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-IC was seen.[71]

In contrast to these findings, Albella *et al.* reported that *ex vivo* expansion of PCB $CD34^+$ 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 PCB $CD34^+$ 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 PCB,[51,52,55,62,74–76] 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 chimerism 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 5×10^3 expanded cells survived the first three weeks after transplantation whereas results from an earlier study showed that mice receiving 1×10^5 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 (1×10^6, 1×10^4, 5×10^3 or 1×10^3) was compared to the survival of recipients (0 of 8) transplanted with BM from mice that originally had received 1×10^6 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 study[77] 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 occured 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 quarternary transplants in contrast to unmanipulated cells that only sustained secondary and ter-