

Background

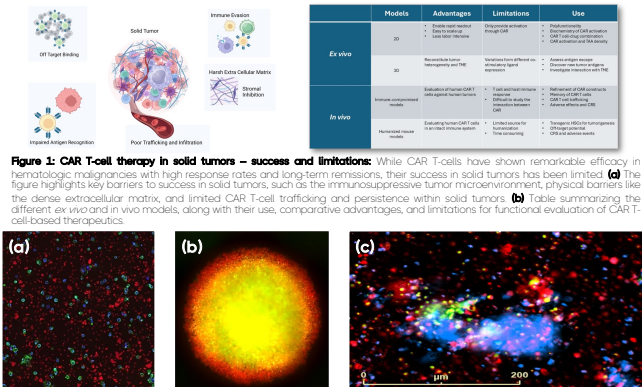


Figure 2: Role of the Tumor Microenvironment (TME) in CAR T-Cell Therapy Success: The tumor microenvironment (TME) in solid tumors is known to be highly immunosuppressive, with various immune-modulatory cells, including regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), all which can hinder the efficacy of CAR T-cell function. (a) 2D coculture model showing the interaction between CAR T-cells and tumor cells, representing a simplified environment to study CAR T-cell efficacy. (b) Coculture of CAR T-cells and tumor cell spheroids, providing a more complex three-dimensional structure mimicking the tumor architecture. (c) Coculture of CAR T-cells with Nilogen-derived tumoroids, demonstrating a more physiologically relevant model that incorporates the TME's influence on CAR T-cell activity and functionality.

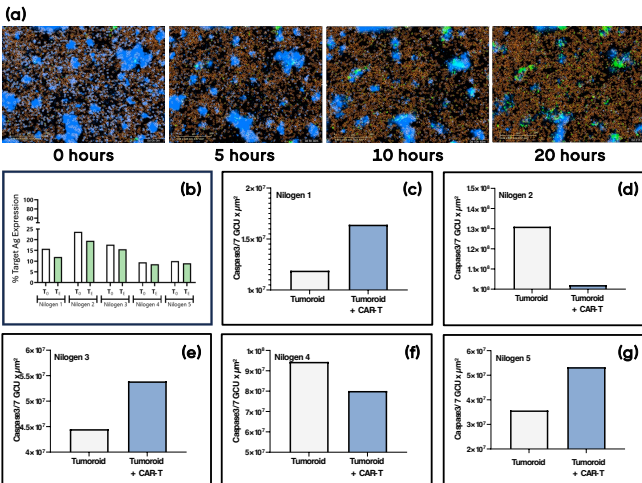


Figure 4: CAR T-Mediated Tumor Cell Killing Assay: Nilogen's tumor cell killing (TCK) assay was performed using high-content confocal imaging to visualize CAR T-mediated changes in tumor cell viability within the live tumoroids. Quantification of relative TCK observed in tumoroids using the indicated treatment conditions and effector to target (E:T) ratios.

Results

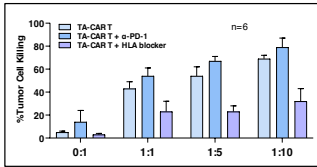


Figure 5: CAR T-Efficiency Assay: Nilogen's tumor cell killing (TCK) assay was conducted using high-content confocal imaging to assess donor-derived variability in CAR T-cell-mediated tumor cell killing within live tumoroids. Quantification of relative TCK observed in tumoroids treated with the indicated conditions.

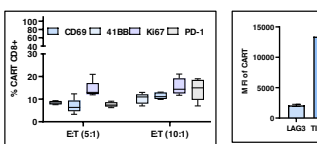
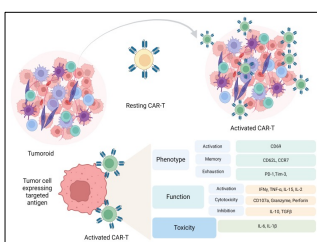
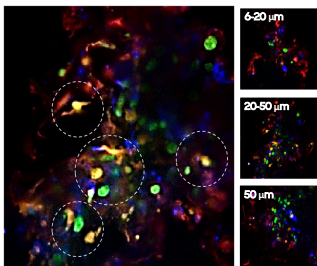


Figure 9: Activation and Exhaustion Marker Expression Levels in CAR T-Cells: This multi-panel box and whisker plot illustrates the expression levels of key markers in CAR T-cells following their interaction with patient-derived ex vivo tumoroids. The panels compare responder versus non-responder samples, highlighting **Activation markers:** CD69, 4-1BB and **Exhaustion markers:** PD-1, Tim-3, and Lag-3.

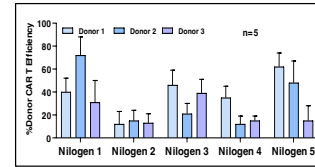
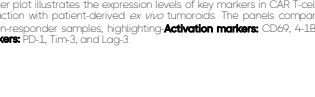
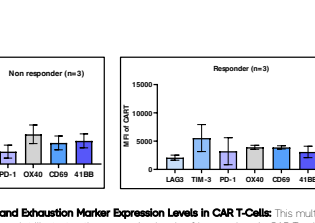
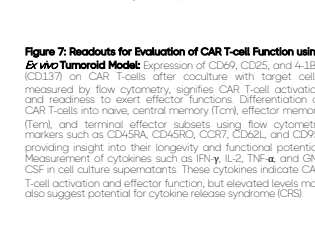
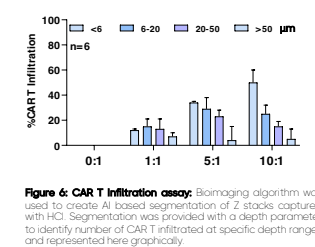


Figure 10: Cytokine Profiling of the Inflammatory Secretome: Culture supernatants post indicated treatment of 3D tumoroids were assayed for expression of cytokines using the MSD Multiplex Assay.



Potential Applications

Applications of CAR T-Cell Therapy Using 3D Tumoroid Models

- Evaluating cytotoxicity:** 3D tumoroid models allow for precise assessment of CAR T-cell-mediated tumor cell killing in a more physiologically relevant environment compared to traditional 2D cultures.
- Modeling tumor microenvironment (TME) interactions:** These models mimic the complex TME, enabling the study of CAR T-cell performance in the presence of immunosuppressive factors such as regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs).
- Predicting patient-specific responses:** Personalized 3D tumoroid models derived from patient samples facilitate testing of CAR T-cell efficacy and resistance mechanisms, helping to predict clinical outcomes.
- Optimizing CAR T-cell design:** By incorporating tumoroid models, researchers can evaluate and refine CAR T-cell constructs to improve targeting, activation, and persistence within the tumor.

The integration of CAR T-cell therapy with 3D tumoroid models for advancing personalized medicine

- Patient-specific tumor modeling:** Tumoroids derived from an individual patient sample retains the unique tumor microenvironment, allowing for a personalized approach to CAR T-cell testing and optimization.
- Tailored CAR T-cell designs:** Based on the specific characteristics of the patient's tumor, CAR T-cells can be engineered to improve targeting and efficacy against the patient's tumor antigens.
- Prediction of therapeutic outcomes:** The use of patient-specific tumoroids enables real-time evaluation of CAR T-cell cytotoxicity, proliferation, and resistance mechanisms, offering insights into how a patient might respond to therapy.
- Precision treatment strategies:** Tumoroid models provide a platform to test multiple CAR T-cell modifications, including those designed to overcome tumor-specific challenges such as antigen escape or immunosuppressive environments.