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Mobilization and selection of CD34-positive hematopoietic progenitors

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High-dose chemotherapy with autologous hematopoietic progenitor-cell support is increasingly used for the treatment of hematologic malignancies and solid tumors. Over the last few years, the major source of progenitor cells for clinical use has shifted from bone marrow to peripheral blood. The current approaches on peripheral blood progenitor-cell mobilization and collection is examined. The isolation of CD34-positive cells from peripheral blood progenitor-cell grafts for tumor purging in the autologous transplant setting and for T-cell depletion in the allogeneic transplant setting is also discussed.

INTRODUCTION

The use of high-dose chemotherapy followed by autologous hematopoietic progenitor cell support (AHPCS) has increased markedly over the last few years.[1] Moreover, the use of peripheral blood progenitor cells (PBPCs) is rapidly replacing bone marrow as the primary source of hematopoietic support.[2] Durable engraftment of nine years or longer has been produced with PBPCs used to support myeloablative regimens including total body irradiation.[3] PBPCs can be collected without pre-treating the patient in a 'steady-state', or after treatment with growth factors and/or chemotherapeutic agents to 'mobilize' the progenitors from the bone marrow to the peripheral blood. Once collected, the PBPCs can be cryopreserved or manipulated further with positive selection procedures which isolate the progenitors expressing CD34, purged with a variety of biologicals or chemicals, and/or expanded ex vivo prior to cryopreservation or transplantation.

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COLLECTION OF PERIPHERAL BLOOD PROGENITOR CELLS

PBPCs are collected during an out-patient leukapheresis procedure, using a continuous-flow blood cell separator such as the COBE-Spectra, Fenwall CS-3000, or Haemonetics V-50. Approximately 9–14 liters of patient blood are processed during each procedure, which takes 3–4 hours. The vast majority of the processed blood is returned to the patient with a final PBPC volume of approximately 200 milliliters, which is cryopreserved following the collection. Typically a total of 2–4 leukaphereses are performed on consecutive days.

Recently a single large volume (14 liters) leukapheresis has been performed in lieu of multiple procedures.[4,5]

A. Collection of peripheral blood progenitor cells without previous chemotherapy or growth factor treatment (steady state)

Kessinger et al[6] and Williams et al[7] have demonstrated that it is possible to collect enough PBPCs in the steady state to rescue patients after high-dose chemotherapy. The negative aspects of this approach

Table 1 Engraftment following transplantation of peripheral blood progenitor cells collected in steady state: results of selected studies

Mobilization regimen	Number of phereses	Median days to ANC ≥ 500/μl	Median days to platelets > 20 000–50 000/μl	Ref
None	8	22	23	6
None	6	15	43	7

Abbreviations: ANC, absolute neutrophil count; Ref, reference.

include the requirement for large numbers of leukapheresis (4–8), and delayed platelet recovery which characterizes engraftment produced by steady state PBPCs.[6-8] Results from representative studies are summarized in Table 1.

B. Collection of peripheral blood progenitor cells after mobilization with chemotherapy

During the recovery phase from non-ablative chemotherapy administration, there is a well-documented increase in the number of circulating hematopoietic progenitors.[9, 10] Chemotherapy-induced mobilization occurs after administration of non-myeloablative high-dose chemotherapy with, for example, single agent cyclophosphamide[11, 12] or etoposide.[13] The phereses are performed beginning with the first day that the leukocyte count recovering from chemotherapy reaches 1–2 × 10^9/L. Following chemotherapy mobilization, generally 3–4 phereses are performed. Chemotherapy-mobilized PBPCs have been shown to contain a significantly higher number of myeloid precursors measured as colony forming-unit granulocyte-macrophage (CFU-GM) than those collected in the steady state.[12] To et al reported that patients who received chemotherapy-mobilized PBPCs had a significantly faster recovery of both granulocytes and platelets (11 and 13.5 days, respectively), than patients who received either autologous marrow support (22 and 32 days, respectively) or allogeneic marrow support (24.5 and 33 days, respectively).[12] Other studies reported similar data with hematopoietic recovery occurring approximately one week earlier when chemotherapy-mobilized PBPCs are compared to marrow support.[14] The undesirable aspects of chemotherapy mobilization include the lack of standardization with respect to the chemotherapy regimens employed. The large inter-patient variability in time to bone-marrow recovery after administration of the mobilizing regimen make it difficult to predict when to schedule the leukapheresis. Finally, the chemotherapy mobilization regimens can be associated with nadir sepsis, which have rarely resulted in patient deaths.[12] Results from representative studies of chemotherapy-mobilized PBPCs are summarized in Table 2.

C. Collection of peripheral blood progenitor cells after mobilization with growth factors with or without concomitant chemotherapy

Several recombinant hematopoietic growth factors have been shown to increase the number of hematopoietic precursors circulating in peripheral blood, thereby providing an alternative to chemotherapy-induced PBPC mobilization.[15, 16] PBPCs are most commonly mobilized with granulocyte colony-stimulating factor (G-CSF)[17-19] or granulocyte–macrophage colony-stimulating factor (GM-CSF).[20-23] Other growth factors which have been used, either alone or in combination with G-CSF or GM-CSF, include interleukin-3 (IL-3),[24] PIXY-321, erythropoietin, and stem-cell factor (SCF).[25] Typically, the growth factor is administered for 6–14 days, with 2–6 leukapheresis performed on the last few consecutive days of therapy (i.e. days 5, 6, and 7 of a 7-day growth-factor course). Growth factors have advantages over chemotherapy for mobilization of PBPCs. The time course for increased circulating neutrophils is more predictable and so it is logistically easier to schedule the leukapheresis. As there is no nadir, the risk of sepsis is markedly reduced. When compared with the use of bone marrow or non-mobilized PBPCs, studies which employed chemotherapy- or growth-factor-mobilized PBPCs demonstrated faster time to platelet recovery. An example is the study reported by Sheridan et al, where the addition of G-CSF mobilized PBPCs to bone marrow after

Table 2 Engraftment following transplantation of peripheral blood progenitor cells collected after mobilization with chemotherapy: results of selected studies

Mobilization Regimen	Number of Phereses	Median days to ANC ≥ 500/μl	Median days to platelets 20, 000–50, 000/μl	Ref
Chemotherapy	NR	17	38	10
Chemotherapy	3–6	11	13.5	12
Chemotherapy	5	13	10	13

Abbreviations: ANC: absolute neutrophil count; Ref, reference.

high-dose chemotherapy shortened the time to a platelet recovery from 39 to 15 days.[17] Other investigators have demonstrated similar improvements in the time-to-platelet recovery in addition to a 2–5 days, improvement in time to neutrophil recovery.[26, 27] The reason platelet recovery is so much faster with mobilized PBPCs compared to marrow has not been definitively explained. It may be due to the infusion of a higher number of progenitors as well as the increased mobilization of megakaryocytic-derived cells.

Strategies to improve PBPC mobilization include the use of chemotherapy plus growth factor[28, 29] combinations of growth factors[25, 30, 31] or newer growth factors such as SCF.[25]

Animal studies demonstrating that SCF stimulates the differentiation of primitive hematopoietic progenitors triggered a number of clinical studies to evaluate its use in PBPCs mobilization.[32] Briddel et al demonstrated that low doses of SCF in combination with G-CSF synergistically increases the number of PBPCs, CFU-GM, and the more primitive high proliferative potential colony-forming cells (HPP-CFC).[33] Based on these animal data, phase I-II clinical trials were performed.[34] Glaspy et al showed that the combination of SCF (5–20 ug/kg) with G-CSF (10 ug/kg/d) increased the number of MNC, CFU-GM and CD34-positive cells in PBPC products when compared to either G-CSF or SCF alone.[35] The use of SCF was associated with local injection site reactions, but not with generalized adverse side-effects. These preliminary clinical studies suggest that the use of SCF in combination with G-CSF may reduce the number of leukaphereses required to collect PBPCs for transplantation. A randomized phase III study of G-CSF versus SCF + G-CSF to confirm these results has been initiated.

Results of selected studies using growth factors with or without chemotherapy for mobilization are summarized in Table 3.

ISOLATION OF CD34-POSITIVE HEMATOPOIETIC PROGENITORS

One of the initial rationales for using PBPC instead of bone marrow for autografting was that this technique could be used in patients with their bone marrow involved with tumor. Although PBPC fractions may have less tumor than bone marrow, several studies over the last five years have shown that breast cancer, non-Hodgkin's lymphoma, Hodgkin's disease and neuroblastoma cells are detected in the peripheral blood or PBPC collections of 10–78% of the patients.[28, 35–38] Studies where autologous hematopoietic cell products were gene-marked suggest that the tumor cells contained in the graft are associated with relapse in patients with neuroblastoma,[39] acute leukemia[40] or chronic myeloid leukemia.[41] Furthermore, several studies using different purging methods, such as chemicals or monoclonal antibodies, suggest that elimination of contaminating tumor cells from the graft improves the outcome after high-dose chemotherapy.[42–44] These purging methods often produce delayed engraftment, increasing the patient's risk of complications associated with the myelosupression.[45] Investigators have therefore focused on developing methods to select autologous hematopoietic precursors to support high-dose chemotherapy. In 1986, Andrews et al reported that the monoclonal antibody 12.8 reacted with the CD34-positive subset of hematopoietic cells including the CFU-GM and long-term culture initiating cells (LTC-IC).[46] Berenson et al

Table 3 Engraftment following transplantation of peripheral blood progenitor cells collected after mobilization with growth factors with or without chemotherapy: results of selected studies

Mobilization regimen	Median number of phereses	Median days to ANC ≥ 500/ul	Median days to platelets > 20 000–50 000/ul or platelet tranfusion independence	Ref
G-CSF*	3	9	15	17
G-CSF*	3	11	12	19
G-CSF	3	11	10	19
G-CSF	4	10	13	26
G-CSF	3	12.7	13.3	27
GM-CSF	4	14	12	20
GM-CSF	6	28.5	39	22
IL-3	NR	32	NR	24
SCF	NR	25	38	25
SCF+G-CSF	NR	10	11	25
GM-CSF+CT*	2–4	9.1*	10.7*	21
GM-CSF+CT*	NR	NR	10	23
G-CSF+CT	3	12	12	29

Abbreviations: ANC, absolute neutrophil count; Ref, reference; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; SCF, stem cell factor; IL-3, interleukin 3; CT, chemotherapy; * mean values.

*These patients received autologous bone marrow together with peripheral blood progenitor cells.

Table 4 Allogeneic peripheral blood progenitor cells transplants

Ref	N	Dose of G-CSF (ug/kg/d)	Days to platelets > 20 000 mm3 (median)	Days to ANC> > 500 mm3 (median)	AGVHD
57	8	16	10.5	14.5	3/8
58	9	12	12	9	3/9
59	8	5–10	19.5	15.5	5/8

Abbreviations: Ref, reference; N, number of patients; G-CSF, granulocyte colony-stimulating factor; ANC, absolute neutrophil count; AGVHD, acute graft-versus-host disease.

reported that concentrates of CD34$^+$ cells enriched by an avidin–biotin immunoadsorption method employing 12.8 could-successfully engraft lethally irradiated baboons[47] and a small number of patients with neuroblastoma or breast cancer.[48] A larger study involving more than 150 patients with breast cancer demonstrated that CD34$^+$ cells isolated from marrow growth factor-mobilized PBPC are capable of reconstituting hematopoiesis after high-dose chemotherapy.[49] Engraftment rates were comparable to patients who received unmanipulated hematopoietic cell grafts.[19] Immunohistochemical staining for breast cancer was performed on all grafts before and after CD34-selection.[50] The disease-free survival (DFS) of the first 47 Stage IV patients with immunohistochemical evidence of breast cancer in the graft was analyzed. Thirteen patients had immunohistochemically negative hematopoietic fractions after CD34$^+$ selection, and their DFS is 45%. In contrast, the 34 patients that had fractions remaining positive despite CD34-selection, had a disease-free survival of only 13% (P=0.035). A multi-variate analysis showed that the purification of a graft to negativity and enrolment on a Phase II study as opposed to Phase I studies were the only two covariates which independently predicted for a significantly better DFS.[49]

More recently, other groups have reported clinical results using the immunoadsorption technique to isolate CD34$^+$ cells in patients with non-Hodgkin's lymphoma, [51] and multiple myeloma.[29] Gorin et al transplanted 15 patients with NHL using CD34$^+$ bone marrow. Nine of fourteen marrow samples tested had evidence of NHL by polymerase chain reaction (PCR) before selection: eight of them became negative after purification. Schiller et al treated 37 patients with advanced multiple myeloma with high-dose busulfan and cyclophosphamide followed by CD34-selected PBPC rescue.[29] Median time to engraftment was comparable to that obtained with the use of unselected PBPCs. A 2.7 to > 4.5 log reduction in the number of contaminating myeloma cells was demonstrated following the selection process. A randomized study of autologous unselected vs CD34$^+$ PBPCs in patients with multiple myeloma is currently ongoing.

Other positive selection methods involve immunomagnetic separation and high-speed flow cytometry.[52] Civin et al reported results in eight children with solid tumors that received purified CD34$^+$ cells obtained by immunomagnetic separation of the bone marrow, that were cryopreserved and then later infused following high-dose chemotherapy. Engraftment rates in this study were comparable to those obtained using unselected bone marrow.[53]

Miltenyi et al developed a magnetic-cell separator that uses paramagnetic nanoparticles as the solid phase for collection of the target CD34$^+$cells.[52] Preclinical studies have shown that bone marrow and PBPC fractions with 80–95% of CD34$^+$ cells can be routinely obtained. Clinical studies with this device will be initiated soon.

Tricot et al reported their preliminary clinical results using a high-speed cell sorter to separate CD34$^+$ cells from PBPC products of patients with multiple myeloma undergoing a double autologous transplant.[54] The engraftment rates for the first transplant in the first three patients appeared to be successful. Significant depletion of myeloma cells from the unmanipulated PBPC fraction was demonstrated.

TRANSPLANTATION OF ALLOGENEIC PERIPHERAL BLOOD PROGENITOR CELLS

In 1989, Kessinger et al used allogeneic PBPC support in a patient with acute lymphoblastic leukemia.[55] More recently, several groups reported their experience with transplantation of PBPCs from allogeneic donors.[56–61] All these studies used G-CSF-mobilized PBPCs at doses ranging from 5–16 μg/kg/d. Their results suggest that time-to-platelet engraftment is faster than that of historical controls receiving bone-marrow support. No evident increase in the incidence of acute graft-versus-host disease (GVHD) was seen in these studies. However, Anderlini et al from M D Anderson reported a group of 47 patients that received allogeneic PBPC transplants and that survived at least 100 days.[62] The median follow up for the group was 7 months. The actuarial rate of clinically extensive chronic GVHD at 1 year was 48%, compared to 35% of 35 historical controls that received allogeneic bone marrow (P=0.015). Also, the clinical presentation of the chronic GVHD apperared to be

different in both groups, with less mouth and lung, and more liver and gastrointestinal manifestations in the PBPC group. A randomized study comparing blood and bone-marrow stem cells transplantation has been initiated.[63] Results from selected studies using allogeneic PBPCs are summarized in Table 4.

A. Use of CD34 selection in allogeneic transplantation

The main cause of morbidity and mortality after allogeneic bone-marrow transplantation is graft-vs-host disease. T-cell depletion of the graft, which can be achieved by a variety of methods, effectively reduces the incidence of GVHD. Since T-lymphocytes do not express the CD34-antigen, CD34-selection methods can potentially be used for T-cell depletion of allogeneic grafts. Link et al reported a pilot study on the use of CD34-selected allogeneic stem cells for rescue after myeloablative treatment in ten patients with hematologic malignancies.[64] The time to engraftment appeared slightly shorter than that for historical controls, and no increase in the incidence of GVHD was evidenced. Using the same immunoadsorption technique, Schiller et al showed that 2–3 logs of T cells can be depleted from allogeneic PBPC grafts.[65] Bensinger et al demonstrated that CD34-selection using immunomagnetic beads can remove a median of 4 logs of T cells from an allogeneic PBPC graft, without compromising engraftment in seven patients.[66] Similar results were obtained by other investigators using different CD34-selection methods.[67–69]

CONCLUSIONS

In the next several years, we will see an increasing use of PBPC support in a variety of clinical settings. These approaches will likely contribute to improvements in the clinical outcome of patients receiving high-dose chemotherapy.

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