Enhancing Transduction Efficiency of Chimeric Antigen Receptor T Cells With RetroNectin and Poloxamer 407

Pedram B. Bayat[1], Ivan J. Cohen, PhD[2], Audrey C. Bochi-Layec[2], Jean Lemoine, MD[2], Puneeth Guruprasad, PhD[2], and Marco Ruella, MD[2,3]

[1]SEAS 2027, Department of Bioengineering, University of Pennsylvania, Philadelphia PA; [2]Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [3]Division of Hematology-Oncology, Hospital of the University of Pennsylvania, Philadelphia, PA

Introduction

- Chimeric antigen receptor (CAR) T cell therapy targeting B-lymphocyte antigen CD19 (CART19) has been effective in treating leukemias and lymphomas. However, many patients experience resistance or relapse to CART19 (**Figure I**).[1]
- One alternative is targeting B cell receptors on malignant B cells which share the same immunoglobulin heavy chain variable region (IGHV). Particularly, anti-IGHV4-34 CAR T cells (**Figure II, III**) have shown increased specificity in targeting diffuse large B cell lymphomas (DLBCL).

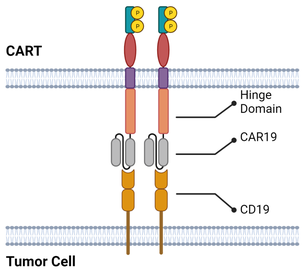

Figure I. CD19-directed CAR T cells

- In fact, anti-IGHV4-34 CAR T cells (CART4-34) have shown the ability to target IGHV4-34+ tumor cells while sparing healthy B cells, while CART19 target both malignant and healthy B cells.[2]

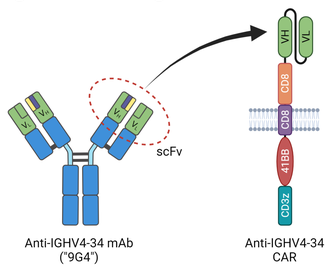

Figure II. CAR4-34 construct

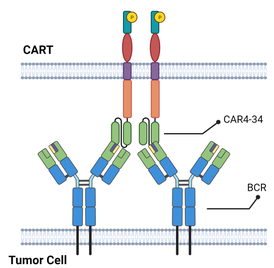

Figure III. IGHV4-34-directed CAR T cells

- Yet, achieving high transduction efficiency when manufacturing these CAR T cells remains challenging. Therefore, this project investigates different methods for developing and expanding CART4-34 cells.

Methods

- To investigate transduction efficiency, different commercially available reagents were used. CART4-34 was manufactured using various transduction efficiency boosters (**Figure IV**) and RetroNectin, which facilitates proximity between virus and target cell (**Figure V**). Finally, transduction efficiency was measured using flow cytometry.
- Lentiviral transduction of the CAR4-34 construct (**Figure II**) was carried out for each booster with or without RetroNectin. The construct contained a truncated EGFR reporter gene, so transduction efficiency was detected using flow cytometry after staining with an anti-EGFR antibody.
- After flow cytometry analysis, the conditions yielding the highest EGFR positivity were selected and CART4-34 expansions were maintained for up to 16 days to observe cell growth and EGFR positivity.

Experimental Design

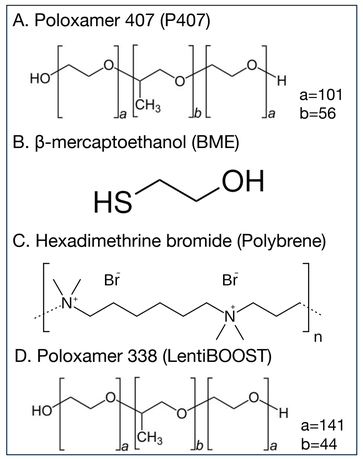

Figure IV. Transduction efficiency boosters.

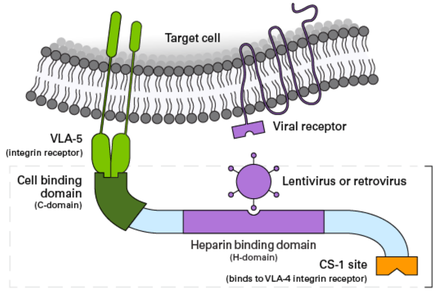

Figure V. RetroNectin facilitates proximity between lentivirus and target cell.

- CART4-34 was manufactured using each transduction efficiency booster, once with RetroNectin (RN) and once without. Therefore, a total of eight transductions were analyzed for EGFR positivity (**Figure VI**).

Results: Transduction Efficiency Improvements

I. RetroNectin and P407 Improve Transduction Efficiency

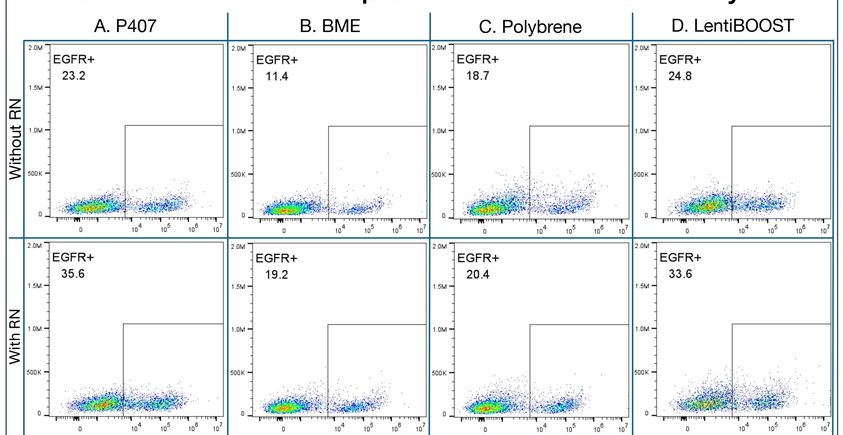

Figure VI. Flow cytometry results showing EGFR positivity in each condition used to examine transduction efficiency in CART4-34 manufacturing. Conditions not treated with RetroNectin (RN) were compared to a control of 13.6% EGFR positivity and conditions treated with RN were compared to control of 18.7%.

- Flow cytometry results indicate that the combination of RetroNectin and Poloxamer 407 yield the highest transduction efficiency (35.6%).
- Based on these results, an expansion of CART4-34 was carried out for 16 days (**Figure VII**).

Results: CART4-34 Expansion

II. CART4-34 Expansions with RetroNectin and P407

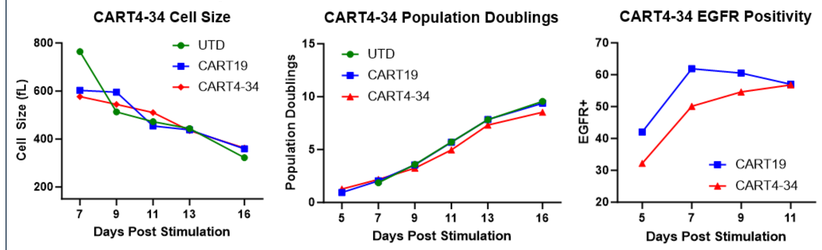

Figure VII. CART4-34 cell size and population doublings observed throughout a 16-day expansion. CART4-34 was compared to CART19 and untransduced T cells (UTD). EGFR positivity for CART4-34 and CART19 was also recorded.

- Strong growth and high EGFR positivity values were maintained for the duration of the expansion, showing the effectiveness of RetroNectin and P407 in producing CAR T cells.

Conclusion & Discussion

- CART4-34 cells manufactured using RetroNectin and P407 have demonstrated high transduction efficiency and strong performance in long-term expansions.
- It is hypothesized that the observed improvements in transduction efficiency are caused by a combination of the close physical proximity of CAR4-34 construct and T cells facilitated by RetroNectin along with the nonionic surfactant properties of P407. Both T cells and lentivirus particles have a negative surface charge, so P407 could improve transduction efficiency by reducing repulsion effects.
- Further investigation is required to validate the use of transduction efficiency boosters in creating human CART4-34 cells targeting DLBCL *in-vivo*. For example, human tumor xenograft models in mice will be used in the future to evaluate CART4-34.

Acknowledgments & References

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