are alive (5 with autologous recovery) with 25 thalassemia free with a mean and median follow up time of 392 and 257 days respectively (range 7-1,760 days) as of April 28, 2006. The median day to hospital discharge was day +58 (range 22-137 days). An analysis was undertaken to compare experienced centers (>5 cases) with less experienced centers, as well as compare units that were post-thaw washed versus units that were not post-thaw washed (Table 5). These results show that when cell dose is optimal and post-thaw wash is not used, at experienced centers, unrelated CBT may be a promising approach for the curative therapy of thalassemia major.

## 14

### REDUCED INTENSITY CONDITIONING IN STEM CELL TRANSPLANTATION FOR NON-MALIGNANT DISORDERS

*Shenoy, Shalini. Washington University School of Medicine and Saint Louis Children's Hospital, Saint Louis, MO.*

**Background:** Hematopoietic stem cell transplantation (HSCT) is curative in several non-malignant disorders including hemoglobinopathies. It is limited by donor availability, regimen related toxicities, graft rejection, and GVHD. HSCT is often restricted to myeloablative regimen and matched related donors.

**Study objective:** To determine whether children with non-malignant disorders achieve donor engraftment with lowered toxicity following reduced intensity conditioning (RIT).

**Study design:** Fifty-two children received related or unrelated donor transplants after conditioning with Campath-1H, fludarabine, and melphalan (140 or 70 mg/m$^2$). GVHD prophylaxis included cyclosporine or tacrolimus, short course methotrexate (3 doses), and methyl prednisone. Patients were 8 months to 20 years old; 4 had failed previous transplants; 12 had received transfusion therapy for 8 months to 12 years. Indications for HSCT included hemoglobinopathy (6), bone marrow failure (7), immune dysfunction (25), metabolic disorders (13) and autoimmunity (1). Marrow/peripheral blood (41) was 7-8/8 allele matched and cord blood (11) was 4-5/6 antigen matched.

**Results:** All but 2 patients, both with hemoglobinopathy who received the lower melphalan dose, engrafted. Myeloid (ANC >0.5 × 10$^9$/L) and platelet (>50 × 10$^9$/L) engraftment occurred at a median of 13 (10-36) and 26 (12-82) days. At median follow up of 14 months (5-52), overall and event free survival were 87% and 77%. Treatment related mortality was 11%. Grade 2-4 acute GVHD developed in 15%; chronic GVHD in 11% between 6-9 months post transplant. Post transplant complications were predominantly bacterial and viral infections; all in the first 6 months post HSCT. Hemoglobinopathy patients tolerated conditioning without toxicity and successfully engrafted donor cells only with the higher dose of melphalan.

**Conclusions:** This RIT regimen was well tolerated and achieved donor engraftment irrespective of stem cell source in non-malignant disorder patients at high risk for graft rejection with acceptable rates of TRM and GVHD.

## 15

### STANDARDIZATION OF CFU ASSAYS FOR CORD BLOOD (CB) PRODUCTS

*Clarke, E. for the NMDP Proficiency Testing Committee. StemCell Technologies Inc., Vancouver, BC, Canada.*

The advantages of using CB derived cells for the treatment of leukemia and other hematological malignancies are now well recognized. However, the selection of the most appropriate CB product could be enhanced if functional data, e.g., a colony forming cell (CFC) assay was available. Such data may only be of utility if centers elect to use a standardized protocol and agree to report on similar criteria, so in association with the NMDP, a proficiency testing program was designed specifically to evaluate the challenges and variability of CFC assessment from both fresh and frozen CB samples. Unprocessed fresh CB cells from a designated bank were shipped to 28 participants who were asked to perform a total nucleated cell count (TNC) and viability assessment and transfer a fixed number of viable cells to the tube of MethoCult™ provided. Following 14 days, participants were asked to score and report BFU-E, CFU-GM and total CFC numbers. All centers reported data for all variables. In the second program, 41 participants received a vial of frozen CB cells from a designated bank and asked to perform similar analyses. All centers reported values for TNC and viability but only 60% of participants reported growth of progenitors. The coefficient of variation (CV) for cell assessment from fresh CB was significantly lower than that from frozen CB, though the CVs for CFCs were similar.

**Table 6.** *Mean Cell Values Obtained by CB Centers*

| | CVs from Fresh CB (n = 28) | CVs from Frozen CB (n = 41) |
|---|---|---|
| TNC (×10$^6$/mL) | 6.1% | 16.5% |
| Viability | 3.2% | 35.7% |
| Total viable cells (×10$^6$/mL) | 6.4% | 40.9% |
| BFU-E | 40.6% | 40.4% |
| CFU-GM | 65.0% | 55.0% |
| Total CFC | 34.4% | 35.3% |

Investigation as to the reported lack of growth at 40% of centers is underway. There was no statistically significant association between shipping method (cryo-shippers or dry ice) and CFC growth, but the reported TNC numbers correlated with CFC proliferation, suggesting transient warming of the samples or an extended time frame between thawing, sampling and plating. These data suggest that adherence to standardized protocols for cell assessment and colony enumeration may facilitate global applicability of data generated at various banks.

## 16

### TRANSPLANTATION OUTCOME OF PLASMA DEPLETED CORD BLOOD UNIT AND THE EFFECT OF POST-THAW WASHING

*Chow, Robert,[1,8] Nademanee, Auayporn,[2] Rosenthal, Joseph,[2] Karanes, Chatchada,[2] Jaing, Tang-Her,[3] Graham, Michael,[4] Wang, Brian,[5] Gjertson, David,[5] Chan, Lee Lee,[6] Eames, Gretchen,[7] Tan, Ah Moy,[9] Tan, Poh-Lin,[10] Lin, Hai-Peng,[11] Tan, Patrick,[12] Petz, Lawrence.[1,5] [1]StemCyte International Cord Blood Center, Arcadia, CA, & StemCyte Taiwan National Cord Blood Center, Linko, Taiwan; [2]City of Hope National Medical Center, Duarte, CA; [3]Chang Gung University & Children's Hospital, Linko, Taiwan; [4]University of Arizona Medical Center, Tuscon, CA; [5]UCLA Medical Center & School of Public Health, Los Angeles, CA; [6]University of Malaya Medical Center, Kuala Lumpar, Malaysia; [7]Cook Children's Hospital, Fort Worth, TX; [8]StemCyte Research Institute, Arcadia, CA; [9]KK Women & Children's Hospital, Singapore; [10]National University Hospital, Singapore; [11]Subang Jaya Medical Center, Kuala Lumpar, Malaysia; [12]Mount Elizabeth Hospital, Singapore.*

We studied the outcome of cord blood transplantation (CBT) using two approaches to maximize cord blood (CB) cell dose—depletion of plasma (PD) but not of red blood cells during CB processing, and no post-thaw washing (NW). All 237 known PD CB used in 215 patients up to 3/2006 were analyzed; median age 9 yo, range 0.3-59 with 33% >16 yo; median weight 30 kg, range 4.5-112 with 35% >50 kg; male 60%; median #HLA ABDR matches 4.0; median TNC dose 5.3 × 10$^7$/kg; median CD34 dose 1.8 × 10$^5$/kg; transplant center reported median post-thaw TNC dose of 4.4 × 10$^7$/kg; malignant indications 70%; transplants outside U.S. 39%; double transplant 27%; and non-myeloablative 16%. The incidence of grade III-IV aGVHD and extensive cGVHD was 13% and 14% respectively. Unadjusted engraftment rate of ANC500, platelet 20,000 and 50,000 engraftment were 88 ± 3%, 82 ± 4% and 76 ± 4% respectively. The median time to engraftment for ANC 500, platelet 20,000 and 50,000 was 22, 48, and 63 days respectively. Relapse rate was 23 ± 4% and TRM was 29 ± 3%. With a median follow-up of 325 days, 1-year overall survival and disease-free survival are 59 ± 4% and 54 ± 4% respectively. Stratification analysis showed worse engraftment and survival outcome at CD34$^+$ cell dose below 0.7 × 10$^7$/kg.

There were 113 washed (W) and 95 non-washed (NW) PD CBTs. No significant adverse events occurred when the recommended DMSO threshold of 1g per kg recipient weight was not exceeded. TNC recovery after thawing as reported by transplant centers is higher for NW (median 89% vs 75%).

Unadjusted engraftment rates were higher and median time to engraftment was earlier for NW than W PD CB: 91 ± 4% and 20 days for NW vs 88 ± 4% and 24 days for W for ANC 500 ($P$ = 0.03), 86 ± 6% and 44 days for NW vs 78 ± 5% and 58 days for W for platelet 20,000 ($P$ = 0.004), 85 ± 6% and 57 days for NW vs 72 ± 6% and 75 days for W for platelet 50,000 ($P$ = 0.01). Acute grade III-IV GvHD incidence was 12% (NW) and 13% (W), and extensive chronic GvHD was 4% (NW) and 19% (W). Relapse rates were 16 ± 5% for NW and 28 ± 5% for W ($P$ = 0.15), with TRM for at 25 ± 5% for NW and 34 ± 5% for W ($P$ = 0.52). One-year OS was 63 ± 6% vs 49 ± 5% ($P$ = 0.40), and 1-year DFS was 62 ± 6% vs 36 ± 7% for NW and W ($P$ = 0.21), respectively.

Outcome for PD CBT compare favorably to published data of outcomes using RBC depleted CBT and trended better with respect to engraftment, TRM and survival. There appears to be no clear benefit for post-thaw washing of PD CB, and not washing may be better than post-thaw washing of PD CB with respect to neutrophil and platelet engraftment rate and speed to engraftment, TRM, relapse rate, 1-year OS and DFS.

## 17

### COMPARISON OF CORD BLOOD PRODUCT THAWING METHODS ON CELL RECOVERY AND PROGENITOR INTEGRITY

*Regan, D.M.,[1] Grunzinger Nelms, L.M.,[1] Wofford, J.D.,[1] Alonso, J.F. III,[1] Creer, M.H.[2] [1]St. Louis Cord Blood Bank @ SSM Cardinal Glennon Children's Hospital, St. Louis, MO; [2]St. Louis University Health Sciences Center, St. Louis, MO.*

Umbilical cord blood (UCB) products have traditionally been thawed using a conventional washing (CW) method intended to stabilize the cells, reduce DMSO toxicity and remove potentially ABO-incompatible RBC stroma and plasma. Concerns with CW include total nucleated cell (TNC) loss, bag breakage during centrifugation and poor reproducibility by transplant centers unfamiliar with this technique. We compared CW, albumin reconstitution without centrifugation (AR), and direct thaw (DT, no dilution or wash) methods by assessing viability and TNC, CD34 and colony-forming cell (CFC) recovery post-thaw. Ten cryopreserved UCB products were thawed, split equally into three parts, processed by CW, AR and DT methods and post-thaw tests performed at multiple time intervals up to 48 hours. Mean TNC recovery by CW was lower than DT ($P$ < 0.01) throughout the 48 hour interval, however, CW and AR recoveries were not different ($P$ > 0.05). CD34 and CFC in CW, AR and DT did not differ up to 2 hours but CFC recovery in DT progressively declined with no CFC recovered at 32 hours. In DT, CD34 recovery declined progressively after eight hours compared to AR and CW ($P$ = 0.0009). Throughout the entire evaluation, CW and AR methods performed equally well with no significant differences observed in viability, TNC, CD34 or CFC recovery. Subsequent to the success of this study, AR has been used for 6 patients with no apparent effect on engraftment compared to CW.

We conclude that removing DMSO, RBC stroma and plasma post thaw using CW is not necessary when CB products are RBC and plasma depleted before cryopreservation and have implemented the practice of reconstituting units in our laboratory where environmental conditions are controlled and the infused product characterized. Reconstituting products is safe, easily standardized and comparable to conventional wash in maintaining cellular and progenitor integrity while ensuring recipient safety during infusion.

## 18

### GRAFT-VERSUS-LEUKEMIA EFFECT AFTER ALLOGENEIC TRANSPLANTATION FOR ACUTE LEUKEMIA

*Eapen, Mary. Center for International Blood and Marrow Transplant Research (CIBMTR), Milwaukee, WI.*

It is widely accepted that donor lymphocytes can eradicate tumor cells that survive preparative regimens and the occurrence of chronic graft-versus-host disease (GVHD) associated with fewer relapses. This lower risk does not always translate into a survival advantage, as chronic GVHD is associated with higher transplant-related mortality (TRM).

We examined the influence of GVHD on relapse and mortality in an era where peripheral blood grafts are increasing used for allogeneic transplantation. We performed a retrospective analysis of 1754 recipients of HLA-matched sibling donor transplants in 1996-2003 for acute lymphoblastic (ALL) or acute myeloid (AML) leukemia. Compared to patients with ALL, those with AML were older, more likely to be in 1st clinical remission and receive a non-total body irradiation containing regimen. All patients received non-manipulated grafts and approximately 50% received peripheral blood grafts.

After adjusting for other significant factors, relapse rates were similar in those with and without acute GVHD. The influence of chronic GVHD on relapse, TRM and overall mortality varied by type of leukemia. Chronic GVHD was associated with lower relapse rates in ALL (RR 0.83, $P$ = 0.013) but relapse rates were not statistically different in AML (RR 0.96, $P$ = 0.496). Among patients with AML, TRM was higher in those with both acute (RR 1.45, $P$ = 0.005) and chronic (RR 1.65, $P$ = 0.003) GVHD, a trend not seen amongst those with ALL. While overall mortality rates did not differ significantly in patients with and without acute GVHD, rates were significantly lower in patients with ALL who developed chronic GVHD (RR 0.80, $P$ < 0.001). This survival advantage contrasts most reports and requires confirmation in a larger data set. Though GVHD rates were higher after transplantation of peripheral blood relative to bone marrow, the influence of GVHD on relapse and mortality did not differ by graft type suggesting that there may be a GVHD threshold beyond which there is no additional benefit with respect to either recurrence or survival.

## 19

### NK CELLS AND KIR MISMATCH IN CORD BLOOD TRANSPLANTATION

*Miller, Jeffrey S., Brunstein, Claudio G., McKenna, David H., Wagner, John E. Blood and Marrow Transplant Program, University of Minnesota, Minneapolis, MN.*

We have shown that NK cell killer Ig-like receptor (KIR) interactions predict better survival in recipients of adult donor unrelated transplant. We studied KIR reconstitution from recipients at day +100 after transplant from different allogeneic cell sources. Adult marrow unrelated donor grafts resulted in significantly lower KIR recovery than from UCB sources (27.31 ± 2.06%, n = 36 vs 37.99 ± 2.54%, n = 49, $P$ = .0027), which may have clinical consequences. We have recently found that NK cell receptor expression defines maturation on developing NK cells and hypothesized that NK cell alloreactivity would predict oucomes in recipients of UCBT. We therefore assessed the effect of KIR-L MM in 243 recipients of UCB transplanted at the University of Minnesota. KIR-L MM in the GVH direction was found in 70 (29%) donor-recipient pairs.

Using this strategy, there was no difference in 2-year survival, or GVHD. In contrast, relapse was less in recipients of single UCB transplants who were KIR-L MM but for the entire group, the incidence of TRM was higher among recipients of KIR-L MM grafts [26% vs 13%, $P$ = 0.01] complicating detection of any possible benefit. Although we fail to support a specific search for UCB units with a KIR-L MM, we are exploring methods to better activate NK cells in vivo to optimize NK cell effects to decrease relapse after UCB transplant. Based on our experience using haploidentical NK cells from adult donors, we find that anti-tumor activity in AML patients corresponds with the in vivo expansion of NK cells stimulated by a surge in endogenous IL-15 but NK cells. In an attempt to improve on these outcomes, NK cell precursors from a third UCB unit are being expanded in vivo after a myeloablative conditioning to test the NK cell therapy potential of UCB in patients with poor prognosis AML.

## 20

### DIFFERENTIATION OF NAIVE CORD BLOOD T CELLS INTO CD19-SPECIFIC CYTOLYTIC EFFECTORS FOR POST-TRANSPLANTATION ADOPTIVE IMMUNOTHERAPY

*Cooper, Laurence J.N. MD Anderson Cancer Center, Houston, TX.*

Disease relapse is a barrier to achieving therapeutic success after unrelated umbilical cord blood transplantation (UCBT) for B-lineage acute lymphoblastic leukemia (B-ALL). While adoptive transfer of donor-derived tumor-specific T cells is a conceptually