DMSO-Free Cryopreservation of Human T Lymphocytes in Chemically Defined Systems

Application Note

Introduction

Engineered human T lymphocytes are commonly cryopreserved before patient administration. Maintaining the integrity and potency of the cells during the cryopreservation process is essential for product efficacy and safety. Traditionally dimethyl sulfoxide (DMSO) is used to prevent intracellular and extracellular crystal formation that can lead to cell membrane rupture and cell death, however there are well-documented significant side effects associated with the DMSO use that can result in adverse reactions in patients [1], as well as inhibit T cell activation and functionality [2].

InVitria has developed a DMSO-free cryopreservation media, OptiFRZ, to provide optimal conditions for cryopreservation without the need or risks of DMSO. Compared to DMSO, OptiFRZ has been shown to improve the recovery of viable cells post-thaw while providing high viability and improved cell proliferation in chemically defined conditions. OptiFRZ can be used throughout the cell therapy manufacturing process, showing improved and more consistent performance with both starting PBMC material as well as the final expanded and/or modified cell product.

Materials

Cryopreservation Process

- OptiFRZ DMSO-Free Cryopreservation Media (InVitria 555OFR0067)
- Nalgene 1 mL Cryogenic Tubes (Thermo Fisher #5000-0012)
- 50 mL Conical Tubes
- Dulbecco's Phosphate-Buffered Saline (Thermo Fisher #14190136)
- Controlled rate freezer (ex: Mr. Frosty – Thermo FIsher #5100-0036)

Expansion Process

- OptiPEAK T Lymphocyte XPR (InVitria 555CDK0045)
- CD3 Antibody, Anti-Human (Miltenyi #130-093-387)
- CD28 Antibody, Anti-Human (Miltenyi #130-093-375)
- Recombinant Il-2 (Peprotech #200-02)
- Falcon T-25 flasks (Corning #353109)
- Leukopak
- Lymphoprep Density Gradient (Stemcell #07801)

Protocol

Freezing Fresh Apheresis (PBMC) Material:

1. Isolate PBMC from leukopak by diluting leukapheresis 1:4 with DPBS and layering diluted material over density gradient in a 50 mL conical tube.
2. Centrifuge contents for 20 minutes at 2600 rpm with the brake off.
3. Harvest PBMC layer and wash with 25 mL of DPBS and centrifuge for 5 minutes at 1600 rpm.
4. Take a count and resuspend cell suspension in required volume of cold OptiFRZ DMSO-Free cryopreservation media to obtain a density of 1e8 cells/mL.
5. Transfer 1 mL of the cell suspension directly into each cryovial.
6. Freeze cells using a controlled cooling rate protocol to achieve approximately -1°C per minute.
7. After 24 hours, transfer cryovials to liquid nitrogen (-135°C) for long-term storage.

Expansion of Cryopreserved Apheresis Material:

1. To thaw, place cryovials in a 37°C water bath until there appears to be no frozen cell pellet.
2. Transfer thawed cell suspension to a tube and dilute with DPBS at a 1:10 ratio.
3. Centrifuge for 5 minutes at 1600 rpm, remove supernatant, and resuspend in 1 mL of OptiPEAK T Lymphocyte XPR growth media.
4. Take a cell count and seed in 10 mL of OptiPEAK T Lymphocyte XPR growth media supplemented with 400 ng/mL anti-CD3, 200 ng/mL anti-CD28, and 10 ng/mL IL-2 in a T-25 flask at a starting density of 1e6 cells/mL.
5. After 3 days of activation, spin cells down and replace media with 10 mL regular OptiPEAK T Lymphocyte XPR supplemented with 10 ng/mL IL-2 at a starting density of 5e5 cells/mL in a T-25 flask.
6. Feed cells every other day, setting the density back to 5e5 cells/mL each time. Determine total fold expansion after 7 days of expansion

Cryopreservation of Expanded T Lymphocytes:

1. Collect expanded T lymphocytes for cryopreservation and obtain a cell pellet by centrifugation.
2. Remove supernatant and resuspend cell pellet in cold OptiFRZ DMSO-free cryopreservation media at a volume to achieve a density of 1e7 cells/mL.
3. Transfer 1 mL of the cell suspension directly into each cryovial.
4. Freeze cells using a controlled cooling rate protocol to achieve approximately -1°C per minute.
5. After 24 hours, transfer cryovials to liquid nitrogen (-135°C) for long-term storage.
6. To thaw, place cryovials in a 37°C water bath until there appears to be no frozen cell pellet.
7. If desired, wash cells with DPBS before centrifuging for 5 minutes at 1600 rpm.
8. Remove supernatant and resuspend cell pellet for viability assessment and post-recovery functional assays.

Results and Conclusions

Isolated PBMC from fresh leukapheresis material were cryopreserved in OptiFRZ DMSO-free cryopreservation media, a commercially available DMSO-free media, 10% DMSO media formulation, and 5% DMSO media formulation at 1e8 cells/mL as described. Frozen vials were thawed and seeded for activation and expansion in serum-free, chemically defined OptiPEAK T Lymphocyte media. As shown in Figure 1, the total fold expansion after 7 days of the PBMC frozen in OptiFRZ DMSO-free media was significantly greater than the other cryopreservation medias tested. T lymphocytes expanded 5.18 ± 1.57 fold when starting material was cryopreserved in OptiFRZ versus 1.69 ± 0.15 fold, 1.56 ± 0.37 fold, and 1.60 ± 0.04 fold when cryopreserved in a commercial DMSO-free media, 10% DMSO media, and 5% DMSO media respectively.

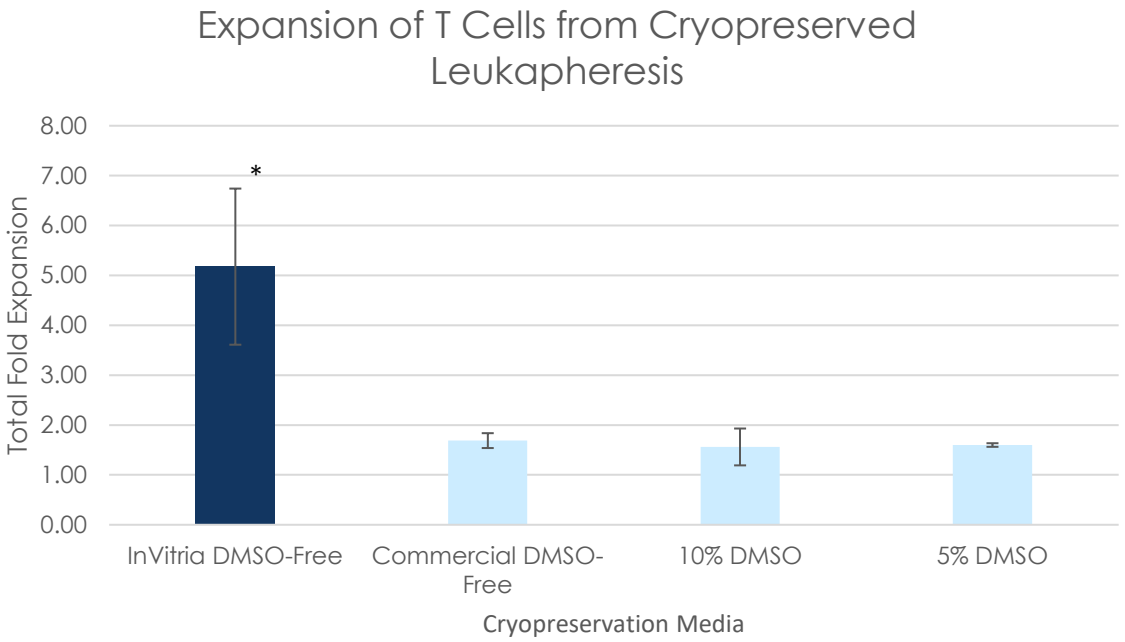

Figure 1. Total fold expansion of cryopreserved PBMC isolated from fresh leukapheresis over a 7 day period in chemically defined OptiPEAK T Lymphocyte growth media.
**, p = 0.026 by One Way ANOVA.*

To also demonstrate compatibility with the final expanded cell product, T lymphocytes isolated from fresh peripheral blood were expanded in chemically defined OptiPEAK T Lymphocyte media for 7 days and then cryopreserved at 1e7 cells/mL in OptiFRZ DMSO-free media, a commercial DMSO-free media, and 10% and 5% DMSO media conditions. Vials were thawed and viable cells determined through calcein-AM staining on the flow cytometer. Percent recovery was calculated as the fraction of viable cells at thaw versus the viable cells frozen per vial (Figure 2).

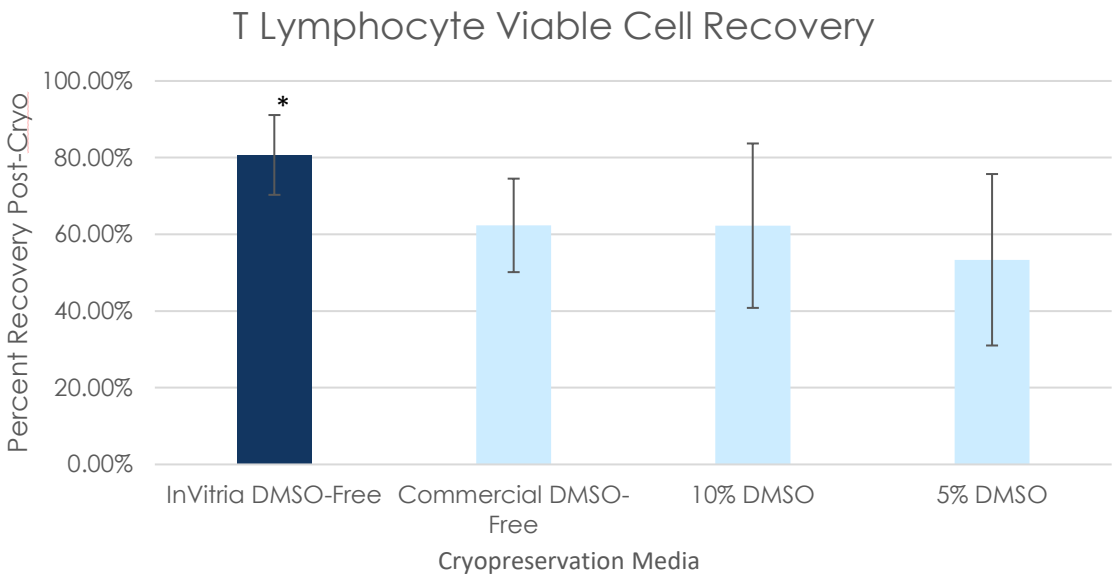

*Figure 2. Percent recovery of viable T lymphocytes cryopreserved after 7 days of expansion in chemically defined conditions. *, p = 0.015 by One Way ANOVA.*

References

1. B. Kollerup Madsen, M. Hilscher, D. Zetner, and J. Rosenberg, “Adverse reactions of dimethyl sulfoxide in humans: a systematic review,” F1000Research, vol. 7, p. 1746, Aug. 2019, doi: 10.12688/f1000research.16642.2.
2. L. Holthaus et al., “CD4+ T cell activation, function, and metabolism are inhibited by low concentrations of DMSO,” J. Immunol. Methods, vol. 463, pp. 54–60, Dec. 2018, doi: 10.1016/j.jim.2018.09.004.

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