Evidence of improved transduction and preservation of relevant T cell subpopulations using different media conditions: Towards a novel CAR-T media formulation

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Abstract

The field of cell therapy is shifting away from animal origin components used in the culture or preservation of cells and replacing them with human or chemically defined components. However, this shift often leads to diminished cell proliferation and functionality due to the lack of physiologically relevant proteins. Therefore, there is an important need for generation of media supplements to optimize cell therapy. Physiologix™ XF Human Growth Factor Concentrate (hGFC) is a cGMP, xeno-free media supplement that can be used as a replacement to supplements such as human serum. In this study, T cells were isolated from blood and cultured for 14 days in OpTmizer or X-VIVO 15 supplemented with 5% human serum or 2% Physiologix™ XF. Population doublings and cell size were defined, as well as CD4/CD8 and memory markers (CCR7 and CD45RO) to assess phenotype. We detected an increase in naïve and central memory populations in 2% Physiologix™ XF compared to human serum across all tested basal media. These populations have been described as highly relevant for the outcome of patients undergoing CAR-T cell therapy. We compared the transduction efficiency of a lentiviral-GFP in OpTmizer, X-VIVO 15 or RPMI, supplemented with 2% Physiologix™ XF or 5% human serum at different multiplicities of infection (MOIs). Higher transduction efficiency was observed when using 2% Physiologix™ XF at a high MOI of 4 and was conserved across all dilutions including a MOI of <1. These results indicate that Physiologix™ XF can be used to optimize the generation of relevant T cell subpopulations and maximize transduction efficiency even at a low MOI, thus increasing the efficiency of vector usage. These results may lead to better clinical efficacy while reducing overall costs.

Unmet Needs

Transduction Efficiency

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™ XF Human Growth Factor Concentrate (hGFC) is a cGMP, xeno-free media supplement made for stem cells and T cells that replaces standard serum supplements such as fetal bovine serum or human serum. Previous work shows that the optimal concentration of Physiologix™ is 2-5%, lower than most supplements.

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

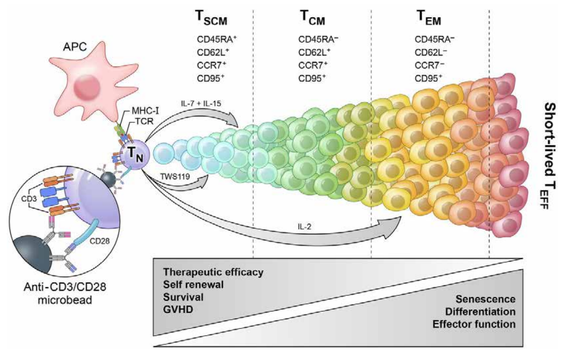
- Sterility
- Endotoxin
- Mycoplasma
- pH

- 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

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

Refining Endpoints

Recent progress in CAR-T therapy has identified several T cell subpopulations that play an important role in therapy outcomes. In particular, therapeutic efficacy has been linked to high capacities of self-renewal and survival, while the presence of highly differentiated and terminal effector T cells correlates with lower therapeutic potential and poor outcome. Among the markers used to define these populations are CD45RA/RO, CCR7 and CD62L. Due to this correlation with outcome, it is of importance to analyze the ratios of these populations after expansion in order to optimize the therapeutic dose and provide an enhanced therapeutic effectiveness.



Programming T-cell fates for therapeutic use. After antigen encounter or stimulation with anti-CD3 and anti-CD28 antibody-conjugated microbeads, naive T cells (T_N) enter a program of proliferation and differentiation that culminates in the generation of terminally differentiated short-lived effector T cells (T_{EFF}). During this process of maturation, T cells progressively acquire effector functions but simultaneously lose their capacities for self-renewal and survival, diminishing their therapeutic effectiveness. Figure originally published by Restifo et al. in Blood 2013 121:567-568.

Experimental Design

Proliferation and Phenotype: Bulk T cells (CD4+ and CD8+) from three healthy donors were activated for 48-72 hours using CD3/CD28 Dynabeads. After 24-48 hours, the media was changed. The two media conditions used were:

- Basal Media: OpTmizer (Thermo Fisher), X-VIVO 15 (Lonza) or RPMI 1640 (with glucose and L-glutamine)
- Supplementation: 5% Human Serum (HS), 2% Physiologix™ XF hGFC serum replacement, or 10% fetal bovine serum (FBS)

T cells were expanded for 10 days post-activation and monitored for population doublings 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 CD4+ and CD8+ populations were then examined for levels of CCR7 and CD45RO expression.

Transduction Efficiency: Bulk T cells (CD4+ and CD8+) from three healthy donors were activated using CD3/CD28 Dynabeads for three days while being grown in OpTmizer or X-VIVO 15 supplemented with 5% HS or 2% Physiologix™ XF prior to the addition of a green fluorescent protein (GFP) lentiviral reporter plasmid at several different multiplicities of infection (MOI). Four days later, GFP expression was examined using FACS as a readout for transduction efficiency. For GD-2 CAR expression, T cells were transduced in the described media and then stained using an anti-CAR antibody followed by a secondary antibody.

In vitro killing assay. Cells transduced with GD-2 CAR were added in culture to target cells expressing GD-2 at different ratios. Percent killing was calculated using a luciferase reporter assay after 20 hours.

Virus production: HEK 293T cells were grown in either 10% fetal bovine serum (FBS) or 2% Physiologix™ XF for virus production. T ells were then transduced using different supernatant dilutions to define the virus amount.

Results

Proliferation

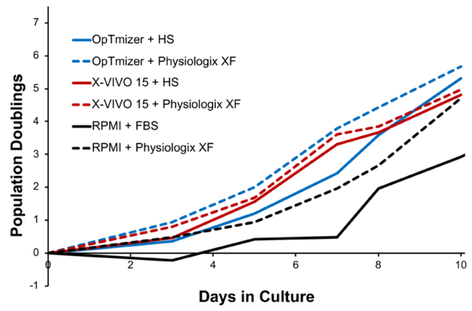


Figure 1. Representative data of population doublings of bulk T cells in different basal media supplemented with either 5% human serum (HS) or 2% PhysiologixTM XF. Regardless of the basal media used, the population doublings were enhanced by Physiologix™ XF.

Transduction Efficiency

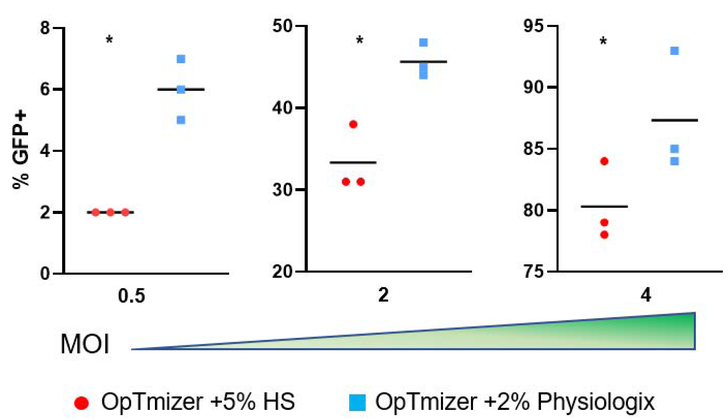


Figure 2. Transduction efficiency (FACS data of positive GFP expression) of bulk T cells (3 healthy donors) grown in OpTmizer supplemented with either 5% HS or 2% Physiologix™ XF. In all MOI conditions tested (0.5 to 4), transduction efficiency was markedly enhanced when Physiologix™ XF was used as a serum replacement.

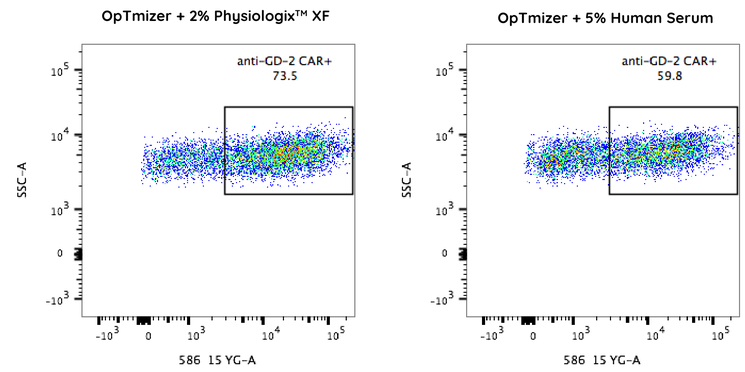


Figure 3. Transduction efficiency of anti-GD-2 CAR in OpTmizer media (C). This could significantly reduce the cost of CAR-T cell manufacturing by increasing the number of clinical dosages that can be produced using a particular amount of viral vector.

Maintaining Relevant Phenotypes

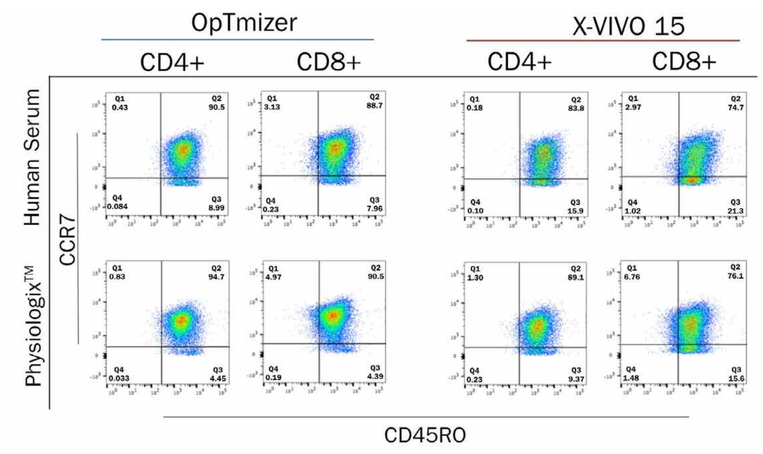


Figure 4. Representative flow cytometry data from one donor showing phenotype of bulk T cells grown for 10 days in different media conditions. All three donors followed this same trend. Naïve T cells were identified as CD45RO-/CCR7+ while central memory T cells are CD45RO+/CCR7+. Gating was used to look at the phenotypes of both the CD4+ and CD8+ subpopulations. When using either OpTmizer or X-VIVO 15 as the basal media, replacement of 5% human serum with 2% Physiologix™ XF enhanced the amount of naive and central memory (Q1+ Q2) T cells in both the CD4+ and CD8+ populations. Preventing loss of these phenotypes correlates to higher persistence and durability leading to better clinical outcomes.

In vitro killing assay

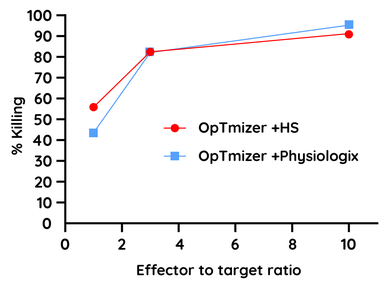


Figure 5. Killing efficiency of transduced GD-2 T cells grown in OpTmizer supplemented with either 5% human serum (HS) or 2% Physiologix™ XF. T Cells were added at different ratios to target cells expressing GD-2. Percent killing was calculated using a luciferase assay.

Virus production

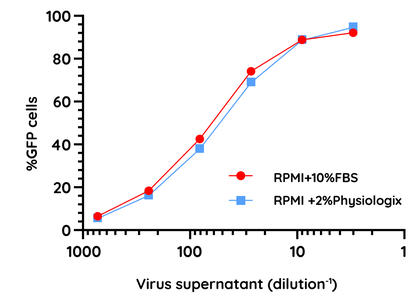


Figure 6. Titration of GFP lentivirus produced in HEK-293T cells growing in RPMI supplemented with either 10% fetal bovine serum (FBS) or 2% Physiologix™ XF for virus production. T cells were then transduced using different supernatant dilutions. Virus production was comparable when cells were maintained in Physiologix™ XF.

References

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[6] Blood 2013 121:567-568; doi: https://doi.org/10.1182/blood-2012-11-468660

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 while maintaining in vitro functionality. These features may also lead to significantly lower cost of goods for cell therapy manufacturers.