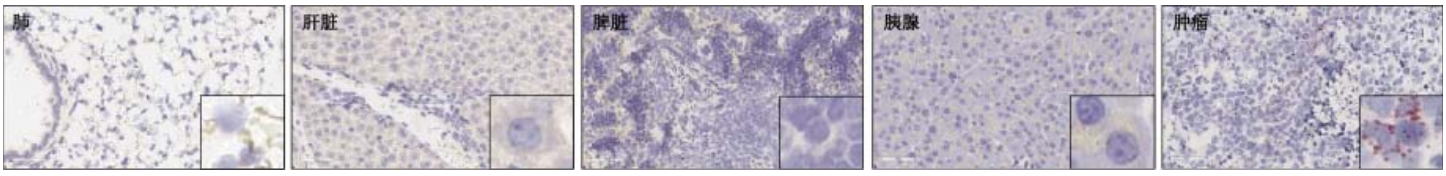
Figure 2 Schematic Diagram of Transcript Expressed by CAR Vector and RNAscope Probe Design



Result

Figure 3. In vivo expression of CAR-T cell target gene products

Mice in the Anti-BCMA CAR-T Cell Injection Group
BCMA Probe



Mice in the Anti- ROR1 CAR-T Cell Injection Group
ROR1 Probe

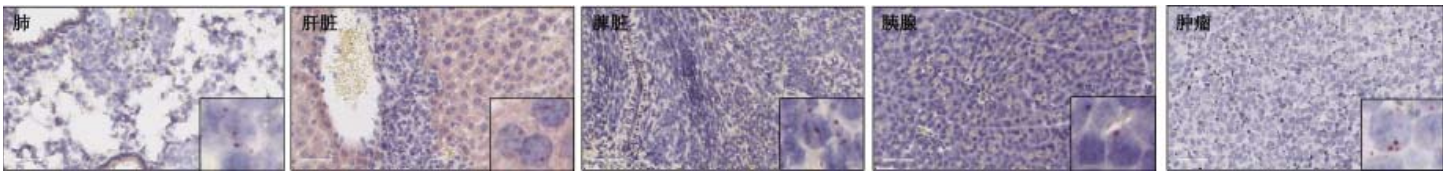
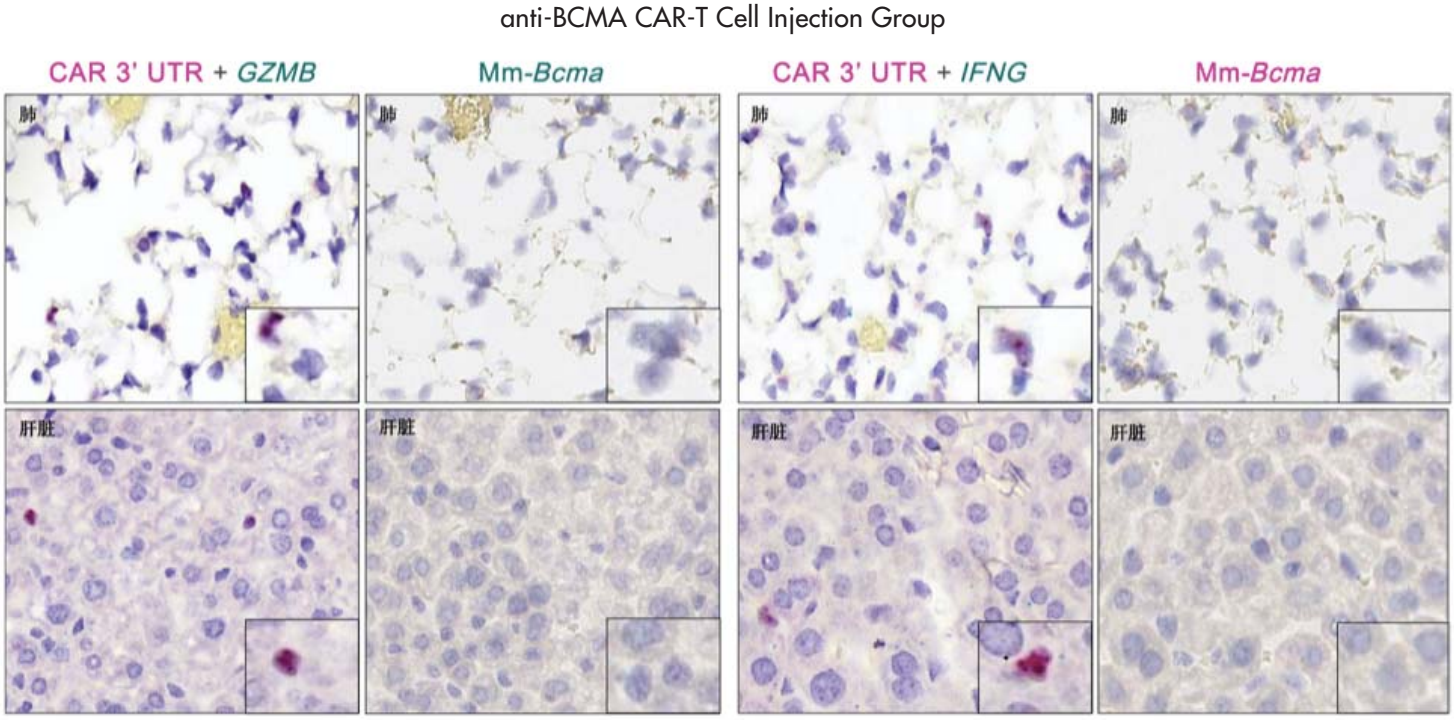


Figure 4 Active anti-BCMA CAR-T cells were detected only in the transplanted tumor tissues, showing the effect of targeted therapy within the tumor anti-BCMA CAR-T Cell Injection Group



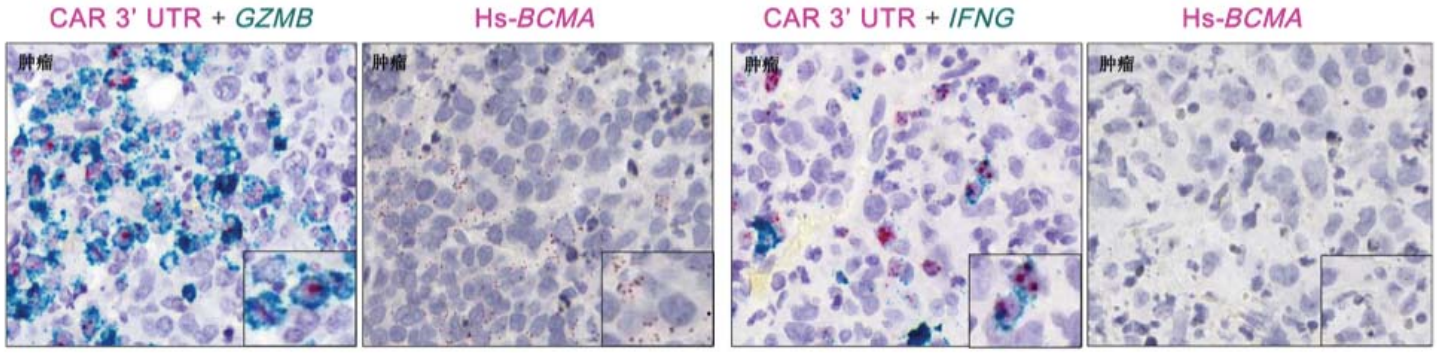


Figure 5 Active anti-ROR1 CAR-T cells were detected in transplanted tumor tissues in lung and liver, showing the characteristic of targeted attacks outside the tumor.

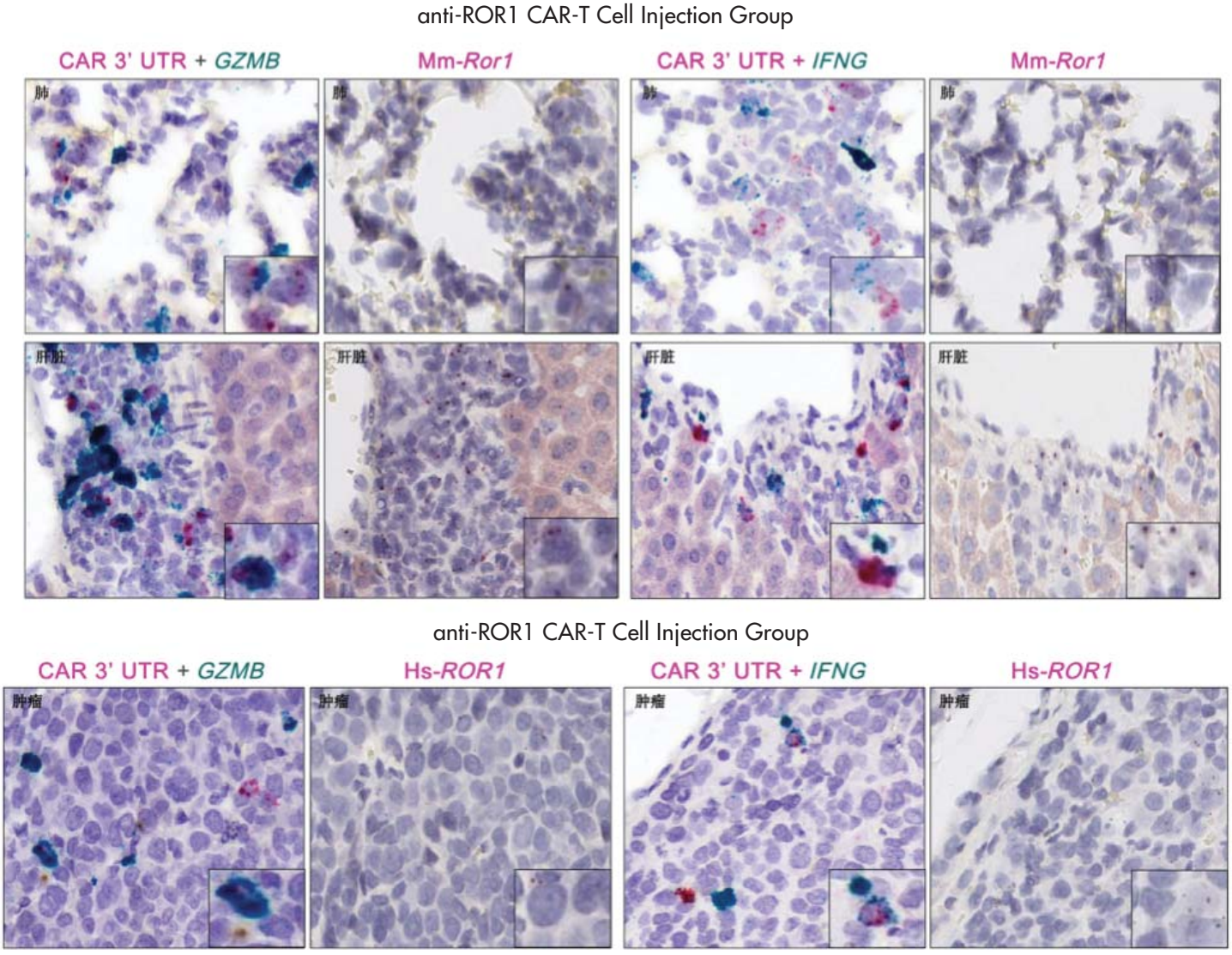
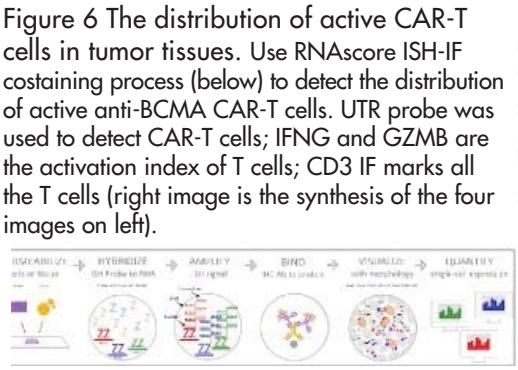
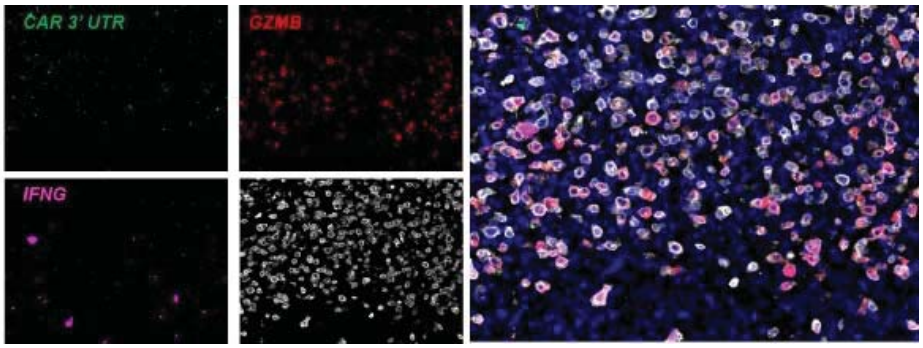


Figure 6 The distribution of active CAR-T cells in tumor tissues. Use RNAscore ISH-IF costaining process (below) to detect the distribution of active anti-BCMA CAR-T cells. UTR probe was used to detect CAR-T cells; IFNG and GZMB are the activation index of T cells; CD3 IF marks all the T cells (right image is the synthesis of the four images on left).





Summary: The data above shows how to use RNAscope in situ hybridization technology in preclinical animal models, by detecting the distribution of target gene products with low expression levels in tumors and non-tumor tissues, and at the same time detecting the pharmacokinetics and viability of CAR-T cells, to evaluate the safety/toxicity and therapeutic effect of CAR-T cell therapy.