Improved T cell Proliferation and Transduction Efficiency *ex vivo* for CAR-T therapy in a Xeno- and Serum-Free medium.

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Introduction

Chimeric antigen receptor (CAR) T cell immunotherapy has proven to be very promising for cancer treatment. This therapy consists of using T cells isolated from a patient's PBMCs (peripheral blood mononuclear cells). During this process T cells are maintained in cell culture media, activated and then transduced with a lentivirus to express a CAR (chimeric antigen receptor) gene. Once acquired, T cells expressing the CAR will have the capacity to recognize and attack the malignant cells once transfused back to the patient (1).

Despite the successes achieved to date, additional efforts are needed to improve and develop a completely defined and xeno-free cell culture medium. Accordingly, components of animal origin used in the culture of T cells should be replaced with relevant protein sources and concentrations. **Transparency** is a required component of these efforts, and we strive to include this concept in our media solutions. Using a combination of metabolomics and process optimization **Nucleus Biologics** has identified a new xeno- and serum-free cell culture medium formulation named **NB ROC**.

In this study, we tested **NB ROC** on T cells isolated from the blood of three donors using standard protocols. Our data show that **NB ROC** allowed cells to grow 50% more compared to OpTmizer (*Fisher Scientific*). This increase in proliferation did not compromise early memory phenotypes. Additionally, transduction using a GFP lentivirus was ~40% higher when cells were grown and activated in **NB ROC**, which results in optimization of virus usage and reduction of manufacturing costs.

Critical Parameters for CAR-T Cell Culture Medium

The first parameter for developing CAR-T cells, after PBMC harvesting and T cell separation, is T cell enrichment. During this process T cells proliferate after a CD3/CD28 co-stimulation. At this level, the *ex vivo* cell culture medium in which the cells are maintained is of crucial importance. Indeed, T cell proliferation is highly dependent on the available metabolites and cytokines provided by the *ex vivo* cell culture medium.

Here at **Nucleus Biologics** we developed **NB ROC**, which is a complete xeno- and serum free T cell culture medium able to provide T cells with all the required metabolites with no addition of supplementary cytokines. The use of Physiologix™ XF Human Growth Factor Concentrate as a supplement brings to the medium all the required elements to ensure sustained cell proliferation compared to competitors, without altering T cell phenotype. Furthermore, the second parameter for CAR-T therapy is the preservation of naïve and memory phenotypes. T cell exhaustion is associated with impaired CAR-T therapy outcomes.

After T cell proliferation, the introduction of the genes coding for the CAR is delivered by lentiviral transduction (2), the third parameter. Several studies pointed to the importance of cell culture media for cell transduction. Our serum-free medium, **NB ROC**, improved over 3 times T cell lentiviral transduction compared to OpTmizer + 5% Human Serum.

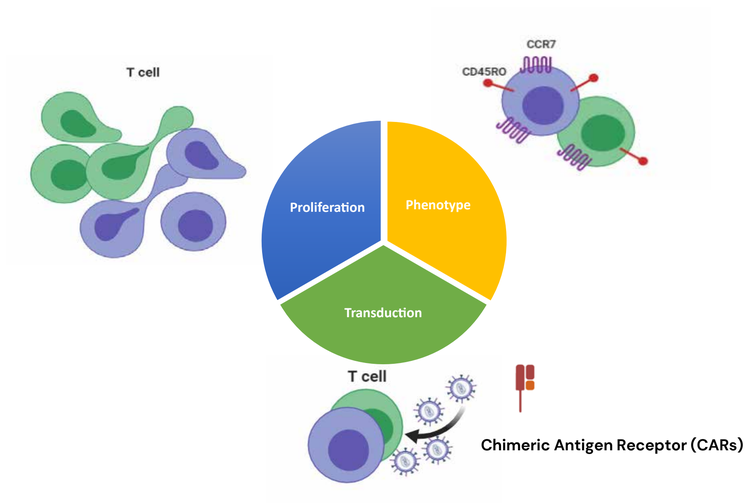

Figure 1. Critical parameters for CAR-T cell culture medium.

CAR-T cell therapy parameters used to evaluate the quality of the manufactured CAR-T cells are: T cell proliferation, phenotype and transduction (3). T cell proliferation is evaluated through cell count over a period of 14 days. Phenotype is identified based on antibody recognition of surface markers. Transduction efficiency is assessed by the use of a GFP virus forT cell transduction.

NB ROC
T Cell Culture Medium

NB ROC is designed to be used along with Physiologix™ XF Human Growth Factor Concentrate (hGFC); a cGMP, xeno-free media supplement made for stem cells and T cells and is able to replace standard serum supplements such as FBS and human serum. **NB ROC** is a medium that improves T cell expansion and generation of CAR-T cells with a preserved memory-like phenotype, thus avoidingT cell exhaustion.

- Improved T cell proliferation (50% more cells)
- Highly improved T cell transduction (~40% more positive cells, higher MFI)
- Preserved T cell naïve & memory phenotype (similar % CCR7+ cells)

Experimental Design

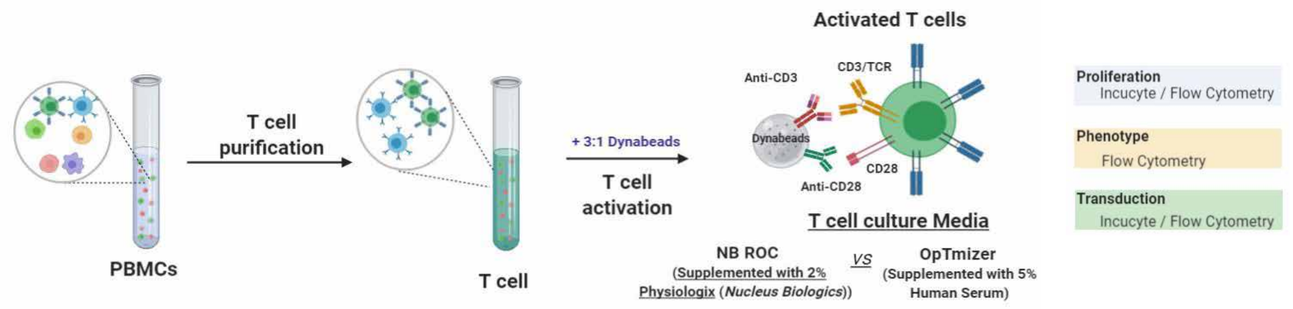

Figure 2. Experimental procedure and key parameters analyzed.

Briefly, T cells were cultured for up to 14 days in NB ROC (+2% Physiologix) vs OpTmizer (+ 5% human serum) and assayed using flow cytometry and IncuCyte to evaluate proliferation and phenotype (CCR7 and CD45RO) after Dynabead activation.To assess transduction efficacy, T cells were transduced using different dilutions of a GFP lentivirus.

Results

T Cell Proliferation

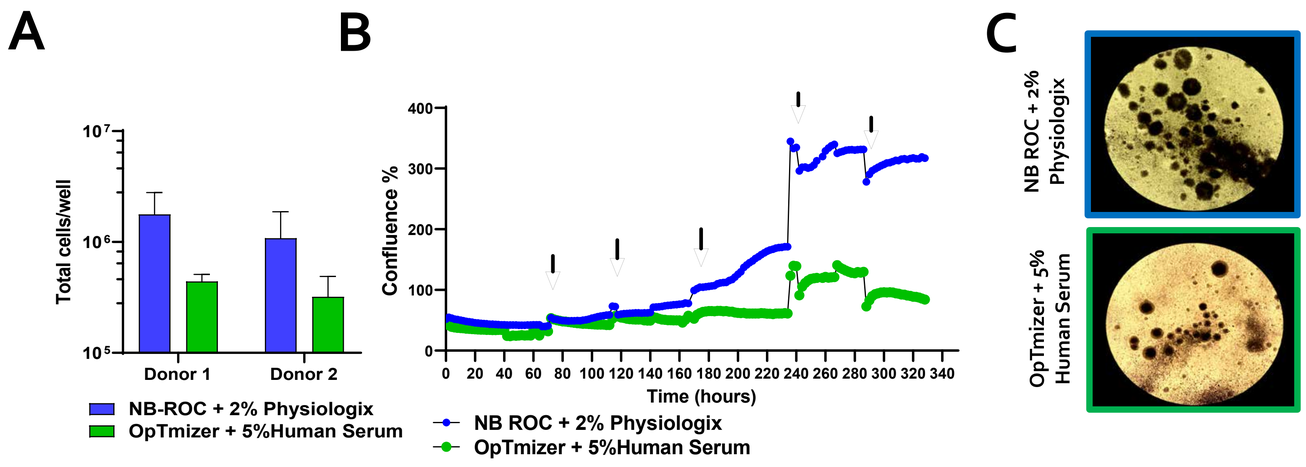

Figure 3. Comparison of T cell proliferation in NB ROC + 2% Physiologix vs OpTmizer + 5% human serum.

A: HemocytometerT cell count 5 days after activation in two different healthy donors.
B: T cell proliferation follow-up from activation to day 14. Data represented in this graph is provided by one donor. Comparable data was generated with two other healthy donors.
C: Echo Revolve Microscopy capture of T cell blasts formation at day 7 post-activation. T cells cultivated and activated in **NB ROC** show higher proliferation and blast formation levels compared to OpTmizer.

T cell Transduction

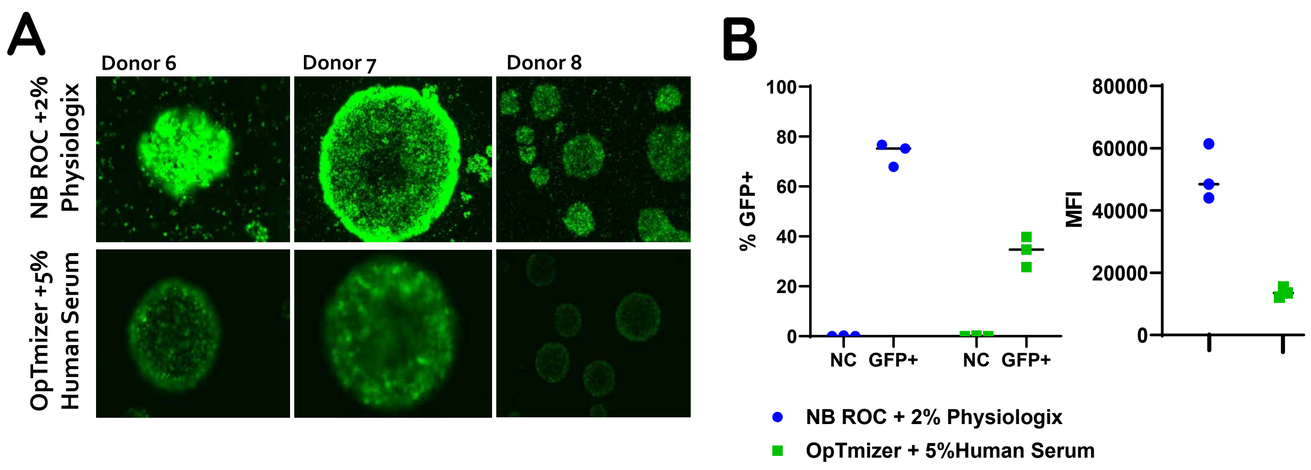

Figure 4. Comparison of T cell transduction efficiency in NB ROC + 2% Physiologix vs OpTmizer + 5% human serum.

A: Incucyte captures of transduced T cells in three different donors at day 5 post-transduction. The green fluorescent staining corresponds to the expression of the GFP gene introduced by lentiviral transduction.
B: Flow cytometry analysis of GFP positive cells and their corresponding MFI at day 5 post-transduction.

T cell Phenotype

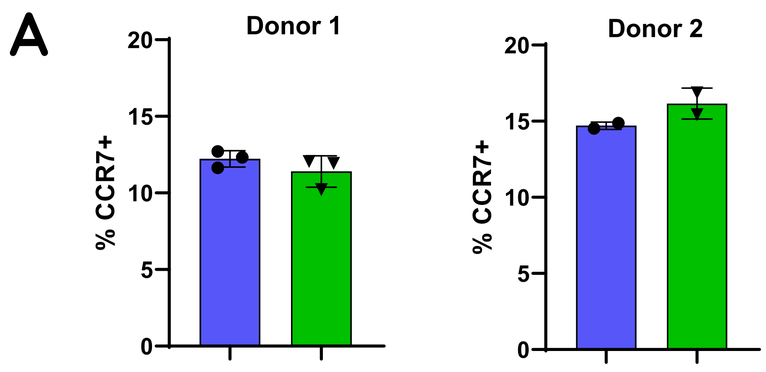

Figure 5. CCR7+ T cell antibody mediated identification at day 7 post-activation *ex vivo* in NB ROC + 2% Physiologix vs OpTmizer + 5% human serum.

Activated T cells were kept in culture to proliferate. The expression of the surface marker CCR7+ was evaluated using a fluorescence marked anti-CCR7+ antibody and analyzed by flow cytometry. CCR7+ T cells include central memory, naïve and stem cell memory T cells

Conclusion

Nucleus Biologics' main mission is to improve cell culture medium by providing researchers and clinicians with a product able to solve the major problems in growing cells *in vitro* and *ex vivo*. We aim to improve cell growth and proliferation, phenotype preservation, and cell transduction by controlling different parameters including lot-to-lot variability and the use of xeno-free and serum-free protein sources. CAR-T therapy is among the most promising immunotherapies to address B cell leukemia and lymphoma. Despite the successes achieved to date, additional efforts are needed to improve outcomes. T cell culture media plays a crucial role to ensure the success of the therapy. Unfortunately, the media available for CAR-T therapy lacks **transparency** and **consistency**. Therefore, at **Nucleus Biologics** we developed **NB ROC, a serum- and xeno-free T cell culture medium. Using NB ROC for T cell culture improved T cell expansion and generation of transduced cells with a memory-like phenotype**. Based on these data, we believe that NB ROC brings a considerable improvement to CAR-T cell therapies.

Acknowledgment

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References

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