Physiologix™ Serum Replacement and NB-ROC™ T-Cell Culture Media Combination Shows Enhanced T-Cell Transduction Efficiency for CAR-T Therapy

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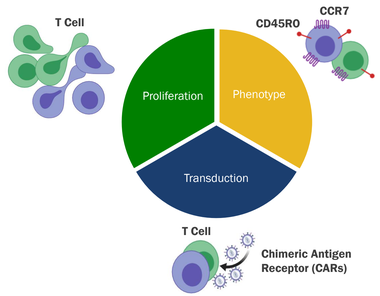
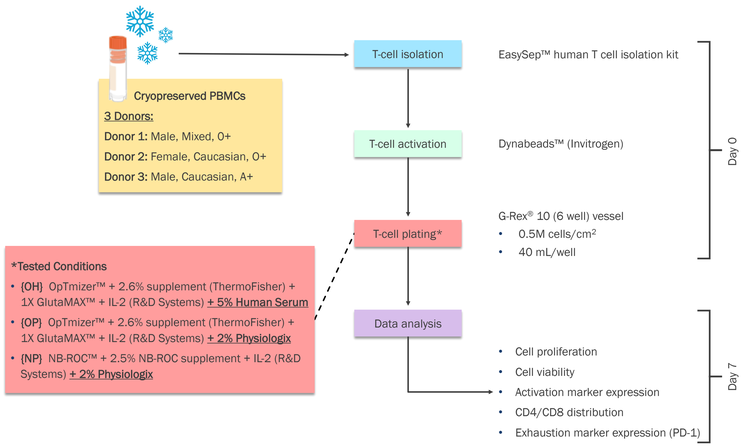
Introduction

CD19 chimeric antigen receptor (CAR) T-cell therapy has shown a great potential for the treatment of B-cell malignancies and lymphoma, and an increased number of studies are in progress for its translation to solid tumors (1,2). Success of this treatment is unfortunately limited by multiple factors such as donor-to-donor variability, patients' response to the therapy, and manufacturing related issues. Cell culture media is a critical factor to consider when improving the process as it is intimately linked to cell function and metabolism (3). In this study, we tested a new cell culture media, NB-ROC™, developed specifically to support activated T-cell proliferation and lentivirus transduction. Based on our previous data generated with the University of Pennsylvania on T cells, we supplemented NB-ROC with 2% Physiologix™ XF (human growth factor concentrate), a cGMP, xeno-free media supplement made for stem cells and T cells that can replace standard serum supplements such as FBS and human serum.



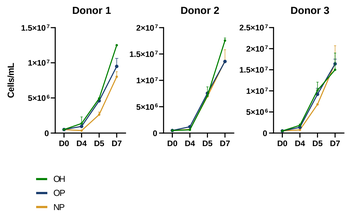
- Maintain proliferation rate
- Significantly improve T-cell transduction (>30% more positive cells)
- Preserve T cell naïve and memory phenotype

Experimental Setting

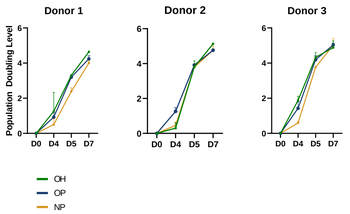


Data Analysis

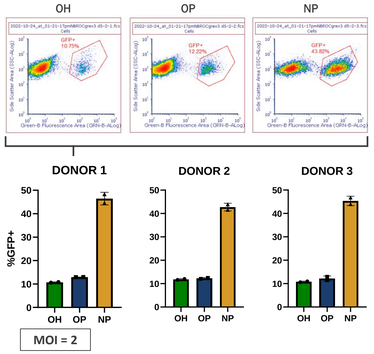
A Cell Proliferation



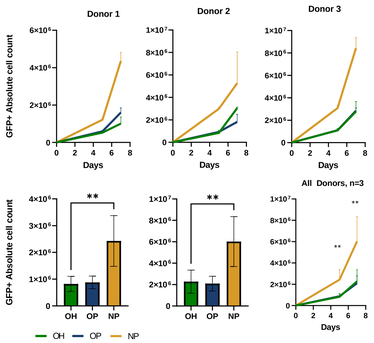
B Population Doubling



C Transduction Efficiency



D Calculated Total Viable CD19 CAR-T Cells



Results

- NB-ROC offers equivalent cell population doubling to off-the-shelf proprietary media (Figures A and B)
- NB-ROC leads to a higher transduction efficiency (40-50%) vs 10% in off-the-shelf proprietary formula (Figure C)
- Using NB-ROC as a cell culture media significantly increases the absolute cell count of GFP+ cells (Figure D)

Discussion

Compared to CTS™ OpTmizer T-cell expansion supplemented with 5% human serum, NB-ROC shows on a Dynabeads-activated T cell model a maintained proliferation rate and population doubling (Figures A and B) with a preserved phenotype - determined through the evaluation of surface expression markers such as PD-1, CCR-7, and CD45RO (data not shown) over a period of 7 days. Lentivirus transduction data is more compelling as it shows an increased level by 20-30% compared to OpTmizer supplemented with human serum (Figures C and D). Our data also confirms previous observations on Physiologix, as supplementing CTS OpTmizer with 2% Physiologix shows significant transduction efficiency improvement compared to when it is supplemented with 5% human serum. All in all, our data show that NB-ROC, as a serum free media, combined with Physiologix offers a very promising alternative for an improved T-cell transduction efficiency with a maintained phenotype and proliferation rate on both 6 well plate and G-Rex model. This set of data holds the promise for enhancing transduction efficiency for CAR-T therapy which will help ameliorate the process and bring more success to the field.

References

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2. Denning et al. (2013). Optimization of the transductional efficiency of lentiviral vectors: effects of sera and polycations. Mol. Biotechnol.
3. Schmueck-Henneresse et al. (2017). Comprehensive approach for identifying the T-cell subset origin of CD3 and CD28 antibody-activated chimeric antigen receptor-modified T-cells. J. Immunol.

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