2013 INTERNATIONAL CORD BLOOD SYMPOSIUM

POSTER RECEPTION

Thursday, June 6
5:30 - 6:30 PM

Market Street Foyer

The annual poster reception fosters the research and development of the fields of regenerative medicine, cord blood banking, and stem cell transplantation. The reception provides an opportunity to network with an engaged audience.
What are the Opportunities for Cord Blood Derived Products in Regenerative Medicine?

Mahendra Rao, MD, PhD, National Institutes of Health Center for Regenerative Medicine

Cord Blood cells have shown remarkable utility for replacement of bone marrow transplants in a variety of clinical settings. In recent years investigators have investigated the utility of cord blood for a variety of additional uses. These include treatment of neurological disorders, cardiac infarcts as well as isolation of specific subtypes of cells from a cord blood sample. More recently investigators have observed that cord blood is an excellent source of naïve cells for making induced pluripotent cells. I will describe efforts made by different investigators along these lines and potential pitfalls and business models that appear to be developing.

Deriving Mesenchymal Stem Cells from Human Amniotic Fluid: Potential for an Allogeneic Cellular Therapy Product

Julie G. Allickson, PhD, MS, MT(ASCP), Wake Forest University

Amniotic fluid has been used for more than 70 years for prenatal diagnosis generally collected at 14-20 weeks of gestation to assess for chromosomal abnormalities. Amniotic fluid contains a unique, heterogeneous population of cells derived from exfoliating tissues of the fetal skin, respiratory, digestive and the urinary tract. Amniotic fluid may provide an important source of immature cells derived from the fetus during development. The primitive nature of the cell may lend itself to a large number of applications due to its plasticity. There is no need for ectopic induction for pluripotency, no tumorigenic potential, and no ethical considerations associated with the amniotic fluid stem cells.

Within the heterogeneous population of cells are characterized stem cells capable of more than 250 population doublings, pluripotent able to differentiate into 3 germ cell layers and demonstrate an immunomodulatory capacity (1). Atala et al. in 2007 reported on the highly proliferative nature of a subset of amniotic fluid cells that are pluripotent and could differentiate at minimum into adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages (2). Ditandi et al. in 2009 was able to demonstrate the hematopoietic activity as demonstrated by erythroid, myeloid and lymphoid cells of amniotic fluid cells selected for c-kit which were also lineage negative. In a mouse model they could demonstrate reconstitution of hematopoietic cells after a primary and secondary transplant with selected amniotic fluid cells (3). These cells demonstrated self renewal and therefore the potential for future use of amniotic fluid cells in therapeutic applications.

These studies and others point to the possibility that amniotic fluid may be important source of cells to store for future use as a tissue replacement. Potentially 99 percent of the U.S. population could conceivably find genetic matches for tissue regeneration or engineered organs from 100,000 unique cryopreserved amniotic fluid samples (4). The amniotic fluid cell source may be revolutionary in the field of healthcare as a highly proliferative immature cell that is capable of immune modulation and assessed in several functional models (5).

References:

MSC-like Cells from Human Umbilical Cord Tissue: Roadmap to Clinical Utility in Regenerative Therapies

Morey Kraus, CSO, PerkinElmer Diagnostics/ViaCord

Newborn stem cells from Umbilical Cord Tissue are gaining increasing attention as a potential raw material for regenerative medicine. This talk will discuss methods of collecting, processing and storing Cord Tissue MSC-like cells (CT-MSCs), the status of preclinical studies utilizing CT-MSCs in relevant animal models of disease, and a roadmap for evaluating the clinical utility of Autologous or Related CT-MSC units in the Private, or Family banking model.

This talk will include data related to alternative methods of processing CT-MSCs from Cord Tissue; functional properties of CT-MSCs including co-administration, immune-modulation, broncho-pulmonary dysplasia, hypoxia, and other relevant animal models; characteristics of CB and CT collected for premature infants (<34 wks GA) as compared with collections from a normal distribution of newborns. Finally the speaker will propose a roadmap to evaluate the clinical utility of CT-MSCs in newborns and haplo-identical (or identical) family members for a wide range of degenerative diseases.

Ex Vivo Modulation Strategies to Enhance the Therapeutic Potential of Cord Blood

Dan Shoemaker, PhD, Fate Therapeutics

Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells (HSCs) for use in allogeneic transplantation. However, UCB contains an inherently limited HSC count, which is associated with delayed time to engraftment, high graft failure rates and early mortality. 16,16 dimethyl prostaglandin E2 (dmPGE2) was previously identified to be a critical regulator of HSC homeostasis and we hypothesized that a brief ex vivo modulation could improve patient outcomes by increasing the “effective dose” of HSCs. Molecular profiling approaches were used to determine the optimal ex vivo modulation conditions (e.g., temperature, time, concentration and media) to enhance HSCs in a clinical setting. A phase I trial was performed to evaluate the safety and therapeutic potential of ex vivo modulation of a single UCB unit using dmPGE2 (ProHema) prior to reduced-intensity double UCB transplantation (dUCBT). Results from this study demonstrated durable, multi-lineage engraftment of the ProHema units with no safety signals. We also observed encouraging trends in efficacy, with accelerated neutrophil recovery (17.5 vs. 21 days (historical), p=0.045), coupled with preferential, long-term engraftment of the ProHema unit in 10 of 12 treated subjects. We have recently extended molecular characterization experiments beyond the stem cell compartment (e.g., Tregs, CD4+ cells, CD8+ cells, monocytes, dendritic and NK-cells) to elucidate additional mechanism(s) by which a pulse treatment with dmPGE2 may impact cord dominance and rates of viral reactivation.

In December 2012, we initiated a randomized, controlled, Phase 2 multi-center clinical trial for ProHema in adult patients undergoing dUCBT for hematologic malignancies. In parallel to advancing the clinical trial, Fate has continued development efforts on ways to enhance the ex vivo manufacturing process. We have recently developed a novel media formulation that results in significant improvements in the potency as well as increased viability of the ProHema product. Specifically, treating human CD34+ cells with dmPGE2 in the new media results in an additional 5-fold increase in CXCR4 gene expression levels (20-fold total) along with significant improvements in homing and engraftment in mouse models. We are currently working to introduce the new media formulation into our Phase2 study. We also plan to initiate additional clinical studies using ProHema in patients with non-malignant diseases.

Fate is continuing to screen for second generation ex vivo modulators to further improve the homing and engraftment properties of HSC to both the bone marrow and the CNS of patients with non-malignant disease. We are also exploring the use of ex vivo enhanced CD34+ cells to treat a variety of non-transplant indications (e.g., cerebral palsy, CLI, stroke and wound healing), grade 2).
Efficacy of Cord Blood Stem Cells in a Baby Rabbit Model of Hypoxic-Ischemic Encephalopathy

Sidhartha Tan, MD, University of Chicago

Although cerebral palsy (CP) has significant impact on both patient and society, there is very limited therapy for this disease. Human umbilical cord blood cells (HUCBC), containing various stem cells, have been used for targeting wild range conditions, including CP. An advantage of the rabbit CP model is that therapy of severe cases of newborn CP can be investigated. In this study, we infused HUCBC to hypertonic newborn rabbit kits following near-term hypoxia-ischemia (H-I) in utero. Intravenous administration of high dose HUCBC (5x10⁶) significantly alleviated the abnormal neurobehavioral abnormalities including posture, righting reflex, locomotion, tone, and dystonia. Half of the dose showed mild but still significant improvement. Tracing HUCBCs with either MRI marker or PCR test for human DNA found no correlation between presence of HUCBC in the brain and therapeutic effect. These proof-of-concept studies show that HUCBC may be a viable therapy for severe cases of CP in the newborn period although the exact mechanism of action still needs to be determined.

Combined Hematopoietic Stem Cell Transplantation, Gene and Substrate Reduction Therapy Show Superior Results to HSCT in a Murine Model of Krabbe Disease

Mark Sands, PhD, Washington University School of Medicine in St. Louis

Krabbe disease (Globoid Cell Leukodystrophy, GLD) is an invariably fatal inherited demyelinating disease primarily affecting children. It is caused by a deficiency in the lysosomal enzyme galactosylceramidase (GALC). Galactosylceramidase is responsible for degrading galactosylceramide, a major myelin lipid. The enzyme is also responsible for degrading the highly cytotoxic sphingolipid, galactosylsphingosine (psychosine). In the absence of GALC activity, psychosine accumulates in the central (CNS) and peripheral (PNS) nervous systems and causes the preferential death of oligodendrocytes. Affected children begin to miss developmental milestones at ~6 months of age, develop motor defects, have cognitive decline, intractable seizures and die prematurely at 2-5 years of age. There is currently no cure for Krabbe disease.

Bone marrow transplantation (BMT) is the only currently used treatment for Krabbe disease. However, BMT results in only a partial correction and simply slows the progression of the disease. Data from pre-clinical experiments using BMT in the murine model of Krabbe disease (Twitcher mouse) mimic the human results. Similarly, other experimental treatments in the Twitcher mouse such as gene therapy, anti-inflammatory drugs, anti-oxidants, and substrate reduction therapy led to minimal to modest improvements. This may not be surprising given the rapidly progressive nature of the disease and the relatively large number of secondary consequences of the disease. Therefore, it seems unlikely that a single therapeutic approach will effectively treat the primary and secondary defects associated with this disease.

We initially combined BMT with CNS-directed AAV-mediated gene therapy in an attempt to target multiple aspects of the disease in the Twitcher mouse. We also initiated the therapies during the neonatal period in order to prevent the onset of disease rather than reverse preexisting disease. By themselves, BMT and AAV-mediated gene therapy increased the median life span of Twitcher mice from 40 days to 45 and 70 days, respectively. Interestingly, the animals treated with both BMT and gene therapy had a median life span of ~130 days. Clearly there is dramatic synergy between these two disparate therapeutic approaches. If the two therapies were simply additive, the predicted median life span would be ~75 days.
Further analysis of this synergy revealed that AAV-mediated gene therapy provided a persistent source of GALC to the CNS. Interestingly, there were very few GALC-positive donor-derived microglia/macrophages in the brains of Twitcher mice receiving BMT. In fact, the level of GALC in the CNS following BMT was virtually undetectable. Rather, we showed that BMT virtually eliminated the AAV- and disease-specific inflammatory responses. Therefore, we believe that the immunomodulatory effects of BMT synergize with AAV-mediated gene therapy to provide the dramatic increase in efficacy observed with this combination.

Although the combination of BMT and CNS-directed gene therapy increases the median life span of Twitcher mice from 40 to 130 days, this is still far from normal. Therefore, we added an additional treatment to the AAV/BMT combination. Substrate reduction therapy with the small molecule drug L-cycloserine increases the median life span of Twitcher mice to ~55 days. However, when Twitcher mice are treated with AAV, BMT and L-cycloserine the life span is further increased. Although this experiment is still in progress, some treated mice have lived to at least 450 days and the earliest death was recorded at 165 days of age. Therefore, our preliminary data suggest that by targeting yet another aspect of Krabbe disease we see even greater synergy. These data suggest that targeting other secondary aspects of Krabbe disease could provide even further benefit, beyond what would be predicted from targeting that disease aspect alone.

Umbilical Cord Blood Mononuclear Cell Therapy of Spinal Cord Injury, Stroke, Myocardial Infarction, and Optic Nerve Atrophy

Wise Young, MD, PhD, Rutgers University

Umbilical cord blood (UCB) is the richest immune-compatible source of human stem and progenitor cells. Over 500,000 UCB units are stored around the world in public banks, ready for HLA-matching and transplantation. UCB contains mononuclear cells that are ~40% monocytes (macrophage precursors), ~40% lymphocytes, 10% neurotrophils and other types of leukocytes, and the remaining 10% are stem cells and progenitor cells, including CD34+ endothelial progenitor cells, CD133+ pluripotent stem cells, and CD105+ mesenchymal stem cells. About 0.6% of umbilical cord blood mononuclear cells are a special multipotent form of mesenchymal stem cells called MUSE (multipotent stress-enduring) cells. Many laboratories have reported the UCB mononuclear cells (UCBMC) are beneficial when transplanted into injured spinal cords of rats and dogs. We assessed the safety and efficacy of transplanting 1.6, 3.2, and 6.4 million UCMBC into the spinal cords of people with chronic ASIA A (complete) spinal cord injury (4-20 years after injury) in two clinical trials. The first trial was carried out at the Chinese University of Hong Kong (CUHK) and Hong Kong University (HKU). Four subjects received 1.6 million and another four subjects received 3.2 million cells. Magnetic resonance diffusion tensor imaging (MR/DTI) revealed a gap in white matter (WM) at the injury site in all subjects before and up to a year after treatment. However, at 1.0-1.5 years after treatment of two of five subjects who had clear MR/DTI images, long fiber tracts appeared to grow across the injury site. None of the subjects in Hong Kong received locomotor training and none recovered walking. The second trial was carried out in the Chengdu People’s Liberation Army General Hospital in Kunming. A total of 20 subjects received 4, 8, or 16 µliter injections of cells four times into the spinal cord above and below the injury site, one group received 16 µliters plus a bolus dose of methylprednisolone (MP), and a final group receive four 16 µliters plus MP and a 6-week course of lithium. The subjects then received 3-6 months of intensive (6 hours a day, 6 days a week) locomotor training. At 6-12 months after treatment, 75% (15/20) subjects recovered ability to engage in stepping and walking long distances using a rolling walker with minimal assistance. Neither motor nor sensory scores corresponded with the walking recovery. Based on these results, we have proposed to do further double-blind randomized placebo-controlled phase III trials in China (n=120) and the rest of the world trial in India (n=40), Norway (n=40), and the United States (n=40). The trials will compare four groups of subjects with: A. Untethering surgery and a 6-week course of oral placebo, B. Untethering surgery and a 6-week course of oral lithium, C. Untethering surgery, transplantation of 6.4 million UCMBC into the spinal cord, and a 6-week course of oral placebo, and D. Untethering surgery, transplantation of 6.4 million UCMBC into the spinal cord, and a 6-week course of oral lithium. The primary outcome measure will be locomotor recovery. Secondary outcome measures include motor and sensory scores, spasticity, neuropathic pain, and spinal cord independence measure (SCIM).
successful, the trial determine whether UCBMC transplants improve locomotor function compared to untethering surgery alone and whether a 6-week course of lithium improves that recovery. This will be the first double-blind randomized surgically and placebo-controlled clinical trial for a combination drug-transplant therapy.

**High Resolution Tracktography is a Highly Sensitive Biomarker to Study Myelination in the Brain**

Jessica Sun, MD, Duke University

Diffusion-Tensor Magnetic Resonance Imaging (DTI) is a valuable tool to investigate tissue microstructure and brain anatomy. Using DTI tractography, information regarding connectivity between different regions of the brain, segmentation of neural fibers, and tract trajectories can be obtained. Changes in DTI have been described in multiple disease states. In children with cerebral palsy, we have demonstrated a reduction in total white matter connectivity and inter-regional connectivity. We also observed a preferential decrease in long-range connectivity associated with increasing severity of cerebral palsy.

Utilization of tractography in conjunction with other complimentary techniques has the potential to reveal an even greater degree of detail about brain structures and pathology. For example, incorporating functional MRI and tractography can provide valuable information regarding the relationships between structure and function in the brain, and Susceptibility Tensor Imaging (STI) can yield more tissue-specific information such as myelination. As these techniques continue to be developed and refined, they have the potential to noninvasively acquire information regarding brain microstructure that cannot be obtained via conventional methodologies.
Overcoming Allogeneic Barriers to Cellular Therapies with Banked Cord Blood

A. E. Willing, K. R. Pennypacker, P. R. Sanberg
Center of Excellence for Aging & Brain Repair, Department of Neurosurgery & Brain Repair,
University of South Florida Morsani College of Medicine

Human umbilical cord blood (HUCB) mononuclear cells (MNCs) have consistently been found to rescue neurons and oligodendrocytes from apoptotic cell death in both in vitro and in vivo models of stroke. These effects are most pronounced when HUCB MNCs are administered intravenously at delayed time points after the stroke, when apoptotic and inflammatory post-stroke injury cascades predominate. The intravenous route of delivery ensures that the HUCB cells are subject to full immune surveillance, making it essential to address issues of immune interactions between the donor HUCB cells and the recipient’s immune cells in our preclinical development of a HUCB cell therapy. Potential mechanisms of HUCB-induced brain repair and how these may interact with the immune/inflammatory system will be discussed.

This project was supported in part by: NINDS #R01NS52839 (AEW), James and Esther King Biomedical Research program, #07KB-07 (AEW), and the American Heart Association, #0555266B (AEW). AEW is a consultant to Saneron CCEL Therapeutics, Inc. and is an inventor on cord blood patents licensed to Saneron CCEL Therapeutics, Inc.
Deriving Induced Pluripotent Stem Cells from Small Samples of Cryopreserved Cord Blood

Scott Noggle, PhD, New York Stem Cell Foundation Research Institute

Umbilical cord blood cells have been applied for decades to treat a number of disorders. However, the availability of optimal numbers of cells from matched donors for transplantation remains a significant barrier to treatment access; moreover, the difficulty in expanding the relevant functional and normal populations in vitro hinders the progress towards many potential therapies and disease studies. Induced pluripotent stem (iPS) cells represent a potential source of patient-specific cells with the capacity to differentiate into all the cell types of the human body. Recent progress in reprogramming blood cells into iPS cells may provide the possibility to expand the utility the cord blood cell banks through their conversion to iPS cell banks providing suitable and matched cells for cell therapy, disease modeling, and drug screening. Yet, it is important to note that there are some limitations of current cord blood cell reprogramming methods including requirement of large amount of materials, low efficiency, possibility of xenogenic contamination, and high cost. Therefore, development of novel approaches for efficient reprogramming in a safe and cost-effective manner are necessary. We are developing a new automated technology platform for derivation and manipulation of pluripotent stem cell lines in a high-throughput, parallel process. Standardization and scale-up capabilities achieved through this automated process are critical in our efforts to reduce methodological variability to uncover true biology and functionalize human genetics. We are further developing this system to allow for derivation of iPS cells from small numbers of cord blood derived cells to enable their use as a potential renewable source for therapeutic application. Our aim is to accelerate the development of safe and effective stem cell derived interventions.
Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease

Shalini Shenoy, MD, Washington University School of Medicine in St. Louis

Sickle cell disease (SCD) has devastating vascular complications that increase with age and result in morbidity, poor quality of life and premature mortality. The unpredictable involvement of vital organs such as the brain, lungs and kidneys make this non-malignant disorder formidable and systematic natural history studies have demonstrated the vast scope of the problem in recent years. Though conservative management has improved over the years, the only curative therapy remains successful hematopoietic stem cell transplantation (HSCT). Yet, HSCT remains underutilized in this population with less than 800 transplants reported to the Center for International Blood and Marrow Transplant Research to date despite a SCD prevalence of >100,000 in the United States. Deterrents to transplant include lack of knowledge and consequently misunderstanding regarding HSCT, paucity of suitably HLA matched marrow donors, side effects of conditioning therapy overlaid on disease induced organ damage, and risks of HSCT such as GVHD, infections and death. Transplant studies have sought to improve outcomes by tackling each of these obstacles. This presentation and subsequent discussion will focus on key areas of study aimed at providing better HSCT options for patients with sickle cell disease by:

• overcoming transplant related education barriers and working with affected families
• focusing on availability of stem cell products for SCD transplants; strategies undertaken to expand stem cell sources such as umbilical cord products
• reducing the intensity and/or toxicity of conditioning even with cord transplants to limit organ toxicities and offset late effects especially in those already compromised by SCD related organ damage
• facilitating immune reconstitution post HSCT to offset infection risks

In this regard, ongoing studies and preliminary observations will be presented and the promise of future directions will be discussed.
Late Outcomes of Cord Blood Transplantation for Patients with Hurler Syndrome

Jaap Boelens, MD, PhD, University Medical Center Utrecht
Co-authors: Aldenhoven M, Escolar, ML, Poe, MD, Wynn, RF, Wraith, E, MD, O’Meara, A, Veys, P, Orchard, P, Kurtzberg, J

**Background:** Hurler syndrome (HS), the most severe phenotype in the spectrum of Mucopolysaccharidosis type I, is caused by a deficiency of the lysosomal enzyme alpha-L-iduronidase. HS is clinically characterized by a progressive and ultimately fatal multi-system deterioration with involvement of the central nervous system. At present, hematopoietic cell transplantation (HCT) is the only treatment that prevents disease progression in the central nervous system and is therefore considered the treatment of choice in HS. Long-term follow-up of outcomes of HCT for HS are sparse and risk factors for favorable long-term outcomes are still largely unknown. Therefore, an international multicenter study was initiated to describe the long-term outcomes of successfully transplanted HS patients.

**Methods:** HS-patients transplanted between 1980 and 2007 within the leading transplantation centers in Europe and the United States were include in this study. Patient, donor, and transplantation-related variables which may influence long-term outcome were analyzed. Patients who were ‘alive and engrafted (donor-chimerism >10%)’ with a follow up of at least three years after HCT were included. The functional outcomes assessed for the various organ systems - orthopedic, cardiac, ophthalmologic, respiratory and audiologic - were analyzed using multivariate Cox proportional hazards and logistic regression models.

**Results:** 197 Hurler patients were included from 8 different transplant centers. This is estimated to be about 70-80% of the successfully transplanted HS patients worldwide during that time period. These patients had a median age of 16 (2-80) months at HCT with a median follow up of 88 (36-258) months after successful HCT. Seventy-nine % of the patients received a graft from an unaffected (non-carrier) donor. Seventy-two % of the patients achieved full (>95%)-donor-chimerism and 28% mixed-chimerism. Full-donor chimerism was noted in all cord blood recipients, while mixed-chimerism was noted in 30-40% of the matched sibling donors and unrelated donors. After HCT, normal enzyme-levels (EL; according to the local reference range) were found in 75% of the patients while 25% had EL below lower limit of normal; either due to mixed-chimerism or carrier-donorship). Multivariate analyses (table 1) showed having a “normal EL” after HSCT and younger (below the median age of 16mths) “age at transplantation” were associated with less serious orthopedic complications requiring surgical interventions; e.g. cord compression, genu-valgum surgery, carpal tunnel surgery. Genotype (double non-sense vs. any other genotype) was associated with a lower probability of requiring hip dysplasia surgery as well as with the occurrence of retinopathy. For other endpoints; e.g progression valve insufficiency, progression corneal clouding and development of retinopathy and the need for hearing aids having a normal EL as well as age at HSCT (<16mths) were predictors for better outcome. Furthermore, growth at the age of 60mths was influenced by EL (-1.93 SDS vs. -1,09 SDS; p=0.042).

**Conclusion:** The long-term outcome of clinical manifestations in HS-patients after successful H SCT is promising although residual disease burden remains. Predictors, favorably influencing the long-term outcomes are suggested to be 1) enzyme level (normal vs. below LLN) after HCT, 2) genotype and 3) age at HCT. Achieving normal enzyme levels at an early age might significantly impact the prognosis of Hurler syndrome patients. Newborn screening (resulting in early HCT), the use of non-carrier donors and achieving full-donor chimerism (as seen in cord blood transplants) may be crucial in optimizing long-term outcomes.
## Multivariate analyses

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Refining the Indications for Cord Blood Transplantation

Daniel J. Weisdorf, MD, University of Minnesota

The broad array of indications for allotransplantation is confounded by the differing goals of a transplant. For malignant disease, potent graft vs. tumor effects without excess non-relapse mortality dominate the treatment options while for nonmalignant disease, satisfactory sustained and prompt lymphohematopoietic recovery with the least morbidity is essential. The choices of matched sibling (for 1/3), allele matched unrelated donors for 3/4 or more of those with well represented racial and ethnic subpopulations, but haploidentical allografts for nearly everyone in addition to umbilical cord blood are all graft options that can be considered. Cord blood transplantation has barriers to engraftment based on the small cell dose, but expansion approaches may overcome that limitation. Cord blood transplantation is associated with less frequent and less severe acute GVHD, notably less frequent chronic GVHD and thus reduced risks of later posttransplant non-relapse morbidity and mortality. Disease states with intrinsic resistance to engraftment including myelofibrosis, splenomegaly, hypercellular marrows associated with hemoglobinopathies or thalassemia might be circumstances where cord blood options are less preferred. Alternatively, situations where patients may be particularly burdened by the ongoing hazards of serious acute and particularly chronic GVHD (older patients or diabetics who are poorly tolerant of extended steroids) might be better served with the lesser late morbidity of cord blood grafting. Comparative judgments of the antineoplastic potency between the different graft sources must also be considered and most importantly must be studied. These all represent new challenges for the field; and opportunities for our patients.

Unrelated Cord Blood Transplantation in Adults with Hematological Malignancies: Eurocord Results

Vanderson Rocha, MD, PhD, Oxford University
Annalisa Ruggeri, Luciana Tucunduva, Marie Robin, Celso Arrais, and Eliane Gluckman, on behalf of Eurocord

In recent years, unrelated cord blood transplantation (UCBT) have been used more frequently in adults, due to better results with children, improved cord blood unit selection, more clearly defined indications for transplantation, new developments in the cord blood field and more experience of transplant centers, Reviews focusing on the clinical results of unrelated donor UCBT in adults have been published. To date, more than 10000 UCBT have been performed and reported to Eurocord; and around 4200 UCBT have been performed in adults.
The number of adults have increased after 2004 and since 2006 has surpassed the number of children transplanted with UCB. This increase is mainly due to a) studies that have shown similar outcomes of UCBT with HLA matched BM or PB donors, b) use of double CBT units to circumvent the problem of cell dose and c) the use of reduced intensity conditioning regimen that decreases the mortality related to transplantation. Most of the UCBTs are performed in adults with malignant disorders using double cord blood units. Table below shows the overall results for adults with specific diseases.

### High Resolution Typing for A, B, C, DR1 in Single-Unit Cord Blood Hematopoietic Stem Cell Transplantation

Mary Eapen, MBBS, DCH, MRCPI, MS, Medical College of Wisconsin

We studied the effect of allele-level matching in 1568 single umbilical cord blood transplantations for hematologic malignancy. The primary endpoint was non-relapse mortality. Only 7% of donor-recipient pairs were matched at HLA-A, -B, -C, -DRB1; 15% were mismatched at one, 26% at two, 30% at three, 16% at four and 5% at five alleles. Only 54% of units matched at HLA-A, -B and –DRB1 were actually matched at the allele-level at all loci. Non-relapse mortality was higher with units mismatched at one- (HR 2.79; 26%), two- (HR 2.69; 26%), three- (HR 3.60; 34%), four- (HR 3.48; 37%) or five-alleles (HR 4.61; 41%) compared to HLA-matched units (9%; p<0.001). Transplantation of UCB with cell dose < 3 x 10^7/kg was associated with higher non-relapse mortality independent of HLA-match. Compared to HLA-matched units, neutrophil recovery was lower with mismatches at three (OR 0.56, p=0.011), four (OR 0.55, p=0.014) or five (OR 0.45, p=0.009) but not at one or two alleles. Overall survival was not significantly different except for transplants mismatched at five-alleles (HR 1.63; p=0.01). These data support allele-level HLA-matching in the selection of single UCB units. Mismatches at one or two HLA-loci are better tolerated than three or four HLA-loci, and mismatches at five HLA-loci should be avoided.
Selected Abstract Presentation: Improved Virus-Specific Immune Reconstitution After Cord Blood Transplantation Using Cord Blood-Derived Virus-Specific T cells

Caridad Martinez, MD, Baylor College of Medicine

Martinez C,1 Hanley PJ,1 Leung K1, Brenner M1, Savoldo B1, Dotti G1, Rooney CM1, Heslop H1, Krance R1, Shpall EJ2, Bollard CM1.

1Center of Cell and Gene Therapy, Texas Children’s Cancer Center, Baylor College of Medicine, Houston, TX; 2University of Texas MD Anderson Cancer Center, Houston, TX.

Background: CMV, Adenovirus (Ad) and EBV (triVirus) are leading causes of mortality after cord blood (CB) transplantation (CBT). Virus-specific CTL (mCTL) from peripheral blood (PB) donors have been shown to treat and prevent viral-infections after bone marrow transplantation, but CB-derived CTL have never been administered to patients after CBT. We have now extended these studies by expanding mCTL from CB (CBmCTL) to restore triVirus immunity.

Methods and Patients: Development of CBmCTLs for CBT patients requires the priming of naive T-cells rather than the simple expansion of pre-existing memory T-cells. We have developed a novel protocol stimulating T-cells with autologous CB-derived dendritic cells and EBV-LCL transduced with an adenovirus carrying CMV-pp65. We have created a phase 1 study for CBmCTLs to be given after CBT. We report the outcome following CBmCTL in 7 patients with: malignant (n=3) and non-malignant (n=4) diseases. The median age at transplant was 1 year (range, 0.5-5 years). Two patients received a 6/6 matched cord and five patients received a 5/6 mismatched cord. All patients received a full ablative regimen consisting of busulfan, cyclophosphamide and fludarabine. No serotherapy was given. GvHD prophylaxis combined cyclosporine and MMF.

Results: Median time of neutrophil engraftment was 15 days (range; 11-21 days). Median day of CTL infusion was 83 days (range; 63-146). Five of 7 pts had no initial infection or reactivation episodes, remaining free of CMV, EBV, and Ad from 2 months to 2 years post-CBT. Of the two remaining pts, pt 1 was transiently viremic for CMV pre-infusion and became viremic 4-weeks post-CBmCTL. The pt received a 2nd dose of CBmCTLs and CMV-DNA/antigen became undetectable in the PB within 16 days of the 2nd dose. Analysis of this pt’s PB showed a rise 31-fold expansion of CMV-T-cells by 4 weeks after the initial CTLs. This pt also had AdV in his stool, which resolved without additional therapy. Shortly after CTL infusion, pt 4 had detectable EBV-DNA in the PB that was controlled without additional antiviral therapy.

None of the recipients of CBmCTL developed viral disease; in two pts with viral infections, the infections resolved without progression to disease, coinciding with the appearance of virus-specific T-cells in peripheral blood.

Conclusions: Administration of CBmCTL to pts after CBT has so far been safe and can facilitate reconstitution of virus-specific T-cells and control viral reactivation/infection in vivo.
Anti-HLA Antibodies and Outcomes after Cord Blood Transplantation

Annalisa Ruggeri, MD, Eurocord International Registry
Vanderson Rocha, Eliane Gluckman, Pascale Loiseau, on behalf of Eurocord, Haematologica. 2012

Unrelated cord blood transplantation (UCBT) is an alternative option for patients without a suitable HLA matched donor. (1) Tolerance of some degree of HLA mismatch, with relatively low rates of both acute and chronic graft-versus-host disease (GvHD) makes UCBT an interesting source of hematopoietic stem cells for both pediatric and adult transplants.

Different studies (2-4) comparing UCBT to matched unrelated donor transplant showed no significant difference in disease free survival in children and adults; however probability of neutrophil and platelets engraftment is lower and delayed after UCBT. With the aim to improve engraftment after UCBT many approaches are currently available such as the use of double UCBT (dUCBT) and reduced intensity conditioning regimen (RIC). (5) However, the hematopoietic engraftment remains lower after double UCBT when compared to other stem cell sources. (6)

Other strategies (7, 8) are under investigation to prevent graft failure (GF) by the intrabone infusion of UCB and the co-infusion of expanded single UCB unit. (9) Nevertheless, delayed engraftment and GF continue to be a major source of concern after UCBT.

Importantly, the detection of patient-, donor-, disease and transplant-related factors that are associated with engraftment may help clinicians to choose the best UCB unit and to improve the techniques of transplantation. Since most of UCBT are performed with HLA mismatched CB units (10) the presence of anti-HLA (donor specific antibodies, DSA) in the patients against the UCB unit can be an issue for engraftment. Anti-HLA antibodies before transplant may occur due to the alloimunization to HLA through blood transfusions, pregnancy and also in some unexposed individuals. (11-14) In unrelated donor recipients, Spellman et al reported pre-transplant anti HLA antibodies associated to GF and higher mortality. Interestingly, in this case control study, higher frequency of DSA anti HLA DP was reported. (13)

In the UCBT setting, few studies with controversial results are available on the impact of DSA and outcomes. Two series reporting respectively 386 (15) and 73 patients (15), receiving single or double UCBT showed an increased risk of GF and lower survival for patients with positive DSA. However, another report showed no association between the presence of DSA and transplant outcomes in 126 dUCBT recipients. (16)

In order to address the impact of the anti-HLA antibodies on outcomes after UCBT, in an independent dataset and using standardized methodologies for detection of DSA, we performed a retrospective registry based analysis including 294 patients given a RIC UCBT in 16 French transplant centers, from 2000 to 2011. The majority of the patients (82%) were transplanted for malignancies, 60% with dUCBT, 63% were HLA mismatched. Retrospectively, pre-UCBT serum was tested for HLA-Ab using LuminexTM platform. Results were interpreted as mean fluorescence intensity (MFI) against donor-specific mismatch.

Among 62 recipients (23%) who had anti-HLA-antibodies before UCBT, 14 patients had donor specific anti-HLA-antibodies (DSA) (7 were donor-specific-anti-HLA-antibodies for single-UCBT and 7 for double-UCBT). Donor-specific-anti-HLA-antibodies threshold ranged from 1620-17629 of mean fluorescence intensity (MFI). Cumulative incidence of day-60 neutrophil engraftment was 76%. It was 44% for recipients with donor-specific-anti-HLA-antibodies and 81% in those without donor-specific-anti-HLA-antibodies (p=0.006). The cumulative incidence of 1-year transplant related mortality was 46% in patients with donor-specific-anti-HLA-antibodies and 32% in those without antibodies (p=0.06). The presence of donor-specific-anti-HLA-antibodies was associated with a trend for decreased survival rate (42% vs. 29%, p=0.07).
Conclusion: Donor-specific-anti-HLA-antibody in recipients of unrelated cord blood transplant is associated with graft failure and decreased survival. Patient’s screening for donor-specific-anti-HLA-antibodies before unrelated cord blood transplantation is recommended before choosing a HLA mismatched cord blood unit. Whenever possible it is important to avoid selecting a unit when the patient has donor-specific-anti-HLA-antibodies against.

References
The Significance of the Direction of the HLA Mismatch in Cord Blood Matching and the Implication of Graft-Specific Anti-HLA Antibodies

Marcelo Fernandez-Vina, PhD, D(ABHI), Stanford University School of Medicine

Optimal selection criteria for unrelated donors (UD) and cord blood units (CBU) for allogeneic stem cell transplantation may result in beneficial outcomes including reduced incidence of primary graft failure (PGF), severe acute graft versus host disease (aGvHD) and patient survival. Histocompatibility factors play major roles determining these outcomes.

Some studies have shown that in CBU transplantation donor-recipient human HLA mismatches in the graft-versus-host only direction produce engraftment and survival comparable to HLA-matched CBU that in turn are superior than transplants mismatched in the rejection-only direction. These studies showed transplants where the CBU unit is homozygous for one HLA antigen/allele and the patient is heterozygous at the same locus had neutrophil and platelet engraftment rates that were comparable to recipients of transplantsations matched in HLA-A, -B, and -DRB1. In addition patients with hematologic malignancies given GvH only mismatch had lower transplantation-related mortality, overall mortality and treatment failure compared with those with one bidirectional mismatch or with mismatches in the HvG mismatch. Similar findings were made for MUD transplantation in CML without the infusion of ATG pre-transplantation. More recent studies have shown no differences in incidence of PGF between HLA mismatched transplants in which the mismatch occurs in the GvH vector only (homozygous patient) or in transplants with bi-directional mismatches.

The potential role of anti-HLA antibodies was investigated in several recent studies providing evidence of a significant role in causing graft rejection. Modern sensitive methodologies can rapidly evaluate the patient’s anti-HLA humoral sensitization status; evaluation of mismatches associated with increased graft rejection can be used to prioritize the selection of CBU or UD. It has been observed that patients who are homozygous in a specific locus have higher chances of presenting allo-antibodies that react with the mismatched antigen of the donor; therefore the impact of the unidirectional mismatch in the rejection direction may result from broad anti-HLA sensitization.

Mismatches in the GvH vector associate with increased risk of severe GvH and transplant related mortality while at the same time associate with reduced risk of disease relapse in patients with hematologic malignancies.

The direction of the HLA mismatch may have different impact in outcomes depending on the graft type and manipulation, the conditioning regimen, the immunosuppressive therapy the patient’s primary disease and the preservation of the patient’s immune system at the time of transplant.
Increased Relapse Associated with Mixed Donor-Donor Chimerism Following Double-Unit Cord Blood Transplantation

Michael Verneris, MD, University of Minnesota

We previously showed lower rates of relapse in recipients of dUCBT, compared to patients treated with single UCBT (Verneris, Blood, 2009). One unit predominates by D+100 in ~90% of patients and recent studies show that single unit dominance is associated with T cell recognition of the rejected unit (Gutman, Blood, 2010). In addition, higher rates of acute graft-vs-host disease (aGVHD) have been observed after dUCBT compared to single UCBT, regardless of age (MacMillian, Blood, 2009). Thus, we hypothesized that graft vs. graft interactions that occur during dUCBT may drive both GVL and GVHD reactions. To test this hypothesis, we reviewed the records of patients with hematological malignancies transplanted with a dUCBT from 2000 to 2011 at the University of Minnesota. Myeloablative (MA) conditioning was with cyclophosphamide (Cy 120 mg/kg), fludarabine (Flu 75 mg/m2) and total body irradiation (TBI 13.2 Gy) (n=178) and non-MA conditioning was with Cy (50 mg/kg), Flu (200 mg/m2) and TBI (2 Gy) (n=282). Acute GVHD prophylaxis consisted of cyclosporine and mycophenolic acid. At D+100 316 patients were evaluable and had either BM (n=273) and/or PB (n=178) donor engraftment data. Engraftment was assessed using short tandem repeat analysis. “Mixed donor-recipient chimerism” was defined as >5% host cells and “dual chimerism” was defined as complete donor chimerism (donor 1 + donor 2), with a minimum contribution of 5% by each unit (5% is the lower limit of detection for this clinical test). As expected, patients with evidence of mixed donor-recipient chimerism in the blood or marrow at D+100 (n=35) had high rates of relapse (69% [95% CI, 49-89%] at 2 years) and were excluded from further analysis. Of the patients with evaluable peripheral blood chimerism data, 90.4% (n=161) had hematopoiesis derived from a single unit, while 9.6% (n=17) showed dual chimerism after dUCBT. In multivariate analysis, dual chimerism at D+100 was associated with higher relapse (HR=1.97 [95% CI, 1.08-3.59], p=0.01), less grade II-IV aGVHD (HR=0.36 [95% CI, 0.17-0.78], p=0.01) and a trend toward worse DFS (HR=1.68 [95% CI, 0.9-3.13], p=0.1). Dual chimerism was not associated with age (p=0.32), nor non-MA conditioning (p=0.15). Recipients of two HLA 6/6 matched units (with each other and the recipient), were more likely to have dual chimerism (p<0.01). Nearly identical results were obtained in a separate model examining BM engraftment (single (n=247) and dual chimerism (n=26)) and transplant outcomes. While these findings should be confirmed in a separate dataset, they suggest that an unexpected consequence of selecting well matched units for dUCBT is higher mixed donor-donor chimerism, less aGVHD and more relapse. Further study of dUCBT and GVL is needed.
Outcomes of Double-Unit Cord Blood Transplantation in Patients with Malignant Disorders: Eurocord Registry Analysis

Vanderson Rocha, MD, PhD, Oxford University
Annalisa Ruggeri and Eliane Gluckman, on behalf of Eurocord

Because cell dose is considered to be a critical determinant of outcomes in umbilical CB transplantation, the Minneapolis group has demonstrated that transplantation of two partially HLA matched cord units may overcome the problem of cell dose and make the transplantation of heavier adult patients feasible. This strategy has led to an increased number of adult patients receiving UCB transplantation. To-date around 10,000 unrelated cord blood transplants (UCBT) have been reported to Eurocord. Of these 1400 UCBT have been performed with two cord blood units (dUCBT) from March 1999 to December 2012. The vast majority of patients were adults (n=1055) transplanted with hematological malignant disorders. The median age at transplant was 45 years (18-76), median weight 71 kg (40-151) median number of collected nucleated cells 4.9x10^7/kg (2.1-14.8) and median follow-up: 14 months (1-85). Considering the highest HLA disparities between CB units and patients, 73% were transplanted with 4/6 CB. Conditioning regimen was reduced intensity (RIC) in 67% (mostly CY+FLU+TBI2Gy) and 33% were myeloablative (MAC) (70% TBI based). Five hundred seventy eight patients were transplanted with acute leukemias (68% AML and 32% ALL) in CR1 (n=230) CR2 (n=177) or advanced phase (n=127). For patients with AL, overall neutrophil recovery was 88% and it was 93% after MAC and 85% for RIC. Two year overall survival for patients transplanted with RIC in CR1 (n=136), CR2 (n=102) and advanced disease (n=72) was 66%, 54% and 33% respectively. For those patients transplanted with MAC, 2-year overall survival for patients transplanted in CR1 (n=90), CR2 (n=75) and advanced disease (n=54) was 58%, 42% and 21% respectively. Around 15% of dCBT were performed in children (median weight 45kg). For those children transplanted with acute leukemias (n=117) in CR1, CR2 and advanced phase of the disease, overall survival at one year was 65%, 53% and 39% respectively.

DCBT has extended the use of cord blood cells in patients for whom a cell dose in a single CB unit is not sufficient and is associated with higher mortality. Despite short follow up in adults and children the results of dCBT are encouraging. Double cord blood units selection, GVHD prophylaxis and type of conditioning regimen are factors that have to be analyzed with more details with the aim to improve final outcomes.
Cord Blood Transplantation Supported by Third-Party Donor Cells: Rationale, Results and Applications

Koen Van Besien, MD, PhD, Weill-Cornell Medical College

Transplantation of unrelated umbilical cord blood stem cells is an effective method of HLA-mismatched transplant with low rates of graft vs host disease and powerful GVL effects. Support by third party donor cells, through transient engraftment of adult hematopoietic stem cells largely overcomes the problem of delayed engraftment. We summarize the available data on US studies of co-infusion of adult CD34 selected hematopoietic stem cells, largely from two institutions. With the earliest patients now followed for up to five years after transplant, the incidence of chronic GVHD remains low. We also briefly discuss current models of the fetal immune system which provide plausible underpinnings for the powerful GVL effects and low GVHD of UCB grafts.

Single-Unit Cord Blood Combined with HLA-Mismatched Third-Party Donor Cells: Comparable Results to Matched Unrelated Donor Transplantation in High-Risk Patients with Hematologic Disorders

Mi Kwon, MD, Hospital General Universitario Gregorio Marañón

Matched unrelated donor (MUD) transplantation is the first alternative in the absence of a matched sibling donor. For patients without a suitable adult donor, we have adopted the dual or haplo-cord stem cell transplantation (SCT) protocol consisting of cord blood (CB) in combination with CD34+ cells from a third party HLA-mismatched donor. The objective of this study was to analyze toxicity and survival rates of adults who underwent dual SCT in our center and to compare these rates with those in a cohort of patients who underwent myeloablative MUD SCT in the same time period. Starting in 2004, a total of 20 patients with high-risk disease underwent 22 dual transplants and 25 patients underwent myeloablative MUD transplantation. The conditioning regimen for dual SCT included fludarabine 30 mg/m2 (days -8 to -5), antithymocyte globulin (ATG) 2 mg/kg (days -2 and -1), cyclophosphamide 60 mg/kg (days -4 and -3), and i.v. busulfan 3.2 mg/kg (days -6 and -5) or 10 Gy of fractionated total body irradiation (TBI). CB cells were infused on day 0, followed by the TPD cells either the same day or on day +1 posttransplantation. As graft-versus-host disease (GVHD) prophylaxis, patients received cyclosporine (CsA) from day -5, and metilprednisolone 2 mg/kg from day -2 and tapered until suspension on day +10. Conditioning for MUD transplantation included i.v. busulfan 3.2 mg/kg for 4 days and either cyclophosphamide 60 mg/kg (days -3 and -2), fludarabine 40 mg/m2 for 4 days, or 12 Gy of fractionated TBI. ATG 2.5 mg/kg on days -3, -2, and -1 was included in all but 2 MUD transplantations. GVHD prophylaxis was provided by CsA starting on day -7 and a short course of methotrexate (15 mg/m2 on day +1 and 10 mg/m2 on days +3, +6, and +11).

Patient characteristics were not statistically different between the 2 groups. The 30-day cumulative incidence of neutrophil engraftment was similar in both groups (91% and 95%), with a median time to engraftment of 14 and 16 days, respectively (Figure 1, A). For the dual group, the cumulative incidence of platelet recovery at 60 days was 78%, with a median time of platelet engraftment of 27 days (range, 9-84 days). For the MUD group, platelet engraftment occurred in a median of 12 days (range, 9-41 days), with a cumulative incidence of 95% at 30 days and 87% at 60 days. (Figure 1, B).
Grade II-IV acute graft-versus-host disease was more frequent in the MUD group (40% versus 5%, Figure 2).

Except for a tendency toward a higher incidence of viral hemorrhagic cystitis in the dual transplantation group, posttransplantation infectious events were comparable in the 2 groups.

The 3-year cumulative incidence rates of relapse (41% versus 44%) and nonrelapse mortality (30% versus 25%) were similar in the MUD and dual transplantation cohorts (Figure 3). Estimated 3-year overall survival and disease-free survival were 47% and 41%, respectively, with no survival advantage for either group (Figure 3).

In our experience, single CB transplantation together with the coinfusion of CD34+ cells from a third party HLA-mismatched donor in high-risk patients offers time to engraftment and survival rates comparable to those seen with myeloablative 8/8 HLA-matched MUD transplantation, with significantly lower GVHD rates. Thus, this CB transplantation approach merits broader exploration and validation in different hematologic diseases and centers.
Cord Blood Transplantation Using Cells Expanded In Vitro

Colleen Delaney, MD, Fred Hutchinson Cancer Research Center

The low cell dose available in a cord blood graft is directly correlated with a significant delay in hematopoietic recovery and a lower incidence of sustained donor engraftment. Furthermore, cord blood transplant (CBT) patients are at a higher risk of early non-relapse mortality (NRM), with the highest risk of NRM being in those patients with a time to neutrophil engraftment of ≥26 days, the median time to engraftment. Thus, strategies aimed at decreasing the prolonged period of neutropenia post CBT are likely to have an impact on survival. With this goal, we have successfully developed a novel and clinically feasible methodology utilizing an engineered Notch ligand for the ex vivo generation of increased numbers of CD34+ cells that is not only safe, but also resulted in earlier neutrophil engraftment post myeloablative CBT. In fact, a significant reduction in the median time to an absolute neutrophil count (ANC) of 500/l to just 11 days was observed compared to 25 days in a concurrent cohort of patients not receiving expanded cells (updated data).

However, this approach of real time, on demand, cGMP-compliant expansions starting from previously cryopreserved units that are at least 4/6 HLA-matched to the recipient is neither clinically feasible outside of highly specialized transplant centers, nor economically cost effective as it would require maintenance of a very large inventory of stored cord blood units to ensure finding an appropriate match to initiate the expansion cultures. This approach is further complicated by manufacturing on a tight timeline dictated by the patient’s treatment, not allowing for efficient usage of resources, facilities and personnel. In contrast, development of an “off-the-shelf” pre-expanded cellular therapy would obviate the requirement for real time production, and with no requirement for HLA-matching (or even minimally matched at one loci), would permit maintenance of a minimal inventory of expanded products that are immediately available (and easily shipped) for most patients regardless of ethnicity.

We have now successfully demonstrated the safety and clinical feasibility of using a non-HLA matched, ex vivo expanded and cryopreserved cell product in the myeloablative CBT setting. Our data using ex vivo expanded cord blood stem and progenitor cell products, both partially matched and as a third party donor product, in the setting of myeloablative cord blood transplantation will be discussed. The limitations of each approach will be presented, and plans for the future included. In addition, a brief overview of the current state of the art in the area of cord blood graft manipulation will also be discussed.

References

Myeloablative Cord Blood Transplantation for Hematologic Malignancies is Comparable to Unrelated Donor Transplantation: A Retrospective Single-Center Study

Filippo Milano, MD, PhD, Fred Hutchinson Cancer Research Center

**Background:** The number of cord blood transplants (CBT) is rapidly increasing with suggestion of outcomes comparable to those obtained after unrelated donor transplantation (URD). We conducted, a retrospective analysis comparing post-transplant outcomes between myeloablative CBT and myeloablative URD at our Institution. Methods: Between January 2006 and December 2011 a total of 488 patients received either a CBT (n=88) or URD (n=400). Of these 400, 358 (90%) received a 10/10 HLA-matched (MURD) and 42 (10%) a ≤9/10 HLA-mismatched (MMURD) graft. All patients received a double CB graft except for 12 patients (13%) who received a single CB unit. In addition, 25 (28%) patients received an ex vivo expanded graft as part of either a single or double CBT. Mycophenolate mofetil and cyclosporine were used for graft-versus-host disease (GVHD) prophylaxis in all CBT recipients, while FK506 + methotrexate was preferentially used among URD patients (n=339, 84%). Conditioning regimens for both groups are summarized in Table 1. Time-to-event outcomes were compared between groups using Cox regression, and logistic regression was used for acute GvHD. All models were adjusted for age, disease risk and CMV serostatus.

**Results:** Patient characteristics are shown in Table 1. Differences between groups included higher median age in URD recipients and a higher proportion of non-caucasian and CMV seropositivity in CB recipients. Disease risk was similar between the 2 groups. Peripheral blood stem cells (PBSC) was used for the majority of URD grafts (61%). The median time to neutrophil [URD 19 days vs CBT 23 days; hazard ratio (HR) 1.91 (1.46-2.51, p<0.0001)] and platelet recovery [URD 19 days vs CBT 45 days; HR=2.76 (2.05-3.71, p<0.0001)] was significantly shorter for URD recipients. In multivariate analysis, the risk of mortality was similar in URD vs CBT (HR=1.11 (0.71-1.73, p=0.64)). When HLA-match status was considered in the URD group, the risk of death was higher in the MMURD group compared to CBT, although the difference was not statistically significant (HR=1.37 (0.85-2.26, p=0.22)). The risk of relapse was suggestively higher in the URD group overall relative to CBT (HR=1.90 (0.94-3.84, p=0.07)), and this difference was enhanced when HLA matching and source of stem cells in URD were considered. In particular, recipients of unrelated (matched or mismatched) PBSC had a higher risk of relapse relative to CBT (HR=2.33 (1.11-4.91, p=0.03)), as did the MMURD (BM or PBSC) group (HR=2.34 (1.07-5.11, p=0.03)). Furthermore, unrelated recipients of matched or mismatched PBSC each had a higher risk of relapse relative to CBT (matched PBSC: HR=2.44 (1.11-5.38, p=0.03); mismatched PBSC: HR=3.89 (1.63-9.30, p=0.002)). The combined results for mortality and relapse led to an increased risk of relapse-free survival (RFS) failure (earliest of relapse or death) for patients receiving PBSC from amismatched URD (HR=1.88 (1.08-3.27, p=0.03)); the risk of failure was also increased for PBSC recipients from a matched URD, but the difference was not statistically significant (HR=1.42 (0.87-2.32, p=0.16)). The risk of non-relapse mortality (NRM) was similar between URD and CBT (HR=0.89 (0.52-1.53, p=0.67)), and while there was less chronic GvHD in the URD group, the difference was not statistically significant, and this slight reduction was largely due to the effect in BM recipients (URD BM vs CBT, HR=0.59 (0.35-0.98, p=0.04); URD PBSC vs CBT, HR=1.01 (0.62-1.66, p=0.95)). The risks of grades 2-4 and 3-4 acute GvHD were similar between URD and CBT groups (odds ratio (OR) 1.10 (0.57-2.11, p=0.78)), and (OR=0.70 (0.38-1.31, p=0.26)), respectively.

**Conclusions:** Our data suggest that OS, RFS and NRM after CBT are not inferior to those observed after URD transplantation, and OS and RFS might be higher when the URD group is restricted to recipients of PBSC, particularly those who are mismatched with their unrelated donor. Relapse occurred less frequently for CBT recipients especially when compared to MMURD or URD with PBSC. The retrospective nature of this study and the heterogeneity of the population do not allow us to draw definitive conclusions, however, our results reinforce the need for a randomized study to definitively address these comparisons.
### TABLE 1

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<th>CORD (N=88)</th>
<th>URD (N=400)</th>
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<td>Median Weight in kg (range)</td>
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<td>- High-dose (1320/1200 cGy)</td>
<td>88 (100)</td>
<td>211 (52)</td>
<td>0.33</td>
</tr>
<tr>
<td>- Low-dose (200/400 cGy)</td>
<td>65 (74)</td>
<td>151 (72)</td>
<td></td>
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<tr>
<td>Donor match</td>
<td></td>
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<tr>
<td>Unrelated-Matched (MURD)</td>
<td>-</td>
<td>297 (74)</td>
<td>-</td>
</tr>
<tr>
<td>Unrelated-Mismatched (MMURD)</td>
<td>-</td>
<td>103 (26)</td>
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Expenses as an Issue Inhibiting the Use of Cord Blood Transplantation

Michael Boo, JD, National Marrow Donor Program

Unrelated donor cord blood transplant has been shown to provide equivalent outcomes and lower GVHD compared to other sources of unrelated donor grafts. It has also expanded access for those who do not have an acceptable adult donor. However, it has been noted in many presentations on the economics of cord blood banking and transplantation that the cost of cord blood transplantation exceeds the cost of other stem cell source based transplant processes, including other unrelated adult donor sources and haplo-identical transplants. Some cite this cost as an additional barrier to further adoption or expansion of cord blood activity at transplant centers. As noted below, there is justification for this view, but strategies can be developed to address many of these cost based concerns.

The costs associated with cord blood transplantation can be divided into two areas, the cost of the product itself and the cost of the procedure.

Product related costs. There are many factors that contribute to the product costs. These factors include:

- Cost of collection: Factors that contribute to the cost of collection including the collection model used, whether OB/GYN volunteer service based or cord blood bank staff based, resource commitment at the collection institution, cost of kits and other components of the collection activity itself, and restrictions placed upon the collector by the institution.

Other costs of collection are demographically related. To insure a diverse registry, collection must be targeted to all ethnicities that require collection across all socio-economic groups. Communities in lower social economic areas tend to experience higher incidents of deferral through the screening process. In addition, within certain populations, a higher rate of rejection of units is experienced based on TNC.

- Costs of banking: Factors that impact costs of banking include the TNC cut off decision by the bank for an acceptable unit, the source of testing services and the costs associated with those services, and the costs of processing. It is interesting to note that automated or enclosed systems have not been found to be more cost effective than manual processing, but have improved quality controls and verification of processes.

- Cost of regulation: In countries where regulations have been adopted, these typically have imposed additional responsibilities on the banks to maintain good tissue processes or good manufacturing processes (cGMPs). Often, as in the United States, compliance with licensure has resulted in higher capital costs associate with facility improvements to meet cGMP requirements and higher staffing costs to comply with necessary quality oversight requirements imposed by the licensing entity.

- Other factors that have contributed to the cost of banking include the research interest of the institution and the amount of that cost imposed upon the bank, the costs of overhead associated with the sponsoring institution. Higher costs of overhead imposed by academic medical centers may be different than costs imposed by blood centers, for instance. And the scale of operations may contribute to higher costs as well.

Procedure related costs. The cord blood transplant has higher procedure related costs including:

- Expense of CBU. CBUs are priced across quite a range as each bank sets its own price, and additional cost may be imposed for further testing, registry related services, and transportation. In addition, to the extent more than one cord blood unit is needed for the patient, this can double or triple the costs of graft source.

- Time to engraftment.

Cord blood transplant requires longer time to neutrophil and platelet recovery, which requires a longer hospital stay and increased utilization of resources associated with complications during the period of time until engraftment, as well as thereafter. In addition, UCB transplant is associated with a higher risk of non-engraftment contributing to costs of managing the patient at the transplant center. Contracting by third party payors for transplantation services generally pays a global fee for the first hospitalization associated with preparation for transplantation, the procedure itself and initial recovery. As duration of hospital stays are longer for recipients of UCBT, the global fee doesn’t always cover the costs to provide these services.

Other considerations are also at play. Low demand for the product and the need for large inventories to improve
match rates for patients require cord blood banks to spread the cost over a relatively small sales opportunity. With current trends that include fewer cord blood transplants and/or less double cord blood transplants, demand pressure will increase on the cord blood banks.

In spite of these concerns, UCB transplant continues to provide a needed option to patients and many of these issues can be addressed. For instance, efforts to reduce the time to engraftment through expansion or other technologies have shown promise. And studies are underway that use UCB to treat other diseases that may create greater demand. These efforts and others should be pursued to assure that current market conditions do not inhibit the growth of this valuable cell source.

**Confronting the Cost of Cord Blood Transplants**

Sergio Querol, MD, PhD, Anthony Nolan Cord Blood Bank & Barcelona Cord Blood Bank, Banc Sang i Teixits

The fact that a CB donation becomes a long-term available product if adequately stored in liquid nitrogen tanks makes unnecessary a continuous growth of the inventories. When fully established, a CB bank should focus on the renewal of the CBUs used for transplantation and therefore equating the cost of collection and processing to the revenues by the use. This change in the objective, from a continuous growth to a maintenance phase, becomes critical to make affordable the CB transplantation. In order to be competitive, it is our opinion that cost of CB procurement should not exceed that of other similar alternatives particularly that adult volunteer donor provision. Especially important is to evolve the concept of costing per procedure independently if it requires one or two units. Double CB transplantation was developed as a solution to improve safety of CBT and looks reasonable to discount cost of a CBU to correct this kind of “incomplete graft” to guarantee a successful engraftment. But, what is the cost of a CBU shipped for transplantation? This depends on two major factors: the unitary cost of production and the likelihood of being selected for transplantation. For instance, with a model of use of 5% in a defined period, an inventory of 10,000 units will generate 500 shipments. If the cost of production of a CBU is in the region of €1,500, the estimated fee for a CB procured will be in the region of €30,000. This is likely the current situation. But, any further increase in size of the CBB will result in a decrease in the probability of use and a subsequent increase of the cost of the unit provided (the growth paradox, see figure 1).

Figure 1. Match prediction according match level and inventory size (data taken from Querol S, Rubinstein P, Marsh SG, Goldman J, Madrigal JA. Cord blood banking: ‘providing cord blood banking for a nation’. Br J Haematol. 2009 Oct;147(2):227-35. Review) (Y axis, probability of match according 4± or 5+/6 HLA-A, -B 2-digits and DRB1 4-digits resolution; X axis, size of the CB inventory)

Under a nation perspective, we proposed an optimal size of 50,000 for UK considering a 5+/6 match as the right target for an acceptable clinical outcome. But the optimal target might be unaffordable because the probability of use of each individual unit decrease too much when growing far above 10,000 units (we projected that cost of unit provided by a 50,000 inventory will cost 2 times more than that of a 10,000 one for a
projected transplantation of 100 units per year). For that reason, we propose that the correct target of a CBB bank should be based not in optimal inventories but in minimal size that guarantees an economical affordability but serving the objectives pursued. For instance, Figure 1 shows how an inventory of around 15,000 highly cellular CB units can provide a 4+/6 match donor for almost all patients (95% of searches either in UK and Spain but also ensuring 50% of grafts being on the 5+/6 category).

So, if we use the current scenario of worldwide size (500,000 CBUs) and use (4,000/year), fees for a CB unit on the basis of covering renewal of the inventory with only highly cellular unit (more than 1.5E9) to substitute those transplanted and those becoming obsolete (we estimate that a renewal rate of 5% (meaning storing 25,000 new units worldwide per year) of the current inventory could suffice) would result in a cost of provision of around 10,000 per unit that will probably make CB banking and transplantation sustainable. For instance, with the current worldwide inventory, 75% of pediatric and 50% of adult patients are transplanted already with 5+/6 matched units (data taken from the WMDA annual report). This global approach can be transferred to any single CBB. To complete the goal of a donor for everyone, international networking of high quality, highly-diverse units will still be necessary.

**Combined Haploidentical Cord Blood Transplantation to Shorten Engraftment Time and Hospitalization Period**

**Koen Van Besien, MD, PhD, Weill-Cornell Medical College**

Umbilical Cord Blood Transplantation, compared with transplants from adult donors, is more costly. Major determinants of cost include the frequency of complications and the length of stay. The economic impact of long term care and management of chronic GVHD are unclear, but likely to be considerable. Haplo Cord Transplantation is associated with reduced duration of admission compared to historical controls undergoing double UCB SCT and the potential implications for cost are discussed.

Lower UCB cell doses, may be acceptable with haplo cord SCT and the potential implications for long term outcomes and for cord banking will be discussed.
Comparison of Cord Blood Transplantation and Haploidentical Stem Cell Transplantation

Richard Champlin, MD, University of Texas MD Anderson Cancer Center

Despite the international network of registries for unrelated donors, an HLA matched donor cannot be identified for a substantial fraction of patients, particularly for non-Caucasian patients and patients of mixed ethnic backgrounds.

There has been major progress in performing cord blood transplants and also for haploidentical transplants. Cord blood transplants can be successfully performed from donors matched for 4 or 5 HLA A, B, and DR antigens, and such a donor can be identified for >90%1. Results depend on cell dose and HLA match2. The relatively low cell dose is associated with a relatively slow rate of hematopoietic recovery, but ex vivo expansion of hematopoietic progenitor cells can accelerate engraftment.3 Transplantation of two cord blood units provides and adequate cell dose to allow treatment of larger adult patients4,5. Recent studies have shown comparable results of double cord blood with matched unrelated donor transplants, and some studies report a lower risk of malignancy relapse with cord blood, consistent with a more potent graft-vs-malignancy effect4,.

There has been a major advance in haploidentical hematopoietic transplantation using post transplant cyclophosphamide, tacrolimus and mycophenolate for GVHD prophylaxis6. Recent studies have reported a low rate of graft-vs-host disease and treatment related mortality. Some studies indicate comparable results as with transplants from matched unrelated donors7,8. Nearly all patients will have a potential haploidentical donor.

Phase 2 studies have indicated similar overall survival with cord blood or haploidentical hematopoietic transplantation9. The pros and cons for each approach will be discussed. Both methods have an advantage to use of unrelated adult donors, in that the transplants can be provided more promptly. A prospective randomized study is ongoing to compare these two approaches.

References:
Graft-Versus-Host Disease (GVHD) in Cord Blood Transplantation

Amin M. Alousi, MD, University of Texas MD Anderson Cancer Center

Umbilical Cord Blood transplantation (UCBT) has expanded hematopoietic allogeneic transplantation to patients who lack a human leukocyte antigen (HLA)-matched sibling or unrelated donor. This is primarily due to the fact that HLA mismatches are more “permissible” resulting in rates of graft-versus-host disease (GVHD) comparable to matched unrelated donors. This reduction in GVHD has not come at the expense of graft-versus-malignancy as relapse rates following UCBT have been comparable or superior to other donor sources. However, despite a reduction in GVHD risk, outcomes have been hampered by increased rates of treatment-related mortality (TRM) following UCBT. This lecture will serve as a clinical overview of GVHD occurring following UCBT and will include a review of the current understanding regarding the unique immune-biology of cord blood. Additionally, a review of risk factors for acute and chronic GVHD following UCBT along with controversies in the field related to its prevention will be illustrated. The current literature related to non-relapse mortality (NRM) resulting from GVHD following UCBT and how it compares to GVHD-specific NRM following other donor sources will be presented including the impact and timing of severe bacterial, fungal and viral infections. Lastly, the talk will conclude with a discussion of future strategies using adoptive cellular therapy with umbilical cord blood expanded regulatory T cells (Tregs).

Multicenter Study of Third-Party Virus-Specific T cells to Treat Adenovirus, Epstein-Barr Virus or Cytomegalovirus Infections after Hematopoietic Stem Cell Transplantation

Ann M. Leen, PhD, Baylor College of Medicine

Adoptive transfer of virus-specific T-cells (VSTs) can reconstitute antiviral immunity to Epstein-Barr virus, cytomegalovirus and adenovirus in allogeneic HSCT recipients. However, the time taken to prepare patient-specific products and the lack of virus-specific T-cells in cord blood and seronegative donors restricts application. As part of the NHLBI-SCCT program we evaluated, in a multicenter setting, whether infusion of “off the shelf” VSTs from third party donors would overcome this limitation and prove feasible, safe and effective in HSCT recipients with refractory infections. Using the NHLBI-PACT program we prepared a bank of 32 virus-specific lines from individuals with common HLA polymorphisms who were immune to Epstein-Barr virus, cytomegalovirus or adenovirus. Eighteen lines were administered to 50 patients with severe, refractory illness due to infection with one of these viruses after hematopoietic stem cell transplant. The cumulative rates of complete or partial responses at 6 weeks post-infusion were: 74% (95% CI: 58.5%-89.5%) for the entire group (n=50), 73.9% (51.2-96.6%) for cytomegalovirus (n=23), 77.8% for adenovirus (n=18), and 66.7% (36.9-96.5%) for Epstein-Barr virus (n=9). Only four responders had a recurrence or progression. There were no immediate infusion-related adverse events, and only two subjects developed de-novo graft-versus-host disease. Despite the disparity between the lines and their recipients, the mean frequency of virus-specific T-cells rose significantly post-infusion, coincident with striking decreases in viral DNA and resolution of clinical symptoms. These results show that it is feasible and safe to implement adoptive immunotherapy for Epstein-Barr virus, cytomegalovirus and adenovirus using banked allogeneic VSTs, and that the approach may be both practicable and effective for a high proportion of recipients who otherwise lack options for the treatment of intractable virus infections after HSCT.
Cord Blood Cells for Immunotherapy Using CAR+ T cells

Laurence James Neil Cooper, MD, PhD, University of Texas MD Anderson Cancer Center

T cells can be manipulated ex vivo for improved therapeutic potential in vivo. One approach to redirecting T-cell specificity is through the enforced expression of a chimeric antigen receptor (CAR) to target CD19 on malignant B cells, independent of human leukocyte antigen. Clinical trials at MD Anderson Cancer Center (MDACC) and elsewhere are infusing CD19-specific CAR+ T cells that are derived from peripheral blood. Our methodology at MDACC is based on the electro-transfer of DNA plasmids coding for the Sleeping Beauty (SB) system and selectively propagating CAR+ T cells on artificial antigen presenting cells. We have now adapted this manufacturing process to generate CD19-specific T cells from umbilical cord blood (UCB). These CAR+ T cells recognize CD19, as can generate cytokines in response to and kill malignant B cells, as well as can sustain proliferation in a CAR-dependent manner. We have opened a clinical trial to test the human application of allogeneic UCB-derived CD19-specific T cells (IND #2010-0835, NIH-OBA #1001-1022, IRB# 14739, ClinicalTrials.gov Identifier NCT01362452). This trial is based on harvesting small numbers of mononuclear cells (MNC) at the time of thawing the UCB unit(s) used to reconstitute a recipient’s hematopoiesis. In compliance with current good manufacturing practice, these MNC are used to generate CAR+ T cells based on genetic modification with SB system and their numeric expansion by repeated addition of g-irradiated aAPC in the presence of soluble interleukin (IL)-2 and IL-21. In summary, we provide a clinically-appealing approach to targeting CD19 after UCB transplantation based on the timed infusion of donor-derived CAR+ T cells.

Autoimmune Diseases following Cord Blood Transplantation

Annalisa Ruggeri, MD, Eurocord International Registry

Thomas Daikeler, Eliane Gluckman, Dominique Farge, Vanderson Rocha, on behalf of Eurocord, Blood. 2013

Existing data on the nature, treatment and outcome of autoimmune diseases (AD) occurring following the autologous and/or allogeneic haematopoietic stem cell transplantation (HSCT) for different indications have been summarized in recent reviews 1-2. Mostly, these ‘new’ AD are autoantibody mediated organ specific, e.g. autoimmune thyroiditis, autoimmune hemolytic anaemia (AIHA) or immune thrombocytopenia (ITP) and more rarely multisystemic, e.g. systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Data on incidence and risk factors are only emerging, and this has been first investigated in a larger cohort for AD occurring after HSCT used as treatment option for primary autoimmune disease3,4. Besides bone marrow and peripheral blood, hematopoietic stem cells originating from umbilical cord blood are being used for HSCT. Cord blood derived lymphocytes are naïve in contrast to adult lymphocytes, resulting in decreased stringency of HLA match and the relatively lower risk of graft versus host disease (GvHD). The application of ‘double’ cord blood transplantation (CBT) to overcome the dose limit of single units in adults might modify the early lymphoid reconstitution5. These immunological and genetic properties may impact on the development of AD after CBT. Reports of AD occurring after CBT are scarce and based on single case reports and small series, the largest including 10 patients6, with the most prevalent being AIHA6-7, EVANS syndrome8, ITP6,9, thyroiditis10 and bullous dermatoses (pemphigus and pemphigoid)11,12.

This study was undertaken to characterize the nature, incidence and risk factors for autoimmune diseases developing after CBT in a large patient population. CBT were performed between 1992 and 2008 in EBMT centers and reported to Eurocord. Fifty-two out of 726 reported patients developed at least one AD within 212 (27-4267) days following CBT. AD occurred in more than 6% of patients from 5 weeks up to more than 10 years after CBT. Most were organ-specific autoimmune diseases such as cytopenias followed by autoimmune diseases of the thyroid and few cases of miscellaneous multisystemic AD. AD target haematopoietic [autoimmune haemolytic anaemia (n=20), EVANS syndrome (n=9), autoimmune thrombocytopenia (n=11), immune neutropenia (n=1)] and other tissues [thyroiditis (n=3), psoriasis (n=2), Graves’ disease (n=1), membranous glomerulonephritis (n=2), rheumatoid arthritis (n=1), ulcerative colitis (n=1) systemic lupus erythematosus (n=1)]. Four patients developed two AD (3 ITP followed by AIHA and 1 EVANS syndrome with rheumatoid arthritis). By multivariate analysis, the main risk factor for developing
an AD was non-malignant disease as indication for CBT (p = 0.0001). Haematological AD were most often treated with steroids, rituximab and cyclosporine. With a median follow-up of 26 months (2-91 months), 6/52 patients died as a consequence of AD.

In conclusion, CBT may be followed by potentially life-threatening, mainly haematological AD. Patients transplanted for non-malignant diseases are considered to be at an increased risk for developing post-transplant AD. AIHA and ITP were most commonly observed and therefore have to be considered in any patient at any time following CBT as a possible reason for an unexplained cytopenia. In patients not responding to initial treatment with steroids, rituximab may be an effective second line treatment option. Both the clinical and the mechanistic aspects of new AD occurring after hematopoietic stem cell transplantation merit further attention and research.

References:

Cord Blood Natural Killer Cells for Immunotherapy

Nina D. Shah, MD, University of Texas MD Anderson Cancer Center

Natural killer (NK) cells are important mediators of anti-tumor immunity and are active against several hematologic malignancies. NK cells can be activated by cytokines, antibodies or a shift in the balance between their activating and inhibitory receptors. Specifically, NK cells are thought to be cytotoxic to cells lacking appropriate self-major histocompatibility complex (MHC) class I molecules via disinhibition of the killer immunoglobulin-like receptor (KIR). Umbilical cord blood (CB) is a promising source of allogeneic NK cells but large scale ex vivo expansion is required for generation of clinically relevant CB-derived NK (CB-NK) cell doses. In addition, naïve CB-NK cells require activation for cytotoxicity against tumor targets. In this presentation, we will discuss novel, GMP-compliant strategies for expanding NK cells for adoptive cellular therapy. In addition the phenotypic evolution and anti-tumor activities of expanded CB-NK cells will be presented. Finally, we will discuss the rationale for allogeneic (versus autologous) adoptive NK cell therapy and, specifically, the potential advantage of CB-derived NK cells over peripheral blood sources.
Dipeptidyl peptidase-4 (DPP4) Regulation of Cell Growth as Related to Stem Cells and Regenerative Medicine Potential

Hal E. Broxmeyer, PhD, Indiana University School of Medicine

The physiology and pathology of organs and tissues depends respectively on normal or disordered regulation of their inherent cell types. Hematopoietic stem (HSC) and progenitor (HPC) cell functions are regulated in paracrine fashion by cell released cytokines, chemokines, and other growth modulating factors which induce their effects through specific receptor-mediated intracellular signaling.1-3 Such proteins also regulate other stem and progenitor cell types, and influence functions of more mature cells. While many studies have elucidated activities of these proteins for cell and intracellular effects, little effort has gone into understanding how changes in the biomolecules themselves influence steady state or stressed cell functions in health and disease, and the roles that enzymes may play in modifying biomolecule activity. This presentation focuses on Dipeptidylpeptidase (DPP) 4, recently shown to influence the functional activity of a number of chemokines, colony stimulating factors (CSFs), and interleukins (ILs) for effects on HSCs, HPCS, and hematopoiesis.4-6 We believe that DPP4 plays an important, under recognized role in modifying the activities of many different proteins that influence a multitude of different cell, tissue and organ responses via protein truncation. DPP4, discovered in 1966, is a 110 Kd member of the prolyl oligopeptidase family,7 whose crystal structure has been described in complex with a substrate analog.8 DPP4 functions as a serine protease, selectively cleaving the N-terminal, penultimate proline or alanine of proteins; other amino acids, such as serine, in the second position, may also be able to be cleaved.9 DPP4 is found as both a type II cell surface protein (CD26), and as a soluble molecule lacking intracellular and transmembrane domains.10 DPP4 is found in serum/plasma, cerebrospinal fluid, synovial fluid, and semen.11,12 The transmembrane form of the protein is ubiquitously expressed in many tissues including bone marrow, venular end of blood vessels, lung, spleen, pancreas, kidney, liver, intestines and epithelial cells.10,13 It is expressed on embryonic stem cells (ESC),14 HSCs and HPCs, as well as on other more mature blood cells such as memory T cells,15 and may be expressed on other stem and progenitor cell populations. Two recent papers have reviewed information on the enzymatic activities of DPP4,16,17 providing a large but not necessarily all inclusive list of chemokines, other cytokines, and growth modulatory proteins with putative DPP4 truncation sites focusing on proteins that are known to or may in the future be shown to influence blood cells. In this talk, studies on DPP4 regulation of hematopoiesis will be summarized, and suggestions will be presented as to the means by which DPP4 changes, or might change, the activities of biomolecules that act on many different stem and progenitor cell types.

References:

Analysis of Cord Blood Unit Segment for CD34+ Cell Viability & Hematopoietic Progenitor Cell Content Correlates with the Post-Thaw Cord Blood Unit after Albumin-Dextran Reconstitution

A. Scaradavou 1,2, M.S. Albano 2, L. Dobrila 2, K. Smith 1, M. Lubin 1, J. Tonon 1, D. Sung 2, CE. Stevens 1, J.N. Barker 1

1 Adult and Pediatric Bone Marrow Transplant Programs, Memorial Sloan-Kettering Cancer Center, NY;
2 National Cord Blood Program (NCBP), New York Blood Center, NY

Background: The segment attached to the freezing bag is considered an important source of cells for testing the quality of a cryopreserved CBU prior to release for transplantation. CB banks test cells from the segment for CD34+ cell count, viability and colony forming units (CFU) as surrogates of potency of the frozen products. The NCBP reported results of 384 segments of CBU processed with the AutoExpress (AXP) system (Albano et al, ASH 2011). Average segment CD34+ viability was 96% (SD: +/- 3.3%) and correlation with the CBU pre-cryopreservation viable CD34+ counts (vCD34; R2:0.9) and CFU (R2:0.6) were excellent. Segment vCD34+ and CFU also correlated highly with each other (R2:0.69). As of 08/2012, 1056 segments from NCBP AXP CBU have been evaluated with average CD34+ cell viability of 96% (SD: +/- 3.1). However, how the segment results compare to those obtained from the CBU at the transplant center is not established.

Methods: To evaluate whether the segment could predict the post-thaw CBU vCD34+ counts, viability and CFU at MSKCC, we compared the post-thaw results of 37 NCBP CBU, AXP-processed and stored in BioArchive freezers, shipped, and thawed at MSKCC, with the information from their respective segments tested at NCBP prior to CBU release and the pre-cryopreservation data. Segment CD34+ counts and viability were evaluated by flow cytometry and 7-AAD exclusion using a single platform and the ISHAGE gating strategy. Segment CFU were evaluated using the NCBP CFU high resolution digital imaging technology (Albano et al, ASH 2008). The segment viable cells/ul were used to estimate the total vCD34 and CFU for the respective CBU. At MSKCC, CBU underwent thaw and albumin reconstitution with 8-fold dilution (10% Dextran 40; 25% albumin) as previously reported (Barker et al, BBMT 2009;15(12):1596-602). Duplicate samples were evaluated by flow cytometry within two hours. Four color flow cytometry using a dual platform was performed to measure CD45+/CD34+/CD3+ cell counts; CD34+ cell viability was assessed using a modified ISHAGE strategy (Scaradavou et al, BBMT 2010;16(4):560-8). CFU assays were performed using 1x105 cells plated in duplicate and growth was evaluated at 14 days. All CBU were part of double unit grafts.

Results: Consistent with prior NCBP data, segment vCD34+ cell counts correlated well with segment CFU (R2:0.89, p< 0.01; N=21). Additionally, high correlation of vCD34+ cell counts and CFU were seen between the pre-cryopreservation CBU and the segment (p<0.01, Table).

Importantly, despite the differences in testing laboratories and gating strategies, the number of vCD34+ cells in the CBU post-thaw correlated with the pre-cryopreservation vCD34+ counts, as well as those from the segment (Table). Average decrease in post-thaw CBU CD34+ cell viability compared to that of the segment was 1.4% (SD: +/- 3.7%, N: 37). Although statistically significant (p: 0.029), this difference was not clinically relevant: the range of change was -10% to +4.8% and the lowest CBU CD34+ viability was 86% (Figure). Post-thaw CBU total CFU and CFUGM

did not correlate with pre-cryopreservation values (Table), probably reflecting high inter-laboratory assay variability. Segment CFU and CFUGM correlation with the post-thaw values was significant although the R² was weak. The ratio of post-thaw to segment vCD34 cells (median: 1.3; SD: +/- 0.47) indicated that the segment calculation may underestimate CBU cell content. The median ratio of post-thaw to segment colony-forming cells was 0.54 for total CFU (SD: +/- 0.63) and 0.5 for CFUGM (SD: +/- 0.96). The lower CFU than vCD34 ratio may be explained, in part, by the fact that 7-AAD detects dead cells but not apoptotic; apoptotic cells are counted as alive by flow cytometry but do not have functionality and do not generate CFU in culture.

**Conclusions:** Our results indicate that testing of CBU segments can measure accurately the potency of the frozen CB products. Moreover, the results demonstrate that CBU quality can be maintained following albumin-dextran reconstitution. These findings reflect the cryopreservation procedures, freezing bags and reconstitution method described; whether they can be applied to other CB banks or transplant center laboratories requires further investigation.

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**Challenges in Performing Cord Blood Transplants in Developing Countries**

**Revathi Raj, MBBS, DCH, MRCP, Apollo Specialty Hospital**

Umbilical cord blood stem cells are a unique source of hematopoietic stem cells. There are 49481 births registered every day in India. Over 20 cord blood banks have been established in the country over the past 10 years to harness the potential of this huge biological waste. I would like to discuss here my experience with use of cord blood stem cells in a total of 50 children.

**Sibling cord program** - We have performed a total of 14 sibling transplants in 13 patients with thalassemia major and one with myelodysplasia using both cord and bone marrow from their fully HLA matched sibling. Cord blood from various cord blood banks in India were used in the sibling program. The children were aged between 2 and 13 years and the donors from 7 months to 3 years. Cord infusion was associated with DMSO toxicity in all children in the form of hypertension and fever. Bone marrow was used in addition to cord for two reasons – cord nucleated cell count alone was inadequate in all our cases and data was not available in our country regarding post thaw nucleated cell count from private cord banks. Total nucleated cell count ranged from 0.5 x 10⁶ / kg to 1 x 10⁷ / kg in these children and the CD34 ranged from 0.18 x 10⁴ / kg to 1.8 x 10⁵ / kg. Bone marrow harvest yielded 1 to 7 x 10⁶ / kg CD34 count
after harvesting less than 5 ml/kg of the recipient body weight. Cord was infused first followed by bone marrow in all of these children on the day of transplantation. There was on average 38% cell loss after thawing from different private cord blood banks in India. Cord viability ranged from 21% to 92% in our 14 patients after thawing. All patients engrafted between days 12 to day 17 with persistent donor chimerism between 85 to 100% after more than a year follow up. One child rejected his graft after initial engraftment but has been subsequently transplanted successfully using the donor’s bone marrow stem cells. Graft versus host disease was mild in three children and no cytomegalovirus reactivation was seen in any of these children. A total of 6 children received 3/6 matched cord stem cells only as the stem cell source. Five out of six children that had a haploidentical cord failed to engraft and had autologous reconstitution. There were 3 thalassaemia patients and 3 high risk children with leukemia. Mortality in this group was 33%.

We had learnt that the use of cord and bone marrow helps cure thalassaemia with the benefit of durable engraftment with a trend towards lower incidence of graft versus host disease. The donor needed to donate far lower doses of stem cells which helped us plan transplantation early. We needed to watch for DMSO toxicity as we did not routinely wash our units and hence we devised our own protocol for safe infusion of cord units using a low dose sodium nitroprusside infusion during transfusion to prevent acute hypertension. Mismatched cords from siblings carried a high rejection and mortality rate and the use of these units for high risk malignancies is not to be recommended.

Unrelated cord blood program

We have performed a total of 30 unrelated cord blood stem cell transplants between April 2007 and April 2013. All cords were obtained after searches from the National Marrow Donor Programme in the USA. The units were chosen based on a total nucleated cell dose of over 3 x 107 / kg body weight and an allele level match at DRB1 and antigen match at A and B. Matching criteria could not be met with in all patients as some were high risk children who needed an urgent transplant and had no other match in all international registries. Search requests came entirely from the transplant physicians in India as there are no designated search teams. This involved about 4 hours physician time per child to help the units arrive in India. These are extremely difficult transplants to perform and require a team of specialists including infectious disease consultants, pediatric intensive care support, a blood bank and apheresis unit and adequate lab support for rapid diagnosis of bacterial, fungal and viral infections.

The cost of each transplantation was about 75 to 100,000 US dollars most of which was the price of the cord unit itself. Our public cord blood banks need to be funded adequately to upgrade facilities and procure FACT accreditation. At present, only 5000 cord units are available for searches and that with only low resolution typing. The biggest hurdle has been financial, as the per capita annual income in India is only 1549 US dollars as compared to the US with 42693 dollars. Health spending is one of the important causes of poverty in HYPERLINK “http://www.worldbank.org/en/country/india.html” \t “ blank” India. The country’s public financing for health care is less than 1 percent of the world’s total health expenditure, although it is home to over 16 percent of the world’s population. Families meet almost 70 percent of their health expenses out of their own pockets, placing considerable financial burden on poor households, often pushing them deeper into poverty. Over the last five years, government-sponsored schemes have contributed to a significant increase in the population covered by health insurance in the country, scaling up at a pace possibly unseen elsewhere in the world. Over 300 million people, or more than 25 percent of India’s population, gained access to some form of health insurance by 2010, up from 55 million in 2003-04. More than 180 million of these were people below the poverty line.

More than 630 million persons, or about half of the country’s population, will be covered by health insurance by 2015 and this would still be only 8.4 percent of the total health expenditure. However, most of these schemes do not cover genetic disorders or tertiary care procedures such as unrelated cord blood transplants.

Our small series highlights the value of serial estimation of serum ferritin as a simple tool to predict engraftment failure, brisk engraftment syndrome and possible grades 3 to 4 graft versus host disease. Possible interventions like early introduction of TNF alpha blockade could help reduce mortality. We have also seen that use of a lactose free elemental enteral nasogastric tube feeding helps reduce sepsis rates as compared to total parenteral nutrition. Mortality rates have been high in our series with 100 day mortality of 43%. Gram negative sepsis and acute graft versus host disease of the gut have been the major causes of mortality. Providing supportive care for a child with marrow failure for over a 3 to 4 week period is a daunting task. The use of treosulphan in conditioning and avoiding the use of serotherapy like antithymocyte globulin in all immune deficiency transplants resulted in excellent outcomes. Higher resolution matching with 8/8 allele level matched cord units had shown better results as all of our fully matched cords had an
uneventful peritransplant period. The use of cord for conditions like thalassemia and aplastic anaemia where the children come in alloimmunised from multiple transfusions of non leucodepleted and irradiated blood products will result in high rejection rates and will be done strictly under trial basis. Babies with primary immune deficiency, Hurler syndrome and children with high risk leukaemia have benefitted from cord transplantation in our country.

Despite these challenges, umbilical cord blood stem cell transplantation is poised to be one of the main sources of stem cells in our country where very few marrow registries are in existence. Education of our nursing staff, lab support staff is lead by the transplant physician. Over the last 5 years, there has been a tremendous team effort to help make these advances become a reality.

Acknowledgements

Physicians and nurses of our BMT unit, infection control team, apheresis unit, Pediatric Intensive care team, cord banks worldwide

Impact of Storage Temperature & Processing Delays on Cord Blood Quality: Discrepancy Between Functional In Vitro & In Vivo Assays

Isabelle Louis, PhD, CHU Sainte-Justine Research Center


*EW and IL contributed equally to this work, ** These authors share the senior authorship

Background: Optimal conditions of cord blood (CB) storage, processing, cryopreservation and thawing are critical for banking and transplantation. Nevertheless, standardized procedures are still awaited. We hypothesize that the quality of CB unit including cell recovery, viability, and hematopoietic stem cell function may be affected by differences in: (i) processing methods prior to cryopreservation, (ii) cryopreservation methods, and (iii) thawing methods. Our aim is to better define the optimal processing conditions for maximal recovery of viable and functional reconstituting cells.

Materials and Methods: We evaluated the impact of pre-processing storage and temperature on recovery, viability and functional differentiation capacities of cord blood derived hematopoietic progenitor cells. We compared units stored for 72 hours at room temperature (RT) or at 4°C prior to cryopreservation to units processed shortly after collection (< 12 hours).

Results: Post-thaw results showed similar in vitro characteristics between immediate processing and 4°C storage for cell recovery and viability, both significantly higher than RT storage. Surprisingly, we demonstrated that storage of CB units at RT prior to processing and cryopreservation profoundly altered in vivo hematopoietic reconstitution in mice, although in vitro hematopoietic colony-forming unit potential was unaltered.

Conclusion: Our findings challenge current CB storage practices and suggest standard in vitro quality assessments may not always be indicative of CB engraftment potential.
Training, Advisory and Quality Management System for Collection Sites in a Nationwide Cord Blood Banking Program

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**Background:** Banking of cord blood has to be performed in a highly regulated ambience. Following national and international law and directives cord blood banks have to invest in significant quality management efforts. Beyond this NetCord-FACT International Standards apply. Collection sites and their staff represent an important part in the collection procedure and have to be included in the quality management and training system.

**Materials & Methods:** In 2012 DKMS Lifeline CBB recruited cord blood donors nationwide in 165 collection sites with an overall potential of 163,000 annual deliveries. As the bank does not employ its own collectors the collection sites´ staff has to be trained and the collections´ quality data evaluated. A system regarding aspects of training and quality control was implemented to oversee the action of more than 2,000 employees.

**Results:** The collection site advisory team consists of 6 employees who visit each collection site annually for training and quality purposes. A presentation of 45 minutes is held with a subsequent competency test. Audits are performed regarding facility, hygiene and storing conditions. Each collection site has to appoint a training designee who can train absent or newly employed staff on his own and is in regular contact with the CBB.

Quality reports are generated biannually reflecting all aspects of each site´s activity and are discussed with the staff. Collection sites not enhancing the quality of cord blood collections are trained additionally.

Implementation and continuous improvement of the advisory program resulted in higher collection rates and lower quality issues in the collected units. Unfortunately these results do not refer to each collection site.

**Conclusions:** Maternity staff not employed by the CBB is not sufficiently aware of all aspects of cord blood collection. Frequent contact with the CBB is a fundamental precondition to increase awareness and motivation to collect more cord blood units with a sufficient quality.
Multiparametric Immunophenotyping of Human Hematopoietic Stem Cells and Progenitor Cells in Cryopreserved Cord Blood Samples

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Cord blood transplantation has grown significantly in the past 10 years due to less stringent requirements for HLA matching and ready availability of frozen grafts. However, there is still a 20% failure rate associated with cord blood transplantations. In order to gain a better understanding of the functional components in cord blood units, we previously developed an 8-color flow assay (RUO) to characterize and enumerate hematopoietic stem cells (HSCs), multipotent progenitor cells, and common myeloid, lymphoid, and megakaryocyte erythroid progenitor cells, as well as regulatory T cell (Treg) and mesenchymal stem cell (MSC) populations. These cell populations are reported to be functionally important in a cord blood unit, and may influence engraftment success and the development of graft-versus-host disease in the cord blood recipient.

In the current study, we collected 30 cryopreserved attached segments from cord blood units and investigated the aforementioned cell populations by using our 8-color flow assay. The frequency of each of the cell populations in the attached segments reflects the frequency of cells in the cord blood units themselves.

Our results showed that the percent viable CD45+ counts ranged from 65% to 90%, which is consistent with values published by other cord blood banks. The percent viable CD34+ in total viable CD45+ cells ranged from 0.11%–1.03%. Cell count data for the cell populations we investigated revealed variations in the cell numbers of these cell populations for different cord blood samples, which may correlate with the difference in the neutrophil and platelet recovery rates in recipients after cord blood transplantation. Our assay could be a useful research tool for obtaining valuable information about cord blood units, and for better understanding the cellular phenotypes important for engraftment of a cord blood unit in the transplantation setting.
Cell Condensation with Versican Expression, Hedgehog Signaling, and Changes in BMP-4 Secretion Drive the Development of Hair-Like Structures In Vitro

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Background: Previously, we generated CK 19 positive cells and hair-like structures using Wharton’s jelly mesenchymal stromal cells (WJMSCs) seeded on a decellularized Wharton’s jelly matrix (DWJM) scaffolds and subjected to osteogenic differentiation. Herein, we investigated potential mechanisms that explain the formation of the hair-like structures in our model. We hypothesized that WJMSCs seeded on DWJM scaffolds would develop cell condensation associated with versican expression. Given the role of bone morphogenetic protein-4 (BMP-4) and sonic hedgehog (shh) in both; osteogenic differentiation and hair follicle development, we hypothesized that expression of shh and BMP-4 would play a role in hair follicle development in our model.

Materials/methods: WJMSCs were seeded on DWJM scaffolds and osteogenic differentiation was induced by exposure to osteogenic differentiation medium. Supernatant was collected weekly during culture for BMP-4 measurement by enzyme-linked immunosorbent assay (ELISA) and formalin fixed seeded DWJM scaffolds were stained for BMP-4, shh and versican immunoexpression. Expression of versican was also examined by western blotting.

Results: We observed that WJMSCs arranged themselves to develop an area of cell condensation upon contact with DWJM scaffolds. This area of cell condensation demonstrated strong staining for versican and shh by immunohistochemistry. Versican expression was also confirmed by Western blotting. Spindle-like cells in multilayers overlaid the cell condensation area resembling the hair placode. The surface layers of spindle-like cells faintly expressed BMP-4 by immunohistochemistry, while BMP-4 secretion, measured by ELISA, significantly decreased over time.

Conclusion: Cell condensation with versican and shh expression, as well as changes in BMP-4 secretion, supported the development of hair-like structures in our model.
Hyperbaric Oxygen Improves Engraftment in a Murine Model of Human Umbilical Cord Blood Transplantation

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Background: Delayed engraftment and graft failure represent major obstacles to successful umbilical cord blood (UCB) transplantation. Despite progress made in understanding the biology of UCB engraftment, there is an urgent need to identify safe and practical interventions to enhance UCB engraftment. We evaluated the use of hyperbaric oxygen (HBO) as an intervention to improve human UCB stem/progenitor cell engraftment.

Materials/methods: Six- to eight-week old NSG mice were sublethally irradiated 24 hours prior to CD34 selected UCB cell transplant. The irradiated mice were separated into a non-HBO group (where mice remained under normoxic conditions) and HBO group (where mice received two hours of HBO therapy; 100% oxygen at 2.5 atmospheres absolute). Four hours after completing HBO therapy, both groups received 1x10⁵ CD34 selected human UCB cells via tail vein injection. The infused CD34+ cells were transduced with a lentivirus carrying luciferase gene and expanded for in vivo imaging. Mice were imaged and then sacrificed at various time points up to 4.5 months post-transplant to determine retention and engraftment in peripheral blood, spleen and bone marrow (N=4 for each group-time). Engraftment of myeloid, B-cell, and T-cell subsets was examined.

Results: HBO treated mice demonstrated significantly improved bone marrow (p=0.0067), peripheral blood (p=0.0131), and spleen (p=0.0295) retention and engraftment of human UCB measured by flow cytometry. In addition, HBO significantly improved blood, spleen and bone marrow engraftment of myeloid and B-cell subsets as measured by flow cytometry. In vivo imaging demonstrated that HBO mice had significantly higher ventral and dorsal bioluminescence values. The HBO effect was more pronounced toward later post-transplant time points of 3 and 4 months.

Conclusion: We demonstrated for the first time that HBO treatment of NSG mice prior to UCB CD34+ cell infusion significantly improved engraftment, especially later engraftment, of transduced and ex vivo expanded UCB CD34+ cells.
Testing the Knowledge of Umbilical Cord Blood Banking

Amina Rafique

**Study Goal:** To quantify the knowledge of obstetricians, pediatricians, and family doctors about umbilical cord blood (UCB) banking.

**Background:** The placenta, previously discarded after birth, is now recognized as a rich source of hematopoietic stem cells. In 1988, umbilical cord blood was found to contain enough hematopoietic stem cells to be used in reconstitution of diseased or damaged bone marrow. 1,2,3 Expecting parents are tasked with the decision of whether or not to bank their umbilical cord blood and, if they choose to bank, whether they should bank with a private or public company. Of the 100 cord blood banks available, about 25% are commercial/private and advertise their banks as a “biologic insurance” for their child. 1 This is problematic as the chances of a child actually requiring their own cord blood cells range between 0.000005 and 0.1%. 4

Allogenic cord blood donations have more indications than autologous cord blood donations (See Table 1). 2,4,5 Private cord blood banking companies suggest and advertise the opposite. 6,7 To date there have only been two reported cases of autologous stem cells used out of an estimated 500,000 stored. 8,9 For down payments of $1,500-2000 upfront and yearly storage fees of $125-200 thereafter, these private companies store the umbilical cord blood to be only used by the donor. 6,7 Institutions such as the American Association of Pediatrics (AAP) and the American College of Obstetricians and Gynecologists (ACOG), therefore, recommend altruistic donation to public cord blood banks, where every child has access to the stem cells, over private cord blood banking. 2,4 Even with clear evidence and recommendations against private cord blood banking, families are still choosing to store their child’s cord blood in private banks. It is apparent that there is a need for further data and discussion to help guide obstetricians, pediatricians, family doctors, and others involved with accurately informing families on their cord blood banking options.

**Materials and Methods:** We hypothesize that there will be a significant difference between what those participating in the survey believe and what is generally recommended for umbilical cord blood banking. We believe that this difference is due to misinformation being advertised about umbilical cord blood banking.

We studied practitioner specialty (Pediatrician, Obstetrician, Family Doctor) age, sex, years in practice, and personal experience with umbilical cord blood banking as variables for agreement or no agreement to statements regarding umbilical cord blood banking.

Survey was conducted by the Co-PI sampled 56 medical students, residents, nurses, and attending physicians. The survey consisted of questionnaire with True/False choices for each answer. Correct answer for each question should be ‘False.’ The questions correspond as follows:

- Umbilical stem cells have been used to treat over 70 conditions including: Alzheimer’s, Parkinson’s, and spinal cord degeneration.
- The chance that a child will need to use his/her own stored cord blood in the future is 1-2%.
- Patients’ own stored umbilical cord blood have been used for themselves (autologous) more than 3000 times to treat malignancies and other disorders.
- Acquiring fetal hematopoietic stem cells is a one time opportunity (at delivery).

The American Academy of Pediatrics (AAP) and the American College of Obstetricians and Gynecologists (ACOG) recommend private cord blood banking. A further survey conducted by the Co-I over a similar time frame indicates that knowledge of official opinions of the AAP and ACOG is only 64%. (Survey size: 86).
Results: To our knowledge, this study is the first to investigate and survey obstetricians, pediatricians, and family doctors regarding knowledge of the strengths, weaknesses, and misconceptions of umbilical cord blood banking. It is the goal of this study to quantify the general knowledge of obstetricians, pediatricians, and family doctors about umbilical cord blood banking and compare these results to what is recommended by experts. Preliminary informal surveys by the Co-PI collected over three years showed indications of misinformation and general lack of knowledge regarding umbilical cord blood banking (See Figure 1). It should be noted that only THREE surveys were returned 100% correctly and only SIX survey were returned with over three correct answers.

Conclusion: According to the results of our analysis, we have found that the knowledge of the participants is significantly different than what is generally recommended for UCB banking. According to the results of our analysis, we have found that there is not only general lack of knowledge but also misinformation regarding UCB banking. We believe that this is due to misrepresentation being advertised about UCB banking. The data lead us to the conclusion that further study is necessary to determine the full implications of this sample survey.

Table 1: Indications for Allogenic and Autologous Stem Cell Transplant in Children

<table>
<thead>
<tr>
<th>Disease</th>
<th>Allogenic Transplantation</th>
<th>Autologous Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemias</td>
<td>Effective</td>
<td>Controversial; no better than conventional therapy</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>Effective</td>
<td>Used rarely and only when marrow not involved.</td>
</tr>
<tr>
<td>Bone and soft tissue sarcomas, Wilms tumor, brain tumor</td>
<td>Very rarely indicated</td>
<td>Barely indicated &amp; effectiveness unproven</td>
</tr>
<tr>
<td>Aplastic anemia, other cytopenias</td>
<td>Effective</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Immune deficiency</td>
<td>Effective</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Hemoglobinopathies</td>
<td>Effective</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Metabolic storage disorders</td>
<td>Controversial; may be effective in selected patients</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Neuroblastoma (Stage IV)</td>
<td>Controversial</td>
<td>Controversial</td>
</tr>
</tbody>
</table>

References
Evaluating the Factors Affecting Cord Blood Banking Quality and their Correlation with CD34+ cells

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**Background:** Umbilical cord blood (UCB) has become an alternative to bone marrow and peripheral blood as a source of progenitors for hematopoietic stem cell (HSC) transplantation and its quality basically defined by: Total nucleated cells (TNC), CD34+ cells and colony forming units (CFU) content. In this study we evaluated some factors affecting banking quality of umbilical cord blood for transplantation and their correlation between CD34+ cell doses. These factors are including: hematocrit, the number of TNC, the time between collection and processing and method of processing.

**Material and methods:** Data collection - Royan Cord Blood Bank was founded in 2005 to take, proceed and fulfill the required steps in the realm of cord blood banking. This center has covered more than 35000 samples. In this study we analyzed 1973 samples of them. Cord blood processing - The cord blood units were processed using two different methods which are including manual technique and Sepax (CBU automate separation). Flowcytometery protocol - Monoclonal CD34 and CD45 antibodies labeled with PE and FITC were used to quantify CD34+ cells were measured according to the ISHAGE protocol.

**Results:** After omitting outlier data and transforming the data to log (gamma), the data were analyzed by MINITAB software to ensure which parameters affect the rate of CD34+ cells. The data were categorized to 2 clusters according to type of processing. 1- Manual: In manual form we use regression model to assess the effect of parameters. The results have shown in tables 1 and 2 and figure 1.

**Conclusion:** This article investigates on the assessing of the parameters which can be effect on CD34+. Our Computational results revealed that time to start processing has no significant effect on the rate of CD34+ cells. On the other hand they have been shown that TNC affects CD34+ cells indirectly in both manual and Sepax while hematocrit directly affects the rate of CD34+ cells in Sepax.
According to Table 1, it is obvious that time to start of processing has not significant effect on the rate of CD 34+ cells. So this variable can be omitted. After omitting this variable, the data were analyzed. The results are tabulated in Table 3 and 4 and have been shown in Figure 2.

Figure 1. Residual plots for log CD 34+ cells. Manual According to Table 1, it is obvious that time to start of processing has not significant effect on the rate of CD 34+ cells. So this variable can be omitted. After omitting this variable, the data were analyzed. The results are tabulated in Table 3 and 4 and have been shown in Figure 2.
Table 3. Regression model of manual form of processing

The regression equation is: \( \log\text{-CD34} = 2.21 - 0.0119 \times \text{TNC Count} \)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.20893</td>
<td>0.06945</td>
<td>31.81</td>
<td>0.000</td>
</tr>
<tr>
<td>TNC Count</td>
<td>-0.011889</td>
<td>0.005832</td>
<td>-2.04</td>
<td>0.042</td>
</tr>
</tbody>
</table>

\( S = 0.843098 \quad R\text{-Sq} = 0.4 \% \quad R\text{-Sq(adj)} = 0.3 \% \)

Table 4. Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>2.9543</td>
<td>2.9543</td>
<td>4.16</td>
<td>0.042</td>
</tr>
<tr>
<td>Residual Error</td>
<td>1090</td>
<td>774.7869</td>
<td>0.7108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1091</td>
<td>777.7412</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Residual plots for \( \log\text{-CD34} + \text{, Manual 2- Sepax} \). In the Sepax form, we use a regression model to assess the effect of parameters. The results are shown in tables 5 and 6 and figure 3.
Figure 3. Residual plots for log CD34+. According to table 5, it is obvious that time to start of processing has not significant effect on the rate of CD34+ cells. So this variable can be omitted. After omitting this variable, the data were analyzed. The results are tabulated in table 7 and 8 and have been shown in figure 4.

Table 7. Regression model of Sepax form of processing

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coef</th>
<th>SE Coef</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.1039</td>
<td>0.2501</td>
<td>8.41</td>
<td>0.000</td>
</tr>
<tr>
<td>hematocrit</td>
<td>0.01198</td>
<td>0.005591</td>
<td>2.01</td>
<td>0.046</td>
</tr>
<tr>
<td>TNC Count</td>
<td>-0.02358</td>
<td>0.01040</td>
<td>-2.27</td>
<td>0.024</td>
</tr>
</tbody>
</table>

S = 0.908087  R-Sq = 2.2%  R-Sq(adj) = 1.6%

Table 8. Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>6.5766</td>
<td>3.283</td>
<td>4.17</td>
<td>0.016</td>
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<tr>
<td>Residual Error</td>
<td>376</td>
<td>310.0580</td>
<td>0.8246</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>316.9346</td>
<td></td>
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</table>
Cord Blood Separately Stored in a Tube is Better Than Cells From Attached Bag Segments for Cell Viability and Functionality Assessments of Cord Blood Units

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²Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden

Background: We examined whether an aliquot of cord blood (CB) stored in a separate tube could be used to assess stem cell viability and functionality, and whether the values obtained, better than the attached segment, reflected the values of the bag.

Materials & Methods: CBU (n=20) not acceptable for banking because of low cell numbers (<10x10⁸ TNC; range 4.3x10⁸ – 9.7x10⁸) were processed (Sepax®) and cryopreserved. An aliquot (0.5 mL) of the processed CBU was frozen in a sterile 2 mL microtube (Sarstedt) at -80°C and transferred the next day to the N2 vapor phase of an isothermal freezer. The bag and four attached segments (CryoSc-Db cryobag; Biosafe) containing CB were frozen at a controlled rate down to -50°C and stored in liquid N2 in a BioArchive® (Thermogenesis). The viability of cells from the CBU tube, segment and bag of a CBU was determined by flow cytometry using 7- aminoactinomycin D (7-AAD) gating on CD45+ and CD34+ cells. The number of colonyforming units - granulocyte macrophage (CFU-GM) in the different compartments was determined by seeding 2x10⁴ viable NC/mL of Methocult™ medium with erythropoietin (STEMCELL technologies). CFU-GM were counted under the microscope after 14 days of cultivation. Results are given as the number of CFU-GM/TNC.

Results: The mean number of CFU-GM in the bag, tube and segment was 4.3x10⁵, 3.3x10⁵ and 2.3x10⁵. The Pearson correlation coefficients (SPSS, IMB) for the bag/tube and bag/segment combinations were 0.88 (p = 0.002; Fig. 1) and 0.67 (p = 0.001; Fig. 2). Also the CD34 viability appeared to correlate better between bag and tube (R=0.72;p0.0001) than between bag and segment (R=0.66;p0.0001).

Conclusions: According to these results the CB CD34 viability and CFU-GM of the tube aliquot correlated better to those of the bag, and can therefore be used instead of segments to assess CBU quality.
Reduced-Intensity Cord Blood Transplantation for Mycosis Fungoides

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Rosenzweig, Kenneth, Radiation Oncology Department, Icahn School of Medicine at Mount Sinai, New York, NY
Grosskreutz, Celia, Tisch Cancer Institute, Mount Sinai Medical Center, New York, NY

Background: Mycosis fungoides (MF) is a T-cell derived lymphoma characterized by infiltration of mature CD4+ T cells into the upper dermis and epidermis. Allogeneic stem cell transplantation (SCT) with reduced-intensity conditioning (RIC) has been successful in rare cases of advanced MF. We here report a unique case of advanced refractory MF treated with RIC cord blood transplantation who achieved a complete remission.

Material & Methods: A 33-year-old male with generalized erythroderma, disseminated skin nodules, bilaterally palpable axillary lymph nodes, and elevated LDH was originally diagnosed with MF at age 22. He received several lines of chemotherapy, only obtaining partial responses (PR) followed by progressive disease. No full siblings were available and an unrelated donor search was initiated but due to rapidly progressive disease 4/6 HLA compatible umbilical cord blood (UCB) units were identified. After obtaining PR with ifosfamide, carboplatin and etoposide he received RIC (cyclophosphamide, fludarabine, TBI) followed by UCB infusion. The biopsy of persistent scalp lesions, present since prior to SCT, showed residual MF. Immunosuppression (IS) was decreased to stimulate graft-versus-lymphoma effect and he received electron beam therapy to the scalp with complete resolution of the lesions. At day +313 he remains clinically well, with no skin lesion or recurrent disease, and not on IS therapy.

Conclusions: Advanced MF is known to poorly respond to conventional treatment and has a dismal prognosis. Auto-SCT was shown to be effective in case series but with short lived CR, and the experience with allo-SCT remains limited. To our knowledge this is the first reported case of UCB transplantation for advanced MF with no relapse at day +313. UCB transplantation should be considered as a therapeutic option for MF patients, not only in view of its positive results but also for its availability in a timely fashion, especially for patients lacking a suitable donor.

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**Background:** Development of novel (immune) therapies is of utmost importance to improve survival in relapsed pediatric-AML (acute myeloid leukemia). We aim to develop a powerful and safe therapy consisting of 2 synergistic components: Cord Blood (CB) HSCT and vaccination with CB-derived Wilms T umor-1 (WT1) mRNA-electroporated dendritic cells (DCs).

**Materials & Methods:** After isolation, the CD34+ CB stem cells were cultured using a two-step protocol. First, they were expanded using a combination of (growth) factors (Flt3L, SCF, IL-3 and IL-6). Next, the cells were differentiated towards DCs for one week using medium containing Flt3L, SCF, GM-CSF, IL-4 and human serum followed by a CYTOMIX (IL-1–, IL-6, TNF-– and PGE2)-induced maturation for the last 24 hours. Finally, the CB-DC culture was electroporated with WT1-mRNA and their phenotype (cell surface markers) and function (migration and antigen presentation) were assessed.

**Results:** Using the two-step protocol a total cell expansion of 300-500 fold was achieved. Based on surface marker expression, at least 5 different DC subsets could be distinguished in our CB-DC cultures. Since no differences in antigen presentation capacity between the DC subsets were detected, the whole CB-DC culture was used in all phenotypic and functional assays. The maturation using CYTOMIX induced upregulation of costimulatory molecules and CCR7. These cells were also functional, showing enhanced CCR7-dependent migration towards CCL21 in a trans-well migration assay. Finally, the stimulation of WT1-specific T cells by the CB-DCs, matured using CYTOMIX and electroporated with WT1 mRNA, proofed presentation of WT1 antigens.

**Conclusion:** We have developed and tested an in vitro system for culturing large amounts of DCs from the CD34+ CB stem cells. Both the phenotypic and functional data support the use of the whole CB-DC culture as vaccine. The next step will be to translate our preclinical protocol to GMP production of a clinical grade vaccine.
Antimicrobial Components in Cord Blood Plasma

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Background: Currently, there is no standardized method for testing cord blood (CB) units for bacterial contamination. Some banks use the CB plasma to inoculate culture bottles whereas others recover the residual cells remaining in the transfer bag or collect a small aliquot from the final product. Preliminary experiments allowed us to observe that plasma extracted from several CB did not allow growth of certain bacterial species. In this work, we have characterized the antimicrobial properties of plasma extracted from CB units.

Methods: The antimicrobial activity of plasma from 60 CB units and 20 adult whole blood samples (controls) was analyzed using an inhibition test similar to that of an antibiogram. Blood agar dishes were inoculated with ten bacterial strains known as frequent CB contaminants. Thirty µL of plasma or PBS (control) were deposited onto 13 mm disks previously placed onto the agar surface. Zones of inhibition were measured after an overnight incubation at 37°C. Plasma showing an antibacterial activity were heated to 56°C for 30 minutes to inhibit complement proteins or treated with a -lactamase to abolish -lactam antibiotic activity.

Results: Antimicrobial activity was observed in 33% of CB samples, whereas none of the adult plasma samples inhibited bacteria growth. Only Gram-positive bacteria were growth-inhibited by CB plasma samples. The inhibitory activity remained after heating at 56°C, indicating that complement proteins are not involved. However, treatment of plasma samples with -lactamase completely abolished the inhibitory effect in 62% of CB samples, supporting the presence of -lactam antibiotics in those CB units.

Conclusions: In obstetric practice, antibiotics are commonly used to prevent the risk of contamination during childbirth. This study shows that antibiotic concentration in CB units can be sufficient to inhibit bacterial growth. Additional work will be required to measure the impact of antibiotics on the sterility tests.
Evaluation of a Multiplex PCR Assay for the Detection of Bacterial Contamination in Cord Blood

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Background: Nowadays, the standard method to detect microbial contamination of blood products relies on culture-based techniques which are time-consuming and above all require high inoculum volumes. Recently, Patel and coworkers (Transfusion, 2012) reported a new molecular strategy to detect bacterial contaminations in platelet concentrates. In this study, a modified qPCR assay was used to detect the presence of bacterial contaminants in umbilical cord blood (UCB) samples.

Methods: UCB samples were first spiked with four bacterial strains at 10, 100 and 500 colony-forming units (CFUs). DNA extractions were performed using 1 mL of spiked UCB using a commercial pathogen DNA extraction kit (Molzym, Bremen, Germany). PCR amplifications were done with a set of universal primers and a probe targeting a conserved region of the bacterial 16S ribosomal DNA. Reliability of the assay was monitored by amplifying a region of the human 12S mitochondrial DNA. The qPCR detection limits was compared to the one of the BacT/ALERT system (bioMérieux, Marcy l’Etoile, France).

Results: Bacterial DNA extraction yield was affected by the presence of human DNA and inhibitors present in the UCB matrix. After determining cut-off values for the qPCR assay, the assay detection limit ranged from 150 to 430 CFUs/mL and no differences were observed between the detection of Gram-negative and Gram-positive bacteria. qPCR assay use only 1 mL of sample and is realized within four hours which was much shorter than culture detection with the BacT/ALERT system.

Conclusions: Current qPCR technology does not yet demonstrate the required sensitivity and precision for being use by cord blood banks to detect microbial contaminants in UCB samples. Further work is required to improve the reliability of bacterial contaminations using a qPCR method. Meanwhile, automated culture systems like the BacT/ALERT system remain more sensitive to detect contaminations in UCB samples.
Evaluation of Potential Factors Affecting Umbilical Cord Blood Total Nucleated Cell Viability

G. Epstein; A. Mangal; E. Stacey; V. Sun
Insception Lifebank Cord Blood Program, Burnaby British Columbia Canada

**Background:** Umbilical cord blood (UCB) is an important source of hematopoietic stem and progenitor cells. Assays that must be performed on UCB units prior to cryopreservation are now specified by NETCORD-FACT and AABB Standards. These include enumeration and viability of total nucleated cells (TNC), which are fundamental for the determination of suitability and hematopoietic potential of the UCB unit. At Insception Lifebank (IL) >70% viable TNC in a unit of UCB is considered an important indicator of potency for long term storage.

**Study and Design Methods:** During 2012, 32 UCB units (2.4% of total units collected) demonstrated TNC viability <70%. We evaluated for potential offending factors including: UCB weight, transport distance from collection facility to laboratory (TD), transport temperature (TT), time of collection to freeze (TTF), bacteriology test results, time of birth to collection, method of delivery and collection, gestational and maternal age and ethnicity. The above factors were compared to control group (CG) of 32 consecutive UCB units with TNC viability >70%. UCB units were transported to laboratory at ambient temperature (AT) 15-25°C and processed according to standard protocol. 7-AAD stain was used to determine the TNC viability, performed on a Beckman Coulter flow cytometer using a single platform assay.

**Results:** The UCB units with TNC viability of <70% demonstrated average TTF of 43.0 hours (31.5-52.5) and TT of 18.0°C while the CG had an average TTF of 29.7 hours (7.5–43.0) and TT of 19.7°C. All other parameters were comparable in both groups.

**Conclusion:** The TTF emerged as the single independent factor affecting the TNC viability (p<0.0001) regardless of all other parameters considered.
Reduced Engraftment Times using the PremierMaxCBSM Processing Methodology

Donald Hudspeth, Denise Clifton and Sara Irrgang, MD – Lifeforce Cryobank Sciences, Inc.

Background: Following clinical trial participation to evaluate PrepaCyte®-CB, Lifeforce Cryobanks (LC) switched its Cord Blood Unit (CBU) processing methods from traditional hetastarch to PrepaCyte-CB in mid-2008, and began using it exclusively in 2010. This change was intended to maximize stem cell recovery while depleting the CBU of red cells, resulting in a superior stem cell product vs. traditional LC methods.

Materials and Methods: In late-2008 LC switched from a home-made DMSO and Gentran cryopreservation solution to CryostorCS10® to provide superior cellular protection during the freezing and thawing processes as well as to evaluate the reduction of the final DMSO concentration from 10% to 5%. These changes in reagents, coupled together with individual sample QC, were branded as PremierMaxCBSM.

Results: Post processing lab results of 2225 CBU processed with PremierMaxCB show increased recovery of TNC count (91.4%/84.8%), mean CFU results (20.4/ 11.7), and mean CD34+ count (5.7/4.9) and a markedly decreased hematocrit (7.8%/ 44.1%) vs. our traditional method. Viability data continued to be acceptable.

A retrospective evaluation was performed to further determine the clinical significance of the new processing method. Using outcomes data from the 8 PremierMaxCB-processed transplanted CBU, the mean neutrophil engraftment time is 11.8 days and the mean platelet engraftment time is 33.8 days. These times show a significant reduction when compared to the LC historical data (TNC – 22.0 days and plt – 51.2 days). Further, no adverse events or product-related infusion reactions have been demonstrated from PremierMaxCB processed CBU.

Conclusions: While the number of CBU transplanted is quite low, the data is encouraging and mimics that seen and reported by Regan, Donna, et.al. from St. Louis CBB using PrepaCyte-CB. Due to the significance of the preliminary findings, LC plans to continue to evaluate PremierMaxCB-processed CBU data.
Stabilization of a Pediatric Patient with a Devastating Relapsing Remitting Form of Multiple Sclerosis After Unrelated Cord Blood Transplantation

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² UMC Utrecht, Pediatric Blood and Marrow Transplantation Program, Utrecht, The Netherlands

Background: Multiple sclerosis (MS) is an idiopathic autoimmune inflammatory demyelinating disease of the central nervous system. We recently decided to transplant a pediatric patient with MS within our open “unrelated CBT in multi-agent refractory auto-immune diseases” study protocol.

At the age of 5 years the patient (girl) experienced bilateral optic neuritis and vomiting and headaches. At the age of 6 she had an EDSS score of 5.5. Despite of several treatment options (Methylprednisolone, Interferon-beta, Glatiramer acetate, Natulizumab, Rituximab and Cyclophosphamide) to have relapses resulting in progressive clinical disability. At the age of 10 years she was almost blind, with only light perception, was not able to walk without assistance and she experienced focal seizures. The first MRI at diagnosis showed focal abnormalities in the periventricular white matter with gadolinium enhancement. The follow-up scans showed numerous new gadolinium enhancing lesions in the periventricular white matter. Also lesions in the spinal cord were shown.

CBT - We transplanted the patient (in 2011) with a 6/6-matched unrelated-CB after an ATG-Fludarabine/Busulfan myeloablative conditioning. She received Cyclosporine-A, MMF and steroids as GvHD-prophylaxes.

Clinical course after CBT - No CBT associated complications were noted. She is off immunosuppression since 9 months after CBT. Currently, 2 years after CBT she has experience no relapses of the disease. Furthermore she has improved and regained some colour vision (binocular vision is still severely impaired) and she is now able to walk with the assistance of one person. Cognitively she continues to be slow and emotionally unstable. She is attending school and is having a much better quality-of-life. Her current EDSS score is 6.5 (+1 compared to pre-CBT).

Imaging follow up - MRI at 1 and 1,8 year after bone marrow transplantation showed extensive loss of white matter, and on T2 an abnormal high signal intensity in the remaining white matter (diffuse abnormal white matter). In contrast to all pre-transplant MRIs the images at these two time points did not show any acute disease activity in terms of gadolinium enhancement.

Conclusion: With unrelated cord blood transplantation we were able to stabilize radiological and clinical deterioration of a pediatric patient with a devastating unresponsive relapsing remitting form of Multiple sclerosis. Allogeneic-CBT may be a treatment option in severe cases of MS.
Exploring the Popularity of Cord Blood Banking in China

Hung-Chieh Chang
Research Fellow, Department of Anthropology, School of Global Studies, University of Sussex

The storage of cord blood is rapidly growing in China. Operating since 1996, Beijing Cord Blood Bank is the first cord blood bank in China, with the capacity to store a half million of cord blood units. The storage rate in Beijing raised from 2% to 20% between 2002-2010. The total storage units have exceeded 150,000 units while the ratio of public donation and private banking is 1/10. Taking Beijing as an example, this paper focuses on two issues: (1) structural and cultural explanations behind the popularity of cord blood banking, and (2) how private banking helps the sustainability of public banking under its context.

The data is drawn from documentary analysis, observations, and informal interviews in Beijing in March 2013. This paper presents six correlated explanations in the popularity of cord blood banking: 1) increased morbidity and matching difficulty, 2) parents’ attitude prefers family banking to public donation, 3) national license that creates monopolized market, 4) health system and medical insurance, 5) Finance and payment method, 6) Active recruitment. In addition, because of the regulation and context in China, the storage of public donation is financially supported by its private banking. Western literature traditionally condemned private cord blood banking for families without foreseeable risk. However, it is necessary to keep the operation of both private and public banking, and find a balance between them in China. In conclusion, the ecology of private and public cord blood banking is deeply rooted in its context. Private cord blood banking plays a crucial role to maintain public banking in the context of China, as well as in countries that lack government-funded public cord blood banks.
Selection of Cord Blood Units for Transplant and Recipient Outcome at the Sydney Cord Blood Bank: An Update

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² UMC Utrecht, Pediatric Blood and Marrow Transplantation Program, Utrecht, The Netherlands

Background: Ensuring the quality of cord blood units (CBU) banked is reliant on analysis of recipient outcomes following cord blood transplantation (CBT). This study analysed data on CBU selected for CBT from the Sydney Cord Blood Bank (SCBB) and the associated recipient outcomes.

Materials & Methods: 505 CBU released for CBT were evaluated for progenitor cell function, viability and engraftment potential. Recipient outcome data requested at 1 week, 3 months, 6 months and annually post transplant was analysed for engraftment, incidence of GvHD and survival.

Results: 505 CBU have been released for 477 patients (51% paediatric [≤15], 49% adult), most for single CBT (59%) versus dual (41%). 66% of CBU were released to overseas recipients: 20% USA, 11% France, 5% Italy. Over half (54%) were transplanted for Acute Myelogenous Leukemia or Acute Lymphoblastic Leukemia. 20% were transplanted for non-malignant diseases.

Median post processing TNC and CD34+ were 145.55x10⁷ and 5.35x10⁶ respectively, median infused were 3.61x10⁷/kg and 0.14x10⁶/kg respectively. Median time to neutrophil and platelet engraftment for paediatric patients was 23 and 59 days respectively; for adult patients, 24 and 60 days respectively. 18% of recipients experienced grade III/IV aGvHD (n=391).

Overall survival was 48% at three years (n=427). Survival at three years for paediatric versus adult was 58% (n=228) and 36% (n=199) respectively. 63% received a CBU with an HLA match of 5/6 or better (12% received a 6/6 match).

Age of released CBUs ranged from 0-14 years. 28% of released CBUs were stored for ≥7 years, 33% for 4-6 years and 39% for ≤3 years. Data showed little difference in neutrophil engraftment for the 3 CBU storage durations.

Conclusions: CBT using CBU from the SCBB continues to successfully treat paediatric and adult patients worldwide for a range of diseases. Data showed little cell loss through cryopreservation. Also, no correlation was observed between age of CBU and engraftment success.
Why Umbilical Cord Blood Units Fail to Qualify for Public Banking: A Report from the Carolinas Cord Blood Bank

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2 EMMES Corporation, Rockville, MD

Background: The goal of the CCBB, a public cord blood (CB) bank, is to procure and store high quality cord blood units (CBU) for hematopoietic stem cell transplantation. The banking process is comprised of donor screening and cord blood collection, processing, cryopreservation and testing. At each step, CB eligibility is determined and units not meeting specifications are discarded. We explored reasons for CBU disposal to inform strategies to maximize successful CB collection and banking.

Study design and methods: The CCBB database was retrospectively analyzed to characterize and quantify reasons for CBU ineligibility. Reasons were collated and assigned to a step on the process chain. Specifications for banking included a minimum volume of 40ml (to 2005) and 60ml (to present), a pre-processing total nucleated cell count (TNCC) of 1x10⁹ cells, and a post-processing viability of >90%.

Results: From 1/1998-8/2012, 109,177 total CBU donors were registered in the database. From these, a total of 71,618 CBUs were collected, 40,986 (57%) of which were shipped to the processing lab. Twenty-two percent of the CBUs sent for processing were subsequently discarded. Reasons for disposal of 77,149 registrations or CBUs (Table 1) included 40% for low TNCC, 23% for low volume and 2% for low viability. The financial impact of CBU loss at various stages of the process was determined (Table 2).

Conclusion: We identified reasons for banking failures in a large public CB bank. Small volumes/low TNCC represented the major reasons for failure of collected CBUs to be banked. Enhancing MD and technician training to reinforce optimal collection procedures may lead to procurement of larger volume CBUs. Expanding program collection hours, expediting shipping of collected CBUs to the lab, and improving donor screening could impact overall banking success. We can realize cost savings by identifying unqualified CBUs at earlier time points in the banking process.
### Table 1: Top 10 reasons for CBU or Donor Registration Disposal

<table>
<thead>
<tr>
<th>Reasons for CBU/Registration Disposal</th>
<th>Count</th>
<th>% of Total Disposed CBU/Registrations (n=77489)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low TNCC</td>
<td>30815</td>
<td>39.8%</td>
</tr>
<tr>
<td>Low Volume</td>
<td>17415</td>
<td>22.5%</td>
</tr>
<tr>
<td>Placenta Problem</td>
<td>7279</td>
<td>9.4%</td>
</tr>
<tr>
<td>Not collected</td>
<td>5356</td>
<td>6.9%</td>
</tr>
<tr>
<td>Umbilical Cord Problem</td>
<td>3693</td>
<td>4.8%</td>
</tr>
<tr>
<td>Maternal history or paperwork problem</td>
<td>2680</td>
<td>3.5%</td>
</tr>
<tr>
<td>Low Viability</td>
<td>1404</td>
<td>1.8%</td>
</tr>
<tr>
<td>Processing complications</td>
<td>1159</td>
<td>1.5%</td>
</tr>
<tr>
<td>Maternal Infectious Disease</td>
<td>1137</td>
<td>1.5%</td>
</tr>
<tr>
<td>Umbilical cord clot/ hemolysis</td>
<td>872</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

### Table 2: Costs of materials for Registration through CBU Storage

<table>
<thead>
<tr>
<th>Disposal Time Point</th>
<th>Number of CBUs</th>
<th>Summary costs per CBU</th>
<th>Total costs by disposal time point ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After barcode assigned/before</td>
<td>37559</td>
<td>$2.39</td>
<td>$89,766</td>
</tr>
<tr>
<td>collection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to processing</td>
<td>30632</td>
<td>$49.73</td>
<td>$1,523,329</td>
</tr>
<tr>
<td>During Processing/</td>
<td>3862</td>
<td>$245.20</td>
<td>$946,962</td>
</tr>
<tr>
<td>Cryopreservation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After cryopreservation/before</td>
<td>5436</td>
<td>$749.11</td>
<td>$4,072,161</td>
</tr>
<tr>
<td>release to registry/after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>release to registry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>$6,632,218</td>
<td></td>
</tr>
</tbody>
</table>
Mesenchymal Stem Cells from Umbilical Cord Tissue Inhibit Myelin and Insulin Specific Auto-Reactive Human T Cell Responses to Auto-Antigenic Peptide

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Background: Mesenchymal stem cells (MSC) have been shown to possess immunomodulatory properties that highlight their potential as a cellular therapy for autoimmune disease. We examined the in vitro ability of umbilical cord tissue-derived MSC (TC-MSC) to suppress the effector functions of human auto-reactive T cells. While the mechanism(s) of suppression of T cell function are not fully understood, it has been hypothesized that MSC-derived immunosuppressive soluble factors and cell-to-cell contact are important.

Methods & Materials: We developed an in vitro assay to assess the effects of umbilical cord derived MSC on T cell function. Dose titrations of low-passage TC-MSCs from multiple TC-MSC lines were adhered to collagen-coated 96 well plates or in the lower chamber wells of transwell plates. HLA-matched EBV transformed B cells were pulsed +/- with appropriate auto-antigenic peptide and cultured with adherent MSC or in the upper transwell chambers with the appropriate T cell clone (myelin-reactive or islet-reactive human T cell clones). After 48 hours, T cells were stained for CD4 and stained intracellularly for IFN-γ and analyzed by flow cytometry.

Results: We observed decreased T cell effector function with TC-MSC co-culture as determined by reduced intracellular IFN-γ; this was partially restored by separation of MSC and T cell+B cell+peptide in the transwell. Inclusion of a COX-2 inhibitor (SC-58125, an inhibitor of prostaglandin E2 synthesis) in the co-cultures led to partially restored T cell effector function.

Conclusions: We conclude that TC-MSC-derived soluble factor(s) and TC-MSC:T cell contact both contribute to the TC-MSC’s immunosuppressive effects. Further studies will pinpoint the functional mechanisms of the TC-MSC immunomodulatory properties on T cell effector function and may suggest avenues of enhancing MSC function in the treatment of autoimmune disease.
Umbilical Cord Blood-Derived Mesenchymal Stem Cells for the Treatment of Steroid-Refractory Acute or Chronic Graft-Versus-Host Disease

Soo Hyun Lee¹, Hong Hoe Koo¹, Keon Hee Yoo¹, Na Hee Lee¹, Dong Hwan Kim¹
Ki Woong Sung¹, Soo Jin Choi², Wonil Oh², Yoon-Sun Yang², Ji Won Lee³

¹Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine
²Medipost Biomedical Research Institute, MEDIPOST Co., Ltd
³Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea

Background: Steroid-refractory graft-versus-host disease (GVHD) is a devastating illness both in acute and chronic cases. Mesenchymal stem cells (MSCs), due to their immuno-regulatory properties, have been tested by many investigators to treat steroid-refractory acute GVHD. However, data on their use in chronic GVHD are scarce. We performed this study to evaluate the safety and efficacy of umbilical cord blood (UCB)-derived MSCs in treating steroid-refractory GVHD mainly focused on chronic cases.

Methods: Culture expanded UCB-MSCs were infused at three time points after patients were enrolled. After the 1st infusion with 1x10⁶/kg of MSCs on day 0, the following doses at the subsequent infusions were sequentially escalated as 2x10⁶/kg on day 14 and 3x10⁶/kg on day 42. For an assessment of chronic GVHD, organ scoring and global scoring were performed according to NIH consensus criteria.

Results: Fourteen patients (2 acute and 12 chronic) were enrolled and their median age was 12 y (range, 1-21). No patients suffered from MSCs infusion-related toxicity. One of the 2 patients with acute GVHD of skin and gut showed complete response (CR), but the other one died of sepsis and multi-organ failure after 11 days of 1st MSCs infusion although she showed partial reduction of her total serum bilirubin level from 30.5 to 24.4 mg/dL. Among the 12 patients with chronic GVHD, the median interval between transplantation and MSCs infusion was 23 mo (range, 7-110), and 8 patients (67%) showed partial response (PR), 2 stable disease (SD), and 2 progressive disease (PD) on day 56 of MSCs infusion. Of the 10 patients who survived 6 mo from enrollment, 6 patients showed PR and 4 patients remained SD.

Conclusion: Infusion of 3rd party UCB-MSCs was safe and effective in ameliorating chronic GVHD-related symptoms in a significant proportion of patients.
Development of a Cost-effective, Single-use Container to Maximize Protection from High Temperature for Transportation of Fresh Umbilical Cord Blood

Lynda St. Jour, David Popp, Paul Robbins & Linda Kelley, Cryo-Cell International

Hematopoietic stem cells are sensitive to heat which adversely affects viability and potency. Temperature excursions can occur during transportation of freshly collected cord blood as a result of inadequate thermal protection during unexpected circumstances in shipments. We sought to evaluate the current state of the art for single-use transportation containers of cord blood to seek a solution that would guarantee quality while containing size, weight and cost. We surveyed a cross-section of containers currently utilized in the cord blood banking industry. We assessed dimensions, weight, the nature of temperature-insulating materials and their ability to maintain ambient temperature when consistently exposed to extreme heat in a controlled environment. The data demonstrate that a wide range of container sizes and weights are utilized. The insulating material ranges from none, to refined materials with high R value (measure of thermal resistance) used in the packaging and construction industries. Based on the information obtained in our controlled study, we designed a collection and transport kit utilizing materials intended to maximize temperature control while containing size, weight and cost. We subjected the new kit to a real-time validation study using “worst-case” scenario by transporting to distant, warm-climate, domestic and international destinations where the material was unopened and then returned to our facility using a medical courier. The process was repeated allowing for analysis of up to four times the amount of time expected for normal transport. We identified a container of intermediate size and weight that utilizes readily available insulating materials, is cost-effective and provides adequate thermal protection during a challenging, potentially realistic, transportation scenario. Safe guarding the quality of fresh human stem and progenitor cells through conservative container design, when coupled with reliable service from a responsible courier, ensures protection from heat to maximize the utility of the cells for future therapeutic use.
Cytomegalovirus Infection in Seropositive Umbilical Cord Blood Recipients

Meerim Park¹, Young Ho Lee², Soo Hyun Lee³, Keon Hee Yoo³, Ki Woong Sung³, Hong Hoe Koo³, Hyoung Jin Kang⁴, Kyung Duk Park⁴, Hee Young Shin⁴, Hyo Seop Ahn⁴, Nak Gyun Chung⁴, Bin Cho⁵, Hack Ki Kim⁵, Kyung Nam Koh⁵, Ho Joon Im⁵, Jong Jin Seo⁶, Dong Kyun Han⁷, Hee Jo Baek⁷, Hoon Kook⁷, Tai Ju Hwang⁷, Jae Min Lee⁸, Jeong Ok Hah⁸, Yeon Jung Lim⁹, Hyun Joo Jung¹⁰, Jun Eun Park¹⁰, Moon Ju Jang¹¹, So Young Chong¹¹ & Doyeon Oh¹¹ on behalf of the Korean Cord Blood Transplantation Working Party

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Background: Little is known about cytomegalovirus (CMV) infection in unrelated cord blood transplantation (UCBT) recipients in CMV high prevalent populations, such as Korea where over 90% of HSCT patients are CMV-seropositive before transplantation. To gain insight into the natural history of CMV infection following UCBT in seropositive patients, we analyzed the incidence, risk factors and clinical outcomes of CMV infection in UCBT recipients.

Materials and methods: Between 2000 and 2011, data of 349 seropositive patients who received UCBT from 19 medical centers in Korea were reviewed.

Results: The majority (85%) received antiviral prophylaxis with acyclovir. CMV reactivation occurred in 48.7% (170/349) of CMV-seropositive transplant recipients at a median of 31 days post UCBT. Approximately 93% of patients (156/170) received preemptive therapy and most of them (143/156) received ganciclovir. The median duration of CMV infection was 29 days. CI of neutrophil engraftment by 60 days after UCBT was higher in patients with CMV reactivation (90.6% vs. 77.0%, p<0.01). On a multivariate analysis, use of rabbit antithymocyte globulin (p=0.01), acute graft-versus-host disease (p<0.01), and increased body weight (p=0.04) remained independent predictors of CMV reactivation. CMV reactivation did not impact transplantation-related mortality (TRM), relapse, or survival. CMV disease occurred in 36.3% of the CMV reactivated patients and the longer duration of CMV reactivation was the only risk factor for progression from CMV reactivation to CMV disease (p=0.02). CMV disease resulted in higher TRM (56.0% vs. 31.4%, p<0.01) and lower survival (36.1% vs. 55.1%, p=0.02).

Conclusion: Although CMV reactivation showed little demonstrable impact on UCBT outcomes, the development of CMV disease was associated with inferior outcomes. Considering the duration of CMV reactivation is the only risk factor for developing CMV disease, novel prophylactic and preemptive therapies for CMV infection are warranted to improve UCBT outcomes in seropositive patients.
Selecting Optimal Umbilical Cord Blood Donors Before Cord Blood Unit Collection

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**Background:** Previous research has suggested that pre-birth parameters might be useful for identifying potential poor collections. In particular, correlation has been shown between the fetal biometric parameters of biparietal diameter and abdominal circumference on ultrasound exam in the third trimester, at week 34±7 of gestation, and CBU suitability based on volume and total nucleated cell count (TNC). Head circumference at birth has also been correlated with TNC.

**Materials and Methods:** We looked at the reasons for cord blood collections failing to enter storage. A total of 161 public donations collected for the Cord Blood Bank of Arkansas between April 2011 and April 2013 were included in the database. To be accepted for processing the collection needed a minimum volume of 60 g actual cord blood and a TNC count of >15 x 10^9/L before processing.

**Results:** A total of 65 donations failed to have an adequate volume (56.3% in 2011, 35.2% in 2012, and 28% in 2013). A total of 90 collections failed to have an adequate cell count (46% in 2011, 60% in 2012, and 60% in 2013). Overall, 40.4% of all collections in this period were inadequate based on volume and cell count.

**Conclusions:** There is clearly a learning curve in cord blood collection since the volume collected improved over time, but cell count did not improve over time. Thus it seems likely that pre-donation evaluation of the biparietal diameter by ultrasound in the third trimester for women wishing to donate cord blood for public banking would reduce the number of collections with inadequate TNC count and help reduce the number of rejected CBU. Alternatively, head circumference at birth could be used to triage potential unacceptable donations when ultrasound measurement is not available.
Comparison of Objective Measures of Visual Acuity for Assessing Potential Change in Vision following Cord Blood Transplant in Preverbal Children

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Commercial Interests: None

Purpose: To compare Diopsys NOVA Pattern Reversal Visual Evoked Potential (VEP) and Teller Acuity Card (TAC) measures of vision in low (LBW) and higher (HBW) birth weight “at risk” children less than 3 years of age, and determine if either would be suitable to assess vision in infants and preverbal children before and after cord blood transplant for any reason.

Methods: Subjects were 7 to 26 months old with LBW (< 2200g) or HBW (>2800g), without ocular abnormalities, and “at risk” for developmental problems. VEP and TAC testing was conducted binocularly. Analyses compared VEP (0.26 cy/deg stimuli) to TAC (Green, ARVO 2013). Examiners used a 5 point confidence scale to rate confidence in VEP and TAC results.

Results: Testing was attempted on 24 children. One LBW child was unable to complete TAC testing and one HBW child was unable to complete VEP testing. Tester confidence in VEP results was low (< 3) for 3/14 LBW children and was low for none (0/8) of the HBW children (NS). Tester confidence in TAC results was high (≥3) for all children. TAC and VEP results were positively correlated (r²=0.21, p = 0.02), and results were similar for the LBW and HBW groups (r²=0.31, p=0.15 and r²=0.20 p = 0.11, respectively). The HBW group on average had better acuity, larger amplitude VEPs, and higher examiner confidence in VEP results, but none of these effects were significant. TAC confidence was significantly higher for the LBW group (p < 0.02).

Conclusions: TAC and VEP results agreed for both LBW and HBW children. VEP provides objective measures of vision, as does the TAC procedure. Binocular age norms exist for TAC but not for VEP. Thus, for this population, the TAC procedure appears to be more appropriate for the serial evaluation of vision in children having cord blood transplant.

Support: Research to Prevent Blindness Center Grant (Joseph Miller); Cord Blood Registry (CBR) grant to Newborn Possibilities Program (Hugh Miller).
The Cure of HIV with Hematopoietic Cell Transplantation

Lawrence D. Petz, MD, StemCyte International Cord Blood Center

Hematopoietic cell transplantation (HCT) has produced the only known cure of HIV infection in a patient. The patient had AML and HIV infection and was transplanted in 2007 using peripheral blood stem cells from an adult CCR5-delta32/delta32 donor. The patient, now known as “The Berlin Patient”, does not require antiretroviral drug therapy and, in the analysis of peripheral blood cells and numerous tissue samples, no proviral DNA can be detected. However, this successful HCT has not been repeated because the frequency of the CCR5-delta32/delta32 mutation is less than 1% in Caucasians and much less in other ethnic groups, and patients in need of an HCT generally have only a few potential donors. Moreover, a very close HLA match between donor and patient is required when an adult donor is used. In marked contrast, cord blood HCT requires a significantly less stringent HLA match between donor and patient making it much more feasible to find an appropriate unit for an HIV infected patient. We have tested more than 20,000 cord blood samples from our cord blood bank and collaborating cord blood banks (Duke University, St. Louis Cord Blood Bank, University of Colorado, MD Anderson Cord Blood Bank, Barcelona Spain, and Sydney Australia Cord Blood Bank) and have identified 180 cryopreserved CCR5-delta32/delta32 units that are available for HCT (1). An adequate cord blood cell dose need be only 1 x 10^7/kg if a combined haploidential/cord blood transplant is performed. Projections of HLA match rates for an inventory of 300 homozygous units indicates a probability of finding an adequately matched cord blood unit with an adequate cell dose 82.1% of the time for Caucasian adults and for 85.6% for Caucasian pediatric patients. For adult African-Americans, Mexican-Americans and Chinese-Americans the potential HLA match rates are 31.6%, 48.9% and 13.9%, respectively.

In 2012 a hematopoietic cell transplant was performed in the Netherlands on a patient with acute leukemia who was HIV positive. The transplant used a CCR5-delta32/delta32 cord blood unit from the inventory developed by StemCyte and collaborating cord blood banks as described above. A dual haploidential and cord blood transplant was carried out. Engraftment occurred and the patient’s condition was satisfactory until about 2 months post-transplant when his leukemia relapsed and caused his death.

Recent press releases from the University of Minnesota erroneously state that a transplant that was performed at that institution in April 2013 was the first cord blood transplant that was done with intent to cure HIV. Also, since cord blood banks throughout the United States and in Spain and Australia have contributed significantly to our ability to test more than 20,000 cord blood samples for the development of our inventory of 180 CCR5-delta32/delta32 units, it is evident that the intent to cure HIV with cord blood transplants is a well-known concept rather than a recent novel idea.

**Conclusion:** Patients who have an indication for a hematopoietic cell transplant for a hematologic malignancy or other disorder, and who are infected with HIV should be considered for transplantation with a CCR5-delta32/delta32 cord blood unit with the intent to cure the HIV infection as well as the underlying disorder.

Transient Myeloproliferative Disorder in a Normal Infant Cord Blood Donor

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**Background:** Transient Myeloproliferative Disorder (TMD) is usually associated with Trisomy 21 (T21) and is present at birth in ~10-20% of Down Syndrome (DS) infants, but rarely observed in non-DS neonates. We report a case of TMD in a phenotypically normal infant and cord blood donor. TMD in neonates is associated with clonal chromosome aberrations in the blast cells, and usually resolves spontaneously with the disappearance of the clone. Risk of recurrence and conversion to acute megakaryoblastic leukemia is high in DS, but unclear for non-DS patients.

**Materials and Methods:** Cord Blood was collected from an apparently normal infant in whom TMD was discovered postnatally. An automated complete blood count was performed on the cord blood upon arrival at the laboratory. After TMD was diagnosed, flow cytometry and karyotyping were performed on peripheral blood samples, and microarray analysis (NimbleGen CGX) was performed on the whole genome.

**Results:** CBC of the cord blood showed an elevated monocyte count (7.57 x10³) with an inverted lymphocyte to monocyte ratio (L/M=0.51, normal average L/M=~ 3.5). On postnatal day 2 a diagnosis of TMD was made with 17% myeloblasts in peripheral blood with relative neutropenia. There were no features of DS. Initial and repeated karyotyping revealed a normal 46XY male. The myeloblasts from peripheral blood were identified by flow cytometry as megakaryoblastic with the following markers: CD 4, 7(dim subset), 16(dim), 33, 38(dim), 41, 42B, 45(dim), 61, and 123. The postnatal course was otherwise unremarkable and the myeloblastosis resolved by postnatal day 27. Microarray analysis has been negative to date.

**Conclusions:** TMD is common in DS neonates with potential for later recurrence and a~20-30% probability of progression to acute megakaryoblastic leukemia. Although TMD in non-DS infants is apparently rare, its appearance and resolution within the neonatal period suggests some cases may go undetected. Monitoring the L/M ratio of cord blood could be useful as an early screen for myeloproliferative disorders, as well as for immunodeficiency syndromes resulting in significant lymphopenia.
Impact of Maternal Thrombophilia on Donated Umbilical Cord Blood Cellular Content

Pinar Yurdakul, A. Seval Ozgu Erdinc, Handan Karakaya, Seda Ozkul, Klara Dalva, Doruk Katlan, Feride Çelebi, M. Sinan Beksac, Meral Beksac

Background and Aim: In cord blood banking every effort to avoid transmission of serious life threatening acquired or genetic disorders to cord blood recipients have to be taken. FACT/NETCORD and WMDA standards have defined the maternal acceptability criteria based on medical questionnaire and IDM test results. Thrombophilic morbidities are observed frequently ie 2-10 % among apparently healthy people and is a more frequent observation in tertiary Maternity Clinics. The aim of this retrospective analysis was to evaluate the rate of acceptability among cord blood donations from families with a previous history of fetal loss, documented thrombophilia or anti cardiolipin/auto antibodies in comparison to those who lack such findings.

Patients and Methods: 546 cord blood donations between June 2011- January 2013 were included in the analysis. The acceptability criteria is collection /pre processing volume>70 mL, TNC > 50 x10E7, CD 34> 1x10E6, viability >90%. IDM tests are obtained immediately postpartum with results obtained post processing. Thrombophilia was diagnosed by the obstetricians based on the presence of these findings: recurrent fetal/ preterm or early post term loss, intrauterine growth retardation not attributable to other causes, presence of Factor V Leiden, Prothrombin, MTHFR mutations, hyperhomocysteinemia, anticardiolipin antibodies or presence of antiplatelet /antinuclear antibodies, low levels of natural anticoagulants. 188 of the donations met these criteria.

Results: The cellular content of the CBUs are summarized in the Table.

<table>
<thead>
<tr>
<th></th>
<th>Thrombophilia (+) N: 188</th>
<th>P value (Mann-Whitney U)</th>
<th>Normal N:358</th>
</tr>
</thead>
<tbody>
<tr>
<td>viability</td>
<td>95 +/-5%</td>
<td>n.s.</td>
<td>95 +/-5%</td>
</tr>
<tr>
<td>volume</td>
<td>92,1±23,6</td>
<td>0,009</td>
<td>98,7±23,9</td>
</tr>
<tr>
<td>TNC(x10e7)</td>
<td>77,5±32,4</td>
<td>0,001</td>
<td>91,1±44,4</td>
</tr>
<tr>
<td>CD 34 counts (x10e6)</td>
<td>2,6±1,8</td>
<td>n.s.</td>
<td>2,4±1,9</td>
</tr>
<tr>
<td>Unacceptable rate</td>
<td>35 %</td>
<td>0.01</td>
<td>7.8%</td>
</tr>
</tbody>
</table>

Conclusion: The rate of below acceptability is higher among subjects with a maternal history of thrombophilia. However if they meet the volume and TNC criteria hematopoietic stem cell counts are similar to those who present with healthy obstetric history. The colony formation capacities will be reported at the meeting.
Mesenchymal Stem Cells in Injectable Hydrogel for Muscle Regeneration

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Background: The type of stem cells and the clinical source to be used for muscular dystrophy therapy is proposed to have the following properties: (1) They must be easy to isolate from human biological material; easy to multiply in the laboratory to amounts necessary for a systemic treatment of children; (2) systemic delivery into the blood circulation should be possible; (3) they have to be able to migrate from the blood circulation into the muscles; (4) they must give rise to large amounts of functional muscle cells with dystrophin and with functional satellite cells inside the dystrophic muscle tissue and should not produce any serious side effects.

Methods: In this study, we have evaluated the above published data points with lipo aspirated adipose source, processed for mesenchymal stem cells with a novel trypsin method for medium scale clinical production on batches up to 100 million per batch, characterized in vitro for their potency. As also, the biocompatibility of produced progenitor cells with injectable PEGF-Hydro gel was assessed based on time dependent cell kinetics v/s hydro gel concentration.

Results: The trypsin method of harvesting multifold mesenchymal stem cells from lipoaspirates was a clean approach which resulted in purified forms of multipotent lineages forming progenitor population. These purified batches showed around 13% of adipogenic differentiation potential and an average 60% of muscle cell forming capabilities in-vitro. For the first time, we report feasible composite grafting method of adipose derived mesenchymal stem cells in particular with injectable hydro gel, proposing viable progenitor cell load ratio with no toxicity induced by the contact.

Conclusion: Our in-vitro data support the hypothesis that a human lipo aspirate derived multipotent mesenchymal stem cells loaded on injectable PEGF-hydro gel may represent a promising progenitor cell grafting holistic approach to regenerate and build damaged muscle cell an matrix integrity.
Cord Blood Transplant as Salvage Therapy Following Graft Failure or Relapse After Prior Allogeneic Transplant

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**Background:** For patients with graft failure or relapse following hematopoietic cell transplant (HCT) a second HCT may be lifesaving. Prior studies have shown a survival advantage of this approach over non-HCT treatment options, although data remains limited. Cord blood (CB) as a donor source in this setting offers the advantages of rapid availability and no risk to the donor. Herein, we report the outcomes of patients undergoing second HCT with CB.

**Methods:** Fourteen patients with hematologic malignancies (median age of 34 years; range 4-68) underwent a second HCT with CB. First HCT donors were matched related (N=6), unrelated (N=4), haploidentical (N=2) and CB (N=2). Prior conditioning was myeloablative in eight patients and non-myeloablative in six. Primary indication for second allograft was relapse (N=11) and graft failure (N=3), and patients were conditioned with fludarabine (200mg/m²), cyclophosphamide (50mg/m²), 2-4 Gy TBI (N = 8) or treosulfan (14mg/m²), fludarabine (150mg/m²), 2Gy TBI.

**Results:** Of the 13 patients alive at day 21, 12 engrafted at a median of 23 days (range day 6-49). Death prior to day 100 was seen in 4 patients. Acute Grade II-IV and III-IV GVHD was diagnosed in 10 and 3 of 11 evaluable patients respectively, and 3 of 9 evaluable patients developed chronic GVHD. At 1-year post-transplant, 9 of 14 patients (64%) were alive and 8 of 14 (57%) were in remission. At two years post-transplant, 5 of 14 (36%) were alive. Of the 9 deaths, 4 died of disease relapse and 5 of infectious complications. One patient undergoing transplant for graft failure survived.

**Conclusions:** Use of CB for second HCT in this high-risk population resulted in a favorable incidence of engraftment at 92% and 1-year OS of 64%. Expected outcomes are poor for patients undergoing second HCT. However, our outcomes support further evaluation of second HCT with CB after failing a prior HCT.
Estimation of Serum Ferritin is a Useful Market to Predict Adverse Outcome in Unrelated Cord Blood Transplantation in Children

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Inflammatory cytokines play a key role in engraftment syndrome and graft versus host disease in unrelated transplantation. Mismatched cord units are particularly known to produce a peculiar engraftment syndrome with swinging fever, diffuse macular rash, capillary leak syndrome with respiratory distress. This is seen between days 5 to 25 after infusion of the cord unit. Early therapy with methylprednisolone at 2 to 4 mg / kg / day and fluid restriction are the mainstay of therapy. A complete lack of this immune response may be an early predictor of non engraftment and help plan the request for a back up cord unit. Hypercytokinaemia seen during engraftment has also been linked as a trigger for graft versus host disease. Serum ferritin is a good marker of acute inflammation.

From April 2007 to December 2012 we have performed 27 unrelated cord blood transplantation at our centre. We had prospectively performed serum ferritin in a serial manner in all our 27 unrelated cord transplantation. The primary diagnosis was relapsed / high risk leukaemia (n=11), Fanconi anaemia (n=6), primary immune deficiency (n=4), pure red cell aplasia (n=1), sickle cell anaemia (n=1) and Hurler syndrome (n=3), thalassaemia major (n=1). Serial ferritin data is available on 24 out of 27 patients. Ferritin levels remained less than 500 in the two children who failed to engraft. Marked elevation of serum ferritin from baseline to up to 194041 was noticed in our patients within the first 28 days of graft. Children who had a serum ferritin of over 50,000 died early due to steroid refractory graft versus host disease or sepsis.

These results in a small series highlight the value of serial estimation of serum ferritin to predict engraftment failure, brisk engraftment syndrome and possible grades 3 to 4 graft versus host disease. Possible interventions like early introduction of TNF alpha blockade could help reduce the high mortality seen in our series.
Sibling Cord and Bone Marrow to Cure Beta Thalassaemia Major

Dr Revathi Raj, Dr Deenadayalan M, Dr Vipin Khandelwal, Dr Vimal Kumar, Dr Karuna Sri, Dr Madhan Kumar, Dr Lakhmanan V, Dr Jose Easow, Dr Ranjan Kumar Mohapatra, Dr Raja T, Dr Ramesh Nimmagadda - Blood and Marrow Transplantation Unit, Apollo Speciality Hospital, Chennai, India

There are 10,000 new births of beta thalassaemia major in India each year. The burden of long term transfusion and chelation in such children is huge and the majority of such patients in our country face early death in their teenage due to cardiac or liver haemosiderosis. Private cord blood banks have been operational in India since 2002. Does cord blood make a difference to the outcome in children undergoing fully matched sibling allograft for thalassaemia major?

We present a series of 13 children with thalassaemia major who have been cured of their disease by their saviour sibling. All 13 children were transfusion dependent with age ranging from 2 years to 11 years. Nine children received thiotepa, treosulphan and fludarabine conditioning and four had busulphan and cyclophosphamide. The cord was thawed and infused first followed by bone marrow harvested from the sibling. The sibling donors were between 7 months to 3 years of age.

Bone marrow was used in addition to cord for two reasons – cord nucleated cell count alone was inadequate in all our 13 cases and data was not yet available in our country regarding post thaw nucleated count from private cord banks. Total nucleated cell count ranged from $0.5 \times 10^6$/kg to $1 \times 10^7$/kg in these eight children and the CD 34 ranged from $0.18 \times 10^{4}$/kg to $1.8 \times 10^{5}$/kg. Bone marrow harvest yielded 1 to $7 \times 10^6$/kg CD 34 count after harvesting less than 5 ml/kg marrow of the recipient body weight. There was on average 38% cell loss after thawing from different private cord blood banks in India. All patients engrafted between days 12 to day 17 with persistent donor chimerism between 85 to 100% after more than a year follow up. One child rejected his graft after initial engraftment but has been subsequently transplanted successfully using the donor’s bone marrow stem cells. Graft versus host disease was mild in one child and no cytomegalovirus reactivation was seen in any of these children.

We conclude that the use of cord and bone marrow helps cure thalassaemia with the benefit of durable engraftment with a trend towards lower incidence of graft versus host disease. The donor needs to donate far lower doses of stem cells which helps us plan transplantation early. Cord is an ideal source of stem cells for transplantation and should be used even if the cell dose seems suboptimal and balanced with addition of cells from bone marrow.
Quality of Functional Hematopoietic Stem/Progenitor Cells from 2 to 6 Year Cryopreserved Human Umbilical Cord Blood

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As a leading public bank in Korea since 2006, we have been focusing on the quality of the cord blood (CB) units. Because long-term cryopreservation is critical for CB banking and transplantation, in this study, we evaluated hematopoietic stem cell function in vivo and in vitro with individual CBs cryopreserved for 2 (as the group 1, n=5), 4 (as the group 2, n=5), and 6 years (as the group 3, n=5) respectively in Seoul Metropolitan Government Public Cord Blood Bank, Republic of Korea. The mean percentage of viable cells after thawing was 86.2 ± 2.9%, 86.6 ± 2.3% and 87.8 ± 1.3% for Group 1, 2 and 3, respectively. The recovery rate of total nucleated cells was 104.2 ± 7.9%, 92.8 ± 6.5%, and 98.9 ± 6.0% for Group 1, 2 and 3, respectively. We compared the post-thaw colony-forming unit granulocyte-macrophage (CFU-GM) number between the three Groups. The number of post-thaw CFU-GM was 8.6 ± 1.68, 10.2 ± 5.00 and 12.5 ± 3.83 for Group 1, 2 and 3, respectively, indicating that the colony forming potentials from the three Groups are intact.

In addition, we could find functionally responsive CD4+ and CD8+ T lymphocytes which were isolated from each TNC of Group 1, 2 and 3.

To assess the hematopoietic stem cell capability, the thawed cells from single collections were bead-separated into CD34+ cells and infused into 2.7 Gy sublethally irradiated NOD/SCID mice. Human CD45+ cell engraftment with phenotypes of T lymphocyte (huCD3+ cells) was clearly detected in mice in 6 to 8 wk, the engrafting levels of huCD34+ HSC from Group 1, 2 and 3 were comparable to that reported with fresh CBs. These results indicate that long-term cryopreservation for at least 6 years does not negatively affect the quality of CBs for clinical transplantation.
Intramyocardial Delivery of Porcine Umbilical Cord Blood Derived Mononuclear Cells to Determine Safety and Feasibility Required for Right Ventricle Regeneration within Congenital Heart Diseases


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Background/Objectives: Hypoplastic left heart syndrome (HLHS) is a severe form of CHD that consists of multiple obstructions to flow through the left heart and aorta, as well as hypoplasia of left ventricle. This requires the affected children to undergo in a multistage surgical palliation within the first days of life and most of them end in a cardiac transplantation.

Our goal is to re-engineer the native RV of HLHS children with stem cell-based regenerative strategies using autologous UCB-MNC from children with HLHS and delivered into the RV myocardium.

Material and Methods: A long-term study using a porcine model was designed for autologous cell testing. This safety study required timed caesarian delivery of individual piglets for collection of UCB. Upon meeting clinical-grade release criteria, the oldest animals (n=12) that passed a veterinarian medical exam were randomized in a double-blinded procedure before surgical delivery of test article injection into the RV at age of 4 weeks. Table 1 shows animal characteristics. After 3-month of cardiovascular and biochemical follow-up, animals were sacrificed for terminal necropsy with comprehensive histology. We also maintained a satellite group of animals that contained labeled cells to allow for cell-tracking and biodistribution data.

Results: The study was technically (cell processing/cryopreservation, cardiac surgery and monitoring) feasible, and was conducted in the spirit of GLP to mimic potential clinical studies.

The data demonstrated no evidence for adverse events that included clinical monitoring, cardiovascular performance, clinical chemistry, and terminal multi-system histological analysis. There were no abnormalities or discrepancies between cell-therapy and control cohorts (Table 2). All animals survived the procedures without medical complications.

Conclusions: The overall large animal long-term safety study demonstrated no evidence of adverse risk due to intramyocardial injection of UCB-MNC. Demonstrating that autologous UCB-MNC can be safely administered will establish the foundation to advance new therapeutic modalities leading towards FDA-approval for clinical testing within CHD.
2013 Most Outstanding Abstract Award Recipient
Caridad Martinez, MD, Baylor College of Medicine

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