from 1-11 months post transplant. Favorable engraftment and survival was seen as compared to a control group from the COBLT study. The cummulative incidence of neutrophil engraftment by day +42 (median = 17 days) and platelet engraftment by day 100 (median 50 days) were 90.9% (95% CI 78-100) [p=0.001) and 79.5% (95% CI 63.6-95.5%) [p=0.003]. Overall survival at 180 days was 92.8% (95% CI 63.6-95.5) [p=0.001]. CD4 recovery was accelerated. Isolation and priming of ALDHbr cells is feasible and safe. Favorable effects on engraftment and survival were observed.

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VALIDATION STUDY OF MONONUCLEAR CELL RECOVERY USING THE AXP™ AUTOXPRESS™ PLATFORM

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BACKGROUND: The AXP AutoXpress Platform has been developed for automatically processing cord blood stem cells before cryopreservation. The platform consists of a microprocessor-controlled device, docking station, functionally closed sterile disposable bag set and supporting XpressTRAK™ software. Cord blood is sterilely transferred to the bag set and centrifuged in the device at high and low speeds. During the high speed spin, the cells separate into layers according to density and during the low speed spin the device transfers the red blood cells (RBC) into the RBC bag, mononuclear cells (MNC) into the freezing bag and retains the plasma in the processing/plasma bag. The MNC volume in the freezing bag is operator selected and achieved with an analytical balance built into the AXP device. After being mixed with 5 ml cryoprotectant, the 25 ml MNC solution in the freezing bag can be directly frozen at a controlled rate and stored in the robotic BioArchive® System.

STUDY DESIGN: The validation study was designed and performed to determine the efficiency of MNC recovery with and without addition of 20% HES before processing. High speed centrifugation was 1400xg for 20 minutes and low speed spin 80xg for 10 or 20 min. Human peripheral blood was used in the study to serve as a surrogate for cord blood. Blood volumes investigated were 60, 120 and 170 ml. In addition to MNC recovery, other parameters such as WBC recovery, red cell reduction, hematocrit, final volume in the freezing bag, and cell mass balance in the freezing, RBC and processing bags were also measured.

RESULTS: Results are presented as the mean ± S.D. for MNC recovery value. The following table summarizes MNC cell recovery from human peripheral blood in the freezing bag with 60, 120 and 170 ml of initial blood volume in the presence or absence of 20% HES.

CONCLUSION: The AXP AutoXpress Platform consistently yields MNC recovery greater than 90% with processing blood volume at 60 and 120 ml without HES addition. Adding 20% HES remarkably enhances average MNC recovery to greater than 90% when blood volume processed is 170 ml. The results from this study demonstrate that the AXP AutoXpress Platform can achieve automated volume reduction of blood with high efficiency of MNC recovery.

N	Low speed centrifuge	Volumes (mL)	MNC recovery (%)	HES addition
12	80xg, 20min	60	91.5 ± 4.8	20% HES
12	80xg, 20min	170	92.3 ± 5.4	20% HES
8	80xg, 10min	60	96.0 ± 6.0	20% HES
8	80xg, 10min	170	98.7 ± 5.4	20% HES
8	80xg, 10min	60	98 ± 5	no HES
8	80xg, 10min	120	92 ± 14	no HES
8	80xg, 10min	170	78 ± 15	no HES

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A COMPARISON OF THE TRADITIONAL WATER-BATH VS. DRY THAWING METHOD FOR THAWING CRYOPRESERVED PERIPHERAL STEM CELL COMPONENTS WITH A GOAL OF REDUCING THE CONTAMINATION RISKS

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The thawing of cryopreserved products has traditionally been done using a water-bath. The potential contamination risks involved with thawing cryopreserved stem cell products are always a concern. A safer, more standardized method of thawing cryopreserved products while reducing the contamination risk associated with water-baths was investigated. A comparison study was performed using the Equitherm Model 299-733 water-bath and the Cytotherm® Model D1 Dry Plasma Thawing unit. Two equivalent cryopreserved bags from each of nine peripheral stem cell collections were thawed using both water-bath and dry thawing methods. Bag volumes, storage, cell concentrations, initial product temperature and initial equipment temperature were identical. Measured parameters included post-thaw cell viability, product temperature, Lactate Dehydrogenase (LDH) levels and temperature recovery time of the equipment. All products were placed into an over-wrap bag. Viabilities found using each method were unremarkable with a variation range of less than 5% and an average variation of less than 1%. The differences noted were: 1) the products thawed in the water-bath had a temperature range of –2.0°C to 24.4°C post-thaw versus a temperature range of 6.0°C to 13.6°C following dry thawing method, 2) LDH levels of products thawed in the water-bath had LDH levels averaging 37.5% higher with a range of 5.8% to 87.8% higher than dry thawing method, 3) temperature recovery of the water-bath to desired temperature averaged 8.9 minutes compared to 4 minutes using the dry thawing unit. After performing this comparison, it was observed that the dry thawing method may have benefits over the traditional water-bath method. The benefits of the dry thawing method include: 1) the reduced risk of product contamination, 2) lower LDH levels on the thawed products which may indicate less cell destruction or lysis, 3) a consistent post-thaw temperature of the product ensuring that the product is not over-warmed or under-thawed, 4) faster temperature recovery of the equipment following each thaw allowing for a consistent starting temperature when performing multiple thaws, 5) elimination of the need to hand manipulate the product during the thawing process, 6) lastly, the dry thawing unit was very portable with no water to maintain.

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RIGOROUS PROTOCOLS USING ALEMTUZUMAB TO T CELL DEPLETE STEM CELL PRODUCTS

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The therapeutic effect of allogeneic peripheral stem cell transplantation (alloPSCT) is related to an immune-mediated graft-versus-tumor (GVT) effect. However, alloPSCT is limited by T-cell mediated graft-versus-host disease(GVHD). Approaches used to treat or prevent GVHD include *in vitro* or *in vivo* purging of T cells using monoclonal antibodies such as Alemtuzumab, which is directed against CD52 on B and T lymphocytes, monocytes, and some dendritic cells (DCs). Alemtuzumab has been injected systemically to treat established GVHD, and *ex vivo* to purge T cells from stem cell products (SCPs) to reduce GVHD induction. T-cell depletion with Alemtuzumab infusion is an effective method to treat GVHD but is also associated with relapse and infectious disease. *Ex vivo* purging by the addition Alemtuzumab to a SCP, shaking for 15 minutes and infusion is also effective. However, the efficacy of this T cell purging protocol can not be assessed and potentially results Alemtuzumab infusion. The present study examined the effect of Alemtuzumab co-incubation on cellular subsets and the mechanism of purging i.e. antibody dependant cellular cytotoxicity (ADCC) vs. antibody-complement toxicity. Our studies revealed that cellular concentration is critical

to purging activity, such that high cellularity (10e8 cells/ml) results in a significant loss (65%) of T and B cells with only 1 hr co-incubation, raising to 85-90% at a 6 hr co-incubation. Co-incubation at a log lower cellularity results in a 20-30% T-cell loss. ADCC is the primary purging mechanism; however, serum is required to retain cellular viability as is DNAse to reduce cellular clumping. Co-incubation temperature has little effect on purging, although cellular viability is higher at 4C compared to 37C. T, B and NK cells are sensitive to Alemtuzumab ADCC. DCs have a lower sensitivity and there is a differential sensitivity of DC subsets to alemtuzumab-mediated ADCC, such that $CD123^+$ DCs have a lower sensitivity as compared to the $CD11c^+$ DCs. Further, immature myeloid suppressor cells (IMSCs) that are $Lin^-DR^-CD11b^+$ or $Lin^-DR^-CD14^-CD11b^+$ cells are refractory to alemtuzumab toxicity. This novel observation is of interest as IMSCs can induce T cell tolerance and thus have the potential to control GVHD. Critically, alemtuzumab purging of a hematopoietic stem cell products has no effect on CD34+ cell numbers and their (CFU-c) as assessed in vitro.

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COMPARISON OF CELL DOSE AND OUTCOME FOR CORD BLOOD TRANSPLANTATION (CBT) SOURCED FROM PLASMA DEPLETED (PD) VERSUS RED CELL DEPLETED (RD) CORD BLOOD INVENTORIES

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To discern whether cord blood processing influences cord blood bank (CBB) inventory cell dose and CBT outcome, we (1) performed a large scale cell dose comparison of the NMDP CBBs that practice red cell depletion (RD) versus plasma depletion (PD), (2) performed an analysis comparing RD CBT results from an Institute of Medicine (IOM) study to audited outcome from a PD cord blood (CB) inventory, and (3) searched the literature for outcome of CBBs that practice PD and RD processing. Two of the CBBs in the NMDP network practice PD processing (n=10,912 CB) whereas all others practice RD (n=38,819 CB). PD processing results in less than 1% cell loss in the discarded fraction (excluding testing and archival samples), and various studies have reported different recovery rates for RD; however, no data exist for a rigorous comparison of actual inventories. Using data from NMDP's Cord Blood Bank Performance Report (6/30/06), the percentage of high cell dose units among the two types of CBBs were compared. Table 1A shows the proportion of CB with high TNC counts was significantly higher for PD CBU at all three levels ($p < 0.0001$; test for difference in proportions). To our knowledge, this is the largest cell dose comparison of cord blood banks.

The IOM conducted a study that analyzed the outcome of 3 large CB inventories in order to find determinants of engraftment and patient survival (Gibbons 2005). The IOM study used RD CB. All 205 patients transplanted with PD CB through 5/06 with survival and/or engraftment data were divided into three groups - alive, dead, and engraftment failure - which were then compared to the 755 transplants from the IOM study using identical methodologies (alive=engrafted & alive, dead=engrafted & dead, and engraftment failure=never engrafted). Table 1B summarizes the proportion of patients in each category and chi-square test of homogeneity yielded a p-value of 0.002, suggesting that the PD CBT had a significantly higher proportion of survivors and lower proportion of engraftment failures.

Various cord blood bank studies from the literature were also summarized, which showed that transplants using PD CB may have higher cell doses than those using RD CB. Moreover, various RD CB inventories appeared to have similar outcome, which may be different from transplants using PD CB inventories though patient and disease profiles appear to match.

Cell Dose & Outcome Comparison Between PD & RD CB Inventories

Table 1A	NMDP PD CBB	NMDP RD CBB	Test for Difference
CBU with TNC	# & % of PD CB	# & % of RD CB	in Proportions
$\geq 125 \times 10^7$	3,772 (35%)	8,172 (21%)	$p < 0.0001$
$\geq 150 \times 10^7$	2,157 (20%)	3,871 (10%)	$p < 0.0001$
$\geq 200 \times 10^7$	703 (6%)	822 (2%)	$p < 0.0001$

Table 1B	# & % Alive	# & % Dead	# & % Engraftment Failure
PD CB	116(56.6%)	47(22.9%)	42(20.5%)
IOM CB	323(42.8%)	214(28.3%)	218(28.9%)

HEMATOPOIESIS/MESENCHYMAL CELLS

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TRANSPLANTATION OF VASCULAR ENDOTHELIAL CELLS MEDIATES THE HEMATOPOIETIC RECOVERY AND SURVIVAL OF IRRADIATED MICE

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During embryogenesis, Flk-1+ endothelial precursors contribute critically to the definitive onset of hematopoiesis. Adult sources of endothelial cells have also been shown to support the expansion of hematopoietic stem cells in vitro. However, the in vivo contribution of vascular endothelial cells to adult hematopoiesis has been less well characterized. In this study, we sought to determine whether transplantation of primary endothelial cells could enhance the hematopoietic recovery and survival of lethally irradiated mice. C57BL/6J mice were given sublethal (700 cGy) or lethal (1050 cGy) irradiation and subsequently transplanted with murine brain-derived ECs (MBECs) or fetal blood-derived ECs (FBECs) for 5 days. Mice transplanted with MBECs alone demonstrated accelerated BM cellular recovery, radioprotection of BM $c\text{-}kit^+sca\text{-}1^-lin^-$ progenitors and enhanced regeneration of $c\text{-}kit^+sca\text{-}1^+lin^-$(KSL) stem/progenitor cells following irradiation compared to controls. MBEC transplantation also facilitated the recovery of circulating white blood cells and platelet counts following radiation exposure. Remarkably, 57% of mice transplanted with MBECs survived long-term (>60 days) following 1050 cGy irradiation, which was 100% lethal in control mice. Irradiated mice that were transplanted with FBECs also showed significantly increased survival, although these animals did not survive long-term. Conversely, transplantation with mesenchymal stem cells (MSCs) had no impact on the survival of irradiated mice, suggesting that an endothelial cell-specific activity accounted for the observed effects. These data suggest that vascular endothelial cells can mediate hematopoietic recovery following myeloablative injury and that transplantation of endothelial cells alone can improve the survival of lethally irradiated animals.

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MESENCHYMAL STEM CELLS ARE EFFECTIVE AT PREVENTING BUT NOT AT TREATING GVJD

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Mesenchymal stem cells (MSC) are progenitor cells of stromal origin which can differentiate into multiple lineages. MSC also