Delineating the Critical Process Parameters for Cell Therapy Cryopreservation
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Cell-based therapies require specialized handling to ensure their successful transport from the manufacturing facility to the patient. To provide logistical flexibility, many current commercialization models employ a frozen cell product that can be delivered to the clinic and stored, then thawed and infused on demand. However, post-thaw viability and expansion of many cell types dramatically suffer from cryopreservation-induced stresses, collectively known as Delayed Onset Cell Death (DOCD). As such, it is assumed that some cell types cannot be cryopreserved and successful commercial models should be based on fresh cell culture and delivery. Cryopreservation critical process steps overlap with manufacturing process immediately prior to and after the end of the cell culture processing. As such, the efficiency and efficacy of the final therapeutic dose, as well as the manufacturing process, are directly affected by cryopreservation critical process parameters. Hence, thorough understanding of the cryopreservation impact on cells is vital for successful commercial manufacturing of cellular therapies. In this study, we investigated the impact of some of the most well-known, and also some of the more obscure, critical process parameters (CPPs) on post-thaw viability and proliferation in a Jurkat T cell model. Our results suggest that, next to incorporation of Biopreservation Best Practices for cryomedia formulation, other seemingly unimportant and irrelevant process parameters, that may generally be neglected, can have a significant impact on cell viability and proliferation post-thaw.

METHODS

Cell Culture: The Jurkat (Clone E6-1) human acute T-cell leukemia (ATCC, VA) was cultured in complete growth medium (CGM) comprised of RPMI 1640 (Lonza, MD) supplemented with 10% v/v fetal bovine serum (FBS, Atlas Biologicals, CO).

Cryopreservation Media: Two major types of cryomedia were used in this study: (1) Home-brew mix was prepared by dissolving Human Serum albumin (HSA, Cellantim, USA) in PlasmaLyte-A at 10% w/v and supplementing with 5% v/v Dimethyl Sulfoxide (DMSO). The solution was then sterile-filtered before use; and (2) GMP-manufactured, serum-free and protein-free CryoStor CS5 (contains 5% v/v DMSO).

Cryopreservation: Jurkat T cell pellets were suspended in the indicated cryomedia and cell density, then placed in 2 ml FluidX cryovials (Brooks Life Sciences, MA), and were incubated at 2-8°C for 15 min. Cryovials were then transferred to a LN2-free controlled-rate freezer, and cryopreserved at a rate of -1°C/min. After reaching -70°C, the vials were transferred to LN2 storage for a minimum of 24 h.

Statistical Analysis: For all measurements, data represent the mean of 3-11 independent experiments and are normalized to pre-freeze healthy culture prior to cryopreservation (with 93% viability), the 3-day old healthy culture (with 91.5% viability), and decreasing variability in the results.

CONCLUSIONS

Immediate post-thaw analysis of viability and count is not reflective of a successful cryopreservation process. Adverse effects of non-optimized cryomedia may not be detectable until 24-48 hours post-thaw, attributed to cryopreservation-induced Delayed Onset Cell Death (DOCD). Incorporation of optimized cryopreservation media, on the other hand, can protect against DOCD; hence, resulting in decreased variability and a more accurate estimation of post-thaw survival and proliferation.

Timing of feeding and media change prior to cryopreservation can have a significant impact on outcome of the cryopreservation process. This may be attributed to a number of parameters including accumulation of stresses that does not appear in pre-freeze viability assay, but combined with DOCS, result in significant loss of viability and function post-thaw.

The stochastic nature of the ice nucleation inherently results in variations in cryopreservation induced stresses endured by the cells during the cryopreservation process. Hence, proper nucleation has a significant impact on viability and proliferation in model T cells.

Dilution in 37°C media (similar to patient administration) is the optimal post-thaw dilution practice. It is highly recommended that for assaying purposes, the cells also be diluted in warm media to minimize the osmotic cell swelling and lysis during the wash process.