that included all autologous HPC Transplant (HPCT) patients with hematological malignancies seen by our HPCT program from February 2003 to January 2007. Costs were compiled from the Institution's Charge Description Master and included costs from additional filgrastim administration through collection. The threshold was increased incrementally from 4 to 9 CD34+ cells/μl to determine the most cost effective strategies while maximizing the number of successful collections. **Results:** There were 302 autologous patients with 463 peripheral CD34 assays and 572 collections. Of these, 149 patients and 322 observations had CD34+ counts <21 CD34+ cells/μl. 175/322 were assays with collections, while 147 were assay only. The analysis of the decision tree indicates that at a CD34+ level of 7, the costs of the collection are moderately reduced by 3.3%, and 87% of the patients meet the minimum cell goal. At a level of 8 CD34+ cells/μl, the costs are reduced 4.5% and 100% of patients meet the minimum cell goal. **Discussion:** The results suggest that the most cost effective collection occurs at peripheral CD34+ count of 8 CD34+ cells/μl or above. This is not unexpected, as better collectors have fewer collections. However, this results in an unacceptably high number of patients that will fail to collect.

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DETERMINING FINAL APHERESIS VOLUME FOR HEMATOPOIETIC PROGENITOR CELL (HPC) COLLECTION BASED ON INTRA-PROCEDURAL SAMPLING

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When collecting HPC by apheresis, sufficient CD34 cells must be collected to effect a timely and stable graft. Donor safety must also be protected, requiring that apheresis not be longer than necessary. A correlation between circulating and collected CD34 cells has been reported, but variability between donors in apheresis collection efficiency may limit the accuracy of this measure for determining blood volume to process. Apheresis efficiency and circulating CD34 cell levels must be similar throughout the collection procedure. Thus, we hypothesized that CD34 cell collection, measured early in the apheresis process, could be used to extrapolate the final apheresis volume needed to meet collection targets. In 47 normal HPC donors, undergoing apheresis using the Gambro Spectra and PBSC protocol version 6.1, the collection bag was sampled after one (n = 18) or two (n = 29) total body blood volumes (TBV) had been processed. CD34 cells collected was determined, and total apheresis volume was projected as:

Final apheresis vol. = vol. at sampling × [CD34 required/CD34 at sampling]

To insure adequate collection, apheresis was continued until a blood volume at least 10% greater than projected was processed. Even so, three collections (6%) failed to meet targets. To determine the validity of the hypothesis that CD34 count at early sampling predicts final CD34 content, dependent only on apheresis volume, expected CD34 content for the final volume actually collected was computed as

Expected CD34 = sample CD34 × [vol. collected/vol. at sampling]

and was compared to the observed final CD34/Kg content. Using simple linear regression and correlation analysis to measure the strength of the association between the expected and the observed CD34/Kg content, significant linearity between expected CD34 content and observed CD34 content for both 1xTBV and 2xTBV sampling was found, with Pearson correlation coefficients of 0.914 and 0.945 respectively. F-test revealed $p < 0.0001$ for projections from both sampling intervals. In comparison, similar analysis comparing projected CD34 collection from circulating CD34 cells yielded a Pearson correlation coefficient of 0.754 with F-test $p < 0.0001$. These results support the practice of using the CD34 content of samples drawn from partially collected products, as early in apheresis processing as 1–2 × TBV, to compute the necessary donor blood volume to process to achieve sufficient HPC collection without undue risk to the donor.

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CHARACTERISTICS AND CELL COMPOSTION OF PRIVATELY BANKED AUTOLOGOUS UMBILICAL CORD BLOOD (UCB) UNITS UTILIZED FOR AUTOLOGOUS UCB INFUSION IN CHILDREN WITH TYPE I DIABETES

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Private cord blood banks process and store umbilical cord blood (UCB) for potential future use by that child or perhaps a sibling. Despite increasing numbers of UCB units banked in private banks, very few have ever been used besides those banked intentionally for siblings with high risk malignancies or other diseases treated with stem cell transplant (SCT). There is high variability in the characteristics, cell dose, as well as processing and storage of the UCB units in these banks. Families pay fees for the banking and continued storage of the units, unaware of the number of cells or other characteristics of the UCB unit that is banked. At the University of Florida a study of autologous UCB infusion in children with newly diagnosed type 1 diabetes (T1D) is underway. Children with T1D who happened to have UCB stored at birth received an infusion of their autologous UCB that was thawed in standard fashion. The UCB units were all tested prior to infusion for viability, sterility, and identity confirmation via HLA typing, and standard cell enumeration and flow cytometry were performed. Twelve children have had their autologous UCB infused at a median age of 6 (range 2–10) years. Eleven of the UCB units were stored in 8 private banks and 1 unit had been donated to a public bank and was retrieved for the study. The median weight of patients was 24 (range 12–45) kg. The average nucleated cell dose per kilogram infused was 1.89×10^7 (range 0.27–4.39), with an average CD34 dose per kilogram of 4.4×10^4 and viability of 96%. At most transplant centers, the minimum desired cell dose for standard UCB transplant is 3×10^7 per kg body weight. Of the 12 UCB units, only 3 would have exceeded this minimum, despite the fact that the average weight was only 24 kg. One of the 3 units of sufficient size was the unit that had actually been banked in the public bank. In addition, half of the units were stored in cryovials ranging from 0.5 ml to 5 mls. The vials leave no room to add any media for the thawing process. This resulted in a 15% decrease in recovery when compared with units stored in bags. In addition, the risk of bacterial contamination is higher when the cells are in vials and have to be transferred to larger vials or bags for processing and infusion. Private UCB banks advertise to families to store a child's UCB for future use, charging a significant fee, however it appears that many units may be under the acceptable cell dose for standard transplant.

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ELEVATED CD14+ CELL DOSE IN MARROW GRAFT CORRELATES WITH INCREASED MORTALITY AFTER ALLOGENEIC TRANSPLANTATION

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Background: The role of donor antigen presenting cells (APC) in human bone marrow grafts in post-transplant clinical outcome is not well established, although data from animal models suggest that they may regulate GVH responses and immune reconstitution early after transplant. **Aim:** In this retrospective study we tested whether marrow composition of donor APC was associated with outcome after allogeneic transplantation. **Patients and Methods:** 81 consecutive patients undergoing allogeneic bone marrow transplantation in our institution from June 1999 through June 2006 were analyzed, provided that graft composition data were available. All but 1 patient received the marrow from HLA matched unrelated donors. Of 81 patient, 67 received a myeloablative and 14 a reduced intensity conditioning regimen. ATG was also administered in 79/81 patients. Graft numbers of CD11c+ myeloid DC (mDC), CD123+ plasmacytoid DC (pDC) and CD14+ monocytes were determined based on flow cytometry analysis. **Results:** After a median follow up of 346 days (interquartile 132–968), the following transplant-related events were recorded: acute GVHD II-IV in 18 and extensive chronic GVHD in 22 patients and relapse in 19 patients. Transplant-related mortality (TRM) occurred in 23, and relapse-related mortality in 12 patients. Patients were divided based

on median donor graft mDC (0.81×10^6/kg recipient weight), or pDC (0.67×10^6/kg), or monocyte (6.6×10^6/kg) counts. No correlation was observed between acute and chronic GVHD as well as relapse and APC counts. However, the mortality rate was significantly higher in patients who received a greater dose of monocytes (24/41 vs 11/41) ($p = 0.003$), with a trend for a more elevated TRM (15/41 vs 8/41) ($p = 0.08$), as compared to patients who received a lower dose of monocytes. mDC and pDC counts did not correlate with survival after transplant. In univariate analysis, TRM correlated with advanced disease ($p = 0.03$), age ($p = 0.039$) and graft monocyte and T cell counts (both $p = 0.03$), whereas overall mortality correlated with advanced disease ($p < 0.0001$) and graft monocyte counts ($p = 0.02$). In multivariate analysis, monocyte counts correlated with both TRM ($p = 0.06$) and overall mortality ($p = 0.01$). **Conclusion:** Donor CD14+ cell dose in bone marrow grafts independently correlates with mortality following allogeneic transplantation suggesting a possible role of donor mature accessory cells in the regulation of post-transplant immunologic events.

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CLINICAL ISOLATION AND EX VIVO CULTURING OF CORD BLOOD-DERIVED T CELLS FOR DONOR LYMPHOCYTE INFUSION

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Umbilical cord blood (CB) is a promising source of hematopoiesis for patients lacking a human leukocyte antigen (HLA)-matched donor. CB can be collected easily and safely, is readily available, and leads to successful long-term engraftment. Greater HLA disparity can be tolerated between the recipient and donor CB compared with bone marrow (BM) or peripheral blood (PB). CB T cells are naive and express an immature phenotype, and CB grafts have lower total nucleated cell (TNC) doses compared with BM. These factors contribute to delayed time to engraftment and poor immune reconstitution in CB recipients.

CB transplantation has also been reported to be associated with a lower risk of graft-vs-host disease (GVHD), which then reduces the graft-vs-leukemia (GVL) effect. T cells are believed to be responsible for the GVL effect. These cells now are used clinically as donor lymphocytes to control minimal residual disease or reinduction of remission in relapsed leukemia after Allogenic bone marrow or peripheral blood (PB) stem cell transplantation. It has been shown that Allogenic ex vivo expanded suicide gene-modified PB T cells also have the potential to cause GVHD and GVL effects. This raised the question of whether ex vivo expanded CB lymphocytes (CBL) also can be used as a tool for adoptive cellular immunotherapy.

Preliminary Results: We have just recently managed to establish a protocol for massive expansion of CB derived T cells. We have been able to show that cells can be expanded very efficiently more then 100 fold, making them usable in the clinic suitable in number for DLI after CB transplantation. We have further shown that the expansion protocol doesnt skew the T cell population regarding the phenotype, CD4:CD8 ratio as well as TCR usage profile. By activating the expanded cells we have further confirmed the expanded T cells capacity to efficiently produce pro-inflammatory cytokines. **Clinical Significance:** Cord blood is increasingly used as stem cell source in allogeneic SCT. However, in patients with malignant disease we hesitate to use CB since there is no possibility to increase the GVL effect after CB SCT. If we can develop methods for expansion of donor T cells from the CB unit given to the patient this may have a dramatic impact on the treatment of rejection and relapse of the underlying disease after CB transplantation. Today we lack treatment options if the patient is showing signs leukemia relapse or rejection after CB transplantation.

HEMATOPOIESIS/MESENCHYMAL CELLS

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GLYCOSAMINOGLYCANS AS ANTICOAGULANTS IN MUCOPOLYSACCHARIDOSIS TYPE I (MPS I)

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MPS IH (Hurler syndrome) is caused by severe mutations in a-L-iduronidase (IDUA) gene, leading to accumulation of glycosaminoglycans (GAG). The disorder is lethal unless treated with hematopoietic stem cell transplantation (HSCT). When compared to other recipients of HSCT, MPS IH patients have an increased incidence of bleeding complications. To investigate whether this bleeding propensity is related to the GAG accumulation we evaluated five MPS IH patients (mean age: 1.1 years) prior to any therapy. We observed that three of the five patients had an elevated activated partial thromboplastin time (aPTT). We reasoned that the accumulation of heparin sulfate, a hallmark of MPS IH, may serve as a heparin-like anticoagulant. Consistent with this assumption, we have observed a clear dose – effect relationship between GAG load and level of anticoagulation. In this context, it is important to note that the patient with the second highest value of urinary GAG and aPTT experienced pulmonary bleeding on day 12 after HSCT requiring intubation and ventilatory support for 25 days. We hypothesized that endogenous GAG heparin sulfate exerts its antithrombotic effect through inhibition of factor Xa. To test this hypothesis, we have assessed anti-Xa levels in mice with IDUA deficiency, an animal model of MPS IH. As expected, the anti-Xa level were not significantly different in wild type C57Bl/6 mice and heterozygous mice: 0.11 ± 0.005 IU/cc plasma versus 0.10 ± 0.004 IU/cc plasma ($p = 0.2$). Anti-Xa values of MPS IH mutant mice, however, were significantly elevated when compared to the age-matched sex-matched wild type controls (0.19 ± 0.05 IU/cc plasma versus 0.11 ± 0.005 IU/cc plasma; $p < 0.001$). In addition, we have observed that GAG anticoagulant activity was neutralized by addition of the heparinase. In conclusion, the current study links GAG produced in excess as a result of IDUA deficiency and a hemostatic defect in MPS IH mice and humans. The implications of the current study are that an impaired hemostasis may exist in untreated patients with MPS IH, and that MPS IH patients with high urinary GAG are likely to experience bleeding complications. This information may be useful in identifying MPS IH patients at higher risk for bleeding, especially in the peri-transplant period, when GAG levels have not yet normalized and additional, HSCT-related factors (e.g., thrombocytopenia and hepatic dysfunction affecting clotting factors) contribute to the high risk of bleeding.

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INTERACTION OF $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS WITH HEMATOPOIETIC PROGENITOR CELLS REVEALS TWO DISTINCT PATHWAYS OF REGULATION

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Naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells are responsible for a physiologically essential peripheral mechanism to maintain self tolerance. Several Treg cytokines which mediate immune regulation concomitantly possess hematopoietic modulating activity, for example: transforming growth factor β1 (TGF- β1) and interleukin 9 (IL-9) have been found to have multiple effects during early and late stages of myelopoiesis. Based on the inhibition of CFU-IL3 observed after co-culture of BMC with anti-CD3/CD28+IL-2 activated Tregs, we hypothesize that this regulation occurs in a contact dependent manner through a mechanism involving TGF-β1. Inhibition was not observed in transwell cultures and we found that Tregs fail to inhibit CFU-IL3 activity from MHC class II deficient BMC *in vitro*. Interestingly, CFU-IL3 numbers were enhanced following transplant of syngeneic B6-wt but not B6-Class $II^{-/-}$ BM in lethally conditioned recipients depleted of Tregs, further supporting the notion that cognate interaction is required for this negative regulation to take place. IL-9 is a T cell factor which can promote both growth and survival signals capable of augmenting erythroid progenitor cell (PC) activity. Activated Treg cells co-cultured with syngeneic BMC populations as well as Lin^- fractions routinely resulted in a 3–4 × augmentation of CFU-E vs. control cultures lacking Tegs. Anti-IL-9 specific mab but not isotype control Ig abolished the enhancement of CFU-E activity. Moreover, Tregs from IL-9 KO mice inhibited