

Umbilical Cord Blood Stem Cell Literatures

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Abstract: The definition of stem cell is “an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell”. Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on umbilical cord blood stem cell.

[Smith MH. **Umbilical Cord Blood Stem Cell Literatures**. *Stem Cell* 2012;3(2):83-103] (ISSN 1545-4570). <http://www.sciencepub.net/stem>. 5

Key words: stem cell; life; gene; DNA; protein; umbilical cord blood

Introduction

Stem cell is the origin of an organism's life. Stem cells have the potential to develop into many different types of cells in life bodies, that are exciting to scientists because of their potential to develop into many different cells, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science.

The definition of stem cell is “an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell” (Stedman's Medical Dictionary, 2002).

Five key stem cells have been isolated from human: (1) Blastocysts; (2) Early embryos; (3) Fetal tissue; (4) Mature tissue; (5) Mature cells that can be grown into stem cells.

Up to today, only stem cells taken from adults or children (known generically as "adult stem cells") have been used extensively and effectively in the treatment of degenerative diseases.

Literatures

Amano, H., M. Kurosawa, et al. (2000). "Cultured human mast cells derived from umbilical cord blood cells in the presence of stem cell factor and interleukin-6 cannot be a model of human skin mast cells: fluorescence microscopic analysis of

intracellular calcium ion mobilization." *J Dermatol Sci* **24**(2): 146-52.

To know whether cultured human mast cells raised from umbilical cord blood cells in the presence of stem cell factor (SCF) and interleukin-6 (IL-6) can be a model of human skin mast cells, the cells were stimulated, and intracellular calcium ion ($[Ca^{2+}]_i$) mobilization was analyzed by fluorescence microscopic techniques in parallel with a measurement of histamine released from the cells. When IgE-sensitized mast cells were activated by anti-IgE, $[Ca^{2+}]_i$ elevation began at the periphery and subsequently proceeded toward the center of the cells. The increase in $[Ca^{2+}]_i$ in calcium ionophore A23187-stimulated mast cells began at the center and spread to the periphery of the cells. Significant histamine release was observed by each stimulation. However, either compound 48/80 or substance P failed to increase $[Ca^{2+}]_i$ with no appreciable histamine release. This study shows that there is heterogeneity of $[Ca^{2+}]_i$ mobilization in the activated human mast cells, and that cultured human mast cells derived from umbilical cord blood cells in the presence of SCF and IL-6 can not be a model of human skin mast cells.

Arora, M., S. Nagaraj, et al. (2007). "Chronic graft-versus-host disease (cGVHD) following unrelated donor hematopoietic stem cell transplantation (HSCT): higher response rate in recipients of unrelated donor (URD) umbilical cord blood (UCB)." *Biol Blood Marrow Transplant* **13**(10): 1145-52.

We present a comparative analysis of clinical presentation and response to treatment in 170 patients with chronic graft versus host disease (cGVHD) (123 following transplant from an unrelated donor [URD] and 47 from umbilical cord blood [UCB]). URD

transplant recipients were significantly younger (median age 25 versus 39 years, $P = .002$; and the donor grafts were mostly HLA matched (67% versus 10%, $P < .0001$). UCB recipients had more frequent responses (complete remission [CR] + partial remission [PR]) to treatment (URD 48% versus UCB 74% at 2 months [$P = .005$]; 49% versus 78% at 6 months [$P = .001$] and 51% versus 72% at 1 year [$P = .03$] in the URD and UCB groups, respectively). Nonrelapse mortality (NRM) after diagnosis of cGVHD was worse after URD grafts. (1 year NRM 27% [19%-35%] URD versus 11% [2%-20%] UCB, $P = .055$). Separate multivariate analyses were performed in each cohort. In both, thrombocytopenia and no CR or PR at 2 months were independently associated with increased mortality. In addition, progressive onset of cGVHD was a significant predictor of increased mortality in URD cohort. These data suggest that cGVHD following UCB transplant may be more responsive to therapy and also lead to a lower NRM.

Aufderhaar, U., W. Holzgreve, et al. (2003). "The impact of intrapartum factors on umbilical cord blood stem cell banking." *J Perinat Med* **31**(4): 317-22.

AIMS: Umbilical cord blood can be used as an alternative source for related and unrelated allogeneic stem cell transplantation. This study was undertaken to determine whether intrapartum factors have an influence on the hematopoietic cell compartment of cord blood. METHODS: Cord blood samples were obtained from 102 normal full-term deliveries for the banking of stem cells. We analyzed the influence of intrapartum factors on the count of CD34+ cells, total nucleated cells, colony forming units and total volume of collection. Fluorescence-activated cell sorting was used to measure CD34+ cell numbers. Statistical analysis was undertaken using Pearson correlation test and multiple regression analysis. RESULTS: The higher the infants' birthweight the larger was the volume. A lower arterial umbilical pH and a larger blood volume resulted in an increased number of CD34+ cells. A large blood volume, long duration of labor, lower arterial and venous pH were correlated with more nucleated cells. A higher birthweight, larger blood volume and lower arterial pH resulted in an increased number of colony forming units. CONCLUSIONS: Some intrapartum factors have an impact on the characteristics of collected cord blood cells. Stress during delivery may influence the number of hematopoietic cells, through altered cytokine production. This knowledge may facilitate the selection of optimal cord blood samples for unrelated banking and the early discarding of suboptimal cord

blood samples thus resulting in the saving of costs related to expensive further processing.

Barriga, F. and M. A. Wietstruck (2007). "Search for unrelated donor umbilical cord blood units for allogeneic stem cell transplantation: results in two time periods." *Transplant Proc* **39**(3): 629-30.

Umbilical cord blood (UCB) banks have increased their stock worldwide in the past years. There are more than 230,000 available units today. The ideal UCB graft is a unit that is matched in five or six of six HLA. A, B (low-resolution) and DRB1 (high-resolution) alleles and which has over 2.5×10^7 nucleated cells/kg body weight (BW). Four of six matched units are also used specially if the cell dose gives more than 3×10^7 nucleated cells/kg BW. Our unrelated donor UCB transplant program was started in 1996 searching international cord blood banks (Netcord, New York) for patients with a definitive or potential indication for stem cell transplantation who lacked a matched family donor. PATIENTS AND METHODS: From 1995 to 1996, a search was initiated for 87 patients with malignant ($n = 56$, 37 acute leukemia) and nonmalignant conditions (16 congenital diseases, 14 aplastic anemia). Patient data along with low-resolution A, B, and DR typing were sent to the New York Blood Center, along with a blood sample for high-resolution DRB1 typing. Parallel searches were done in the Netcord database among UCB units with reported high-resolution DRB1 typing. Forty-eight searches were done between 1995 and 2000 (31 with high resolution) and 39 were done between 2000 and 2005 (33 with high resolution). UCB units were considered adequate if they had more than 2.7×10^7 cells/kg BW. RESULTS: During the first period, four patients (13%) matched five of six high-resolution unit and 21 (67%) a four of six match. During the second period, 15 patients (46%) found a five of six match and 16 (48%) a four of six match ($P = .012$). CONCLUSION: Nearly half of our patients find an optimal matched UCB unit for transplantation in international banks. The creation of a local UCB bank in our country is supported by these data.

Broom, M. A., L. L. Wang, et al. (2006). "Successful umbilical cord blood stem cell transplantation in a patient with Rothmund-Thomson syndrome and combined immunodeficiency." *Clin Genet* **69**(4): 337-43.

The ATP-dependent DNA helicase Q4 (RECQL4) belongs to a family of conserved RECQ helicases that are felt to be important in maintaining chromosomal integrity (Kitao et al., 1998, *Genomics*: 54 (3): 443-452). Deletions in the RECQL4 gene located on chromosome 8 region q24.3 have been

associated with Rothmund-Thomson syndrome (RTS, OMIM 268400), a condition characterized by poikiloderma, sparse hair, small stature, skeletal abnormalities, cataracts and an increased risk of malignancy. We present a patient with a molecularly confirmed diagnosis of RTS with two unique genetic alterations in RECQL4 (IVS16-2A>T and IVS2+27_51del25), who at the age of 7 months nearly succumbed to *Pneumocystis carinii* pneumonia. Evaluation of his immune system demonstrated a T-B+ NK- phenotype with agammaglobulinemia consistent with combined immunodeficiency (CID). Studies to evaluate for known genetic causes of CID were not revealing. The patient received an umbilical cord blood (UCB) transplant with complete immune reconstitution. This report represents the first description of a CID phenotype and UCB transplantation in a patient with RTS.

Buzanska, L., M. Jurga, et al. (2006). "Neuronal differentiation of human umbilical cord blood neural stem-like cell line." *Neurodegener Dis* 3(1-2): 19-26.

The expanding population of neural stem/progenitor cells can be selected from human cord blood nonhematopoietic (CD34-negative) mononuclear fraction. Due to repeated expansion and selection of these cells we have established the first clonogenic, nonimmortalized human umbilical cord blood neural stem-like cell (HUCB-NSC) line. This line can be maintained at different stages of neural progenitor development by the presence of trophic factors, mitogens and neuromorphogens in culture media. Neurogenic potential of HUCB-NSC was established for serum-free and low-serum cultured cells. Commitment of HUCB-NSC by serum was shown to be important for the optimal response to the signals provided by surrounding environment in vitro. Enhanced neuronal differentiation induced by dBcAMP treatment was accompanied by expression of several functional proteins including glutamatergic, GABAergic, dopamine, serotonin and acetylcholine receptors, which was shown by microarray, immunocytochemistry and electrophysiology. Electrophysiological studies, whole-cell patch-clamp recordings, revealed in differentiated HUCB-NSC two types of voltage-sensitive and several ligand-gated currents typical for neuronal cells. The above HUCB-NSC characteristic conceivably implicates that cord blood-derived progenitors could be effectively differentiated into functional neuron-like cells in vitro.

Buzanska, L., M. Jurga, et al. (2006). "Neural stem-like cell line derived from a nonhematopoietic population of human umbilical cord blood." *Stem Cells Dev* 15(3): 391-406.

The ability of stem and progenitor cells to proliferate and differentiate into other lineages is widely viewed as a characteristic of stem cells. Previously, we have reported that cells from a CD34(-) (nonhematopoietic) adherent subpopulation of human cord blood can acquire a feature of multipotential neural progenitors in vitro. In the present study, using these cord blood-derived stem cells, we have established a clonal cell line termed HUCB-NSCs (human umbilical cord blood-neural stem cells) that expresses several neural antigens and has been grown in culture for more than 60 passages. During this time, HUCB-NSCs retained their growth rate, the ability to differentiate into neuronal-, astrocyte-, and oligodendrocyte-like cells and displayed a stable karyotype. DNA microarray analysis of HUCB-NSCs revealed enhanced expression of selected genes encoding putative stem and progenitor cell markers when compared to other mononuclear cells. dBcAMP-induced HUCBNSCs were further differentiated into more advanced neuronal cells. This is the first report of the establishment and characterization of a nontransformed HUCB-NSC line that can be grown continuously in a monolayer culture and induced to terminal differentiation. These cells should further our understanding of the regulatory mechanisms involved in NSC self-renewal and differentiation.

Cicutini, F. M., C. G. Begley, et al. (1992). "The effect of recombinant stem cell factor (SCF) on purified CD34-positive human umbilical cord blood progenitor cells." *Growth Factors* 6(1): 31-9.

We describe the effect of soluble c-kit ligand (stem cell factor, SCF) on highly purified CD34-positive hemopoietic progenitors from human umbilical cord blood. Progenitor cells were purified from cord blood mononuclear cells by immune rosetting with lineage specific antibodies and subsequent sorting of the rosette-negative population for CD34(BI3C5)-positive cells. This procedure enriched greater than 100-fold for colony forming cells (CFC). Using optimal concentrations of colony-stimulating factors (CSF) without added SCF approximately 2.5% of cells formed colonies. SCF also had CSF activity on this population, up to 0.5% of cells forming small colonies in response to SCF alone. In contrast, the addition of SCF to optimal concentrations of the other growth factors produced a greater than 10-fold increase in colony number. However, the most notable effect was an approximately 100-fold increase in the number of cells in each colony. Equally striking was the very high proportion (50-80%) of mixed colonies (CFU-MIX). These findings suggest the progenitor cell pool in cord blood is skewed towards very early cells.

However, when day 14 colonies formed in response to SCF and other factors were assessed for their re-cloning potential they did not contain significant numbers of CFC, implying that SCF did not support the self-renewal of these CD34 positive cord blood progenitor cells. These findings support a role for SCF as an enhancing factor for hemopoietic progenitor cells but it does not promote self-renewal in these populations.

Cohen, Y. and A. Nagler (2003). "Hematopoietic stem-cell transplantation using umbilical-cord blood." *Leuk Lymphoma* **44**(8): 1287-99.

In the recent years, umbilical cord blood (UCB) has emerged as an alternative source of hematopoietic progenitors (CD34+) for allogeneic stem cell transplantation, mainly in patients lacking an HLA-matched marrow donor. Since 1998, about 2500 patients have received UCB transplants for a variety of malignant and non-malignant diseases. The vast majority of recipients were children with an average weight of 20kg, however, more than 500 UCB transplantations (UCBT) have already been performed in adults. The "naive" nature of UCB lymphocytes may explain the lower incidence and severity of graft vs. host disease (GvHD) encountered in UCBT compared to the allogeneic transplant setting. Furthermore, UCB is rich in primitive CD16-CD56++ NK cells, which possess significant proliferative and cytotoxic capacities and can be expanded using IL-12 or IL-15, so as to mount a substantial graft vs. leukemia (GvL) effect. The major disadvantage of UCB is the low yield of stem cells, resulting in higher rates of engraftment failure and slower time to engraftment compared to bone marrow transplantation (BMT). A rational approach thus involves ex vivo expansion of UCB derived hematopoietic precursors. All these issues are discussed in detail in this review.

Dasari, V. R., D. G. Spomar, et al. (2008). "Umbilical cord blood stem cell mediated downregulation of fas improves functional recovery of rats after spinal cord injury." *Neurochem Res* **33**(1): 134-49.

Human umbilical cord blood stem cells (hUCB), due to their primitive nature and ability to develop into nonhematopoietic cells of various tissue lineages, represent a potentially useful source for cell-based therapies after spinal cord injury (SCI). To evaluate their therapeutic potential, hUCB were stereotactically transplanted into the injury epicenter, one week after SCI in rats. Our results show the presence of a substantial number of surviving hUCB in the injured spinal cord up to five weeks after transplantation. Three weeks after SCI, apoptotic cells were found especially in the dorsal white matter and gray matter, which are positive for both neuron and

oligodendrocyte markers. Expression of Fas on both neurons and oligodendrocytes was efficiently downregulated by hUCB. This ultimately resulted in downregulation of caspase-3 extrinsic pathway proteins involving increased expression of FLIP, XIAP and inhibition of PARP cleavage. In hUCB-treated rats, the PI3K/Akt pathway was also involved in antiapoptotic actions. Further, structural integrity of the cytoskeletal proteins alpha-tubulin, MAP2A&2B and NF-200 has been preserved in hUCB treatments. The behavioral scores of hind limbs of hUCB-treated rats improved significantly than those of the injured group, showing functional recovery. Taken together, our results indicate that hUCB-mediated downregulation of Fas and caspases leads to functional recovery of hind limbs of rats after SCI.

De Bruyn, C., A. Delforge, et al. (2000). "Characterization of CD34+ subsets derived from bone marrow, umbilical cord blood and mobilized peripheral blood after stem cell factor and interleukin 3 stimulation." *Bone Marrow Transplant* **25**(4): 377-83.

We characterized CD34+ cells purified from bone marrow (BM), mobilized peripheral blood (PB) and cord blood (CB) and we tried to establish correlations between the cell cycle kinetics of the CD34+CD38- and CD34+CD38+ subpopulations, their sensitivity to SCF and IL-3 and their expression of receptors for these two CSFs. At day 0, significantly fewer immature CD34+CD38- cells from CB and mobilized PB are in S + G2M phases of the cell cycle (respectively 2.0 +/- 0.4 and 0.9 +/- 0.3%) than their BM counterpart (5.6 +/- 1.2%). A 48-h incubation with SCF + IL-3 allows a significant increase in the percentage of cycling CD34+CD38- cells in CB (19.2 +/- 2.2%, P < 0.0002) and PB (14.1 +/- 5.5%, P < 0.05) while the proliferative potential of BM CD34+CD38- progenitors remains constant (8.6 +/- 1.0%, NS). CD123 (IL-3 receptor) expression is similar in the three sources of hematopoietic cells at day 0 and after 48-h culture. CD117 (SCF receptor) expression, although very heterogeneous according to the subpopulations and the sources of progenitors evaluated, seems not to correlate with the difference of progenitor cell sensitivity to SCF nor with their proliferative capacity. Considering the importance of the c-kit/SCF complex in the adhesion of stem cells to the microenvironment, several observations are relevant. The density of CD117 antigen expression (expressed in terms of mean equivalent soluble fluorescence, MESF) is significantly lower on fresh PB cells than on their BM (P < 0.017) and CB (P < 0.004) counterparts, particularly in the immature CD34+CD38- population (560 +/- 131, 2121 +/- 416 and 1192 +/- 129 MESF respectively); moreover,

when PB and BM CD34+CD38- cells are stimulated for 48 h with SCF + IL-3, the CD117 expression decreases by 1.5- and 1.66-fold, respectively. This reduction could modify the functional capacities of ex vivo PB and BM manipulated immature progenitor cells.

De Felice, L., T. Di Pucchio, et al. (1998). "Flt3L enhances the early stem cell compartment after ex vivo amplification of umbilical cord blood CD34+ cells." *Bone Marrow Transplant* **22 Suppl 1**: S66-7.

Umbilical cord blood (UCB) transplant represents a promising therapeutic approach, nevertheless this procedure has been so far almost exclusively used in pediatric patients because of the reduced volume of UCB units. The availability of larger numbers of early and late hematopoietic progenitors by ex vivo amplification procedure may allow the use of UCB in adults and improve the rate and time to engraftment. We describe a stroma-free liquid culture system that induces a 10-fold increase of CD34+ cells and hematopoietic progenitors after 8 days in vitro amplification. The presence of flt3L is essential to preserve and amplify the early stem cell compartment identified by the phenotype CD34+Thy-1+CD45RO+.

Dravid, G. and S. G. Rao (2002). "Ex vivo expansion of stem cells from umbilical cord blood: expression of cell adhesion molecules." *Stem Cells* **20**(2): 183-9.

Expansion of stem cells from cord blood has been demonstrated to increase the numbers of CD34+ cells, CD34+ subsets, long-term culture-initiating cells, and severe combined immunodeficient mouse, repopulating cells. However, reports suggest that the ex vivo expanded population behaves differently than freshly isolated cells and shows a delayed or diminished engraftment. In this study, we investigated the effects of the cytokines flt3 ligand, stem cell factor, and thrombopoietin on expansion of CD34+ and CD34+/CD38- cells. In addition, we studied the expression of adhesion molecules, very late activation antigen-4 (VLA-4) and leukocyte function antigen-1 (LFA-1), on CD34+ cells from cord blood by flow cytometry. We also looked at the expression of an adhesion receptor, namely, vascular cell adhesion molecule-1 (VCAM-1) on bone marrow stromal cells by Western blot analysis after exposure to low dose gamma irradiation. After culturing for 7 days, increases in the absolute numbers of CD34+, CD34+/CD38-, CD34+/VLA-4+, and CD34+/LFA-1+ cells were 5.67 +/- 2.91 (mean +/- standard deviation) fold, 7.21 +/- 4.38 fold, 99.56 +/- 101.5 fold, and 101.39 +/- 83.25 fold, respectively. There was a transient upregulation in the expression levels of VCAM-1 on stromal cells, which peaked at 4 hours.

Though there was an increase in the absolute numbers of CD34+ cells expressing the adhesion molecules, the expression levels (antigen density) of the adhesion molecules on the CD34+ cells remained unaffected.

Dvorak, C. C., A. L. Gilman, et al. (2009). "Primary graft failure after umbilical cord blood transplant rescued by parental haplocompatible stem cell transplantation." *J Pediatr Hematol Oncol* **31**(4): 300-3.

AB Graft failure and aplasia are known complications of umbilical cord blood (UCB) hematopoietic stem cell transplantation (HSCT). In the absence of a second HSCT, graft failure is generally a fatal complication due to overwhelming infection. We describe 2 children with primary graft failure and life-threatening infections after UCB HSCT who were rescued by the use of related haplocompatible T-cell-depleted peripheral blood stem cell transplants. The rapid availability of haplocompatible donors and the brisk neutrophil recovery after haplocompatible peripheral blood stem cell transplant with high numbers of CD34+ cells make this an attractive rescue strategy for patients with graft failure after UCB HSCT.

Feldmann, R. E., Jr., K. Bieback, et al. (2005). "Stem cell proteomes: a profile of human mesenchymal stem cells derived from umbilical cord blood." *Electrophoresis* **26**(14): 2749-58.

Multipotent mesenchymal stem cells (MSCs) derived from human umbilical cord blood (UCB) represent promising candidates for the development of future strategies in cellular therapy. To create a comprehensive protein expression profile for UCB-MSCs, one UCB unit from a full-term delivery was isolated from the unborn placenta, transferred into culture, and their whole-cell protein fraction was subjected to two-dimensional electrophoresis (2-DE). Unambiguous protein identification was achieved with peptide mass fingerprinting matrix-assisted laser desorption/ionization - time of flight - mass spectrometry (MALDI-TOF-MS), peptide sequencing (MALDI LIFT-TOF/TOF MS), as well as gel-matching with previously identified databases. In overall five replicate 2-DE runs, a total of 2037 +/- 437 protein spots were detected of which 205 were identified representing 145 different proteins and 60 isoforms or post-translational modifications. The identified proteins could be grouped into several functional categories, such as metabolism, folding, cytoskeleton, transcription, signal transduction, protein degradation, detoxification, vesicle/protein transport, cell cycle regulation, apoptosis, and calcium homeostasis. The acquired proteome map of nondifferentiated UCB-MSCs is a useful inventory

which facilitates the identification of the normal proteomic pattern as well as its changes due to activated or suppressed pathways of cytosolic signal transduction which occur during proliferation, differentiation, or other experimental conditions.

Gluckman, E., V. Rocha, et al. (2001). "Results of unrelated umbilical cord blood hematopoietic stem cell transplant." *Transfus Clin Biol* **8**(3): 146-54.

The number of umbilical cord blood transplants (UCBT) is increasing worldwide, and the purpose of Eurocord is to evaluate the results and compare the outcome of UCBT with allogeneic bone marrow transplants (BMT). Data have been reported to Eurocord by many transplant centers. Close links have been established with cord blood banks through Netcord. BMT data have been provided by transplant centers and also by the European Blood and Marrow Transplant (EBMT) and International Bone Marrow Transplant Registries (IBMTR). Eurocord has analyzed the outcome of unrelated UCBT from 121 transplant centers and 29 countries. The results have shown that survival with unrelated mismatched UCBT was comparable to that with unrelated BMT. Engraftment with cord blood was delayed, resulting in an increased incidence of early transplant complications. The incidence of acute and chronic graft-versus-host-disease (GVHD) was reduced with cord blood grafts even in HLA mismatched transplants and in adults. In patients with leukemia, the rate of relapse was similar to that after BMT. The overall event-free survival with UCBT was not statistically different when compared to BMT. In conclusion, this large registry study confirms the potential benefit of using umbilical cord blood hematopoietic stem cells for allogeneic transplants.

Gluckman, E., V. Rocha, et al. (2001). "Results of unrelated umbilical cord blood hematopoietic stem cell transplantation." *Rev Clin Exp Hematol* **5**(2): 87-99.

The number of umbilical cord blood transplants is increasing worldwide. The purpose of Eurocord is to evaluate the results and to compare the outcome of umbilical cord blood transplants with allogeneic bone marrow transplants. Data have been reported to Eurocord by multiple transplant centers. Close links have been established with the cord blood banks through Netcord. Bone marrow transplant data have been provided by transplant centers and also through the European Group for Blood and Marrow Transplantation (EBMT) and International Bone Marrow Transplant Registries (IBMTR). Eurocord has analyzed the outcome of unrelated umbilical cord blood transplants from 121 transplant centers and 29 countries. The results showed that survival with

unrelated mismatched umbilical cord blood transplants was comparable to that with unrelated bone marrow transplants. Engraftment with cord blood was delayed, resulting in an increased incidence of early transplant complications. The incidence of acute and chronic graft-vs.-host disease was reduced with cord blood grafts even in human leukocyte antigen (HLA)-mismatched transplants and in adults. In patients with leukemia, the rate of relapse was similar to the rate of relapse after bone marrow transplant. The overall event-free survival with umbilical cord blood transplantation was not statistically different when compared to bone marrow transplants. This large registry study confirms the potential benefit of using umbilical cord blood hematopoietic stem cells for allogeneic transplants.

Hayashi, N., K. Takahashi, et al. (2009). "Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells support hematopoietic recovery of X-irradiated human CD34+ cells." *Life Sci* **84**(17-18): 598-605.

AIMS: The potential of human mesenchymal stem cell-like stroma prepared from placental/umbilical cord blood for hematopoietic regeneration by X-irradiated hematopoietic stem cells is herein assessed. MAIN METHODS: Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells were applied to a regenerative ex vivo expansion of X-irradiated human CD34(+) cells in a serum-free liquid culture supplemented with a combination of interleukine-3 plus stem cell factor plus thrombopoietin. KEY FINDINGS: The total number of cells and of lineage-committed myeloid hematopoietic progenitor cells generated in the co-culture of both non-irradiated and X-irradiated cells with stromal cells was significantly higher than those in the stroma-free culture. In addition, the number of CD34(+) cells and CD34(+)/CD38(-) cells, immature hematopoietic stem/progenitor cells also increased more than the stroma-free culture. The stromal cells produced various types of cytokines, although there was little difference between the co-cultures of non-irradiated and X-irradiated cells with stromal cells. Furthermore, when X-irradiated cells came in contact with stromal cells for 16 h before cytokine stimulation, a similar degree of hematopoiesis was observed, thus suggesting the critical role of cell-to-cell interaction. SIGNIFICANCE: The present results showed the potential efficacy of human mesenchymal stem cell-like stroma for hematopoietic regeneration from irradiated hematopoietic stem/progenitor cells.

Hexner, E. O., G. A. Danet-Desnoyers, et al. (2007). "Umbilical cord blood xenografts in immunodeficient

mice reveal that T cells enhance hematopoietic engraftment beyond overcoming immune barriers by stimulating stem cell differentiation." Biol Blood Marrow Transplant **13**(10): 1135-44.

Clinical experience and animal models have shown that donor T cell depletion (TCD) adversely affects engraftment of hematopoietic stem cells (HSCs). Although it is known that donor T cells are acting to overcome residual host immune barriers, they may also exert effects independent of host resistance via direct or indirect interactions with donor stem cells, their microenvironment, or key differentiation events. To more precisely define the effect of T cells on engraftment, we have performed human umbilical cord blood (UCB) transplantation into immunodeficient mice under limiting dilution conditions. UCB mononuclear cells (MNC) or TCD UCB were transplanted into NOD/LtSz-scid/scid B2m(null) (NOD/SCID-beta(2)m(-/-)) mice. Cohorts of mice received UCB MNC or TCD UCB at 5 dose levels between 5×10^4 and 5×10^6 cells. At dose levels at or above 10^5 cells, engraftment was higher in the MNC recipients ($n = 32$) than the TCD recipients ($n = 31$) in a dose-dependent manner. Despite this difference, limiting dilution analysis to determine functional stem cell frequency revealed that SCID repopulating cells in TCD UCB was not significantly less than in CB MNCs, suggesting that T cells may facilitate engraftment at stages beyond the stem cell. Add-back of CD3/CD28 costimulated T cells restored and appeared to enhance engraftment, both in NOD/SCID-beta(2)m(-/-) as well as NOD/LtSz-scid IL2Rgamma(null) (NOG) recipients. These results, in a model where there are minimal host immune barriers to overcome, suggest T cells possess additional graft-facilitating properties. CD3/CD28 costimulation of UCB T cells represents a potential strategy for enhancing the engraftment of UCB.

Hill, A. J., I. Zwart, et al. (2009). "Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats." Stem Cells Dev **18**(3): 399-409.

This study investigated the ability of mesenchymal stem cells (MSCs) derived from full-term human umbilical cord blood to survive, integrate and differentiate after intravitreal grafting to the degenerating neonatal rat retina following intracranial optic tract lesion. MSCs survived for 1 week in the absence of immunosuppression. When host animals were treated with cyclosporin A and dexamethasone to suppress inflammatory and immune responses, donor cells survived for at least 3 weeks, and were able to spread and cover the entire vitreal surface of the host retina. However, MSCs did not significantly

integrate into or migrate through the retina. They also maintained their human antigenicity, and no indication of neural differentiation was observed in retinas where retinal ganglion cells either underwent severe degeneration or were lost. These results have provided the first in vivo evidence that MSCs derived from human umbilical cord blood can survive for a significant period of time when the host rat response is suppressed even for a short period. These results, together with the observation of a lack of neuronal differentiation and integration of MSCs after intravitreal grafting, has raised an important question as to the potential use of MSCs for neural repair through the replacement of lost neurons in the mammalian retina and central nervous system.

Kashiwakura, I., O. Inanami, et al. (2003). "Protective effects of thrombopoietin and stem cell factor on X-irradiated CD34+ megakaryocytic progenitor cells from human placental and umbilical cord blood." Radiat Res **160**(2): 210-6.

In previous studies we characterized the radiosensitivity of CFU-megakaryocytes from human placental and umbilical cord blood and the effects of various early-acting cytokines. We found that the maximal clonal growth of CFU-megakaryocytes in vitro and maximal protection against X-ray damage were supported by a combination of thrombopoietin and stem cell factor. However, the mechanism by which the two cytokines exert a synergistic effect remained unclear, so we extended these studies to investigate the radioprotective action of synergistic thrombopoietin and stem cell factor on the survival of X-irradiated CD34(+) CFU-megakaryocytes. A combination of thrombopoietin and stem cell factor led to activation of mitogen-activated protein kinase and extracellular signal-regulated protein kinase and to suppression of caspase 3 in X-irradiated CD34(+) cells. When PD98059 and various synthetic substrates-specific inhibitors of these proteins were used, the combination had less effect on the clonal growth of X-irradiated CD34(+) CFU-megakaryocytes. However, the addition of wortmannin, a specific inhibitor of the phosphatidylinositol-3 kinase pathway, did not alter the synergistic action of thrombopoietin plus stem cell factor. We suggest that part of this synergistic effect can be explained by activation of mitogen-activated protein kinase and extracellular signal-regulated protein kinase and by suppression of the caspase cascade.

Katzenstein, H. M., E. R. Morgan, et al. (1997). "Haploidentical related umbilical cord blood stem cell transplant in a child with acute non-lymphocytic leukemia." Bone Marrow Transplant **19**(8): 765-9.

Umbilical cord blood stem cells (UCBSC) were used to reconstitute hematopoiesis following myeloablative therapy in a 13-month-old infant with acute nonlymphocytic leukemia (ANLL):FAB-M5 who had failed to sustain a chemotherapeutic remission. The patient's mother was 18 weeks pregnant with her second child at the time of diagnosis. Amniocentesis revealed that the fetus was HLA-haploidentical with the patient at the paternally inherited allele. The umbilical cord blood was harvested and processed by Ficoll centrifugation with 100% recovery of 5×10^7 mononuclear cells/kg and then cryopreserved. Two weeks after collection the cells were thawed and then infused into the patient following conditioning with total body irradiation, cyclophosphamide, and etoposide. Graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. The patient experienced clinical grade I GVHD consisting of skin involvement only that resolved within 2 weeks following the addition of corticosteroids. Engraftment was achieved with an absolute neutrophil count (ANC) above $0.5 \times 10^9/l$ on day 16, a platelet count above $50 \times 10^9/l$ on day 56, platelet transfusion independence on day 32 and red blood cell transfusion independence after day 44. Three months following transplantation restriction fragment length polymorphism (RFLP) revealed no discernible difference between the donor and the recipient. The patient remains in remission without evidence of GVHD 23 months post-transplant.

Kim, S. W., H. Han, et al. (2006). "Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model." *Stem Cells* **24**(6): 1620-6.

Buerger's disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vasoocclusive disease. It is characterized pathologically as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially the distal extremities (the feet and the hands). There is no curative medication or surgery for this disease. In the present study, we transplanted human leukocyte antigen-matched human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) into four men with Buerger's disease who had already received medical treatment and surgical therapies. After the stem cell transplantation, ischemic rest pain suddenly disappeared from their affected extremities. The necrotic skin lesions were healed within 4 weeks. In the follow-up angiography, digital capillaries were increased in number and size. In addition, vascular resistance in the affected extremities, compared with

the preoperative examination, was markedly decreased due to improvement of the peripheral circulation. Because an animal model of Buerger's disease is absent and also to understand human results, we transplanted human UCB-derived MSCs to athymic nude mice with hind limb ischemia by femoral artery ligation. Up to 60% of the hind limbs were salvaged in the femoral artery-ligated animals. By in situ hybridization, the human UCB-derived MSCs were detected in the arterial walls of the ischemic hind limb in the treated group. Therefore, it is suggested that human UCB-derived MSC transplantation may be a new and useful therapeutic armament for Buerger's disease and similar ischemic diseases.

Kleen, T. O., S. Kadereit, et al. (2005). "Recipient-specific tolerance after HLA-mismatched umbilical cord blood stem cell transplantation." *Transplantation* **80**(9): 1316-22.

BACKGROUND: Lower incidence and severity of acute graft versus host disease (GVHD) has been observed in leukemia patients receiving HLA-mismatched umbilical cord (UCB) transplants. However, despite the increased use of UCB in stem cell transplantation, the mechanisms underlying these favorable outcomes are not well delineated. **METHODS:** We analyzed antigen specific lymphocyte responses after transplant to determine whether the decreased allogeneic responsiveness of UCB lymphocytes is attributable to pan-unresponsiveness, lymphocyte repressive or recipient-specific tolerance. **RESULTS:** Circulating lymphocytes collected early (3 months) after UCB transplant demonstrate a less naive phenotype compared with that in the infused graft. Additionally, after transplant, circulating peripheral blood UCB-derived lymphocytes produced normal levels of interferon-gamma and proliferated normally when stimulated with mitogen or third party alloantigen. In contrast, when stimulated with recipient antigen, circulating lymphocytes emerging posttransplant did not proliferate nor produce interferon-gamma. Moreover, analysis of interleukin-4 production revealed a Th2 response to recipient antigens. These data indicate early induction of immune tolerance of naive UCB graft lymphocytes with skewing of transplant recipient-specific immune response towards Th2 cytokine profile. **CONCLUSIONS:** UCB graft lymphocyte immune naivety and observed early tolerance induction may contribute to the observed favorable GVHD incidence, despite infusion of HLA mismatch grafts in the unrelated allogeneic setting.

Koestenbauer, S., A. Zisch, et al. (2009). "Protocols for hematopoietic stem cell expansion from umbilical cord blood." *Cell Transplant* **18**(10): 1059-68.

The reconstitution of adult stem cells may be a promising source for the regeneration of damaged tissues and for the reconstitution of organ dysfunction. However, there are two major limitations to the use of such cells: they are rare, and only a few types exist that can easily be isolated without harming the patient. The best studied and most widely used stem cells are of the hematopoietic lineage. Pioneering work on hematopoietic stem cell (HSC) transplantation was done in the early 1970s by ED. Thomas and colleagues. Since then HSCs have been used in allogeneic and autologous transplantation settings to reconstitute blood formation after high-dose chemotherapy for various blood disorders. The cells can be easily harvested from donors, but the cell number is limited, especially when the HSCs originate from umbilical cord blood (UCB). It would be desirable to set up an ex vivo strategy to expand HSCs in order to overcome the cell dose limit, whereby the expansion would favor cell proliferation over cell differentiation. This review provides an overview of the various existing HSC expansion strategies—focusing particularly on stem cells derived from UCB—of the parameters that might affect the outcome, and of the difficulties that may occur when trying to expand such cells.

Kraai, R., A. G. Reymer, et al. (2001). "Hemopoietic stem and precursor cell analysis in umbilical cord blood using the Sysmex SE-9000 IMI channel." *Cytometry* **46**(2): 114-8.

Circulating hematopoietic progenitor cells (HPCs) are routinely measured by flow cytometry using CD34 expression. As an alternative, the "immature information" (IMI) channel measurement of the automated hematology analyzer Sysmex SE machines was developed. We tested the IMI channel HPC method with umbilical cord blood specimens. The IMI-HPCs were compared with CD34 counts and numbers of colony-forming units (CFUs). The IMI-HPC data were reproducible and dilution experiments yielded a log-linear relationship. The mean percentage of CD34(+) cells in 50 umbilical cords was 0.43 versus 0.11 of HPCs in the IMI channel (correlation coefficient $r = 0.67$). Absolute numbers yielded $96.79 \times 10^6/L$ CD34(+), $33.17 \times 10^6/L$ IMI-HPC, and $35.04 \times 10^6/L$ CFU-HPC. Receiver operating characteristics curves were made at various cutoff levels for CD34(+) cells to visualize sensitivity and specificity profiles. With median values of $13.56 \times 10^6/L$ for IMI-HPC and $20 \times 10^6/L$ for CD34(+) as cutoff points (the levels used in the laboratory to start stem cell pheresis), the percentage of false-negative observations was 70.4%. To exclude the influence of storage time, tests were repeated until 72 h after umbilical cord collection. Total white blood cell count

decreased in most cases, whereas absolute number of IMI-HPC tended to increase in time. In conclusion, if HPC measurements in the IMI channel are used to monitor circulating stem cells during mobilization, one has to be aware of a very low correlation between these results and those of other methods such as CD34(+) analysis and colony growth. False-negative results do occur, but if events are seen in the IMI channel, this simple monitoring technique is useful to predict the presence of circulating stem cells.

Kreja, L., S. Thoma, et al. (1993). "The effect of recombinant human stem cell factor and basic fibroblast growth factor on the in vitro radiosensitivity of CD34+ hematopoietic progenitors from human umbilical cord blood." *Exp Hematol* **21**(11): 1436-41.

Human umbilical cord blood (CB) cells selected by immunomagnetic beads for expression of the CD34 antigen were irradiated with increasing doses of x-rays (72 cGy/min). Clonogenic survival of the hematopoietic progenitors, including mixed colony-forming cells (Mix-CFC), erythroid burst-forming units (BFU-E), and granulocyte-macrophage colony-forming cells (GM-CFC), was determined in methylcellulose cultures containing placenta conditioned medium (PCM) and erythropoietin (Epo). Exponential survival curves were fitted to the data of all the colonies, resulting in $D_0 = 95$ cGy for Mix-CFC, 136 cGy for BFU-E, and 136 cGy for GM-CFC. Additionally, the radiosensitivity of CD34+ cells was studied employing cultures containing either recombinant human stem cell factor (rhSCF) or basic fibroblast growth factor (b-FGF) in combination with PCM and Epo. It was found that the colony-forming efficiency (CFE) of non-irradiated CD34+ cells of 5.5% (range 1.4 to 14.4%) did not increase after the addition of SCF or b-FGF to the culture. The radiation response characteristics showed, however, that in the presence of SCF, the D_0 value and the extrapolation number n increased significantly. This suggests the stimulation of what operationally is termed "recovery from potentially lethal damage." In contrast, no response modifying effect could be seen for b-FGF.

Larcher, C., V. A. Nguyen, et al. (2005). "Human herpesvirus-8 infection of umbilical cord-blood-derived CD34+ stem cells enhances the immunostimulatory function of their dendritic cell progeny." *Exp Dermatol* **14**(1): 41-9.

CD34(+) progenitor cells carrying human herpesvirus-8, Kaposi's sarcoma-associated herpesvirus (HHV-8/KSHV), have been described in the peripheral blood of AIDS patients suffering from Kaposi's sarcoma (KS). In this study, we investigated the influence of HHV-8 on the differentiation of CD34(+) progenitor cells. Native CD34(+) cells

derived from cord blood could be infected by a laboratory strain of HHV-8, as shown by immunofluorescence staining and polymerase chain reaction, but no significant initial maturation/differentiation effects were observed. In addition, these infected cells were differentiated into immature and mature dendritic cells (DCs) using cytokine induction with recombinant human granulocyte-macrophage colony-stimulating factor (rhGm-CSF), recombinant human tumor necrosis factor (rhTNF-alpha) and recombinant human stem cell factor (rhSCF). Double immunofluorescence and flow cytometry studies demonstrated that virus infection did not impair the development of immature and mature DC populations. Subsequently, the immunostimulating capacity of DC populations was tested in a mixed lymphocyte reaction using allogeneic T-cells. The HHV-8-infected CD34(+) progenitor cell-derived mature DC population showed a significantly enhanced antigen-presenting capacity, compared to non-infected DCs, which was not observed with the immature DCs. This suggests stimulation of DC function by HHV-8 infection. Because there are only a small percentage of HHV-8-positive DCs in the preparations and because it is not clear whether infection is abortive or productive to some extent, this seems to be most likely due to an indirect viral effect.

Lee, E., H. K. Min, et al. (2003). "Recombinant human (rh) stem cell factor and rhIL-4 stimulate differentiation and proliferation of CD3+ cells from umbilical cord blood and CD3+ cells enhance FcepsilonR1 expression on fetal liver-derived mast cells in the presence of rhIL-4." *Cell Immunol* **226**(1): 30-6.

We previously reported that rhIL-4 induced apoptosis and rhIL-6 mediated protection of human mast cells derived from cord blood mononuclear cells. Based on the result, we attempted to obtain the phenotypes and differentiation of CD3+ cells from cord blood by investigating their cell surface markers in the presence of rhSCF plus rhIL-4. The effect of co-cultured CD3+ cells on fetal liver mast cells (FLMCs) was also determined. Phenotypes from cord blood-derived cells were analyzed by flow cytometry and cell numbers were determined. Fetal liver mast cells were cultured with cord blood-derived cells (mainly CD3+) in the presence of rhSCF and/or rhIL-4 and were analyzed to determine cell number and expression of Kit+ and FcepsilonR1. The percentage of CD3+ cells from cord blood-derived cells on day 0 was about 41 +/- 13.5%, following monocytes and granulocytes. CD3+ cells increased in number (1.5-fold) and purity (90%), whereas other cell types did not survive. More than 60% of CD3+ cells from cord

blood at day 0 were CD4(-)CD8-. These double-negative cells dramatically decreased by 1 week of culture, while CD4+CD8+ cells increased in number and purity through 3 weeks of culture, and then decreased as greater numbers of single-positive T cells emerged. We also found that FcepsilonR expression on FLMC increased in the presence of rhIL-4, but was not affected by the T cells that developed from cord blood mononuclear cells. The results indicate that IL-4, a Th2 type cytokine, together with rhSCF, can induce T cell proliferations, differentiation, and maturation from cord blood progenitor cells.

Lei, P., Y. He, et al. (2007). "Effect of human WEE1 and stem cell factor on human CD34+ umbilical cord blood cell damage induced by chemotherapeutic agents." *Acta Biochim Biophys Sin (Shanghai)* **39**(8): 599-607.

Myelosuppression is one of the major side-effects of most anticancer drugs. To achieve myeloprotection, one bicistronic vector encoding anti-apoptotic protein human WEE1 (WEE1Hu) and proliferation-stimulating stem cell factor (SCF) was generated. In this study, we selected human umbilical cord blood CD34+ cells as the in vitro model in an attempt to investigate whether WEE1Hu, rather than conventional drug-resistant genes, can be introduced to rescue cells from the damage by chemotherapeutic agents such as cisplatin, adriamycin, mitomycin-c and 5-fluorouracil. Cell viability and cytotoxicity assay, colony-forming units in culture assay and externalization of phospholipid phosphatidylserine analysis showed that the expression of WEE1Hu and SCF in CD34+ cells provided the cells with some protection. These findings suggest that the expression of WEE1Hu and SCF might rescue CD34+ cells from chemotherapy-induced myelosuppression.

Lepus, C. M., T. F. Gibson, et al. (2009). "Comparison of human fetal liver, umbilical cord blood, and adult blood hematopoietic stem cell engraftment in NOD-scid/gammac-/-, Balb/c-Rag1-/-gammac-/-, and C.B-17-scid/bg immunodeficient mice." *Hum Immunol* **70**(10): 790-802.

Immunodeficient mice bearing components of a human immune system present a novel approach for studying human immune responses. We investigated the number, phenotype, developmental kinetics, and function of developing human immune cells following transfer of CD34(+) hematopoietic stem cell (HSC) preparations originating from second trimester human fetal liver (HFL), umbilical cord blood (UCB), or granulocyte colony-stimulating factor-mobilized adult blood (G-CSF-AB) delivered via intrahepatic injection into sublethally irradiated neonatal NOD-scid/gammac(-/-), Balb/c-Rag1(-/-)

) γ mac(-/-), and C.B-17-scid/bg mice. HFL and UCB HSC provided the greatest number and breadth of developing cells. NOD-scid/ γ mac(-/-) and Balb/c-Rag1(-/-) γ mac(-/-) harbored human B and dendritic cells as well as human platelets in peripheral blood, whereas NOD-scid/ γ mac(-/-) mice harbored higher levels of human T cells. NOD-scid/ γ mac(-/-) mice engrafted with HFL CD34(+) HSC demonstrated human immunological competence evidenced by white pulp expansion and increases in total human immunoglobulin following immunization with T-dependent antigens and delayed-type hypersensitivity-infiltrating leukocytes in response to antigenic challenge. In conclusion, we describe an encouraging base system for studying human hematopoietic lineage development and function utilizing human HFL or UCB HSC-engrafted NOD-scid/ γ mac(-/-) mice that is well suited for future studies toward the development of a fully competent humanized mouse model.

Lin, H., E. De Stanchina, et al. (2009). "Maitake beta-glucan enhances umbilical cord blood stem cell transplantation in the NOD/SCID mouse." *Exp Biol Med (Maywood)* **234**(3): 342-53.

Beta glucans are cell wall constituents of yeast, fungi and bacteria, as well as mushrooms and barley. Glucans are not expressed on mammalian cells and are recognized as pathogen-associated molecular patterns (PAMPS) by pattern recognition receptors (PRR). Beta glucans have potential activity as biological response modifiers for hematopoiesis and enhancement of bone marrow recovery after injury. We have reported that Maitake beta glucan (MBG) enhanced mouse bone marrow (BMC) and human umbilical cord blood (CB) cell granulocyte-monocyte colony forming unit (GM-CFU) activity in vitro and protected GM-CFU forming stem cells from doxorubicin (DOX) toxicity. The objective of this study was to determine the effects of MBG on expansion of phenotypically distinct subpopulations of progenitor and stem cells in CB from full-term infants cultured ex vivo and on homing and engraftment in vivo in the nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse. MBG promoted a greater expansion of CD34+CD33+CD38-human committed hematopoietic progenitor (HPC) cells compared to the conventional stem cell culture medium (P = 0.002 by ANOVA). CD34+CXCR4+CD38- early, uncommitted human hematopoietic stem cell (HSC) numbers showed a trend towards increase in response to MBG. The fate of CD34+ enriched CB cells after injection into the sublethally irradiated NOS/SCID mouse was evaluated after retrieval of xenografted human CB from marrow and spleen by flow cytometric analysis.

Oral administration of MBG to recipient NOS/SCID mice led to enhanced homing at 3 days and engraftment at 6 days in mouse bone marrow (P = 0.002 and P = 0.0005, respectively) compared to control mice. More CD34+ human CB cells were also retrieved from mouse spleen in MBG treated mice at 6 days after transplantation. The studies suggest that MBG promotes hematopoiesis through effects on CD34+ progenitor cell expansion ex vivo and when given to the transplant recipient could enhance CD34+ precursor cell homing and support engraftment.

Majhail, N. S., D. J. Weisdorf, et al. (2006). "Comparable results of umbilical cord blood and HLA-matched sibling donor hematopoietic stem cell transplantation after reduced-intensity preparative regimen for advanced Hodgkin lymphoma." *Blood* **107**(9): 3804-7.

We compared the safety and efficacy of allogeneic stem cell transplantation (allo-SCT) after reduced-intensity conditioning using either unrelated umbilical cord blood (UCB) donors or matched-sibling donors (MSDs) for 21 adults at high risk with advanced Hodgkin lymphoma (UCB, n = 9; MSD, n = 12). Both groups were comparable except for younger age in the UCB cohort (median, 28 vs 42 years; P = .02). Neutrophil recovery occurred earlier in the MSD group (median, 7 vs 10 days; P = .02). All patients had sustained donor engraftment by day 60. Cumulative incidence of acute severe graft-versus-host-disease (33% vs 33%; P = .99), chronic graft-versus-host-disease (11% vs 33%; P = .24), and 100-day treatment-related mortality (11% vs 17%; P = .80) were comparable. With median follow-up periods of 17 and 24 months, the 2-year progression-free survival rates were 25% (95% confidence interval [95% CI], 0%-55%) for UCB and 20% (95% CI, 0%-44%) for MSD allo-SCT (P = .67). Our results suggest comparable outcomes for reduced-intensity allo-SCT using UCB or MSD in adults at high risk with advanced Hodgkin lymphoma.

Martin, C. H., P. S. Woll, et al. (2008). "Differences in lymphocyte developmental potential between human embryonic stem cell and umbilical cord blood-derived hematopoietic progenitor cells." *Blood* **112**(7): 2730-7.

Hematopoietic progenitor cells derived from human embryonic stem cells (hESCs) develop into diverse mature hematopoietic lineages, including lymphocytes. Whereas functional natural killer (NK) cells can be efficiently generated in vitro from hESC-derived CD34(+) cells, studies of T- and B-cell development from hESCs have been much more limited. Here, we demonstrate that despite expressing functional Notch-1, CD34(+) cells from hESCs did

not derive T cells when cocultured with OP9 cells expressing Delta-like 1, or in fetal thymus organ culture. hESC-derived CD34(+) cells also did not produce B cells in vitro. In contrast, CD34(+) cells isolated from UCB routinely generated T and B cells when cultured in the same conditions. Notably, both undifferentiated hESCs, and sorted hESC-derived populations with hematopoietic developmental potential exhibited constitutive expression of ID family genes and of transcriptional targets of stem cell factor-induced signaling. These pathways both inhibit T-cell development and promote NK-cell development. Together, these results demonstrate fundamental differences between hESC-derived hematopoietic progenitors and analogous primary human cells. Therefore, hESCs can be more readily supported to differentiate into certain cell types than others, findings that have important implications for derivation of defined lineage-committed populations from hESCs.

Miniero, R., A. Busca, et al. (1995). "HLA-haploidentical umbilical cord blood stem cell transplantation in a child with advanced leukemia: clinical outcome and analysis of hematopoietic recovery." *Bone Marrow Transplant* **16**(2): 229-40.

Growing attention has been focused on cord blood as a source of transplantable hematopoietic stem cells. However, clinical experience is rather limited. In this study we describe a child with advanced acute lymphoblastic leukemia who received an HLA-haploidentical cord blood transplant. The patient was transplanted in third complete remission after conditioning with fractionated total body irradiation, thiopeta and cyclophosphamide. Forty-one milliliters of cryopreserved umbilical cord blood, containing 0.15×10^8 nucleated cells/kg and 0.25×10^4 CFU-GM/kg, were infused. Cyclosporine and prednisone were administered for graft-versus-host disease (GVHD) prophylaxis. The patient received G-CSF from day +1 to day +35, but no improvement in granulocyte counts was observed. Therefore, administration of GM-CSF was started on day +36 to day +59, which resulted in a significant increase in white blood cells and granulocyte counts. Sustained myeloid engraftment was evidenced by a granulocyte count $> 0.5 \times 10^9/l$ by day +41. The presence of donor-derived cells could be documented in the peripheral blood and bone marrow of the patient by cytogenetic analysis, HLA phenotyping and DNA studies. Forty-one days after transplant, clonogenic bone marrow assays showed the presence of low frequencies of primitive hematopoietic progenitor cells (BFU-E = 19/10(5) and CFU-GM = 8/10(5)). The chimerism was complete and no host-derived cells could be detected. However, the engraftment was

restricted to the myeloid lineage whereas lymphoid and megakaryocytic engraftments were inadequate. The immunophenotype of the patient's peripheral blood showed the presence of T lymphocytes expressing an immature phenotype (CD2+ CD3-) at day +21.(ABSTRACT TRUNCATED AT 250 WORDS)

Miniero, R., U. Ramenghi, et al. (1993). "Umbilical cord blood stem cell transplantation." *Int J Artif Organs* **16 Suppl 5**: 113-5.

Human umbilical cord blood as an alternative source of hematopoietic stem cells for bone marrow reconstitution, has recently been demonstrated to yield successful HLA-matched placental blood grafts in children. It has been shown that cord blood contains sufficient progenitor cells to effect hematological reconstitution. Since then, more than 25 cord blood stem cells (CBSCs) transplants have been performed worldwide for the treatment of a variety of malignant and nonmalignant diseases. The majority of the grafts performed thus far have utilized CBSCs from HLA-identical siblings. However, much of the interest in this setting is devoted to the potential use of CBSCs for HLA-mismatched and unrelated transplants. Preliminary results suggest that allorecognition and graft-versus-host disease may be less intense in CBSCs transplants than in recipients of similarly compatible bone marrow. This review summarizes the results and potential future applications of cord blood transplantation.

Mino, E., R. Kobayashi, et al. (2004). "Umbilical cord blood stem cell transplantation from unrelated HLA-matched donor in an infant with severe congenital neutropenia." *Bone Marrow Transplant* **33**(9): 969-71.

We report here a 6-month-old boy with severe congenital neutropenia (SCN) successfully treated by cord blood stem cell transplantation (CBSCT) from an unrelated donor. He had recurrent life-threatening respiratory infection due to severe neutropenia that was refractory to recombinant human granulocyte colony-stimulating factor (rhG-CSF). Because he had no HLA-matched sibling and no time to wait for unrelated donor, he received HLA-matched unrelated CBSCT as determined by DNA typing. A total of 6.4×10^7 CB nucleated cells/kg was infused after conditioning with busulfan/horse antihuman thymocyte serum/cyclophosphamide. No GVHD developed under the treatment with cyclosporin A and methyl prednisolone. The neutrophil count reached $0.5 \times 10^9/l$ on day 14, reticulocyte 1% on day 13 and platelet count over $50 \times 10^9/l$ on day 31. We conclude that unrelated CBSCT can be an indication for some cases of SCN, who have recurrent life-

threatening infections and are refractory to rhG-CSF, and have no HLA-matched sibling.

Nagayama, H., K. Misawa, et al. (2002). "Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim." *Bone Marrow Transplant* **29**(3): 197-204.

We performed stem cell rescue and allogeneic skin transplantation on a lethally neutron-irradiated nuclear accident victim. HLA-DRB1 mismatched unrelated umbilical cord blood cells (2.08×10^7 /kg recipient body weight) were transplanted to an 8-10 Gy equivalent neutron-irradiated patient because of a lack of a suitable bone marrow or peripheral blood donor. Pre-transplant conditioning consisted of anti-thymocyte gamma-globulin alone, and GVHD prophylaxis was a combination of cyclosporine (CYA) and methylprednisolone (mPSL). Granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), and thrombopoietin (TPO) were concurrently administered after transplantation. The absolute neutrophil count reached $0.5 \times 10^9/l$ on day 15, the reticulocyte count rose above 1% on day 23, and the platelet count was over $50 \times 10^9/l$ on day 27, respectively. Cytogenetic studies of blood and marrow showed donor/recipient mixed chimerism. Rapid autologous hematopoietic recovery was recognized after withdrawal of CYA and mPSL. Repeated pathological examinations of the skin revealed no evidence of acute GVHD. Eighty-two days after the irradiation, skin transplantation was performed to treat radiation burns. Almost 90% of the transplanted skin engrafted. Immunological examination after autologous hematopoietic recovery revealed an almost normal T cell count. However, immune functions were severely impaired. The patient died from infectious complication 210 days after the accident.

Narimatsu, H., T. Matsumura, et al. (2005). "Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients." *Biol Blood Marrow Transplant* **11**(6): 429-36.

Bloodstream infection (BSI) is a significant problem after cord blood transplantation (CBT). However, little information has been reported on BSI after reduced-intensity CBT (RI-CBT). We retrospectively reviewed the medical records of 102 patients. The median age of the patients was 55 years (range, 17-79 years). Preparative regimens comprised fludarabine 125 to 150 mg/m², melphalan 80 to 140 mg/m², or busulfan 8 mg/kg and total body irradiation 2 to 8 Gy. Prophylaxis against graft-versus-host disease comprised cyclosporin or tacrolimus. BSI developed within 100 days of RI-CBT in 32 patients. The cumulative incidence of BSI was 25% at day 30

and 32% at day 100. The median onset was day 15 (range, 1-98 days). Causative organisms included *Pseudomonas aeruginosa* (n = 12), *Staphylococcus epidermidis* (n = 11), *Staphylococcus aureus* (n = 6), *Enterococcus faecium* (n = 4), *Enterococcus faecalis* (n = 4), *Stenotrophomonas maltophilia* (n = 4), and others (n = 7). Of the 32 patients with BSI, 25 (84%) died within 100 days after RI-CBT. BSI was the direct cause of death in 8 patients (25%). Univariate analysis failed to identify any significant risk factors. BSI clearly represents a significant and fatal complication after RI-CBT. Further studies are warranted to determine clinical characteristics, identify patients at high risk of BSI, and establish therapeutic strategies.

Nishihira, H., K. Ohnuma, et al. (1998). "Unrelated umbilical cord-blood stem cell transplantation: a report from Kanagawa Cord Blood Bank, Japan." *Int J Hematol* **68**(2): 193-202.

Umbilical cord-blood (CB) has been used as a source of hematopoietic stem cells in pediatric patients with sibling donors. As a result of the success with CB transplantation from sibling donors, pilot programs for the banking of unrelated donor CB were initiated in the organization of Kanagawa Cord Blood Bank, Japan in 1995. As of December 1997, unrelated donor CB was used to reconstitute hematopoiesis in seven patients aged 0.7-12.8 years, weighing 7-36 kg with high-risk leukemia (n = 5), myelodysplastic syndrome (n = 1), and immunodeficiency syndrome (n = 1). Engraftment of CB was achieved in six patients. The absolute neutrophil count reached 500/microliter in a median of 27 days; a platelet count of 20,000/microliter was reached by a median of 64 days in three patients who could be evaluated. Five patients are currently surviving. Grade I GVHD developed in three patients and grade III in one patient; no GVHD developed in three patients. Although only a small number of patients have been studied and the period of observation is too short to determine long-term survival, HLA-matched or HLA-mismatched CB from unrelated donors can provide an alternative source of hematopoietic reconstitution for clinical transplantation.

Park, J., V. Setter, et al. (2009). "Umbilical cord blood stem cells: induction of differentiation into mesenchymal lineages by cell-cell contacts with various mesenchymal cells." *Tissue Eng Part A* **15**(2): 397-406.

CD133(+) cells isolated from bone marrow, peripheral blood, or umbilical cord blood (UCB) represent an established source of transplantable hematopoietic progenitors. Further, there is increasing evidence that such CD133(+) cell isolates comprise subpopulations capable of differentiating into several

mesenchymal lineages. In this study, we investigated conditions under which mesenchymal differentiation can be induced, particularly the role of cell-cell contacts with mesenchymal cells. A purified, nearly homogeneous CD133(+) population of human UCB cells was expanded by stimulation with platelet-derived growth factor and epidermal growth factor, labeled with the fluorescent marker DiI and cocultivated with rat osteoblasts, C2C12 myoblasts, or rat cardiomyocytes, respectively. In control experiments, the two cell types were separated by microporous membranes to avoid cell-cell contacts. Direct coculture of DiI-labeled UCB cells with the different mesenchymal cell populations resulted in both significant morphological changes and upregulation of lineage-specific markers. Expression of osteocalcin, myosin heavy chain, or alpha-actinin confirmed differentiation of the UCB cells into an osteoblastic, myoblastic, or cardiomyocytic phenotype, respectively. In contrast, coculture of UCB cells with the respective inducer cells under conditions preventing cell-cell contacts yielded minor, if any, evidence for such differentiation. Our data, thus, indicate that UCB cell expansion *in vitro* and subsequent direct cell-cell contacts with mesenchymal cells can induce their differentiation into mesenchymal lineages specific to the cell type they are in contact with. This finding has important implications for understanding the homing of adult stem cells and the promise of UCB as a cell source for tissue engineering and regenerative medicine.

Reed, S. A. and S. E. Johnson (2008). "Equine umbilical cord blood contains a population of stem cells that express Oct4 and differentiate into mesodermal and endodermal cell types." *J Cell Physiol* **215**(2): 329-36.

Mesenchymal stem cells (MSCs) offer promise as therapeutic aids in the repair of tendon, ligament, and bone damage suffered by sport horses. The objective of the study was to identify and characterize stem-like cells from newborn foal umbilical cord blood (UCB). UCB was collected and MSC isolated using human reagents. The cells exhibit a fibroblast-like morphology and express the stem cell markers Oct4, SSEA-1, Tra1-60 and Tra1-81. Culture of the cells in tissue-specific differentiation media leads to the formation of cell types characteristic of mesodermal and endodermal origins. Chondrogenic differentiation reveals proteoglycan and glycosaminoglycan synthesis as measured histochemically and Sox9 and collagen 2A1 gene transcription. Osteocytes capable of mineral deposition, osteonectin and Runx2 transcription were evident. Hepatogenic cells formed from UCBs express albumin and cytokeratin 18. Multinucleated myofibers

that express desmin were observed indicating partial differentiation into mature muscle cells. Interestingly, conventional human protocols for UCB differentiation into adipocytes were unsuccessful in foal UCB and adult horse adipose-derived MSC. These results demonstrate that equine UCB can be induced to form multiple cell types that underlie their value for regenerative medicine in injured horses. In addition, this work suggests that subtle differences exist between equine and human UCB stem cells.

Rizvanov, A. A., A. P. Kiyasov, et al. (2008). "Human umbilical cord blood cells transfected with VEGF and L(1)CAM do not differentiate into neurons but transform into vascular endothelial cells and secrete neuro-trophic factors to support neuro-genesis-a novel approach in stem cell therapy." *Neurochem Int* **53**(6-8): 389-94.

Genetically modified mono-nuclear cell fraction from human umbilical cord blood (HUCB) expressing human vascular endothelial growth factor (VEGF) and mouse neural L(1) cell adhesion molecule (L(1)CAM) were used for gene-stem cell therapy of transgenic (G)93(A) mice adopted as an animal amyotrophic lateral sclerosis (ALS) model. We generated non-viral plasmid constructs, expressing human VEGF(165) (pcDNA-VEGF) and mouse neural L(1) cell adhesion molecule (pcDNA-mL(1)CAM). Mono-nuclear fraction of HUCB cells were transiently transfected by electro-poration with a mixture of expression plasmids (pcDNA-VEGF+pcDNA-mL(1)CAM). Sixteen transgenic female and male mice were randomly assigned to three groups: (1) transplantation of genetically modified HUCB cells expressing L(1) and VEGF (n=6), (2) transplantation of un-transfected HUCB cells (n=5), and (3) control group (n=5). In first two experimental groups 1x10⁶ cells were injected retro-orbitally in pre-symptomatic 22-25-week-old (G)93(A) mice. Our results demonstrate that HUCB cells successfully grafted into nervous tissue of ALS mice and survived for over 3 months. Therefore, genetically modified HUCB cells migrate in the spinal cord parenchyma, proliferate, but instead of transforming into nerve cells, they differentiate into endothelial cells forming new blood vessels. We propose that: (A) expression of mouse neural L(1)CAM is responsible for increased homing and subsequent proliferation of transplanted cells at the site of neuro-degeneration, (B) expression of human VEGF directs HUCB cell differentiation into endothelial cells, and (C) neuro-protective effect may stem from the delivery of various neuro-trophic factors from newly formed blood vessels.

Saulnier, N., C. Di Campi, et al. (2005). "From stem cell to solid organ. Bone marrow, peripheral blood or umbilical cord blood as favorable source?" Eur Rev Med Pharmacol Sci **9**(6): 315-24.

Adult stem cells (ASC) have becoming a great domain of research by their promising interest for the regenerative medicine. For some years, the number of publications has been increasing, displaying the potential of ASC to differentiate in all tissue-lineages, challenging the previous dogma that ASC were restricted to give rise only to specific cells from their tissue of origin. Among the diversity of ASC, hematopoietic stem cells (HSC) have been the most studied and their use in the clinical setting is largely documented. Commonly, HSC have been harvested from the bone marrow, but for some years, two others sources, the peripheral blood and the umbilical cord blood have been introduced. All these HSC possess their own molecular characteristics and degree of maturity and represent a more or less good candidate to participate in the cellular-based tissue regeneration. We have reviewed the different parameters allowing to define which subset could be the more favorable such as the accessibility to the pool of HSC; the quantity of available cells; the tolerability of host-engraftment and the capacity of the cells to home correctly to the required site of damaged. Besides, recently, the molecular profiling of HSC has allowed identifying which subset possesses the more promising characteristics.

Savarese, T. M., W. C. Strohsnitter, et al. (2007). "Correlation of umbilical cord blood hormones and growth factors with stem cell potential: implications for the prenatal origin of breast cancer hypothesis." Breast Cancer Res **9**(3): R29.

INTRODUCTION: Prenatal levels of mitogens may influence the lifetime breast cancer risk by driving stem cell proliferation and increasing the number of target cells, and thereby increasing the chance of mutation events that initiate oncogenesis. We examined in umbilical cord blood the correlation of potential breast epithelial mitogens, including hormones and growth factors, with hematopoietic stem cell concentrations serving as surrogates of overall stem cell potential. **METHODS:** We analyzed cord blood samples from 289 deliveries. Levels of hormones and growth factors were correlated with concentrations of stem cell and progenitor populations (CD34+ cells, CD34+CD38- cells, CD34+c-kit+ cells, and granulocyte-macrophage colony-forming units). Changes in stem cell concentration associated with each standard deviation change in mitogens and the associated 95% confidence intervals were calculated from multiple regression analysis. **RESULTS:** Cord blood plasma levels of insulin-like growth factor-1

(IGF-1) were strongly correlated with all the hematopoietic stem and progenitor concentrations examined (one standard-deviation increase in IGF-1 being associated with a 15-19% increase in stem/progenitor concentrations, all $P < 0.02$). Estriol and insulin-like growth factor binding protein-3 levels were positively and significantly correlated with some of these cell populations. Sex hormone-binding globulin levels were negatively correlated with these stem/progenitor pools. These relationships were stronger in Caucasians and Hispanics and were weaker or not present in Asian-Americans and African-Americans. **CONCLUSION:** Our data support the concept that in utero mitogens may drive the expansion of stem cell populations. The correlations with IGF-1 and estrogen are noteworthy, as both are crucial for mammary gland development.

Schwinger, W., C. Urban, et al. (1999). "Transplantation of related and unrelated umbilical cord blood stem cells in Austria. Austrian Working Party for Stem Cell Transplantation. Austrian Society of Hematology and Oncology." Wien Klin Wochenschr **111**(9): 348-53.

Allogeneic bone marrow transplantation is limited by the availability of suitable HLA-matched donors and the risk of graft versus host disease (GvHD). In an attempt to overcome these limitations umbilical cord blood (UCB), has become a further alternative. UCB transplantations in Austria were started in 1991. As of September 31, 1998, six patients have been transplanted. Diagnoses were severe aplastic anaemia (SAA) (n = 2), acute lymphoblastic leukaemia (ALL) (n = 1), familial hemophagocytic syndrome (FHL) (n = 2) and chronic myelomonocytic leukaemia (CMML) (n = 1). Three patients received UCB grafts from HLA-identical siblings and three patients from unrelated donors, of whom two were disparate at two HLA loci (A/B) and one mismatched at one locus (C). Five patients were engrafted with complete donor hematopoiesis, with a median time of 26.5 days (range 14 to 39 days) to an ANC count of $> \text{or} = 0.5 \times 10^9/\text{L}$ and a median time of 42.5 days (range 24 to 67 days) to a platelet count of $> \text{or} = 20 \times 10^9/\text{L}$. One patient with FHL had partial engraftment and died due to reactivation of cytomegalovirus (CMV) infection and CMV pneumonia on day +25. Of the five patients surviving the post-transplant period, one with CMML had a relapse on day +128 and died after a HLA-matched bone marrow transplantation from the same sibling donor in the second relapse. Another patient with ALL relapsed on day +200 but is still alive under palliative treatment; one patient with SAA showed graft rejection and autologous hematopoietic reconstitution and later had a successful CD34(+)-selected allogeneic peripheral

stem cell transplant from a C-locus mismatched unrelated donor. Two patients (one with SAA and one with FHL) are alive with complete remission of the underlying disease. This report reflects the experience and results of UCB transplantation in Austria and discusses the position of UCB transplantation in the context of the other stem cell alternatives available today.

Szabolcs, P., K. D. Park, et al. (2004). "Superior depletion of alloreactive T cells from peripheral blood stem cell and umbilical cord blood grafts by the combined use of trimetrexate and interleukin-2 immunotoxin." *Biol Blood Marrow Transplant* **10**(11): 772-83.

Acute graft-versus-host disease, a major obstacle to the overall success of allogeneic hematopoietic stem cell transplantation, is primarily induced by a subset of donor T cells. Most strategies to prevent acute graft-versus-host disease target all T cells regardless of their specificity, and this leads to prolonged posttransplantation immunodeficiency. Selective depletion of alloreactive T cells could spare protective immunity and facilitate engraftment and graft-versus-leukemia effects. Recently described depletion strategies target activation markers such as CD25 that are expressed by alloreactive T cells. However, incomplete depletion may occur when a single surface epitope or pathway of apoptosis is targeted that may not be fully and concurrently expressed among all alloreactive cells. We now report on a novel strategy effective in both cord blood and peripheral blood stem cell alloreactive T cells that simultaneously induces 2 independent pathways of apoptosis after stimulation by recipient dendritic cells or Epstein-Barr virus-transformed B cells. First, we demonstrate that the folate antagonist trimetrexate selectively depletes proliferating alloreactive precursors in vitro in a dose- and time-dependent manner. Similarly, a second agent, denileukin difitox, kills activated alloreactive T cells expressing CD25. Most importantly, these 2 agents can exert their effects in concert with superior efficacy while sparing resting bystander T cells, which remain available to mount antimicrobial or third-party responses.

Thierry, D., R. Traineau, et al. (1990). "Hematopoietic stem cell potential from umbilical cord blood." *Nouv Rev Fr Hematol* **32**(6): 439-40.

We studied the conditions of collection and isolation of hematopoietic cells from cord blood in order to optimise the sampling. A statistically significant correlation was found between the total stem cell content of the samples and the time of delivery suggesting that the quantity of hematopoietic stem cells available is higher when cord blood

collection is performed earlier during pregnancy. Attempt to isolate the white cells resulted in a dramatic loss of stem cells. Factors affecting cell recovery and purification must be investigated in order to optimize cord blood cell banking.

Toren, A., A. Nagler, et al. (1999). "Successful human umbilical cord blood stem cell transplantation without conditioning in severe combined immune deficiency." *Bone Marrow Transplant* **23**(4): 405-8.

A 2-month-old girl with severe combined immunodeficiency (SCID), presented with mild staphylococcal skin infection, lymphopenia, low T cell number, absence of B cells, high number of NK cells, and a negligible response to mitogens. Since her older brother died as a result of SCID 2 years earlier, cord blood was harvested from a sister born 2 1/2 years earlier, who was normal and fully matched both by serology and molecular typing. In view of her clinical condition and in spite of a high number of NK cells with normal activity, HUCBT without preparative conditioning was performed. No G-CSF was administered. Engraftment with mixed chimerism was evident 3 weeks post transplantation. There were no peritransplantation complications. Eighteen months post transplantation, the girl is in excellent condition, blood counts are normal, T cell engraftment is complete, B cell engraftment is proceeding gradually, and the mitogen stimulation tests are normal. Due to the unique nature of HUCB hematopoietic cells, engraftment without conditioning may be possible in patients with SCID with fully matched donors. This is the first HUCBT performed without conditioning.

Traycoff, C. M., M. R. Abboud, et al. (1994). "Rapid exit from G0/G1 phases of cell cycle in response to stem cell factor confers on umbilical cord blood CD34+ cells an enhanced ex vivo expansion potential." *Exp Hematol* **22**(13): 1264-72.

Currently, the most commonly used grafts of progenitor and stem cells for patients undergoing bone marrow transplantation (BMT) are derived from large collections of autologous or allogeneic adult human bone marrow (BM). The feasibility of using human umbilical cord blood (HUCB), normal peripheral blood (PB), and smaller collections of BM as sources of hematopoietic stem cell grafts for adult patients remains questionable. We investigated the ex vivo proliferative potential of HUCB CD34+ cells as a means of expanding HUCB grafts, thereby making them more acceptable for clinical transplantation. HUCB-derived CD34+HLA-DR+ cells, maintained for 5 days in suspension cultures supplemented with 10% HUCB plasma and a combination of stem cell factor (SCF) and interleukin-3 (IL-3), displayed a 10-fold increase in the total number of CD34+ cells. In

contrast, only a four-fold increase was observed in identical cultures initiated with BM-derived CD34+HLA-DR+ cells. Whereas BM CD34+ cells failed to proliferate in response to SCF alone, HUCB CD34+ cells expanded 5.6-fold by day 5, thus demonstrating an enhanced response to SCF. When the effects of SCF on the exit of HUCB cells from G0/G1 phases of cell cycle were investigated, we found that although HUCB CD34+HLA-DR+ cells were more quiescent than BM CD34+HLA-DR+ and BM CD34+HLA-DR- cells (97.5% of HUCB CD34+HLA-DR+ in G0/G1 vs. 88.6% of BM CD34+HLA-DR+ and 92.0% of BM CD34+HLA-DR- [p < 0.005]), HUCB CD34+HLA-DR+ cells exited from dormancy more rapidly than BM cells, such that by 36 to 48 hours following exposure to SCF, only 55% remained in G0/G1.

van de Ven, C., D. Collins, et al. (2007). "The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration." *Exp Hematol* **35**(12): 1753-65.

Stem cells have been isolated from human embryos, fetal tissue, umbilical cord blood (UCB), and also from "adult" sources. Adult stem cells are found in many tissues of the body and are capable of maintaining, generating, and replacing terminally differentiated cells. A source of pluripotent stem cells has been recently identified in UCB that can also differentiate across tissue lineage boundaries into neural, cardiac, epithelial, hepatocytic, and dermal tissue. Thus, UCB may provide a future source of stem cells for tissue repair and regeneration. Its widespread availability makes UCB an attractive source for tissue regeneration. UCB-derived stem cells offer multiple advantages over adult stem cells, including their immaturity, which may play a significant role in reduced rejection after transplantation into a mismatched host and their ability to produce larger quantities of homogenous tissue or cells. While research with embryonic stem cells continues to generate considerable controversy, human umbilical stem cells provide an alternative cell source that has been more ethically acceptable and appears to have widespread public support. This review will summarize the in vitro and in vivo studies examining UCB stem cells and their potential use for therapeutic application for nonhematopoietic tissue and cell regeneration.

Wadhwa, P. D., H. M. Lazarus, et al. (2003). "Hematopoietic recovery after unrelated umbilical cord-blood allogeneic transplantation in adults treated with in vivo stem cell factor (R-MetHuSCF) and filgrastim administration." *Leuk Res* **27**(3): 215-20.

Transplantation with unrelated umbilical cord blood (UCB) is marked by delayed hematologic recovery. This report summarizes two adults with chronic myelogenous leukemia (CML), who received myeloablative conditioning followed by infusion of a non-expanded single UCB graft. These CML patients were enrolled in a clinical trial incorporating concomitant in vivo administration of stem cell factor (R-MetHuSCF) and filgrastim from day of UCB infusion until attained hematopoietic recovery. Each patient engrafted fully with donor UCB, with days to absolute neutrophil count (ANC) >500/microl being 13 and 29 days, respectively. Both patients remain in cytogenetic remission at 28 months follow-up. 'In vivo UCB expansion' with administration of concomitant R-MetHuSCF and filgrastim may facilitate prompt hematologic engraftment.

Xiao, J., Z. Nan, et al. (2005). "Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury." *Stem Cells Dev* **14**(6): 722-33.

Umbilical cord blood (UCB) is a rich source of hematopoietic stem cells (HSCs). We have isolated a novel cell line population of stem cells from human UCB that exhibit properties of self-renewal, but do not have cell-surface markers that are typically found on HSCs. Analysis of transcripts revealed that these cells express transcription factors Oct-4, Rex-1, and Sox-2 that are typically expressed by stem cells. We refer to these novel cells as nonhematopoietic umbilical cord blood stem cells (nh-UCBSCs). Previous studies have shown that the intravenous infusion of UCBCs can ameliorate neurological deficits arising from ischemic brain injury. The identity of the cells that mediate this restorative effect, however, has yet to be determined. We postulate that nh-UCBSCs may be a source of the UCB cells that can mediate these effects. To test this hypothesis, we intravenously injected one million human nh-UCBSCs into rats 48 h after transient unilateral middle cerebral artery occlusion. Animals in other experimental groups received either saline injections or injections of RN33b neural stem cells. Animals were tested for neurological function before the infusion of nh-UCBSCs and at various time periods afterwards using a battery of behavioral tests. Some human nuclei-positive cells were co-labeled for NeuN, indicating that the transplanted cells expressed markers of a neuronal phenotype. Cells expressing the human nuclei marker within the brain, however, were rather scant, suggesting that the restorative effects of nh-UCBSCs may be mediated by mechanisms other than cell replacement. To test this hypothesis, nh-UCBSCs were directly transplanted into the brain parenchyma after ischemic brain injury. Sprouting of

nerve fibers from the nondamaged hemisphere into the ischemically damaged side of the brain was assessed by anterograde tracing using biotinylated dextran amine (BDA). Animals with nh-UCBSC transplants exhibited significantly greater densities of BDA-positive cells in the damaged side of the brain compared to animals with intraparenchymal saline injections. These results suggest that restorative effects observed with nh-UCBSC treatment following ischemic brain injury may be mediated by trophic actions that result in the reorganization of host nerve fiber connections within the injured brain.

Yasutake, M., M. Sumita, et al. (2001). "Stem cell collection filter system for human placental/umbilical cord blood processing." *Vox Sang* **80**(2): 101-5.

BACKGROUND AND OBJECTIVES: The hydroxyethyl starch method and the Top & Bottom method have been used worldwide for the volume reduction of human placental/umbilical cord blood (PCB) units. To simplify the preparation of nucleated cell (NC) concentrates, we developed a new filter device--the stem cell collection filter system (SCF SYSTEM)--which can collect mononuclear cells (MNC) at a high recovery rate. **MATERIALS AND METHODS:** The SCF SYSTEM consisted of a filter and two bags. Multilayered polyethylene terephthalate non-wovens, coated with a hydrophilic polymer, were used as filter media. PCB units were filtered by gravity (n = 12). Red blood cells, platelets and plasma were drained into the drain bag, and the NC trapped on the filter media was collected in the recovery bag by reverse washing. Recovered cell fractions were evaluated. **RESULTS:** The volume of cell concentrate recovered was 27.4 +/- 2.2 ml (mean +/- SD, n = 12). The whole time required for processing was less than 30 min, and handling was very simple. The viability of recovered NC was 97.8 +/- 3.2%. The recovery of lymphocytes, monocytes and granulocytes was 79.5 +/- 16.9%, 79.8 +/- 20.4% and 39.0 +/- 19.5%, respectively. The recovery rate of granulocytes was significantly lower than that of monocytes and lymphocytes (P < or = 0.0001). The recovery rates of CD3+ cells, CD19+ cells and CD56+ cells were almost the same as that of MNC. The recovery rates of CD34+ cells, total colony-forming cells and long-term culture-initiating cells were 81.7 +/- 27.0% (n = 11), 80.8 +/- 27.7% (n = 12) and 75.0 +/- 18.4% (n = 2), respectively. **CONCLUSION:** The new filter system was shown to be efficient for PCB processing, encompassing a very simple handling procedure with a good recovery of haematopoietic progenitor cells. Hence, the SCF SYSTEM is potentially useful for the volume reduction of PCB units for cord blood banking.

Yasutake, M., Y. Zheng, et al. (2005). "SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem and/or progenitor cells in a nonobese diabetic/Shi-SCID mice serial xenotransplantation model and immune cell activities in vitro: a comparative study of the filter method and the hydroxyethyl starch method." *Transfusion* **45**(12): 1899-908.

BACKGROUND: A novel filter system was developed for umbilical cord blood (UCB) volume reduction. The aim of this study was to compare the functions of cryopreserved UCB cells processed by the filter and by the hydroxyethyl starch (HES) sedimentation method from the aspect of the graft quality. **STUDY DESIGN AND METHODS:** UCB specimens were divided into two portions, processed in parallel by the filter or HES, and then cryopreserved in the clinical setting. The thawed UCB specimens containing 1×10^5 CD34+ cells were injected into nonobese diabetic/Shi-SCID mice, and the engraftment capacity in primary and secondary transplants was assessed. The functions of natural killer (NK) cells and monocyte-derived dendritic cells (DCs) were also assayed in vitro. **RESULTS:** The percentage of recovery of CD34+ cells by both methods was equivalent. In the marrow of the primary transplant recipients, the percentage of hCD45+ cells in the filter group and HES group was 58.2 +/- 31.6 and 46.5 +/- 28.4 percent, respectively (p = 0.016). The engraftment capacity and multilineage differentiation in the secondary transplantations were equal in both groups. The cytotoxic activity of the NK cells and phagocytosis activity of the DCs from both the groups were similar. **CONCLUSION:** The filter yielded a desirable percentage of recovery of hematopoietic cells with engraftment ability in the clinical setting. Thus, it is considered that the filter system may be useful for UCB banking for cord blood transplantation.

Zhao, Y., Z. Huang, et al. (2007). "Immune regulation of T lymphocyte by a newly characterized human umbilical cord blood stem cell." *Immunol Lett* **108**(1): 78-87.

Previous work identified a novel type of stem cell from human umbilical cord blood, designated cord blood-stem cells (CB-SC). To further evaluate their immune characteristics, we cocultured CB-SC with allogeneic peripheral blood lymphocytes in the presence of phytohaemagglutinin (PHA) or interleukin-2 (IL-2). Results showed that CB-SC could significantly inhibit lymphocyte proliferation and reduce tyrosine phosphorylation of STAT5 in both PHA- and IL-2-stimulated lymphocytes, along with the regulation on the phenotypes of CD4+ and CD8+ T cells. Additionally, CB-SC also suppressed

the proliferation of IL-2-stimulated CD4+CD25+ regulatory T cells. Mechanism studies revealed that programmed death receptor-1 ligand 1 (PD-L1) expressed on CB-SC membrane, together with a soluble factor nitric oxide (NO) released by PHA-stimulated CB-SC, not prostaglandin E2 (PGE2) and transforming growth factor-beta1 (TGF-beta1), mainly contributed to the T cell suppression induced by CB-SC, as demonstrated by blocking experiments with a nitric oxide synthase inhibitor (Nomega-nitro-l-arginine, l-NNA) and a neutralizing antibody to PD-L1. Our findings may advance our understanding of the immunobiology of stem cells and facilitate the therapeutic application of cord blood stem cells.

Zheng, Y., A. Sun, et al. (2005). "Stem cell factor improves SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem/progenitor cells in xenotransplanted NOD/SCID mouse model." *Bone Marrow Transplant* **35**(2): 137-42.

Poor in vivo homing capacity of hematopoietic stem/progenitor cells (HS/PCs) from umbilical cord blood (UCB) can be reversed by short-term ex vivo manipulation with recombinant human stem cell factor (rHuSCF). This study was designed to evaluate the effect of ex vivo manipulation of UCB-derived HS/PCs with rHuSCF on human cell engraftment rates in xenotransplanted NOD/SCID mouse model. The human cell engraftment rates in xenotransplanted primary and secondary NOD/SCID mice were characterized using four-color flow cytometric analysis and progenitor assay. Grafts of rHuSCF-treated UCB CD34(+) cells resulted in significantly higher levels of human cell engraftment than that of nontreated ones in both xenotransplanted primary and secondary NOD/SCID recipients. Fresh UCB CD34(+) cells did not express either of the matrix metalloproteinase (MMP) family members MMP-2 or MMP-9. rHuSCF-treated UCB CD34(+) cells expressed significant levels of MMP-2 and MMP-9. Pretreatment of UCB CD34(+) cells with the specific MMP inhibitor completely blocked human cell engraftment in xenotransplanted NOD/SCID recipients. Our results indicate that ex vivo manipulation of human HS/PCs with rHuSCF might provide an optimal approach to develop more effective stem cell-based therapies in situations where engraftment is delayed due to limiting HS/PCs number, for example, UCB transplantation.

Zheng, Y., N. Watanabe, et al. (2003). "Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their

transmigratory potential." *Exp Hematol* **31**(12): 1237-46.

OBJECTIVE: The cause of delayed hematopoietic reconstitution after umbilical cord blood transplantation (UCBT) remains controversial. We hypothesized that hematopoietic stem/progenitor cells (HS/PCs) from UCB have some defects of the homing-related molecules responsible for their slow engraftment. UCB-derived CD34(bright) cells expressed significantly lower levels of CD49e, CD49f, and CXCR-4 than their mPB and BM counterparts. CD34+ cells from UCB (and BM) exhibited significantly lower ex vivo transmigration than those from mPB, which were largely blocked by neutralizing antibodies to CD49e or CD49f. Recombinant human tumor necrosis factor-alpha treatment enhanced ex vivo transmigration of CD34+ cells from UCB and BM by inducing expression of the matrix metalloproteinases MMP-2/MMP-9. Short-term treatment of UCB-derived CD34+ cells with rHu-stem cell factor (rHuSCF) up-regulated levels of the homing-related molecules with their increased ex vivo transmigratory and in vivo homing potential. **CONCLUSION:** Our results indicate that disadvantageous transmigratory behavior of HS/PCs from UCB, which might partly explain the delayed reconstitution after UCBT, can be reversed by ex vivo manipulation with rHuSCF.

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