REVIEW


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Abstract. Placental/umbilical cord blood (PCB) stem cells for transplantation provide a potentially useful alternative for patients who do not have an HLA-matched family or unrelated bone marrow donor. Concerns regarding this source of stem cells include the limited number of stem cells in a PCB unit and the delayed time to platelet engraftment. Because of the limited number of stem cells, there is a very clear cell dose effect for both success of engraftment and time to engraftment. As a result, many transplant centers will only consider PCB stem cells as a second choice for transplanting adults, despite the very favorable profile of post-transplant graft-versus-host disease (GvHD). This has resulted in considerable interest in the development of ex vivo stem cell expansion strategies. This review outlines the current status of PCB transplant outcomes as well as the status of our understanding of stem cell expansion with the currently available technologies. A stem cell dose-limiting effect on outcome will result in a narrower window of clinical indications for the use of this stem cell source, despite the acknowledged reduction in GvHD. The trade-offs between poor engraftment and reduction in fatal or severe chronic GvHD remain to be quantitated. (Keio J Med 49 (4): 141-151, December 2000)

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Introduction

Hematopoietic stem cell (HSC) transplantation in the form of bone marrow transplantation (BMT) to treat inherited or acquired hematologic diseases is well established.1,2 Because not all families had an HLA-matched related donor when needed, the National Marrow Donor Program (NMDP) was established in the United States in 1986 to provide a registry of volunteer BM donors. The registry has now grown to more than 3.5 million potential donors and similar registries have been established in many other countries. Nevertheless, the length of time required to find a suitable donor (median 3.5 months; range 1 month to six years),3 the limited numbers of donors of ethnic minorities, as well as the rigor of the BM donation process and the risk to the donor, have led to studies of alternative HSC sources.

The potential use of placental/umbilical cord blood (PCB) as a source of transplantable HSC was first suggested by Boyse in 1983, and “proof of principle” was provided by the finding that lethally-irradiated mice could be rescued and hematopoiesis completely reconstituted by transplantation of perinatal blood.4 In 1988, Gluckman et al. successfully treated a patient with Fanconi anemia by using PCB from the patient’s sibling.5 That patient, the first to have received a related PCB stem cell transplantation, remains alive and free of disease to the present.

In addition, mobilization of HSC into the peripheral blood (PB) and their collection by leukapheresis became feasible and this source of HSC has been used since the late 1980’s to reconstitute marrow function after high-dose chemotherapy for solid tumors or hematologic malignancies.6-8 These advances over the past two decades have extended the field of HSC
transplantation and now provide treatment options for malignancies, marrow failure syndromes, hereditary immunodeficiency states, inborn errors of metabolism, and certain solid tumors, as well as providing a platform for gene therapy.

Among the various HSC sources for transplantation, PCB offers a number of potential advantages: (1) PCB is a waste product after childbirth and its procurement is relatively easy; (2) the ability to ensure ethnic diversity and rare HLA constellations among donors; (3) low risk of transmissible infectious diseases; (4) no risk to the donor; (5) no contamination by residual tumor cells. Analysis of the clinical results in the first 44 patients transplanted with sibling PCB established the fact that PCB contains sufficient numbers of stem and progenitor cells to engraft at least small recipients and that such transplants were associated with a very low risk of acute and chronic graft-versus-host-disease (GvHD).

As a result of the early success with PCB, programs for the banking of unrelated donor PCB were initiated both in the U.S. and Europe. At present, the Placental Blood Program at the New York Blood Center, established in 1992, is the largest PCB bank in the world with approximately 11,000 units collected, HLA-typed, tested for infectious transmissible diseases and cryopreserved. Such PCB stem cell preparations can be made available rapidly, with some units identified and transplanted within one week after the initial search request. To date, the Placental Blood Program of the New York Blood Center has provided nearly 1000 PCB units for unrelated transplantation (P. Rubinstein, personal communication).

**PCB Progenitor Cells: In Vitro Properties**

PCB, mobilized PB and BM display differences in numbers and phenotypes of stem and progenitor cells as well as differences in their proliferative response to cytokines. The different characteristics of PCB, BM and mobilized PB may be due to their different ontogenetic stages or to maturation-related differences in the expression of different surface markers. Such differences may influence homing and engraftment potential following transplantation. In contrast to BM and mobilized PB, PCB progenitor cells have: (1) a higher proliferative potential reflected by the size of hematopoietic colonies growing in semisolid medium; (2) a higher clonogenic potential measured as the percentage of colony-forming cells (CFC) among nucleated cells or CD34+ cells, and by replating capacity of CFC; (3) a greater proportion of cells with a more primitive phenotype represented by the presence of stem cell surface markers such as CD34 and c-kit and the absence of lineage-specific markers such as CD33, transferrin receptor, CD 41, thy 1 and CD 38; (4) a higher content of mixed-cell CFC with the capacity to give rise to colonies containing up to five types of differentiated cells; (5) a higher engraftment potential in the nonobese diabetic severe combined immunodeficient (NOD-SCID) mouse; (6) longer telomeres.

**PCB in Clinical Transplantation: Outcomes and Possible Limitations**

Over the past several years, a number of studies have established PCB as a viable alternative source of HSC for transplantation in the allogeneic related and unrelated settings. The patients in these studies have been heterogeneous with respect to diagnosis and stage of disease. While the range of diagnoses was similar to large studies of BMT, a substantial number of PCB recipients had advanced or relapsed disease and had no other treatment option or suitably-matched family or unrelated BM donor. Between 1995 and 1999, the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR) reported that, of the first patients transplanted with unrelated PCB for leukemia, only 26% were in early stage and 50% in an intermediate stage of their disease. This was contrasted with a similar group of patients who received unrelated BM transplants where 43% and 35% were in early or intermediate stage, respectively.

For both stem cell sources, the largest percentage of all transplants—49% for PCB and 43% for BM—were carried out for treatment of acute leukemia. The second largest category comprised chronic leukemia for BMT (30%) and congenital disorders for PCB transplants (18%). Most (69%) of the PCB transplants were performed on patients under the age of 10 (and, therefore, with low body weight). The use of PCB transplantation decreased progressively with increasing patient age. In contrast, the use of BMT is evenly distributed throughout all age groups (range 17–26%) (M. Horowitz, personal communication).

Observation endpoints reported following transplantation vary widely from several months to several years so that the interpretation of clinical outcomes is difficult, especially for rare diseases with data collected on only a few patients. The comparison of results from recent PCB transplantation studies to historical results with BMT also needs to take into account that stem cell transplantation techniques have been subject to constant refinement over the past decades and outcomes are heavily influenced by the experience of the transplant center. Taking this fact into consideration, the precise interpretation of patient outcomes following PCB versus BM transplantation can only be achieved in future trials by matched/paired case-control studies.
The largest study of PCB transplantation from unrelated donors (and comprising most of the PCB transplants carried out worldwide between August 1992 and January 1998) was reported by Rubinstein et al. The study includes data on 562 patients who were transplanted for a variety of malignant and non-malignant blood disorders, genetic diseases and inborn errors of metabolism, as well as hereditary immunodeficiency states. Patients were followed for likelihood of engraftment, speed of neutrophil and platelet recovery, GvHD, event-free survival, viral infection and, as appropriate, leukemia relapse. The large number of patients in the study, as well as the integration of preliminary data published earlier on smaller patient groups, resolved differences in three areas: (1) engraftment dependent on cell dose infused, (2) delayed platelet recovery following PCB transplantation, and (3) CMV-antibody status predictive of GvHD and survival. Three months post-transplantation, the overall rate of transplantation-related events was 46% and these events correlated with the recipient's diagnosis, age, number of leukocytes infused, extent of HLA-disparity, and the location of the center.

The class of HLA mismatch and the presence or absence of CMV-antibodies before transplantation were not associated with transplantation-related events. In accordance with other studies, it is generally accepted that the degree of GvHD seen with PCB transplantation is less severe than with BMT. Thus, 31% of patients showed no acute GvHD, 47% grade I–II and 22% grade III–IV. In contrast, a similarly large study of unrelated BMT reported in 1993 by the NMDP gave the probability of severe GvHD (grade III–IV) to be almost 0.5. In general, GvHD following PCB transplantation was more prominent in patients >12 years old, with a greater degree of HLA-incompatibility, with infection, and in transplant centers outside the U.S. The frequency of acute grade III–IV GvHD in PCB transplantation was lower in patients matched for 6 of 6 HLA antigens than in patients with one or more HLA mismatches, but did not correlate with the degree of HLA mismatch. Of patients who had previously had grade III–IV acute GvHD, 80% developed chronic GvHD; in comparison, only 18% of those patients who did not have severe acute GvHD developed chronic GvHD. The generally reported low incidence of GvHD after PCB transplantation is a major concern in leukemic patients as the absence of GvHD might be associated with the absence of a graft-versus-leukemia (GvL) effect. However, a study carried out by the Eurocord-Cord Blood Transplantation Group on 102 children with acute leukemia in early and advanced stage of disease, good and poor risk, reported no remarkable differences in the overall two year event-free survival (interval from PCB transplantation to relapse or death in complete remission) when compared to the two year disease-free posttransplant outcomes of children receiving an unrelated BMT. The two year event-free survival was 30% after PCB transplantation and 33% after BMT.

Another study of BMT reported 47% and 20% disease-free survival in patients with acute lymphoblastic leukemia in first or second remission, respectively. Considering the significantly lower incidence of GvHD seen with PCB transplantation and the detrimental impact of GvHD on a growing organism, the results on overall survival are encouraging. Apparently, the low incidence and severity of GvHD with PCB transplantation are not necessarily associated with a reduced GvL effect in leukemic patients. In fact, the prompt availability of PCB stem cell preparations may eventually permit transplants at a more favorable disease state, and the better tolerated HLA-disparity could be associated with a greater GvL effect.

With adult BM or cytokine-mobilized PB stem cell transplantation, the time to neutrophil engraftment correlates with CD34+ cell dose. In addition, preliminary reports have claimed a correlation between the number of megakaryocytic (Meg)-CFC given and time to platelet engraftment following PB transplants. Previously, the New York Blood Center and others reported on the correlation between nucleated cell dose and the success and speed of both neutrophil and platelet engraftment following PCB transplants. It is generally accepted that the degree of GvHD seen with PCB transplantation is less severe than with BMT. Thus, 31% of patients showed no acute GvHD, 47% grade I–II and 22% grade III–IV. In contrast, a similarly large study of unrelated BMT reported in 1993 by the NMDP gave the probability of severe GvHD (grade III–IV) to be almost 0.5. In general, GvHD following PCB transplantation was more prominent in patients >12 years old, with a greater degree of HLA-incompatibility, with infection, and in transplant centers outside the U.S. The frequency of acute grade III–IV GvHD in PCB transplantation was lower in patients matched for 6 of 6 HLA antigens than in patients with one or more HLA mismatches, but did not correlate with the degree of HLA mismatch. Of patients who had previously had grade III–IV acute GvHD, 80% developed chronic GvHD; in comparison, only 18% of those patients who did not have severe acute GvHD developed chronic GvHD. The generally reported low incidence of GvHD after PCB transplantation is a major concern in leukemic patients as the absence of GvHD might be associated with the absence of a graft-versus-leukemia (GvL) effect. However, a study carried out by the Eurocord-Cord Blood Transplantation Group on 102 children with acute leukemia in early and advanced stage of disease, good and poor risk, reported no remarkable differences in the overall two year event-free survival (interval from PCB transplantation to relapse or death in complete remission) when compared to the two year disease-free posttransplant outcomes of children receiving an unrelated BMT. The two year event-free survival was 30% after PCB transplantation and 33% after BMT.

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cells not only from PCB but also with other stem cell sources.

**Ex Vivo Expansion of Human HSC**

Numerous strategies for the expansion of murine and human HSC and progenitor cells have been reported in the past several years and the studies have included PCB, PB and BM cells. Consistently, the numbers of hematopoietic progenitor cells (CFC of various classes) can be expanded in culture with a variety of cytokines in the presence or absence of serum components and/or stromal feeder layers. The use of early-acting cytokines such as Flt-3 ligand, IL-11, IL-3, stem cell factor (SCF), IL-6 and thrombopoietin (Tpo), as opposed to lineage-restricted cytokines such as granulocyte (G)-colony stimulating factor (CSF), GM-CSF and erythropoietin (Epo), was hoped to promote expansion of the HSC pool rather than differentiation of progenitor cells into mature cells. Theoretically, application of different cytokines would expand different subsets of HSC and progenitor cells having different functions in the post-transplant period: accelerated neutrophil and platelet recovery, short-term engraftment (radioprotection) and long-term engraftment (permanent donor-derived reconstitution of hematopoiesis). However, there is growing evidence that, regardless which cytokines are used, ex vivo cell expansion conditions largely facilitate the maturation of HSC into lineage-restricted progenitor cells as well as changes in homing features associated with loss of long-term repopulating capacity.

It is generally accepted that the function of a stem cell is defined as the ability to produce cells of all myeloid and lymphoid lineages and, following transplantation, to home to the bone marrow and permanently establish hematopoiesis. Candidate cells with "stem-cell like" characteristics have been identified by in vitro assays through features that are thought to distinguish HSC from progenitor cells: (1) long-term culture-initiating cells (LTC-IC) grown on bone marrow stromal feeder layers with sustained ability to generate more LTC-IC as well as CFC of various types for several weeks; (2) cobblestone area-forming cells that form cell patches with cobblestone morphology on bone marrow stromal feeder layers (the latter are thought to provide stromal niches for HSC homing); (3) high proliferative potential-CFC forming extremely large colonies (several thousand cells per colony) in semisolid culture.

While helpful, these in vitro assays should only be considered as surrogate assays for HSC function. As growth characteristics of candidate HSC in vitro might not translate into HSC function in vivo—especially once HSC have been subjected to ex vivo exposure to cytokines—in vivo models to quantitate HSC numbers and monitor HSC function have been established. One recently developed model is the NOD/SCID mouse. This model permits the study of human HSC function in the mouse, where human HSC are transplanted into a severely immunocompromised host with no graft rejection. While allowing semiquantitative assessment of primitive normal and leukemic human stem cells (SCID repopulating cells; SRC), the model has several limitations. First, malignancies of the lymphohematopoietic system and infections following radiation and transplantation of hematopoietic cells result in a short lifespan of only several weeks to a few months in these mice, precluding studies of long-term (>4-6 months) engraftment. Second, human HSC function is tested under xenogeneic conditions that don't mirror the homologous post-transplant environment in man or animal. Some investigators try to mitigate these disadvantages by administering human cytokines post-transplant or by placing human bone marrow fragments subcutaneously in order to facilitate homing and to support human HSC function in the murine background. Third, neutrophil and platelet recovery cannot be assessed because the proportion of human cells in circulation is very low (typically <3%).

Piacibello et al. carried out extensive studies on the ex vivo expansion of PCB with subsequent transplantation into NOD/SCID mice. Her studies provided evidence that CD34+ cells from PCB can be expanded in vitro for several months in serum-containing cultures. Combinations of early acting cytokines (Flt-3 ligand, IL-6, Tpo, SCF, IL-3) and the absence of stroma were advantageous by administering human cytokines post-transplantation of hematopoietic cells result in a short lifespan of only several weeks to a few months in these mice, precluding studies of long-term (>4-6 months) engraftment. While allowing semiquantitative assessment of primitive normal and leukemic human stem cells (SCID repopulating cells; SRC), the model has several limitations. First, malignancies of the lymphohematopoietic system and infections following radiation and transplantation of hematopoietic cells result in a short lifespan of only several weeks to a few months in these mice, precluding studies of long-term (>4-6 months) engraftment. Second, human HSC function is tested under xenogeneic conditions that don't mirror the homologous post-transplant environment in man or animal. Some investigators try to mitigate these disadvantages by administering human cytokines post-transplant or by placing human bone marrow fragments subcutaneously in order to facilitate homing and to support human HSC function in the murine background. Third, neutrophil and platelet recovery cannot be assessed because the proportion of human cells in circulation is very low (typically <3%).

In a follow-up study, stem cell function following expansion was tested in sublethally irradiated NOD/SCID mice that were injected with either 20,000 uncultured CD34+ cells or the progeny of 20,000 CD34+ cells that had been expanded for several weeks in the presence of combinations of the aforementioned cytokines (Flt-3 ligand and Tpo; SCF, Flt-3 ligand and Tpo; IL-6, SCF, Flt-3 ligand and Tpo). No engraftment was observed with uncultured cells whereas ex vivo expanded cells engrafted nearly all the mice, although to variable degrees. The frequency of SRC in fresh CD34+ PCB cells was found to be 1 in 29,800 whereas,
after 7–8 weeks of expansion, the frequency of SRC was 1 in 471 initial CD34+ cells as calculated by Poisson statistics.

Bathia et al. expanded and transplanted CD34+CD38− cells from PCB into NOD/SCID mice and reported 4- and 10-fold increases in CD34+CD38− cells and CFC, respectively, as well as a 4-fold increase in SRC when cells were cultured for 4 days with SCF, Flt-3 ligand, G-CSF, IL-3 and IL-6. However, after 9 days of culture, all SRC were lost.70

Similar results were reported by Mobest et al. who expanded CD34+ cells from cytokine-mobilized PB with Flt-3 ligand, SCF and IL-3. Cells expanded past day 4 lost their engraftment potential in NOD/SCID mice even though an increase in the numbers of LTC were seen.71

In contrast to these findings, Albella et al. reported that ex vivo expansion of PB CD34+ cells with SCF, IL-11 and Flt-3 ligand preserved, but did not enhance, the ability of the grafts to repopulate lymphohematopoiesis in NOD/SCID mice.72 Similarly, Guenecha et al. reported the maintenance of engraftment potential of PB CD34+ cells expanded with either IL-3, IL-6 and SCF or IL-11, SCF and Flt-3 ligand with a significant delay in early (day 20) marrow reconstitution when expanded cells were used.73

Other studies of ex vivo expansion of human PB,51,32,55,62,74–76 have mostly employed early-acting cytokines such as Flt-3 ligand, IL-11, IL-3, Tpo, SCF and IL-6. While there has been amplification of committed progenitor cells as well as amplification or maintenance of cells with surrogate in vitro HSC characteristics, the studies have not assessed stem cell function in vivo following expansion. Therefore, the results of these studies must be interpreted cautiously.

Other transplantation models have been developed to better understand HSC engraftment kinetics following ex vivo cell expansion and transplantation including syngeneic mouse transplants that allow a distinction between donor and recipient-derived hematopoiesis. Such models employ mice that are either congenic for the white blood cell marker CD45.1 or CD45.2, or sex markers, and the models were established to be able to determine percent donor engraftment or to quantitate stem cell-like cells. In these models, the stem cell source used for transplantation is mostly BM because of the difficulty obtaining cord or perinatal blood.

Long term HSC function, defined as the ability of a cell to establish and sustain multilineage hematopoiesis for at least 6 months post-transplantation, has been tested mostly in competitive repopulation assays and/or by applying sublethal myeloablative regimens. The injection of sufficient numbers of cells allows for the survival of all animals, and donor chimera can be followed over time. However, with regard to ex vivo expansion, these models fall short of the rigors of clinical transplantation. The transplantation of limited numbers of HSC in comparison to transplantation of their expanded progeny following lethal radiation is more indicative of the radioprotective capacity of the manipulated cells.

To date, the evidence for ex vivo expansion of HSC responsible for multilineage long-term engraftment is inconclusive, but there are data that support ex vivo expansion as a means to accelerate the speed of engraftment and to provide short-term radioprotection. Muench et al. claimed that expansion of BM cells (harvested one day after administration of 5-fluorouracil [FU] in order to enrich for more primitive non-cycling precursors) with IL-1 and SCF greatly reduced the number of transplanted cells needed to provide radioprotection monitored 30 days post-transplantation, and these expanded cells also showed better survival in secondary transplants.77,78 This conclusion was based on the observation that mice transplanted with $5 \times 10^3$ expanded cells survived the first three weeks after transplantation whereas results from an earlier study showed that mice receiving $1 \times 10^5$ fresh cells all died within the first two weeks. In secondary transplants, the combined survival of recipients (6 of 32) transplanted with BM from donors that had originally received different numbers of expanded cells ($1 \times 10^3$, $1 \times 10^4$, $5 \times 10^3$ or $1 \times 10^3$) was compared to the survival of recipients (0 of 8) transplanted with BM from mice that originally had received $1 \times 10^6$ unmanipulated cells. The results suggested that the stem cell properties (radioprotection and ability to successfully transplant secondary recipients) were equivalent between the two populations of cells.

These studies also were among the first to investigate the engraftment potential of ex vivo-expanded cells. However, the first study77 was carried out with only 3 mice per group and did not account for the possibility of death caused by infection rather than bone marrow failure, especially when death occurred within the first two weeks. Nevertheless, these studies provided the first evidence that the rate of neutrophil, platelet and red cell recovery might be accelerated following transplantation with expanded cells.

Positive results with regard to radioprotection were reported by Holyoake et al. with transplantation of unseparated BM cells expanded with SCF and IL-11.79 Mice receiving ex vivo-expanded cells showed significant improvement in survival at day 30 compared with those receiving unmanipulated cells, but no differences were observed at three months and nine months post-transplantation or two months following secondary transplantation. However, ex vivo-expanded BM cells supported quaternary transplants in contrast to unmanipulated cells that only sustained secondary and ter-
tary transplants. When transplanted in cell doses above threshold for engraftment, BM cells expanded \textit{in vivo} gave significantly more rapid hematopoietic recovery.

Ratajczak \textit{et al.} showed faster platelet recovery after BM cells were primed for 48 hours with Tpo, SCF, IL-1a and IL-3—a combination that gave a substantial increase in Meg-CFC numbers.\textsuperscript{80} Szilvassy \textit{et al.} also reported accelerated platelet recovery following transplantation of a highly enriched cell population (Thy-1$^{lo}$, Sca-1$^{-}r$, H-2K$^{h}$) purified from BM one day after 5-FU-treatment and cultured with IL-3, IL-6, G-CSF and SCF for 7 days.\textsuperscript{81} Albella \textit{et al.} also transplanted lethally-irradiated mice with BM cells harvested from 5-FU-treated donors and expanded in culture with IL-6 and IL-3, IL-6, G-CSF and SCF for 7 days.\textsuperscript{81} They observed faster recovery of peripheral blood leukocytes as well as femoral and splenic GM-CFC numbers. However, in animals transplanted with cells expanded with SCF and IL-3, a detrimental effect on long-term engraftment was observed 300 days post-transplantation when compared with animals transplanted with unmanipulated cells.

Peters \textit{et al.} showed a profound engraftment defect with murine BM cells expanded with SCF, IL-3, IL-6 and IL-11 in two competitive repopulation studies using either CD45.1/CD45.2 markers (C57 Bl/6J) or sex markers (BALB/c).\textsuperscript{83} In the first model, \textit{ex vivo}-expanded CD45.2 cells that competed with unmanipulated CD45.1 cells led to 4% ± 2% engraftment at 22 weeks post-transplant for the cytokine-exposed cells. Unmanipulated CD45.2 cells when competed with cultured CD45.1 cells led to 93% ± 2% engraftment at 22 weeks. In the second model, male cells cultured with cytokines provided 2% ± 1% engraftment at 14 weeks post-transplant, whereas non-cultured cells led to 95% ± 2% engraftment. Two other studies, in which limited numbers of either purified Sca-1$^{-}Lin^{-}$ and Thy1$^{-}Lin^{-}$ murine BM cells or their expanded progeny were transplanted into lethally-irradiated recipients, found a significantly decreased survival rate in mice transplanted with expanded cells.\textsuperscript{84,85}

Similar studies in larger animals also have shown no benefit of \textit{ex vivo} expansion prior to transplantation. Mobilized peripheral blood CD34$^{+}$ cells, expanded in the presence or absence of an autologous stromal monolayer with IL-3, IL-6, SCF, ±Flt-3 ligand prior to transplantation into rhesus monkeys, generated lower short-term and long-term engraftment in all animals compared with unexpanded cells.\textsuperscript{86} Expansion of mobilized baboon PB CD34$^{+}$ cells on porcine microvascular endothelial cells, followed by reinfusion after myeloablation, resulted in delayed platelet and neutrophil recovery in comparison to reinfusion of freshly isolated, unexpanded CD34$^{+}$ cells, but did provide a graft capable of rescuing a myeloablated autologous host.\textsuperscript{87} Abkowitz \textit{et al.} demonstrated in a feline autologous BMT model that the transplantation of five times as many cells as needed for hematopoietic reconstitution, generated by \textit{ex vivo} expansion with G-CSF, Epo and SCF, resulted in impaired stem cell function; only two of nine cats engrafted.\textsuperscript{88}

\textbf{Ex \textit{Vivo} Expansion and Clinical Transplantation}

\textit{Cord blood}

The first report of the use of \textit{ex vivo}-expanded PCB cells in transplantation was from Kurtzberg \textit{et al.}\textsuperscript{89} This Phase I trial included 28 patients with leukemias or non-malignant diseases. Aliquots of PCB (100–300 × 10$^{6}$ cells) were expanded with PIXY 321 (an IL-3/GM-CSF fusion protein), Flt-3 ligand and Epo over a period of 12 days in an automated, continuous perfusion culture device (Aastrom Biosciences). The expanded cells were then transplanted as a boost to the conventional graft (1.5–2 × 10$^{7}$ cells). No enhancement in the rates of neutrophil or platelet engraftment was seen. However, recipients of \textit{ex vivo}-expanded cells had a superior 100 day survival when compared to historical controls receiving similar unmanipulated PCB stem cell preparations. Although the desired outcome of more rapid hematological recovery was not achieved, the study showed that the administration of expanded cells was safe, at least in the short term.

\textit{Mobilized peripheral blood}

Only recently has PCB transplantation become an increasingly popular source of stem cells, whereas the idea to shorten neutrophil and platelet recovery emerged far earlier with hematopoietic cell-supported high dose chemotherapy trials as a treatment option for various malignancies. Therefore, there is more information available on the clinical use of \textit{ex vivo}-expanded mobilized PB cells. As a sufficient number of endogenous HSC survive non-myeloablative treatment regimens, there was less concern about the long-term function of \textit{ex vivo}-expanded cells. Ex \textit{vivo} expansion strategies were moved quickly from the experimental stage into the clinic, with the idea that the generation of large numbers of mature progenitor cells would provide more rapid hematopoietic recovery following infusion. Brugger \textit{et al.} were the first to report the transplantation of \textit{ex vivo}-expanded cells alone for autografting in patients following high-dose chemotherapy.\textsuperscript{90} A fixed number (11 × 10$^{6}$) of mobilized CD34$^{+}$ PB cells were expanded with SCF, IL-1, IL-3, IL-6 and Epo in large-scale liquid culture. Infusion of the expanded progeny rapidly restored hematopoiesis, and short-term engraftment was comparable but not superior to historical controls. The contribution of the \textit{ex vivo}-expanded cells
to long-term engraftment is impossible to determine, however, given that no cell marking was used to allow the contribution of the ex vivo-expanded cells to be distinguished from endogenous stem cell recovery.

Ex vivo expansion of mobilized PB CD34+ cells as well as BM cells and their use as the sole graft was reported, respectively, by Alcorn et al.,91 who used the same cytokine combination as Brugger, and Stiff et al.,92 who used PIXY-321, Flt-3 ligand and Epo. Transplantation of the ex vivo-expanded mobilized PB cells resulted in rapid hematopoietic recovery in 3 of 4 patients but failed to provide stable long-term engraftment in at least two patients.91 Transplantation of the expanded autologous BM cells into a group of 15 patients undergoing treatment for breast cancer provided stable engraftment, suggesting — although not proving — that ex vivo-generated cells might be capable of providing long-term engraftment.92 Several other groups administered ex vivo-expanded CD34+ cells, together with a sufficient number of unmanipulated CD34+ cells, following high-dose chemotherapy for solid tumors.93–96 These studies showed no accelerated hematopoietic recovery, but demonstrated the feasibility of the expansion procedures and the clinical safety of the approach.

In contrast, McNiece et al. reported significantly accelerated neutrophil recovery in patients treated for breast cancer with high-dose chemotherapy when expanded cells alone or together with uncultured CD34+ cells were infused and compared to historical controls reconstituted with unmanipulated CD34+ cells alone.97 Cell expansion was achieved using SCF, G-CSF and Tpo.

The choice of cytokines and culture conditions may be critical in the different clinical outcomes in such studies. A well recognized advantage in culturing either BM cells or mobilized PB cells before autologous reinfusion for therapy of malignancy is the elimination of contaminating tumor cells.98–100 In addition to possibly accelerating speed of neutrophil recovery, ex vivo expansion provides an environment for efficient purging of tumor cells as these cells have been shown not to be viable under culture conditions fostering hematopoietic cell expansion.

Summary and Future Direction

Most of the animal studies designed to mimic or predict future clinical application of HSC ex vivo expansion strategies (see above) showed a loss of engraftment potential and long-term repopulation capacity under the conditions employed. However, once enough cells were transplanted to pass the threshold for survival, the infusion of expanded cells appeared to accelerate hematopoietic recovery, at least in the murine transplant model. This effect may very well be dependent on the combination of cytokines used for expansion. For mobilized PB and BMT, time to neutrophil engraftment is highly correlated with the number of CD34+ cells infused per kg body weight, and for PCB transplantation with the number of nucleated cells and CFC per kg body weight.38,41,42,44,45 In the case of PCB, the development of strategies for ex vivo expansion of HSC and progenitor cells may be beneficial by shortening the time to hematological recovery, thus reducing the likelihood for infection and the need for continued platelet transfusion. For PB or BM, the use of cytokine-expanded cells may be able to reduce the number of leukapheresis collections needed to obtain sufficient numbers of CD34+ cells or the amount of BM harvested from donors, respectively. The modulating effect of the diverse combinations of cytokines on the degree of expansion as well as the impact of ex vivo culture conditions on the engraftment ability and longevity of HSC remain to be clarified. For near-term clinical applications, the most promising concept is the expansion of aliquots rather than total grafts. Injection of the expanded aliquot in addition to the conventional graft should provide a sufficient number of unmanipulated HSC to guarantee long-term engraftment while also providing an increased number of cells capable of accelerating hematopoietic recovery.

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