DEVELOPMENT OF ANTI-CD3 CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS FOR ALLOGENEIC CELL THERAPY OF PERIPHERAL T-CELL LYMPHOMA (PTCL)

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INTRODUCTION

Infusion of CAR-T cells results in major clinical responses in B-cell leukemias, B-cell lymphomas, and multiple myeloma, but cell-based and potentially curative therapies for PTCL are not available.

PTCL develops from mature T-cells and tumors from most subtypes retain high and uniform CD3 expression. CD3 expression is also specific to the hematological compartment, making it an attractive CAR-T target antigen.

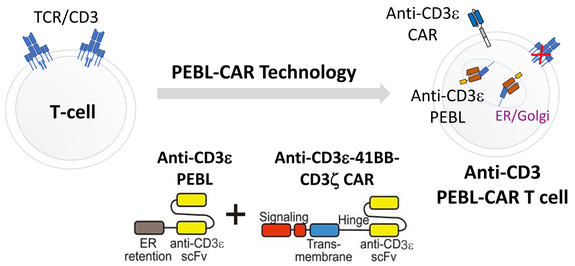
CD3, however, is highly expressed in T lymphocytes. Therefore, expression of an anti-CD3 CAR results in T-cell self-killing.

AIM

To develop an effective CAR T-cell therapy for PTCL by targeting CD3ε

METHODS

- To downregulate CD3, we developed an anti-CD3ε PEBL (Protein Expression Blocker)[1,2] constituted by the single-chain variable fragment (scFv) of an anti-CD3ε monoclonal antibody (UCHT1) and an intracellular retention domain that anchors the cognate antigen to the endoplasmic reticulum and Golgi apparatus before degradation.
- The anti-CD3ε CAR is constituted by the same scFv with a CD8α hinge and transmembrane domain linking it to 4-1BB and CD3ζ.
- Sequential transduction of anti-CD3ε PEBL and anti-CD3ε CAR in human T cells produces anti-CD3 PEBL-CAR T cells.



RESULTS

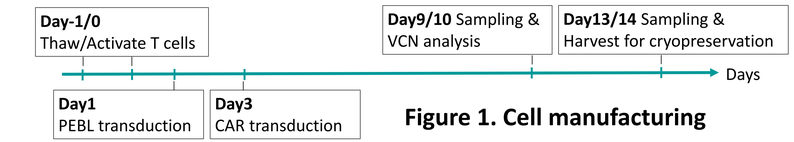


Figure 1. Cell manufacturing

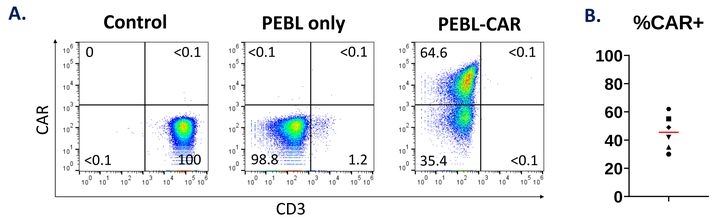


Figure 2. Phenotype of anti-CD3 PEBL-CAR T cells. **A.** High CAR expression and no surface CD3 ("Control" = non-transduced T cells); **B.** Percentage of CAR expression in T cells from 6 donors

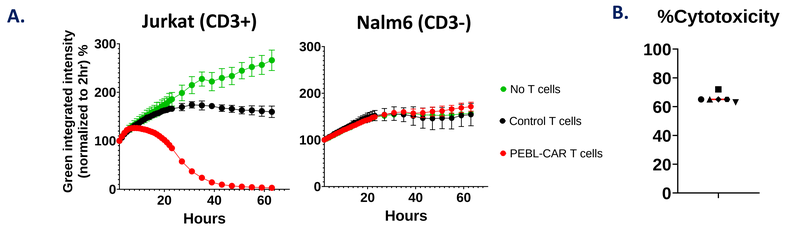


Figure 3. Potent and specific cytotoxicity against CD3+ target cells. **A.** Long-term cytotoxicity assay using IncuCyte live cell analysis; **B.** 24-hour cytotoxicity assay (E:T=1:10; n=6)

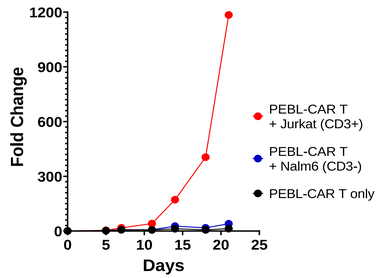


Figure 4. Sustained proliferation stimulated by CD3+ target cells

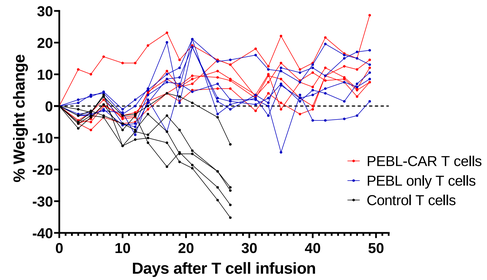
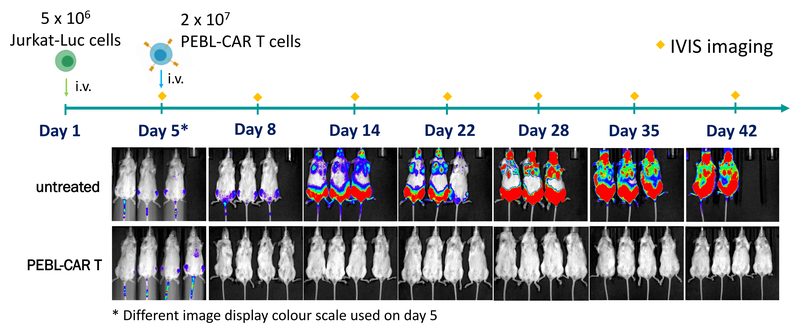


Figure 5. No xenoreactivity in NSG mice
("Control T cells" = non-transduced T cells)



* Different image display colour scale used on day 5

Figure 6. Anti-CD3 PEBL-CAR T cells kill CD3+ tumor cells in a tumor xenograft NSG mouse model. Bioluminescence imaging was used to track the growth of Jurkat cells expressing firefly luciferase.

CONCLUSIONS

- Sequential transduction with anti-CD3ε PEBL and CAR enables generation of high numbers of viable anti-CD3 PEBL-CAR T cells
- Powerful and specific cytotoxicity against CD3+ cells in vitro and in vivo
- Vigorous long-term proliferation in the presence of CD3+ target cells
- No xenoreactivity in a mouse model suggesting suitability for allogeneic off-the-shelf application
- The results support clinical testing of anti-CD3 PEBL-CAR T cells in patients with PTCL and CTCL

REFERENCES

1. Y.T. Png et al. Blockade of CD7 expression in T cells for effective CAR targeting of T-cell malignancies. *Blood Adv 2017; 1: 2348–2360*
2. T. Kamiya et al. A novel method to generate T-cell receptor–deficient chimeric antigen receptor T cells. *Blood Adv 2018; 2: 517–528*

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