

Harnessing Tumoroids to Advance CAR-T Cell Therapies

Nilogen Oncosystems Tampa FL 33612

Background



Figure 1: CAR T-cell therapy in solid tumors - success and limitations: While CAR T-cells have shown remarkable hematologic malignancies with high response rates and long-term remissions, their success in solid tumors has been limited. (a) Th figure highlights key barriers to success in solid tumors, such as the immunosuppressive tumor microenvironment, physical barriers lik the dense extracellular matrix, and limited CAR T-cell trafficing and persistence within solid turnors. (b) Table summarizing the different *ex vivo* and in vivo models, along with their use, comparative advantages, and limitations for functional evaluation of CART



Figure 2: Role of the Turnor Microenvironment (TME) in CAR T-Cell Therapy Success

nmunosuppressive, with various immune-modulatory cells ; known to be highly immunosuppressive, with various immune-modulatory cells, suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), all which including regulatory T-cells (Treas), myeloid-c wing the interaction between CAR T-cells and tumor cells, Julture of CAR T-cells and tumor cell spheroids, providing a cture. can hinder the efficacy of CAR T-cell function. (a) oculture representing a simplified environment to study CART-cell efficacy. (b) C more complex three-dimensional structure mimicking the tumor arch ecture. (c) (tumoroids, demonstrating a more physiologically relevant model that incorporates the TME's influence on CAR T-cell activity and functionality

(a)



Figure 3: Timelapse Imaging of Apoptosis in Tumoroids:

sequential images captured over time display apoptosis events occurring within RCC (renal cell carcinoma) tumoroids following itment with anti-TA-CAR therapy. The time-lapse highlights key apoptotic changes as the therapy interacts with the tumoroids, nstrating progressive cell death.

Expression levels of the target antigen are shown in patient samples both before tumoraid plating at TO (represented in white) and the experimental endpoint TE (represented in pale green). The figure illustrates changes in antigen levels across these two time

tomparative quantification of caspase-3/7 activity as a marker of apoptosis is presented for five distinct patient samples. The (c)-(g) bar graphs depict apoptosis induction through the interaction between tumoraids and CART therapy, revealing varying degrees of caspase-3/7 release in response to the treatment across different patient-derived tumoraids.

Results



Figure 4: CAR T-Mediated Tumor Cell Killing Assay: Nilogen's tumor cell killing (TCK) assay was performed using high-conten 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 (ET) ratios.







Figure 8: Activation and Exhaustion Marker Expression Levels: A multi-panel box and whisker plot showing the proliferation marker Ki67, expression levels of activation markers (CD69, 4-1BB) and exhaustion markers (PD1) in CAR T-cells post-interaction with patient derived ex vivo tumoroids, comparing different E:T ratios.



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-cellmediated tumor cell killing within live tumoroids. Quantification of relative TCK observed in tumoroids treated with the indicated conditions



Figure 6: CAR T Infiltration assay: Bioimaging algorithm was used to create Al based segmentation of Z stacks captured with HCl. Segmentation was provided with a depth parameter to identify number of CAR T infiltrated at specific depth ranges and représented here graphically.

Figure 7: Readouts for Evaluation of CAR T-cell Function using Figure 2: reactions of Evaluation II can clear real multicular learning RV:woTumoroid Model: Evaluation of Contraction and 4-188 (CD137) on CAR T-cells offer coulture with target cells, measured by flow cytometry, signifies CAR T-cell activation and readiness to evert effector functors. Differentiation of CAR T-cells into nave, central functors. Differentiation of CAR T-cells into nave. central multicular intercent memory. (Tem), and terminal effector subsets using flow cytometry, markers such as CD45RA, CD45RO, CCR7, CD62L, and CD95, Industry and functional potential. Measurement of cytokines such as IPN- $\gamma_{\rm I}$ IL-2, TNF- $\alpha_{\rm c}$ and GM-CSF in cell culture supernatants. These cytokines indicate CAR T-cell activation and effector function, but elevated levels may also suggest potential for cytokine release syndrome (CRS).



Figure 9: Activation and Exhaustion Marker Expression Levels in CAR T-Cells: This mult 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, 4-1BE and **Exhaustion markers:** PD-1, Tim-3, and Lag-3.







TNF-a

Figure 10: Cytokine Profiling of the Inflammatory Secretome:

Culture supernatants post indicated treatment of 3D tumoroids were assaved for expression of cytokines using the MSD Multiplex Assav

Potential Applications

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

Evaluating evidence with the second state of the second se mediated tumor cell killing in a more physiologically relevant environment compared to traditional 2D cultures.

CAR T

- Modeling tumor microenvironment (TME) interactions: These models mimic the complex TME, enabling the study of CART-cell performance in the presence of mmunosuppressive 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 ilitate testing of CAR T-cell efficacy and resistance mechanisms, helping to predict
- 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 testina and optimization.
- Tailored CAR T-cell designs: Based on the specific characteristics of the patient's tumor, CAR -cells can be engineered to improve targeting and efficacy against the patient's tumor
- 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 Tcell modifications, including those designed to overcome tumor-specific challenges such as antigen escape or immunosuppressive environments.



