Regeneration of patients after infusion of T Cells

**WBC T Cells**

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% human plasma derivate in Ringer’s solution) or intracellular-like CryoStor® CS10 (CS10; contains 10% human plasma derivate in Ringer’s solution). Some issues observed in traditional use of an isotonic/extracellular-like media such as saline as the vehicle for the cryoprotectant.

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.

**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.