tiary transplants. When transplanted in cell doses above threshold for engraftment, BM cells expanded *ex vivo* gave significantly more rapid hematopoietic recovery.

Ratajczak *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.[80] Szilvassy *et al.* also reported accelerated platelet recovery following transplantion of a highly enriched cell population (Thy-1 lo, Sca-1+, H-2Khl) purified from BM one day after 5-FU-treatment and cultured with IL-3, IL-6, G-CSF and SCF for 7 days.[81] Albella *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 or SCF and IL-3.[82] 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 *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).[83] In the first model, *ex vivo*-expanded CD45.2 cells that competed with unmanipulated CD45.1 cells led to 4% $\pm$ 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% $\pm$ 2% engraftment at 22 weeks. In the second model, male cells cultured with cytokines provided 2% $\pm$ 1% engraftment at 14 weeks post-transplant, whereas non-cultured cells led to 95% $\pm$ 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.[84,85]

Similar studies in larger animals also have shown no benefit of *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, $\pm$Flt-3 ligand prior to transplantation into rhesus monkeys, generated lower short-term and long-term engraftment in all animals compared with unexpanded cells.[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.[87] Abkowitz *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 *ex vivo* expansion with G-CSF, Epo and SCF, resulted in impaired stem cell function; only two of nine cats engrafted.[88]

Ex Vivo Expansion and Clinical Transplantation

Cord blood

The first report of the use of *ex vivo*-expanded PCB cells in transplantation was from Kurtzberg *et al.*[89] This Phase I trial included 28 patients with leukemias or non-malignant diseases. Aliquots of PCB ($100–300 \times 10^6$ 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 ($1.5–2 \times 10^7$ cells). No enhancement in the rates of neutrophil or platelet engraftment was seen. However, recipients of *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.

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 *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 *ex vivo*-expanded cells. *Ex 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 *et al.* were the first to report the transplantation of *ex vivo*-expanded cells alone for autografting in patients following high-dose chemotherapy.[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 *ex vivo*-expanded cells