

Umbilical Cord Blood Hematopoietic Stem Cell Transplantation, an Alternative to Bone Marrow

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Abstract

Umbilical cord blood (UCB) is an alternative hematopoietic stem cell (HSC) source. It is widely applicable for ameliorating several diseases through HSC transplants. One of the few disadvantages of this source of stem cells is the limited amount of HSCs that can be extracted from each cord blood unit (CBU). Single CBU transplantation seems to be more ideal in lower weight, younger patients. Adult clinical studies comparing HSCs from a single CBU and HSCs from bone marrow indicate that umbilical cord blood transplantation is a viable method when a matched bone marrow transplant cannot be identified. Further clinical studies using two CBUs suggest better engraftment and lower risk of relapse. However, double cord blood transplantation has been faced with the challenge of single unit dominance in most studies. *Ex vivo* expansion of UCB HSC is another promising method to overcome limited HSC counts.

Keywords: Umbilical cord blood, Hematopoietic stem cells, Umbilical cord blood transplantation, Double cord blood transplantation, *Ex Vivo* expansion

1. Introduction

Originally thrown away after the delivery of the baby, umbilical cord blood (UCB) was first considered as a source of stem cells in the 1970s (Mayani, Alvarado-Moreno & Flores-Guzmán, 2003). Recent advances in the potential of UCB have revealed therapeutic uses of HSCs (Eapen *et al.*, 2007; Gluckman *et al.*, 1989; Rocha *et al.*, 2001). The presence of hematopoietic progenitor cells in human UCB was first evidenced by Knudtzon in 1974 (Knudtzon, 1974). Approximately 10 years later, the presence of more primitive hematopoietic progenitor cells was observed by Leary and Ogawa (Leary & Ogawa, 1987). UCB has the potential to be used as a therapeutic tool due to its abilities to engraft (Broxmeyer *et al.*, 1989). In comparison to bone marrow (BM) hematopoietic stem cells (HSCs), UCB is easier to attain, more available, and requires less compatibility with the patient.

UCB has been a successful alternative to BM as a source of HSCs for treating a variety of hematologic disorders and malignant/non-malignant diseases such as leukemia, lymphoma, and sickle cell anemia (Broxmeyer, 2010; Higuchi *et al.*, 2010). UCB was first tested on a young boy who had Fanconi Anemia, an autosomal recessive disorder. Fanconi Anemia is characterized by hematological abnormalities and BM failure. The transplant of UCB from a female HLA-identical sibling allowed the boy to regenerate all hematopoietic efficacies that were limited by the disease (Gluckman *et al.*, 1989). This success has been a vital motivation to all subsequent research on UCB. The first unrelated umbilical cord blood transplant (UCBT) was performed in 1993 on patients with malignant and non-malignant diseases (Kurtzberg *et al.*, 1996). In 1996, Laporte *et al.* reported successful UCBT in an adult with chronic myelogenous leukemia from an unrelated donor (Laporte *et al.*, 1996). This innovative use of stem cells has led to a wide increase of UCB banking. As a result, expecting mothers are now more likely to bank their newborn's UCB in cord blood banks (CIBMTR) (Pasquini & Wang, 2010).

Here, we provide a review of the most recent research performed on UCBTs. We first examine the essential constituents of UCB including HSCs and mesenchymal stem cells (MSCs). We further review the advantages and disadvantages of UCBT HSCs as opposed to bone marrow transplant (BMT) HSCs by examining clinical trials. Since there are less HSCs in UCB than in BM, we discuss methods that overcome the limitation of HSCs from a single cord through double cord blood transplantation (DCBT) and *ex-vivo* expansion. Additionally, we analyze clinical studies performed on DCBT which use various types of conditioning regimens including reduced intensity conditioning (RIC), myeloablative conditioning (MAC), and non-myeloablative conditioning (NMAC). Lastly, we discuss the processes of UCB extraction, cryopreservation and banking.

2. Essential constituents of Umbilical Cord Blood

The umbilical cord (UC) is the basic life-line support for all newborns. It is the essential tube in which all nutrients and nourishments are transported from the mother to the embryo. Following delivery, the umbilical cord is usually clamped and discarded. The human umbilical cord begins to form in the first 5 weeks of pregnancy. The allantois, a membrane formed out of the gut, contains umbilical vessels that attach the fetus to the developing placenta. Blood vessels typically develop from the yolk sac, which will then combine with the umbilical vessels. The amnion or the membrane surrounding the amniotic fluid envelops the allantois and the yolk sac, forming the developing umbilical cord. In the following months leading up to birth, the umbilical cord begins to insert deeper into the abdomen of the fetus (Kurt Benirschke & Peter Kaufmann, 2000). The umbilical cord consists of two arteries and one vein arranged in a helical structure (Figure 1). The two arteries of the umbilical cord spiral around the umbilical vein, making them stiffer and more difficult to rupture. Opposite to the arteries and veins of the human heart (except for the pulmonary arteries), umbilical cord arteries and veins carry deoxygenated blood to the placenta and oxygenated blood from the placenta to the developing fetus, respectively. The three vessels of the umbilical cord are embedded in a connective tissue gelatinous base known as the Wharton's Jelly which insulates the umbilical cord.

A striking difference between the umbilical arteries and vein is observed in the endothelial cells at 10 weeks gestation. The endothelial cells of the arteries become richer in glycogen (Parry & Abramovich, 1972). At 15 weeks gestation, venous endothelium contains the most amount of rough endoplasmic reticulum and thereafter decreases until almost none is seen by mid-pregnancy. Arterial endothelium contained rough endoplasmic reticulum that progressively increased over the course of the pregnancy.

2.1 Hematopoietic Stem Cells

HSCs are multipotent and have the capability to self-renew and differentiate into all mature blood cells including myeloids such as granulocytes, monocytes, leukocytes, erythrocytes, and megakaryocytes, and lymphoids such as T-lymphocytes and B-lymphocytes (Figure 2) (Gunsilius, Gastl, & Petzer, 2001; Higuchi *et al.*, 2010). HSCs in UCB are similar to those in BM and peripheral blood in their capacity for differentiation. They can self-renew and they contain erythroid and granulocyte-macrophage progenitor cells when examined in an *in vitro* colony assay (Broxmeyer *et al.*, 1989).

Current scientific investigation is attempting to determine the best marker for HSCs. The most recent significant constituents of UCB are erythrocytes, endothelial cells, MSCs, HSCs, and Tregs. Many other mononuclear cells are found in UCB, making it difficult to identify HSCs. A common marker used is cell determinant-34 (CD34), a glycoprotein found on the surface of the HSCs and progenitor cells that is important for adhesion with neighboring cells (Soiffer, 2008). Simple combinations of markers that efficiently select for HSCs have not yet been determined. Protocols experimenting with complex combinations of markers have provided a more efficient method of detecting these stem cells. One such protocol begins by reducing the amounts of cells that portray any lineage-specific markers and then using the remaining cells to positively select for Sca-1⁺ Lineage-c-kit⁺ cells (Osawa, Hanada, Hamada, & Nakauchi, 1996). However, this protocol only gives 20% multilineage reconstitution (Morrison, Hemmati, Wandycz, & Weissman, 1995; Osawa *et al.*, 1996; Spangrude, Brooks, & Tumas, 1995; Wagers, Sherwood, Christensen, & Weissman, 2002). More recently, a protocol using the signaling lymphocyte activation molecule family of markers were recognized to distinguish between 'true' HSCs and primitive progenitor cells. Microarray analysis was performed on three markers from the signaling lymphocyte activation molecule family, and the most purified HSCs pertained to CD150⁺CD244⁺CD48⁻ cells (Kiel *et al.*, 2005).

2.2 Mesenchymal Stem Cells

MSCs have multipotential capabilities to self-renew and differentiate into various cellular lineages including adipocytes, osteocytes, chondrocytes, cardiomyocytes, hepatocytes, and nerve cells (Chen, Shao, Xiang, Dong, & Zhang, 2008; Kang *et al.*, 2006; Kestendjieva *et al.*, 2008; Romanov, Svintsitskaya, & Smirnov, 2003). Their

proliferative properties and their ability to migrate to sites of inflammation make MSCs highly desirable for tissue engineering and cell-based therapy (Chen *et al.*, 2008; Romanov *et al.*, 2003).

To date, BM is the most common source of MSCs (Kestendjieva *et al.*, 2008). BM MSCs have certain disadvantages such as the invasive nature of aspirating BM, the drop in cell number (1 MSC in 10^4 nucleated BM cells at birth to 1 in 2×10^6 at the age of 80) and reduction of proliferative potentials with donor's age (Kestendjieva *et al.*, 2008). UCB provides an alternative source of MSCs that possess better proliferative capabilities than BM MSCs (Rodeck & Whittle, 2009; Wang *et al.*, 2004).

Previous research by Panepucci *et al.* (Panepucci *et al.*, 2004) and Jeong *et al.* (Jeong *et al.*, 2005) showed that MSCs from UCB have similar differentiative properties to MSCs from BM. Similarities include abilities to differentiate into adipose tissue, neuronal tissue, bone forming capacity, hepatocyte-like cells, neuroglial-like cells, bone and cartilage (Rodeck & Whittle, 2009). Moreover, MSCs from UCB show similar cell morphology and immune phenotype to MSCs from BM (Kern, Eichler, Stoeve, Kluter, & Bieback, 2006; Romanov *et al.*, 2003). However, previous attempts to isolate MSCs from UCB have been unsuccessful or have resulted in low yields (compared to yield rates of 100% from BM) (Bieback, Kern, Kluter, & Eichler, 2004; Kern *et al.*, 2006). Recent research has shown that UCB MSCs may be expanded due to their higher proliferation rate and long culture period (Kern *et al.*, 2006).

Similar to HSCs, surface markers used to identify MSCs are not specific to MSCs but also to other cellular lineages (Bieback & Kluter, 2009; Li, Gollapudi, Patterson, Huang, & Tuan, 2010; Westwood & Clements, 2007). Therefore, a combination of expressed and non-expressed markers are used to identify MSCs. Positive MSC markers include CD29, CD44, CD73 (SH-3, SH-4), CD90 (Thy-1), CD105 (SH-2, Endoglin), and CD166 whereas negative markers include CD11, CD14, CD19, CD31, CD34, CD45, and CD79 (Bieback & Kluter, 2009; Li *et al.*, 2010; Westwood & Clements, 2007).

2.2.1 MSCs as immunoregulators

MSCs are considered to assist in lowering graft-versus-host-disease (GVHD) in UCBT (Ringdén *et al.*, 2006). This may be attributed to the ability of MSCs in reducing T-cell proliferation and cytokine production (Aggarwal & Pittenger, 2005; Glennie, Soeiro, Dyson, Lam, & Dazzi, 2005; Maitra *et al.*, 2004). Exact mechanisms of how MSCs perform their immunosuppression remain unknown. It has been suggested that factors such as transforming growth factor- β , nitric oxide, and interleukin-10 co-function with MSCs to cause immune suppression (Batten *et al.*, 2006; Groh, Maitra, Szekely, & Koç, 2005; Sato *et al.*, 2007). Further research is required to determine the feasibility of using MSCs along with UCB HSCs to lower GVHD and improve engraftment rates.

2.3 Regulatory-T cells as immunoregulators

T-regulatory cells (Tregs) are immune suppressor cells that restrain allogeneic immune responses. Their ability to respond to foreign or self-antigens allows them to maintain the immune system at a homeostasis (Sakaguchi, Yamaguchi, Nomura & Ono, 2008). Tregs are typically characterized as $CD4^+CD25^{hi}$ T cells that express the fork head box transcription factor (Fontenot *et al.*, 2005). Fork head box transcription factor is crucial for T cell activation and in regulating a variety of immune reactions. It is also the most reliable marker for Tregs.

Tregs in UCB may play a crucial role in hematopoietic stem cell transplants (HSCT). Studies on mice administered with allogeneic HSCs have determined that the depletion of Tregs triggers an increased T cell response to tumors, self-antigens, and pathogens, resulting in increased GVHD in recipients (Anderson *et al.*, 2004; Chen *et al.*, 2007; Cohen, Trenado, Vasey, Klatzmann, & Salomon, 2002; Taylor, Lees, & Blazar, 2002). Conversely, the presence of Tregs resulted in fewer cases of GVHD (Taylor *et al.*, 2004; Trenado *et al.*, 2003). Patients who receive BM transplants are at a higher risk of developing GVHD as compared to patients who receive UCBT (Kim & Broxmeyer, in press). Reasons for such results remain unknown but it is speculated that the lower percentages of GVHD in UCBT recipients may be due to the Tregs present in UCB (Kim & Broxmeyer, in press). The lack of human clinical trials, mainly due to the complexity of isolating human T populations, makes it difficult to validate such a speculation.

3. Hematopoietic Stem Cell Transplantation

Once a HSC unit has been identified for a patient, he/she must first be administered on a conditioning regimen 5-10 days before the HSC transplant (Carrier & Ledingham, 2004). Conditioning regimens are chemotherapy only or chemotherapy combined with total body irradiation (TBI) treated to patients undergoing allogeneic HSCTs (Vriesendorp, 2003). The conditioning regimen destroys diseased cells in the patient's body, opens up space in the bone marrow for new cells, and reduces the risk of rejection by suppressing the immune system

(Bacigalupo *et al.*, 2009; Casper *et al.*, 2004). The intensity of the conditioning regimens given to patients is crucial and may vary according to age or comorbidities (Nakamae *et al.*, 2009). One of the most common conditioning regimens for patients with acute leukemia or high-risk lymphoma is cyclophosphamide combined with total body irradiation (Cy/TBI). TBI is radiation performed to the entire body as opposed to the specific infected area. This therapy aids in suppressing the patient's immune system to allow for reduced rejection of the transplant. Another common type of conditioning regimen includes busulfan and cyclophosphamide (Bu/Cy) for patients with chronic myelogenous leukemia (CML). Along with the conditioning regimen, the patient receives chemotherapy treatments. Many of these preparatory therapies for transplant are associated with side effects and risk factors that patients must be informed of beforehand.

3.1 Conditioning regimens

There are three common categories of conditioning regimens: (1) myeloablative conditioning (MAC), (2) non-myeloablative conditioning (NMAC) and (3) reduce intensity conditioning (RIC).

MAC regimens are associated with irreversible pancytopenia and myeloablation. Such conditioning requires stem cell support (Bacigalupo *et al.*, 2009). MAC is administered to young patients (<50 years of age) without comorbidities due to its high toxicity (Mielcarek & Storb, 2003). Even in young patients, MAC is associated with high treatment related mortality rates (Kanda *et al.*, in press; Vriesendorp, 2003). Common MAC regimens include either cyclophosphamide 120 mg/kg and TBI (12 to 14.4 Gy) or 16 mg/kg busulfan (Mielcarek & Storb, 2003). Such combination of high doses of TBI and/or alkylating agents is so intense that autologous hematologic recovery is disabled and thus hematopoiesis in patients can only be restored by transplanted HSCs (Bacigalupo *et al.*, 2009).

Bacigalupo *et al.* defined NMAC regimens as those that cause minimal cytopenia and do not require stem cell support (Bacigalupo *et al.*, 2009). NMAC regimens are developed to reduce toxicity in HSCTs. Such reduction in cytotoxicity provides better results in HSCTs with older or medically weak patients (Mielcarek & Storb, 2003). NMAC is associated with minimally myelosuppressive regimens combined with intensive immune suppression post-transplant to ensure engraftment (Nakamae *et al.*, 2009). NMAC regimens include the use of fludarabine and/or reduced chemotherapy and radiation (Bacigalupo *et al.*, 2009). Fludarabine is important in NMAC due to its ability to reduce high rate of graft rejection (Horwitz & Chao, 2010). Common NMAC regimens include combinations of busulfan/fludarabine or TBI 200 cGy/fludarabine (Horwitz & Chao, 2010). In addition, patients are treated with cyclosporine and mycophenolate mofetil as post-graft immunosuppression (Horwitz & Chao, 2010; Nakamae *et al.*, 2009).

RIC regimens can neither be defined as MAC or NMAC. Compared to MAC, RIC has a minimum TBI or alkylating agent dosage reduction of 30% (Bacigalupo *et al.*, 2009). It differs from NMAC in that RIC causes prolonged cytopenia and requires stem cell support (Bacigalupo *et al.*, 2009). According to the Center for International Blood and Marrow Transplant Research (CIBMTR) and National Marrow Donor Program, RIC regimens have to meet one or more of the following criteria (Giralt, 2005):

1. ≤ 500 cGy total-body irradiation,
2. ≤ 9 mg/kg total busulfan dose,
3. ≤ 140 mg/m² total melphalan dose,
4. ≤ 10 mg/kg total thiotepa dose and
5. Includes a purine analog—fludarabine, cladribine, or pentostatin.

The aim of RIC regimes is to prevent graft rejection and to establish stable donor-derived hematopoiesis while maintaining low rates of non-hematologic toxicity (Giralt, 2005; Satwani, Cooper, Rao, Veys, & Amrolia, 2008).

The donor HSCs are transplanted after a patient has been placed on a conditioning regimen. The donor HSCs are infused into the patient's bloodstream through a typical central line. The HSCs will travel along the bloodstream throughout the body for several days, after which they begin to dwell into the spaces of the patient's bone marrow. At this point, the HSCs will grow and will begin producing new blood cells after 1-2 weeks. White blood cells will be formed first, then red blood cells, and lastly platelets. The engraftment, or the process by which the patient's body accepts the new HSCs, will follow 1-3 weeks after transplantation.

3.2 BM vs. UCB

UCBTs are currently one of the most common types of transplants in the United States, as evidenced by the increasing number of transplantation centers (Figure 3) (National Marrow Donor Program, 2010b). The outcomes of transplants from 6/6 matched BM vs. unrelated UCB have been compared in various studies (Table

1) (Laughlin *et al.*, 2004; Rocha *et al.*, 2004; Takahashi *et al.*, 2004).

A study performed by Laughlin *et al.* included 150 patients with 1 or 2 HLA mismatched CBUs (Laughlin *et al.*, 2004). The study also included 367 patients receiving 6/6 HLA matched bone marrow units and 83 patients receiving 5/6 HLA matched BM units. UCBT patients had a median body weight less than BMT patients (68kg vs. 76kg). UCB patients received ten-fold less total nucleated cells (TNCs) compared to BMT patients (0.22×10^8 vs. 2.4×10^8 per kg of body weight). The recovery times for neutrophils (>500 cells/mm³) and platelets (20 000 cells/mm³) were both delayed for UCBT recipients compared to BMT recipients (27 days vs. 18 days, 95% CI and 60 days vs. 29 days, 95% CI). Similarly cumulative incidences for neutrophil recovery at 100 days and platelet recovery at one year after matched BM transplants were significantly higher than in UCBT. However, compared to 1-HLA mismatch BMTs, UCBTs resulted in similar cumulative incidences of neutrophil recovery at 100 days ($p=0.29$) and platelet recovery at one year ($p=0.16$). The rate of acute II-IV GVHD was similar between matched BMT and UCBT (48% vs. 41%, 95% CI). Patients who survived after 90 days were assessed for chronic GVHD which was higher in UCBT patients than in matched BMT patients (35% vs. 51%). However, UCBT patients developed less acute II-IV GVHD occurrences than mismatched BMT patients (95% CI) while chronic GVHD rates were similar. TRM was significantly higher in UCBT patients than in matched BMT patients (63% vs. 46%). Treatment failures developed in 69% of matched BMTs and 81% of UCBTs. Leukemia relapse was observed in 26 out of 150 UCBT patients and 83 out of 367 BMT patients. The DFS rate after 3 years was 23% and 33% for UCBT and BMT patients, respectively. After 100 days, the rates of death in UCBT patients were significantly higher than in matched BMT patients (70% vs. 50%, $p<0.001$). The proportion of deaths due to infection was higher in UCBT patients than in both matched and mismatched BMT patients. Although TRM, rate of treatment failure, and overall mortality were significantly higher in UCBT patients than in matched BMT patients, the rates were similar with BMTs that have 1 HLA mismatch.

A four-year retrospective study by Rocha *et al.* compared 98 UCBTs and 584 BMTs in patients diagnosed with acute leukemia (Rocha *et al.*, 2004). UCBT patients were diagnosed with more advanced leukemia at the time of transplantation ($p<0.001$). The median age and body weight of UCBT patients were both less than BMT patients (24.5 years and 58kg vs. 32 years and 68kg). All BMTs were 6/6 HLA matched while 90% of UCBTs were 1-2 HLA mismatched. Similar to the study by Laughlin *et al.* (2004), UCBT recipients received ten-fold less TNCs compared to BMT recipients (0.23×10^8 vs. 2.9×10^8 per kg of body weight). ANC (>500 cells/mm³) engraftment was significantly delayed in UCBT (26 days vs. 19 days). Primary graft failure occurred in 43 BMT patients and 20 UCBT patients while secondary graft failure occurred in four BMT patients and one UCBT patient. UCBT patients were significantly less likely to experience acute II-IV GVHD when compared to BMT patients (26% vs. 39%, $p=0.008$) while there was no significant difference in chronic GVHD between the two groups (30% vs. 46%, $p=0.07$). A two-year follow-up demonstrated that TRM and DFS were insignificant between the two groups (44% vs. 38%, $p=0.13$, and 33% vs. 38%, $p=0.06$).

A single center study was performed in Japan by Takahashi *et al.* in 2004 comparing 68 UCBTs and 45 BMTs (Takahashi *et al.*, 2004). Median age of UCBT patients was higher than BMT patients while median weight was higher in BMT patients (36 years and 55.1kg vs. 26 years and 59.6kg). Out of 68 UCBT patients, 75% received 4-5/6 matched CBUs. Most of BMT patients received matched BMTs while only six patients received 5/6 HLA matched BMTs. The patients underwent MAC regimens consisting of cyclophosphamide and TBI. UCB grafts contained more than 2.0×10^7 cells per kg and a median of 0.9×10^5 CD34⁺ progenitor cells per kg. Within 28 days after transplantation, 4% of UCBT patients died while 8% experienced primary graft failure. UCBT recipients had a delayed ANC (>500 cells/mm³) engraftment than BMT recipients (22 days vs. 18 days). Platelet (>20 000 cells/mm³) recovery was observed after 40 and 25 days for UCBTs and BMTs, respectively. The overall myeloid engraftment rates on day 42 were 92% for UCBT and 100% for BMT. Despite higher HLA mismatches in UCBTs, acute II-IV and III-IV GVHD incidences were lower than BMTs. Among the patients surviving 100 days after transplantation, 78% of patients receiving UCBTs and 74% of those receiving BMTs were affected by chronic GVHD. The hazard ratio for one-year TRM incidence was lower in UCBT patients compared to BMT patients (0.32 vs. 1.0, 95% CI). However, although there is no apparent risk of relapse in the two groups ($p=0.73$), relapse was slightly lower in UCBT recipients (hazard ratio of 0.76 vs. 1.0, 95% CI). DFS was significantly better among patients receiving UCBTs than BMT patients (74% vs. 44%). Deaths of BMT patients were more likely due to GVHD while no correlation between death and GVHD was observed in UCBT patients. TRM and DFS rates were surprisingly better in UCBT patients than in BMT patients compared to other studies (Laughlin *et al.*, 2004; Rocha *et al.*, 2004). A possible explanation for the improved TRM and DFS rates in this study is that it evaluated patients only from a single center. Lower incidences of acute II-IV GVHD may be explained due to lower degree of diversity for HLA and minor histocompatibility antigens among more

homogenous population in Japan. Moreover, it has been shown that the Japanese population expresses a higher frequency of the gene that induces interleukin-10 production which may also contribute to lower acute II-IV GVHD incidences (Lin *et al.*, 2003).

3.2.1 Advantages vs. disadvantages

Based on the UCBT and BMT studies performed one can determine the advantages and disadvantages of both transplant types. Compared to HSCs from BM, HSCs from UCB are able to engraft not only with reduced GVHD, but also with less severe cases of GVHD (Rocha *et al.*, 2004). A possible reason may be due to immune regulators such as Tregs or MSCs, as described earlier, that are mixed in with UCB. In addition, UCB allow for more diverse transplants due to its ability to engraft despite a higher degree of HLA mismatch. BMTs require at least a 5/6 HLA match while UCBTs require at least a 4/6 HLA match (although a 3/6 match has also proved to be functional)(J. N. Barker *et al.*, 2002). This allows for a much higher probability for finding a patient-donor match. A statistical analysis has shown that patients seeking a CBU will be able to find a 4/6 HLA match 99% of the time (Beatty, Boucher, Mori, & Milford, 2000; Stevens, Scaradavou, Carrier, Carpenter, & Rubinstein, 2005). The probability of finding a 5/6 CBU with a sufficient cell dose ($> 2 \times 10^7$ TNC/kg) is 80% given that at least 170 000 CBUs are available. Moreover, if a patient is identified for an HSC transplant, the process of finding a CBU match is quicker (days-weeks) than identifying a match for a BMT (weeks-months). The average time for identifying a donor of a CBU is 13.5 days while it is 45 days to identify a BM donor (Barker *et al.*, 2002). It is faster to identify a matching CBU because it will be available for transplant as soon as the unit is identified. It takes longer to acquire a BMT because first, a donor must be identified (average of 19 days), and second, a 30-day period necessary to clear the donor (J. N. Barker *et al.*, 2002). Extraction of HSCs from UCB is noninvasive compared to extraction of HSCs from BM. While BMTs require the use of pervasive extraction procedures (aspiration) which will exert some pain to the donor, cord blood permits easy access to stem cells with no pain to the mother or the newborn. Furthermore, extracting HSCs from BM can lead to donor infection.

A disadvantage of UCB is that the patient has both a longer time-to-neutrophil recovery and time-to platelet recovery. Clinical trials have shown that UCB displayed a time-to-neutrophil recovery (neutrophils > 500 cells/mm³) ranging from 22-27 days as opposed to an average of 18 days for unrelated BMTs (Taghizadeh & Sherley). Moreover, UCB presented a time-to-platelet (platelets > 20000 cells/mm³) recovery of 60 days compared to 29 days for BM transplants (Laughlin *et al.*, 2004). Similarly, another clinical trial exhibited a time-to-neutrophil recovery (neutrophils > 500 cells/mm³) average of 27 days for HLA mismatched UCB and 19 days for HLA matched BM recipients (Rocha *et al.*, 2004).

The TNC dose transplanted per kg of body weight is directly correlated with the outcomes of the transplantation (Lim *et al.*, 1999; Tung, Parmar, Robinson, De Lima, & Shpall, 2010). TNC dose is critical in neutrophil recovery and higher CD34⁺ cell counts, which is associated with higher engraftment rate, TRM, and improved survival (Barker *et al.*, 2005; Tung *et al.*, 2010). Patients receiving UCBTs of cell doses less than 1.8×10^7 TNCs and 1.7×10^5 CD34⁺ cells per kilogram of the recipient body weight had inferior engraftment and survival rates (J. E. Wagner *et al.*, 2002). On average, TNCs in UCB are approximately 10 fold lower than BM. These low amounts of CD34⁺ cells may provide an explanation for the delayed engraftment of UCBTs (Eapen *et al.*, 2007; Laughlin *et al.*, 2004; Rocha *et al.*, 2004; Takahashi *et al.*, 2007). Laboratory techniques as well as purification procedures are currently being explored to determine the most efficient methods of collecting or culturing CD34⁺ cells.

4. Double Cord Blood Transplantation

Due to the limited cell dose available from one CBU, UCBTs are more ideal for younger, lower weight, patients. Even in UCBTs with small children, delays in engraftment and immune reconstitution are observed compared to other stem cell sources (Tung *et al.*, 2010). Additionally, patients with a total body weight of more than 45 kg who receive only one CBU are associated with decreased neutrophil and platelet recovery and higher rates of engraftment failure (Tung *et al.*, 2010). In order to overcome low cell dose in UCB HSCs, two methods are often employed, DCBT and *ex vivo* expansion.

In DCBT, two CBUs, each of which has no more than 2/6 HLA mismatches (low resolution typing of HLA-A and -B or high resolution typing of HLA-DRB1), are infused together to transplant into adults and large children (Barker *et al.*, 2005; Brunstein *et al.*, 2007; Rocha *et al.*, 2010; Wagner, 2009). The two CBUs chosen for transplantation must have at least a 4/6 HLA match between the units themselves and with the patient.

When a CBU is selected for transplantation, the TNC dose is used to determine if a single unit is sufficient for treatment (Figure 4) (Barker *et al.*, 2005; Brunstein, *et al.*, 2007; Wagner, 2009). A minimum TNC dose of $2.5-3 \times 10^7$ per kg of body weight is recommended for each of the two closely matched (5/6 or 6/6 matches) UCB

units (Tung *et al.*, 2010). A greater mismatch between the two CBUs requires a higher TNC count (Tung *et al.*, 2010). Research suggests use of two CBUs if a single HLA-matched unit has TNC dose of $< 3.0 \times 10^7$ per kg (Brunstein *et al.*, 2007; Wagner, 2009). However, Barker *et al.* (Barker *et al.*, 2005) performed DCBTs on patients when the TNC dose of a single unit was less than 3.5×10^7 per kg in order to increase graft cell dose for patients. Barker *et al.* (Barker, Scaradavou, & Stevens, 2010) further suggested that if the CBU is only 4/6 HLA matched, then the cell dose should be as high as 5.0×10^7 per kg.

4.1 DCBTs using different conditioning regimens

Various DCBT studies have been performed separately on MAC, NMAC, and RIC (Table 2) (Ballen *et al.*, 2007; Barker *et al.*, 2005; Barker *et al.*, 2009; Brunstein *et al.*, 2007; Cutler *et al.*, 2010; Kanda *et al.*, in press; Majhail, Brunstein, & Wagner, 2006; Rodrigues *et al.*, 2009; Verneris *et al.*, 2009; Yoo *et al.*, 2011). The largest MA study performed by Verneris *et al.* included 93 DCBT patients and 84 single UCBT patients all diagnosed with acute leukemia or transplantation in first or second complete remission (CR1-2) (Verneris *et al.*, 2009). Median age and weight of DCBT patients were 24 years (range 9-57 years) and 69kg, respectively. At least 90% of DCBT and single cord recipients received two 4-5/6 HLA matched units. The majority of AML and ALL patients were in CR1 or CR2, and a sub-majority who underwent transplantation in CR1 had high risk clinical features. DCBT recipients received a median infused TNC dose of 3.6×10^7 per kg of body weight while single cord recipients received a median dose of 3.3×10^7 per kg of body weight. ANC engraftment recovery was similar between the two groups. Grade II-IV acute GVHD occurred more frequently in DCBT patients than single cord patients (48% vs. 29%, $p < 0.01$). A similar trend is observed with chronic GVHD (18% vs. 10%, $p = 0.06$). Relapse was significantly lower in CR1-2 DCBT patients than single cord blood transplant patients (16% vs. 31% $p < 0.03$). Verneris *et al.* have determined that two risk factors were associated with relapse, (1) disease state and (2) use of two partially matched CBUs. DFS was similar among the two groups after 5 years (51% vs. 40%, $p = 0.35$). A DCBT study by Barker *et al.* included recipients of a similar age group. They received similar amounts of TNCs, which resulted in analogous ANC engraftment (Barker *et al.*, 2005). Correspondingly, more patients were likely to have acute II-IV GVHD than chronic GVHD (65% and 23%). DFS after 10 months was achieved in 57% of patients. Similar occurrences were observed in other clinical studies (Barker *et al.*, 2009; Kanda *et al.*, in press).

The largest NMA study using two units of UCB was performed by Brunstein *et al.* and included 93 patients diagnosed with high risk hematologic diseases (Brunstein *et al.*, 2007). Median age and weight of patients were 51 years and 76 kg, respectively. The majority of recipients received two 4-5/6 matched units. Patients received combined median TNC dose of 3.7×10^7 and median CD34+ dose of 1.2×10^7 per kg of body weight. ANC engraftment recovery was achieved after 12 days. There were fewer cases of chronic (23%) than acute II-IV GVHD (59%). Acute GVHD did not associate with HLA matching. A three-year follow up assessed a 38% DFS and TRM of 26%. Preexisting high-risk clinical features seemed to be the primary predictors of increased TRM as patients lacking these features showed low TRM. Overall survival was not influenced by age but was rather attributable to severe GVHD and preexisting high-risk clinical features. Chimerism of the two UCB units was observed in 81 patients. Chimerism from each CBU decreased over time (43% at day 21, 9% at day 100, and 0% at one year) until one unit predominated. The contribution of donor-derived cells to the predominant unit was 7% at day 21 and 0% at day 100. Factors including cell doses and HLA matching were not predictive of the dominating unit. A possible reason that a relationship was not observed might be attributable to the sequential (~1 hour) infusion of the two units. Ballen *et al.* allowed 3.5-4.5 hours between the infusion of the first and second unit, and observed that the first unit infused predominated 76% of the time (Ballen *et al.*, 2007).

In a previous study performed by Ballen *et al.*, 21 patients were treated by same RIC with fludarabine/melphalan/rabbit antithymocyte globulin but different GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil. The median age and weight were 49 years and 78 kg respectively. The median infused TNC dose was 4.0×10^7 and that of CD34+ was 1.9×10^5 per kg. After transplantation, two patients experienced primary graft failure. Among the remaining patients, the median time to ANC (> 500 cells/mm³) and platelet count (> 20000 cells/mm³) were 20 days and 41 days respectively. Grade II-IV acute GVHD developed in 40% of the patients. Among 16 patients evaluable, 31% suffered chronic GVHD. TRM was 14% at day 100 and 19% at 6 months. The two year follow up for OS and DFS were 71% and 55%. Chimerism was evaluated among 17 patients. Both donor units were initially identified in 6 patients. However, after 12 weeks post-transplantation, single unit predominance was observed in 13 patients, double cord chimerism was observed in 1 patient, and host hematopoiesis along with single cord blood was observed in 3 patients. Of all the patients with single unit predominance, the predominant unit infused first ($p = 0.049$) and generally had higher TNC ($p = 0.071$) and CD34+ ($p = 0.120$) counts.

Cutler *et al.* recently performed a study describing their experiences with 32 patients who underwent DCBT. All

patients received RIC of fludarabine (180mg/m²), melphalan (100mg/m²) and rabbit antithymocyte globulin (6.0mg/kg) and received sirolimus, which is a potent immunosuppressant that prevents T-cell mediated alloimmunity and tacrolimus to prevent acute GVHD. The median age and weight of the patients were 53 years and 75.9 kg respectively. The majority of patients (91%) received two 4-6/6 units. UCB units engrafted had a minimum required combined cell dose of 3.7x10⁷ TNC/kg before cryopreservation and each individual unit had 1.5x10⁷ TNC/kg pre-cryopreservation. The median pre-cryopreserved combined doses of TNC and CD34⁺ progenitors were 5.16x10⁷ and 1.9x10⁵ per kg respectively. The two CBUs were administered sequentially 1 and 6hr apart, with the unit with higher pre-cryopreservation TNC dose being administered first. Patients achieved ANC (>500 cells/mm³) ranging from 13-70 days (median = 21 days) and platelet engraftment at 42 days. Grade II-IV acute GVHD developed in three of 32 patients and chronic GVHD developed in four of 32 patients. Non-relapse mortality at 100 days and two years were 12.5% and 34.4% respectively. Chimerism was evaluated among 29 patients who survived, to determine the relative contribution to hematopoiesis. At day 100, 62% of the surviving patients showed evidence of single CBU dominance while both units contributed to hematopoiesis in the remaining patients. The first infused unit was observed to be responsible for the majority of hematopoiesis in 61% of the patients who showed single CBU dominance. Among the patients with single cord predominance at day 100, 67% had only single unit contributing to hematopoiesis while the remaining patients had evidence of single unit early graft rejection or loss. The median time to relapse after transplantation was 12.6 months while DFS and OS at two years were 31.2% and 53.1% respectively. Comparing to the study performed by Ballen *et al.*, there was no significant difference between engraftment rates. However, the incidences of acute GVHD were significantly lower ($p=0.035$). It is speculated that the use of sirolimus and tacrolimus GVHD prophylaxis reduced the GVHD occurrence since both studies used the same RIC and UCB selection algorithm. Similar results were achieved in studies performed by Ballen (2007) and Rodrigues (2009) in which DFS rates were 55% and 57%, respectively.

In most patients, only one of the two units gave rise to donor hematopoiesis (Ballen *et al.*, 2007; Brunstein *et al.*, 2007; Cutler *et al.*, 2010). However, this strategy has been shown to improve engraftment (Scaradavou *et al.*, 2010). Moreover, a reduced risk of relapse but higher incidence of acute GVHD may be associated with DCBT compared to single UCBT (Rocha *et al.*, 2010; Scaradavou *et al.*, 2010).

4.1.1 MA Double Cord Blood Transplants vs. MA single UCBT - Clinical

There is great difficulty in drawing conclusions from data results when there isn't much data to consider. DCBT TNCs were normally greater than 3.5x10⁷ which is approximately two fold greater than single UCBTs. ANC engraftment rates were similar between double and single UCBTs that use MA conditioning regimens. The ANC engraftments for DCBT ranged from 23-25 days, which are comparable to results using single UCBTs. Prevalence of acute II-IV GVHD were diverse among studies in both single and DCBTs, however, the rate of acute II-IV GVHD seemed higher in DCBTs. Chronic GVHD rates were also diverse, but overall seemed to be lower in DCBTs. The percentage of DFS following one or two years in DCBTs was ranged from 51-57%, which appeared to be lower than single UCBTs.

5. *Ex Vivo* Expansion

An alternative method of overcoming low cell dose of UCB is *ex vivo* expansion of UCB HSCs. Primitive hematopoietic progenitor cells such as CD133⁺ and CD34⁺ from UCB are cultured with cytokines, growth factors and other growth promoting compounds in liquid culture (Tung *et al.*, 2010). The growth factors that are cultured with hematopoietic progenitor cells usually include stem cell factor, interleukin -3, -6, granulocyte colony-stimulating factor, thrombopoietin, and Flt-3 Ligand (Tung *et al.*, 2010). Several other techniques have been attempted to further improve expansion by using histone deacetylases to promote HSC self-renewal, glycogen synthase kinase-3 inhibitors to maintain pluripotency of stem cells, or tetraethylenepentamine to stimulate *ex vivo* expansion of hematopoietic progenitors (Peled, Landau, Prus, Treves, & Fibach, 2002; Sato, Ozaki, Oh, Meguro, Hatanaka & Nagai, 2007; Young *et al.*, 2004).

Another technique to expand UCB is to provide a microenvironment for HSCs. This technique will regulate differentiation and proliferation of HSCs and provide cues that direct hematopoiesis (Tung *et al.*, 2010). One potential attempt to accomplish this is by co-culturing HSCs with MSCs. MSCs derived from the Wharton's jelly of an UC provide stromal support for cord blood HSCs (Bakhshi *et al.*, 2008). In the long-term culture-initiating cell assay, UC MSCs were proven to effectively support the growth of CD34⁺ cells from UCB. Other research suggests co-transplanting MSCs with HSCs in UCBTs (Noort *et al.*, 2002). Co-transplantation may promote engraftment of UCB CD34⁺ cells. Mc Niece *et al.* (Mc Niece, Harrington, Turney, Kellner, & Shpall, 2004) isolated and cultured mononuclear cells from UCB on MSC layers *ex vivo* and observed a 10-20 fold increase in

TNC, a 7-18 fold increase in committed progenitor cells, a 2-5 fold increase in primitive progenitor cells, and a 16-37 fold increase in CD34⁺ cells after 14 days. Based on previous research, co-culturing HSCs with MSCs may prove to be a potentially effective therapeutic application in HSC transplantation.

6. Blood extraction and processing

UCB is currently collected for one of two reasons: (i) donation for public use or (ii) storage for self or family members who are suffering from a disease that can be cured by HSC transplantation (Ballen, Barker, Stewart, Greene, & Lane, 2008). Expecting mothers must meet certain criteria to be eligible to donate UCB of the newborns. The general eligibility guidelines for expectant mothers, according to the National Marrow Donor Program (National Marrow Donor Program, 2010a), are listed below:

- (i) Be at least 18 years of age
- (ii) Have no history of hepatitis B or C, HIV, medication-dependent diabetes and cancer
- (iii) Have no history of organ transplantation
- (iv) Have no history of sexually transmitted diseases within the last 12 months
- (v) Have not had tattoos, non-sterile piercings or acupuncture done in the previous 12 months
- (vi) Have no malaria history within last three years

UCB is collected after delivery either before placental separation from the uterus wall or after placenta delivery (Ballen *et al.*, 2008). Life force Cryobanks, ViaCord and New England Cord Blood Bank have similar procedures of collecting UCB (Life force Cryobanks, 2011; New England Cord Blood Bank, 2011; Via Cord, 2011). The cord of the baby is clamped and cut postnatally. After the UC is rinsed with antiseptic solution, the umbilical vein is punctured with a needle connected to a standard blood collection bag that contains anticoagulant. Blood flows into the bag by gravity, and after flow stops, the bag is detached and stored away for further processing. This simple procedure used by UCB banks corresponds with the UCB collection technique by Adami *et al.* (Adami *et al.*, 2005). In this method, the UC was double clamped within 30sec after newborn delivery, and blood was collected while placenta was *in utero*. The anticoagulant used in the collection bag was citrate-phosphate-dextrose. A similar procedure has been done by Elchalal *et al.*, except that the cord was ligated before clamping to avoid tissue crushing. The UC was then cut between the clamp and ligation. Moreover, massaging or “milking” of the cord was performed at the end of blood flow (Elchalal *et al.*, 2000).

It is important to be cautious when collecting UCB to obtain high-quality CBUs for transplantation. The major issues involved with UCB collection include the volume of UCB collected, microbial infection, delay in blood collection, and blood coagulation (Ballen *et al.*, 2008).

6.1 Cryopreservation

The storage process for HSCs is termed cryopreservation and is outlined in Figure 5. UCB is first treated with chemicals such as starch or ammonium chloride to reduce plasma and red blood cell (RBC) counts. RBCs typically lyse easily when they are frozen or thawed. Research suggests that these cell lysates may have detrimental effects when the HSCs are used for transplants (Regidor *et al.*, 1999). Chemical treatment additionally helps reduce blood volume, allowing for easier storage and reduction of cellular debris upon thawing. Once RBCs are depleted; a freezing medium containing dimethyl sulfoxide is added. Dimethyl sulfoxide is a cryoprotectant that helps preserve the cells from the sub-zero temperatures used during the cryopreservation process. The HSCs will freeze at a controlled rate of 1°C /1 min until -80°C. This process is usually carried out overnight. Several studies have indicated that freezing cells at a controlled rate increases cell viability (Perez-Oteyza *et al.*, 1998 (Balint *et al.*, 2006). The HSCs will then be transferred into a liquid nitrogen tank at a temperature of -196°C. The nitrogen tank typically consists of numerous compartments that help in the labeling and identification process.

6.2 Banking

There are two types of UCB banks: public and private (Table 3). Public banking is the more widely used of the two and stores donated stem cells to the public registry. Access to stem cells is provided on a first-come first-serve basis. The donation is done with the consent of the mother and at no cost to the family. When a patient needs an UCBT, he/she will have up to a 90% chance of finding a compatible transplant in a public bank (Novello-Garza *et al.*, 2008). The cost of the transplant, which is generally covered by insurance, ranged from \$15 000-\$25 000.

Private blood banking stores cord blood exclusively for the family of the newborn. Mothers who have had a

history of a particular disease in their family that may require a stem cell transplant in the future typically choose this type of banking. The cord blood transplant is guaranteed to be 100% compatible with the child of the mother. However, this percentage decreases drastically to 25% compatibility with any relative (J. Wagner *et al.*, 1996). Banking privately costs about \$1500 in down payment and \$150-\$200 annually. When a transplant is needed, the CBU is released at no cost to the patient.

7. Conclusion

DCBT and *ex vivo* expansion of HSCs have expanded transplantation into all patients, despite of size or ethnicity. The results of these expansion techniques require a better understanding of the biological processes that occur. The spread and rise of HSC transplantation centers promises further research in the field. Such research may reveal better methods for improving overall GVHD and survival rates in patients who receive HSC transplantations. One implication for future research is to compare the different types of conditioning regimens. It seems that MA conditioning regimens in the analyzed DCBT studies resulted in greater than 50% DFS in all studies. Co-transplantation of HSCs with MSCs has shown to improve patient outcomes but requires additional study (Blanc *et al.*, 2007; in't Anker *et al.*, 2003; Maitra *et al.*, 2004). Further investigation for using UCB to aid in remodeling of the myocardium following an acute myocardial infarction may also be executed. It has been shown that heterogeneous cell populations of bone marrow containing hematopoietic and mesenchymal progenitor cells are able to improve post-infarction remodeling processes (Britten *et al.*, 2003; Lunde *et al.*, 2006; Schächinger *et al.*, 2006) UCB contains similar progenitor cells and thus may have the potential to aid in the restoration of the contractile functions of the myocardium.

References

- Adami, V., Malangone, W., Falasca, E., Marini, L., Risso, A., Crini, S., *et al.* (2005). A closed system for the clinical banking of umbilical cord blood. *Blood Cells, Molecules, and Diseases*, 35 (3), 389-397. <http://dx.doi.org/10.1016/j.bcmed.2005.07.008>
- Aggarwal, S. & Pittenger, M. F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*, 105 (4), 1815-1822. <http://dx.doi.org/10.1182/blood-2004-04-1559>
- Anderson, B. E., McNiff, J. M., Matte, C., Athanasiadis, I., Shlomchik, W. D., & Shlomchik, M. J. (2004). Recipient CD4+ T cells that survive irradiation regulate chronic graft-versus-host disease. *Blood*, 104 (5), 1565-1573. <http://dx.doi.org/10.1182/blood-2004-01-0328>
- Bacigalupo, A., Ballen, K., Rizzo, D., Giralt, S., Lazarus, H., Ho, V., *et al.* (2009). Defining the intensity of conditioning regimens: Working definitions. *Biology of Blood and Marrow Transplantation*, 15 (12), 1628-1633. <http://dx.doi.org/10.1016/j.bbmt.2009.07.004>
- Bakhshi, T., Zabriskie, R. C., Bodie, S., Kidd, S., Ramin, S., Paganessi, L. A., *et al.* (2008). Mesenchymal stem cells from the wharton's jelly of umbilical cord segments provide stromal support for the maintenance of cord blood hematopoietic stem cells during long-term ex vivo culture. *Transfusion*, 48 (12), 2638-2644. <http://dx.doi.org/10.1111/j.1537-2995.2008.01926.x>
- Balint, B., Paunovic, D., Vucetic, D., Vojvodic, D., Petakov, M., Trkuljic, M., *et al.* (2006). Controlled-rate versus uncontrolled-rate freezing as predictors for platelet cryopreservation efficacy. *Transfusion*, 46 (2), 230-235. <http://dx.doi.org/10.1111/j.1537-2995.2006.00706.x>
- Ballen, K. K., Barker, J. N., Stewart, S. K., Greene, M. F., & Lane, T. A. (2008). Collection and preservation of cord blood for personal use. *Biology of Blood and Marrow Transplantation*, 14 (3), 356-363. <http://dx.doi.org/10.1016/j.bbmt.2007.11.005>
- Ballen, K. K., Spitzer, T. R., Yeap, B. Y., McAfee, S., Dey, B. R., Attar, E., *et al.* (2007). Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biology of Blood and Marrow Transplantation*, 13 (1), 82-89. <http://dx.doi.org/10.1016/j.bbmt.2006.08.041>
- Barker, J. N., Scaradavou, A., & Stevens, C. E. (2010). Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood*, 115 (9), 1843-1849. <http://dx.doi.org/10.1182/blood-2009-07-231068>
- Barker, J. N., Abboud, M., Rice, R. D., Hawke, R., Schaible, A., Heller, G., *et al.* (2009). A "No-wash" albumin-dextran dilution strategy for cord blood unit thaw: High rate of engraftment and a low incidence of serious infusion reactions. *Biology of Blood and Marrow Transplantation*, 15 (12), 1596-1602. <http://dx.doi.org/10.1016/j.bbmt.2009.08.009>
- Barker, J. N., Weisdorf, D. J., DeFor, T. E., Blazar, B. R., McGlave, P. B., Miller, J. S., *et al.* (2005).

Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*, 105 (3), 1343-1347. <http://dx.doi.org/10.1182/blood-2004-07-2717>

Barker, J. N., Krepski, T. P., DeFor, T. E., Davies, S. M., Wagner, J. E., & Weisdorf, D. J. (2002). Searching for unrelated donor hematopoietic stem cells: Availability and speed of umbilical cord blood versus bone marrow. *Biology of Blood and Marrow Transplantation*, 8 (5), 257-260. <http://dx.doi.org/10.1053/bbmt.2002.v8.pm12064362>

Batten, P., Sarathchandra, P., Antoniow, J. W., Tay, S. S., Lowdell, M. W., Taylor, P. M., & Yacoub, M. H. (2006). Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: Relevance to tissue engineering human heart valves. *Tissue Engineering*, 12 (8), 2263-2273. <http://dx.doi.org/10.1089/ten.2006.12.2263>

Beatty, P. G., Boucher, K. M., Mori, M., & Milford, E. L. (2000). Probability of finding HLA-mismatched related or unrelated marrow or cord blood donors. *Human Immunology*, 61 (8), 834-840. [http://dx.doi.org/10.1016/S0198-8859\(00\)00138-5](http://dx.doi.org/10.1016/S0198-8859(00)00138-5)

Bieback, K. & Kluter, H. (2009). Non-hematopoietic stem and progenitor cells derived from human umbilical cord blood. In N. Bhattacharya, & P. Stubblefield (Eds.). *Frontiers of cord blood science* (1st ed., pp. 123-161). London: Springerlink.

Bieback, K., Kern, S., Kluter, H., & Eichler, H. (2004). Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. *Stem Cells*, 22 (4), 625-634. <http://dx.doi.org/10.1634/stemcells.22-4-625>

Britten, M. B., Abolmaali, N. D., Assmus, B., Lehmann, R., Honold, J., Schmitt, J., et al. (2003). Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): Mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation*, 108 (18), 2212-2218. <http://dx.doi.org/10.1161/01.CIR.0000095788.78169.AF>

Blanc, K. L., Samuelsson, H., Gustafsson, B., Remberger, M., Sundberg, B., Arvidson, J., et al. (2007). Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells; MSCs to enhance engraftment of allogeneic HSCs. *Leukemia*, 21, 1733-1738. <http://dx.doi.org/10.1038/sj.leu.2404777>

Broxmeyer, H. E. (May 26, 2010). Cord blood hematopoietic stem cell transplantation. *StemBook* (1st ed.). The stem cell research community.

Broxmeyer, H. E., Douglas, G. W., Hangoc, G., Cooper, S., Bard, J., English, D., et al. (1989). Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proceedings of the National Academy of Sciences of the United States of America*, 86 (10), 3828-3832.

Brunstein, C. G., Barker, J. N., Weisdorf, D. J., DeFor, T. E., Miller, J. S., Blazar, B. R., et al. (2007). Umbilical cord blood transplantation after nonmyeloablative conditioning: Impact on transplant outcomes in 110 adults with hematological disease. *Blood*, 110 (8), 3064-3070. <http://dx.doi.org/10.1182/blood-2007-04-067215>

Carrier, E. & Ledingham, G. (2004). *100 questions & answers about bone marrow and stem cell transplantation* (1st ed.). Massachusetts: Jones and Bartlett Publishers, Inc.

Casper, J., Knauf, W., Kiefer, T., Wolff, D., Steiner, B., Hammer, U., et al. (2004). Treosulfan and fludarabine: A new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. *Blood*, 103 (2), 725-731. <http://dx.doi.org/10.1182/blood-2002-11-3615>

Chen, X., Vodanovic-Jankovic, S., Johnson, B., Keller, M., Komorowski, R., & Drobyski, W. R. (2007). Absence of regulatory T-cell control of TH1 and TH17 cells is responsible for the autoimmune-mediated pathology in chronic graft-versus-host disease. *Blood*, 110 (10), 3804-3813. <http://dx.doi.org/10.1182/blood-2007-05-091074>

Chen, Y., Shao, J., Xiang, L., Dong, X., & Zhang, G. (2008). Mesenchymal stem cells: A promising candidate in regenerative medicine. *The International Journal of Biochemistry & Cell Biology*, 40 (5), 815-820. <http://dx.doi.org/10.1016/j.biocel.2008.01.007>

Cohen, J. L., Trenado, A., Vasey, D., Klatzmann, D., & Salomon, B. L. (2002). CD4+CD25+ immunoregulatory T cells. *The Journal of Experimental Medicine*, 196 (3), 401-406. <http://dx.doi.org/10.1084/jem.20020090>

Cutler, C., Stevenson, K., Kim, H. T., Brown, J., McDonough, S., Herrera, M., et al. (2010). Double umbilical cord blood transplantation with reduced intensity conditioning and sirolimus-based GVHD prophylaxis. *Bone Marrow Transplantation*. [Epub ahead of print], 1-9. <http://dx.doi.org/10.1038/bmt.2010.192>

Eapen, M., Rubinstein, P., Zhang, M., Stevens, C., Kurtzberg, J., Scaradavou, A., et al. (2007). Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: A

- comparison study. *The Lancet*, 369 (9577), 1947-1954. [http://dx.doi.org/10.1016/S0140-6736\(07\)60915-5](http://dx.doi.org/10.1016/S0140-6736(07)60915-5)
- Elchalal, U., Fasouliotis, S. J., Shtockheim, D., Brautbar, C., Schenker, J. G., Weinstein, D., *et al.* (2000). Postpartum umbilical cord blood collection for transplantation: A comparison of three methods. *American Journal of Obstetrics and Gynecology*, 182 (1), 227-232. [http://dx.doi.org/10.1016/S0002-9378\(00\)70517-5](http://dx.doi.org/10.1016/S0002-9378(00)70517-5)
- Fontenot, J. D., Rasmussen, J. P., Williams, L. M., Dooley, J. L., Farr, A. G., & Rudensky, A. Y. (2005). Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity*, 22 (3), 329-341. <http://dx.doi.org/10.1016/j.immuni.2005.01.016>
- Giralt, S. (2005). Reduced-intensity conditioning regimens for hematologic malignancies: What have we learned over the last 10 years? *Hematology*, 2005 (1), 384-389. <http://dx.doi.org/10.1182/asheducation-2005.1.384>
- Glennie, S., Soeiro, I., Dyson, P. J., Lam, E. W. F., & Dazzi, F. (2005). Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood*, 105 (7), 2821-2827. <http://dx.doi.org/10.1182/blood-2004-09-3696>
- Gluckman, E., Broxmeyer, H. E., Auerbach, A. D., Friedman, H. S., Douglas, G. W., Devergie, A., *et al.* (1989). Hematopoietic reconstitution in a patient with fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *New England Journal of Medicine*, 321 (17), 1174-1178. <http://dx.doi.org/10.1056/NEJM198910263211707>
- Groh, M. E., Maitra, B., Szekely, E., & Koç, O. N. (2005). Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. *Experimental Hematology*, 33 (8), 928-934. <http://dx.doi.org/10.1016/j.exphem.2005.05.002>
- Gunsilius, E., Gastl, G., & Petzer, A. L. (2001). Hematopoietic stem cells. *Biomedicine & Pharmacotherapy*, 55 (4), 186-194. [http://dx.doi.org/10.1016/S0753-3322\(01\)00051-8](http://dx.doi.org/10.1016/S0753-3322(01)00051-8)
- Higuchi, A., Yang, S., Li, P., Tamai, M., Tagawa, Y., Chang, Y., *et al.* (2010). Direct ex vivo expansion of hematopoietic stem cells from umbilical cord blood on membranes. *Journal of Membrane Science*, 351 (1-2), 104-111. <http://dx.doi.org/10.1016/j.memsci.2010.01.034>
- Horwitz, M. E. & Chao, N. (2010). Non-myeloablative umbilical cord blood transplantation. *Best Practice & Research Clinical Haematology*, 23 (2), 231-236. <http://dx.doi.org/10.1016/j.beha.2010.06.001>
- in 't Anker, P. S., Noort, W. A., Kruisselbrink, A. B., Scherjon, S. A., Beekhuizen, W., Willemze, R., *et al.* (2003). Nonexpanded primary lung and bone marrow-derived mesenchymal cells promote the engraftment of umbilical cord blood-derived CD34+ cells in NOD/SCID mice. *Experimental Hematology*, 31 (10), 881-889. [http://dx.doi.org/10.1016/S0301-472X\(03\)00202-9](http://dx.doi.org/10.1016/S0301-472X(03)00202-9)
- Jeong, J. A., Hong, S. H., Gang, E. J., Ahn, C., Hwang, S. H., & Yang, I. H. (2005). Differential gene expression profiling of human umbilical cord blood-derived mesenchymal stem cells. *Stem Cells*, 23 (4), 584-593. <http://dx.doi.org/10.1634/stemcells.2004-0304>
- Kanda, J., Rizzieri, D. A., Gasparetto, C., Long, G. D., Chute, J. P., Sullivan, K. M., *et al.* Adult dual umbilical cord blood transplantation using myeloablative total body irradiation (1350 cGy) and fludarabine conditioning. *Biology of Blood and Marrow Transplantation, In Press, Corrected Proof.* <http://dx.doi.org/10.1016/j.bbmt.2010.09.009>
- Kang, X., Zang, W., Bao, L., Li, D., Xu, X., & Yu, X. (2006). Differentiating characterization of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Cell Biology International*, 30 (7), 569-575. <http://dx.doi.org/10.1016/j.cellbi.2006.02.007>
- Kern, S., Eichler, H., Stoeve, J., Kluter, H., & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*, 24 (5), 1294-1301. <http://dx.doi.org/10.1634/stemcells.2005-0342>
- Kestendjieva, S., Kyurkchiev, D., Tsvetkova, G., Mehandjiev, T., Dimitrov, A., Nikolov, A., *et al.* (2008). Characterization of mesenchymal stem cells isolated from the human umbilical cord. *Cell Biology International*, 32 (7), 724-732. <http://dx.doi.org/10.1016/j.cellbi.2008.02.002>
- Kiel, M. J., Yilmaz, Ö. H., Iwashita, T., Yilmaz, O. H., Terhorst, C., & Morrison, S. J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*, 121 (7), 1109-1121. <http://dx.doi.org/10.1016/j.cell.2005.05.026>
- Kim, Y. & Broxmeyer, H. E. Immune regulatory cells in umbilical cord blood and their potential roles in transplantation tolerance. *Critical Reviews in oncology/hematology, In Press, Corrected Proof.*

<http://dx.doi.org/10.1016/j.critrevonc.2010.07.009>

Knudtzon, S. (1974). In vitro growth of granulocytic colonies from circulating cells in human cord blood. *Blood*, 43 (3), 357-361.

Kurt Benirschke & Peter Kaufmann. (2000). Anatomy and pathology of the umbilical cord and major fetal vessels. *Pathology of the human placenta* (4th. ed., pp. 335). New York: Springer-Verlag New York, Inc.

Kurtzberg, J., Laughlin, M., Graham, M. L., Smith, C., Olson, J. F., Halperin, E. C., et al. (1996). Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *New England Journal of Medicine*, 335 (3), 157-166. <http://dx.doi.org/10.1056/NEJM199607183350303>

Laporte, J., Gorin, N., Rubinstein, P., Lesage, S., Portnoi, M., Barbu, V., et al. (1996). Cord-blood transplantation from an unrelated donor in an adult with chronic myelogenous leukemia. *New England Journal of Medicine*, 335 (3), 167-170. <http://dx.doi.org/10.1056/NEJM199607183350304>

Laughlin, M. J., Eapen, M., Rubinstein, P., Wagner, J. E., Zhang, M., Champlin, R. E., et al. (2004). Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *New England Journal of Medicine*, 351 (22), 2265-2275. <http://dx.doi.org/10.1056/NEJMoa041469>

Leary, A. & Ogawa, M. Blast cell colony assay for umbilical cord blood and adult bone marrow progenitors. *Blood*, 69 (3), 953-956.

LifeforceCryobanks. (2011). *The collection process*. Retrieved 2/25, 2011. [Online] Available: <http://www.lifeforcecryobanks.com/what/collection/>

Lim, F. T. H., Beckhoven, J., Brand, A., Kluin-Nelemans, J., Hermans, J. M. H., Willemze, R., et al. (1999). The number of nucleated cells reflects the hematopoietic content of umbilical cord blood for transplantation. *Bone Marrow Transplantation*, 24 (9), 965.

Lin, M., Storer, B., Martin, P. J., Tseng, L., Gooley, T., Chen, P., et al. (2003). Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *New England Journal of Medicine*, 349 (23), 2201-2210. <http://dx.doi.org/10.1056/NEJMoa022060>

Lunde, K., Solheim, S., Aakhus, S., Arnesen, H., Abdelnoor, M., Egeland, T., et al. (2006). Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *New England Journal of Medicine*, 355 (12), 1199-1209. <http://dx.doi.org/10.1056/NEJMoa055706>

Maitra, B., Szekely, E., Gjini, K., Laughlin, M. J., Dennis, J., Haynesworth S. E., et al. (2004). Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplantation*, 33 (6), 597-604. <http://dx.doi.org/10.1038/sj.bmt.1704400>

Majhail, N. S., Brunstein, C. G., & Wagner, J. E. (2006). Double umbilical cord blood transplantation. *Current Opinion in Immunology*, 18 (5), 571-575. <http://dx.doi.org/doi:10.1016/j.coi.2006.07.015>

Mayani, H., Alvarado-Moreno, J. A., & Flores-Guzmán, P. (2003). Biology of human hematopoietic stem and progenitor cells present in circulation. *Archives of Medical Research*, 34 (6), 476-488. <http://dx.doi.org/10.1016/j.arcmed.2003.08.004>

McNiece, I., Harrington, J., Turney, J., Kellner, J., & Shpall, E. J. (2004). Ex vivo expansion of cord blood mononuclear cells on mesenchymal stem cells. *Cytotherapy*, 6, 311-317. <http://dx.doi.org/10.1080/14653240410004871>

Mielcarek, M. & Storb, R. (2003). Non-myeloablative hematopoietic cell transplantation as immunotherapy for hematologic malignancies. *Cancer Treatment Reviews*, 29 (4), 283-290. [http://dx.doi.org/10.1016/S0305-7372\(03\)00004-5](http://dx.doi.org/10.1016/S0305-7372(03)00004-5)

Morrison, S. J., Hemmati, H. D., Wandycz, A. M., & Weissman, I. L. (1995). The purification and characterization of fetal liver hematopoietic stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, 92 (22), 10302-10306. <http://dx.doi.org/10.1073/pnas.92.22.10302>

National Marrow Donor Program (2010a). *Learn if you can donate cord blood*. Retrieved 12/5, 2010. [Online] Available: http://www.marrows.org/HELP/Donate_Cord_Blood_Share_Life/How_to_Donate_Cord_Blood/Learn_If_You_Can_Donate/index.html

National Marrow Donor Program (2010b). *U.S. NMDP transplant centers*. Retrieved 11/20, 2010. [Online] Available:

- http://www.marlow.org/PATIENT/Plan_for_Tx/Choosing_a_TC/US_NMDP_Transplant_Centers/tc_list_by_state.pl
- Nakamae, H., Kirby, K. A., Sandmaier, B. M., Norasetthada, L., Maloney, D. G., Maris, M. B., *et al.* (2009). Effect of conditioning regimen intensity on CMV infection in allogeneic hematopoietic cell transplantation. *Biology of Blood and Marrow Transplantation*, 15 (6), 694-703. <http://dx.doi.org/10.1016/j.bbmt.2009.02.009>
- New England Cord Blood Bank. (2011). *Cord blood*. Retrieved 2/25, 2011. [Online] Available: <http://www.cordbloodbank.com/cord-blood.php>
- Noort, W. A., Kruisselbrink, A. B., in't Anker, P. S., Kruger, M., van Bezooijen, R. L., de Paus, R. A., *et al.* (2002). Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Experimental Hematology*, 30, 870-878. [http://dx.doi.org/10.1016/S0301-472X\(02\)00820-2](http://dx.doi.org/10.1016/S0301-472X(02)00820-2)
- Novello-Garza, B., Limon-Flores, A., Guerra-Marquez, A., Luna-Bautista, F., Juan-Shum, L., Montero-Ines, *et al.* (2008). Establishing a cord blood banking and transplantation program in Mexico: A single institution experience. *Transfusion*, 48, 228-236. <http://dx.doi.org/10.1111/j.1537-2995.2007.01529.x>
- Osawa, M., Hanada, K., Hamada, H., & Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-Low/Negative hematopoietic stem cell. *Science*, 273 (5272), 242-245. <http://dx.doi.org/10.1126/science.273.5272.242>
- Panepucci, R. A., Siufi, J. L., Silva, W. A., Proto-Siquiera, R., Nader, L., & Orellana, M. (2004). Comparison of gene expression of umbilical cord vein and bone marrow-derived mesenchymal stem cells. *Stem Cells*, 22 (7), 1263-1278. <http://dx.doi.org/10.1634/stemcells.2004-0024>
- Parry, E. W. & Abramovich, D. R. (1972). The ultrastructure of human umbilical vessel endothelium from early pregnancy to full term. *Journal of Anatomy*, 111 (1), 29-42.
- Pasquini, M. C. & Wang, Z. (2010). *Current use and outcome of hematopoietic stem cell transplantation: CIBMTR summary slides*. Retrieved November 26, 2010. [Online] Available: <http://www.cibmtr.org>
- Peled, T., Landau, E., Prus, E., Treves, A. J., & Fibach, E. (2002). Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34+ cells. *British Journal of Haematology*, 116 (3), 655-661. <http://dx.doi.org/10.1046/j.0007-1048.2001.03316.x>
- Perez-Oteyza, J., Bornstein, R., Corral, M., Hermosa, V., Alegre, A., Torradabella, M., *et al.* (1998). Controlled-rate versus uncontrolled-rate cryopreservation of peripheral blood progenitor cells: A prospective multicenter study. Group for cryobiology and biology of bone marrow transplantation (CBTMO), Spain. *Haematologica*, 83 (11), 1001-1005.
- Regidor, C., Posada, M., Monteagudo, D., Garaulet, C., Somolinos, N., Forés, R., *et al.* (1999). Umbilical cord blood banking for unrelated transplantation: Evaluation of cell separation and storage methods. *Experimental Hematology*, 27 (2), 380-385. [http://dx.doi.org/10.1016/S0301-472X\(98\)00016-2](http://dx.doi.org/10.1016/S0301-472X(98)00016-2)
- Ringdén, O., Uzunel, M., Rasmusson, I., Remberger, M., Sundberg, B., Lönnies, H., *et al.* (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*, 81 (10), 1390-1397.
- Soiffer, R. J. (2008). Biology of transplantation. In Soiffer, R. J. & Karp, J. E. (Eds.), *Hematopoietic stem cell transplantation* (2nd ed., pp. 3). New Jersey: Humana Press.
- Rocha, V., Cornish, J., Sievers, E. L., Filipovich, A., Locatelli, F., Peters, C., *et al.* (2001). Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood*, 97 (10), 2962-2971. <http://dx.doi.org/10.1182/blood.V97.10.2962>
- Rocha, V., Crotta, A., Ruggeri, A., Purtill, D., Boudjedir, K., Herr, A., *et al.* (2010). Double cord blood transplantation: Extending the use of unrelated umbilical cord blood cells for patients with hematological diseases. *Best Practice & Research Clinical Haematology*, 23 (2), 223-229. <http://dx.doi.org/10.1016/j.beha.2010.07.005>
- Rocha, V., Labopin, M., Sanz, G., Arcese, W., Schwerdtfeger, R., Bosi, A., *et al.* (2004). Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *New England Journal of Medicine*, 351 (22), 2276-2285. <http://dx.doi.org/10.1056/NEJMoa041469>
- Scherjon, S. & Anker, I. E. (2009). In utero stem cell transplantation. In C. H. Rodeck & M. J. Whittle (Eds.). *Fetal medicine: Basic science and clinical practice*, 2nd ed., pp. 678-684. New York: Churchill Livingstone Else.

- Rodrigues, C. A., Sanz, G., Brunstein, C. G., Sanz, J., Wagner, J. E., Renaud, M., *et al.* (2009). Analysis of risk factors for outcomes after unrelated cord blood transplantation in adults with lymphoid malignancies: A study by the eurocord-netcord and lymphoma working party of the european group for blood and marrow transplantation. *Journal of Clinical Oncology*, *27* (2), 256-263. <http://dx.doi.org/10.1200/JCO.2007.15.8865>
- Romanov, Y. A., Svintsitskaya, V. A., & Smirnov, V. N. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate MSC-like cells from umbilical cord. *Stem Cells*, *21* (1), 105-110. <http://dx.doi.org/10.1634/stemcells.21-1-105>
- Sakaguchi, S., Yamaguchi, T., Nomura, T., & Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell*, *133* (5), 775-787. <http://dx.doi.org/10.1016/j.cell.2008.05.009>
- Sato, K., Ozaki, K., Oh, I., Meguro, A., Hatanaka, K., Nagai, T., *et al.* (2007). Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood*, *109* (1), 228-234. <http://dx.doi.org/10.1182/blood-2006-02-002246>
- Sato, N., Meijer, L., Skaltsounis, L., Greengard, P., & Brivanlou, A. H. (2004). Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nature Medicine*, *10* (1), 55-63. <http://dx.doi.org/10.1038/nm979>
- Satwani, P., Cooper, N., Rao, K., Veys, P., & Amrolia, P. (2008). Reduced intensity conditioning and allogeneic stem cell transplantation in childhood malignant and nonmalignant diseases. *Bone Marrow Transplantation*, *41* (2), 173-182. <http://dx.doi.org/10.1038/sj.bmt.1705923>
- Scaradavou, A., Smith, K. M., Hawke, R., Schaible, A., Abboud, M., Kernan, N. A., *et al.* (2010). Cord blood units with low CD34+ cell viability have a low probability of engraftment after double unit transplantation. *Biology of Blood and Marrow Transplantation*, *16* (4), 500-508. <http://dx.doi.org/10.1016/j.bbmt.2009.11.013>
- Schächinger, V., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., *et al.* (2006). Intracoronary bone Marrow-Derived progenitor cells in acute myocardial infarction. *New England Journal of Medicine*, *355* (12), 1210-1221. <http://dx.doi.org/10.1056/NEJMoa060186>
- Spangrude, G., Brooks, D., & Tumas, D. (1995). Long-term repopulation of irradiated mice with limiting numbers of purified hematopoietic stem cells: In vivo expansion of stem cell phenotype but not function. *Blood*, *85* (4), 1006-1016.
- Stevens, C. E., Scaradavou, A., Carrier, C., Carpenter, C., & Rubinstein, P. (2005). An empirical analysis of the probability of finding a well matched cord blood unit: Implications for a national cord blood inventory. *ASH Annual Meeting Abstracts*, *106* (11), 2047.
- Taghizadeh, R. R. & Sherley, J. L. Expanding the therapeutic potentials of umbilical cord blood hematopoietic stem cells. In Cetrulo, C. L., Cetrulo, K. J., & Cetrulo, C. L. Jr. (Eds.), *Perinatal stem cells* (1st ed., pp.21-35), John Wiley & Sons, Inc.
- Takahashi, S., Iseki, T., Ooi, J., Tomonari, A., Takasugi, K., Shimohakamada, Y., *et al.* (2004). Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*, *104* (12), 3813-3820. <http://dx.doi.org/10.1182/blood-2004-03-1001>
- Takahashi, S., Ooi, J., Tomonari, A., Konuma, T., Tsukada, N., Oiwa-Monna, M., *et al.* (2007). Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood*, *109* (3), 1322-1330. <http://dx.doi.org/10.1182/blood-2006-04-020172>
- Taylor, P. A., Lees, C. J., & Blazar, B. R. (2002). The infusion of ex vivo activated and expanded CD4+ CD25+ immune regulatory cells inhibits graft-versus-host disease lethality. *Blood*, *99* (10), 3493-3499. <http://dx.doi.org/10.1182/blood.V99.10.3493>
- Taylor, P. A., Panoskaltis-Mortari, A., Swedin, J. M., Lucas, P. J., Gress, R. E., Levine, B. L., *et al.* (2004). L-selectinhi but not the L-selectinlo CD4+25+ T-regulatory cells are potent inhibitors of GVHD and BM graft rejection. *Blood*, *104* (12), 3804-3812. <http://dx.doi.org/10.1182/blood-2004-05-1850>
- Trenado, A., Charlotte, F., Fisson, S., Yagello, M., Klatzmann, D., Salomon, B. L., *et al.* (2003). Recipient-type specific CD4+CD25+ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. *The Journal of Clinical Investigation*, *112* (11), 1688-1696. <http://dx.doi.org/10.1172/JCI200317702>

- Tung, S. S., Parmar, S., Robinson, S. N., De Lima, M., & Shpall, E. J. (2010). Ex vivo expansion of umbilical cord blood for transplantation. *Best Practice & Research Clinical Haematology*, 23 (2), 245-257. <http://dx.doi.org/10.1016/j.beha.2010.06.004>
- University of Alabama at Birmingham Medicine. 2008. *Fetal Circulation*. Retrieved 9/12, 2010. [Online] Available: <http://www.health.uab.edu/14535/>
- University of Kansas Medical Center. (2011). *Umbilical Cord*. Retrieved 9/12, 2010. [Online] Available: <http://www.kumc.edu/instruction/medicine/anatomy/histoweb/ct/ct14.htm>
- Verneris, M. R., Brunstein, C. G., Barker, J., MacMillan, M. L., DeFor, T., McKenna, D. H., et al. (2009). Relapse risk after umbilical cord blood transplantation: Enhanced graft-versus-leukemia effect in recipients of 2 units. *Blood*, 114 (19), 4293-4299. <http://dx.doi.org/10.1182/blood-2009-05-220525>
- ViaCord. (2011). The cord blood banking process overview. Retrieved 2/25, 2011. [Online] Available: <http://www.viacord.com/cord-blood-banking-process.htm>
- Vriesendorp, H. M. (2003). Aims of conditioning. *Experimental Hematology*, 31 (10), 844-854. [http://dx.doi.org/10.1016/S0301-472X\(03\)00229-7](http://dx.doi.org/10.1016/S0301-472X(03)00229-7)
- Wagers, A. J., Sherwood, R. I., Christensen, J. L., & Weissman, I. L. (2002). Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*, 297 (5590), 2256-2259. <http://dx.doi.org/10.1126/science.1074807>
- Wagner, J. E., Barker, J. N., DeFor, T. E., Baker, K. S., Blazar, B. R., Eide, C., et al. (2002). Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: Influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood*, 100 (5), 1611-1618. <http://dx.doi.org/10.1182/blood-2002-01-0294>
- Wagner, J., Rosenthal, J., Sweetman, R., Shu, X., Davies, S., Ramsay, N., et al. (1996). Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: Analysis of engraftment and acute graft-versus-host disease. *Blood*, 88 (3), 795-802.
- Wagner, J. E. (2009). Should double cord blood transplants be the preferred choice when a sibling donor is unavailable? *Best Practice & Research Clinical Haematology*, 22 (4), 551-555. <http://dx.doi.org/10.1016/j.beha.2009.09.003>
- Wang, J., Wang, L., Wu, Y., Xiang, Y., Xie, C., Jia, B., et al. (2004). Mesenchymal stem/progenitor cells in human umbilical cord blood as support for ex vivo expansion of CD34+ hematopoietic stem cells and for chondrogenic differentiation. *Haematologica*, 89 (7), 837-844.
- Yoo, K. H., Lee, S. H., Sung, K. W., Koo, H. H., Chung, N. G., Cho, B., et al. (2011). Current status of pediatric umbilical cord blood transplantation in Korea: A multicenter retrospective analysis of 236 cases. *American Journal of Hematology*, 86 (1), 12-17. <http://dx.doi.org/10.1002/ajh.21886>
- Young, J. C., Wu, S., Hanstee, G., Du, C., Sambucetti, L., Remiszewski, S., et al. (2004). Inhibitors of histone deacetylases promote hematopoietic stem cell self-renewal. *Cytotherapy*, 6 (4), 328-336. <http://dx.doi.org/10.1080/14653240410004899>

Table 1. Clinical studies comparing unrelated myeloablative single UCBT vs. 6/6 HLA matched BMT in adults

Study	N-UCB N-BM	Median age (years)	Diagnosis	Median infused TNC x 10 ⁶ /kg	Engraftment			GVHD (%)		DFS (%) (follow up in months)
					ANC (Day)	Platelets (Day)	Primary failure (%)	Acute II- IV	Chronic	
Rocha [¶] (2004)	98	24.5	AML, ALL	0.23	26	N/S	20	26	30	33 (24)
	584	32		2.9	19	N/S	7	39	46	38 (24)
Laughlin [#] (2004)	150	N/S	AML, ALL, CML, MDS	0.22	27	60	30	41	51	23 (36)
	367	N/S		2.4	18	29	N/S	48	35	33 (36)
Takahashi [‡] (2004)	68	36	AML, ALL, CML, MDS, NHL	0.25	22	40	8	50	78	74 (24)
	39	26		N/S	18	25	0	75	74	44 (24)

Abbreviations: ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, CML = chronic myelogenous leukemia, NHL = non-Hodgkin's lymphoma, MDS = myelodysplastic syndrome, TNC = total nucleated cell, ANC = absolute neutrophil count, GVHD = graft versus host disease, DFS = disease free survival, N/S = not stated

[¶] For UCBTs, 6 patients received 6/6 HLA-matched units, 85 patients received 4-5/6 HLA-matched units, and 4 patients received 3/6 HLA-matched units.

[#] All UCBT patients received 4-5/6 HLA-matched units.

[‡] BMT outcomes also include 6 patients who received 5/6 HLA-matched units. For UCBTs, 51 patients received 4-5/6 and 17 received 2-3/6 HLA-matched units.

Table 2. Clinical results of DCBTs using MA/NMA/ or RIC conditioning regimens in adults

Study	N- DCBT	Median age(years)	Diagnosis	Conditioning regimen	Median infused TNC x 10 ⁷ /kg	Engraftment			GVHD (%)		DFS (%) (follow up in months)
						ANC (Day)	Platelets (Day)	Primary failure (%)	Acute II- IV	Chronic	
Barker (2005)	23	24	ALL, AML, CML	MA	3.5	23	N/S	0	65	23	57 (10)
Cutler (2010)	32	53	ALL, AML, CML, NHL, HD, MDS, CLL, MD	RIC	5.16*	21	42	0	9	13	31.2 (24)
Ballen (2007)	21	49	ALL, AML, CLL, MDS, NHL, HD, AA	RIC	4.0	20	41	10	40	26	55 (24)
Kanda [*] (2010)	27	33	ALL, AML, CML, MDS, NHL, HD	MA	3.8	24	N/S	12	37	32	52 (24)
Brunstein [~] (2007)	93	51	High risk hematologic disease	NMA	3.7	12	N/S	6	59	23	38 (36)
Majhail (2006)	9	28	HL	RIC	3.8	10	N/S	0	67	11	25 (17)
Barker (2009)	35	42	Acute leukemia, NHL, HD, MDS	MA	4.46	25	50	6	44	26	56 (12)
	19			NMA		10	38	5			
Rodrigues (2009)	26	41	NHL, HL, CLL	RIC	3.02	17	N/S	N/S	32	19	57 (18)
Yoo [‡] (2010)	64	10.3	ALL/ABL, AML, MDS/JMML, SAA, CML	RIC	4.37	19	46	N/S	41	39	36.9 (60)
Vemeris (2009)	93	24	ALL, AML, High- risk CR1	MA	3.6	25	N/S	N/S	48	18	51 (60)

Abbreviations (also see Table 1): HD = Hodgkin's disease, CLL = chronic lymphocytic leukemia, MD = myeloproliferative disorder, AA = aplastic anemia, HL = Hodgkin's lymphoma, ABL = acute biphenotypic leukemia, JMML = juvenile myelomonocytic leukemia, SAA = severe aplastic anemia, MA = myeloablative, NMA = non-myeloablative, RIC = reduced intensity conditioning, N/A = not available

*Pre-cryopreservation

^{*}Article in Press.

[‡]Pediatric study

[~]Outcomes include single and DCBTs.

Table 3. Comparison between public vs. private UCB banking

Categories	Public Bank	Private Bank
Purpose of storage	Donate stem cells to public	Keep stem cells for own children
Compatibility	Percentage of finding a match for a transplant is up to 90%	Assures 100% match for transplantation
Probability that you will need a stem cell transplant	N/A	Probability to use your own UCB is 0.0005% - 0.04%
Costs to store	Generally covered by insurance	Must pay \$1000-2000 to begin banking and \$100 - \$200 annually
Costs to retrieve stem cells from the bank	Generally covered by insurance	Does not cost money for retrieval

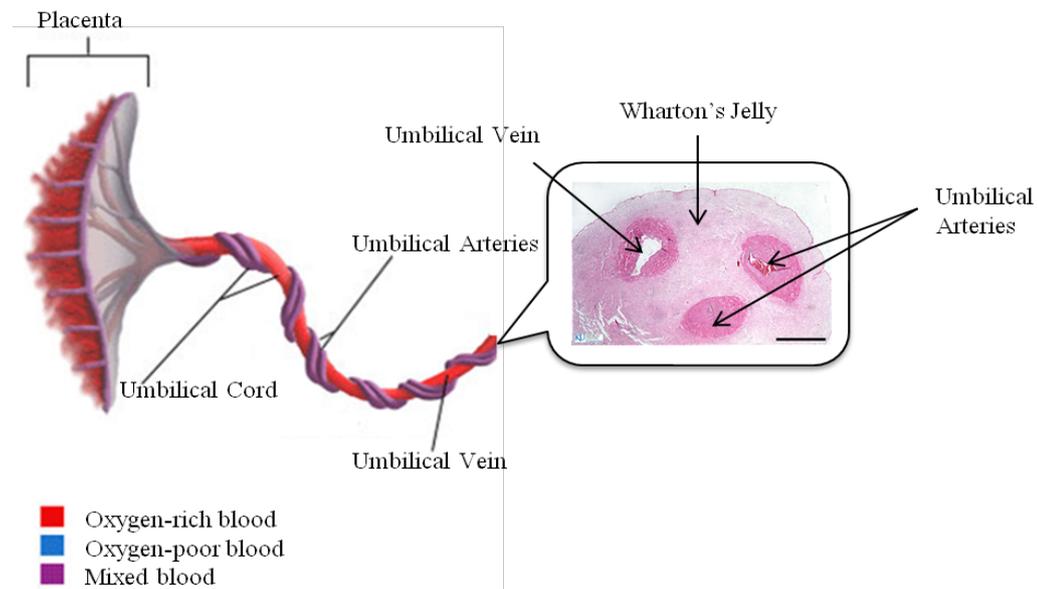


Figure 1. The anatomy of the umbilical cord (Courtesy of University of Alabama at Birmingham Medicine, 2008 and University of Kansas Medical Center, 2011)

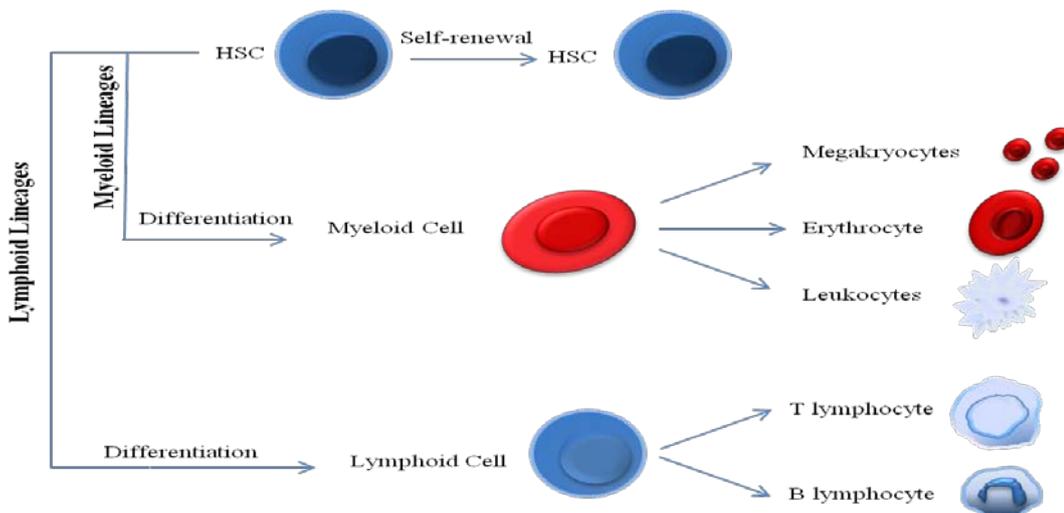


Figure 2. Differentiation pattern of HSCs

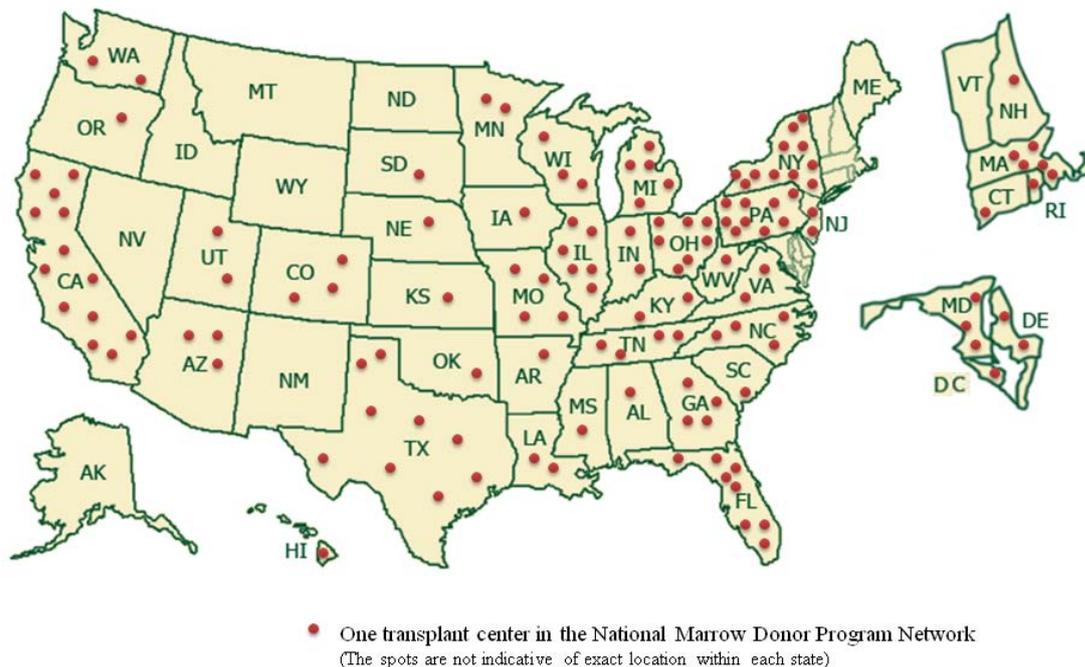


Figure 3. Locations of HSC transplantation centers in the United States (National Marrow Donor Program, 2010b)

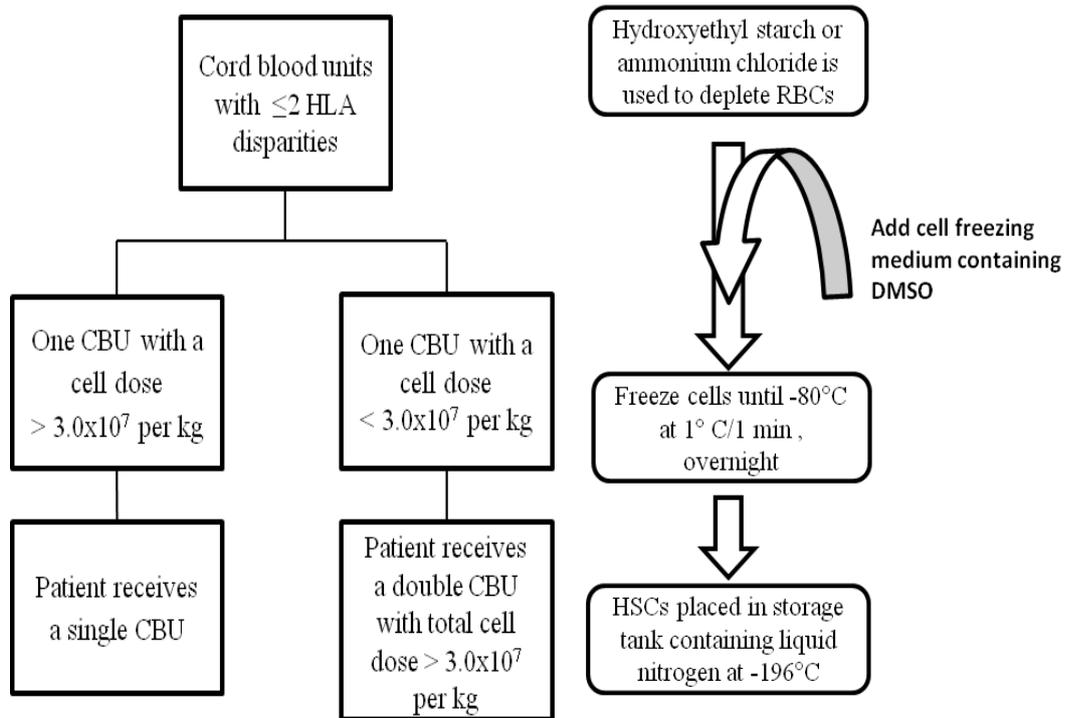


Figure 4. The common algorithm used for UCB selection (Adapted from Wagner, 2009)

Figure 5. The common cryopreservation process of UCB