Chapter 14
Cord Blood *Ex Vivo* Expansion

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1 Introduction

Over the last three decades, umbilical cord blood (UCB) has become a frequently used source of hematopoietic stem cells (HSCs) for transplantation. The rapid growth in the rates of UCB transplantation (UCBT) is a reflection of the advantages this modality has over bone marrow (BM)- or peripheral blood (PB)- sourced grafts including rapid availability of stored UCB units, decreased rates of acute and chronic graft-versus-host disease (GVHD), and less stringent histocompatibility requirements. These characteristics have established UCBT as an alternative for patients who lack a suitable matched related donor (MRD) [1].

The main disadvantage of UCB is the presence of delayed engraftment kinetics and a higher rate of engraftment failure than BM transplants (BMT) or PB transplants (PBT). Initial reports of pediatric matched sibling UCBT showed primary graft failure occurred in 15 % of patients [2]. In a matched-pair analysis of pediatric unrelated UCBT and human leukocyte antigen (HLA)-matched unrelated BMT, Barker and colleagues [3] observed neutrophil engraftment was significantly delayed after UCBT although the rates of primary graft failure were not statistically different. Subsequent pediatric studies confirmed that UCBT was associated with delayed engraftment and increased early transplant-related mortality (TRM; [4, 5]). The studies that followed clarified the relevance of cell dose and HLA matching on the outcomes of pediatric UCBT. In an observational study including 102 patients undergoing UCBT, grafts with an HLA match of at least 4/6 and a minimum dose of 1.7×10^5 CD34+ cells/kg of recipient body weight had significantly improved rates of survival [6]. Several reports from the Eurocord registry indicated pediatric patients receiving a total nucleated cell (TNC) dose above 3.7×10^7 cells/kg had a higher probability of engraftment and survival [7, 8].

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Early experiences in adult UCBT confirmed the problem of delayed engraftment. In the initial report of UCBT in 68 high-risk hematologic malignancy patients by Laughlin and colleagues [9], primary graft failure among patients surviving more than 28 days was 10 %, but mortality 100 days after transplant was 51 %. A higher TNC dose ($\geq 2.4 \times 10^7$ cells/kg) was associated with improved event-free survival (EFS). Recognition of cell dose as a critical barrier to engraftment and improvements in supportive care have resulted in major improvements in adult UCBT outcomes. A study performed by the Eurocord and the Center for International Blood & Marrow Transplant Research (CIBMTR) [10] compared outcomes of recipients of unrelated BMT or PBT (7–8/8 HLA matched donors) and UCBT (4–6/6 HLA matched units with a minimum TNC dose of 2.5×10^7 cells/kg). UCBT had slower neutrophil recovery (29 vs. 14, PB, and 19, BM, days, $p = 0.01$), and lower neutrophil engraftment rate by day 42 (80 vs. 96, PB, and 93 %, BM, $p < 0.0001$). TRM was higher with UCBT, while GVHD and relapse rates were lower, leading to comparable leukemia-free survival rates. This report and other studies would suggest that shortening time to hematopoietic engraftment could lead to reduced early TRM and improved overall survival after UCBT.

Adding a second UCB unit (double UCBT, dUCBT), has been investigated as a means to increase cell dose and improve engraftment. Initial studies from the University of Minnesota indicated possibly expedited engraftment (median 23 days, range 15–41) [11]. However, dUCBT appears to lead to a higher incidence of GVHD [12]. In a recent retrospective study, Brunstein and colleagues compared outcomes of dUCBT with those of MRD, matched unrelated donor (MUD), and mismatched unrelated donors [13]. While leukemia-free survival was similar in all four groups, dUCBT was still associated with higher rates of nonrelapse mortality (NRM), slower hematopoietic recovery, and higher incidence of engraftment failure.

UCB is remarkable in that the hematopoietic progenitor cells (HPCs) have a higher proliferative potential than those in the BM [14]. As a result, UCBT leads to engraftment despite CD34 + cell doses that are only 10 % of BM grafts and 5 % of PB grafts [15]. Therefore, the central hypothesis of *ex vivo* expansion strategies is to exploit the high proliferative potential of UCB to increase the cell dose before transplantation. As graft engineering methods continue to advance [15, 16], UCB expansion has become a reality that has reached clinical applications, many of which are currently being tested in clinical trials.

2 Initial *Ex Vivo* Expansion Studies

2.1 Hematopoietic Growth Factors

Shpall and colleagues reported in 2002 the first clinical study of *ex vivo* cord blood expansion [17]. In this trial, a CD34-selected portion of UCB units that were originally frozen in two aliquots was exposed for 10 days to liquid culture conditions with stem cell factor (SCF), granulocyte-stimulating factor (G-CSF) and megakaryocyte

growth and differentiation factor (MGDF). These conditions had been previously reported to lead to 100-fold expansion of myeloid and erythroid colony-forming cells and a 500-fold expansion of megakaryocyte progenitors [18]. A prior study exposing autologous stem cell grafts to these culture conditions had resulted in accelerated neutrophil engraftment [19]. CD34 selection was used due to poor expansion in these conditions when unselected UCB cells were used. The expanded portion was either co-infused with unmanipulated UCB, or given on day 10 after transplantation. Thirty-seven patients (25 adults and 12 children) with hematologic malignancies and breast cancer were treated. The median weight was 61 kg (range 9–116 kg). The median CD34 + cell dose was 10.4×10^4 cells/kg (range $0.97–311 \times 10^4$ CD34 + cells/kg). There was no acute toxicity associated with the infusion of expanded or unmanipulated UCB. Four patients died of disseminated fungal infections before day 28, whereas three additional patients died with extensive BM relapse and pancytopenia on days 41, 51, and 78. None of the remaining patients ($n = 30$) had engraftment failure. The median time to neutrophil engraftment was 28 days. Subjects receiving a cell dose higher than 5×10^4 CD34 + cells/kg presented a more rapid time to neutrophil engraftment, but the difference was not statistically different.

A major concern with expansion approaches is that of stem cell exhaustion. It is believed that most current techniques lead to stem cell differentiation and lineage commitment, thereby decreasing "stemness." Shpall and collaborators then hypothesized that using a dUCBT platform in which one unit was 100 % *ex vivo* expanded, combined to an unmanipulated UCB unit, would allow for early engraftment of the expanded unit, while a safety net would be provided by the unmanipulated unit, which was expected to provide long-term engraftment. The authors then performed a randomized study at the MD Anderson Cancer Center comparing infusion of two unmanipulated UCB units (standard dUCBT) versus the combination of one unmanipulated unit and an *ex vivo* expanded unit [20]. The liquid culture system was similar to that employed by Shpall and collaborators in their preliminary work [17]. In this dUCBT platform, CD133 selection was used instead of CD34, due to the possibility of selection of earlier HPCs. In addition, the unselected cell fraction obtained after CD133 selection was frozen after separation and reinfused at the time of transplant. The authors hypothesized that this cell population could contain engraftment-facilitating cells. The liquid culture was performed in two stages, with culture flasks transferred to larger culture bags after 7 days. Forty-eight hematologic malignancy patients were enrolled. The median TNC dose was 0.36×10^8 cells/kg in both cohorts, whereas CD34 + cell dose was 0.16×10^6 and 0.13×10^6 cells/kg in the expanded and unmanipulated cohorts, respectively. There were no statistically significant differences in hematopoietic engraftment or survival between both cohorts. One UCB unit dominated engraftment in all patients; among those receiving expanded units, the unmanipulated unit predominated in all but three subjects. These data suggested that cytokine-based liquid culture systems push progenitor cells towards a more differentiated stage of maturation, depleting the stem cell potential.

2.2 Coculture with Mesenchymal Stem Cells

The proliferation and differentiation of HPCs is regulated by microenvironmental signals and interaction with cells from the BM stroma [21–23]. Mesenchymal stromal cells (MSCs) are precursors of mesodermal tissues and give rise to the BM stromal compartment [23]. Preclinical studies demonstrated coculture of UCB with BM-derived MSCs [24] required less graft manipulation (i.e., no CD34 + or CD133 + selection), while at the same time, producing higher TNC and HPC numbers than *ex vivo* liquid culture methods previously investigated. The first clinical trial of infusion of UCB expanded by MSC coculture was recently reported [25]; 31 high-risk hematologic malignancies received two UCB units, one of which had undergone *ex vivo* expansion through MSC coculture. Expansion increased TNC numbers by a median factor of 12.2 and CD34 + cells by a median factor of 30.1, leading to grafts with median TNC dose of 5.8×10^7 cells/kg. The median time to neutrophil and platelet engraftment was 15 and 42 days, respectively. Chimerism studies indicated that the expanded unit provided early engraftment, while the unmanipulated unit was responsible for long-term engraftment in the majority of patients. Only a minority of patients had evidence of long-term expanded-unit hemopoiesis, with unmanipulated unit predominance. These encouraging initial clinical results provided the rationale for an ongoing international randomized multicenter study comparing unmanipulated dUCBT versus dUCBT containing one *ex vivo* expanded MSC cocultured unit and an unmanipulated unit.

2.3 Notch-Mediated Expansion

The Notch gene family encode for transmembrane proteins that participate in cell-cell interactions and have a pivotal role in cell-fate decisions of progenitor cells in several organisms and organ systems. Milner and colleagues demonstrated HPCs express Notch1 (TAN1) [26] and subsequent studies showed that constitutive activation of this gene was capable of establishing pluripotent cytokine-dependent hematopoietic progenitor cell lines [27]. Incubation of murine HPCs with engineered Notch ligand and hematopoietic growth factors resulted in inhibition of differentiation and a several log increase in number of HPCs [28]. Delaney and colleagues demonstrated that incubation of human UCB HPCs with Notch ligand resulted in *ex vivo* expansion by approximately 100-fold, with resultant-improved hematopoietic engraftment when transplanted to immunodeficient mice [29]. In a phase I clinical trial conducted at the Fred Hutchinson Cancer Center, 10 hematologic malignancy patients had myeloablative conditioning (fludarabine, cyclophosphamide, and total body irradiation) followed by infusion of one unmanipulated UCB unit and one *ex vivo*-expanded UCB unit. Notch ligand-mediated expansion resulted in a 164-fold expansion of CD34 + cells and 562-fold expansion of TNC. The median time to neutrophil engraftment was 16 days (range 7–34). One subject failed to engraft. GVHD rates were acceptable, and there were no infusional toxicities. One-year actuarial survival was 70 %.

3 Expansion with Preservation of Long-Term Engraftment Potential

An important limitation to expansion of hematopoietic cell grafts with the methods discussed above is the generation of committed HPCs at the expense of less-differentiated progenitor cells, responsible for long-term hematopoietic recovery. It should be noted that the use of dUCBT platforms preclude a direct evaluation of engraftment potential of expanded cells, but the fact that in most cases the expanded UCB lost the "competition" to the unmanipulated unit suggests poorer long-term engraftment potential or reflect loss of engraftment facilitating cells. Additional developments in graft manipulation have identified methods that could potentially allow HPC expansion without loss of long-term engraftment potential.

3.1 Copper Chelation

Intracellular copper concentration regulates differentiation of HPCs. The copper chelator tetraethylenepentamine (TEPA) reduces intracellular copper concentration and prevents HPC differentiation *in vitro* [30]. Culture of UCB cells with TEPA in conjunction with cytokines increases the numbers of early progenitors without differentiation to less-pluripotent cells. Grafts cultured with TEPA led to improved engraftment in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice [31]. The feasibility of *ex vivo* expansion using tetraethylenepentamine (TEPA) in a liquid *ex vivo* expansion system was investigated in a phase I/II study that included ten advanced hematologic malignancy patients [32]. UCB units that were frozen in two fractions were used, and the smaller fraction was cultured for 21 days in a cytokine-based liquid culture system. The median CD34 + cell increase was sixfold, and the mean times to neutrophil and platelet engraftment were 30 and 48 days, respectively. One patient failed to engraft. Grade II–IV acute GVHD occurred in 44 % of cases, while 100-day survival was 90 %. These results led to the design of a multicenter trial investigating the use of a single UCB unit partially expanded with the copper chelation-based system (Stem Ex ®). Expanded unit transplant outcomes were compared with historical controls receiving double UCBT [33]. One hundred and one patients received expanded UCBT. Median TNC and CD34 + expansion were 400-fold and 77-fold, respectively. The median TNC and CD34 + doses were 2.2×10^7/kg and 9.7×10^5/kg, respectively. One-hundred-day survival after transplantation was significantly higher in patients receiving a single expanded unit (84.2 % vs. 74.6 %, $p = 0.035$), while neutrophil and platelet engraftment were faster in patients who received expanded units: 21 days vs. 28 days ($p < 0.0001$) and 54 days vs. 105 days ($p = 0.008$), respectively.

3.2 Nicotinamide

Nicotinamide is the precursor for nicotinamide adenine dinucleotide (NAD^+), and is a potent inhibitor of enzymes that require NAD^+, including sirtuins, protein/histone deacetylases. SIRT1 is a sirtuin whose deficiency was associated with increase *in vitro* proliferative activity of HPCs [34]. Exposure to nicotinamide has been shown to delay differentiation, while increasing the expansion, migration, and engraftment of *ex vivo* expanded UCB CD34 + cells [35]. The authors reported TNC increase of 400-fold, while CD34 + expanded 80-fold. A phase I/II study of infusion of one UCB unit expanded on a nicotinamide-based system combined with one unmanipulated UCB unit was recently completed [36]. Eleven hematologic malignancy patients received the planned UCB infusion after myeloablative conditioning. Eight of the patients engrafted with the nicotinamide-expanded unit, two with the unmanipulated unit, whereas one patient experienced primary engraftment failure. Median neutrophil engraftment time was 12.5 days (range 7–26), and 10.5 days (range 7–18) for the whole cohort and for patients who had engraftment of the expanded unit, respectively. One-hundred-day mortality was 10 %. Three patients had grade I/II acute GVHD, while none had more severe GVHD. These results suggested that nicotinamide-based *ex vivo* expansion could achieve shortened hematopoietic recovery time with preserved long-term engraftment. A recently opened phase I trial (ClinicalTrials.gov identifier NCT01816230) is investigating the infusion of a single unit, 100 % expanded with this nicotinamide-based system.

3.3 Aryl Hidrocarbon Receptor Antagonist

The purine derivative StemRegenin 1 (SR1) can increase the number of CD34 +, CD133 + and CD90 + stem and progenitor cells when added to cytokines in culture media. SR1 does not stimulate proliferation, and importantly, causes a reversible arrest in differentiation [37]. Expansion with SR1 resulted in higher engraftment rates of human hematopoietic cells in a NOD/SCID mouse model. Boitano and colleagues demonstrated that SR1 binds and inhibits aryl hydrocarbon receptor (AHR), a nuclear receptor implicated in the induction of drug-metabolizing enzymes as well as in the regulation of several pathways involved in hematopoiesis, including c-MYC, CCAAT-enhancer-binding proteins (C-EBP), C-X-C chemokine receptor type 4 (CXCR4), among others [37]. Khan and colleagues have recently reported that hypoxic culture conditions can increase the expansion rates in the presence of SR1 and cytokines [38]. Investigators at the University of Minnesota are conducting a phase I study consisting of infusion of one unmanipulated UCB unit and one CD34- selected, SR1-expanded UCB unit. The median CD34 + expansion was 248-fold (range 66–446). Five of nine patients that engrafted with the expanded unit had faster neutrophil engraftment (16 days vs. 24 days).

3.4 *Dimethyl Prostaglandin E_2*

Screening studies showed that agents that increased prostaglandin E_2 (PGE_2) levels in zebra fish resulted in higher numbers of HSCs [39]. Dimethyl prostaglandin E_2 ($dmPGE_2$), a stable derivative of PGE_2, acts through G-protein-coupled receptors and the second messenger cyclic adenosine monophosphate (AMP) to increase expression of genes involved in homing, proliferation, survival, and self-renewal of HSC. It has been reported that this agent induces expansion and long-term maintenance of the HSC population [40]. Based on these data, the Dana Farber Cancer Institute and Massachusetts General Hospital conducted a phase I clinical trial evaluating the safety of a $dmPGE_2$-treated UCB unit co-transplanted with an unmanipulated unit. In contrast with the trials summarized above, $dmPGE_2$ treatment did not consist of *ex vivo* expansion, but of exposure to this agent for a short period of time after UCB units were thawed. Two cohorts of hematologic malignancy patients were treated. In the first cohort (nine patients), UCB units were incubated with 10 μM of $dmPGE_2$ for 60 min at 4 °C; only two patients presented engraftment of the $dmPGE_2$-treated units and neutrophil engraftment times were not improved (median = 24 days). Based on the lack of improvement in engraftment parameters, the investigators sought to optimize the conditions for *ex vivo* treatment with $dmPGE_2$, by increasing the incubation time to 120 min and the temperature to 37 °C. The second cohort included 12 patients who received one of two units treated under the optimized conditions. Median time to neutrophil engraftment was then 17.5 days (range 14–31), with ten patients engrafting with the $dmPGE_2$-treated UCB unit. Grade I–II infusion reactions were observed in four patients, and one patient presented with grade IV ST segment elevation and myocardial ischemia [41].

4 Limitations and Future Directions of UCB Expansion

UCB graft manipulation is coming of age. It is postulated, but not yet proven in a randomized fashion, that improved engraftment time will lead to decreased TRM, less infections, and shorter hospitalizations. However, graft manipulation could lead to the loss of some of the attributes of UCB that permit its application to patients who do not have a matched family donor, in particular the easy and rapid availability of procurement. The use of the dUCBT platform adds to the overall cost of the transplant, and it remains to be proven that expansion will actually decrease transplant costs by decreasing transfusion rates, or by shortening hospitalization, for example. On the other hand, it is likely that once cell dose and engraftment kinetics barriers are overcome, the main limiting characteristic of UCBT will be slower immune and lymphoid reconstitution and long-term infection risk [42, 43].

Alternative approaches to expedite engraftment are now under active investigation. Improving stem cell homing is an alternate approach being studied in a phase I/II clinical trial. Investigators at the MD Anderson Cancer Center have postulated that fucosylation of UCB cells would lead to better homing and to faster engraftment. Preliminary results are promising [44].

The scientific advances in our understanding of hematopoietic cell proliferation and differentiation have led to the early stages of clinical implementation of multiple UCB graft engineering technologies. However, the majority of the reported clinical trials have been nonrandomized. A large, randomized controlled study comparing unmanipulated dUCBT versus the combination of one MSC-expanded UCB unit and one unmanipulated unit (ClinicalTrials.gov identifier NCT01854567) is ongoing. Therefore, the efficacy of UCB expansion methodologies and their applicability in a large number of patients is yet to be formally determined. Moreover, combinations of multiple expansion and homing methods may yield even better results, but the feasibility and efficacy of such an approach will need to be clarified by rigorous research.

References

1. Barker JN, Byam CE, Kernan NA, et al. Availability of cord blood extends allogeneic hematopoietic stem cell transplant access to racial and ethnic minorities. Biol Blood Marrow Transplant. 2010;16:1541–8.
2. Wagner JE, Kernan NA, Steinbuch M, et al. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. Lancet. 1995;346:214–9.
3. Barker JN, Davies SM, DeFor T, et al. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. Blood. 2001;97:2957–61.
4. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood. 2001;97:2962–71.
5. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. Lancet. 2007;369:1947–54.
6. Wagner JE, Barker JN, DeFor TE, 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. 2002;100:1611–8.
7. Locatelli F, Rocha V, Reed W, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. Blood. 2003;101:2137–43.
8. Gluckman E, Rocha V. Cord blood transplantation for children with acute leukaemia: a Eurocord registry analysis. Blood Cells Mol Dis. 2004;33:271–3.
9. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med. 2001;344:1815–22.
10. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol. 2010;11:653–60.
11. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005;105:1343–7.
12. MacMillan ML, Weisdorf DJ, Brunstein CG, et al. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors. Blood. 2009;113:2410–5.
13. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. Blood. 2010;116:4693–9.
14. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013;122:491–8.

15. Delaney C, Bollard CM, Shpall EJ. Cord blood graft engineering. Biol Blood Marrow Transplant. 2013;19:S74–8.
16. Dahlberg A, Delaney C, Bernstein ID. Ex vivo expansion of human hematopoietic stem and progenitor cells. Blood. 2011;117:6083–90.
17. Shpall EJ, Quinones R, Giller R, et al. Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant. 2002;8:368–76.
18. Gehling UM, Ryder JW, Hogan CJ, et al. Ex vivo expansion of megakaryocyte progenitors: effect of various growth factor combinations on CD34 + progenitor cells from bone marrow and G-CSF-mobilized peripheral blood. Exp Hematol. 1997;25:1125–39.
19. McNiece I, Jones R, Bearman SI, et al. Ex vivo expanded peripheral blood progenitor cells provide rapid neutrophil recovery after high-dose chemotherapy in patients with breast cancer. Blood. 2000;96:3001–7.
20. de Lima M, McMannis J, Komanduri K, et al. Randomized study of double cord blood transplantation (CBT) with versus ex vivo expansion (exp). Blood. 2007;110:2014. (ASH Annual Meeting Abstracts 2007).
21. Chitteti BR, Cheng YH, Poteat B, et al. Impact of interactions of cellular components of the bone marrow microenvironment on hematopoietic stem and progenitor cell function. Blood. 2010;115:3239–48.
22. Cao H, Oteiza A, Nilsson SK. Understanding the role of the microenvironment during definitive hemopoietic development. Exp Hematol. 2013;41:761–8.
23. Shen Y, Nilsson SK. Bone, microenvironment and hematopoiesis. Curr Opin Hematol. 2012;19:250–5.
24. Robinson SN, Ng J, Niu T, et al. Superior ex vivo cord blood expansion following co-culture with bone marrow-derived mesenchymal stem cells. Bone Marrow Transplant. 2006;37: 359–66.
25. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. N Engl J Med. 2012;367:2305–15.
26. Milner LA, Kopan R, Martin DI, Bernstein ID. A human homologue of the Drosophila developmental gene, Notch, is expressed in CD34 + hematopoietic precursors. Blood. 1994;83:2057–62.
27. Varnum-Finney B, Xu L, Brashem-Stein C, et al. Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling. Nat Med. 2000;6:1278–81.
28. Varnum-Finney B, Brashem-Stein C, Bernstein ID. Combined effects of Notch signaling and cytokines induce a multiple log increase in precursors with lymphoid and myeloid reconstituting ability. Blood. 2003;101:1784–9.
29. Delaney C, Heimfeld S, Brashem-Stein C, et al. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med. 2010;16:232–6.
30. Peled T, Landau E, Prus E, et al. Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34 + cells. Br J Haematol. 2002;116:655–61.
31. Peled T, Landau E, Mandel J, et al. Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34 + cells and increases their engraftment potential in NOD/SCID mice. Exp Hematol. 2004;32:547–55.
32. de Lima M, McMannis J, Gee A, et al. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. Bone Marrow Transplant. 2008;41:771–8.
33. Montesinos P, Peled T, Landau E, et al. StemEx® (copper chelation based) ex vivo expanded umbilical cord blood stem cell transplantation (UCBT) accelerates engraftment and improves 100 day survival in myeloablated patients compared to a registry cohort undergoing double unit UCBT: results of a multicenter study of 101 patients with hematologic malignancies. Blood. 2013;122:295. (ASH Annual Meeting Abstracts 2013).
34. Narala SR, Allsopp RC, Wells TB, et al. SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity. Mol Biol Cell. 2008;19:1210–9.

35. Peled T, Shoham H, Aschengrau D, et al. Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. Exp Hematol. 2012;40:342–55.
36. Horwitz ME, Stiff PJ, Chao NJ. Nicord® expanded hematopoietic progenitor cells (HPC) are capable of outcompeting the unmanipulated (UM) cord blood unit and of prolonged myeloid and lymphoid engraftment following myeloablative dual umbilical cord blood (UCB) transplantation. Biol Blood Marrow Transplant. 2013;19:S118.
37. Boitano AE, Wang J, Romeo R, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science. 2010;329:1345–8.
38. Stewart AL, Mukherjee S, Scheiber SL, et al. Ex vivo expansion of umbilical cord blood CD34 + cells under hypoxic conditions using novel compound#999 with cytokines. Blood. 2013;122:4508. (ASH Annual Meeting Abstracts 2013).
39. North TE, Goessling W, Walkley CR, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature. 2007;447:1007–11.
40. Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. Blood. 2009;113:5444–55.
41. Hoggatt J, Mohammad KS, Singh P, Pelus LM. Prostaglandin E2 enhances long-term repopulation but does not permanently alter inherent stem cell competitiveness. Blood. 2013;122:2997–3000.
42. Brown JA, Boussiotis VA. Umbilical cord blood transplantation: basic biology and clinical challenges to immune reconstitution. Clin Immunol. 2008;127:286–97.
43. Kanda J, Chiou LW, Szabolcs P, et al. Immune recovery in adult patients after myeloablative dual umbilical cord blood, matched sibling, and matched unrelated donor hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2012;18:1664–76.
44. Oran B, Hosing CM, Kebriaei P, et al. Ex vivo fucosylation of cord blood accelerates neutrophil and platelet engraftment. Blood. 2013;122:691. (ASH Annual Meeting Abstracts 2013).