A Comprehensive Serial Killing Workflow for Assessment of CAR T Cells

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Introduction

Adoptive cell therapy with chimeric antigen receptor T cells (CAR T cells) has succeeded highly in individuals with hematological malignancies. However, its efficacy against most solid tumors remains elusive due to short-term persistence and inadequate expansion of CAR T cells within the tumor microenvironment. Traditional in vitro cytolysis assays often fail to reflect the true antitumor potential of the CAR T cells, as they typically involve brief co-culture periods and assess CAR T cell performance only from a single perspective. To address these limitations, we developed a long-term multiplexed workflow that can robustly and comprehensively assess CAR T cell products.

In our study, the EpCAM CAR T cells were repeatedly challenged with an EpCAM-expressing breast cancer cell line, T47D cells, over seven consecutive rounds. During each challenge, the EpCAM T cells were directly added to the T47D cells cultured on an E-Plate VIEW 96 (E-Plate) for 24 hours. This was followed by effector addition at the effector to target ratios (E:T) of 2:1, 1:1, 0.5 :1, and 0.25:1. The CAR T cell cytotoxicity against T47D cells was continuously measured over three days using an Agilent xCELLigence RTCA eSight system (xCELLigence RTCA eSight). After each coculture round, the CAR T cell metabolism, immunophenotype, and percentage were evaluated using an Agilent Seahorse XF Pro analyzer and NovoCyte flow cytometer. Following assessment, the challenged CAR T cells were transferred to a new tumor cell-containing E-Plate to repeat the assay sequence.

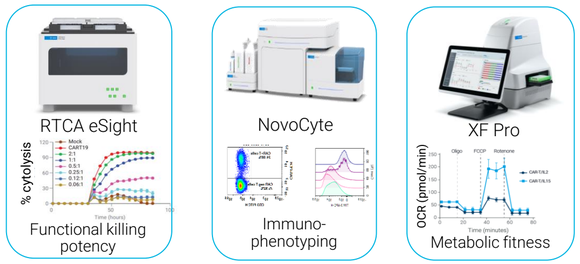
Experimental

Cells

Target cells: T47D, breast cancer cell line expressing EpCAM antigens on the surface. They were purchased from ATCC.

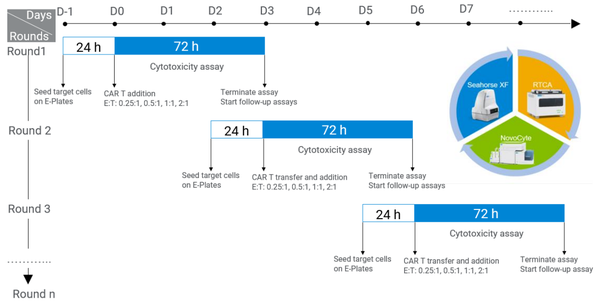
CAR T cells: EpCAM CAR T cells were produced from CD4+/CD8+ enriched T cells, which were activated, transduced, and expanded in the Lonza Cocoon system for 10 days.

Agilent analytical tools



Experimental

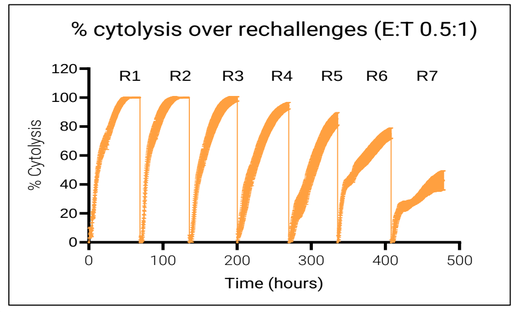
Experimental workflow for characterization of the repeatedly challenged CAR T cells



Results and Discussion

Cytolytic activity of CAR T decreased over repeated challenges.

A.



B.

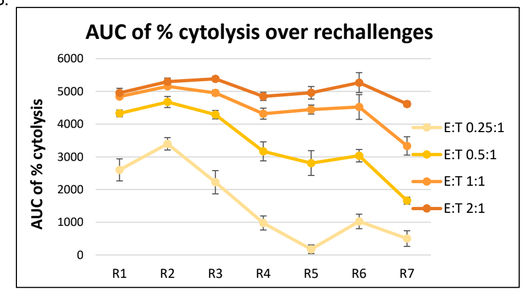


Figure 1. EpCAM CAR T cell cytotoxic capacity was evaluated over seven rounds of target tumor cells (T47D) challenge assays using an Agilent xCELLigence RTCA system. A. Time course of percent cytolysis of T47D at E:T ratio of 0.5:1. B. The area under curve (AUC) of percent cytolysis was obtained at E:T ratios of 0.25:1, 0.5:1, 1:1, and 2:1 over serial killing assays. R: rounds of killing

Results and Discussion

Dynamic changes in percent CAR+ and CAR T cell's proliferation over repeated challenges

A. B.

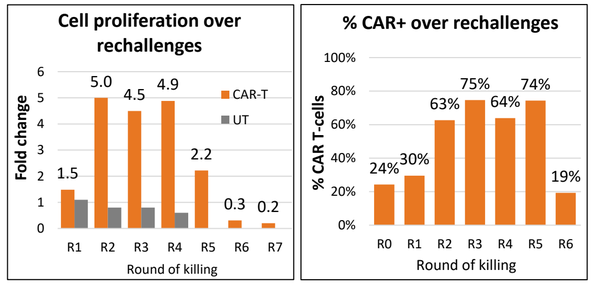
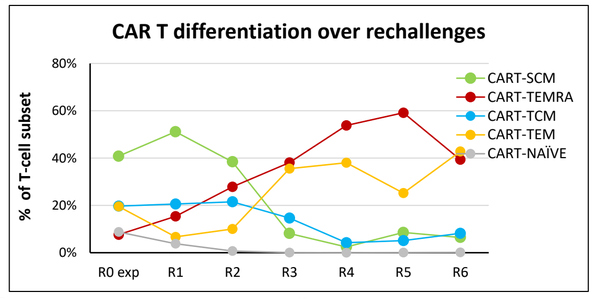


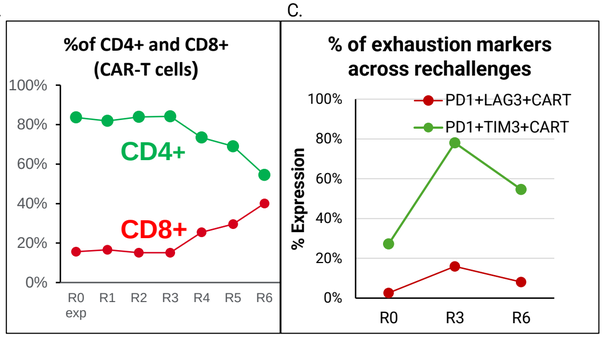
Figure 2. CAR T cell proliferation, survival, and percentage of CAR-positive cells were examined over seven rounds of target tumor cell challenges using an Agilent NovoCyte flow cytometer. A. Fold change of CAR and untransduced T cell numbers. B. Percentage of CAR-positive cells. R0: after CAR T revival.

CAR T differentiation and exhaustion over repeated challenges

A.



B. C.



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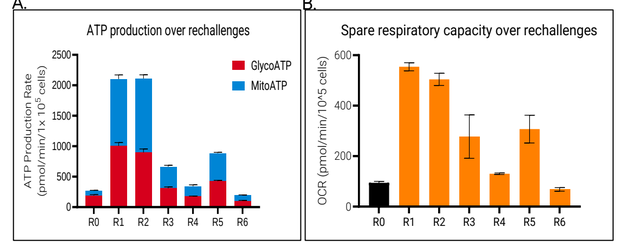
Results and Discussion

Figure 3. CAR T cell differentiation and exhaustion over repeated challenges were assessed using a NovoCyte flow cytometer. Dynamic changes in (A) the percentage of each T cell subset, (B) the percentage of CD4+ and CD8+, and expression levels of PD1+TIM3+ and PD1+LAG3+ (C) in CAR-positive T cells over serial killing assays. R: The round of rechallenge. R0: Right after CAR T revival.

The changes in the metabolic profile of CAR T cells over repeated challenges

A. B.



C.

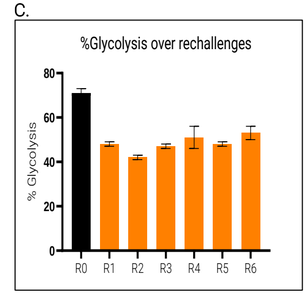


Figure 4. CAR T cell metabolism was assessed over six rounds of target cell challenges using an Agilent Seahorse XF Pro analyzer and T cell metabolic profiling kits. Dynamic changes in normalized ATP production rate (A), normalized spare respiratory capacity (B), and percent glycolysis (C) over serial killing assays.

Conclusions

1. CAR T cells exhibited a gradual reduction in proliferation and cytotoxicity against tumor cells, along with an increase in PD1+ expression across rechallenges.
2. Although the percentage of CAR T cells progressively increased from 25% to 80% by the fifth challenge round, a significant decline was observed in the sixth round.
3. CAR T cells demonstrated a distinct metabolic profile, characterized by progressively decreased maximal respiration, spare respiratory capacity (SRC), and ATP production rate throughout serial killing assays.
4. The multiplexed workflow enables comprehensive evaluation of CAR T cell proliferation, killing potency, exhaustion phenotype, and metabolic profile over rechallenges.
5. The multimodal workflow for T cell characterization during serial killing assays can be applied to CAR T cell design, preclinical, and clinical development.