



# Annotated Bibliography

## XVIII. Ex-Vivo Expansion of Hematopoietic Progenitor Cells



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For further information on mesenchymal cells, see [Annotated Bibliography, XIX. Miscellaneous Topics](#).

### 1. **Ex vivo expansion of umbilical cord blood stem cells for transplantation: Growing knowledge from the hematopoietic niche.**

Hofmeister CC, Zhang J, Knight KL, Le P, Stiff PJ. *Bone Marrow Transplant* 2007;39:11-23. [Abstract](#)

This is a useful review for those interested in the past, present and possible future of ex vivo expansion of UCB stem cells for transplantation.

Initial efforts to expand UCB progenitors ex vivo have resulted in expansion of mature rather than immature HSC, confounded by the inability to accurately and reliably measure long-term reconstituting cells. **Ex vivo expansion of UCB HSC has failed to improve engraftment** because of the resulting defects that promote apoptosis, disrupt marrow homing and initiate cell cycling.

In this review, the authors discuss the future of ex vivo expansion after providing a review of early clinical experience, current needs for successful HSC expansion and recent attempts to optimize HSC culture conditions. They suggest that *ex vivo* expansion could be enhanced by manipulating newly discovered signaling pathways (Notch, Wnt, bone morphogenetic protein 4 and Tie2/angiopoietin-1) and intracellular mediators (phosphatase and tensin homolog and glycogen synthase kinase-3) in a manner that promotes HSC expansion with less differentiation.

Recent preclinical/clinical HSC expansion investigations are reviewed including transcription inhibition, copper chelation, and Wnt pathway activation. Finally, the authors discuss current/planned HSC expansion clinical trials, and conclude by stating that the ultimate clinical value of infusing expanded UCB is yet to be realized.

### 2. **Ex vivo expansion does not alter the capacity of umbilical cord blood CD34+ cells to generate functional T lymphocytes and dendritic cells.** Kobari L, Giarratana C, Gluckman JC, Douay L, Rosenzweig M. *Stem Cells*. 2006;24:2150-7. [Abstract](#)

The authors examined whether *ex vivo* expansion of umbilical cord blood progenitor cells affected their capacity to generate immune cells such as T lymphocytes (TLs) and dendritic cells (DCs). The capacity to generate TLs from cord blood CD34(+) cells expanded for 14 days (d14) was compared with that of nonexpanded CD34(+) cells (d0) using fetal thymus organ cultures or transfer into nonobese diabetic/severe combined immunodeficient mice.

**The cell preparations yielded comparable percentages of immature (CD4+)CD8(-), CD4(+)CD8(+) TLs and functional mature (CD3+)CD4(+), CD3(+)CD8(+) TLs with an analogous TCR (T-cell receptor)-Vbeta repertoire pattern. As regards DCs, d0 and d14 CD34(+) cells also yielded similar percentages of CD1a(+) DCs with the same expression levels of HLA-DR, costimulatory and adhesion molecules, and chemokine receptors.** DCs derived from either d14 or d0 CD34(+) stimulated allogeneic TLs to the same extent, and the cytokine pattern production of these allogeneic TLs was similar with no shift toward a predominant Th1 or Th2 response.

Even though the intrinsic capacity of d14 CD34(+) cells to generate DCs was 13-fold lower than that of d0 CD34(+) cells, this reduction was offset by the prior amplification of the CD34(+) cells, resulting in the overall production of 15-fold more DCs.

The authors conclude that their **data indicate that ex vivo expansion of CD34(+) cells does not impair T lymphopoiesis nor DC differentiation capacity.**

### 3. **Rapid and massive expansion of cord blood-derived cytokine-induced killer cells: an innovative proposal for the treatment of leukemia relapse after cord blood transplantation.** Introna M, Franceschetti M, Ciocca A, Borleri G, Conti E, Golay J, Rambaldi A. *Bone Marrow Transplant*. 2006; 38:621-7. [Abstract](#)

CB transplantation is gaining increasing attention for the several advantages that it can offer with respect to the traditional adult BM transplant. However, one major disadvantage lies in the unavailability of the donor for patients experiencing graft failures nor of DLI for recipients who suffer leukemia relapse.

Cytokine-induced killer (CIK) cells are naturally occurring cytotoxic cells active against a variety of leukemia and lymphoma targets and with low or absent activity against normal BM stem cells and tissues. Their *in vitro* expansion is easily obtained applying standardized protocol which can be utilized under strict adherence in GMP conditions, as already has been established for the autologous setting. **This paper presents the idea that a salvage CIK infusion may be potentially planned for patients experiencing leukemia relapse after CB transplantation.** Indeed, the authors demonstrated that simply washing the CB unit bag at the end of the infusion is sufficient to recover a number of functionally active CIK cells, which could be infused.

**The authors used a standardized 21-day expansion protocol to produce CIK cells starting from very small amounts of nucleated cells (approximately 15 x 10<sup>6</sup> cells) isolated from cord blood.**

Mononuclear cells are stimulated with anti CD3 (OKT3) and IFN $\gamma$  and then expanded with IL-2. Washouts of cord blood units bags (at the end of the infusion) may be sufficient to yield almost 500 x 10<sup>6</sup> CIK by the same expansion protocol.

CIK cells show strong cytotoxic activity against a variety of tumor target cell lines including B and T lymphomas and myeloid

leukemias. More importantly, expanded cord blood-derived CIK cells are cytotoxic against fresh leukemic blasts and express perforin, granzyme and NKG2D molecule at high levels.

The same *in vitro* protocol has already been used to expand CIK cells from peripheral blood of adult donors under GMP conditions. CIK cells have been used in a phase I study at  $10 \times 10^6/\text{kg}$  or above in the autologous setting. **The present observations open up the possibility of imagining a future clinical application of leukemia relapse following cord blood transplantation with CIK cells obtained from the same cord blood unit.**

#### 4. **Ex vivo expansion of umbilical cord blood stem cells for transplantation: growing knowledge from the hematopoietic niche.**

Hofmeister CC, Zhang J, Knight L, Le P, Stiff PJ. Bone Marrow Transplant. 2007;39:11-23. [Abstract](#)

Initial efforts to expand UCB progenitors *ex vivo* have resulted in expansion of mature rather than immature HSC, confounded by the inability to accurately and reliably measure long-term reconstituting cells. **Ex vivo expansion of UCB HSC has failed to improve engraftment** because of resulting defects that promote apoptosis, disrupt marrow homing and initiate cell cycling.

In this rather detailed review the authors discuss the future of *ex vivo* expansion, which they suggest will include the isolation of immature hematopoietic progenitors on the basis of function rather than surface phenotype and will employ both cytokines and stroma to maintain and expand the stem cell niche. They suggest that *ex vivo* expansion could be enhanced by manipulating newly discovered signaling pathways (Notch, Wnt, bone morphogenetic protein 4 and Tie2/angiopoietin-1) and intracellular mediators (phosphatase and tensin homolog and glycogen synthase kinase-3) in a manner that promotes HSC expansion with less differentiation.

The authors predict that improved methods for *ex vivo* expansion will make UCBT available to more patients, decrease engraftment times and allow more rapid immune reconstitution post transplant.

#### 5. **International Forum: 1** Engelfriet, C. P. & Reesink., H. W. Vox Sanguinis 2005;89:172-173.

This is an **International Forum regarding ex vivo expansion of hematopoietic precursor cells (HPCs)**. The authors of the Forum state that there are several interesting questions related to *ex vivo* expansion. For example, there seems to be a danger that expansion culture may be harmful to HPCs and there is the question of whether CD34+ cells should be isolated for expansion.

The authors posed 6 questions to 4 experts. The questions were:

**Question 1.** Do you agree that there is a danger that expansion culture may be harmful to haematopoietic stem cells? In view of this problem, if you practice the *ex vivo* expansion of HPCs:

- Do you use a fraction of a CB unit for expansion, infusing the remainder unmanipulated? If so, what is the time-span between infusion of the latter and that of the expanded cells?
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**Question 2.** Do you isolate CD34+ cells for expansion? If so, which techniques do you use for isolating the CD34+ cells?

**Question 3.** Which growth factors do you use for expansion? And for what reason?

**Question 4.** Do you think that single or different cells are responsible for neutrophil-platelet and red cell expansion?

**Question 5.** Do you think that adding mesenchymal cells or intrabone transfusion facilitates *in vivo* engraftment?

**Question 6.** Do you think that infusing two unmanipulated CB units solves the problem? It has been reported that in that case, ultimately only one of the two CB units gives long-term engraftment. Have you any experience concerning this problem?

The experts who responded to the survey were:

- E Dickmeiss, Copenhagen, Denmark
- L Lazzarri, T Montemurro, and P. Rebutta, Milan, Italy
- Joan Garcia, Barcelona, Spain
- J. Wagner, Minneapolis, Minnesota, USA

A summary of the responses of the experts, which was to a large extent supplied by the authors of the Forum, is as follows:

Although there are no further actual data in the answers to show that *ex vivo* expansion may be harmful to HPCs, in those centers where expansion is practiced, protocols have been adopted that take this possibility into account: either only part of the CB unit is used for expansion, or an unexpanded unit is co-infused with an expanded unit. The Danish group do not practice *ex vivo* expansion because, to their knowledge, it is not possible to expand primitive HPCs *ex vivo* and they do not find expansion of short-term repopulating committed HPCs to the expense of the primitive HPCs a viable solution for the problem.

CD34+ cells are, in fact, isolated for expansion in Italy and Spain by using the CliniMACS from Miltenyi Biotech (Glodbach, Germany). In Minnesota, this selection step is being evaluated.

In contrast to the opinion of the Danish experts, the Italian and Spanish groups are of the opinion that an optimal combination of growth factors leads to the expansion of primitive HPCs.

Because primitive HPCs are expanded, it is postulated that all cell lineages are derived from a single expanded primitive HPC.

Some recently published data indicate that culture of CB stem cells on a mesenchymal all-feeding layer supports the *ex vivo* expansion of HPCs and that the co-transfusion of CB and mesenchymal cells may facilitate *in vivo* engraftment. In Spain, mesenchymal cells are co-transfused, especially in cases in which the micro-environment has suffered through treatment regimes.

Dr. Wagner cited [published data on double cord blood transplants](#) and stated that, to date, 37 such transplants have been performed after myeloablative conditioning at the University of Minnesota. All of the patients engrafted at a median of 23 days. Although he states that he believes that treatment with cyclophosphamide, fludarabine and TBI (1320 cGy) followed by double

cord blood transplant has addressed the issue of engraftment and universal availability of umbilical cord blood for adult patients, the question of whether the speed of engraftment can be improved by this approach remains to be proven.

The authors of the Forum concluded that, although the *ex vivo* expansion of CB HPCs is now obviously routinely practiced in some centers, questions concerning important aspects of this procedure remain. "Perhaps we have endeavored to approach this subject at too early a stage, as several aspects of the procedure are still in the experimental phase."

**6. Direct evidence for *ex vivo* expansion of human hematopoietic stem cells.** Ando K, Yahata T, Sato T, Miyatake H, Matsuzawa H, Oki M, Miyoshi H, Tsuji T, Kato S, Hotta T. *Blood*. 2006;107:3371-7. [Abstract](#)

Over the past 15 years many investigators have undertaken experiments to study *ex vivo* expansion of HSCs. These studies have led to a number of clinical trials to evaluate *ex vivo*-expanded cells in patients. However, no significant clinical benefit has been demonstrated to date.

To characterize human hematopoietic stem cells (HSCs), xenotransplantation techniques such as the severe combined immunodeficiency (SCID) mouse repopulating cell (SRC) assay have proven the most reliable methods thus far. While SRC quantification by limiting dilution analysis (LDA) is the gold standard for measuring *in vitro* expansion of human HSCs, LDA is a statistical method and does not directly establish that a single HSC has self-renewed *in vitro*. This would require a direct clonal method and has not been done.

The authors used lentiviral gene marking and direct intra-bone marrow injection of cultured CD34+ CB cells to provide the first direct evidence for self-renewal of individual SRC clones *in vitro*. Of 74 clones analyzed, 20 clones (27%) divided and repopulated in more than 2 mice after serum-free and stroma-dependent culture. Some of the clones were secondary transplantable. This indicates symmetric self-renewal divisions *in vitro*. On the other hand, 54 clones (73%) present in only 1 mouse may result from asymmetric divisions *in vitro*.

The authors conclude that their data demonstrate that current *ex vivo* expansion conditions result in reliable stem cell expansion, and the clonal tracking employed is the only reliable method that can be used in the development of clinically appropriate expansion methods.

**7. *Ex vivo* expansion of umbilical cord blood.** Robinson S, Niu T, de Lima M, Ng J, Yang H, McMannis J, Karandish S, Sadeghi T, Fu P, del Angel M, O'Connor S, Champlin R, Shpall E. *Cytotherapy*. 2005;7:243-50. [Abstract](#)

To potentially improve the efficacy of CB transplantation, approaches have been taken to increase the cell dose available. One approach is the transplantation of multiple cord units, another the use of *ex vivo* expansion. Evidence for a functional and phenotypic heterogeneity exists within the HSC population and one concern associated with *ex vivo* expansion is that the expansion of lower 'quality' hematopoietic progenitor cells (HPC) occurs at the expense of higher 'quality' HPC, thereby impacting the reserve of the graft. There is evidence that this is a valid concern while other evidence suggests that higher quality HPC are preserved and not exhausted.

*Ex vivo* expansion can be performed on whole CB units or a fraction of a CB unit that is combined with its unmanipulated fraction at the time of transplantation, or transplanted a period of time after the unmanipulated fraction. Clinical protocols that explore these approaches are currently under way at a number of medical centers.

Currently, *ex vivo* expansion processes include: (1) liquid expansion: CD34<sup>+</sup> or CD133<sup>+</sup> cells are selected and cultured in medium containing factors targeting the proliferation and self-renewal of primitive hematopoietic progenitors; (2) co-culture expansion: unmanipulated CB cells are cultured with stromal components of the hematopoietic microenvironment, specifically mesenchymal stem cells (MSC), in medium containing growth factors; and (3) continuous perfusion: CB HPC are cultured with growth factors in 'bioreactors' rather than in static cultures. These approaches are discussed in this review and preliminary data that are available are provided.

Ultimately, the goal of *ex vivo* expansion is to increase the available dose of the CB cells responsible for successful engraftment, thereby reducing the time to engraftment and reducing the risk of graft failure.

**Disclaimer:** The Cord Blood Forum endorses collegial discussion among cord blood transplantation professionals, patients and donors. However, the Cord Blood Forum does not necessarily endorse, nor take any responsibility for the specific views and opinions expressed in the forum. The forum is not intended as a substitute for legal and/or medical advice and the content should not be relied upon for medical and/or legal purposes. Readers should make their own determinations as to: (i) what constitutes appropriate medical, technical, and administrative practices, and (ii) how best to comply with laws and regulations relevant to their questions. For the latter, they should consider consulting with an attorney familiar with related state and federal laws.



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8. **Ex vivo expansion of umbilical cord blood hemopoietic stem and progenitor cells.** McNiece I, Gluckman E, Wagner JE, Verfaillie CM. *Exp Hematol.* 2004;32:409-413.

In a series of three short editorials the authors review the current status of ex-vivo expansion of umbilical cord blood stem and progenitor cells. McNiece points out that clinical trials have demonstrated that ex-vivo-expanded cells can be cultured and safely infused, but no significant clinical benefit has been demonstrated to date. A number of challenges remain regarding ex-vivo expansion of cord blood (CB) cells: (1) The CB products in the majority of CB banks are frozen as single products so that the infusion of the expanded cells occurs 10 to 14 days after the unmanipulated product, thus minimizing the potential to demonstrate a significant effect on engraftment, (2) CD34 selection is required for optimal ex-vivo expansion, (3) clinical grade growth factors are required by regulatory agencies, and (4) regulation by the FDA will increase the complexity and cost of undertaking ex-vivo expansion trials. No reports have demonstrated the feasibility of expanding human stem cells (HSCs) and, in contrast, the clinical ex-vivo expansion studies performed to date most likely result in decreased levels of HSCs. However, since neutrophils, platelets and the cells involved in immune recovery may arise from distinct cells, the optimal graft may require a mixture of neutrophil progenitors, megakaryocyte progenitors and T cell precursors.

Gluckman points out that several options are under discussion to improve the speed of engraftment of cord blood transplants: (1) ex-vivo expansion with cytokine cocktails, (2) multi-cord transplants or intra-bone infusion of CD34 cells, and (3) addition of mesenchymal cells which may facilitate cell proliferation and engraftment both in vitro and in vivo and decrease the risk of GVHD. They state that there are very few studies ongoing and that there is a clear need for prospective clinical trials.

Wagner and Verfaillie point out that it is unknown which cell should be expanded (i.e., stem cell, primitive progenitor, or more committed progenitor) but, in any case, it would seem optimal that stem cells not be lost at the expense of generating progenitors incapable of self-renewal. They insist that it is critical that the manufacture of the expansion culture media be performed in a GMP cell therapy facility staffed with technologists experienced in clinical cell therapy. They further emphasize that it has been difficult to determine how expansion culture might be tested clinically, since studies have placed only a proportion of the UCB graft into expansion culture, and the lack of a genetic marker prevents any ability to track the expanded population. To solve this problem the authors suggest transplanting two partially HLA-matched UCB units as a model for testing the safety and potential efficacy of expansion culture.

9. **Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine.** Peled T, Mandel J, Goudsmid RN, et al. *Cytotherapy* 2004;6:344-355. [Abstract](#)

The authors indicate that they have previously demonstrated that the copper chelator tetraethylenepentamine (TEPA) enables preferential expansion of early hematopoietic progenitor cells (CD34+ CD38-, CD34+CD38-Lin-) in human umbilical cord blood-derived CD34+ cell cultures. In the present study they established a clinically applicable protocol for large-scale ex vivo expansion of cord blood-derived progenitors. CD133+ cells purified from cord bloods were cultured for 3 weeks in a clinical-grade closed culture bag system, using the chelator-based technology in combination with early-acting cytokines (SCF, thrombopoietin, IL-6 and FLT-3 ligand). The median output value of CD34+ cells increased 89-fold, CD34+CD38- cells increased by 30-fold, and CFUc increased by 172-fold over the input value. Transplantation into sublethally irradiated NOD/SCID mice indicated that the engraftment potential was significantly superior to that of unexpanded cells. The chelator-based ex-vivo expansion technology is currently being tested in a phase 1 clinical trial in patients undergoing cord blood transplantation for hematological malignancies.

10. **Improved haematopoietic recovery following transplantation with ex vivo-expanded mobilized blood cells.** Prince HM, Simmons PJ, Whitty G, Wall DP, Barber L, Toner GC, Seymour JF, Richardson G, Mrongovius R, Haylock DN. *Br J Haematol.* 2004;126:536-45. [Abstract](#)

The authors demonstrated that CD34+ cells expanded ex vivo with the cytokine combination of G-CSF, SCF and PEG-rHuMGDFDF resulted in a 33-fold and 2.8 fold expansion of total cells and CD34+ cells, respectively. They tested the efficacy of the ex-vivo-expanded cells in three cohorts of three patients with breast cancer who received three cycles of repetitive high dose chemotherapy supported by either unmanipulated and/or ex-vivo-expanded cells. Efficacy was assessed by an internal comparison of each patient's consecutive high dose chemotherapy cycles, and to 106 historical controls using unmanipulated cells.

Twenty-one cycles were supported by ex-vivo-expanded cells and six by unmanipulated cells alone. Infusions of ex-vivo-expanded cells resulted in fewer days with an absolute neutrophil count (ANC)  $<0.1 \times 10^9/l$  (median 2 vs. 4 d,  $P = 0.002$ ) and 3 d faster ANC recovery to  $>0.1 \times 10^9/l$  (median 5 vs. 8 d,  $P = 0.0002$ ). This resulted in a major reduction in the incidence of febrile neutropenia compared with unmanipulated cycles (0% vs. 83%;  $P = 0.008$ ) and in 66% of historical unmanipulated cycles ( $P = 0.01$ ) and a marked reduction in hospital re-admission. There were also fewer platelet transfusions required (43% vs. 100%;  $P =$

0.009). The investigators considered that the most important effect was the markedly reduced depth of thrombocytopenia following high dose chemotherapy, which resulted in 43% of the cycles not requiring any platelet transfusions.

The authors pointed out that utilization of "internal" controls, with six patients having one of their three high dose chemotherapy cycles supported by unmanipulated cells with the remaining two cycles supported by *ex-vivo*-expanded cells, provides compelling evidence that the *ex-vivo*-expanded cells enhanced neutrophil recovery. This finding was further supported by data provided by the historical control group.

The investigators concluded that *ex-vivo*-expanded cells enhance both neutrophil and platelet recovery and reduce febrile neutropenia, platelet transfusion and hospital re-admission.

11. **Elevated telomerase activity and minimal telomere loss in cord blood long-term cultures with extensive stem cell replication.** Gammaitoni L, Weisel KC, Gunetti M, Wu KD, Bruno S, Pinelli S, Bonati A, Aglietta M, Moore MA, Piacibello W. *Blood*. 2004;103:4440-8. [Abstract](#)

The authors indicate that there is clearly a clinical value for a system that provides extensive *ex-vivo* stem cell expansion without concomitant telomere erosion. They evaluated telomerase activity, telomere length, stem/progenitor cell production, and function of CD34(+) cells from cord blood (CB), bone marrow, and mobilized peripheral blood in long-term cultures. CB cells were cultured either on OP-9 stromal cells transduced with an adenovector expressing thrombopoietin (TPO) or stimulated by a cytokine cocktail in the absence of stroma, with, in one method, CD34(+) cells re-isolated at monthly intervals for passage. Despite extensive proliferation, telomere length initially increased and only at late stages of culture was evidence of telomere shortening noted. This telomere stabilization correlated with maintenance of high levels of telomerase activity in the CD34(+) cell population for prolonged periods of culture.

12. **Clinical application of hematopoietic progenitor cell expansion: current status and future prospects.** Devine SM, Lazarus HM, Emerson SG. *Bone Marrow Transplant* 2003; 31:241-252. [Abstract](#)

In this *review of ex vivo expansion of hematopoietic progenitor cells*, the authors point out that in the past decade, there have been significant advances in *ex vivo* hematopoietic stem cell culture expansion, progressing to the point where clinical trials are being designed and conducted. Reported clinical trials are reviewed in depth; other topics reviewed include, "the path to the present", "defining new clinical targets – umbilical cord blood", "additional biologic advantages of cord blood", "current bottlenecks to clinical development", and "where do we go from here?" They point out that successful clinical application of expanded hematopoiesis will clearly require a greater understanding of human stem cell biology, identification of the proper balance and concentration of the available cytokine cocktails for *ex vivo* culture, and hastening the slow pace of clinical trials.

13. **Ex vivo expansion of hematopoietic progenitor cells and mature cells.** McNiece I, Briddell R. *Exp Hematol*. 2001;29:3-11. [Abstract](#)

The authors of this review state that many investigators have explored methods to culture hematopoietic cells *in vitro* to increase the numbers of these cells. Studies attempting to expand hematopoietic stem cells, progenitor cells, and mature cells *in vitro* have become possible over the past decade due to the availability of recombinant growth factors and cell selection technologies. However, the authors state that, as of the date of this publication, no studies have demonstrated convincing data on the expansion of true stem cells. A number of clinical studies have been performed using a variety of culture conditions and there are evolving data that suggest that there are real clinical benefits associated with the use of the expanded cells. The authors suggest that the next decade should determine what culture conditions and what cell populations are needed for a range of clinical applications.

14. **Ex-vivo expansion of cord blood mononuclear cells on mesenchymal stem cells.** McNiece I, Harrington J, Turney J, Kellner J, Sphall EJ. *Cytotherapy* 2004;6:311-317. [Abstract](#)

The authors point out that the current clinical conditions for *ex vivo* expansion of cord blood (CB) cells require selection of the CD34<sup>+</sup> subset or culture in perfusion systems, as unfractionated or even mononuclear cells (MNC) do not expand well in static culture. CD34<sup>+</sup> cell selection of thawed CB units often results in low CD34<sup>+</sup> cell recoveries (median recovery of 50%) and suboptimal purities, resulting in lower expansion overall. A number of studies have demonstrated the supportive role of stromal cells for hematopoietic stem cells and progenitor cells, and clinical studies have evaluated the potential of mesenchymal stem cells (MSCs) to facilitate engraftment and possibly decrease the incidence of GVHD. Accordingly, the authors evaluated the potential of MSCs to support *ex vivo* expansion of unselected CB products. Their results indicated that *ex vivo* expansion of CB MNC on MSC resulted in 10- to 20-fold expansion of total nucleated cells, seven- to 18-fold expansion of committed progenitor cells, two- to five-fold expansion of primitive progenitor cells and 16- to 37- fold expansion of CD34<sup>+</sup> cells. The authors' current focus is to initiate clinical trials to evaluate the *in vivo* potential of CB cells expanded with these conditions.

15. **Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell System.** Jaroscak J, Goltry K, Smith A, Waters-Pick B, Martin PL, Driscoll TA, Howrey R, Chao N, Douville J, Burhop S, Fu P, Kurtzberg J. *Blood*. 2003;101:5061-5067. [Full Text](#)

The authors performed a phase 1 trial augmenting conventional UCB transplants with *ex vivo*-expanded cells. Twenty-eight patients were enrolled on the trial between October 8, 1997 and September 30, 1998. UCB cells were expanded, then administered as a boost to the conventional graft on posttransplantation day 12. While expansion of total cells and colony-forming units (CFUs) occurred in all cases, the magnitude of expansion varied considerably. The median fold increase was 2.4 (range, 1.0-8.5) in nucleated cells, 82 (range, 4.6-266.4) in CFU granulocyte-macrophages, and 0.5 (range, 0.09-2.45) in CD34<sup>+</sup> lineage negative (lin<sup>-</sup>) cells. CD3<sup>+</sup> cells did not expand under these conditions. Augmentation of UCB transplants with *ex vivo*-expanded cells did not alter the time to myeloid, erythroid, or platelet engraftment in 21 evaluable patients.

16. **Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system.** Lewis ID, Almeida-Porada G, Du J, Lemischka IR, Moore KA, Zanjani ED, Verfaillie CM. *Blood*. 2001;97:3441-3449. [Full Text](#)

The authors describe the ability of the murine fetal liver cell line, AFT024, to maintain/expand human umbilical cord blood CD34<sup>+</sup> repopulating cells assayed in the NOD-SCID mouse and the fetal sheep model. They found that culture of cord blood CD34<sup>+</sup>

cells for up to 28 days in an AFT 024 noncontact system supplemented with a combination of early acting cytokines, including SCF, FL, Tpo, and IL-7, maintains repopulating cells in NOD/SCID as well as fetal sheep. They concluded that their data indicate that ex-vivo expanded cells continue to contain "long-term" repopulating cells because they can be serially passaged to secondary NOD-SCID mice and secondary and tertiary fetal sheep.

**17. Ex vivo expansion of megakaryocyte precursors from umbilical cord blood CD34 cells in a closed liquid culture system.**

Shaw PH, Gilligan D, Wang XM, Thall PF, Corey SJ. Biol Blood Marrow Transplant. 2003 Mar;9(3):151-6. [Abstract](#)

The authors propose to hasten platelet engraftment by expanding the number of megakaryocyte (MK) precursors (CD34/CD41 cells) through cytokine stimulation within a closed, pre-clinical liquid culture system. Thirteen UCB samples from full-term births were Ficoll-separated and frozen for subsequent use. On thawing, the mononuclear cell population was positively selected for CD34+ expression. The cells were cultured in gas-permeable Teflon-coated bags in serum-free medium containing the following cytokines: recombinant human interleukin-3, recombinant human Flt3 ligand, recombinant human stem cell factor, and recombinant human thrombopoietin. MK lineage cell expansion was assessed using mononuclear cell count and flow cytometry (CD34/41, CD41, CD34/61, and CD61 expression) on days 7, 11, and 14. Optimal expansion of CD34/41 and CD41 cells was observed at day 11, with a median 6-fold and 33-fold increase in the starting cell doses, respectively. CD34/61 and CD61 cell expansion at day 11 was 7-fold and 14-fold, respectively. The authors concluded that MK precursors can be successfully expanded from CD34+ UCB cells in a closed liquid culture system. However, they stated that their ex vivo expansion technique needs to be further optimized before it can be used in a pilot UCB transplantation trial.

**18. Transplantation of ex vivo expanded cord blood.** Shpall EJ, Quinones R, Giller R, Zeng C, Baron AE, Jones RB, Bearman SI, Nieto Y, Freed B, Madinger N, Hogan CJ, Slat-Vasquez V, Russell P, Blunk B, Schissel D, Hild E, Malcolm J, Ward W, McNiece IK. Biol Blood Marrow Transplant. 2002;8:368-376. [Abstract](#)

The authors studied the ex vivo expansion of CB in an attempt to improve time to engraftment and reduce the graft failure rate in the recipients. In this feasibility study, 37 patients (25 adults, 12 children) with hematologic malignancies (n = 34) or breast cancer (n = 3) received high-dose therapy followed by unrelated allogeneic CB transplantation. A fraction of each patient's CB allograft was CD34-selected and cultured ex vivo for 10 days prior to transplantation in defined media with stem cell factor, granulocyte colony-stimulating factor, and megakaryocyte growth and differentiation factor. The remainder of the CB graft was infused without further manipulation. Patients received a median of  $0.99 \times 10^7$  total nucleated cells (expanded plus unexpanded) per kilogram. The median time to engraftment of neutrophils was 28 days (range, 15-49 days) and of platelets was 106 days (range, 38-345 days). All evaluable patients who were followed for 28 days or longer achieved engraftment of neutrophils. Grade III/IV acute GVHD was documented in 40% and extensive chronic GVHD in 63% of patients. At a median follow-up of 30 months, 13 (35%) of 37 of patients survived. The authors concluded that their study demonstrates that the CD34 selection and ex vivo expansion of CB prior to transplantation of CB is feasible. Additional accrual will be required to assess the clinical efficacy of expanded CB progenitors.

**19. Human stem-progenitor cells from neonatal cord blood have greater hematopoietic expansion capacity than those from mobilized adult blood.** Tanavde VM, Malehorn MT, Lumkul R, Gao Z, Wingard J, Garrett ES, Civin CI. Exp Hematol. 2002;30:816-823. [Abstract](#)

The authors compared the hematopoietic capacity of CD34+ cell preparations from neonatal cord blood (CB) vs adult mobilized peripheral blood (PBSC) before and after ex vivo culture. CD34+ cell preparations purified from CB or PBSC were cultured in serum-free medium containing FKT: FLT-3 ligand (FL), KIT ligand (KL), and thrombopoietin (TPO). After 1-4 weeks ex vivo culture, CB CD34+ cell preparations had greatly increased numbers of total cells, CD34+ cells, and colony-forming cells (CFC). In contrast, ex vivo-cultured PBSC CD34+ cell preparations generated far less in vitro assessed hematopoietic capacity. Nonobese diabetic severe combined immunodeficient mouse (NOD/SCID) engrafting potential (SEP) was maintained in ex vivo-cultured CB CD34+ cell preparations, whereas it was lost in ex vivo-cultured PBSC. CB CD34+ cells continued to proliferate throughout 3 weeks ex vivo, whereas after 1 week, no additional cell divisions were detected in PBSC CD34+ cells. After 3 weeks in culture, the average CB CD34+ cell had divided more than 5 times, as compared to only 2 times for the average PBSC CD34+ cell. The authors concluded that CB CD34+ cell preparations generated massively increased in vitro assessed hematopoietic capacity and maintained SEP during 1- to 4-week ex vivo cultures. In contrast, ex vivo-cultured PBSC CD34+ cell preparations generated far less in vitro assessed hematopoietic capacity and decreased SEP.

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