DMSO-Free Cryopreservation Formulation As An Alternative Cryopreservation Method For Cell-based Therapies

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OptiFRZ

Background

Cryopreservation remains one of the key steps in manufacturing and delivery of cell-based therapies. Optimal viability and stability post-thaw are critical parameters during cell freezing and can have direct impacts on the outcomes of a cell therapy manufacturing process as well as final cell product efficacy and safety. One of the most common cryoprotective agents currently used in mammalian cell culture is dimethyl sulfoxide (DMSO). However, the use of DMSO introduces well-documented risks associated with DMSO-induced cytotoxicity and adverse reactions in human injections. [1], [2]

As a solution to challenges associated with the use of DMSO, InVitria has designed a DMSO-free chemically defined cryopreservation medium named OptiFRZ that has shown statistically significant improvements in post-thaw viable cell recovery and cell functionality when compared to DMSO-containing solutions. The viability and functionality data was tested in human T lymphocytes as well as bone marrow-derived mesenchymal stem cells.

Methods

Media Development

Components previously described as being safe for injection and included in final formulations of therapeutics were screened for their cryoprotective properties. Recombinant human serum proteins were used to replace all serum components that are known to provide essential stability, buffering capacity, shear stress protection, and aggregation prevention. Optimization was done using a DOE central
composite design platform until the most optimal concentration of each component was determined.

Human T Lymphocytes

Isolated human peripheral blood mononuclear cells (PBMC) from fresh leukapheresis material (6 donors) were cryopreserved in either OptiFRZ DMSO-free cryopreservation media, or a commercially available DMSO-free media, 10% DMSO media formulation, or a 5% DMSO media formulation at cell density of 1e8 cells/mL. Frozen vials were thawed and seeded for activation and
expansion in serum-free, chemically defined OptiPEAK T Lymphocyte medium. Cells were fed every other day and total fold expansion was calculated on Day 7 (Figure 1). To demonstrate compatibility with the final expanded cell product, T lymphocytes isolated from fresh peripheral blood (2 donors) were expanded in chemically defined OptiPEAK T Lymphocyte media for 7 days and then cryopreserved at 1e7 cells/mL in either OptiFRZ DMSO-free media, a commercially available DMSO-free media, or 10% and 5% DMSO-containing 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 total viable cells at thaw versus the viable cells pre-cryopreservation per vial (Figure 2).

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells derived from bone marrow were expanded in chemically defined, serum-free conditions before freezing at a density of 1e6 cells/mL in either OptiFRZ DMSO-free cryopreservation media, a commercially available DMSO-free media, or 10% DMSO media. Frozen vials were then thawed in a water bath, washed twice with DPBS, and resuspended in a complete growth medium. Viable cells were determined via Calcein-AM staining through flow cytometry and percent recovery was calculated as the fraction of total viable cells at thaw divided by the viable cells pre-cryopreservation per vial (Figure 3). MSC markers were analyzed pre- and post-cryopreservation process by staining samples with PE anti-human CD45, PE anti-human CD90, and PE anti-human CD105 antibodies (Figure 4).

T Cell Cryopreservation

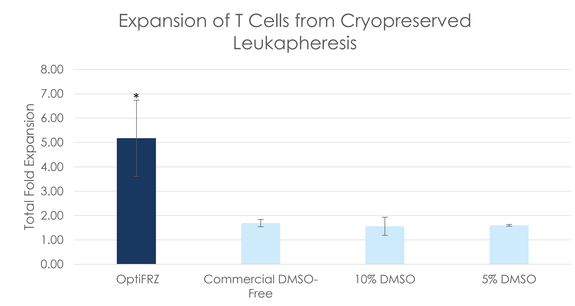

*Figure 1. Total fold expansion of cryopreserved PBMC isolated from fresh leukapheresis over a 7 day period in chemically defined OptiPEAK T Lymphocyte growth media. *, p = 0.026 by One Way ANOVA. Error bars represent standard deviation.*

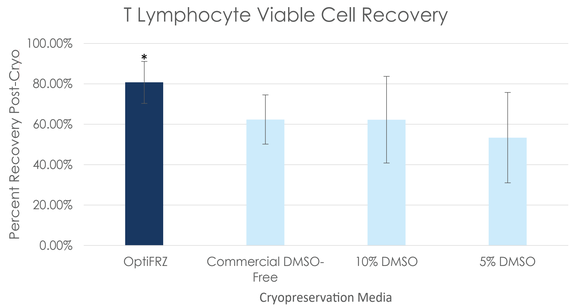

*Figure 2. Percent recovery of viable T lymphocytes cryopreserved after 7 days of expansion in chemically defined conditions. *, p = 0.015 by One Way ANOVA. Error bars represent standard deviation.*

Results

Human T Lymphocytes

To demonstrate functionality of cryopreserved material, 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 commercial DMSO-free media, 10% DMSO, and 5% DMSO cryopreservation conditions. In an average of 6 different donors, expanded T cells cryopreserved in OptiFRZ DMSO-Free media demonstrated significant improvement in percent recovery over the DMSO containing formulations (Figure 2). Additionally, T cells retained high sentinel viability and no morphologic alterations throughout the process.

Mesenchymal Stem Cells (MSCs)

Cryopreserved cells in DMSO-free OptiFRZ displayed no substantial alteration in morphology or attachment kinetics post-thaw while also demonstrating high viability. MSCs cryopreserved in OptiFRZ DMSO-free media demonstrated a statistically significant increase in performance for viable cell recovery coming out of the cryopreservation process (Figure 3). Further, cells maintained target phenotypic characteristics as shown by the CD45 expression of < 1% and both CD90 and CD105 expression > 97% across all media conditions (Figure 4). This would indicate that key markers of stemness were retained.

MSC Cryopreservation

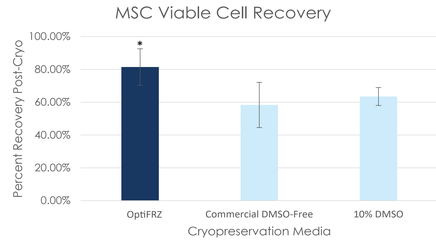

*Figure 3. Percent recovery of mesenchymal stem cells expanded in chemically defined conditions prior to cryopreservation. *, p = 0.01 by One Way ANOVA. Error bars represent standard deviation.*

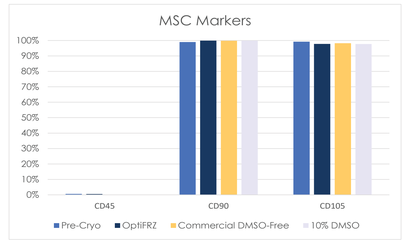

Figure 4. Comparison of CD90, CD45, and CD105 markers in MSCs before cryopreservation and post-thaw in InVitria's OptiFRZ, commercially available DMSO-free and 10% DMSO-containing cryopreservation media.

Discussion / Outlook

Maintaining integrity and target cell count in a cell therapy dose is essential to product efficacy, however, this study demonstrated that the use of DMSO in cryopreservation of a cell therapy can lead to a significant loss of viable cells post-thaw.

The development of OptiFRZ DMSO-free cryopreservation medium has demonstrated that removal of DMSO and use of chemcially defined components tailored for mammalian cell culture and final formulation of biologics directly improves critical paramaters of a cryopreservation process.

This study found statistically significant improvement in post-thaw viable cell recovery of cryopreserved human T lymphocytes and mesenchymal stem cells cryopreserved in OptiFRZ when compared to DMSO-containing and other commercially available DMSO-free cryopreservation media. In addition to enhanced post-thaw viable cell recovery, T lymphocyte expansion data (Figure 1) suggests that removal of DMSO in cryopreservation may significantly improve expansion of primary cells in chemically defined conditions.

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.