# Symposia

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## STEM CELLS AND GROWTH FACTORS: WHAT REGULATES WHAT?

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CSFs are a class of growth factors in that they are necessary for the survival, proliferation and maturation of blood cells without being nutrients. However, their exact role in the control of very early stages or baseline hemopoiesis remain unclear. *In vivo*, peripheral blood cell numbers are often inversely related to the concentration in serum of specific growth factors: circulating monocytes and M-CSF, granulocytes and G-CSF, platelets and thrombopoietin. *In vitro*, the considerable increase in the numbers of lineage-committed CD34+ cells following growth factor treatment usually lead, by promoting differentiation, to a decrease or disappearance of the most primitive lineage uncommitted CD34+ population in which the true self-renewing stem cell likely resides. Until more is known about these most primitive cells, it may prove easier to control *ex vivo* the differentiation of maturing blood cells by appropriate combinations of cytokines (e.g. to generate specific subsets of immunocompetent cells with anti-tumor or anti-viral activity) than to truly expand hemopoietic stem cells. However, the *ex vivo* expansion of progenitor cells to enhance recovery is feasible.

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## *EX VIVO* MANIPULATION AND CLINICAL USE OF PERIPHERAL BLOOD PROGENITOR CELLS IN CANCER PATIENTS

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Peripheral blood progenitor cells (PBPCs) are being used increasingly for autologous stem cell transplantation after high-dose chemotherapy in both solid tumors and hematological malignancies.

To minimize tumor cell contamination of PBPC collections, we have reduced the total volume of blood processed from the patients, followed by expansion of PBPCs *ex vivo*. We have shown that a combination of SCF, IL-1$\beta$, IL-3, IL-6 and Epo mediates the *ex vivo* expansion of clonogenic progenitor cells of various hematopoietic lineages and, moreover, that primitive hematopoletic stem cells, as quantitated by long-term culture initiating cells (LTC-IC), could be preserved. These preclinical studies suggested that *ex vivo* expanded peripheral blood $CD34^+$cells might be able to mediate both, short-term as well as long-term hematopoietic reconstitution following high dose chemotherapy. In a phase I/II trial, we investigated the transplantation potential of *ex vivo* expanded $CD34^+$ PBPCs in solid tumor patients undergoing high-dose chemotherapy. Ten patients were transplanted with *ex vivo* expanded PBPCs, starting from a fixed number of $1.1 \times 10^7$; peripheral blood $CD34^+$ cells, a cell number which corresponds to less than 1/10th of our standard 2-hour leukapheresis preparation. The study showed that this approach is feasible and that *ex vivo* expanded cells mediate rapid and sustained hematopoietic recovery when transplanted after high-dose VIC-chemotherapy. The reconstitution pattern was identical to that of historical control patients who had been treated with unseparated PBPCs or positively selected peripheral blood $CD34^+$cells. Thus, starting from a small number of peripheral blood $CD34^+$cells, *ex vivo* expanded hematopoietic progenitor cells might offer new prospects for cellular therapy, including a reduced risk for tumor cell contamination, the circumvention of leukapheresis, the potential for *ex vivo* manipulation, as well as the potential for repetitive cycles of high-dose therapy.

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## PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) AND TREATMENT OF NON-HODGKIN'S LYMPHOMA

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Autologous stem cell transplantation (ASCT) has been integrated routinely in the treatment of relapsing or histologically aggressive poor prognosis NHL. Comparison of bone marrow (BMT) (72 pts) with PBPC (78 pts) transplantation showed that PBPC was a safer procedure. Hematopoietic recovery was accelerated for neutrophils and platelets as compared with BMT and was associated with lower toxicity. The engraftment persisted in long-term studies and any difference in failure free survival was reported. Recently the therapeutic efficacy of ASCT with PBPC was evaluated in 60 relapsing follicular lymphoma patients. At 2 years overall survival and failure free survival were 86% and 53% respectively. Survival was not affected by the presence or not of residual bone marrow involvement at the time of collection. Purification of blood product after CD34+ selection is presently under study in order to increase the quality of blood product and will be tested to try to improve clinical results.

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## T-CELL DEPLETED HAPLOIDENTICAL "THREE LOCI" MISMATCHED MARROW TRANSPLANTS BY ADDITION OF T-CELL DEPLETED PERIPHERAL BLOOD PROGENITOR CELLS

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We have reported the successful engraftment of T-cell depleted "three loci" HLA-mismatched transplants in a preliminary series of 17 end-stage leukemia patients (Aversa *et al.*, *Blood* 1994, **84**, 3948;). Total of 35 patients (mean age 22 years, range 2–51) with high-risk leukemia (12 AML, 18 ALL and 5 CML) have been transplanted. Fourteen were in hematological remission at the time of transplant and 21 in chemoresistant relapse. All donors were HLA-haploidentical "three loci" incompatible family members (13 siblings, 21 parents and 1 cousin). Five patients rejected the graft, 3 died from aplasia but 2 achieved a stable engraftment after a second transplant from a different mismatched donor. A neutrophil count greater than $1.0 \times 10^9$/L was reached at a mean of 11 days and platelet count $>25 \times 10^9$/L at a mean of 17 days. Acute GvHD > grade II was observed in 4 of the 21 advanced leukemia (Group I) patients and in 1 of the 14 in remission (Group II) at the transplant. Among the Group I, 4 relapses occurred, 14 patients died from transplant related toxicity and 3 survive event-free at a follow-up of 12, 23, and 25 months post-transplant. In the Group II, one patient relapsed, three died from toxicity and 10 survive event-free ad a mean follow-up of 9 months (range 4–13). This approach should be applied to selected high-risk patients who do not have HLA-matched donors. Outcome depends on the stage of disease at the time of transplant, with very encouraging results achieved at hematological remission.

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## MULTIPLE COURSES OF HIGH-DOSE ALKYLATING THERAPY WITH AUTOLOGOUS STEM CELL SUPPORT

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Since the introduction of autologous blood progenitor cell transplantation, (APCT) bone marrow suppression is no longer dose-limiting after high-dose therapy. We have developed a high-dose chemotherapy regimen 'CTC', that includes cyclophosphamide (6 g/m$^2$), thiotepa (480 mg/m$^2$) and carboplatin (1600 mg/m$^2$) administered over four days. This regimen has been shown to be well-tolerated in over 80 (first) courses and multiple courses could be feasible.

28 patients received 2 courses of CTC, CTC-2 beginning on day 28 after the first APCT. One patient died of sepsis, one developed reversible veno-occlusive disease (VOD). Eleven patients received 3 subsequent courses of CTC. There were three toxic deaths: 1 × sepsis, 1 × VOD, 1 × hemolytic uremic syndrome (HUS). Two other patients developed reversible VOD, one had reversible HUS. While two closely spaced CTC courses are feasible, three subsequent courses are associated with frequent unacceptable organ toxicity. Preliminary data of a phase I/II study in advanced breast cancer suggest that three tightly spaced 'tiny CTC' courses—containing 2/3 of the doses of each of the agents in a standard