Umbilical Cord Blood Stem-Cell Transplantations

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One of the biggest concern in blood transplantations is the graft-versus-host disease (GVHD) because its chronicity is a leading cause of morbidity and mortality. In this regard, umblical cord blood transplantations (UCBT) is a preferable source of donor hematopoietic stem cells (HSCs) compared to bone marrow transplantation (BMT). In this report, the authors provide strategies to expand umbilical stem cells and enhance efficacy of transplantation into indicated patients with chronic diseases (e.g. cancers, non malignant hemoglobinopathies).

Keywords: Umbilical stem cells ; Blood cord transplantation ; Hematopoietic stem cell transplantation ; graft-versus-host disease ; Immunity ; Hemoglobinopathies ; Cancers ; Cord Blood Stem Cell Expansion ; Cord Blood Stem Cell Engraftment ; Graf rejection and HLA match

1. Introduction

Umbilical cord blood (UCB) availability as a prospect for therapeutic use was first reported in the British journal, *Lancet*, in 1939 [1]. The proposed use was transfusional, but outside of the neonatology clinic, the concept was slow to be accepted, with standard adult blood transfusions being more available. Many years passed before E. Donnall Thomas eventually achieved bone marrow transplantation (BMT) in the 1950s, leading to his later Nobel Prize. Along with this clinical milestone, became a slow but growing awareness that UCB might also be of interest, but it was not until the 1970s when the medical brothers Ende published the transplantation of multiple units of UCB into an individual [2]. Sadly, this procedure was not successful, most likely because of the complications related to the multiple immunology disparities of the transplant units. However, the procedure did start a new move to investigate cord blood on a more serious level.

Eventually, in 1988, successful transplant for bone marrow replacement of a sibling with Fanconi's anaemia was achieved and then published in 1989 [3]. The growth of this possibility to use what is one of the largest cellular sources available on the planet, but normally discarded, was an exciting move which has now led to UCB being considered an attractive alternative source of donor hematopoietic stem cells (HSCs) in the treatment of both recurrent or refractory malignant hematologic disorders (e.g., leukemia, lymphoma) and nonmalignant blood diseases (e.g., thalassemia, sickle cell disease) [4][5][6]. Indeed, since its successful initial use in 1988, umbillical cord blood transplantation (UCBT), particularly allogeneic-UCBT, from both related and unrelated donors, is increasingly used worldwide to treat patients, mostly pediatrics, with either malignant or nonmalignant disorders [3][7][8][9]. To date, over 20.000 transplantation procedures have been performed from unrelated donor UCB units, and more than 450.000 UCB units have been collected and banked by approximately 50 public cord blood banks worldwide [4][10][11][12].

Globally, UBCT presents the following advantages over BMT [4][11][12][13][14]: (i) lower incidence and lower severity of acute and chronic graft-versus-host disease (GVHD), a leading cause of morbidity and mortality; (ii) possibility of extending the number of HLA-antigen mismatches to 1 to 2 of the 6 HLA loci currently considered in UCB transplantation; (iii) lower risk of transmitting latent virus infections (e.g., cytomegalovirus, Epstein-Barr virus, hepatitis viruses, human immunodeficiency virus); (iv) elimination of clinical risk to the donor during hematopoietic stem cell procurement procedures; (v) higher frequency of rare HLA haplotype representation in the donor pool; (vi) a rapid tempo of immune reconstitution. However, these advantages are balanced by two main disadvantages compared to BMT [4][11][12][13][15]: (i) higher risk of graft rejection because of possible translation of the naive immune system into a blunted allogeneic effect elicited by donor T lymphocytes (i.e., immunologic barriers to engraftment); (ii) delayed hematopoietic recovery after transplantation, due to a reduced number of hematopoietic progenitor cells that can further contribute to serious infections.

Interestingly, children with nonmalignant disorders experienced a higher rate of graft rejection after UBCT compared with children suffering from a malignant disorder [16][17][18]. The reason(s) of such difference might be linked to [8][19][20][21][22][23]: (i) the T-cell depletion, (ii) the total nucleated cell (TNC) dose along with the colony-forming unit (CFU) activity and $CD34^+$ cells (HSC) which has a profound impact on engraftment, transplant-related complications (infection risk, survival), (iii) the

degree of HLA mismatching (i.e., recipients who had greater than 2 HLA mismatches, assessed by low-resolution HLA typing methods at HLA-A and HLA-B loci and by high-resolution at HLA-DRB1, experienced the worst outcomes). The later has a great impact on the incidence and severity of GVHD, engraftment (i.e., neutrophil and platelet count recovery), as well as survival.

Conversely, it was shown that increasing the cell dose of HSC to over 3.5×10^7 TNC/kg could partially overcome those negative consequences, especially if the patients experienced previous autologous stem cell transplantations [10][13]. Nevertheless, in adult recipients, the cell dose constitutes the major limitation which is difficult to overcome if less than two UCB units are used. Indeed, the use of two UCB units, preceded by the application of a reduced intensity preparative regimen, facilitated engraftment and mitigated the difficulties associated with delayed or nonengraftment [24][25][26][27][28].

Eventually, related CBT offers a good probability of success (e.g., possible low occurrence of transplant-related complications and transplant-related mortality (TRM)) as it is mainly associated with a low risk of GVHD [11][12][13][14][29].

2. Hematopoietic Stem Cell Transplantation from Different Sources: Advantages and Disadvantages

2.1. Matched Unrelated Versus Umbilical Cord Blood or Haploidentical Transplantation

Hematopoietic stem cell transplantation (HSCT) is a potentially curative option for many cases of hematologic nonmalignant or malignant diseases such as thalassemia major and acute leukemia. Applicability of HSCT is dependent on the presence of suitable hematopoietic stem cell donor. Unfortunately, many patients do not have suitable HLA match donor in family. Therefore, finding an alternative donor is crucial for such cases. The diversity of HLA antigens in community subsequently led to study several alternative sources HSCT such as the use of (i) unrelated donors bone marrow or peripheral blood hematopoietic stem cells, (ii) cord blood stem cells, (iii) finding a donor between extended family (especially in societies with high rate of consanguitniy in marriage), (iv) unrelated mismatch donor, and (v) haploidentical stem cell donor from a family member [30].

Each modality has its own advantages and disadvantages depending on the source of stem cells. Stem cells from live donor is well studied and shows good results when related HLA match donor HSCT is employed [31][32]. Some advantages are associated with this modality such as (i) potential availability of the donor for further therapeutic maneuvers such as donor lymphocyte infusion (DLI)/boster cell doses or even retransplantation, in case of rejection or relapse, (ii) enough cell doses can be harvested for a successful and safe HSCT, (iii) high chance of finding a suitable donor, especially between white Caucasian race because of more advanced unrelated donor registries and highest number of donors in this population. Nevertheless, those advantages are balanced by some disadvantages such as (i) difficulty of finding a donor between ethnic minorities, (ii) great time consumption (average of 3 months) to find and prepare a donor for HSCT, (iii) unavailability of a potential donor due to personal donor problems, (iv) severe GVHD in case of HLA mismatches, usually greater than 2, (v) high cost of the overall procedure which limit its use in some countries financially limited.

Umbillical cord blood stem cells (UCBSCs) were extensively studied [3][10][33][34][35][36] and constitute an acceptable source of cells for permanent engraftment after transplantation. Further, they can elicit graft versus host/leukemia effect. UCBT has also its own advantages and disadvantages. Usually, there is a waste product of pregnancy deliveries, and so, UCBSCs represent valuable sources for preserving lives. The main advantages of this modality are related to (i) their easy and immediate availability [37], minimizing donor-related problems, (ii) their low risk of GVHD, thus allowing some acceptable degree of HLA mismatch [10][33][34][35], (iv) their greater expansion and division potential than adult cells that makes the use of one Log cell dose lower than adult cells acceptable for a successful transplantation [38], (v) their nature as immunological naïve cells that might explain lower immunological complications than adult stem cells after UCBT [39][40][41]. Disadvantages of UCBSCs for transplantation often concern (i) their harvesting limitation that may be lower than the minimum necessary cells dose for a suitable engraftment, especially in adults with larger body mass [10][33][34][35], (ii) availability of donors for further therapeutic maneuvers such as DLI and, so, in case of rejection/relapse, fewer therapeutic options remain. One of the major disadvantages of UCBT is the delayed engraftment which predisposes patients to severe infectious complications after transplantation [10][33][34][35]. Finally, the cost of harvesting and preserving in frozen condition UCBSCs for several years is high and is not favorable for financially poor patients.

Considering the advantages of HSCT, the improvement of transplantation methods, the better knowledge of transplantation immunology, the development of more potent immunosuppressive drugs and antibiotics, the greater experience with mismatch transplantation as well as the possibility of stem cell purification in clinical setting, UCBSCs transplantation rose as a valuable therapeutic option. This option is generally used from family donor with similarity in HLA antigens in one haplotype [42][43][44]. This is possible by (i) using induction of greater immunosuppression in recipient to

prevent from graft rejection and severe GVHD, (ii) purifying HSCs before HSCT and depletion of alloreactive T cells before transplantation, which can be performed by *ex vivo* T cell depletion or *in vivo* T cell reduction by T cell directed monoclonal antibodoies [45] or cyclophosphamide [46], and (iii) using higher cell doses (or even mega cell doses) to prevent rejection of transplanted cells by persistent recipient immunity [44].

Advantages of haploidentical transplantation are obvious. They include (i) universally availability of sibling donors (i.e., parents) for every therapeutic maneuvers (e.g., DLI or retransplant), (ii) short time for finding a suitable donor, (iii) great immunologic reactions against leukemic cells [47], (iv) acceptable cost which is very important for countries with limited financial resources. The disadvantages of haploidentical transplantation include (i) great possibility of rejection, due to preserved recipient immune system or severe GVHD and, (ii) high rate of infectious complications, [48] or posttransplantation secondary malignancies, because of greater and longer immunosupression necessary for prevention of immunological reactions and rejection, (iii) lesser knowledge and experience to manage the eventual complications associated to this procedure.

Although HSCT performed from all of these sources, there are few studies that compare between these modalities. Because of lack of enough evidence for comparison of these modalities, decision making for patients and choosing one of these options remain difficult.

3. Approaches to Improve Cord Blood Stem Cell Expansion and Engraftment

Increased cell dose and improved homing are two major concerns prevailing in efforts to overcome engraftment delay following UCBT [27]. There is a strong association between these strategies to reconstitute hematopoetic system after UCBT which are discussed here. There are many unknown aspects about the interaction of hematopoietic components. However, designing *ex-vivo* experiments based on *in vivo* conditions shall naturally lead to more findings. Expansion of UCB-HSCs is an approach to increase cell dose and make UCB-HSC applicable for adult transplantation. *Ex vivo* expansion is performed through various ways: modifications in liquid culture, stromal coculture, and perfusion in bioreactors [49]. Reduced intensity conditioning (RIC) regimens, double cord blood transplantation, direct intra-BM injection of CB grafts, notch ligand expansion, as well as SDF-1/CXCR4 targeting represent new promising approaches to shorten CBT engraftment time [12].

3.1. Cytokine-Mediated Expansion

A wide variety of cytokine cocktails, growth factors, or other biological mediators in liquid culture have been assessed. Cytokines such as stem cell factor (SCF), interleukin (IL)-3, IL-6 and granulocyte colony-stimulating factor (G-CSF), thrombopoietin (TPO), and Flt-3 ligand (FL) have been extensively used with various dose or culture length [50]. However, the heterogeneity of CB samples and experimental conditions causes inconsistency among results and there is no specific growth factor cocktail that is universally applicable. Recently, a two-step expansion system proposed by McNiece et al. [51] yielded more than 400-fold increase in TNC and 20-fold increase in $CD34^+$cells, which is more effective than single step expansion [52]. Cytokine-based expansion has not proved any definitive evidence for stem cell expansion for clinical purposes.

3.2. Neuropeptides

The complex hematopoiesis network consists of nonhematopoietic cells, hematopoietic cells, as well as various ranges of biological mediators such as hormones, cytokines, and neurotransmitters. However, until recently, enough evidence regarding the role of neuropeptides on UCB $CD34^+$ cells was not available. Research had indicated that inclusion of biological mediators other than cytokines, such as neuropeptides would be valuable for optimization of UCB-HSC *ex vivo* expansion and shortening engraftment time [53]. Accordingly, once the role of substance P (SP) and calcitonin-gene-related neuropeptides (CGRP) on the expansion of UCB $CD34^+$ cells was investigated [54], results showed maximum expansion in 10^{-9} M of neuropeptides in short time culture. Synergistic and antagonistic effects of both SP and CGRP were dominant at 10^{-9} M and 10^{-7} M dose on total nucleated cells and $CD34^+$ $CD38^-$ cells, respectively [54]. Interestingly, concentration 10^{-9} M of SP leds to optimal production of SCF and IL1 in BM stroma [55]. It seems that the proliferation of immuohematopoietic cells resulted as consequence of these interactions. Based on these preliminary findings, identifying further neuropeptide and UCB-HSC interactions would be helpful to achieve an optimum growth factor cocktail for expansion.

3.3. Coculture and Coinfusion with Stromal Cells

Growth factor cocktails use in *ex vivo* expansion partially compensates lack of natural hematopoietic microenvironment. Mesenchymal stem cells (MSCs)/stromal coculture is an optional modification to resemble the hematopoietic microenvironment. Coinfusion of MSCs—which is suitable for immunomodulation and prevention of GVHD—and employment of HSCs is another potential strategy to facilitate engraftment. Furthermore, immunomodulatory properties of MSCs make them a desirable cell for this purpose. There is little controversial evidence about UCB-derived MSCs and most experiments are performed on marrow-derived cells. Hematopoetic engraftment is supported by MSC through neurogenic and angiogenic mechanisms. Therefore, it has been proposed that coinfusion of MSC and hematopoietic cells accelerate engraftment of UCB [50][56].

3.4. Tetraethylenepentamine- (TEPA-) Mediated Expansion

Reduction of free copper content and oxidative stress level of HSCs is the main suggested reason for induction of *ex-vivo* expansion of HSCs by tetraethylenepentamine (TEPA) treatment. An increase of 89-fold in $CD34^+$ cells was achieved by polyamine copper chelator, -TEPA-in Peled et al. experiment [57]. TEPA mediated expansion studies are in phase I/II clinical trials [12].

3.5. Notch Ligand-Based Expansion

Notch-1 gene expressed in $CD34^+$ hematopoietic precursor cells is involved in self-renewal of repopulating cells. For expansion in static culture an immobilized, engineered notch ligand Delta1 with cytokine cocktail (SCF, FL, IL-6, TPO, and IL-3) was investigated in experiment [58]. Immobilized notch ligand results in improved immune reconstitution and enhanced cell number and phase I/II clinical trials are underway. Delaney et al. showed that coinfusion of unmanipulated UCB and notch-mediated *ex vivo* expanded UCB had faster neutrophil engraftment, 16 days, compared to infusion of unmanipulated double UCBT, which took 26 days [58]. More clinical trials are required to support these results.

3.6. Adhesion Molecules for HSC Homing

Adhesion molecules are involved in the regulation of survival, proliferation and differentiation of progenitor cells. This might occur through interaction with microenvironment components [59] and biological mediators such as cytokines, chemokines, and neuropeptides. Secretion of stromal-derived factor (SDF)-1 by BM stromal cells is crucial for retention/homing of HSC in BM [26]. Additionally, involvement of this axis in survival and proliferation of HSCs has been shown [12]. For HSC engraftment, CXCR4 response to SDF1 and SDF-1 expression in BM microenvironment is important [26]. To improve homing of HSC following CBT, several approaches have been considered. Inhibition of enzymatic activity of CD26/Dipeptidylpeptidase IV (DPPIV) avoids truncation of SDF-1/CXCL12-exclusive ligand for CXCR4, and consequently results in acceleraed UCB-HSC engraftment. Additionally, in order to increase the responsiveness of SDF1/CXCR4, *ex vivo* priming of HSCs prior to transplantation with small molecules including C3 complement fragments, fibronectin, fibrinogen, and hyaluronic acid has been suggested to improve homing/engraftment of UCB-HSCs [26].

Recently, SP and CGRP neuropeptide treated CB stem cells showed increased percentage of $CD34^+$/CXCR4 [60], CD49e, and CD44 [61] subsets in neuropeptide-cytokine treated cells compared to cytokine-treated cells in short time culture, as well as a resistance to frequency decline. Accordingly, since actions of neuropeptides on hematopoeisis are less known, more investigation to clarify underlying mechanisms is required.

4. Conclusion

It is difficult to compare older transplantation outcome reports with more recent studies (i.e., comprehensive metaanalysis) because of changes mainly related to (i) stem cell sources (UCB unit characteristics), (ii) year of transplantation, (iii) time from diagnosis to transplantation, (iv) disease stage, (v) methodology of HLA-typing, (vi) conditioning regimen formulation, and (vii) standard of the cell dose that must be available in a single UCB unit to be infused.

However, UCBT offers an attractive alternative to BMT, in particular because of the low incidence of GVHD. Indeed, although UBCT is associated with a greater risk of graft rejection, due in part to a restricted number of hematopoietic stem cells, nevertheless, this risk can be overcome in part by selecting UCB units that contain a large number of cells and those that are closely matched at the HLA loci.

Alternatively, the use of double UCBT from unrelated donors or the potential collection of HSCs from human placenta might be useful approaches to optimize the donor hematopoietic stem cell content. Interestingly, recent results show excellent outcomes after HLA-identical sibling UCBT, stressing the importance of collecting cord blood in families when a

child is affected by blood disorders. Eventually, recent studies reported that combination of UCB unit collected after a sibling birth with a marrow harvested from the same donor presented excellent results exerted by both low rates of GVHD and graft rejection. Most recent studies aim to optimize UCBT and promising results were obtained once the cell dose was increased and the homing improved taking into consideration several microenvironmental factors (e.g., cytokines, neuropeptides) and cells (e.g., mesenchymal stem cells).

The field of human hematotherapy was transformed with the advent of bone marrow replacement and augmented by the application of umbilical cord blood units. The increasing number of cord blood banks around the world makes sourcing of units an increased potential and has begun to slowly outweigh the need for bone marrow registries. Despite this, the costs involved are still unaffordable to many countries, not least in developing nations. Changes in the processing procedures, our knowledge of the true content of cord blood from children of different backgrounds, and from mothers of different ages and heatlh status, and the advent of new technologies will hopefully make availability of umbilical cord blood transplantation a reality in every nation in the future.

References

1. J. Halbrecht, “Transfusion with placental blood,” The Lancet, vol. 233, no. 6022, pp. 202–203, 1939.
2. M. Ende and N. Ende, “Hematopoietic transplantation by means of fetal (cord) blood. A new method.,” Virginia Medical Monthly, vol. 99, no. 3, pp. 276–280, 1972.
3. E. Gluckman, H. E. Broxmeyer, A. D. Auerbach et al., “Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling,” New England Journal of Medicine, vol. 321, no. 17, pp. 1174–1178, 1989.
4. H. E. Broxmeyer and F. O. Smith, “Cord blood hematopoietic cell transplantation,” in Thomas' Hematopoietic Cell Transplantation, pp. 559–576, Wiley-Blackwell, Malden, Mass, USA, 2009.
5. R. Hough and V. Rocha, “Transplant outcomes in acute leukemia (II),” Seminars in Hematology, vol. 47, no. 1, pp. 51–58, 2010.
6. M. L. MacMillan, M. C. Walters, and E. Gluckman, “Transplant outcomes in bone marrow failure syndromes and hemoglobinopathies,” Seminars in Hematology, vol. 47, no. 1, pp. 37–45, 2010.
7. J. E. Wagner, N. A. Kernan, M. Steinbuch, H. E. Broxmeyer, and E. Gluckman, “Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease,” The Lancet, vol. 346, no. 8969, pp. 214–219, 1995.
8. E. Gluckman, V. Rocha, A. Boyer-Chammard et al., “Outcome of cord-blood transplantation from related and unrelated donors. Eurocord transplant group and the European blood and marrow transplantation group,” New England Journal of Medicine, vol. 337, no. 6, pp. 373–381, 1997.
9. F. Locatelli, V. Rocha, C. Chastang et al., “Factors associated with outcome after cord blood transplantation in children with acute leukemia,” Blood, vol. 93, no. 11, pp. 3662–3671, 1999.
10. V. Rocha and E. Gluckman, “Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors,” British Journal of Haematology, vol. 147, no. 2, pp. 262–274, 2009.
11. H. E. Broxmeyer, “Umbilical cord transplantation: epilogue,” Seminars in Hematology, vol. 47, no. 1, pp. 97–103, 2010.
12. V. Rocha and H. E. Broxmeyer, “New approaches for improving engraftment after cord blood transplantation.,” Biology of Blood and Marrow Transplantation, vol. 16, supplement 1, pp. S126–S132, 2010.
13. G. Cohen, S. L. Carter, K. I. Weinberg et al., “Antigen-specific T-lymphocyte function after cord blood transplantation,” Biology of Blood and Marrow Transplantation, vol. 12, no. 12, pp. 1335–1342, 2006.
14. V. Rocha and F. Locatelli, “Searching for alternative hematopoietic stem cell donors for pediatric patients,” Bone Marrow Transplantation, vol. 41, no. 2, pp. 207–214, 2008.
15. M. Eapen, P. Rubinstein, M. J. Zhang et al., “Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study,” The Lancet, vol. 369, no. 9577, pp. 1947–1954, 2007.
16. V. K. Prasad and J. Kurtzberg, “Umbilical cord blood transplantation for non-malignant diseases,” Bone Marrow Transplantation, vol. 44, no. 10, pp. 643–651, 2009.
17. E. Gluckman, V. Rocha, I. Ionescu et al., “Results of unrelated cord blood transplant in Fanconi anemia patients: risk factor analysis for engraftment and survival,” Biology of Blood and Marrow Transplantation, vol. 13, no. 9, pp. 1073–

1082, 2007.

18. A. Willasch, W. Hoelle, H. Kreyenberg et al., “Outcome of allogeneic stem cell transplantation in children with non-malignant diseases,” Haematologica, vol. 91, no. 6, pp. 788–794, 2006.
19. G. Michel, V. Rocha, S. Chevret et al., “Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis,” Blood, vol. 102, no. 13, pp. 4290–4297, 2003.
20. P. Rubinstein, C. Carrier, A. Scaradavou et al., “Outcomes among 562 recipients of placental-blood transplants from unrelated donors,” New England Journal of Medicine, vol. 339, no. 22, pp. 1565–1577, 1998.
21. J. E. Wagner, J. N. Barker, T. E. DeFor et al., “Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival,” Blood, vol. 100, no. 5, pp. 1611–1618, 2002.
22. V. Rocha, G. Sanz, and E. Gluckman, “Umbilical cord blood transplantation,” Current Opinion in Hematology, vol. 11, no. 6, pp. 375–385, 2004.
23. E. Gluckman, V. Rocha, W. Arcese et al., “Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice,” Experimental Hematology, vol. 32, no. 4, pp. 397–407, 2004.
24. C. G. Brunstein, J. N. Barker, D. J. Weisdorf et al., “Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease,” Blood, vol. 110, no. 8, pp. 3064–3070, 2007.
25. J. N. Barker, D. J. Weisdorf, T. E. DeFor et al., “Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy,” Blood, vol. 105, no. 3, pp. 1343–1347, 2005.
26. C. Delaney, M. Z. Ratajczak, and M. J. Laughlin, “Strategies to enhance umbilical cord blood stem cell engraftment in adult patients,” Expert Review of Hematology, vol. 3, no. 3, pp. 273–283, 2010.
27. A. Stanevsky, A. Shimoni, R. Yerushalmi, and A. Nagler, “Cord blood stem cells for hematopoietic transplantation,” Stem Cell Reviews and Reports, vol. 7, no. 2, pp. 425–433, 2011.
28. J. A. Gutman, S. R. Riddell, S. McGoldrick, and C. Delaney, “Double unit cord blood transplantation: who wins-and why do we care?” Chimerism, vol. 1, no. 1, pp. 21–22, 2010.
29. V. Rocha, J. E. Wagner, K. A. Sobocinski et al., “Graft-versus-host disease in children who have received a cord blood or bone marrow transplant from an HLA-identical sibling,” New England Journal of Medicine, vol. 342, no. 25, pp. 1846–1854, 2000.
30. H. M. Lazarus, “Acute leukemia in adults: novel allogeneic transplant strategies,” Hematology, vol. 17, supplement 1, pp. 47–51, 2012.
31. M.-J. Zhang, S. M. Davies, B. M. Camitta, B. Logan, K. Tiedemann, and M. Eapen, “Comparison of outcomes after HLA-matched sibling and unrelated donor transplantation for children with high-risk acute lymphoblastic leukemia,” Biology of Blood and Marrow Transplantation, vol. 18, no. 8, pp. 1204–1210, 2012.
32. L. E. Perez, “Outcomes from unrelated donor hematopoietic stem cell transplantation,” Cancer Control, vol. 18, no. 4, pp. 216–221, 201
33. M. Eapen, V. Rocha, G. Sanz et al., “Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis,” The Lancet Oncology, vol. 11, no. 7, pp. 653–660, 2010.
34. M. J. Laughlin, M. Eapen, P. Rubinstein et al., “Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia,” New England Journal of Medicine, vol. 351, no. 22, pp. 2265–2275, 2004.
35. J. Kurtzberg, M. Laughlin, M. L. Graham et al., “Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients,” New England Journal of Medicine, vol. 335, no. 3, pp. 157–166, 1996.
36. A. Ghavamzadeh, K. Alimoghaddam, A. Naderi et al., “Outcome of related and unrelated cord-blood transplantation in children at hematopoietic stem cell transplantation research center of Shariati Hospital,” International Journal of Hematology-Oncology and Stem Cell Research, vol. 3, no. 1, pp. 17–20, 2009.
37. J. N. Barker, T. P. Krepski, T. E. DeFor, S. M. Davies, J. E. Wagner, and D. J. Weisdorf, “Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow,” Biology of Blood and Marrow Transplantation, vol. 8, no. 5, pp. 257–260, 2002.
38. H. E. Broxmeyer, E. Gluckman, A. Auerbach et al., “Human umbilical cord blood: a clinically useful source of transplantable hematopoietic stem/progenitor cells,” International Journal of Cell Cloning, vol. 8, supplement 1, pp. 76–91, 1990.
39. S. J. Deacock, A. P. Schwarer, J. Bridge, J. R. Batchelor, J. M. Goldman, and R. I. Lechler, “Evidence that umbilical cord blood contains a higher frequency of HLA class II-specific alloreactive T cells than adult peripheral blood: a limiting

dilution analysis," Transplantation, vol. 53, no. 5, pp. 1128–1134, 1992.

40. M. G. Roncarolo, M. Bigler, E. Ciuti, S. Martino, and P. A. Tovo, "Immune responses by cord blood cells," Blood Cells, vol. 20, no. 2-3, pp. 573–586, 1994.
41. A. Bensussan, E. Gluckman, S. El Marsafy et al., "BY55 monoclonal antibody delineates within human cord blood and bone marrow lymphocytes distinct cell subsets mediating cytotoxic activity," Proceedings of the National Academy of Sciences of the United States of America, vol. 91, no. 19, pp. 9136–9140, 1994.
42. X. J. Huang, D. H. Liu, K. Y. Liu et al., "Treatment of acute leukemia with unmanipulated HLA-mismatched/haploidentical blood and bone marrow transplantation," Biology of Blood and Marrow Transplantation, vol. 15, no. 2, pp. 257–265, 2009.
43. F. Ciceri, M. Labopin, F. Aversa et al., "A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: a risk factor analysis of outcomes for patients in remission at transplantation," Blood, vol. 112, no. 9, pp. 3574–3581, 2008.
44. F. Aversa, A. Terenzi, A. Tabilio et al., "Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse," Journal of Clinical Oncology, vol. 23, no. 15, pp. 3447–3454, 2005.
45. E. Naparstek, M. Delukina, R. Or et al., "Engraftment of marrow allografts treated with Campath-1 monoclonal antibodies," Experimental Hematology, vol. 27, no. 7, pp. 1210–1218, 1999.
46. T. B. Prigozhina, O. Gurevitch, and S. Slavin, "Nonmyeloablative conditioning to induce bilateral tolerance after allogeneic bone marrow transplantation in mice," Experimental Hematology, vol. 27, no. 10, pp. 1503–1510, 1999.
47. L. Ruggeri, A. Mancusi, M. Capanni et al., "Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value," Blood, vol. 110, no. 1, pp. 433–440, 2007.
48. K. Perruccio, A. Tosti, E. Burchielli et al., "Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation," Blood, vol. 106, no. 13, pp. 4397–4406, 2005.
49. Y. Liao, M. B. Geyer, A. J. Yang, and M. S. Cairo, "Cord blood transplantation and stem cell regenerative potential," Experimental Hematology, vol. 39, no. 4, pp. 393–412, 2011.
50. S. S. Tung, S. Parmar, S. N. Robinson, M. De Lima, and E. J. Shpallemail, "Ex vivo expansion of umbilical cord blood for transplantation," Best Practice & Research Clinical Haematology, vol. 23, no. 2, pp. 245–257, 2010.
51. I. McNiece, D. Kubegov, P. Kerzic, E. J. Shpall, and S. Gross, "Increased expansion and differentiation of cord blood products using a two-step expansion culture," Experimental Hematology, vol. 28, no. 10, pp. 1181–1186, 2000.
52. E. J. Shpall, R. Quinones, R. Giller et al., "Transplantation of ex vivo expanded cord blood," Biology of Blood and Marrow Transplantation, vol. 8, no. 7, pp. 368–376, 2002.
53. M. Ebtekar, S. Shahrokhi, and K. Alimoghaddam, Characteristics of Cord Blood Stem Cells: Role of Substance P, (SP) and Calcitonin Gene-Related Peptide (CGRP), Stem Cells and Cancer Stem Cells, vol. 2, Springer Netherlands, Heidelberg, Germany, 2012.
54. S. Shahrokhi, M. Ebtekar, K. Alimoghaddam et al., "Substance P and calcitonin gene-related neuropeptides as novel growth factors for ex vivo expansion of cord blood CD34+ hematopoietic stem cells," Growth Factors, vol. 28, no. 1, pp. 66–73, 2010.
55. P. Rameshwar, D. Ganea, and P. Gascon, "In vitro stimulatory effect of Substance P on hematopoiesis," Blood, vol. 81, no. 2, pp. 391–398, 1993.
56. D. McKenna and J. Sheth, "Umbilical cord blood: current status and promise for the future," Indian Journal of Medical Research, vol. 134, pp. 261–269, 2011.
57. T. Peled, J. Mandel, R. N. Goudsmid et al., "Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine," Cytotherapy, vol. 6, no. 4, pp. 344–355, 2004.
58. C. Delaney, S. Heimfeld, C. Brashem-Stein, H. Voorhies, R. L. Manger, and I. D. Bernstein, "Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution," Nature Medicine, vol. 16, no. 2, pp. 232–236, 2010.
59. C. M. Verfaillie, "Adhesion receptors as regulators of the hematopoietic process," Blood, vol. 92, no. 8, pp. 2609–2612, 1998.
60. S. Shahrokhi, M. Ebtekar, K. Alimoghaddam et al., "Communication of substance P, calcitonin-gene-related neuropeptides and chemokine receptor 4 (CXCR4) in cord blood hematopoietic stem cells," Neuropeptides, vol. 44, no.

5, pp. 385–389, 2010.

61. S. Shahrokhi, K. Alimoghaddam, M. Ebtekar et al., “Effects of neuropeptide substance P on the expression of adhesion molecules in cord blood hematopoietic stem cells,” Annals of Hematology, vol. 89, no. 12, pp. 1197–1205, 2010.