Our program has transplanted 28 patients to date. The first step of our bloodless transplant protocol employs the use of aggressive doses of erythropoietin and intravenous iron to a target Hg of 11 g/dL. Apheresis causes anemia and thrombocytopenia necessitating an average 14 day delay before HDCT allowing the patient's hemoglobin and platelet levels to reach 11 g/dL and 100,000 cells/mm$^3$. Standard doses of chemotherapy with BCV (BCNU, Carboplatin, and VP16) or Melphalan (150-200mg/m$^2$) were administered. Routine blood conservation techniques were utilized. Erythropoietin, intravenous iron, vitamin C, folate, and B complex were given after transplant to facilitate red blood cell recovery. The single mortality was attributed to pre-transplant hemoglobin of 7 g/dL at which time our protocol was amended to require a hemoglobin level of 11g/dL before HDCT. To prevent bleeding, the patients were prophylactically placed on Interleukin (IL)-11 with Amicar and vitamin K added when the platelet count fell below 30,000 cells/mcL. Apheresis catheters were removed the day after reinfusion before thrombocytopenia occurred. No significant bleeding occurred with platelet counts exceeding 5000 cells/mm$^3$. Hematologic data are outlined in table 1: Individuals unwilling to accept blood products can undergo HDCT and APBSCT with an acceptable rate of morbidity and mortality. Blood conservation techniques may limit the number of transfusions thereby further decreasing transplant related complications. Public safety concerns related to transfusion risks, increasing blood shortages and economics may require us to establish better transfusion guidelines.

**Table.** *Hematologic Data*

| | All Patients* (n = 28) | Multiple Myeloma (n = 10) | Lymphoma (n = 16) |
|---|---|---|---|
| Average Hb@ onset of HDCT (g/dL) | 12 | 13 | 11.6 |
| Average change in Hb (g/dL) | 5.3 | 4.7 | 5.9 |
| Average # Days plts < 10 (cells/mm$^3$) | 4.7 | 2.5 | 6.1 |
| Average lowest plts (cells/mm$^3$) (Nadir and Range) | 5.0 (1-36) | 9 (2-36) | 5.8 (1-13) |

*Includes 2 breast cancer patients.

# GRAFT PROCESSING

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### ONE-YEAR FOLLOWUP: PERIPHERAL CD34 COUNTS TO GUIDE LEUKAPHERESIS IN PERIPHERAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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We previously reported at this meeting a multiparameter model to predict CD34 collection on the basis of peripheral CD34 counts obtained on the day of leukapheresis, in conjunction with several clinical indicators (autologous vs allogeneic, collection number, gender, length of collection). The model can be expressed: LOG CD34/kg = CONSTANT + LOG PB_ABS_CD34 + 3_HOUR + AUTO + MALE + COL_NO. Where PB_ABS_CD34 is the absolute peripheral CD34 count in cells/microliter, 3_HOUR indicates length of apheresis collection (0 if 4 hour, 1 if 3 hour), AUTO indicates transplant type (0 if allo, 1 if auto), MALE indicates gender (0 if female, 1 if male), and COL_NO indicates collection number. This model was implemented in 3 phases, with a learning set (n = 60) to create the model, a testing set (n = 203) to validate the model on data not included in the learning set, and a prospective implementation phase of a refined model based on the entire data set. The final model had coefficients of 5.169, 0.986, −0.151, 0.077, −0.058, and −0.028 (corresponding to the above equation). This model was capable of predicting actual collections with an intercept of −0.05, a slope of 1.006, and an $R^2$ of 0.930. During the prospective implementation phase we attempted to use this model to determine whether a patient was eligible to initiate collection. Patients scheduled for a possible first collection reported to the outpatient clinic in the morning and had blood drawn for peripheral CD34 determinations (mean turnaround time < 1 hour). The predicted CD34 collection (CD34/kg) was estimated according to the model and reported to the patient coordinator. Despite the rapid result reporting, implementation of this algorithm disrupted the clinical schedule and proved infeasible. We now report a modified model based on peripheral CD34 counts obtained the day prior to the day of anticipated initiation of leukapheresis. The additional parameters included in this model were disease (multiple myeloma vs other), collection number and transplant type. The learning data set (n = 132) was able to predict CD34 collection with an $R^2$ of 0.811. Although this was not as close a fit as the previous model, analysis of sensitivity and specificity (predicting whether a given collection would yield greater than $5 \times 10^5$ CD34/kg) revealed a positive predictive value of 84% and a negative predictive value of 75%. We are now determining how best to implement this predictive tool in the clinical setting.

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### DMSO REMOVAL REDUCES STEM-CELL INFUSION-RELATED TOXICITY AND ALLOWS EXCELLENT ENGRAFTMENT OF CRYOPRESERVED UNRELATED CORD BLOOD AND AUTOLOGOUS STEM CELLS

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The reinfusion of cryopreserved stem cells (SC) can be associated with allergies, vomiting, seizures and acute renal failure. All these reactions may be attributed to the DMSO that is infused with the stem cells. Since 1999, we have removed the DMSO for all pediatric patients weighting ≤25 kg, with a DMSO dose ≥1 g/kg or with underlying renal or cardiac compromise. **Methods:** DMSO was removed according to the New York Blood Center guidelines for thawing and washing cord blood (CB) units. Briefly, a 1:1 Dextran40 −5% albumin solution was slowly added to the SC product immediately after thawing in a 37°C water bath. The cells were at first washed in 50cc tubes and now, only bags are used. The bags are centrifuged 400g, 4°C for 20 minutes. The buffy coat is re-suspended in the same solution and infused over 30 minutes. The objective of this study is to compare cell yield, viability, sterility, side effects associated to infusion and engraftment in children undergoing BMT with unmanipulated grafts or after DMSO removal. **Results:** Twenty-six patients with underlying malignancies were evaluated; 20 received autologous PBSC, 11 of them with DMSO removal. DMSO was removed from all 6 unrelated CB units. The patients in the groups with and without DMSO removal had a median age of 5 and 4 years and weighted 18 and 17kg, respectively. In CB transplants the mean mononuclear cell (MNC) dose was $3 \times 10^7$/kg (2-6). Viability was 80% (53-96). Cell recovery after thawing was 93% (82-100) and after the DMSO removal, 96% (73-100). All patients engrafted after a mean of 17 days (11-22). In patients undergoing autologous PBSC transplants with and without DMSO removal, the mean MNC dose was 7 (2-10) and 9 (3-21) $\times 10^8$/kg, respectively. After thawing, the mean cell viability was 78% (64-95) and 76% (46-89). Cell recovery was 88% (55-111) and 94% (90-97) and 81% (51-100) after the DMSO removal. All patients engrafted after a mean of 10 (8-13) vs. 11 (8-15) days, respectively. The patients who receive DMSO-free grafts did not have any serious adverse reactions associated to the infusion. Patients for whom the DMSO was not removed had seizures (1), hypervolemia (2), cardiac arrest (1) and all had some degree of nausea, vomiting and chills. **Conclusions:** DMSO removal is safe, reduces the infusion-related toxicity in pediatric BMT without significant cell loss or contamination. Engraftment is satisfactory in both autologous PBSC and unrelated cord blood transplants.