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Fifth Annual International Umbilical Cord Blood Transplantation Symposium, Los Angeles, California, May 11-12, 2007

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Symposium Summary

The 39-member faculty for the Symposium included leaders of the major transplantation centers from a round the globe: France, Italy, Japan, Spain, Australia, and the United States. Attendees were from Belgium, Czech Republic, France, Germany, Italy, Greece, The Netherlands, Poland, Switzerland, United Kingdom, Israel, Cyprus, Kuwait, Saudi Arabia, United Arab Emirates, India, Australia, Hong Kong, Malaysia, Singapore, Japan Korea, Taiwan, Canada, United States, Panama, Brazil, and Columbia.

The program was divided into 8 sessions: (1) Risk factors affecting survival in recipients of cord blood, (2) Strategies to improve engraftment after UCBT, (3) Cord blood banking, manipulation and unit selection, (4) Conditioning regimens, (5) Immune reconstitution and infections, (6) Non-hematopoietic stem cells, (7) The graft versus tumor effect, and (8) Governmental affairs. The following comments emphasize significant aspects of selected presentations. Additional details are provided in the abstracts that follow.

The Fifth Annual International Cord Blood Transplantation Symposium was presented by the California Blood Bank Society and the Cord Blood Forum, and was partially supported by unrestricted educational grants from StemCyte International Cord Blood Center and the National Marrow Donor Program (NMDP). The Symposium was also partially financially supported by a contract between the Health Resources and Services Administration (HRSA), US Department of Health and Human Services, and the California Blood Bank Society. Any opinions, findings, conclusions or recommendations expressed or presented at this conference are those of the presenters and do not necessarily reflect the views of the US Government.

SESSION I. RISK FACTORS AFFECTING SURVIVAL IN RECIPIENTS OF CORD BLOOD

Dr. Vanderson Rocha reviewed the risk factors affecting survival after umbilical cord blood transplantation (UCBT) in children and adults with hematological malignancy. He pointed out that factors associated with outcomes after allogeneic hematopoietic stem cell transplantation (HSCT) are related to the donor, the graft, the patient, the transplant conditioning regimen, and prophylactic graft-versus-host disease (GVHD) therapy. All of these factors affect hematopoietic and immune recovery, GVHD and graft-versus-leukemia (GVL), treatment related mortality (TRM), relapse, and disease-free survival (DFS). Favorable risk factors identified by the authors of a number of significant publications include the diagnosis, cell dose, cytomegalovirus (CMV) negative serology, age, the HLA disparity, favorable remission status, CD34+ cell dose, and absence of acute GVHD (aGVHD). For example, in a study of 191 children with acute myelogenous leukemia (AML) who received unrelated cord blood transplants, DFS at 42 months when transplanted in complete remission (CR) 1 was 67%, in CR2 it was 49% and for later stages of the disease was 24%. A study of 171 adults who received an unrelated cord blood transplant identified favorable risk factors for survival and DFS as remission status, ABO, and sex of patient (female recipients having more favorable outcomes). In another study of 262 adults with hematologic malignancies who received a UCBT using a myeloablative conditioning regimen, favorable factors as determined in a multivariate analysis for DFS were CMV negativity, ABO compatibility, status of the disease at transplant, and the use of total body irradiation (TBI);

also, there was a trend for favorable outcome for patients receiving $>2 \times 10^7$ nucleated cells/kg body weight. In a study of children with acute lymphoblastic leukemia (ALL) in CR2 who received a UCBT, an important factor was whether the first relapse was on or off chemotherapy. The DFS for those whose relapse occurred off therapy (n = 65) was 45% and for those whose relapse was while on therapy (n = 73) the DFS was 26%. In a recent analysis of 925 patients with malignant disorders who received a UCBT, multivariate analysis for TRM identified three favorable prognostic factors: HLA 6 of 6 or 5 of 6, early and intermediate phase of the disease, and the number of cells infused being $>2 \times 10^7$/kg. Multivariate analysis for overall survival (OS) also identified three favorable prognostic factors: early and intermediate phase of the disease, the number of cells infused as $>2 \times 10^7$/kg, and negative CMV serology.

Dr. Guillermo Sanz reviewed the outcomes in 92 adults with hematologic malignancies who underwent a single unit UCBT at a single institution after myeloablative conditioning using thiotepa, busulfan, cyclophosphamide or fludarabine (Flu), and anti-thymocyte globulin (ATG). The cumulative incidence of myeloid engraftment, grade III-IV aGVHD, and nonrelapse mortality (NRM) at 100 days was 90%, 18%, and 25% respectively. The cumulative incidence of relapse and the probability of DFS at 3 years were 21% and 39% (median follow-up, 26 months). A multivariate analysis of prognostic factors of short term outcome endpoints indicated that the absolute number of CD34+ cells infused had a marked effect on neutrophil and platelet engraftment; the type of ATG (lympho vs. thymoglobulin) had a significant effect on platelet engraftment and aGVHD; and the development of aGVHD had a significant effect on platelet engraftment. A multivariate analysis of prognostic factors of long term outcome endpoints indicated that age, stage of disease, CD3+ cells infused, conditioning regimen (Flu vs. no Flu), and development of grade II-IV aGVHD all had a significant effect on NRM. Patients with advanced disease or who had a diagnosis other than CML had a higher relapse rate and the only variable that affected DFS was the stage of disease at transplant. The authors concluded that UCBT of adults with a single and adequate unit and using highly immunosuppressive conditioning can provide the same engraftment rate and similar speed of engraftment to that obtained in children. Also, the number of CD34+ cells in the UCB unit is the most relevant factor for engraftment. This factor must be used for the UCB unit of choice. The degree of HLA mismatching does not seem as important in adults as in children. Several outcomes after UCBT (speed of engraftment and risk of aGVHD, TRM, and relapse) were modulated by the conditioning regimen. Age had a major influence on NRM. The stage of the disease at transplant was the major determinant of DFS. Finally, the criteria for selecting the best CB unit for adults need urgent modification.

Dr. Marcos de Lima reviewed the impact of host-versus-graft (HVG) HLA mismatch on outcomes of CBT. The authors of this presentation developed the hypothesis that HLA mismatches in the HVG direction are likely to influence outcomes of unrelated CBT, in particular engraftment. They performed a retrospective study of 91 patients with malignant or non-malignant disorders who received CBT. The conditioning regimen was myeloablative in 86 patients (95%). In pediatric patients the 1 year survival was 60% versus 70% (zero versus $\geq$1 mismatches, respectively), $P = 0.3$. In adult patients the corresponding values were 45% vs. 39%, respectively. Thus, in this small sample set, there was no statistically significant association between number of graft-versus-host (GVH) mismatches and survival. There was, however, a higher cumulative incidence of grade II-IV aGVHD among adult recipients of larger grafts, with a similar trend apparent in children. The authors concluded that evaluation of mismatches at a higher level of resolution and in other HLA loci (HLA-C, -DQ, -DP) is warranted to better evaluate these histocompatibility effects on engraftment, incidence of GVHD, and immune reconstitution. A larger patient population should be tested.

Dr. Mary Eapen discussed the risks and benefits of UCB vs. bone marrow (BM) unrelated donor HSCT in children with acute leukemia. She pointed out that selection of CB over BM remains controversial. For bone marrow transplants (BMT), studies have shown that there is significantly higher mortality when there is a mismatch at -A, -C or -DR loci. More specifically, the data indicate that there is a 9-10% lower OS with each additional mismatch. That is, with 8 of 8 HLA matched transplants, OS was 52%; with 7 of 8 HLA matched transplants OS was 43% and for 6 of 8 HLA matched transplants the OS was 33%. She reviewed a study of 116 BMT patients who were compared with 503 CBT. All BM patients had an 8 of 8 allele matched donor whereas only 7% of donor-recipient CB pairs were matched (low resolution at -A and -B loci and high resolution at -DR), 40% had a one antigen mismatch and 53% had a 2 antigen mismatch. The results of the study indicated that, relative to matched BM, 1 antigen mismatched CBTs had lower GVHD and similar rates of TRM, relapse, and LFS. Matched CBTs had less GVHD and TRM and higher survival rates. However, small patient numbers prevent definite conclusions. Two antigen mismatched CBTs had lower GVHD, higher rates of early TRM, lower rates of relapse and similar rates of LFS. The authors of the study concluded that the data support use of 0-2 antigen mismatched CB grafts regardless of the availability of a matched BM donor in pediatric patients

with acute leukemia. Also, that larger repositories of CB are needed to meet the increasing demand for larger, partially HLA matched units.

Dr. Eliane Gluckman presented the results of a study of unrelated CBTs in non-malignant diseases (n = 268). The diagnosis in 48% of patients was a BM failure syndrome, 30% with severe combined immunodeficiency disease (SCID), and 22% had a metabolic disorder. HLA matching was done with low resolution for -A and -B and high resolution for -DRB1: 17% of patients were matched, 43% had a one antigen mismatch, 36% had 2 antigen mismatches and 4% had greater than 2 antigen mismatches. For neutrophil recovery, favorable prognostic factors were (1) $\leq$1 HLA disparity, (2) cell dose infused $\geq 3 \times 10^7$/kg, and (3) diagnosis of SCID. For platelet recovery, favorable prognostic factors were (1) $\leq$1 HLA disparity, (2) diagnosis other than BM failure syndrome, and (3) cell dose infused $\geq 3 \times 10^7$/kg. For grade II-IV aGVHD, only one favorable prognostic factor was identified, HLA disparities <3. For survival, favorable factors were (1) negative CMV serology, (2) diagnosis other than BM failure syndrome, (3) cells dose infused $>2 \times 10^7$/kg, and (4) no more than 1 HLA disparity. Further data indicated that the requirements for cell dose and HLA matching are different in malignant and non-malignant diseases. In non-malignant disease the optimal cell dose is $>5 \times 10^7$ at collection and $>4 \times 10^7$ at infusion. Also, HLA mismatches play a major role for engraftment, GVHD, and survival, and the effect of HLA mismatches is partially abrogated by increasing cell dose.

SESSION II. STRATEGIES TO IMPROVE ENGRAFTMENT AFTER UCBT

Dr. John Wagner reviewed factors having an impact on engraftment after UCBT. These include nucleated cell dose, CD34+ dose, HLA match, GVHD, history of prior chemotherapy, graft T cell dose, use of growth factors, host microenvironment, use of post-transplant cytotoxic agents, and development of post-transplant infection. He reviewed transplant data from 1994-2000 at the University of Minnesota (n = 102), which indicated a superiority of CD34+ and CFU-GM dose over nucleated cell dose in predicting engraftment. There was a linear relationship between CD34+ cell dose and the days to neutrophil recovery. Also, patients with the lowest cell doses (specifically $<1.7 \times 10^5$ $CD34^+$/kg) had the highest risk of graft failure. For platelet recovery, CD34+ again had a significant effect as did CMV serostatus. Lessons learned from this early study were that $CD34^+$ cell dose is a major factor. These data led to a change in minimum cryopreserved cell dose from 1 to 2.5×10^7 nucleated cells/kg. HLA match was not a clear factor in this analysis (although too few patients were in the 6/6 and 3/6 categories) and G-CSF may be important. A more recent and larger multi-center study (COBLT) included 316 patients between ages of 0.1 and 17.9 years (median age = 4.6 years) who were transplanted between 1999 and 2004. The results indicated that cell dose, HLA match (5-6/6 versus 4/6) and donor age are significant factors. Eurocord registry data indicate that engraftment is affected by CMV serostatus, stage of the disease, and cell dose infused ($>2 \times 10^7$/kg). There is an interaction between cell dose and HLA match, i.e., one needs more cells to achieve engraftment with increased HLA mismatches. When donor and recipient are matched (6 of 6) there is no clear impact of cell dose, but when there are mismatches, the affect of cell dose becomes apparent. Early results from the NY Blood Center suggest that Flu may have a favorable affect on engraftment. A multivariate analysis in adults indicated that nucleated cell dose $<2.5 \times 10^7$/kg, HLA mismatch, absence of Flu, and use of methotrexate (MTX) adversely affected neutrophil recovery. Different strategies are being developed to enhance engraftment including optimization of the peri-transplant therapy, improving HSC homing (e.g., CD26 inhibition, PTH, intrabone marrow injection), co-infusion of haplo TCD peripheral blood stem cells (PBSC) with UCB, use reduced intensity conditioning (RIC), and increased cell dose through improved collection volumes, ex-vivo expansion, or infusion of two partially HLA matched units.

Dr. Mary Laughlin discussed the influence of HLA disparity and graft lymphocytes on allogeneic engraftment and survival after UCB transplants in adults. She reported the results of a single institution prospective phase II study in adults receiving unrelated UCB following a myeloablative conditioning regimen. The study was designed to test the hypothesis that UCB graft lymphocytes may have an impact on rates and kinetics of donor engraftment. A secondary objective was to prospectively determine the relationship between degree and nature of HLA mismatch in UCB grafts and GVHD/patient survival. Results of this early phase II study indicated that graft nucleated cell and CD34+ hematopoietic progenitor cell dose are predictors of allogeneic engraftment and survival in UCB adult recipients. Univariate analyses demonstrated that UCB graft infused cell doses of CD34+, CD3+ and CD34+HLADR+CD38+ progenitors correlated with donor neutrophil engraftment. Also, allele matching for HLA class II resulted in improved event free survival (EFS) and decreased risk for aGVHD.

Dr. John Wagner reviewed results at the University of Minnesota using double UCBT. As cell dose is a limiting factor in the successful use of UCBT, two CB units were used to increase cell dose. The units must

be partially HLA matched with each other and with the patient. No more than 2 antigen mismatches are allowed with the patient and between the two grafts. The combined cell dose of the two units must be at least 2.5×10^7 cells/kg. The preparative therapy includes TBI, Flu and Cy, and the post-transplant immune suppression is cyclosporine A (CSA) and mycophenolate mofetil (MMF); all patients received growth factor. In the initial phase I study, 23 of 23 patients receiving two units had neutrophil engraftment at a median of 23 days. Transient "double" chimerism occurs and in most, the unit that predominates early on "wins." Predicting which unit will win is not yet possible as there is no association with infusion order, mononuclear cell dose, CD34 dose, CD3 dose, granulocyte-macrophage colony forming units (GM-CFU) dose, HLA, or ABO match. In a multivariate analysis, the only factor predictive of neutrophil engraftment was the combined CD34+ cell dose. Using double UCBT, engraftment is comparable to that in children, 93% of adults are now eligible for UCBT as compared to 30% with a single UCB unit, if the threshold is $\geq 2.5 \times 10^7$/kg).

A retrospective analysis was done to determine what factor – use of Flu or coinfusion of UCB most significantly affected engraftment. The analysis included 147 patients with hematologic malignancy undergoing first transplants between 1994-2005. Results of this study suggested that the combined cell dose is the principal factor responsible for both engraftment and speed of neutrophil recovery. Although Flu is associated with increased incidence of engraftment, it is not statistically significant. In the subgroup of patients receiving Flu/Cy/TBI, HLA-matching as well as cell dose is responsible for the speed of neutrophil recovery.

Dr. Hal Broxmeyer reported on the effect of CD26 inhibition on CB engraftment. Inhibition of CD26/dipeptidylpeptidase IV (DPPIV) with small peptides, such as Diprotin A or Val-Pyr enhance the capacity of human CB $CD34^+$ cells and mouse $Sca1^+c\text{-}kit^+lin^-$ BM cells to respond to the chemotactic activity of stromal cell derived factor *in vitro*, and they enhance donor mouse BM HSC homing and engraftment of lethally irradiated recipient mice in both a competitive and a non-competitive assay. Recent studies indicate that inhibition of CD26/DPPIV in human CB $CD34^+$ cells enhances their engraftment of nonobese diabetic (NOD)/SCID mice. Also, Diprotin A produces enhancement of $CD34^+$ cord blood and G-CSF mobilized adult peripheral blood $CD34^+$ cells, respectively, by pretreating donor CD34+ cells, or treating the recipient mice themselves. Pretreatment of lethally irradiated mice with Diprotin A enhances engraftment of untreated donor mouse cells. Future studies to enhance HSC engraftment will encompass studies in higher animals and human clinical trials.

Dr. Ronald Hoffman discussed the expansion of human UCB SCID-repopulating cells using chromatin-modifying agents. The lack of net stem cell expansion following in vitro exposure to cytokines could result from inability of cytokines to promote HSC divisions. The remaining bone marrow repopulating potential of such expanded cell populations might be from cytokine unresponsive HSC. Previous ex-vivo attempts to alter HSC fate divisions in the presence of a variety of cytokine combinations or feeder layers have resulted over time in a loss of cells expressing the stem cell phenotype and progressive loss of the number of primitive progenitors cells quantified using a variety of surrogate stem cell assays. Further, silencing of genes has been shown to be accompanied by DNA methylation of a gene's promoter and by histone deacetylation in regions controlling the genes of interest. Studies were carried out to investigate whether the addition of two chromatin-modifying agents, 5-aza-2′-deoxycytidine (5azaD) and trichostatin A (TSA), to CB CD34(+) cells in culture results in expansion of the numbers of SCID repopulating cells (SRC). Results indicated that the program of HSC in vitro can be epigenetically modified to change their fate by agents affecting DNA hypomethylation and acetylation. Epigenetics appears to play a role in determining the transcriptional options of HSCs and changing the behavior of these cells in vitro. Finally, the use of chromatin modifying agents favors symmetric cell division of CB HSC. Thus, ex-vivo expansion strategy using chromatin-modifying agents provides a potential avenue by which to expand the number of HSC in single CB unit for use as an alternative source of HSC grafts for adult recipients.

Dr. Tracey O'Brien presented data indicating that GSK-3β regulates ex-vivo expansion and engraftment of UCB HSC through activation of Wnt signaling. Past experience has indicated in animal models that short term reconstituting HPC are expanded at the expense of longer term repopulating cells required for durable engraftment. In clinical trials engraftment has been no faster despite infusion of expanded cell numbers suggesting loss of function and/or homing of HSC/HPC in the expansion process. There are data indicating that Wnt signaling positively regulates self-renewal of murine HSC. An alternate approach to achieve Wnt activation is by inhibition of glycogen synthase kinase-3 β inhibition, which prevents degradation of β-catenin. GSK-3 β inhibition suppresses ex-vivo proliferation of UCB cells induced by cytokines but improves the repopulating capacity of UCB. We have explored the role of GSK-3β inhibition and Wnt activation as a means to expand ex-vivo human UCB cells. We have shown that GSK-3 β inhibition suppresses ex-vivo proliferation of UCB cells induced by cytokines but improves the repopulating capacity of UCB. Using a synthetic GSK-3β inhibitor (6-bro-

moindirubin 3'-oxime; BIO) co-cultured with UCB CD34+ cells, we demonstrate that GSK-3 β inhibition suppresses ex-vivo expansion of committed HPCs, whereas increasing the pool of the most primitive progenitor/stem cells. UCB C34+ cells cultured in BIO results in the accumulation of β-catenin and its relocation from the cytoplasm to the cell nucleus. In addition up-regulation of *c-myc* and *HoxB4* genes both known β-catenin targets involved in the regulation of stem cell renewal, was observed. Using the 'humanized' NOD/SCID mouse model we have demonstrated both expansion and preservation of function with increased numbers of engrafting BIO treated human CD34+ UCB (120-fold expansion) compared to control cells (45-fold expansion). In addition, increased output of myeloid, lymphoid and megakaryocytic progenitor cells was observed in NOD/SCID mice that received BIO-treated UCB cells. Thus, using a humanized NOD/SCID mouse model we have shown that activation of the Wnt pathway through GSK-3β inhibition improves the repopulating ability of human UCB hematopoietic progenitor/stem cells.

Dr. Colleen Delaney discussed Delta1: A notch up on CBT? Notch signaling is generally capable of influencing the ability of a cell to progress from a less to a more differentiated state in response to specific signals, e.g. growth factors. A role for Notch in hematopoiesis was initially suggested by detection of the human Notch1 gene in CD34+ or CD34+lin- human hematopoietic precursors. Retrovirus-mediated expression of a constitutively active form of Notch 1 resulted in enhanced self-renewal of hematopoietic progenitors, and activation of endogenous Notch receptors in primary murine hematopoietic progenitors (LSK cells) by culture in the presence of immobilized Notch ligand (Delta1) resulted in a multi-log increase in short-term lymphoid and myeloid repopulating cells. Similarly, culture of CB progenitors in the presence of Notch ligand results in >100 fold increase in CD34+ cells with short-term and possibly long term NOD/SCID repopulating ability. Optimization of the notch-mediated expansion system was carried out with the goal of providing cells that rapidly engraft and overcome the delay in hematopoietic recovery after HSCT. A clinical trial is underway using double CBT with a fully myeloablative Cy/Flu/TBI conditioning regimen. An unmanipulated unit is infused first followed by expanded cells given 4 hours later. Three patients have been enrolled in the study and data are available on the first two. Both were adults with AML in CR1. Cell expansion resulted in a 791 fold expansion in patient #1 and a 743 fold expansion in patient #2; CD34 expansion was 210 fold for patient #1 and 174 fold for patient #2. Patient #1 engrafted on day +9 and patient #2 engrafted on day +16. Both patients had a consistent absolute neutrophil count (ANC) above 100 by day 7 post-transplant. Platelet engraftment >20K occurred on days +53 and +32, and >50K on days +68 and +41 in the first and second patient, respectively. In patient #1 weekly chimerism data indicated that all cells were from the expanded unit on day +7 and again on day +14; by day +21 there was no longer engraftment by the expanded cells. In patient #2 the non-manipulated unit did not begin to contribute to the engraftment until after day +14 and, until that time, 100% of the engrafted cells were from the expanded unit. Using the same preparative regimen and post-transplant immunosuppression, the median time to engraftment was 27 days for 9 patients receiving double CBTs but without using expanded cells.

Dr. Elizabeth Shpall discussed CB expansion and indicated that to date no infusional toxicity has been associated with transplantation of expanded CB; time to platelet engraftment may be reduced but neutrophil engraftment is still three weeks; CD34+ and total cell losses with positive selection are substantial; and therefore alternative cord expansion strategies are needed. Mesenchymal stem cells (MSC) can differentiate into bone, adipocytes, cartilage, functional muscle, and liver cells. MSC may provide cellular and extracellular components of the stem cell "niche" absent in current liquid expansion protocols, thereby improving CB expansion, engraftment, and potentially reducing the rate or severity of GVHD. Studies are underway in which CB MNC are co-cultured with MSC. The co-culture system, compared with the liquid culture system, has produced superior results in expansion of TNC, CFU, CD34+, CD133+, CAFCwk2 and CAFCwk6. A clinical trial is soon to begin to determine if cells expanded in the co-culture system can improve time to neutrophil and platelet engraftment and engraftment failure rate; secondary endpoints are GVHD, infections, immune reconstitution, day 100, and OS.

Dr. David Scadden discussed methods of enhancing the engraftment of stem cell grafts. The stem cell niche hypothesis suggests that stem cell persistence depends upon engagement of a specialized anatomically defined microenvironment. A niche is an architectural place where stem cell survival and replication are fostered and where interaction between heterologous elements is dynamic and may be transient. Modifying parathyroid hormone (PTH) receptor genetically can lead to an increase the number of stem cells in a mouse by about 2 fold. PTH given as a once daily dose immediately following transplantation in a murine model resulted in improved cellularity in the marrow, an increase in the stem cell pool, and greater ability to tolerate the transplant. Data indicate that PTH in an animal model improves production of stem cells, improves preservation of stem cells after chemotherapy, and improves efficiency of engraftment of stem cells. Experiments in Cynomolgous monkeys (n

= 6) in which conditioning plus allogeneic transplants were performed with and without daily PTH (d −7 to 49) indicated increased cellularity, and marked improvement in immunologic recovery. Patients who had failed mobilization protocols for autologous transplant were treated with PTH; 7 of 15 patients who had failed one prior mobilization had an adequate mobilization after PTH, and 2 of 5 patients who had failed 2 mobilizations had a successful subsequent mobilization procedure. The study of the effects of PTH is being extended to patients who are to receive a dual allogeneic CB transplant to see if PTH can improve the outcome.

Dr. Ka Wah Chan discussed primary graft failure after unrelated donor CB transplants: risk factors and management. The definition of engraftment is recovery of ANC >500/μl with documented donor chimerism. Primary engraftment failure is defined as no evidence of recovery of ANC >500/μl, (except those with autologous recovery), or <5% donor cells on ≥2 chimerism studies (PCR/STR method), performed weekly, beginning 3rd - 4th week. Patients who die before day 28 are not evaluable. Among 100 UCBT transplants 6 died early, 83 engrafted and there were 11 primary graft failures. An analysis was performed of possible risk factors leading to primary graft failure. None of the following features were statistically different among patients who engrafted, and those who did not: patient age or weight; TNC, CD34, or CFU in the graft; a diagnosis of a malignant or non-malignant disorder; inclusion or not of TBI in the preparative regimen; or HLA match of ≥5 of 6 or ≤4 of 6. The approach to primary graft failure at the Texas Transplant Institute is as follows: A backup autologous marrow is not collected. Instead, a second UCBT is used as rescue (sooner rather than later) using the most readily available unit with first priority being cell dose but also important are HLA match and immediate shipment. The patient's marrow is usually aplastic, so emphasis of the preparative regimen is on immunosuppression. The clinical outcomes of 11 patients with primary graft failure were as follows: two were not transplant candidates because of active fungal pneumonia, or recurrent leukemia and these patients died 35 and 62 days post transplant. Nine children went on to second unrelated donor CBT. The median time to the second UCBT was 55 days, range 33-95 days. Six patients were treated with a preparative regime that included CY/Flu/TBI (2Gy), ±ATG. Two patients with aplastic anemia were treated with TBI (6Gy), CY and ECP or Campath. One patient received Campath and Flu. There was one early death (day+12) and the other 8 patients had myeloid engraftment (median day 15), platelet engraftment (median day 92), and complete donor chimerism. However, late infectious complications affected 7 of the 8 survivors. The current status is that 5 patients are alive at a median of 1,095 days (range 758-1,328 days). Chronic GVHD was diagnosed in 6 patients and was extensive in 5; the performance status is 30-90% (median 70% - Lansky scale). Two deaths resulted from respiratory failure, one was from EBV-LPD, and one was due to *Pseudomonas* sepsis. Conclusions reached are that primary graft failure is a serious risk of UCBT. A back-up donor source should be identified before start of procedure; the lack of donor chimerism early on is predictive of GF, an early 2nd UCBT was a feasible therapeutic option, and using an immunosuppressive preparative regimen resulted in high engraftment rate. Survivors experienced a greater risk of extensive chronic GVHD (cGVHD) and multiple infections and viral reactivation post-transplant.

SESSION III. CORD BLOOD BANKING, MANIPULATION AND UNIT SELECTION

Dr. Eliane Gluckman discussed how to choose the best CB unit for unrelated CBT. It is important to consider whether the transplant is for an adult or child and one must also consider the diagnosis and stage of the patient's disease. It is critical to be objective when comparing with other sources of stem cells. The criteria of choice are nucleated cells, CD34+ cells, HLA matching at high or low resolution, and other factors. Eurocord recommendations (2007) are to first look at the number of cells that should be $\geq 3 \times 10^7$ NC/kg and/or $\geq 2 \times 10^5$ $CD34^+$/kg. Second, one should look at HLA matches: 0-1 mismatch is better than 2 (avoid 3-4 mismatches). Class I mismatches are preferable to class II mismatches. Increase the number of cells to partially overcome HLA mismatches. Then one should adopt to the graft indication: For malignant diseases the cell dose is the most important factor for outcome. A minimum of 3×10^7 NC/kg at collection or 2×10^7/kg at infusion must be obtained. HLA mismatches increase the risk of engraftment delays, TRM and cGVHD and decrease the risk of relapse resulting of an absence of the role of HLA mismatches for survival. The type of HLA mismatches did not influence outcomes and increasing cell dose abrogates the effect of HLA mismatches. For non-malignant diseases the requirements for cell dose and HLA matching are different than in malignant disorders. In non-malignant disease cell dose is higher at 4.9×10^7 at collection and 3.5×10^7at infusion; HLA mismatches play a major role for engraftment, GVHD, and survival, and are partially abrogated by increasing cell dose. The CB selection procedures from Dr. J.E. Wagner are quite similar and are as follows: Sort by HLA match showing only those CB units that are potentially 4 of 6 matches, then sort by cell dose within each HLA matched category, and then list only those units that have a cell dose $>1.5 \times 10^7$/kg. Use a

single if 6 of 6 unit is identified with a cell dose >3.0 $\times$ 10^7/kg, use a single if 5 of 6 unit is identified with a cell dose >4.0 $\times$ 10^7/kg, and use a double cord if not enough cells in a single cord, using units as closely matched as possible with a total cell dose >3.0 $\times$ 10^7/kg.

Dr. Juliet Barker's presentation was entitled: A "no wash" albumin-dextran dilution strategy for UCB thaw for adolescent and adult UCB transplant recipients: Superior to wash? Some studies suggest that washing the CB unit is not necessary in adults and that albumin dilution is a good alternative. However, some UCBT centers are resistant to change. The potential advantages of an albumin-dilution thaw strategy over washing are reduced UCB manipulation; it is faster; it is simpler; there is reduced cell loss (washing is responsible for half of post-thaw cell loss); and there is no risk of bag break in centrifuge. Advantages over bedside thaw are that it is performed in the controlled environment of a cytotherapy lab, it avoids prolonged exposure to high concentrations of DMSO at room temperature, it allows for immediately taking and distributing samples for $CD34^+$ analysis and research, and it results in the ability to obtain cell count and perform trypan blue testing prior to infusion. A prospective study was performed with the hypothesis that albumin dilution was comparable to washing in terms of post-thaw TNC/$CD34^+$ cell dose *and* engraftment. The study was initially restricted to RBC depleted units but was subsequently modified to include RBC containing units. Nineteen patients were studied, each of whom received a double CBT. Results indicated that the albumin-dilution thaw of UCB was comparable to wash with centrifugation for TNC yield, infusion reaction profile, and engraftment. It was faster, more efficient, reduces technologist time, speeds time to patient infusion, and reduces potential for cell loss. Therefore, is it superior to wash for adult UCBT? It should facilitate center-center standardization and is to be investigated in CIBMTR sponsored multi-center prospective study of adult double unit UCBT. Finally, reduced cell loss from albumin-dilution strategy makes taking of a small sample for research ethically acceptable. Unanswered questions are whether it should be utilized in smaller children (patients <40 kg were not tested). Also, the incidence of aGVHD seems to be a bit lower so that there is a possibility of qualitative effects on GVHD.

SESSION IV. CONDITIONING REGIMENS

Dr. Richard Champlin reviewed nonmyeloablative SCT for hematologic malignancies – considerations for CBTs. The fundamental hypothesis for nonmyeloablative SCT is to intentionally decrease the intensity of the preparative regimen to reduce toxicity, and allow GVL to eradicate the malignancy. Some regimens are truly nonmyeloablative and the patients are capable of repopulating their marrows without a transplant. Other regimens are RIC but are myeloablative and the patient requires a transplant. Patients receiving truly nonmyeloablative regimens have a lower rate of aGVHD and there is a trend toward a lower rate of cGVHD. The results of nonmyeloablative BMTs have indicated reduced toxicity and reduced GVHD; similar infections occur, but these are generally responsive to therapy. There is a lower rate of TRM and the use of such transplants can extend the use of HSCT to patients up to 75 years of age. Greater immune suppression is needed to reliably achieve engraftment in CBT. Regimens effective for matched sibling and matched unrelated transplants may not be sufficiently immunosuppressive for UCBT. In a Bu/Flu regimen, there was a 3% rejection rate for sibs/matched unrelated donor (MUD), but ~40% with UCBT. RIC effective in UCBT include Cy/Flu/TBI and Melphalan-thiotepa-Flu. There is a potential benefit of HLA mismatches in UCBT in that the frequent use of mismatched units provides a target for GVL and results in a lower relapse rate. Elderly patients with AML have a dismal prognosis, but a trial of reduced intensity BMT indicated no early TRM and a 60% survival at 60 months (patients must be in remission prior to transplant). This is in contrast to ~5% survival at two years for standard chemotherapy. Further studies of reduced intensity transplants in this group of patients are in progress. Another category of patients who have been helped by non-myeloablative conditioning regimes is indolent lymphoma. Using a conditioning regimen of Flu, CSa and rituximab for patients with follicular lymphoma (FL), 90% are alive at 90 days, which is much improved over the results using a myelablative regimen. Studies are also in progress for MCL and chronic lymphocytic leukemia (CLL). Certain diseases, such as chronic myelogenous leukemia (CML), (CLL) and LGL, are more sensitive to graft-versus-malignancy than others and these disorders are the prime candidates for reduced intensity transplants.

Dr. Claudio Brunstein discussed nonmyeloablative UCBT. The hypothesis underlying nonmyeloablative UCB transplantation is that despite the differences in UCB alloreactivity, UCB has enough immune cells to mediate engraftment after nonmyeloablative conditioning. There is a growing number of reports of nonmyeloablative UCB transplants, especially for adult patients. At the University of Minnesota, a study was performed using Cy/Flu/TBI for the conditioning regimen and CSa and MMF for post-transplant immunosuppression. ATG was added for patients who had not received chemotherapy within 3 months of transplantation. Patients receive a double UCB transplant if no single graft is big enough. HLA matching

is done at the antigen level for -A and -B loci and at the allele level for -DRB1. Using this study design about 80-85% of patients received two UCB units. The sustained donor engraftment rate for 161 patients was 87%. The incidence of grade II-IV aGVHD was 54% and Grade III-IV was 18%. The 6 month TRM was 18% and at 3 years it was 25%. The progression-free survival (PFS) for patients who received two units was 39% and for those receiving one unit was 24% (n = 110, $P = .05$). OS was 45% for those receiving either 1 or two units (n = 161). A further study was performed comparing the outcomes after reduced intensity transplantation using UCB versus sibling PBSC for de novo and secondary AML. Results indicated that the OS was not significantly different when comparing the two HSC sources. In summary, UCB is an effective alternative source of HSC for NMA transplantation. Patients who need nonmyeloablative transplant and who do not have a suitable sibling or unrelated donor should be considered for UCB transplantation. Larger numbers of patients will help further determine the role of UCB transplantation in older patients and those with pre-existing high risk clinical features.

Dr. Vanderson Rocha discussed a RIC regimen after single unrelated CBT for adults with hematological malignancies. Unrelated CBT using RIC regimen has been proposed in order to shorten the aplasia period and decrease TRM in adult patients with high risk of dying of toxicity. Since cell dose is a major limiting factor in UCBT, concerns have been raised in the use of CB cells in RIC. Also, another concern is the GVL effect from lower incidence of GVHD after UCBT. There are no data available in single RIC UCBT analyzing risk factors of outcomes. A retrospective study of 142 patients with hematologic malignancies was performed. The median age of patients was 44 years (range, 16-76). Twenty-three conditioning regimens and a number of regimens for GVHD prophylaxis were used in this registry based study. The median days to neutrophil recovery was 20 days (5-56) and for platelet recovery was 38 days (15-171). A multivariate analysis for neutrophil recovery indicated that conditioning with Flu+ENDX+TBI was a significant factor as was HLA 0-1 vs. 2-3-4. Other factors included in the model that were not significant were cell dose, status of the disease and female sex. aGVHD occurred in 29% and cGVHD in 40%. TRM at day 100 was 28% overall and was 14% with a cell dose of $>2.8 \times 10^7$/kg and 39% with a cell dose of $<2.8 \times 10^7$/kg. Multivariate analysis indicated that the conditioning regimen and cell dose were significant factors. Relapse at one year was 53% for patients with advanced disease and 36% for those with early or intermediate disease. OS was 43% and DFS was 32%. The DFS varied depending on the HLA match: 67% when patient and donor were HLA matched, 46% with 1 HLA disparity, 21% with 2 HLA disparities, and 12% with 3 HLA disparities. DFS was 42% when transplanted in remission and 23% if not in remission. With TBI+Flu+ENDX the DFS was 44% and with other conditioning regimens it was 23%. Conclusions reached in this retrospective based registry study and high risk group of patients were that the results of single RIC-UCBT are encouraging. Cell dose and HLA remain important factors in this setting. For this reason probably results can be improved with double cords and better HLA matching. Importantly, the type of conditioning (Flu+ENDX+TBI) is associated with decreased TRM and better DFS. Use of busulfan was associated with increased TRM. Immunosuppression after RIC-UCBT should be carefully evaluated in high risk patients of relapsing without GVHD.

Dr. Karen Ballen presented the results of a study of outcomes of adult patients after double CBT using an RIC regimen which consisted of Flu, melphalan and rabbit ATG. The minimum combined cell dose was 3.7×10^7NC/kg pre-freeze. GVHD prophylaxis in protocol #1 consisted of CSa and MMF (21 patients), and in protocol #2 it consisted of sirolimus and tacrolimus (22 patients with >100 days follow up). The median age for protocol #1 was 49 years (range 24-63) and, for protocol #2 was 53 years (19-64). All patients had hematologic malignancies. For the two protocols the median days to engraftment for ANC >500 were 20 or 21, respectively. Days to platelets >20K were 41 and 47 days; to platelets >100K were 65 and 125, respectively. There were two graft failures in protocol #1 in patients who had aplastic anemia (AA) and MDS and who had not received prior cytotoxic therapy. The aGVHD rate for grades II-IV were 40% and 14%, respectively for the two protocols and, for grades III-IV, the rate was identical at 5%. The cGVHD rate was 34% and 20%, respectively and the extensive cGVHD rate was 20% and 6%, respectively. For protocols #1 and #2, the one year OS was 67% and 73%, one year DFS was 67% and 51%, and relapse rate was 19% and 34%, respectively. The TRM was 14% for both protocols. Chimerism analysis indicated that a single CB donor predominated in 76% of patients by day +30. By day +90 one unit was detectable in 72%, both CB units in 18% and a single CB unit and host cells in 10%. Predictors of the predominant unit were (1) first unit infused, (2) higher CD34+ count and (3) higher NC/kg. A number of factors led to some patients who had an indication for a transplant but who did not receive a transplant: Six patients had no allele level match, two patients had no antigen level match, grafts for 3 patients had an inadequate cell dose, there were insurance issues for 2 patients, progressive disease in 4 patients, and another donor source in 9 patients. In conclusion, double CBT with this RIC regimen are well tolerated with TRM of 14%. Low relapse rate with low risk of GVHD sug-

gests preservation of GVL effect, and the risk of GVHD may be lower with the use of sirolimus/tacrolimus.

SESSION V. IMMUNE RECONSTITUTION AND INFECTIONS

Dr. Bruce Blazar reviewed the topic of infection and immune reconstitution after HSCT: Challenges and opportunities. Despite hematopoietic engraftment and recovery of marrow function, recipients of hematopoietic cell transplants have prolonged defects in generation of functional T and B lymphocytes. Chemoradiotherapy- and GVHD- induced epithelial cell injury introduces a portal for bacterial, fungal, and viral infections. Bacterial products result in macrophage activation and the release of pro-inflammatory cytokines which may contribute to or resemble GVHD. An innate and adaptive immune system response is needed to eliminate infectious pathogens. A major cause of post-BMT immune deficiency is the loss of thymopoietic capacity, and impaired T cell recovery as a result of factors such as age, radiation, or GVHD. Damage to thymic epithelial cells by pre-BMT conditioning impairs the ability of the thymus to generate mature T lymphocytes after BMT. Approaches to lessen the injury or hasten the repair of or replace thymic epithelial cell or stromal elements or their products represent a promising strategy to speed peripheral T cell reconstitution and function.

Dr. Paul Szabolcs presented data regarding a multivariate analysis of patient and graft specific factors among 330 recipients of unrelated cord blood transplant (CBT) to predict risk of death from opportunistic infections in the first 6 months after CBT. Cord blood is unique because of a high frequency of highly proliferative primitive stem cells paired with "naïve" lymphocytes. To determine the impact of patient and graft-specific factors on 6-month post-unrelated CBT OI-related mortality we reviewed all consecutive pediatric patients transplanted at Duke University Medical Center between June 1999 and Oct. 2005. Three hundred thirty (330) consecutive pediatric recipients of single unrelated CB grafts were identified. Those receiving a second transplant for primary graft failure were not analyzed. Two hundred twenty (220) of the 330 patients (67%) were alive at 6 months. Of the 110 children who died by 6 months, 64 patients (58%) were identified with OI (viral, fungal infections) implicated as a cause of death. The 46 patients who died prior to 6 months and for whom OI was not implicated as a cause of death were omitted from the study data set, resulting in 284 patients. Of these 284 patients, 200 patients (77%) were alive at 6 months and 64 (23%) died at or before 6 months with cause of death related to OI. Twenty two (22) patients died related to adenovirus infection and twelve (12) from CMV infection, rendering these two viruses the cause in >50% of all OI related deaths. A logistic regression model was used to investigate the impact of ten demographic and clinical characteristics on the risk of death from OI by 6 months post unrelatd CBT. These potential predictors were sex, race, age at unrelated CBT, CMV serology, HLA mismatches (2-3 vs $\leq$ 1), malignancy, TBI, total graft cell dose/kg, CD34+ graft cell dose/kg and CD3+ graft cell dose/kg. In univariate analyses, sex, race and TBI did not predict 6-month death from OI. Malignancy ($P = .07$) was marginally associated with a greater probability of 6-month death from OI. Malignancy without TBI was also associated with a marginally higher probability of 6-month death due to OI ($P = .04$). A significantly greater probability of 6-month OI-related death was associated with CMV positive serology ($P < .0001$), greater HLA mismatch ($P = .006$), and older age ($P = .0009$). Higher total graft cell dose ($P = .001$), $CD34^+$ cell dose ($P = .014$) and CD3+ cell dose (.014) were associated with lower probability of death from OI at 6 months. Multivariate analyses were performed: Model 1 included; CMV ($P = .0004$), HLA mismatch ($P = .042$) and age ($P = .03$). Model 2 included; CMV ($P < .0001$), HLA mismatch ($P = .005$) and malignancy without TBI ($P = .04$). Since total graft cell dose, CD34+ cell dose and CD3+ cell dose were highly correlated, each of these variables was introduced into models 1) and 2) separately. Total graft cell dose was the strongest predictor when cell dose variables were added to Models 1 ($P = .0097$) and 2 ($P = .004$). $CD34^+$ cell dose contributed less significantly to both models ($P = .02$ both models). CD3+ cell dose did not significantly contribute to Model 1 and was marginally significant in Model 2 ($P = .05$). The percent concordance among these models ranged from 71%-75%. Thus, 6-month death from OI after single cord unrelated CBT in children can be predicted by the following risk factors: older age, positive CMV serology, >1 HLA mismatch, malignancy without TBI, lower graft cell dose (total, CD34+ and CD3+). Sex, race, and TBI alone do not predict 6-month death from OI. In summary, opportunistic infections, (most being viral) are the most prevalent cause of death in the first 6 months after UCBT. This analysis of 330 consecutive patients demonstrates that patient and graft-specific factors have a significant impact on 6-month mortality from OI. Potential strategies to reduce OI related mortality are to increase engraftment with improved homing, an increased cell dose and/or ex-vivo expansion of stem/progenitor cells; enhance immune recovery by improving survival/homeostatic expansion of infused lymphoid cells, boosting thymic output, and reducing GVHD; and increase T cell dose/adoptive therapy by ex-vivo ex-

pansion of broad unprimed T cell repertoire and/or antiviral, antifungal CTL generation in vitro.

Dr. Krishna Komanduri presented data indicating that delayed immune recovery after UCBT is characterized by thymic regeneration failure. A prospective study of T cell immune reconstitution was performed. Samples were obtained from 32 patients (of 34 enrolled), median age: 33.5 years (7-57); 28 patients adults. The median follow-up was 221 days. Diagnoses were AML/MDS (16), CLL (2), CML (2), HD (8), and non-hodgkin lymphoma (NHL) (4). Patients were heavily pretreated. All but one patient received a myeloablative conditioning regimen. Primary immune recovery endpoints were immunophenotypic analysis of T cells, B cells, NK cells; assessment of functional T cell responses using cytokine flow cytometry (stimulation with superantigen and CMV antigens); assessment of thymic function (δTREC assay); and naïve and memory maturation phenotyping. Results indicated decreased to absent thymic function at baseline, no detectable thymic recovery in 24 of 26 subjects, only one subject with significant thymopoietic recovery, thymopoiesis reduced relative to other SCT recipients (despite lower age of CBT recipients, 33 vs. 50). Thus, thymic regeneration failure characterizes this group of cord blood SCT recipients. Improved CBT immune recovery can result from prospective studies characterizing the effects of age, stem cell dose, and HLA matching on T cell recovery; moving beyond "monofunctional" assays to examine functional T cell recovery; optimization of ex vivo expansion strategies to maximize the recovery of T cell progenitors (e.g., using NotchL); use of thymoprotective (KGF) and thymopoietic (GH, leuprolide, IL-7) agents; and infusing antigen-specific T cells in donor grafts.

Dr. Tomohiro Morio discussed a strategy to combat opportunistic infections after UCBT. Current data indicate that the risk of serious infection after pediatric UCBT is comparable to that with unmanipulated BM; there is a marked increased risk of EBV-related complications with the addition of ATG to a nonmyeloablative conditioning prior to URCBT; 24 of 40 adult patients who underwent CBT developed VZV reactivation at a median of 5 months after CBT; there is a high incidence of HHV6 infection (7 of 10) with a high viral load in CBSCT recipients; and positive CMV antigenemia was seen in 19 of 24 sero-positive adults patients at a median of 42 d after CBT. A PCR-based system for rapid and sensitive detection of multiple viruses detects more than 20 viruses in one run; it is rapid with less than 2 hours from extraction to data analysis, sensitive with a detection level of at least 10 copies/sample; and costs less than $25. Experience with the use of this system indicates that it provides a economical, rapid, and sensitive method for monitoring multiple pathogens; that scheduled monitoring would be beneficial for detection of virus infection at early stage; that infection with multiple pathogens was less in CBT-recipients than that in BMT/PBSCT recipients; and all the CBT-recipients with multiple virus infections showed profound immunodeficiency.

SESSION VI. NON-HEMATOPOETIC STEM CELLS

Dr. Mark Weiss presented a review lecture entitled, "Stem cells in the umbilical cord matrix: isolation and characterization." Non-hematopoietic cells in the umbilical cord or CB include mesenchymal stem cells (MSCs), USSCs, BioE's cells, and VSLE. From the umbilical vein one can obtain endothelial cells and MSCs. Wharton's Jelly contains umbilical cord matrix cells and perivascular cells. The amniotic fluid and the placenta and decidua also contain stem cells. The ISCT working group definition of MSCs is that they are plastic-adherent; their surface phenotype is CD34, CD45, HLA-DR negative, and CD29 (SH3) – CD105 (SH2), CD73 (SH4), HLA-class I positive; and they are multipotent, able to differentiate to bone, cartilage, adipose, and muscle. MSCs play a role in immune modulation, hematopoietic support (ex-vivo expansion of HSCs, enable engraftment of HSCs when co-grafted), and homing to pathology. Animal studies suggest that umbilical cord matrix cells may enable engraftment of HSCs and may have therapeutic effects in disorders including breast cancer, Parkinson's disease, global ischemia brain damage (stroke), retinal damage, and myocardial ischemia. The mechanism is not well-understood and may include immune modulation and growth factor/cytokine effects.

Dr. Mariusz Z. Ratajczak discussed the morphological and molecular characterization of a novel population of CXCR4+SSEA-4+Oct-4+ very small embryonic-like (VSEL) cells purified from human CB. Recently we identified in murine BM a homogenous population of rare (~0.01% of BMMNC) Sca-1^+ $CXCR4^+$ lin^- $CD45^-$ cells that express, by RQ-PCR and immuno-histochemistry, markers of pluripotent stem cells. They display several features typical for primary embryonic stem cells such as i) a large nuclei surrounded by a narrow rim of cytoplasm, and ii) open-type chromatin (euchromatin) that is typical for embryonic stem cells. These cells (~2-4 μm in diameter) were named very small embryonic-like (VSEL) stem cells. A new two step isolation procedure is used to purify a similar population of cells from human CB, which is based on isolation of CB mononuclear cells (CB MNC) by hypotonic lysis and multiparameter FACS sorting. In vitro cultures of CB-VSEL are able to grow neurospheres that gave rise to neuronal lineages (beta-III $tubulin^+$, $nestin^+$, $O4^+$, MBP^+, $GFAP^+$) and cardiomyocytes (beta-myosin heavy

chain$^+$, alpha-sarcomeric actin). Based on this we conclude that CB contains VSEL and that the majority of these CB VSEL are lost during routine procedures employed currently for banking of CB MNC. We conclude that CB tissue/organ regenerating potential may be much higher than initially postulated if the proper fraction of CB MNC cells enriched in VSEL is employed.

Dr. Mervin C Yoder discussed high proliferative potential endothelial progenitor cells found in UCB. UCB has been known to contain a population of circulating cells that serve as progenitors for the endothelial lineage (EPCs). We have determined that a rare population of cells in the umbilical cord blood emerges upon in vitro culture that displays clonal colony forming ability. These endothelial colony forming cells (ECFCs) are present in adult peripheral blood as well, though vastly enriched in term and preterm cord blood. ECFCs spontaneously form human blood vessels upon implantation into immunodeficient mice and participate as blood conduits. Recent studies indicate that ECFC can be derived from umbilical arterial and venous endothelial cells as well as endothelium from several other vascular sites. Thus, human CB ECFCs may be useful as a source of cells to regenerate human vasculature in vivo.

Tsuneo A. Takahashi discussed progress in regenerative medicine using CB: MSC isolation, and induction to bone and cartilage. MSC can be isolated from cord blood (CB-MSCs) and from chorionic villi of the placenta (PL-MSCs) using the explant culture method. They may differentiate into osteogenic, chondrogenic, and adipogenic cells. PL-MSCs were induced to chondrocytes in vitro and produced a large amount of extracellular matrix of cartilage in vivo. PL-MSCs could repair the cartilage defect in the knee of a nude rat. MSCs could also be isolated from fresh CB and these cells expanded well with differentiation ability to chondrocytes and osteocytes. Thus, both PL-MSCs and CB-MSCs are potential allogeneic stem cell sources for tissue engineering for bone and cartilage.

SESSION VII. THE GRAFT VERSUS TUMOR EFFECT

Dr. Michael Verneris discussed the impact of multiunit UCB transplantation on leukemia relapse. One of the most common complications of transplantation is leukemia relapse. Variables that impact relapse include patient characteristics, the conditioning regimen, HLA disparity, the type of graft, and graft manipulation. Cell dose has a significant impact on speed of neutrophil recovery, probability of engraftment, the risk of TRM, survival, the influence of HLA disparity, and access to transplantation. The rationale for the use of multi-unit CB transplants is the hypothesis that augmentation of the cell dose by the infusion of two partially HLA matched UCB units will speed hematopoietic recovery, reduce TRM, and improve survival. Early studies showed that the time to engraftment was shifted from 32 days to 23 days with the use of double CBTs vs. historical controls using a single UCB. The mechanism by which two units enhance engraftment could be the augmentation of stem cell dose although immunologic reactions. A study was performed of 126 patients with AML or ALL who received either a single or double UCBT using myeloablative conditioning. Seventy two patients received a single unit and 54 received a double UCBT. Patients received a double CBT if there was not a single unit available with an adequate cell dose. The total infused TNC was 3.2×10^7/kg for the single cord transplants and 3.5×10^7/kg for double CBTs (NS). Total CD34$^+$ was also similar but CD3$^+$ cell dose was larger for the double CBTs (0.9 vs 1.3×10^7/kg). Results indicated that the rates of sustained neutrophil engraftment and TRM were similar between single and double CBTs. However, the incidence of grade II-IV aGVHD was higher with double CBTs, although the incidence of grades III-IV aGVHD was not different. The incidence of cGVHD was low and there was no difference between the two groups. The incidence of relapse for patients in CR1 and CR2 was higher for single UCB recipients than for double and this was not influenced by the diagnosis (AML vs ALL). OS at 3 years was 72% for double CBTs and 47% for single ($P = .16$). Considering patients who survived for at least 9 months, all of the double UCBT patients were alive whereas there were 15 deaths among the single cord transplant patients ($P = .01$) and 13 of 15 events were from relapse. Conclusions are that double UCBT promotes engraftment achieving rates comparable to single UCBT with adequate cell doses; the risk of grades II-IV aGVHD is higher after double UCBT (although there is no difference in risk of grades III-IV aGVHD); and the risk of cGVHD is similar. There is a reduced risk of relapse associated with double UCBT and this was not driven by aGVHD. Patients in early disease status (CR1 and CR2) were the only ones to show benefit.

Dr. Jeffrey Miller discussed a novel triple UCBT strategy to promote natural killer (NK) cell immunotherapy using a fully ablative preparative regimen followed by a double UCBT in patients with refractory AML. The strategy was that appropriately chosen donors will enhance NK cell alloreactivity if they expand in vivo. The best strategy may be to combine adoptive transfer and in vivo expansion followed by HCT. A trial was conducted with 19 poor prognosis AML patients. These patients had primary refractory disease, relapsed disease not in CR after 1 or more cycles of standard re-induction therapy, secondary AML from MDS, relapsed AML $\geq$ 3 months after

HCT, and no active infections. Results indicated that killer cell immunoglobulin-like receptor (KIR) ligand mismatched donor correlated with achieving CR (5 of 19). CR patients had higher numbers of functional NK cells after haplo NK cell infusions. Triple UCB strategy was developed using UCB derived NK cell + double UCB transplant for patients with refractory AML. Results indicated 9 of 10 patients engrafted; all 9 achieved CR at day 14; 4 of 9 engrafted from the NK cell unit #1; 2 are alive and in CR at days +120 and +262; 3 had TRM without evidence of relapse and died on days −2, +62, and +67; and 5 relapses occurred (all were in CR at engraftment). In conclusion, activated NK cells may facilitate engraftment, strategies to better promote NK cells in vivo may improve clinical results, and the DCBT distant from the NK cell unit may not be needed. Better strategies to make primary AML targets susceptible to NK cell killing are being studied.

Dr. Hans G. Klingemann discussed ex-vivo expansion and mRNA transfection of CB derived natural killer cells. NK cells are a third subset of lymphocytes (in addition to T-and B cells). They are independent of V(D)J recombination and receptor, kill virus infected and transformed cells without priming, have an important regulatory function (INF- g and other cytokines), kill MHC class I negative cells, express inhibitory receptors specific for "self" MHC class I, and express many different activating receptors. CB is used because it is a universal source of NK-cells, it is rich in NK cell progenitors, and NK cells are believed to be more active than peripheral blood NK cells. A problem is that there are only a limited numbers in a given CB unit. A study was designed to develop an optimized expansion system for CB NK cells suitable for clinical application, to make expanded cord NK cells target specific by transfection of mRNA for a chimeric antigen receptor recognizing CD19. mRNA is advantageous compared to cDNA transfection in that there is no genomic integration (mRNA stays in cytosol), there is more efficient transfection, expression is highly uniform, expression can be controlled by changing amount of input mRNA, and cells can be efficiently and simultaneously loaded with several different transcripts. Results indicate augmented cytotoxicity of NK cells transfected with mRNA expressing scFV-CD19. This is an important step to make NK cells targeted to tumor cells.

SESSION VIII. GOVERNMENTAL AFFAIRS

Robert Baitty, representing the U.S. Department of Health and Human Services (HHS), Health Resources and Services Administration (HRSA), Division of Transplantation, Blood Stem Cell Transplantation Program, presented an update on the C.W. Bill Young Cell Transplantation Program (the Program) and the National Cord Blood Inventory. The aims of the Stem Cell Therapeutic and Research Act of 2005 are to increase the number of unrelated donor transplants, increase the public inventory of high quality CBUs from diverse populations and increase the number of CBUs available for research. The Act authorizes appropriations of $15 million/year for FY 2007-2010; funding is temporary until 150,000 CBUs are obtained. HRSA's implementation approach is guided by three principles: a single point of access for patients and physicians to all sources of blood stem cells, collection of high-quality diverse CBU expeditiously, and collection of complete data on clinical outcomes of transplants. The National Cord Blood Inventory (NCBI) will be funded through one-time, 10-year contracts awarded competitively with HRSA funds for ≤3 years; funded cord blood banks must participate in the Program for ≥10 years; and the NCBI CBUs must be available through the Program in perpetuity (or until they are no longer viable). Contracts for the first cohort of banks were awarded November 2, 2006, to six banks (New York Blood Center, Duke University, MD Anderson, Puget Sound Blood Center, University of Colorado, and StemCyte). About 10,500 CBU are to be collected with first year of funding for the first cohort and these are to be comprised of ~63% racial and ethnic minorities. Request for Proposals for a second cohort of banks was published April 12 with responses due May 15, 2007. HRSA will hold annual competitions for new cohorts of banks, as funding permits. Infrastructure contracts include a contract for the stem cell therapeutic outcomes database, which was awarded September 15, 2006, to the Center for International Blood and Marrow Transplant Research; contracts for an Office of Patient Advocacy/Single Point of Access, Bone Marrow Coordinating Center, and a Cord Blood Coordinating Center, which were awarded September 25, 2006, to the National Marrow Donor Program. An Advisory Council on Blood Stem Cell Transplantation is being established whose purpose is to consider and make recommendations to the Secretary of HHS on matters related to the Program. Ongoing work includes: complete the transition from the former National Bone Marrow Donor Registry structure to C.W. Bill Young Cell Transplantation Program, launch a web site for the Program (mid-Summer 2007), begin collection of blood stem cell transplant outcomes data (mid-Summer 2007), complete establishment of the Advisory Council and convene its first meeting, and future cycles of funding for NCBI collections. Further, implement the Related Cord Blood Donor Demonstration Project, revisit interim program requirements with Advisory Council and public input, and define targets for composition of NCBI and the size and composition of adult donor registry in consultation

with the Advisory Council. Finally, to encourage research in collaboration with other Federal agencies and the Advisory Council to improve transplant outcomes, refine approaches to CB transplants for adult patients, better define CBU characteristics required for good outcomes, improve reliability and comparability of measures used in CBU selection, and to collect and report data regarding new uses of stem cells from CBU and adult donors.

Dr. Dennis Confer discussed the development of the National Cord Blood Center. The contracts for the Single Point of Access/Office of Patient Advocacy (OPA/SPA) and the Cord Blood Coordinating Center were awarded to the National Marrow Donor Program (NMDP). The contract for the Stem Cell Therapeutic Outcome Database was awarded to the CIBMTR. The OPA/SPA provides a uniform, consolidated search report (all searches are submitted here), provides advocacy services for patients, implements a plan to increase transplants, and facilitates access to transplantation services for patients. The Cord Blood Coordinating Center facilitates transplants of cord blood from NCBI banks and from other participating banks, collects standardized CBU information from the banks and provides this to the OPA/SPA, and coordinates search and distribution of CBUs. The Cord Blood Coordinating Center will also develop an interbank technical proficiency program, support public and professional education and recruitment activities, collaborate with CBBs to make CBUs available for research, improve operating efficiencies in collaboration with the network, support contingency planning for emergencies, and require transplant centers to submit outcomes data to the SCTOD. The Center for International Blood and Marrow Transplant Research (CIBMTR) represents an affiliation between the research programs of former IBMTR/ABMTR at the Medical College of Wisconsin and NMDP to support clinical research in hematopoietic cell transplantation and related fields. The CIBMTR as the SCTOD contractor must establish electronic data capture systems, collect data on all allogeneic transplants with a recipient or donor in the U.S, collect data on other cellular therapies, disseminate data within the program, make transplant data publicly available, perform analyses of optimal size for the adult donor registry and cord blood unit inventory, and conduct and support research. In summary, the C.W. Bill Young Transplantation Program provides a blueprint for organizing and managing of unrelated donor hematopoietic cell transplantation, and establishes a clear emphasis on Cord Blood as an important source of hematopoietic and non-hematopoietic cells for treatment of disease.

Dr. Ellen Lazarus, representing the FDA, Office of Cellular, Tissue and Gene Therapies, Division of Human Tissues, presented an update on FDA Regulatory Activities for Cord Blood. A draft guidance was published January 16, 2007, and represents FDA's current thinking but does not establish legally enforceable responsibilities. Its purpose is to recommend ways for cord blood banks to apply for licensure for specified indications, to explain applicable regulations in Title 21 of the Code of Federal Regulations, and to provide other information about the manufacture of human cord blood progenitor cells (HPC-C) and how to comply with the applicable regulatory requirements. The Guidance may be used when applying for a biologics license. The FDA will review the application, schedule a prelicense inspection as soon as possible after receiving the complete application, and if the application is not complete, will identify and advise the establishment of additional information that they will need to submit. The Draft Guidance Sections include factors of significance include HPC-C description and characterization, manufacturer information, methods of manufacturing, and other information. Applicable regulations and post-marketing activities include applicable CGTPs and CGMPs and clinical outcome data collection. An Advisory Committee meeting was held on 4/30/07, at which CB issues under consideration were addressed. The next steps area will be reviewing comments to the docket and recommendations of the Advisory Committee, and finalization of the Guidance. License applications will be accepted at any time.