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Eighth Annual International Umbilical Cord Blood Transplantation Symposium, San Francisco, California, June 3-5, 2010

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The 39-member faculty for the Symposium included leaders of the major transplantation centers from the United States, Spain, Germany, Taiwan, France, and the Netherlands. Attendees were from Argentina, Australia, Austria, Bolivia, Brazil, Canada, Chile, Colombia, Denmark, Ecuador, Finland, France, Germany, Greece, Hong Kong, India, Israel, Italy, Japan, Korea, Malaysia, Mexico, New Zealand, Panama, Poland, Portugal, Qatar, Saudi Arabia, Singapore, Spain, Sweden, Switzerland, Taiwan, the Netherlands, United Kingdom, United Arab Emirates, and the United States.

The program was divided into 9 sessions. The sessions on the first day concerned *Cord Blood and Regenerative Medicine*, and the subsequent 2 days were devoted to *Cord Blood and Hematopoietic Stem Cell Transplantation*. Sessions were as follows: (I) Basic Science and Preclinical Studies, (II) Emerging Uses of Cord Blood in Hematology and Regenerative Medicine, (III) Clinical Results: Comparisons of CBT with Related and Unrelated Donor Transplants, (IV) Indications and Conditioning Regimens for HSCT, (V) New Concepts in Donor-Recipient Matching for CB Transplants, (VI) Engraftment of Cord Blood Stem Cells, (VII) Infections, Immune Reconstitution and Adoptive Immunotherapy after Cord Blood Transplantation, (VIII) HSCT for Sickle Cell Disease, Thalassemia and HIV-Infected Patients, (IX) Cord Blood Banking.

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CORD BLOOD (CB) AND REGENERATIVE MEDICINE

Session IA. Basic Science and Preclinical Studies

Dr. Hal Broxmeyer delivered the Keynote address: "Cryopreserved cord blood for hematopoietic stem cells, IPS cells, and other nonhematopoietic cells, their regulation, and potential for regenerative medicine." He pointed out that a study in 2003 demonstrated high-efficiency recovery of functional hematopoietic progenitor and stem cells from human CB cryopreserved for 15 years. After thawing, there was >80% recovery of hematopoietic progenitor cells, and their proliferative capacity was intact. A more recent study after cryopreservation for up to 24 years indicates high-efficiency recovery of colony forming unit-granulocyte macrophage (CFU-GM) and colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM). Also, $CD34^+$ cells manifested primary and secondary engraftment of nonobese diabetic/severe combined immunodeficiency (NOD/SCID) IL-2 Ry^{null} mice. Recent studies by a number of investigators have demonstrated that induced pluripotent stem (iPS) cells can be generated from CB. Preclinical studies using a mouse hindlimb ischemia model focused on a role for myeloid

macrophage lineage cells with a myeloid-derived suppressor (MDSC) phenotype of $Gr1^{+}CD11b^{+}$cells, because these cells have been reported to regulate tumorigenesis through induction of angiogenesis. Data derived from studies using this model indicate that $Gr1^{dim}CD11b^{+}$ cells within skeletal muscle are increased in the context of ischemia. Further, that astrocyte-plexus was formed from muscle-derived $Gr1^{dim}CD11b^{+}$cells in Matrigel culture, followed by formation of isolectin and von Willebrand Factor (vWF)-expressing cells, similar to that reported for angiogenesis in the retina. Finally, injection of these cells into ischemic muscle was associated with significantly enhanced recovery of blood flow, and these cells incorporated into vessel cells. However, it remains to be determined whether $Gr1^{dim}CD11b^{+}$–like cells can be found in or generated from CB or other human tissues, and whether they can be used for human regenerative medicine.

Other studies relate to the enhancement of the effectiveness of HSC transplantation. It has been shown that inhibition of CD26 in human CB $CD34^{+}$ cells enhances their engraftment of NOD/SCID mice. In adult patients with malignancy, CD26/DDPOV inhibition was used to enhance engraftment to single CB unit transplantation. Further studies demonstrated that overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation. Overall conclusions regarding the experiments with Rheb2 led to the identification of a pathway that was important in expansion of immature progenitor/stem cells in vitro and in vivo; however, this expansion is accompanied by a loss of activity. Thus, means of regulating the activity of the Rheb-mTOR pathway may be effective at expansion without loss of repopulating ability. Finally, recent studies indicate that up-regulation of nascent mitochondrial biogenesis in mouse hematopoietic stem cells parallels up-regulation of CD34 and loss of pluripotency, which suggests a potential strategy for reducing oxidative risk in stem cells.

Dr. Peter Wernet reported on the production and uses of unrestricted somatic stem cells (USSC) from human CB. He pointed out the proof of the biologic existence of USSC at the clonal level and their in vitro differentiation potential into 3 germ layers. The cells have a normal karyotype and can be separated from mesenchymal stem cells (MSC). USSC lack expression of the pluripotency markers Oct4 and NANOG, which are found in MSC. Osteo-, chondro-, and fat-cell differentiation of USSC can be demonstrated in vitro and in vivo. It is clear that these cells are an early mesodermal cell type. A preimmune fetal sheep model was used to demonstrate the contribution to organogenesis of USSC. Single-cell polymerase chain reaction (PCR) analyses excluded the contribution of cell fusion to formation of the human liver parenchyma cells in this model. Also demonstrated in this model was the in vivo differentiation of USSCs into cardomyocytes and Purkinje cells. Using a pig model of acute myocardial infarction, functional improvement and prevention of scar formation was demonstrated after transplant of USSC. The function of USSC may not be the cell repopulation effect, but it may be an effect that triggers the autonomous regenerative capacity of an organ. Also, USSCs from human umbilical CB can be differentiated into neurons with a dopaminergic phenotype.

Dr. Paul Sanberg indicated that bone marrow (BM) and CB stem cells can be transformed into cells with neural markers and glial markers. Major neurologic diseases in which treatment using cell therapy has been studied include Parkinson's disease, stroke, Huntington's disease, traumatic brain injury, spinal cord injury, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease. CB has advantages over BM stem cells, including that they are easier to obtain with no harm to mother or infant. About one-third of patients with stroke have a chronic deficit, and animal models of stroke have been developed. In a rat model, cells were injected intravenously, and functional improvement was demonstrated in an acute stroke model. Some cells were found in the brain but not enough were demonstrated to indicate that cell replacement provided the therapeutic benefit. The optimal cell dose is difficult to determine as is the optimal time poststroke. The most significant effect occurred at 48 hours poststroke. In addition to stem cells, monocytes may significantly contribute to neovascularization in heart and other ischemic diseases. Removal of monocytes inhibited the effectiveness of CB in treatment of stroke. Cerebral palsy is a brain injury in children that develops for various reasons. A cerebral palsy model has been developed in addition to the human studies currently in progress.

Session IB. Basic Science and Preclinical Studies

Dr. Juan Carlos Izpisúa Belmonte discussed "iPS cells from cord blood." A number of important steps must be accomplished prior to use of iPS cell therapy in humans. Issues include the derivation of human iPS cells (genetic correction, safe protocols, efficient protocols, clinical-grade lines), specific differentiation protocols (differentiation ability of iPS cells, directed differentiation protocols), tissue engineering and animal models, and clinical-grade protocols and manufacturing steps to develop a stem cell product. This is a long journey, and we are at the beginning of that journey. iPS cells, when injected into mice, produce tumors. Reprogramming can be accomplished with numerous cell types, although a number of investigators have pointed out that CB stem cells offer advantages over other types of cells. iPS cells are now

readily obtained from CB. About 30 CB iPS cell lines have been generated under registration at Stem Cell Bank, Barcelona. Further investigations have led to the conclusions that (1) CB cells are readily available, are young cells (minimizing the risk of having accumulated mutations), and are already banked along with immunologic information; (2) CB $CD133^+$ cells can be reprogrammed with only 2 factors (Sox2 and Oct4); (3) CB cells are properly reprogrammed into pluripotent stem cells from both the expression and the epigenetic point of view; (4) CB iPS can be differentiated into cardiomyocytes, dopaminergic neurons, and hematopoietic $CD34^+$ cells; and (5) CB may constitute healthy, off-the shelf, immunologically compatible cells to establish iPS cell lines for future therapy.

Dr. Linzhao Cheng discussed the derivation and differentiation potential of human iPS cells from CB. Human embryonic stem (ES) cells provide an unlimited source of human cells, have the potential to form any cell types, provide a possibility for gene targeting, and create genetic models from human developmental biology or human diseases. However, there are ethical issues with human embryos, and there are only a limited number of lines and they are allogeneic. Human iPS cell lines are highly similar to human ES cells. Human blood-derived iPS cells can differentiate into various cell types as is true with human ES cells: (1) they can generate CFU/(erythroid burst-forming units) BFU-E hematopoietic progenitor cells as well as more mature neutrophils, monocytes/macrophages, and nucleated erythroid cells; (2) they can generate multiple types of vascular cells such as endothelial cells and smooth muscle cells; (3) they can be expanded under a xeno-free culture condition and differentiate into neural stem cells and dopaminergic neurons; and (4) they can differentiate into cardiomyocytes and hepatocytes. A goal is to develop advanced gene and cell therapy (AGCT). Gene correction of recessive mutations in human iPS cell lines requires the following: the use of existing lentivector (clinical grade) expressing an extra copy of the *CYBB* gene that is inserted randomly, allowing clonal analysis from iPS all the way to neutrophils; targeted gene correction, by either correcting the mutation in situ or by inserting a minigene downstream of the ATG start codon; targeted gene addition of a *CYBB* minigene at a nonpathogenic "safe-harbor" locus, if it is much more efficient.

Dr. Mariusz Z Ratajczak discussed CB-derived very small embryonic-like stem cells. BM is the "home" not only of hematopoietic stem cells but data indicate that a population of germ line/epiblast-derived pluripotent very small embryonic-like (VSEL) stem cells reside in BM as well as in other organs.

VSEL stem cells could be released/mobilized from neonatal BM (and other niches?) and circulate in neonatal peripheral blood (CB). As in murine BM-derived VSELs, human umbilical CB (UCB)-derived CD45-negative VSELs could correspond to a population of the most primitive, long-term repopulating HSC (LT-HSC). Of note, currently employed routine UCB processing strategies may lead to an unwanted loss of up to ~50% of these small cells that are endowed with such remarkable hematopoietic activity. The neural and cardiac differentiation of VSELs is currently being explored.

Session IIA. Emerging Uses of CB in Hematology and Regenerative Medicine

Dr. Joanne Kurtzberg discussed the status of a clinical trial of autologous CB stem cells for the treatment of cerebral palsy (CP) and other acquired brain injuries. Autologous unrelated cord blood transplantation (UCBT) is being used in experimental protocols to treat CP, hypoxic ischemic encephalopathy (HIE), congenital hydrocephalus, and other brain injuries. Animal models have demonstrated improvement in neurologic status after infusions. To date, 198 infusions have been done in 184 patients: 76% for cerebral palsy, 12% for congenital hydrocephalus, and 12% for other injuries. CBs accepted for infusions have a total nucleated count (TNC) of $\geq 1 \times 10^7$ cells/kg, have negative bacterial cultures and maternal infectious disease markers, and confirmed identity (HLA). The infusions have been well tolerated, and no clinically apparent infections have been noted. Efficacy has been very hard to determine because of placebo effects. There are many positive anecdotes, leading to impressions of benefit in babies with severe HIE and congenital hydrocephalus. Also, there are impressions of benefit in children with mild to moderate CP. A randomized trial is needed to answer the question of benefit, and such a trial is being developed; outcomes can be expected in about 2.5 years. Interim conclusions are: (1) infusion of autologous UCB is safe and feasible in young children with acquired neurological disorders; (2) quality parameters of CB units (CBUs) stored at private banks are inferior to those stored at public banks (at present, only 14% of private units would qualify for listing as a high-quality unit in the national CB bank Inventory [NCBI])' and (3) if autologous UCB infusion is determined to be clinically efficacious, the quality of privately banked CBUs should be held to the same standards as publicly banked units.

Dr. Wise Young discussed progress in development of treatment of spinal cord injuries (SCIs) with CB stem cells. SCI usually involves a contusion of the spinal cord; relatively few SCI have penetrating wounds. An animal model has been developed using a weight dropped on the spinal cord from a defined height. Regenerative therapy for SCI involves bridging the gap with a loose matrix of glial cells and inflammatory cells to fill the injury site. Many axons grow into the site but do not grow out the other side. Axons grow

very slowly (<1 mm/day). Sustained growth factor support is necessary for long tract regeneration. Blocking growth inhibitors is also necessary and can be accomplished with antibodies and chondroitinase. Treatment of SCI by intravenous infusion of human umbilical cord blood mononuclear cells (UCBMC) has been shown to improve locomotor recovery after clip compression of the rat spinal cord and contused rat cords. Intraspinal UCBMC improves locomotor recovery in hemisected rats and the contused rat spinal cord. A large study network has been developed in China, the "China SCI Network." Several trials are planned: an observational trial with 500 subjects with chronic SCI; a phase 1 lithium trial with 20 subjects with chronic SCI; a phase 2 oral lithium trial (in China) with 40 subjects randomized to lithium or placebo; a phase 2 mononuclear cell transplant trial with 20 subjects; and a phase 3 UCBM cell transplants ± lithium with 400 subjects with chronic SCI. Trials of intradural decompression in over 700 patients with subacute spinal cord injury have shown beneficial results. A multicenter phase 3 trial is planned to compare laminectomy alone against laminectomy and intradural decompression. If shown to be beneficial, this procedure provides an unprecedented opportunity for surgically controlled trials of cell transplants in spinal cord injury. In conclusion, UCBMC and lithium are beneficial in animal SCI models; lithium strongly stimulates UCBMC to proliferate and produce neurotrophin in vitro and in vivo; lithium also stimulates neural stem cells to produce neurons, resulting in more gray matter in brains. Protocols are being developed to test UCBMC and lithium treatment of human chronic spinal cord injury in China and the United States.

Dr. Shinn-Zong Lin discussed a clinical trial of the treatment of stroke with CB stem cells. A human clinical trial on chronic stroke is in progress in Taiwan utilizing subcutaneous granulocyte-colony stimulating factor (G-CSF) injection combined with intracerebral mobilized peripheral blood hematopoietic stem cells ($CD34^+$) transplantation. Subjects are those with middle cerebral artery ischemia with a measurable deficit; subjects must be 35 to 75 years old and have a modified Rankin scale ≥1. Data thus far indicate that there is no remarkable movement of $CD34^+$ stem cells in the brain after implantation. There is regeneration of the corticospinal tracts, and the motor evoked potential of regenerated nerves is good. There is improvement in body motor function recovery after transplantation.

Session IIB. Emerging Uses of CB in Hematology and Regenertive Medicine

Dr. Graça Almeida-Porada discussed the unique characteristics of CB stem cells for tissue repair. The ultimate goal of stem cell therapy is to restore normal function of a tissue or organ through the production of normal cells and/or through the delivery of factors that decrease inflammation/apoptosis, induction of regeneration, or immunomodulation. The ideal cell for cell therapies should be readily available, autologous, or nonimmunogenic, have appropriate differentiative potential, have preferential homing/engraftment to the target, and must be nontumorigenic. A preimmune model to assay human HSC has been developed in sheep and has been used for evaluation of CB-derived HSC for liver regeneration. Human cells are transplanted into 45- to 65-day sheep fetuses. Upon in utero transplantation, endothelial progenitor cells (EPCs) engraft into the liver (0.12%). Donor-derived cells preferentially engraft in and around vasculature, and engrafted cells actively contribute to the liver cytoarchitecture through formation of tight junctions, expression of the hepatocyte marker albumin, production of FVIII, and differentiation into Ov6+ and AFP+ liver cells. Additional results indicate that EPCs engraft in and around the crypts of Lieberkühn (CLR); 23.5% ± 1.7% of the CLR cells are human; EPCs differentiate into mature intestinal cells at low percentages (8.1% ± 1.0% of the interstitial cell population) and contribute (<1%) to the enteroendocrine population. Conclusions are that the unique characteristics and broad potential of CB-derived stem cells suggest that they may be ideally suited for tissue repair. CB-derived EPCs constitute a novel and promising therapy for replenishment of liver and BM vasculature; EPCs contribute to the intestinal stem cell population and therefore represent a novel therapy option for intestinal disorders and, when transplanted individually to correct a singular deficit, or in association with other cells, CB-derived stem cells may be able to correct more complex diseases involving multiple cell types.

Dr. Mitchell S. Cairo discussed CB stem cells for the treatment of epidermolysis bullosa (EB). Heritable forms of EB are a group of mechano-bullous disorders with skin blistering as a unifying diagnostic feature. The estimated incidence is ~1:20,000, there is no ethnic or racial predilection, inheritance is either autosomal dominant or recessive, and the clinical severity is highly variable. The rationale for cell-based therapies is that the gene of interest is already present in the genome of a healthy donor, and there is an ability to infuse large numbers of stem cells for long-term engraftment and repopulation and homing to the skin. This takes advantage of natural properties of adult stem cells to circulate and home to sites of tissue damage and to undergo repair of the skin, gastrointestinal (GI) tract, and esophagus. Advantages of CB as a stem cell source are that there is no risk to the donor, no donor attrition, immaturity of T cell immunity, a lower incidence and severity of graft-versus-host disease (GVHD), and longer telomere length and higher proliferation potential. BM transplantation in an animal model has

been shown to provide improvement of the recessive dystrophic (RD)EB phenotype. An hypothesis is that allogeneic hematopoietic stem cell transplantation (HSCT) using reduced-intensity conditioning (RIC) and family-related donors and unrelated CB donors will be safe and well tolerated in selected patients with RDEB. The primary objectives of a study using allogeneic HSCT are to determine the event-free survival (EFS) and overall survival (OS) following RIC consisting of busulfan/fludarabine/alemtuzumab in selected patients with RDEB.

Dr. Tsuneo A. Takahashi discussed, "Reevaluation of factors leading to the successful isolation of MSC from human CB and their differentiation to mesenchymal lineages." The objective of this study is to determine whether CB-MSC are a potential stem cell source for clinical use. The study will determine the critical factors regarding successful isolation of CB-MSC, characterize the high differentiation capability of the cells to chondrocyte and osteocytes, review their immunosuppressive activity, seek to develop large-scale expansion and production of clinical-grade CB-MSC, and determine the difference between USSC and CB-MSC. Results thus far indicate that, among several possible factors for a successful isolation of CB-MSC, the time between collection and processing was a decisive factor, and volume was also a critical factor. Even though the frequency of CB-MSC was lower initially than BM-MSC, the high proliferation rate of these cells should allow expansion appropriate for clinical use. The high proliferation rate combined with high differentiation capability to chondrocyte and osteoblast, immunosuppresive activity, and the karyotype stability after long culture indicate that CB-MSC should be a potential cell resource for cell therapy and regenerative medicine.

CB AND HSCT

Session III. Clinical Results: Comparisons of CBT with Related and Unrelated Donor Transplants

Dr. Joanne Kurtzberg presented: "A review of UCB—Where do we stand?" The first CBT was performed in France in 1988, and the success of this transplant opened the whole field of CBT. Related transplants were the first to be performed, and the first unrelated transplant was performed in 1993 at Duke University. The first CB bank was established at the New York Blood Center in 1992, and at the present time, more than 20,000 CB transplants have been performed. There are more than 140 CB banks worldwide and ~140,000 cryopreserved units are available in the United States and >500,000 worldwide. Early success include the demonstration of less GVHD than with matched related or unrelated donor transplantation, less stringent HLA matching requirements, increased access to HSCT for minorities, the establishment of public banking, and the application to genetic diseases. Challenges and obstacles include delays in engraftment and graft failure, adequate cell dosing, immune reconstitution, cost, and funding for public banking. Potential solutions to some of these problems include the use of double CBT and/or cell expansion to overcome inadequate cell dose.

Recently, there has been increased emphasis on maximizing efficiencies of CB manufacturing with the goal of producing a high-quality, high-potency unit. There is increasing emphasis on the end user of the CB who receives, stores, thaws, and infuses the product to the patient. Current CBU potency criteria utilize precryopreservation TNC dose combined with HLA matching. CFUs provide a more accurate indication of engraftment probability but the assays are difficult to standardize among laboratories. However, assay of ALDH bright cells correlates with results of CFUs and may provide a more reproducible assay. A study is underway to attempt to correlate available precryopreservation and postthaw data, including TNC, total mononuclear cell count, $CD34^+$, and total CFU, and to establish a score ("Cord Blood Apgar") to use to predict engraftment. Retrospective data show an excellent correlation of the precryopreservation score and engraftment. Future applications of CB stem cells include extended usage for sickle cell disease, thalassemia, and autoimmune diseases. Also, regenerative medicine applications are possible as are cellular therapies using tissue-specific cells (eg, neural, pancreatic, endothelial, cardiac). We need to recognize that we still do not know how to best select CB units for transplantation. More research is necessary to determine whether double cord transplants are better than single CB transplants and whether we can augment immune reconstitution after CBT. We would like to have improved strategies for engraftment, immune reconstitution, sickle cell disease, and reduced-intensity transplants and hope to be successful with novel applications as for stroke, spinal cord injury, cerebral palsy, neonatal brain injury, and type I diabetes.

Dr. Colleen Delaney discussed, "Reduced relapse and similar progression-free survival (PFS) after double-unit cord blood (DUCB) transplantation: comparison of outcomes between sibling, unrelated adult, and unrelated DUCB hematopoietic stem cell donors."

Cell dose remains the main limitation in offering UCB transplantation to larger adolescent and adult patients. To overcome the cell-dose limitation of a single-unit UCB unit transplant, double CB transplants have now been adopted by many transplant centers. A study has been performed to test the hypothesis that recipients of double UCB grafts, that otherwise would have been denied transplant because of inadequate cell dose, would have outcomes similar to those

of adult stem cell sources. The primary objective of this 2-center retrospective analysis was to compare the leukemia-free survival (LFS) of recipients of double UCB to those of sibling and unrelated adult volunteer donor grafts. Diagnoses were acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS). A total of 536 patients were studied: 204 = matched sibling; 152 = 8/8 allele matched unrelated donor (URD); 52 = 7/8 allele matched URD; and 128 = 4-6/6 HLA matched double UCB. Days to neutrophil engraftment for sibling donors was 16 days, for MUDs was 19 days, and for DUCB was 26 days. Platelet engraftment was also delayed, and <50% of patients had a platelet count ≥50,000/μL by day 100. The incidence of grade III-IV acute GVHD (aGVHD) was lowest in sibling and DUCB transplants; chronic GVHD (cGVHD) was also lowest in DUCB transplants. Nonrelapse mortality (NRM) was highest in DUCB. Relapse was significantly lower in DUCB compared with other donor sources (as has been demonstrated in other studies as well). There was no significant difference in the incidence of LFS among the various donor sources. In summary, the data from this study indicate similar LFS with DUCB, sibling, and unrelated adult donors after myeloablation with a cyclophosphamide/total-body irridiation (Cy/TBI)-based conditioning. There is a need to assess the quality of life long term between donor types, as there is less risk of aGVHD and cGVHD after DUCB transplantation compared to unrelated donors.

Dr. Doris Ponce discussed data indicating that progression free survival (PFS) after cord blood transplantation (CBT) is not different to that after related or unrelated donor transplantation in patients with hematologic malignancies. A study was performed comparing the 2-year PFS after related donor, unrelated donor, and DUCB in patients transplanted over the same time period. A total of 367 patients were included in the study: 108 related donor transplants, 184 unrelated donor transplants, and 75 DUCB transplants. The incidence of acute leukemia was similar among the groups, but there was less MDS/CML and more lymphoid malignancies in DUCB transplants compared to the other donor sources. Neutrophil recovery after DUCB transplants was delayed after ablative regimens but not after nonmyeloablative regimens; the speed of neutrophil recovery was similar in all groups after nonmyeloablative conditioning. The incidence of aGVHD after DUCB transplants was the same as unmodified related donor and unrelated donor transplants. Late aGVHD and cGVHD at 1 year was the same with DUCB transplants as with unmodified related donor transplants and was lower than with unmodified unrelated donor transplants. Overall survival after 2 years after DUCB transplants was not different from other donor sources. Conclusions were that early mortality after DUCB transplants was compensated for by reduced late mortality risk so that there was no difference in 2-year PFS after DUCB transplants compared to related donor or unrelated donor transplants. Thus, DUCB transplants provide a promising alternative despite marked HLA mismatch and relatively low cell dose/unit. These data are consistent with those of the University of Minnesota/Fred Hutchinson Cancer Research Center study and Eurocord-Netcord/EBMT studies.

Dr. Vanderson Rocha discussed a comparison of outcomes after unrelated CBT and matched unrelated donor reduced intensity conditioning (RIC) transplantation for lymphoid malignancies. A study has been carried out of patients with malignant lymphoma (both Hodgkin and non-Hodgkin) and chronic lymphocytic leukemia (CLL). A total of 104 patients from 48 centers were included in the study. Eight-five percent had an advanced phase of their disease. A total of 78 patients received a single CBT, and 26 received a double CBT. RIC was used for 57% of the single-unit transplants and 88% of the double CBTs. Engraftment was 85% with CBT and 97% with peripheral blood donors. PFS for mantle cell lymphoma was 75% at 18 months; for indolent non-Hodgkin lymphoma was 60% at 18 months; for Hodgkin disease was 30% at 18 months; and for aggressive non-Hodgkin lymphoma was 25% at 18 months. Conclusions of the study were that UCB transplantation is a valuable alternative for patients with advanced lymphoma and CLL. The most important prognostic factors were: (1) indolent lymphoma; (2) chemosensitive disease; (3) use of low-dose TBI in the conditioning regimen; and (4) UCB units with higher cell doses.

An additional study compared outcomes after unrelated CBT and matched unrelated donor RIC transplantation for lymphoid malignancies. Diagnoses of the patients included indolent NHL, Hodgkin lymphoma, aggressive NHL, mantle cell lymphoma, and CLL. MUD peripheral blood stem cell (PBSC) was used for 284 patients, single CBT for 44 patients, and double CBT for 31 patients. Conclusions of the study were that there were no differences between UCBT and MUD-PBSCT in: (1) NRM; (2) relapse or progression; (3) PFS; or (4) OS. Further conclusions were that UCBTs had delayed neutrophil recovery and a lower incidence of cGVHD.

Session IV. Indications and Conditioning Regimens for HSCT

Dr. Daniel Weisdorf reviewed current indications for hematopoietic cell transplants in adults with hematologic malignancies and the role of CBT. Experience in CBT indicates that in adults graft failure, which occurs in 10%, is still limiting. In CBT, moderate to

severe aGVHD does occur, but grade III/IV aGVHD is uncommon. There is less cGVHD, and it is more responsive to therapy. Earlier discontinuation of immunosuppression is feasible, and there is a lesser need for medical interventions from day 100 to 1 year. Costs are higher with CBT than with matched unrelated donors because of graft failures and costly supportive care. UCB transplants provide graft-versus-leukemia (GVL) effect as evidenced by the fact that the relapse rate is the same with single UCB as with BMT or PBSC transplants. With CB transplants, the GVL is not tied to GVHD. There is less relapse with double UCB compared with matched sibling, matched unrelated donor, and mismatched unrelated donor transplants ($P < .01$). Disease-free survival (DFS) is the same with these various donor sources for HSC ($P = .19$). KIR B/x genotype donors confer improved relapse-free survival in AML unrelated donor HSCT. Studies should take place regarding UCB selection, including permissive HLA mismatches, the significance of KIR genotype and noninherited maternal antigens, and the HSC content and functional capacity of CB units. Other studies should address improving UCB engraftment by improving homing and adhesion to the HSC niche or by ex vivo expansion of HSC or committed progenitors. Improvements should be made in specialized supportive care for UCB HCT to address the question of more prolonged or different antibiotic therapy for infections, and smarter and more economical transfusion support. Important studies are needed to compare haploidentical mismatched transplants with URD transplants, and to find ways to reduce morbidity (infections, GVHD, transfusions, duration of specialized HCT care, and quality of life).

Dr. Vanderson Rocha discussed CBT using myeloablative and RIC regimens. The ideal preparative regimen for marrow transplantation of patients with malignant diseases should be capable of eradicating malignancy, have tolerable morbidity without mortality, and have sufficient immunosuppressive effect to avoid graft rejection. No ideal preparative regimen currently exists. There is a continuum between fully nonmyeloablative and RIC regimens. A risk factor analysis has been carried out regarding neutrophil recovery and mortality after single-unit UCBT using myeloablative conditioning regimen for patients with malignant disorders. The patients (n = 1946) were transplanted with a single-unit UCBT from 1994 to 2009 for malignant disorders after a myeloablative conditioning regimen. Multivariate analysis for neutrophil recovery indicated that significant factors were the number of $CD34^+$ cells, HLA compatibility, use of fludarabine, use of prophylactic HGF, and remission status of the disease. For platelet recovery, significant factors were the number of $CD34^+$ cells, use of fludarabine, use of prophylactic HGF, and transplant year >2004. A study of RIC regimen after single unrelated CBT for adults with hematologic malignancies (n = 176) indicated that significant factors were the type of conditioning (FLU + EDX + TBI) (hazard ratio [HR] = 0.53; $P < .001$) and early and intermediate phase of the disease (HR = 0.63; $P = .02$). A study was carried out comparing RIC regimen versus myeloablative conditioning (MAC) CBT for adults with acute leukemia (a registry-based retrospective analysis). There were 107 RIC patients and 403 MAC patients. Results indicated that in the MAC regimen the addition of fludarabine is associated with myeloid recovery after single-unit CBT. However, it is associated with better survival only in patients transplanted in remission.

RIC regimen (CY + FLU + TBI) is associated with better results in retrospective analysis. RIC is associated with decreased NRM but higher relapse incidence in adults with acute leukemia when compared to MAC. Approaches that decrease the incidence of relapse after RIC may improve outcomes.

Dr. Navneet Majhail discussed RIC for CB transplants in older patients. Hematologic cancers are common in the elderly. Challenges to HCT in older patients include the fact that older AML patients are more likely to have unfavorable cytogenetics, multidrug resistance, a poor performance status, and comorbidities. Also, suitable sibling donors are less frequently available. RIC may alleviate some of these problems. A recently published study has indicated that using RIC, old age has no adverse impact on HCT outcomes. The study concerned 1080 patients with AML complete remission 1 (CR1) and MDS of various ages: 40-54 (n = 409), 55-59 (n = 295), 60-64 (n = 258), and ≥65 (n = 118) years. In all of these age groups, the NRM and DFS were similar using related and unrelated donors. A prospective study has compared the outcomes of UCB transplants versus matched related donor (MRD) HCT in patients 55 to 70 years old. Forty-seven had MRD transplants, and 43 had UCB transplants (88% received 2 CB units). The 3-year PFS, 3-year OS, and the 1-year transplant-related mortality (TRM) were all similar in the 2 groups. Donor engraftment at day 42 was 100% with MRD transplants and 89% with UCB transplants. Grade III-IV aGVHD was 49% with UCB transplants and 23% with MRD transplants, but cGVHD at 2 years was 43% with MRD transplants and only 17% with UCB transplants. Take-home points are that UCB is an alternative graft source for older patients who do not have MRD. Comorbidities should be carefully reviewed when choosing older patients for transplantation. Also, RIC UCB HCT in older patients needs further investigation.

Dr. Mitchell S. Cairo discussed reduced toxicity conditioning (RTC) compared to MAC prior to UCBT in pediatric recipients. A study was performed to test the hypothesis that RTC versus MAC prior to UCBT in pediatric recipients will be associated with

significantly less day 100 NRM and a significant increase in OS. Eighty-eight patients were studied (RTC = 39 and MAC = 49; 24 RTC patients had malignant disorders as did 34 MAC patients). The probability of myeloid engraftment was similar, platelet recovery (>20,000) was better in the RTC group. Grade III-IV aGVHD was higher in the RTC group, and there was no difference in incidence of cGVHD. TRM at 100 was 35% in MAC, whereas it was only 2.5% in patients with RTC. The probability of OS was significantly better with RTC than with MAC at 8 to 9 years posttransplant ($P = .0032$). In summary, the risk of day 100 NRM following UCBT in pediatric recipients was significantly increased by MAC compared to RTC. The probability of OS following UCBT in pediatric recipients is significantly decreased by MAC and in patients with low CD34/kg cell dose. The probability of grade II-IV aGVHD following UCBT in pediatric recipients is significantly increased following MAC versus RTC. A prospective, randomized multicenter trial with a larger cohort with specific regimens and diseases/status will be needed to confirm these preliminary findings.

Session V. New Concepts in Donor-Recipient Matching for CB Transplants

Dr. Andromachi Scaradavou presented data regarding the role of noninherited maternal HLA antigens (NIMA) in CB transplantation and implications for search and unit selection. Fetal exposure to NIMA produces tolerance and has been shown to have a favorable effect in solid organ and related SCT. A study was done to test the hypothesis that reexposure of CB cells to NIMA will affect transplant outcomes. A retrospective analysis was carried out in patients with hematologic malignancies (n = 1121) who received single-unit CB grafts having 0, 1, or 2 HLA-A, -B, -DRB1 mismatches. Conclusions of the study were that matching for the donor's NIMA can improve outcome in unrelated CB transplantation. There is faster engraftment, even in grafts with low TNC dose, improved TRM even in adolescents/adult patients, no increased risk of aGVHD or cGVHD, and possibly reduced relapse rates in patients with myeloid malignancies. Therefore, HLA-mismatched, NIMA-matched CB units can be the graft of choice for patients with hematologic malignancies lacking fully matched donors. Further conclusions are that including the NIMA matches in the search algorithm increases substantially the probability of finding optimal CB grafts for patients. In cases where only HLA-mismatched CB units can be identified, review of the maternal HLA typing of these units may allow selecting a NIMA-matched CB graft. Obtaining maternal HLA typing is an efficient way of expanding manyfold the current global CB Inventory.

Dr. Jeffrey Miller discussed KIR genotyping—thinking beyond HLA for donor choice. Two published studies have reached somewhat different conclusions regarding the significance of killer-immunoglobulin receptor (KIR) genotyping in HSCT. A study was performed to determine whether donor KIR correlates with clinical outcomes. The strategy was to determine whether evaluation of KIR B haplotypes for specific gene motifs will inform selection of "good" KIR donors to improve the effectiveness of unrelated donor HCT. The study cohort consisted of 1409 donor/recipient pairs from URD HCT for acute leukemia. The results of the study indicated that favorable KIR donors are beneficial in T cell–replete and T cell–depleted HCT. This benefit is from relapse protection. There is benefit of some KIR B haplotypes in protecting against GVHD. A prospective plan in URD transplant to choose donors will start in 2010.

Dr. Juliet Barker discussed the combined effect of total nucleated cell dose and HLA matching on transplant outcome in 1061 CB recipients with hematologic malignancies, and the implications for unit selection. Initial analysis indicated that neither TNC dose nor HLA match had any association with relapse risk, but both affected TRM; TRM was the major endpoint of the combined TNC and HLA-match analysis. A study was performed on the influence of cell dose and HLA match on double-unit engraftment (n = 84). All patients had hematologic malignancies; 61 patients received ablative conditioning, and 23 received nonablative conditioning. Results indicated that the TNC dose is a critically important determinant of engraftment, TRM, and survival. HLA match is a critically important determinant of engraftment, GVHD, TRM, and survival. An effect on TRM postengraftment is evident even if there is no GVHD. The best outcome is associated with 0 mismatches (MM) (ie, 6/6 units) without dose effect *to date*; 6/6 CB unit results may rival 10/10 URD. A better match can compensate for a lower dose so that a sliding scale should be used with unit selection: the less well matched the unit, the bigger the required dose (this is not possible in adults). One should avoid single units with 1-2 mismatches and $<2.5 \times 10^7$/kg. In double CBT, there is an increased chance of transplanting at least 1 unit of good quality, *plus* unit versus unit effects may augment engraftment and reduce relapse. In selection of CB units, the TNC should be above 2.0×10^7/kg, and HLA matching should be ≥4/6 A,B antigen, DRB1 allele. Within a given match grade, the largest unit should be chosen. Also consider the bank of origin (speed, reliability, quality). The same rules apply to selecting units 1 and 2. The unit–unit HLA-match criteria should be abandoned.

Dr. Cladd Stevens discussed HLA mismatch and mismatch direction in CBT. The hypothesis is that the direction of HLA mismatch affects outcome. In

the rejection direction (host-versus-graft [HVG]), one would expect decreased engraftment, decreased GVHD, and decreased GVL (increased relapse). In the GVH direction. One would expect improved engraftment, increased GVHD, and persistence of GVL. In the present study, the type of the mismatch and the number of mismatches were considered. The analysis concerned 1202 transplants that were carried out primarily for hematologic malignancies (72%). The number of transplants with the various types of mismatches (MM) were as follows: No MM, 72; 1 MM bidirectional, 364; 2 MM bidirectional, 525; 1 MM GVH only, 51; 2 MM GVH only, 7; 1 MM rejection (Rej) only, 30; 2 MM rejection only, 10; 2 MM (2 GVH, 1 Rej), 83; 2 MM (2 Rej), 1 GVH, 54; and 2 MM (1 GVH + 1 Rej, 5). The outcome of the study was as hypothesized, that is, rejection-only mismatches have a trend for decreased engraftment, a trend for increased GVHD, an increase in relapse, a trend for increased TRM and overall mortality, and a trend for decreased DFS. GVH-only mismatches have increased engraftment, no increase in GVHD compared to other MM transplants, a trend for decreased relapse, a decrease in TRM and overall mortality, and an increase in DFS. There were no effects in mixed direction transplants. Therefore, recommendations for CBU selection are that rejection-only mismatches should be avoided, especially in hematologic malignancies. GVH-only mismatches are preferred. They are as good as 0-mismatched CBUs, especially in hematologic malignancies, and there is no increase in GVHD.

Session VI. Engraftment of CB Stem Cells

Dr. Maruisz Z. Ratajczak discussed novel strategies to enhance homing and engraftment of CB stem cells. Current strategies to accelerate hematopoietic reconstitution after transplantation include transplantation with larger numbers of HSPCs or ex vivo expansion of harvested HSPCs before transplantation. However, the number of HSPCs available for allogeneic or autologous transplantation is often low, and current strategies to expand HSPC ex vivo are unfortunately inefficient. Complement cascade cleavage fragments such as C3a enhance the homing responses of HSPCs to SDF-1 gradient. Also, antimicrobial peptides (AMPs) are host defense peptides and are an evolutionarily conserved component of the innate immune response. Priming of UCB HSPCs homing is a new strategy to accelerate engraftment. Conclusions reached after study of these homing agents are that the complement component fragments as well as small granulocyte-derived cationic peptides that primarily possess antimicrobial functions and are harmless to mammalian cells increase/prime responsiveness of HSPCs to SDF-1 gradient. Their presence in leukopheresis products may explain why mobilized PBSC blood engrafts faster than BM. Cationic peptides could be employed to prime ex vivo HSPCS before transplantation and enhance/accelerate their engraftment.

Dr. Colleen Delaney discussed Notch-mediated expansion of human CB progenitor cells capable of rapid myeloid reconstitution. A number of studies have indicated that there is delayed engraftment in CBT compared with sibling donor or matched unrelated donor transplants. The goal of the present work is to provide cells that rapidly engraft and overcome the delay in hematopoietic recovery following transplantation with CB. Culture with engineered Notch ligands enhances the magnitude and kinetics of marrow repopulation in NOD/SCID mice. A study in patients was done to examine the safety with infusion of ex vivo–expanded CB cells generated via culture in the presence of engineered Notch ligand. Increased absolute numbers of $CD34^+$ cells are reliably generated via Notch-mediated ex vivo expansion. The study examined the kinetics and durability of hematopoietic reconstitution and the relative contribution to engraftment as provided by the expanded and unmanipulated CB grafts. Unmanipulated units provided a mean of 2.4 $CD34^+$ cells, whereas the expanded units averaged 60.3 $CD34^+$ cells/kg. Data are now available for 11 patients who received expanded $CD34^+$ cells. Results indicate that there is a significantly reduced time to absolute neutrophil count (ANC) >500 in patients receiving ex vivo–expanded cells (14.5 days) compared to conventional double CB transplants (26 days). Conclusions to date are: (1) Notch-mediated ex vivo expansion of $CD34^+$ CB progenitors is clinically feasible, resulting in a significant increase in the absolute number of $CD34^+$ cells from a single unit of CB; (2) infusion of the expanded cell product has not resulted in any toxicities; (3) the expanded cells contribute to rapid early myeloid engraftment; and (4) in some cases, the expanded cells have persisted in vivo, suggesting that there may be long-term repopulating ability of the expanded cell product.

Dr. Manuel N. Fernández reviewed the use of adult third-party donor cells to improve outcome of single-unit CB transplants. The objective of the "Dual Transplant" strategy is to produce a "bridge graft" to reduce problems inherent in late engraftment. The dual transplant strategy utilizes myeloablative conditioning and coinfusion of T-depleted mobilized HSC from a third-party donor (TPD). To date, 55 patients have been studied who had high-risk hematologic diseases. Results indicate that there is a short posttransplant neutropenia with early and transient engraftment of the TPD HSC (the "bridge graft"), there is a high rate of full CBT chimerism, and a low morbidity and mortality because of neutropenia-related infections. There is also favorable OS and DFS, not worse than those of HLA-identical family donors, and a relatively low cost. Possibilities for the future include the

possibility that many other TPD cells may be useful to improve outcomes of unrelated CBT, for example, MSC, NK cells, CTLs, T-regs, and γδ-T cells.

Dr. Jonathan Gutman discussed single-unit dominance following double-unit CBT and indicated that $CD8^+$ T cells are associated with the development of single-donor dominance. Clinical observations regarding double-unit CBT indicate fewer graft failures in adults and large children; lower TRM in adults and large children; possible decreased relapse; more (at least mild/moderate) aGVHD; and the fact that 1 unit wins. The reason that 1 unit wins is unclear. The reasons are probably multifactorial. An hypothesis has been developed that the process is immune mediated, that is, that the winning unit rejects the losing unit. A study was developed to investigate the potential role of T (and NK) cells. 14 patients have been studied, 5 receiving 2 unmanipulated units and 4 patients received 1 $CD34^+$–selected and ex vivo–expanded unit and 1 unmanipulated unit. Ten patients developed rapid (by day 28) single donor chimerism, 3 had persistent mixed chimerism, and 1 had graft failure. In patients with a single-donor dominant cell, there was interferon (IFN)-γ–secreting cells reactivity against cells of the losing unit but not against cells of the winning unit in most patients, whereas in patients with persistent mixed chimerism, no such reactivity could be demonstrated. In summary, there is compelling evidence that $CD8^+$ T cells contribute to emergence of dominant units. There is no evidence of functional responses from $CD4^+$ or NK cells. It remains unclear why 3 patients maintained mixed chimerism.

Sessioni VII. Infections, Immune Reconstitution, and Adoptive Immunotherapy after CBT

Dr. Catherine Bollard discussed expansion of CB-derived antigen-specific T cells for clinical use. Fifteen of 25 patients received donor-derived virus-specific T-lymphocytes for viral infections after HSCT. In 13 of 15 patients, there was a decrease in viral load with corresponding elevation in virus-specific cytotoxic T lymphocytes (CTL) detected in the peripheral blood. No other antiviral therapy was required for 23 of the 25 patients. Investigations are being carried out to determine whether virus-specific CTL can be expanded from CB for adoptive immunotherapy to restore antiviral immunity and reduce viral infection post-CB transplantation. Extensive experimentation has indicated: (1) donor-derived multivirus-specific CTL can prevent and treat viral infection after HSCT; (2) virus-specific CTL can also be expanded from naïve CB T cells; and (3) there is a need to determine whether the CB-derived virus-specific CTL will be as efficacious in vivo as peripheral blood–derived CTL. Further conclusions are: (1) CB-derived multivirus-specific CTL can be transduced with a CAR-CD19 to redirect specificity to B cell malignancies; (2) functional CB-NK cells can also be expanded from CB; and (3) there is a need to determine whether the administration of CB-derived NK cells is feasible and safe in patients after CB transplantation.

Dr. Juliet Barker reviewed infections and immune recovery following double-unit CBT. A detailed retrospective study of 72 patients transplanted for hematologic malignancies indicated that: (1) bacterial and fungal infections are common, but death from these is quite unusual; (2) neutopenia following ablative preparative regimens remains a challenge despite a high sustained engraftment rate; (3) the best prophylaxis for bacterial infection is unclear; and (4) the frequency of making a specific diagnosis in cases of bacterial and fungal pneumonia is very low. Further, (1) viruses are the greatest contributor to infectious mortality, but lethal infections are limited to the first 4 months; (2) nonetheless, improved antiviral agents for prophylaxis and treatment and augmentation of antiviral immunity should be a priority; and (3) Epstein-Barr Virus (EBV) viremia and adenoviral enteritis occurred exclusively in the context of GVHD therapy or corticosteroids for another indication. Finally, (1) there is an improved risk after day 120, which is coincident with immune recovery; (2) plans for more sophisticated measures of immune recovery are underway, but ultimately, infection risk is the best measure of immune recovery; and (3) these results support improved infection mortality after ATG-free double-unit CB transplants.

Dr. Paul Szabolcs discussed immune reconstitution following CB transplantation. Death because of infections is the primary cause of death in UCB transplantation in the past and modern (>2002) era alike. Most such deaths occur before 6 months posttransplantation. CB is a unique graft source because of a high frequency of highly proliferative primitive stem cells paired with "naïve" lymphocytes. More robust lymphocyte reconstitution is associated with lower mortality after single-unit UCB transplantation if the mean values are compared over a course of 2 years. Enhanced lymphocyte reconstitution is a composite of B cell, $CD3^+$ T cell, and in particular, $CD4^+$ naïve T cell recovery. If immune recovery is analyzed only from data collected during the first year, B cell, T cell, and even $CD4^+$ T cell recovery lack predictive power even in univariate analysis. In contrast, in univariate analysis, survival is superior in those with more rapid recovery of recent thymic emigrants, Tregs, and plasmacytoid dendritic cells during the first year. Declining activation state (HLA-DR expression) of $CD8^+$ T cells during the first year also predicts better survival in the univariate model. In this pediatric dataset where malignancy was the indication for ~1/3 of UCBT, the only parameter of lymphocyte and dendritic cell reconstitution during the first year post-UCB

transplantation that predicted superior survival in multivariate analysis was the recovery of thymic function measured by $CD45RA^+/CD62L^-/CD4^+$ subset.

Session VIII. HSCT for Thalassemia and HIV-Infected Patients

Dr. Gero Hütter discussed long-term control of HIV by CCR5 Delta32/Delta32 SCT. A 40-year-old man, who had known HIV infection since 1996 and who was treated with highly active antiretroviral therapy (HAART) since 2002, developed AML. Infection with HIV requires the presence of a chemokine receptor 5 (CCR5), and homozygosity for a 32-bp deletion (*delta32/delta32*) in the *CCR5* allele results in an inactive *CCR5* gene product, and consequently confers high resistance against HIV-1 infection. The patient's potential donors were tested for the deletion, which occurs in the homozygous state in about 1% of Caucasians. This patient had a remarkably large number of potential HLA-matched donors (232), and 1 such donor was identified as homozygous for the deletion. HSCT was carried out using PBSCs from this donor, and engraftment was achieved 13 days after the procedure. However, AML relapsed 332 days after transplantation, necessitating a second transplant from the same donor. The second procedure led to a complete remission of the AML, which has remained in remission. The patient has not received HAART therapy since the first transplant was performed. The patient's HIV-1 load was measured with the use of RNA and DNA PCR assays, and since day 61 following the first transplant, serum levels of HIV-1 RNA have remained undetectable. It is now 2 years and 4 months since the first transplant. In summary, allogeneic HSCT in patients with HIV is feasible, and a case of allogeneic HSCT with a *CCR5-Δ32* homozygous donor highlights the decisive role of CCR5 in maintaining HIV infection. More cases are necessary to document the effectiveness of this procedure as are attempts to translate this approach into a more feasible way.

Dr. Lawrence Petz discussed an inventory of CB units homozygous for the *CCR5-Δ32* deletion for potential cure of HIV infections. Investigators have theorized that HSCT using products obtained from individuals homozygous for the *CCR5-Δ32* allele could provide therapeutic benefit for individuals with HIV infections. The case report by Hütter et al. demonstrated long-term control of HIV infection by HSCT from an unrelated adult donor homozygous for *CCR5-Δ32*. However, it is difficult to generalize this approach because there are usually only a small number of very well HLA-matched adult donors available for an individual patient, and there are very few persons who are homozygous for the variant allele. However, an inventory of umbilical CB units that are homozygous for the *CCR5-Δ32* allele could provide an improved probability of finding an appropriately HLA-matched donor for a patient with HIV infection in need of a transplant. We have tested ~6000 Caucasian units in our CB bank to develop an inventory of units homozygous for the *CCR5-Δ32* allele and found 48 homozygous units. Projections of the probability of HLA matches within this special inventory using HLA types of simulated Caucasian patients indicated a 0.04% probability of finding a 6/6 matched unit, a 14.23% for a 5/6 matched unit, and 54.4% probability of a 4/6 matched unit. The median TNC dose of units in the special inventory is 117.5×10^7 (range: 39-351), which is adequate for patients up to 46 kg based on a cell dose of 2.5×10^7 TNC/kg. However, it is eminently feasible to test additional units so that development of an inventory of at least 300 *CCR5-Δ32* homozygous units can be accomplished. With an inventory of 300 units, the probability of finding an HLA-matched unit with a TNC of 2.5×10^7/kg in pediatric patients is 0.01% for 6/6 matches, 10.58% for 5/6 units, and 73.61% for 4/6 matches. For adult patients, the probabilities are 0.01% for 6/6 matched units, 4.49% for 5/6 matched units, and 27.92% for 4/6 matched units. Patients in need of an HSCT and who are infected with HIV should be offered transplantation with stem cells that are homozygous for *CCR5-Δ32*, as should patients with an unsatisfactory course on antiretroviral drug therapy.

Dr. Tang-Her Jaing presented data on transplantation of patients with transfusion-dependent thalassemia. A study has been done in which 40 CBTs were performed in 35 patients with a median age of 5.5 years who had transfusion-dependent thalassemia. A double CBT was performed if no single UCB unit of adequate cell dose was available. All donors were HLA-compatible unrelated CB units that were selected from the same NMDP-affiliated CB bank. CBUs were matched at ≥4/6 HLA antigens based on antigen-level HLA-A and B typing and allele-level HLA-DRB1 typing. The preparative regimens consisted of oral or intravenous busulfan, intravenous cyclophosphamide, and equine ALG. The median number of nucleated cells infused was 7.8×10^7/kg (range: 2.8-14.7) and $CD34^+$ was 4.0×10^5/kg (range: 1.7-19.9). Twenty-two patients were classified as Pesaro I, 11 were Pesaro II, and 2 were unclassified. Twenty-eight of the 35 patients have become transfusion independent and achieved hematopoietic reconstitution after the first transplant. Five patients subsequently underwent retransplantation for graft failure. None required rescue with cryopreserved autologous back-up harvests. Acute GVHD was grade I in 6 patients, grade II in 12, grade III in 15, and grade IV in 1. Fourteen patients developed chronic skin GVHD, which was was extensive in 1 and limited in 13. The 5-year OS and thalassemia-free survival were

88.3% and 73.9%, respectively. The cumulative incidence of TRM at 2 years was 11.7%. Of these patients, 83% were alive and transfusion independent with a Lansky performance score of ≥80% achieved between 6 and 76 months posttransplant (median, 36 months). The conclusion was that this is the largest single institution report of unrelated CBT for transfusion-dependent thalassemia and shows the favorable clinical results that are attainable when TNC dose is optimized with CB units, when no postthaw wash is performed, and when double CBT is utilized when necessary.

Session IXA. CB Banking

Dr. Ellen Lazarus discussed CB licensure. With publication of hematopoietic progenitor cells, cord (HPC-C) guidance documents, the Food and Drug Administration (FDA) also announced that the phase-in implementation period for Investigational New Drug (IND) and Biologic Licensure Application (BLA) requirements for these products will end on October 20, 2011 (2 years after date of publication). Sponsors are encouraged to send in IND and BLA applications as soon as possible. The HPC-C Licensure Guidance provides recommendations to manufacturers applying for licensure of minimally manipulated, unrelated allogeneic placental/umbilical CB, for specified indications. Information contained in the HPC-C Licensure Guidance includes how to use the Guidance when applying for a BLA, applicable regulatory requirements and the license application procedure. Licensure may apply to HPC-C previously manufactured using the same procedure where documentation is provided. Licensure may also apply to HPC-C in inventory that were previously manufactured using different procedures provided that the manufacturer submits a separate validation summary and includes data demonstrating comparability of previously manufactured HPC-C to the currently manufactured HPC-C, and providing evidence that methods, facilities, and controls used for manufacture were compliant with CGMPs. The scope of the guidance was changed to expand the list of intended clinical uses for the HPC-C covered by the Guidance based on public comment and additional data submitted to the docket. The Establishment Description section on Computer Systems includes more information about resources for information on software regulation and validation. Also, references and footnotes have been updated. Draft CB IND Guidance was published for comment in October 2009. An IND is necessary for 2 reasons: (1) a product that does not conform to all BLA requirements (an unlicensed HPC-C) may be the best product from a clinical standpoint; or (2) the clinical use of the HPC-C may be for an indication other than those defined in the Guidance document.

Dr. Michael Boo discussed the use of INDs for nonlicensed CB units and the potential role that NMDP may have in facilitating these procedures. Draft guidance was issued on October 20, 2009, as a companion to the BLA guidance. The IND is intended to provide access to CB units that will not meet license requirements or for indications other than those specified in the document. The comment period has expired, and the industry is now waiting for final guidance. The IND will cover CBUs that are used for minimally manipulated units intended for hematopoietic reconstitution in recipients unrelated to the donor for hematologic malignancies, certain lysosomal storage diseases, primary immunodeficiency diseases, BM failure, and beta thalassemia. Sponsors include CB banks for units from the bank; registries for units accessed through the registry; and transplanters for units used in transplantation of patients. Sponsors are required to submit IND safety reports and annual reports, to ensure that licensed physicians are qualified to administer the investigational drug, to provide licensed physicians with the required information, to maintain an effective IND with respect to the investigations, and to maintain adequate drug disposition records. Investigators must report adverse drug events to the sponsor, ensure that the informed consent requirements are met, ensure that institutional review board review of the use of the HPC-Cs under the IND is obtained, and maintain accurate case histories and drug disposition records. Transplant center responsibilities are to designate a principal investigator, submit a protocol to their institutional review board, obtain research consent from CBU recipients, to continue to report outcomes data to the Center for International Blood and Marrow Transplant Research (CIBMTR) and report adverse events to National Marrow Donor Program (NMDP) and collaborate in investigations of events. International CB banks must be registered with the FDA, preferably with accreditation by AABB or Netcord-FACT, to report adverse events to the NMDP and collaborate in investigations of events, and must comply with NMDP procedures for establishing donor eligibility.

Loren Gragert presented data regarding estimating match rates with adult donors and umbilical CB units in the NMDP "Be the Match" Registry. Past analyses have been limited because of little or no high-resolution haplotype data, only 4 racial/ethnic groups (Caucasian, African American, Asian, and Hispanic), little or no data on generally acceptable mismatches and limited consideration of CB units. Present analyses include extensive high-resolution 4-locus haplotype frequencies within each racial/ethnic group, 21 racial/ethnic groups, stringent matching according to currently accepted standards for CB and adult units, more thorough consideration of CB, and incorporation of adult donor availability and umbilical CB unit

cell dose. Numerous analyses can be carried out, including 8/8 allele (or 7/8 and 6/8 allele) available-match rates in the adult donor registry; adult or pediatric cord match rates in the CB registry with a cell dose of ≥2.5 TNC/kg; "8/8 adult then cord" match rates for adult or pediatric patients, considering availability and dose; "cord only" match rates for adults or for pediatric patients, considering availability and dose; and the increase in match rates with increasing the inventory of adults in the registry or CB units. The data obtained from the analysis are valuable regarding the 4 approaches that will compete for available resources: (1) adult donor recruitment; (2) CB unit "recruitment"; (3) adult donor retention/availability; and (4) clinical research to improve results with suboptimal adult or CB units.

Session IXB. CB Banking

Dr. Kevin Shoulars discussed predicting potency of CBUs with enumeration of ALDH bright ($ALDH^{br}$) cells. CFUs provide a useful potency assay but the usefulness of the assay is limited by the time required (14 days) and variability among laboratories. Alternatives are a shorter CFU assay (5-7 days), a HALO assay that measures ATP produced by dividing cells after 5 days, and an assay for $ALDH^{br}$ cells. A retrospective study of 38 CBUs used for transplantation indicated that rapidly engrafting units could be distinguished from nonengrafting units using CFU-infused dose or $ALDH^{br}$-infused dose. A prospective trial with the NMDP and CIBMTR is planned. Clinical outcomes of 800 transplants (600 single unit and 200 double unit) will be analyzed prospectively to evaluate data from $ALDH^{br}$ and CFU to determine their ability to predict engraftment.

Dr. William Miller discussed the stability of CB cells after processing and storage. Experience has been gained in over 700 transplants using RBC-reduced and plasma-reduced units, some of which are washed at the transplant center, although the latter procedures are not well standardized. A study of units transplanted after various numbers of years of storage indicated that the engraftment rate is essentially the same using units stored up to 12 years prior to transplantation. Further, the CB unit age does not impact the probability of or time to patient neutrophil recovery.

Dr. Joanne Kurtzberg discussed, "Thawing and infusion of CB units; gaining knowledge to prevent adverse infusion events." Over the past several years, increased emphasis has been placed on producing a high-quality CBU, but much less attention has been paid to the end user of the CB. Issues are that CB products and thawing are complex, there is a lack of standardization of thawing methods, new centers do not receive formal training, thawing procedures provided by banks are inconsistently followed by transplant centers, and there is need for improved comprehensiveness of safety monitoring. At the transplant centers, the temperature of the CB unit must be confirmed, the product must be inspected for labeling, the integrity of the bag determined, and the unit must be transferred to a liquid nitrogen freezer. Methods of thawing and infusion include direct infusion, dilution prior to infusion, or dilution with washing before infusion. Such issues are being highlighted because a few serious infusion reactions in recipients of double CB units have been reported in the past few months. Problems with CBU thawing have also been reported from a few laboratories, and there is a lack of "CB thaw preparedness" at transplant centers in general. A list of things that can be done are as follows: (1) formalize guidelines for single- and double-cord infusions; (2) require transplant centers to validate a thaw/wash and wash procedure or to follow the procedure provided by the bank; (3) ask accrediting agencies to create additional standards for CB thawing and administration; (4) improve centralized reporting and review of infusion-related adverse reactions, (5) create a training program for CB thawing; (6) require transplant centers to practice a thaw of a RBC-reduced and RBC-containing CBU before handling a patient sample; (7) consider a randomized trial of thaw versus thaw and wash; and (8) recommend that transplant centers identify a backup unit in advance of the transplant.

Dr. John McMannis discussed preparation of CB units for reinfusion. In the manual procedure that has been used, one thaws the unit, transfers to a 150- to 600-mL transfer pack, dilutes with 10% Dextran40, dilutes with 5% human serum albumin, fills the bag with 10% Dextran40, incubates 5 minutes, centrifuges, removes the supernatant, and resuspends the cells. TNC recovery has been 84% during the last 4 years, and viability of $CD34^+$ cells has been >90% both postthaw and postwash. Analysis of cell recoveries from units from 33 banks indicated that TNC recovery from almost all CB banks was highly consistent, in the range of 80% to 85%. A trial of an automated procedure suggests that there are advantages over the manual method in that there are fewer interventions and there is reduced time for processing and improved reproducibility. TNC recoveries from volume-reduced CBU were similar using the automated procedure. Also, TNC recoveries from plasma-reduced units were lower using the automated procedure although viability of $CD34^+$ cells was >95%.

Donna Regan indicated that a reconstitution protocol was developed and validated for units stored after RBC-reduced process methods. An hypothesis suggested that a simple 1:1 dilution without centrifugation would stabilize the product, reduce the DMSO concentration by 50%, and allow for a controlled

thaw in the laboratory without the risk of cell loss. A study was performed with CB units that were removed from liquid nitrogen storage and thawed in a 37°C water bath. They were split into 3 fractions. One fraction was left unmanipulated to mimic the bedside thaw. The other 2 fractions were diluted with an amount of albumin/dextran solution equal to the volume of product (1:1), resulting in a 5% DMSO concentration. The final third of the product was then centrifuged at 1200 rpm (400 × *g*) for 20 minutes and supernatant expressed. All were held at room temperature and analyzed at the following time points: immediately (time zero), at 2-hour intervals for the first 8 hours, and at 24, 32, and 48 hours postthaw. Results indicated that throughout the entire evaluation, traditional wash and dilution methods performed equally well with no significant differences observed in 7AAD viability, TNC, CD34, or CFC recovery. An additional study indicated that for 163 patients in which diluted products were administered, similar time to engraftment was observed when compared to historic experiences with traditional wash and direct infusion, and that there was no difference in probability of time to neutrophil recovery ($P > .05$) between the 3 methods. The conclusion was that the wash method, intended to remove DMSO, RBC stroma, and plasma postthaw, is not necessary when UCB products are RBC- and plasma-reduced before cryopreservation.

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