Advancing CAR-T Cell Therapies using Nilogen's Ex Vivo 3D Tumoroid Platform

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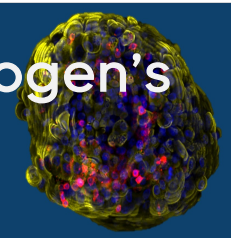
Nilogen Oncosystems Tampa FL 33612



Background

Category	Challenges / Causes of Failure	Example / Detail	Potential Solutions / Strategies
Tumor Antigen Issues	Lack of unique antigens	Many solid tumor antigens are also expressed in normal tissues → on-target toxicity	Dual-target CARs, logic-gated CARs, targeting tumor-restricted antigens
	Antigen heterogeneity	Tumor cells may downregulate or lose target antigens	Bispecific CARs, tandem CARs, or using combination therapies
TME Immunosuppression	Immunosuppressive cytokines	TGF-β, IL-10, adenosine inhibit CAR T function	Use of dominant-negative receptors, gene-editing (e.g., TGFβRKO)
	Inhibitory immune cells	Tregs, MDSCs, TAMs suppress CAR T activity	Combination with checkpoint inhibitors, depletion strategies
	Hypoxia and nutrient depletion	Metabolic competition reduces CAR T cell persistence	Engineering metabolically resilient CAR Ts
Trafficking & Infiltration	Poor CAR Thoming to tumor site	Inadequate expression of chemokine receptors	Engineer CAR Ts to express tumor-matching chemokine receptors
	Physical barriers (dense stroma, ECM)	Fibrotic capsule or ECM limits T cell entry	Co-administer ECM-degrading enzymes (e.g., heparanase)
CAR T-Cell Exhaustion	Chronic stimulation leads to exhaustion phenotype	Upregulation of inhibitory receptors (PD-1, LAG-3, TIM-3)	Checkpoint blockade, gene editing (e.g., PD-1 KO)
Persistence & Expansion	Short-lived CAR T cells	Poor engraftment, rapid apoptosis	Use of memory T cells, cytokine support (e.g., IL-7, IL-15)
	Immunogenicity of CAR constructs	Anti-CAR immune responses in patient	Use of fully human or humanized CARs

Figure 1: *Mechanisms of CAR T-Cell Therapy Failure in Solid Tumors and Potential Solutions*-This table summarizes the major obstacles limiting the success of CAR T-cell therapy in solid tumors, a contrast to its remarkable efficacy in hematologic malignancies. Key failure mechanisms are grouped into categories including tumor antigen challenges, the immunosuppressive tumor microenvironment (TME), trafficking and infiltration barriers, CAR T-cell exhaustion, and limited persistence. Each challenge is accompanied by representative examples and current or emerging strategies aimed at overcoming them. This framework provides a quick-reference guide for understanding the complex interplay between CAR T cells and the hostile solid tumor environment, as well as therapeutic innovations under investigation.

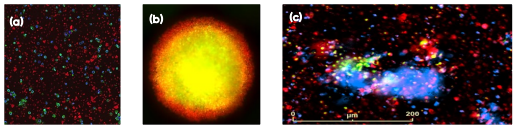
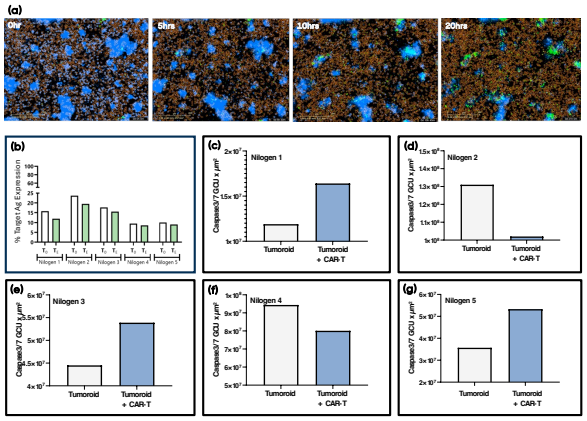


Figure 2: Role of the Tumor Microenvironment (TME) in CAR T-Cell Therapy Success:
The tumor microenvironment (TME) in solid tumors is known to be highly immunosuppressive, with various immune-modulatory cells, including regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated 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.



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Results

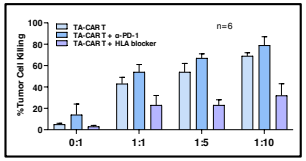


Figure 4: CAR T-Mediated Tumor Cell Killing Assay: Nilogen's tumor cell killing (TCK) assay was performed using high-content confocal imaging to visualize CAR T-mediated changes in tumor cell viability within the live tumoroids. Quantification of relative TCK observed in tumoroids using the indicated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

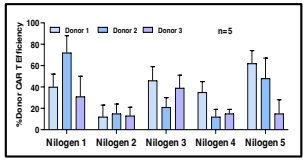
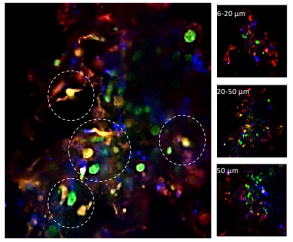


Figure 5: CAR T-Efficiency Assay: Nilogen's tumor cell killing (TCK) assay was conducted using high-content confocal imaging to assess donor-derived variability in CAR T-cell-mediated tumor cell killing within live tumoroids. Quantification of relative TCK observed in tumoroids treated with the indicated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



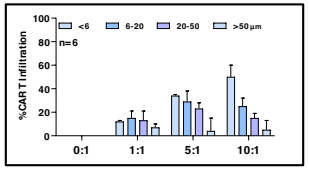


Figure 6: CAR T Infiltration assay: Bioimaging algorithm was used to create AI based segmentation of Z stacks captured with HCI. Segmentation was provided with a depth parameter to identify number of CAR T infiltrated at specific depth ranges and represented here graphically.

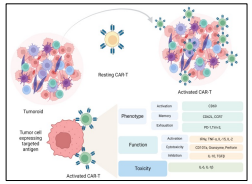


Figure 7: Readouts for Evaluation of CAR T-cell Function using *Ex vivo* Tumoroid Model: Expression of CD69, CD25, and 4-1BB (CD137) on CAR T-cells after coculture with target cells, measured by flow cytometry, signifies CAR T-cell activation and readiness to exert effector functions. Differentiation of CAR T-cells into naive, central memory (Tcm), effector memory (Tem), and terminal effector subsets using flow cytometry markers such as CD45RA, CD45RO, CCR7, CD62L, and CD95, providing insight into their longevity and functional potential. Measurement of cytokines such as IFN-γ, IL-2, TNF-α, 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).

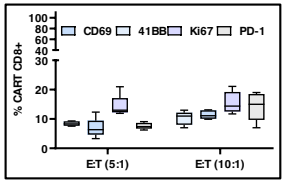
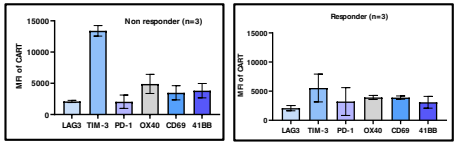


Figure 8: Activation and Exhaustion Marker Expression Levels: A multi-panel box and whisker plot showing the proliferation marker Ki67, expression levels of activation markers (CD69, 4-1BB) and exhaustion markers (PD1) in CAR T-cells post-interaction with patient derived ex vivo tumoroids, comparing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



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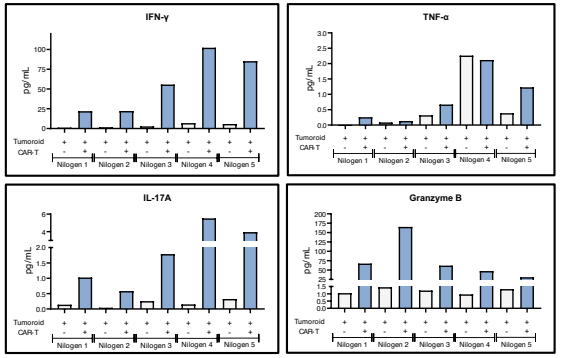

Figure 10: Cytokine Profiling of the Inflammatory Secretome:
Culture supernatants post indicated treatment of 3D tumoroids were assayed for expression of cytokines using the MSD Multiplex Assay.

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.

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