Improved Post-Thaw Stability Validation of Peripheral Blood Cell Products utilizing the Intracellular-like CryoStor® Cryopreservation Solution, and Preliminary Results of Clinical Application

Gerhard Fritsch, PhD[1], Volker Witt, MD[1], Susanne Matthes, MD[1], Michael Dworzak, MD[1], Michaela Artwohl, PhD[1], Dominic Clarke, PhD[2] and Aby J. Mathew, PhD[2]

[1]Children's Cancer Research Institute (CCRI), St. Anna Kinderkrebsforschung, Vienna, Austria and [2]BioLife Solutions, Inc., Bothell, WA, United States

Introduction

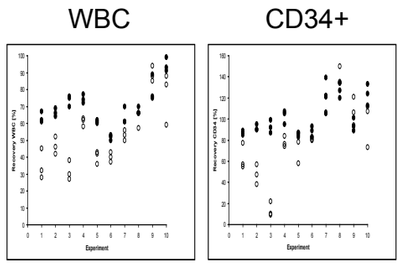
Collection and cryopreservation of autologous stem cells is a routine procedure in a variety of malignant diseases. Further, improved stability of cell products is critical to the development of cell and tissue based therapies as part of the growth in regenerative medicine. A growing body of evidence indicates that one key method for improved cryopreservation efficacy is the utilization of a hyperosmotic/intracellular-like cryopreservation media, as opposed to the traditional use of an isotonic/extracellular-like media such as saline as the vehicle for the cryoprotectant.

The Children's Cancer Research Institute (CCRI) conventional isotonic-based freezing medium (CFM) used for the last 15 years comprised 20% DMSO & 10% human plasma derivate in Ringer's solution. Some issues observed in clinical practice were:

1) Infusion of high volumes was necessary in case of poor mobilizers (DMSO limit: 1g/kg BW)
2) To prevent cell clotting, cells had to be infused cold & rapidly, & immediately after thawing
3) Experienced 2 cases of cell clotting before the end of infusion.

In this evaluation of cryopreservation methods for clinical application, cell samples from apheresis products were cryopreserved in a conventional isotonic-based freeze media (CFM; 20% DMSO, 10% human plasma derivate in Ringer's solution) or intracellular-like CryoStor® CS10 (CS10; contains 10% DMSO, serum-free and protein-free) reported to have favorable properties (Stylianou et al., Cytotherapy 2006, and Clarke et al., Cytotherapy 2009).

Post-Thaw Validation and Stability Results Figures



Triplicate analyses of CS10 [●] & CFM [○]

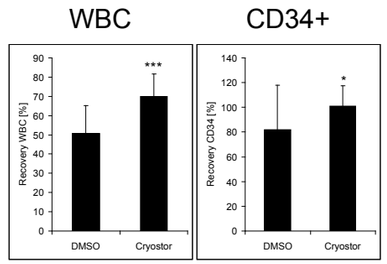


Figure 1

Cell recovery immediately after thawing
(t=0; n=10)
DMSO = CFM
CryoStor = CS10

* = p<0.05
** = p<0.01
*** = p<0.001

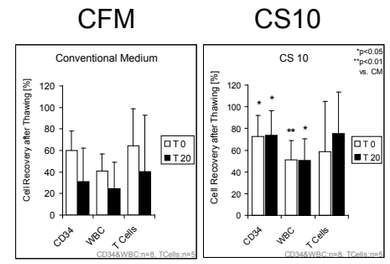


Figure 2

Cell recovery after 20 min
(WBC & CD34: n=8; T-cells: n=5)

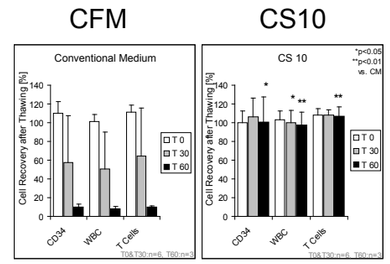


Figure 3

Cell recovery after 30 & 60 min
(t=30: n=5; t=60 n=3)

Post-Thaw Validation and Stability Results Discussion

Immediately after thawing, the percent recovery of WBC was 50.7 (±14.4%) for CFM versus 70 (±11.6%) for CS10 (p<0.001), and that of CD34+ cells was 81.8 (±36.1%) for CFM and 101 (±16.4%) for CS10 (p<0.05).

In CFM, 20 to 60 minutes after thawing there was a dramatic loss in cell viability (-40% to -90%), up to complete clotting in 3/10 samples. By contrast, cells remained viable up to 60 minutes after thawing in CryoStor CS10, and no clotting occurred.

Because of these positive validation results, cryopreservation in the intracellular-like CryoStor CS10 was translated to clinical application for treatment of hematological malignancies.

Methods

Ten cell samples from 6 different apheresis products were mixed (1+1) with either CFM or with CS10, frozen by controlled rate freezing, and stored in the vapor phase over liquid N2. After thawing, viable CD34+, WBC and T-cells were quantified by triplicate single-platform 5-color Flow Cytometric analysis. Analyses were performed immediately after thawing, and after 20, 30, and 60 minutes post-thaw.

Regeneration of patients after infusion of autologous PBSC (n=8)

To date, eight patients have received autologous stem cells cryopreserved in CryoStor CS10. (CD34+ x 10E6/kg = 1.8 to 14; mean 4.9), in a mean volume of 126 ml (65 to 212 ml) containing 0.4g DMSO/kg (0.1 to 0.6). **Infusions were well tolerated, no side effects were observed. Both WBC>1000 and ANC>500 were reached on day +10.5 (WBC:8-12; ANC:7-12)**.

Conclusions

CryoStor CS10 has demonstrated benefits in comparison to the previous standard CFM in terms of cell recovery post-thaw, particularly providing improved stability after 20 to 60 minutes post-thaw at room temperature (RT) in these validation experiments:

1) significantly (> 20%) more viable CD34+
2) 50% less final DMSO concentration
3) advantage for poor mobilizers: higher infusion volumes possible

Preliminary results in patients (n=8 reinfusions) are promising. Physicians report good regeneration kinetics, even after infusion of low CD34+ numbers, and no engraftment failure occurred. Further data will be collected to validate the outcome of hematopoietic regeneration after reinfusion.