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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