A Novel Media Supplementation Strategy for Improved T Cell Culture and Preservation of Naivety

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Introduction

• The rise of CAR-T cell therapies has led to the need for advances in cell culture media to meet the varying demands of cell therapy production (consistency, optimal phenotype etc.).

• Valuable resources are spent optimizing media formulations (e.g. serum free or chemically defined) that are often prohibitively expensive or yield suboptimal growth or function.

• There are challenges with current serum options. FBS is not a viable option for translational cell work. Human AB serum suffers from lack of consistency or availability.

• Cell therapy manufacturers need reagents that allow for them to proliferate and sustain life saving cells in a safe and effective manner.

Experimental Design

Proliferation and Phenotype: Bulk T cells (CD4+ and CD8+) from a health donor were activated for 24 hours using CD3/CD28 Dynabeads. After 24 hours, the beads were removed through magnetic separation and the media was changed. The two media conditions used were:

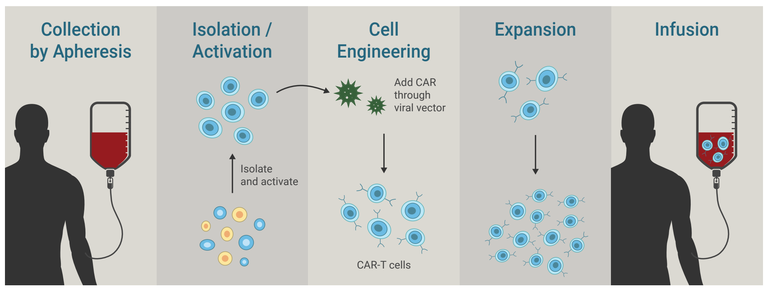
• Control Media: RPMI 1640 (with glucose and L-glutamine) + 1% HEPES + 1% pen/strep + 10% FBS

• Test Media: RPMI 1640 (with glucose and L-glutamine) + 1% HEPES + 1% pen/strep + 2% Physiologix™ XF hGFC

T cells were expanded for 8 days post-activation and monitored for doubling times (FACS), cell size (Coulter Counter) and surface marker expression (FACS). Cells were stained using a viability marker as well as monoclonal antibodies to CD4, CD8, CCR7 and CD45RO. For surface marker expression, cells were gated on size and viability. The CD8+ population was then examined for levels of CCR7 and CD45RO expression. Standard fluorescence minus one (FMO) and compensation controls were completed.

Transduction Efficiency: Bulk T cells (CD4+ and CD8+) from a health donor were activated using CD3/CD28 Dynabeads for three days prior to the addition of a green fluorescent protein (GFP) lentiviral reporter plasmid. Four days later, GFP expression was examined using FACS as a readout for transduction efficiency.

Unmet Need



CAR-T cell manufacturing is expensive & time-consuming

Transduction

Issue: Very expensive, issues with reproducibility.
Solution: Improve transduction efficiency and allow for optimal MOI (more efficient use of vector).
Result: More infected cells, less purification of uninfected cells, higher potency final product.

Expansion

Issue: Replicative capacity is diminished and cells differentiate *ex vivo* leading to low clinical persistence/durability.
Solution: Expand T cells while preserving beneficial phenotypes.
Result: Expand faster and obtain higher potency final product.

Physiologix ™ XF hGFC

Physiologix™ Xeno Free Human Growth Factor Concentrate is a novel serum replacement that is meant to replace fetal bovine serum (FBS) and human serum (HS) in conventional media formulations. Past work has shown that the optimal concentration of 2% Physiologix™ XF is lower than most supplements.

Sourced from transfusion grade donor material, Physiologix™ has been through the following screens:

General
• Sterility/Endotoxin
• Mycoplasma
• pH
• Total protein

Functional proficiency
• Seven day proliferation/growth assay on BM-derived MSCs

Adventitious Agents
• Human Immunodeficiency Virus (HIV) 1 & 2
• Hepatitis B & C Viruses
• Human T-Lymphotropic Virus Types I and II
• Treponema pallidum (Syphilis)
• Trypanosoma cruzi (Chagas disease)
• West Nile Virus & Zika Virus

XENO FREE XF ANIMAL COMPONENT FREE

PROCESSED UNDER cGMP COMP CONDITIONS

Learn more!

Physiologix™ XF hGFC is processed under cGMP conditions and a Drug Master File (DMF) with the FDA is in process.

Results and Discussion

Proliferation

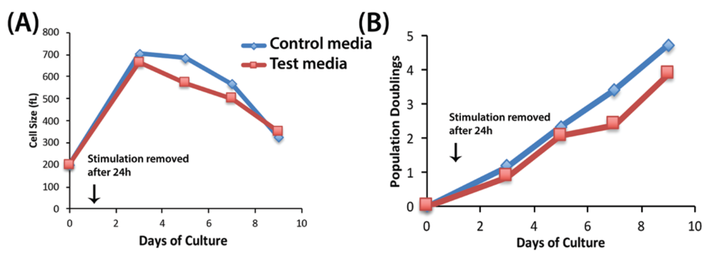


Figure 1.
Representative data of cell size in femtoliters (fL)(A) and population doublings (B) of T cells in FBS containing media (blue) compared to Physiologix™ containing media (red). There is a negligible difference cell size between the control and test media. In graph (B), the test media shows slightly lower population doublings compared to control media.

Transduction Efficiency

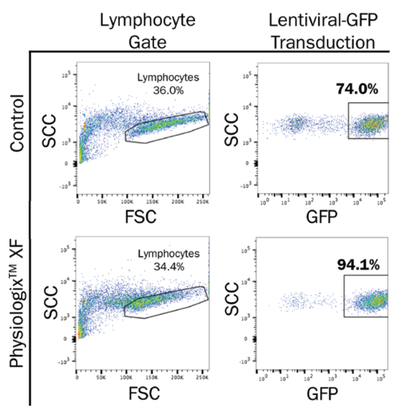


Figure 2.
Flow cytometry data showing transduction efficiency of bulk T cells grown in control media (RPMI 1640 + 10% FBS) compared to test media (RPMI 1640 + 2% Physiologix™ XF hGFC) post-activation. Enhanced transduction efficiency of 94.1% can be seen in the cells grown in Physiologix™ XF. This could significantly reduce the cost of CAR-T cell manufacturing.

Maintaining Naive Phenotype

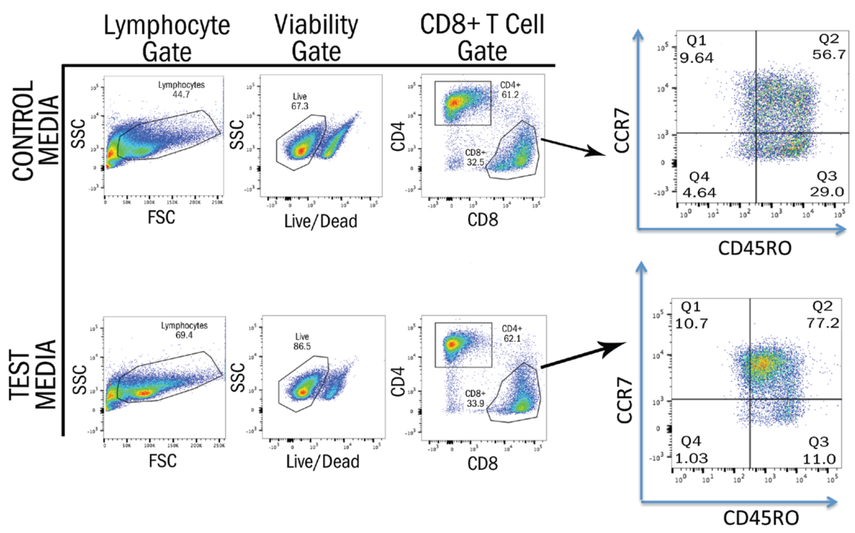


Figure 3.
Flow cytometry data showing phenotype of bulk T cells grown for 8 days in control media (RPMI 1640 + 10% FBS) compared to test media (RPMI 1640 + 2% Physiologix™ XF hGFC) post-activation. Naïve T cells were identified as CD45RA+/CD45RO-/CCR7+ while central memory T cells are CD45RA-/CD45RO+/CCR7+. The CD8+ T cell population grown in the control media exhibits 66.34% naïve and central memory phenotype (Q1+Q2). In contrast, the CD8+ T cell population grown in the test media exhibits 87.90% naïve and central memory phenotype (Q1+Q2). Preventing loss of these phenotypes is thought to correlate with higher persistence and durability leading to better clinical outcomes.

References

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Conclusions

Media formulations for use in CAR-T cell therapy manufacturing have not yet been optimized. Current strategies involve FBS or HS which suffer from lack of consistency or supply. The novel media supplement, Physiologix™ XF hGFC is a serum replacement that could allow for superior clinical outcomes while also reducing the overall manufacturing costs by maintaining more beneficial T cell phenotypes and enhancing transduction efficiency. This functionality may also lead to significantly lower cost of goods for cell therapy manufacturers.