Investigating the role of IL-7 and IL-21 during in vitro CAR T-cell culture

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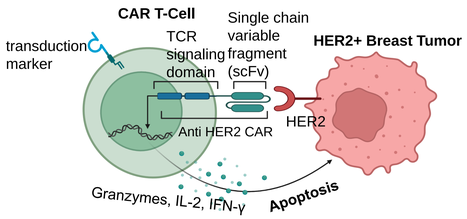
Background

- Expression of a Chimeric Antigen Receptor (CAR) in T-lymphocytes is designed to mimic T cell signaling.
- CAR T-cells can be redirected into patients and specifically kill tumor cells.

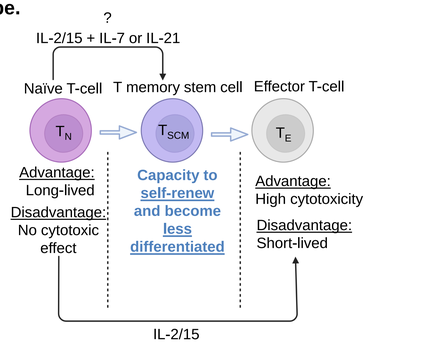


Cytokines & T-Cell Differentiation:

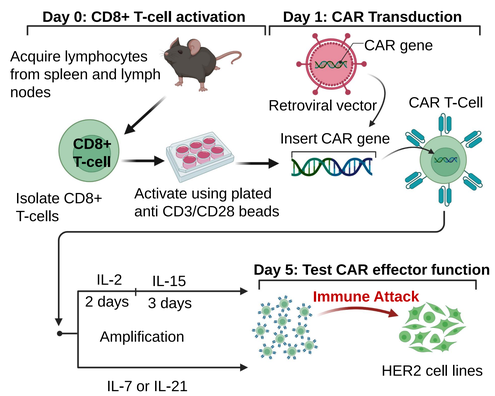
- IL-2 and IL-15 are commonly used for CAR-T cell expansion in vitro.
- However, strong proliferation and differentiation of CAR T-cells in vitro can compromise their longevity in vivo.
- Culturing CAR T-cells with IL-7 and IL-21 has been shown to improve T-cell maintenance and ameliorate anti-tumor response.

Hypothesis:

Adding IL-7 or IL-21 in addition to IL-2/IL-15 will improve CAR expansion, viability and effector function against HER2 cell lines by promoting a stem-like phenotype.



Experimental Design



Targeting Strategy

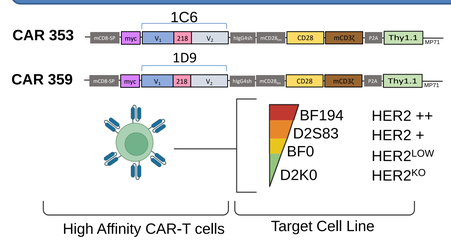


Figure 1: Addition of IL-7 enhances CAR T-cell growth and viability compared to IL-21

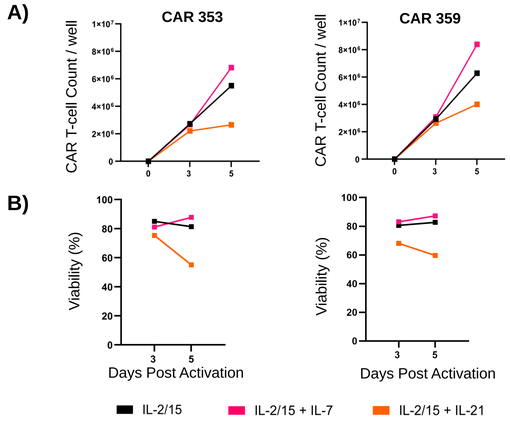
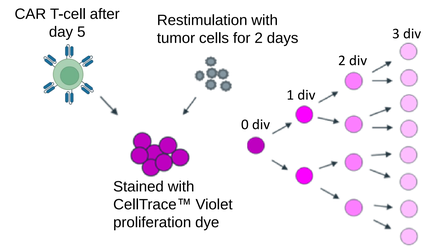


Figure 1: Effect of cytokine cultures on (A) CAR T-cell count and (B) viability.

Figure 2: CAR T-cells show lower proliferation against tumor cells with high HER2 antigen density

A) Cell Trace Violet (CTV) Staining Procedure:



B) CTV Analysis

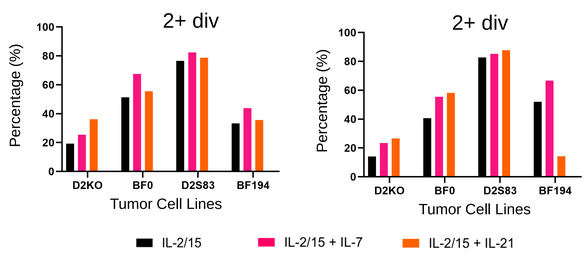
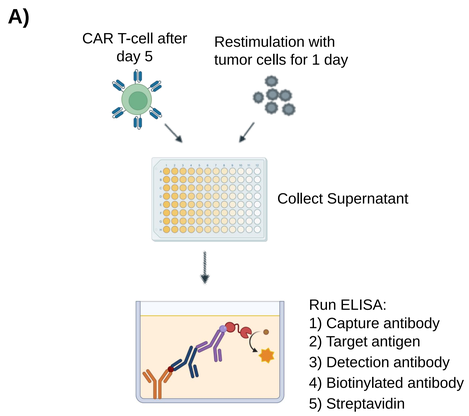


Figure 2: CAR T-cells were collected after 48 hours of coculture. Ratio of effector to target Cells is 2:1. (A) CTV staining procedure. After each cell division, the dye intensity of each cell is halved. (B) Percentage of cells that underwent 2 or more cell divisions.

Figure 3: CAR-T cell expansion in the presence of IL-7 improves cytokine production



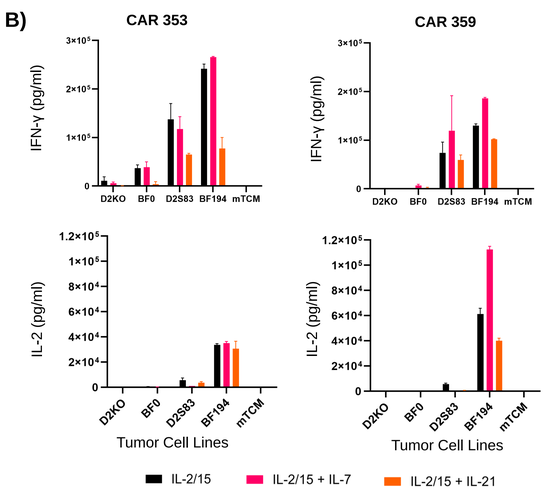
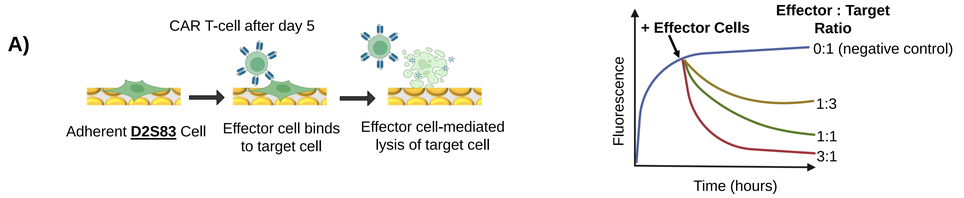


Figure 3: Supernatant of 24 hours co-cultured CAR T-cells with tumor cells. Ratio of effector to target cells was 2:1. (A) ELISA schematic. After solution turned yellow post-reaction, absorbance was measured, and cytokine production was then quantified. (B) IFN-γ and (C) IL-2 quantification using ELISA.

Figure 4: IL-7 and IL-21 improve killing capacity of CAR 359



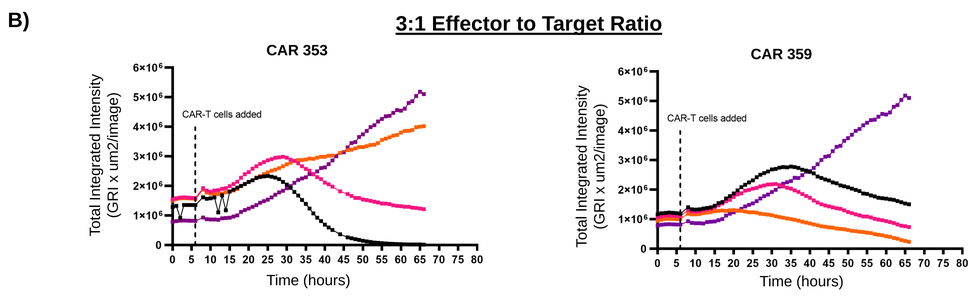


Figure 4: eSight experiment to test CAR T-cell killing when co-cultured with tumor. (A) eSight killing assay schematic. (B) Change in fluorescence over time for CAR T-cells cultured in different cytokine conditions.

Summary

- Addition of IL-7 enhances CAR T-cell growth, viability, and cytokine production compared to IL-21.
- CAR T-cells show lower proliferation against tumor cells with high HER2 antigen density.
- The roles of IL-7 and IL-21 on the killing capacity of CAR T-cells is still unclear.

Future Directions

- To explore how IL-2 and IL-21 interact together.
- To test different cytokine culturing conditions on affinity tuned CAR T-cells.

Acknowledgments

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