CAR-T media development: Novel formulations focused on improved transduction efficiency and preservation of relevant T cell subpopulations

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Unmet Needs in CAR-T Manufacturing

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™ 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:

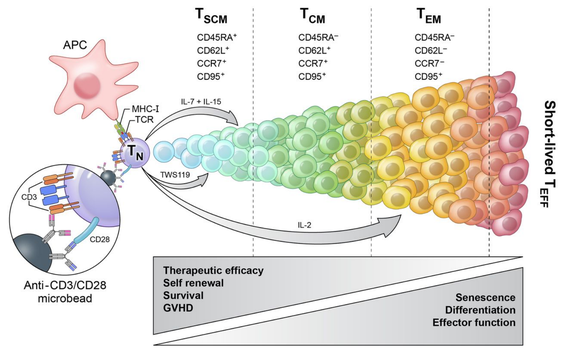
General
- Sterility
- Endotoxin
- Mycoplasma
- pH

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

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

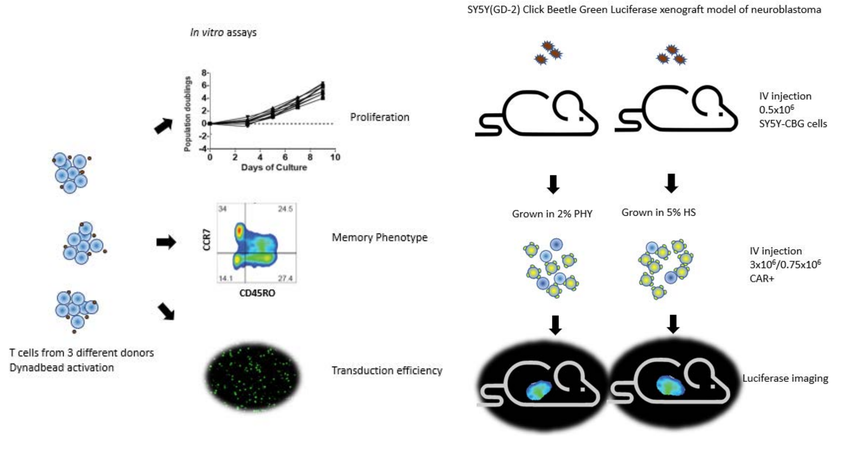
Refining Endpoints

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



Results

Maintaining Relevant Phenotypes

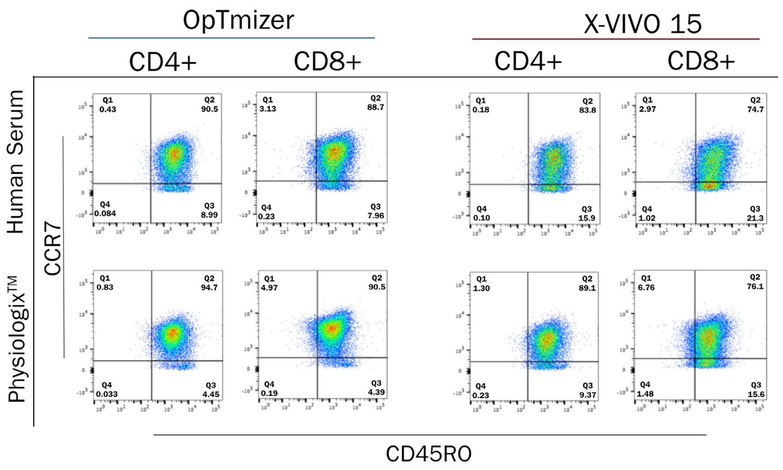


Figure 1. Representative flow cytometry data from one donor showing phenotype of bulk T cells grown for 10 days in different media conditions. All three donors followed this same trend. Naïve T cells were identified as CD45RO-/CCR7+ while central memory T cells are CD45RO+/CCR7+. Gating was used to look 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

Transduction Efficiency

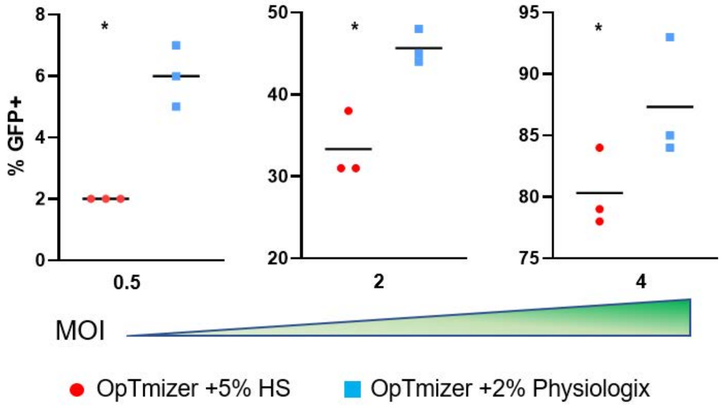


Figure 2. Transduction efficiency (FACS data of positive GFP expression) of bulk T cells (3 healthy donors) grown in OpTmizer supplemented with either 5% HS or 2% Physiologix™ XF. In all MOI conditions tested (0.5 to 4), transduction efficiency was markedly enhanced when Physiologix™ XF was used as a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

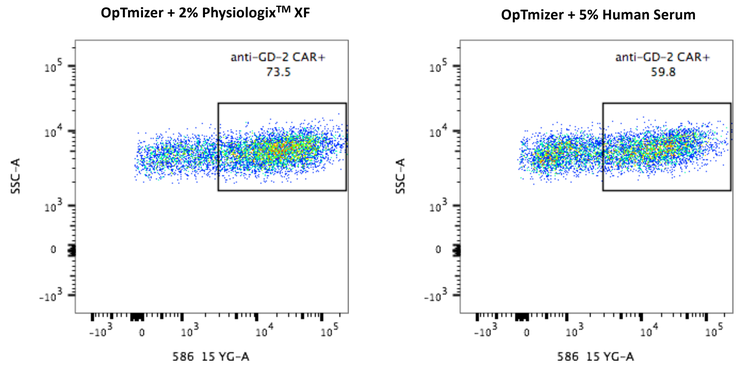


Figure 3. Transduction efficiency of anti-GD-2 CAR in OpTmizer media (C). This could significantly reduce the cost of CAR-T cell manufacturing by increasing the number of clinical dosages that can be produced using a particular amount of viral vector.

In vitro killing assay

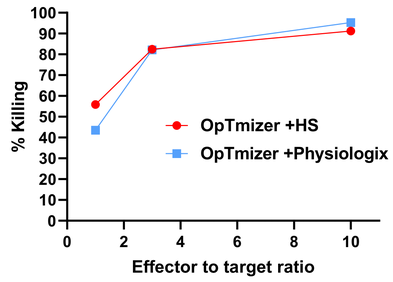


Figure 5. Killing efficiency of transduced GD-2 T cells grown in OpTmizer supplemented with either 5% human serum (HS) or 2% Physiologix™ XF. T Cells were added at different ratios to target cells expressing GD-2. Percent killing was calculated using a luciferase assay.

In vivo neuroblastoma model

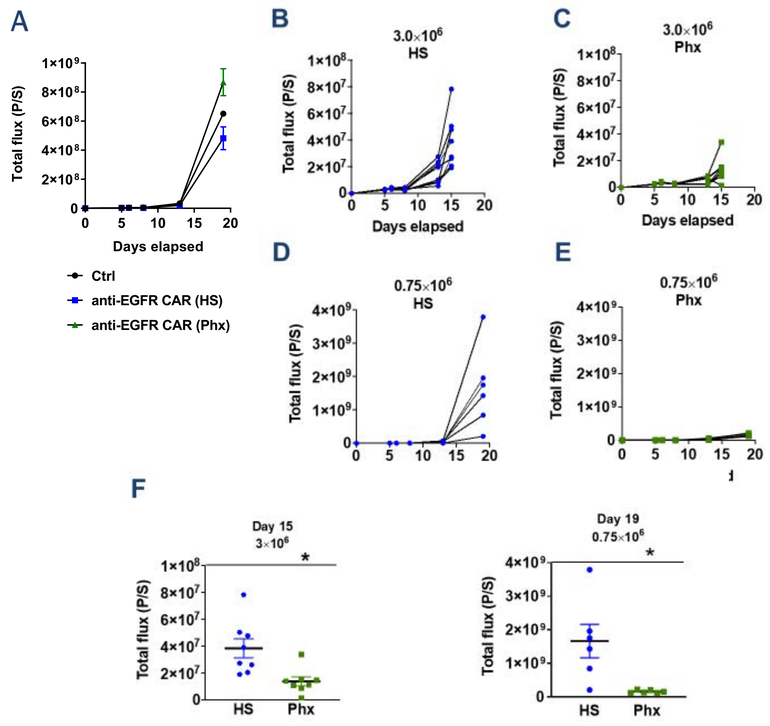


Figure 6. GD-2 neuroblastoma model. **A)** Mice (7-8 per group) were injected via IV 0.5x10^6 SY5Y cells that express luciferase. After 4-6 days, mice were treated with 3x10^6 CAR+ T cells (anti-EFGR, a nonrelated antigen) grown in OpTmizer supplemented with either 2% Physiologix or 5% human serum. **B,C)** Tumor bearing mice were treated with 3x10^6 CAR-positive T cells (specific for GD-2 antigen) grown in OpTmizer supplemented with either 2% Physiologix or 5% human serum. Tumor burden was evaluated over time by administering luciferin. Each line represents an individual mouse. **D,E)** As above, but mice were treated with a limited dose (0.75x10^6) CAR-positive T cells to further evaluate differences in treatment efficacy. **F)** Individual data points from day 15 for 3x10^6 dose and day 19 for the 0.75x10^6 dose. Bar represents mean +/- SD. *P<0.05 using ANOVA.

Virus production

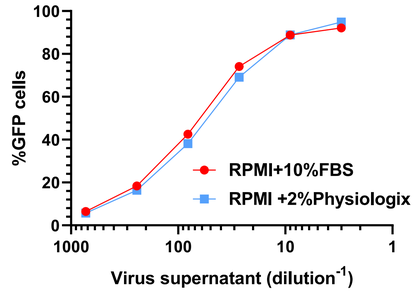


Figure 6. Titration of GFP lentivirus produced in HEK-293T cells growing in RPMI supplemented with 10% fetal bovine serum (FBS). Cells were then either maintained in FBS or transferred to 2% Physiologix™ XF for virus production. T cells were then transduced using different supernatant dilutions. Virus production was comparable when cells were transfected 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 allows for superior outcomes *in vivo* in a solid tumor model. The mechanisms of this enhanced activity are currently under investigation. Physiologix™ XF hGFC also reduces 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.