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.[14,51–58] 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.[59–62] 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 eythropoietin (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[62–64] as well as changes in homing features associated with loss of long-term repopulating capacity.[65]

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.[66] 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.[61,67] 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.[68,69] 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 essential. All cultures were supported by the addition of 10% fetal calf serum or 10% pooled normal human serum. After only 2 weeks—and, therefore, from a clinical perspective potentially interesting—Flt-3 ligand and Tpo, in the presence or absence of SCF, expanded not only CFC but also LTC-IC approximately 100-fold, demonstrating not only expansion of committed progenitor cells but also cells with stem cell-like characteristics with sustained proliferation up to 20 weeks. The addition of IL-3 had a detrimental effect on LTC-IC production beyond week 5.

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,