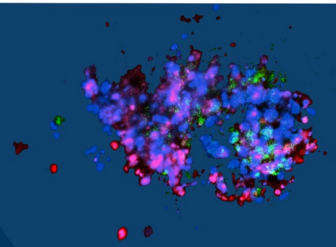
Enhancing T Cell Persistence in Adoptive Cell Therapy: Leveraging Tumoroid-based Ex Vivo Assay for Preclinical Evaluation

Nilogen Oncosystems Tampa FL 33612



Background

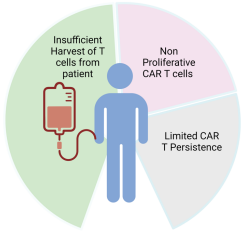


Figure 1: Key Factors Contributing to CAR T Cell Therapy Failure: The three critical limitations of CAR T cell therapy are shown: **Insufficient Autologous T Cells -** Some patients may have an inadequate pool of healthy autologous T cells for CAR modification, reducing the effectiveness of CAR T cell generation and function. **Non-proliferative T Cells -** CAR T cells may fail to expand or proliferate sufficiently after infusion, limiting their ability to achieve sustained tumor clearance. **Limited CAR T Cell Persistence -** Poor long-term persistence of CAR T cells in the patient's body leads to a reduced duration of therapeutic effect and increased risk of tumor relapse. Each of these limitations is represented to underscore the challenges in maintaining CAR T cell efficacy over time.

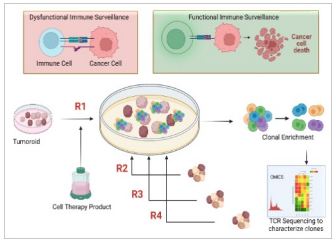


Figure 2. Nilogen's Ex-Vivo Tumoroid Platform for Persistence Assay: The flowchart illustrates the sequential steps for exposing CAR T cells to tumoroids over multiple cycles, highlighting the processes used to assess cytotoxic efficiency and monitor functional changes. Patient-derived tumor cells are cultured into 3D tumoroid structures, which closely replicate the in vivo tumor microenvironment. CAR T cells are then introduced to the tumoroid culture, allowing for their initial interaction with tumor cells, simulating their engagement in the body. After a specified incubation period, CAR T cell-mediated cytotoxicity is evaluated using assays such as flow cytometry and live/dead cell staining to measure the extent of tumor cell killing. Functional assessments are conducted throughout the cycles, monitoring CAR T cell markers of exhaustion, proliferation, and cytokine production, which provide insights into their sustained or declining activity. CAR T cells are repeatedly exposed to fresh tumoroids in multiple cycles, modeling long-term tumor engagement and enabling the evaluation of CAR T cell persistence and functional durability.

Results

1. Time-Lapse Analysis of CAR T Cytotoxic Activity and Persistence with Tumoroid Repeat Challenge

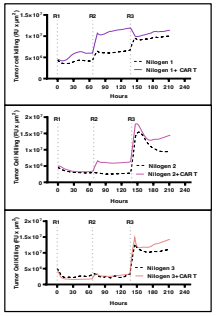


Figure 3: Time-Lapse Imaging of CAR T-Cell and Target Antigen-Expressing Tumoroid Interactions. This time-lapse assay assesses CAR T-cell efficacy and persistence over time, with fresh tumoroids introduced at different time intervals to evaluate the ability of CAR T-cells to sustain their antitumor activity through repeated challenges.Time-lapse image capturing the dynamic interactions between CAR T-cells (CAR Tg) and tumoroids derived from three different patients, each expressing the target antigen for their respective CAR T-cells. Key observations include: **Nilogen 1 -** A robust response was observed, with CAR T-cells maintaining activity and responding to repeated challenges with fresh tumoroids added at 72 hours and 144 hours. **Nilogen 2 -** CAR T-cell activity is delayed, with a notable response only at 144 hours when fresh tumoroids are introduced. **Nilogen 3 -** No significant CAR T-cell response is observed throughout the assay, indicating potential resistance or lack of persistence.

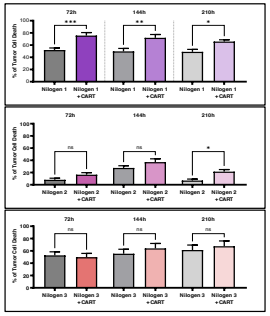


Figure 4: CAR T-Mediated Tumor Cell Killing Assay with Repeat Challenges at 72h and 144h. CAR T-mediated tumor cell killing (TCK) assay was performed using Nilogen's high-content confocal imaging. The assay quantifies relative TCK in tumoroids using the indicated treatment conditions and an effector-to-target (E) ratio of 5:1, providing insights into the persistence, efficacy, and variability of CAR T-cell responses under repeated challenge conditions. The assay visualizes CAR T-cell-induced changes in tumor cell viability within live tumoroids, with data collected at 72, 144, and 210 hours, and evaluated at each timepoint. **Nilogen 1 -** CAR T-cells displayed sustained activity, effectively killing tumor cells with each repeat challenge. Fresh tumoroids were added at 72 hours and 144 hours, and CAR T-cells maintained their efficacy throughout the assay. **Nilogen 2 -** CAR T-cell activity was slower to initiate, with a delayed but notable response, showing significant tumor cell killing only at the final 210-hour timepoint. **Nilogen 3 -** No significant tumor cell killing was observed, indicating limited or no CAR T-cell activity throughout the assay.

2. Longitudinal Assessment of CAR T-Cell Performance

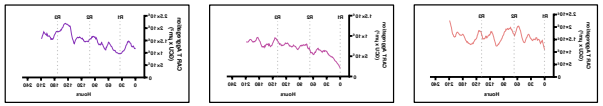


Figure 5: CAR T-Cell Aggregation with Repeat Challenge in Patient Derived Ex Vivo Tumoroids. The differences in CAR T-cell aggregation kinetics across three patients following repeat challenges with fresh tumoroids. CAR T-cell aggregation serves as an indicator of antigen recognition and the trafficking ability of CAR T-cells toward tumor cells: **Nilogen 1** and **Nilogen 2 -** CAR T-cell aggregation is observed, corresponding with their tumor cell killing activity, indicating effective antigen recognition and CAR T-cell movement toward the target. **Nilogen 3 -** Despite not showing significant tumor cell killing after CAR T interaction, CAR T-cell aggregation remains comparable to the other samples, suggesting that antigen recognition and trafficking are intact, but other factors may inhibit cytotoxic activity. This assay highlights the variability in patient responses due to tumor heterogeneity, which can cause a delayed CAR T-cell response despite consistent target antigen expression across samples.

3. Activation and Exhaustion Marker Profiles in CAR T-Cells Post-Challenge and Rechallenge with Tumoroids

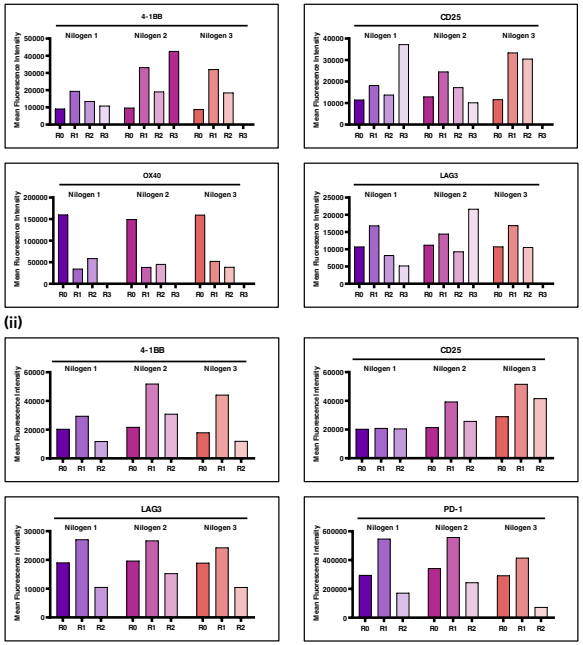


Figure 9: Expression Levels of Activation and Exhaustion Markers in CAR T-Cells. This multi-panel bar graph plot displays the expression levels of crucial activation and exhaustion markers in CAR T-cells following interaction with patient-derived ex vivo tumoroids after repeat challenges with fresh tumoroids. The figure is divided into two panels: panel (i) illustrates data from singlet CAR T-cells, while panel (ii) depicts data from doublet CAR T-cells. **Activation Markers:** CD25 and 4-1BB levels are shown, indicating the degree of CAR T-cell activation following engagement with tumoroids. **Exhaustion Markers:** Expression levels of PD-1 and LAG3 are analyzed, reflecting the extent of T-cell exhaustion, which may impact their long-term efficacy. The results highlight differences in CAR T-cell activation and exhaustion profiles between responders and non-responders, shedding light on factors that may influence therapeutic outcomes.

4. Cytokine Response Dynamics in CAR T-Cell and 3D Tumoroid Interactions

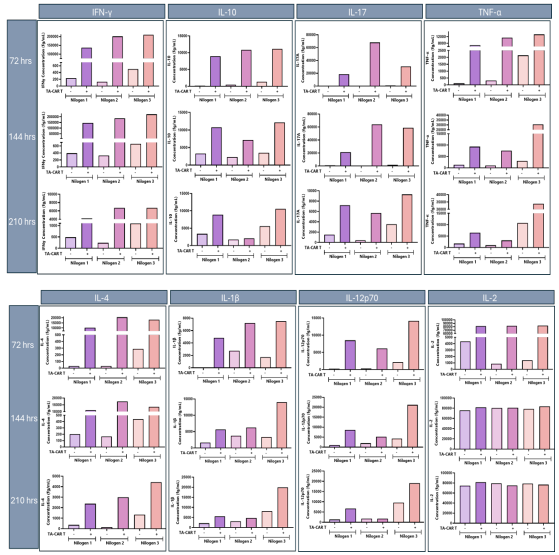
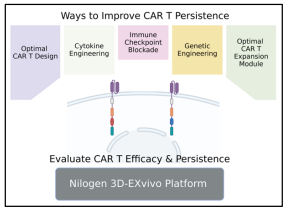


Figure 10: Cytokine Profiling of the Inflammatory Secretome. Cytokine profiling results from culture supernatants collected after initial and repeated challenges of 3D tumoroids. The samples were analyzed for the expression levels of multiple cytokines using the MSD Multiplex Assay. The data provide insights into the inflammatory response dynamics, illustrating changes in cytokine secretion patterns across different conditions and timepoints. These results highlight the variations in immune responses triggered by CAR T-cell interaction with tumoroids, offering a detailed view of the cytokine milieu in the tumor microenvironment during treatment.

5. Evaluate CAR T performance with Nilogen's Platform



Various approaches to improve the persistence and efficacy of CAR T-cells in therapeutic applications, emphasizing the role of the Nilogen 3D-EX vivo platforms in evaluating these strategies:

- **Optimal CAR T Design:** Fine-tuning CAR T-cell design to enhance their targeting and binding efficiency to tumor antigens.
- **Cytokine Engineering:** Modifying cytokine profiles to improve the immune environment and support sustained CAR T-cell activity.
- **Immune Checkpoint Blockade:** Incorporating inhibitors of immune checkpoints to prevent CAR T-cell exhaustion and improve their durability.
- **Genetic Engineering:** Applying genetic modifications to enhance CAR T-cell functions, such as increasing resistance to the immunosuppressive tumor microenvironment.
- **Optimal CAR T Expansion Module:** Developing better expansion protocols to ensure robust CAR T-cell growth and readiness before infusion.

The central pathway highlights the use of the Nilogen 3D-EX vivo platforms for evaluating CAR T-cell efficacy and persistence, serving as a preclinical tool to assess the impact of these modifications in a controlled environment.

NILOGEN ONCOSYSTEMS
Nilogen Oncosystems
3802 Spectrum Blvd., Suite 207
Tampa, FL 33612, USA
www.nilogen.com
Info@nilogen.com
+1 813 440 3588