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