203
OPTIMIZING PBSC COLLECTION FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Purpose: Chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplant is a widely used standard treatment for patients with multiple myeloma and lymphoma. For treatment to be feasible, a target number of stem cells must be collected and stored prior to chemotherapy. At our collection facility, the typical leukapheresis procedure involves processing 5 patient blood volumes (BV). Processing volumes vary widely between programs. This study is being conducted to understand the kinetics of PBSC mobilization and collection with apheresis with the aim of optimizing PBSC collection.

Methods: Patients received a stem cell mobilization regimen consistent with pre-transplant standard of care in our facility. Stem cells were collected during the first leukopheresis procedure for each subject in 3 interconnected bags as follows:

Bag 1: BV1-3
Bag 2: BV4
Bag 3: BV5

Peripheral blood (PB) and PBSC were sampled after completion of collecting each bag. Samples were evaluated by ISHAGE to enumerate CD34+ cells.

Results: 26 subjects consented to participate in this IRB-approved study over a 17 month period. The concentration of CD34+ cells in PB decreased from baseline throughout the leukapheresis procedure, and significantly decreased from baseline to BV4 and BV5 ($p < 0.01$). The number of CD34+ cells collected in the 3 bags increased throughout the leukopheresis procedure ($p<0.05$). Stem cell targets were achieved in 80% of patients after processing 4BVs. Addition of CD34+ cells collected in bag 3 (BV 5) had no effect on achieving stem cell targets.

Conclusions: A typical 5 total blood volume leukapheresis procedure has increased risk of collection related adverse events, and is time consuming. Shortening the leukopheresis procedure has the potential to increase patient safety and satisfaction. This study suggests that decreasing the blood volume processed from 5BV to 4BV does not adversely impact achieving stem cell targets for transplantation.

204
THE USE OF HYDROXYETHYL STARCH (HES) AS A SUBSTITUTE FOR DEXTRAN 40 IN THE THAW AND WASHING OF HPC, APHERESIS PRODUCTS

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Removing DMSO post-thaw of hematopoietic progenitor cell products results in: reduced infusion reactions, improved recovery and stability of viable CD34+ cells, and facilitates better control over the product by enabling thawing within the laboratory setting. Validated methods use 10% Dextran 40 with 2.5 to 4.3% HSA for this purpose. Recent shortages of clinical grade Dextran require identification of suitable alternatives. Here we report on experiments using clinical grade hydroxyethyl starch (Hespan, 6%) as a substitute for Dextran. HPC(A) collections frozen in multiple bags and approved for discard were used to compare a standard 2X wash medium of 5 parts 10% Dextran 40 with 1 part 25% HSA (8.3% Dextran 40/ 4.2% HSA) with Hespan based solutions. Cells from replicate thawed bags (70-100mL) transferred into 300 mL transfer packs, were diluted with equal volume wash solution, equilibrated 5 minutes, filled with wash medium, pelleted and the supernatant expressed. Bags were restored to original frozen volume in wash medium and tested by single platform flow cytometry. Total viability, viable TNC, MNC, and CD34+ cell recovery, and CD34+ cell viability (figure 1) were compared immediately post thaw and after 90 minutes. 5-7 experiments per arm were performed. 5.2% HES/4.2% HSA did not differ from Dextran in CD34 recovery or viability. Due to concerns that HES could affect renal function when infused at higher concentrations we also compared 2.4% HES/4.2% HSA and 0.6% HES/2.5% HSA. Results using 2.4% HES were similar to 5.2% HES, but there was significantly lower CD34+ cell viability and recovery in the 0.6% HES/2.5% HSA arm immediately post thaw that worsened after 90 minutes. Experiments increasing the HSA to 4.2% in the 0.6% HES arm, and to include assessment of colony growth in thawed HPC(A) and HPC(CB) products are underway. At this time we conclude that 2.4% to 5.2% HES with 4.2% HSA is a suitable substitute for Dextran 40 as a washing medium for HPC(A) products.

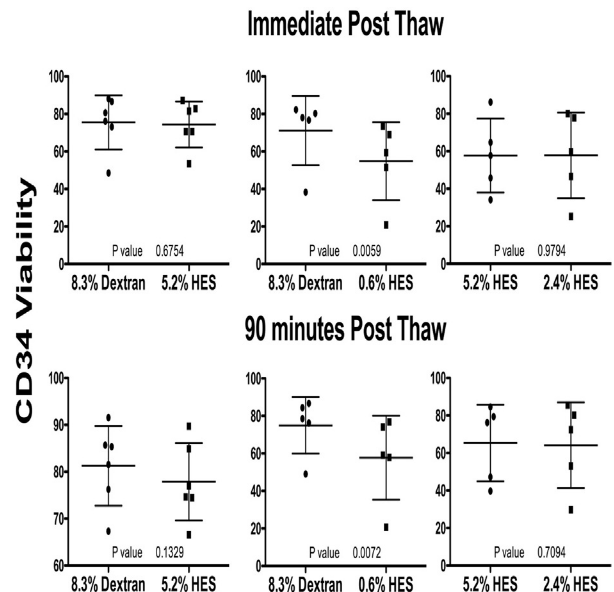

Figure 1.

205
POST-THAW DMSO DEPLETION USING A cGMP-COMPLIANT SPINNING-MEMBRANE SEPARATION DEVICE

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Use of cryopreserved stem cell grafts is still unavoidable, both in the autologous and allogeneic settings. Cryopreserved grafts can be directly thawed at the bedside or thawed and washed at the cell therapy laboratory. Recent study has shown that post-thaw washing does not impair hematopoietic engraftment, in a cohort of 2,930 autologous transplanted patients receiving either unwashed or washed grafts (Calmels B et al, 2014). Post-thaw washing can be implemented using various methods such as manual centrifugation, automated centrifuge-based or spinning-membrane devices such as the Lovo (Fresenius Kabi).

We here report the pre-clinical evaluation of the Lovo for washing cryopreserved stem cell grafts. We compared 15 apheresis products intended for destruction and cryopreserved in 2 identical bags; after dry-thawing (PlasmaTherm, Barkey), bags were connected to the Lovo, diluted at 20 ml/min with +4-8°C 6% hydroxyethylstarch 130/0,4 (Voluven, Fresenius Kabi) and processed using 2-cycle or 3-cycle reduction (a cycle referring to one pass through the spinning membrane). After washing, CD34 and CD45 absolute counts and viability were evaluated (Stem-Kit, Beckman Coulter) and DMSO was quantified by capillary zone electrophoresis (P/ACE, Beckman Coulter). Length of procedures were 15min and 18min for 2-cycle and 3-cycle, respectively. Post-wash data show consistent viability, effective DMSO depletion and relevant CD34 recovery.