A Novel, Chemically Defined, Serum-Free Cell Culture Medium for Expansion of Transfected T Cells

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Introduction

T-cell therapies require *ex vivo* culture and expansion of cells at scales relevant for therapeutic applications. Human AB serum (HAB) or fetal bovine serum (FBS) have been key components of the cell culture media used in T-cell therapy manufacturing processes. Although these components assist in optimal growth and improved gene transfer efficiencies, they have inherent variability and supply challenges.[1] In addition, due to animal origin and traceability issues, they have a potential of introducing pathogens to the process. Without the addition of HAB or FBS, T cells, especially when patient derived, fail to grow adequately and exhibit reduced efficiencies of gene transfer, irrespective of the transfection method applied.

In this study, we present data generated with TheraPEAK® T-VIVO®, a novel, chemically defined, non-animal origin cell culture medium utilized to grow T cells in the absence of serum, before and after transfection with the Nucleofector® Technology. The serum-free medium robustly expanded human T cells and worked well in combination with Nucleofection® Electroporation as demonstrated by transfection efficiency and cell proliferation results.

Materials and Methods

Cell Preparation: Cryopreserved Human Peripheral Blood Mononuclear Cells (**hPBMC**, Lonza CC-2702) were thawed on day 0 and incubated 37°C/5 % CO_2 for 2 hours in the serum-free medium (TheraPEAK® T-VIVO®, Lonza). Cells were then seeded at 1.0 x 10^6 cells/mL and activated with TransAct™ (Miltenyi 130-111-160) 10 µL/mL in medium (TheraPEAK® T-VIVO®, Lonza) containing interleukin 2 (IL2) 100 IU/mL (Prepotech™, 200-02-250UG) and incubated at 37°C/5 % CO_2 for 3 days.

Alternatively, Cryopreserved Human Peripheral Blood **CD3+** Pan T Cells (Lonza, 4W-350), isolated from peripheral blood using negative immunomagnetic selection, were thawed on day 0 and resuspended in serum-free medium (TheraPEAK® T-VIVO®, Lonza). Cells were seeded at 5.0 x 10^5 cells/mL and activated with TransAct™ 10 µL/mL in medium containing IL2, 100 IU/mL. Cells were incubated at 37°C/5 % CO_2. On day 3 cells were transferred in T-75 flasks and fresh medium, with IL2 was added. Cells were cultures at 37°C/5 % CO_2 for 2 days.

Transfection: On day 3 (hPBMC) or day 5 (CD3+), 2.5 or 5.0 x 10^6 cells were resuspended in 100 µL Solution P3 containing 20 µg/mL of pmaxGFP™ Vector (3.4 kb) or 40 µg/mL HA tagged reporter plasmid (7.1 kb). Cells were transferred into the 100 µL Nucleocuvette® Vessel and transfected in the 4D-Nucleofector® X Unit (Figure 1) with program EO-115 or CM-138.

Cell Culture: Immediately after electroporation, cells were diluted with the serum-free medium containing IL2, then transferred into T-25 flasks at the concentration of 1.0 x 10^6 cells/mL. The cell culture media was fully exchanged 48 hours after transfection, and cells seeded at 5.0 x 10^5 cells/mL.

Analysis: On the indicated time points, cells were stained with DAPI (Sigma), and where appropriate with CD3 (Anti Mouse Anti-Human CD3) and HA-Antibody mouse IgG (Miltenyi Biotech) and analysed using flow cytometry (Attune™ NxT Flow Cytometer, Thermo Fisher) to measure transfection efficiency (% GFP positive or HA positive cells vs DAPI negative population of CD3+). Viable cell number and viability (% viable cells vs total cell number) were evaluated using the Nucleocounter® NC-250™ (Chemometec).

A B C

Work-flow	Day 0	Day 3	Day 4	Day 5	Day 7	Day 10
PBMC	Thawing activation	Transfec-tion	Analysis of expression	Media exchange	Media top up	Final time-point

Work-flow	Day 0	Day 3	Day 5	Day 6	Day 7/10	Day 12
CD3+	Thawing activation	Dilution	Transfec-tion	Analysis of expression	Media exchange/ top up	Final time-point

Figure 1: 4D-Nucleofector® System. **A**) 4D-Nucleofector® Core Unit and X Unit for transfection in 20 µL and 100 µL volume. **B**) 100 µL Nucleocuvette® Vessel for transfection of 10^6 – 10^7 cells, **C**) experimental workflow for the different source materials.

Results

Efficient Electroporation and Growth of T Cells Cultured in Serum-free Medium

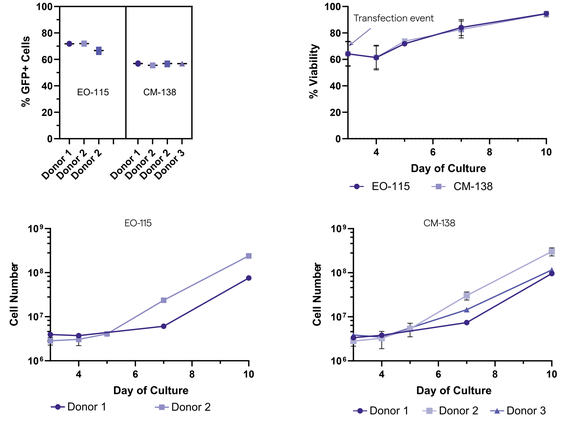
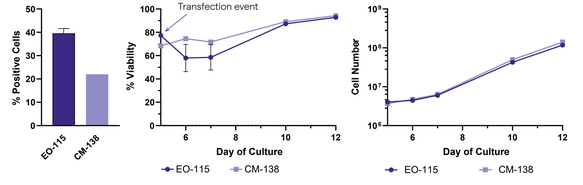


Figure 2: Transfection efficiency, viability and growth profile for T cells initiated from hPBMC and electroporated to deliver model cargo pmaxGFP™ Plasmid. Two recommended programs for activated T cells were applied, EO-115 (2 donors, 3 experiments, 3 replicate/experiment) or CM-138 (3 donors, 3 experiments, 3 replicate/experiment). For Donor 2, two independent experiments are displayed to highlight electroporation reproducibility. The transfection efficiency (%GFP+ cells) achieved with EO-115 is generally higher. However, the cell number over days in culture indicates larger proliferation for the samples electroporated with program CM-138, which is a gentler pulse. Electroporation had an impact on cell viability, which dropped to about 60% immediately after the event. However, even if the medium did not contain serum, viability rose back to the 90's five days after transfection for all conditions tested. The total fold expansion for the three donors in the serum-free medium is recapitulated in Table 1. Data showed great reproducibility both intra and inter donor for the electroporation efficiency and viability.

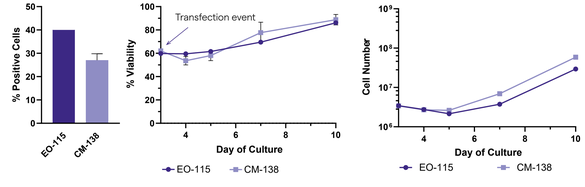
	EO-115	CM-128
Donor 1	19	29
Donor 2	100	110
	73	106
Donor 3	NA	30

Table 1: Fold expansion for cells transfected via plasmid to express GFP. With program CM-138, higher expansion rates can be achieved. Expansion is donor dependent but consistent within the same donor.

Successful Electroporation of Large Plasmid Cargo and Growth of Cells Cultured in Serum-free Medium



Protocol A: Source CD3+	Fold expansion	EO-115	CM-138
	CD3+	29	38



Protocol B: Source hPBMC	Fold expansion	EO-115	CM-138
	hPBMC	9	17

Figure 3: The graphs display transfection efficiency 24 h after transfection and cell viability 30 minute after the Nucleofection® Event and at different subsequent time points. Representative plots for cell expansion after electroporation are also shown. The cargo delivered was a plasmid encoding for a protein with an HA tag. In each experiment, 3 replicate samples were generated, and data represent average numbers. The starting material was purified T cells or hPBMC.
Interestingly, a similar % of transfected cells were generated with both protocols. As previously observed, the strongest pulse EO-115 resulted in a larger ratio of modified cells, while the lighter program slightly favours cell expansion. With hPBMC as source material, viability seems more impacted, and the cells need a longer recovery phase before they start to proliferate. By the end of the expansion phase, viability was close to 90% or above for all conditions tested.

Conclusion

- The new, chemically defined, non-animal origin TheraPEAK® T-VIVO® Medium successfully expanded cryopreserved T cells, from different source materials, after electroporation with the Nucleofector® Technology
- TheraPEAK® T-VIVO® Medium also supported T-cell culture and activation before electroporation, as indicated by the high transfection efficiency obtained with a medium-size and large size cargo like pmaxGFP™ Vector and HA-tagged plasmid respectively
- These results suggest that the new chemically defined, serum-free TheraPEAK® T-VIVO® Medium along with the Nucleofector® Technology can support scalable GMP manufacturing of clinical-grade T-cell therapies

References

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A. Hombach et al. J Immunol 2001; 167: 6123–6131.

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