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MeSH Terms:

- Antigens, CD34*
- Avidin
- Biotin
- Cell Separation/methods*
- Cryoprotective Agents/adverse effects
- Cryoprotective Agents/administration & dosage
- Dimethyl Sulfoxide/adverse effects
- Dimethyl Sulfoxide/administration & dosage
- Graft vs Host Disease/prevention & control
- Hematopoietic Stem Cell Transplantation*
- Hematopoietic Stem Cells/immunology*
- Human
- Immunosorbent Techniques*
- Transplantation, Homologous

Substances:

- Dimethyl Sulfoxide
- Biotin
- Avidin
- Cryoprotective Agents
- Antigens, CD34

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HEMATOPOIETIC STEM CELL TRANSPLANTATION

Transplantation of CD34+ Hematopoietic Progenitor Cells

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INTRODUCTION

Bone marrow transplantation (BMT) was conceived as an allogeneic procedure for the treatment of hematological disorders, such as aplastic anemia and acute and chronic leukemias (1). The first successful allogeneic transplants were performed in the late 1960s (1), followed 10 years later by the first successful autologous transplants in patients with lymphoma (2). Today, autologous BMT is more common than allogeneic BMT as a result of the lack of HLA-matched donors and the lower morbidity associated with the use of less severe conditioning regimens than are required in the allogeneic setting (3). Bone marrow transplantation is indicated in the treatment of a wide variety of malignancies including various solid tumors, lymphoproliferative diseases, and nonmalignant hematological disorders (4).

Recent studies have demonstrated that hematopoietic progenitor cells may be collected from peripheral blood for use as hematopoietic support during high-dose chemotherapy. These results were made possible by the ability of hematopoietic growth factors, such as G-CSF and GM-CSF, to mobilize large numbers of peripheral blood progenitor cells (PBPC). Patients receiving PBPC exhibit complete hematopoietic recovery between cycles of therapy, with rapid engraftment of both platelets and neutrophils (5,6).

The ability to mobilize PBPC may be combined with techniques to manipulate the cellular composition of the graft in a process sometimes referred to as graft engineering (7). Graft engineering offers the potential for further improving disease-free survival in cancer patients and facilitating emerging therapeutic modalities, such as gene therapy.

PROGENITOR CELL SELECTION

Autologous BMT is used more frequently as an adjunct to chemotherapy (8,9). Given the increasing pressure to decrease costs, there has been an impetus to find ways to hasten engraftment and reduce the complications that ac-

company transplant. Many sites monitor patients continuously during the first 24 hr postinfusion owing to the potential for a variety of infusional toxicities (10–16). These complications are an indirect result of the need to cryopreserve the patient's marrow from the time of harvest until the marrow is infused. There are two causes of these complications.

First, the cells are stored in a cryoprotectant, usually dimethyl sulfoxide (DMSO)(41,42). Patients receive 20–50 ml of DMSO with the infusion of a typical marrow preparation (referred to as a buffy coat). Nausea, vomiting, and diarrhea have been reported as common side effects related to DMSO infusion (13,17,18). In addition, anaphylactoid reactions may occur that range in severity from rashes and flushing to hypotension, bronchospasm, pulmonary ede-ma, and respiratory compromise as a result of histamine release induced by DMSO exposure (13,14,19–21). Cardiovascular side effects have also been reported including hypertension, bradycardia, heart block, and in severe cases, cardiac arrest (19,20). The diuretic effects of DMSO have also been reported to contribute to decreased renal function and to the onset of acute tubular necrosis (22).

The second reason why complications occur as a result of cryopreservation is related to damage that occurs to cells during the freeze-thaw process. The cryoprotectant solution is hyperosmolar (approximately 2000 mOsm). High osmolarity is tolerated by marrow progenitors; however, other cells in marrow or peripheral blood such as granulocytes, platelets, and erythrocytes, lyse under these conditions. Since these cells constitute the vast majority of the cells in a typical buffy coat (23,24), a variety of complications occur when they lyse. Renal damage, and in some cases acute renal failure, may occur as a result of release of hemoglobin from erythrocytes (10). Pulmonary emboli have also been reported as a consequence of the cellular debris from damaged platelets, granulocytes, and other nucleated marrow cells, as well as aggregation of damaged cells (14). Soluble products of cell lysis, such as potassium, calcium, and adenosine, have also been postulated to play a role in the development of bradyarrhythmias (25,26).

Similar toxicities of infusion have been observed with transplantation of PBPC as with marrow transplantation (28), again because of the necessity of storing the PBPC product frozen between harvest and reinfusion. In fact, because of the larger volume of a typical PBPC harvest after buffy coating, almost twice as much DMSO is reinfused into the patient as with marrow transplantation.

To summarize, the majority of the toxicities associated with the infusion of autologous bone marrow or PBPC can be attributed to the large volumes of DMSO and cell debris that are coinfused. Attempts to wash out DMSO or remove cellular debris have been unsatisfactory because of the attendant loss of progenitor cells (17). Furthermore, when attempted, these methods have proven clinically ineffective in reducing the side effects of marrow infusion (14).

In view of these data, reducing the volume of the marrow or PBPC product by selecting only those cells required for reconstitution of the hematopoietic system is clinically important. Studies in mice had shown that reconstitution is optimal when both committed progenitor cells and the more primitive, pluripotent stem cells in marrow are transplanted (29,30).

Another reason for selecting only stem cells and committed progenitors is the risk that tumor cells may contaminate an autologous marrow or PBPC product. These cells may lead to relapse if they are reinfused (31). A number of studies have shown that tumor contamination of marrow and PBPC harvests occurs in patients with various types of solid tumors (32–39) and that the number of contaminating cells may increase following mobilization with cytotoxic drugs in at least some of these patients (40).

In the mid-1980s, investigators identified a 115-kDa glycoprotein, now known as the antigen CD34, that is present on 1–3% of human bone marrow cells, including almost all committed progenitor cells, as well as more primitive progenitors (43–45). However, the antigen is not expressed on mature blood cells or on most types of malignant cells (46). The population of cells identified by expression of the CD34 antigen is heterogeneous. Less than 10% of CD34+ cells in marrow are hematopoietic progenitors (43,45); the remainder are committed to lineages beyond the progenitor stage. The level of expression of CD34 declines with maturation; the more primitive stem cells and progenitor cells express the highest amounts of CD34 antigen, while the more differentiated cells express lower levels of CD34 (43,47–49).

CD34+ cells are also present in peripheral blood, although the percentage is lower than in bone marrow (50–53). However, numerous studies have shown that the percentage of CD34+ cells can be increased dramatically by mobilization with chemotherapy, growth factors, or both (27,53–56). Thus, it is possible to collect sufficient numbers of CD34+ cells for transplantation from peripheral blood using one or more apheresis procedures (see discussion below).

We developed an avidin-biotin immunoadsorption technique that enables isolation of large quantities of cells in a time frame that is feasible for laboratory use, called the CEPRATE® SC Stem Cell Concentration System (57–62). A biotinylated anti-CD34 antibody is used to label bone marrow or peripheral blood cells. These cells are captured by passage through a column containing avidin-

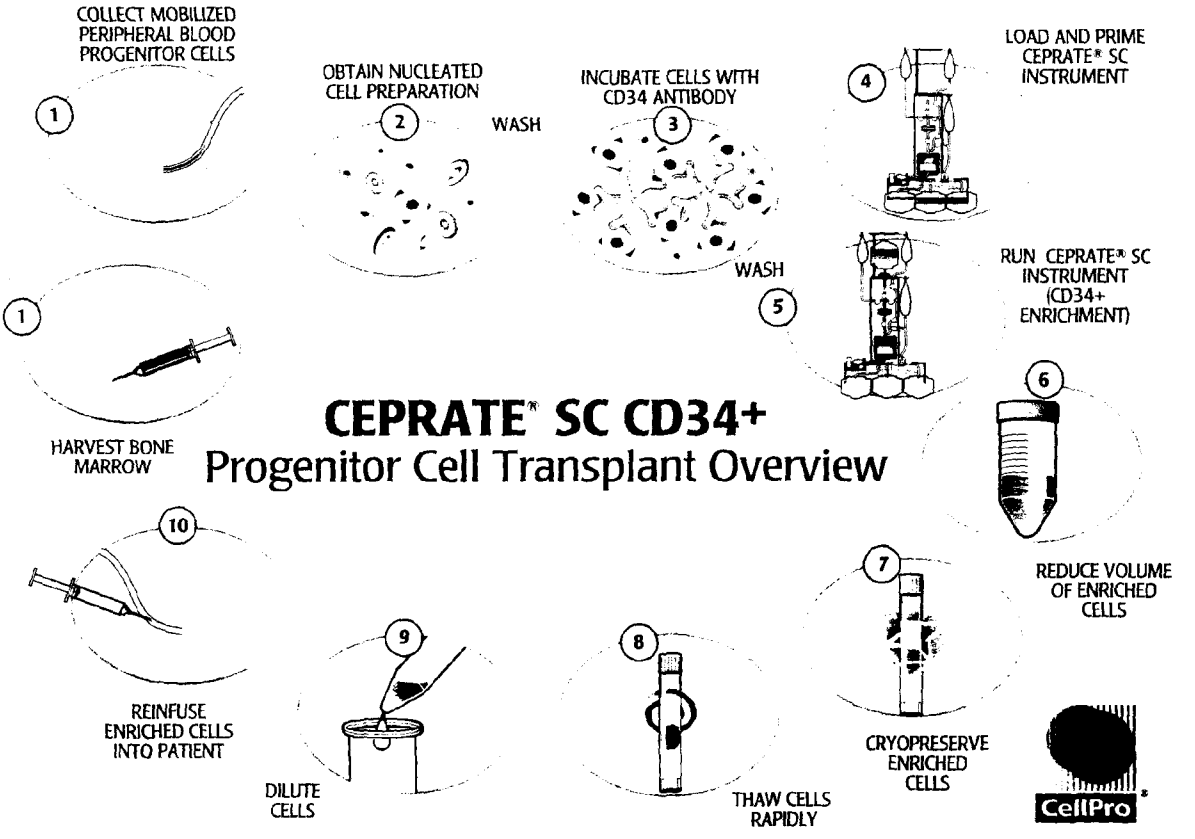

Figure 1. Use of the CEPRATE SC system involves the collection of blood or marrow (step 1), preparation of a nucleated cell preparation from bone marrow (step 2), and incubation of the resulting cell suspension with a biotinylated, mouse monoclonal antibody directed against the CD34 antigen (step 3). The cell suspension is passed through a column of polyacrylamide beads to which avidin has been attached covalently (step 4). The CD34+ cells adhere to the beads, while CD34- cells flow through the column without binding (step 5). The contents of the column are agitated using a magnetically driven stirring bar to release the bound CD34+ cells from the beads; these beads are then washed from the column and collected (steps 5 and 6). The cells are cryopreserved (step 7) until needed, and then they are thawed, diluted, and infused (steps 8–10).

coated beads. The beads are washed to remove unbound cells, and then the bound CD34+ cells are eluted by mechanical agitation (Fig. 1). In a standard marrow buffy coat, 1–2 L of marrow is reduced to a volume of 100–200 ml. After the CEPRATE, the marrow buffy coat is reduced to approximately 5 ml. The result is a 20–40-fold reduction in the amount of DMSO infused into the patient and a 200-fold reduction in the number of nonengrafting cells at risk of lysis.

The avidin-biotin interaction is utilized to bind CD34+ cells labeled with biotinylated antibody to the avidin-coated beads used in the CEPRATE SC system. This interaction has an extremely high dissociation constant ($K_D = 10^{-15}$). There are two important advantages to this high-affinity interaction. First, cells can be selected by continuous flow through the column; this minimizes nonspecific binding of cells to the beads. Second, mechanical agitation of the column bed results in breakage of the link between the cells and the beads at the chain's weakest link: between antibody and antigen, rather than between avidin and biotin. Hence, the cells that are eluted from the column are depleted of antibody. We have measured the amount of residual antibody associated with the CD34+ cells after elution from the column using an enzyme-linked immu-

nosorbent assay. On average, there is less than 80 ng of antibody per infusion (unpublished data). More importantly, there have been no reports of a human anti-mouse antibody (HAMA) or allergic response in the more than 300 patients transplanted to date with CEPRATE-enriched CD34+ cells (unpublished data).

Other methods of positive progenitor cell selection are said to be under development. However, to date, there have been no published reports of successful transplantation of patients with progenitor cells isolated by other methods.

CLINICAL STUDIES WITH CD34+ SELECTED CELLS FROM MARROW

The first clinical study using CD34+ cells selected from marrow was a pilot study performed in 13 cancer patients transplanted after marrow ablative chemotherapy and/or total-body irradiation. The patients received a minimum of 1×10^6 CD34+ cells/kg, and all evaluable patients engrafted (60). On the basis of these results, an automated instrument suitable for laboratory use was developed and a phase I/II clinical study was initiated at the University of Colorado. Forty-three patients with stage II, III, or IV breast cancer were transplanted after marrow ablative therapy (63). All patients were infused with autologous CD34+ cells from marrow ($n = 25$), peripheral blood ($n = 7$), or both ($n = 11$). Forty-one of the 43 patients achieved trilineage engraftment, as defined by recovery of peripheral blood counts. These results were similar to those of historical controls who received marrow buffy coats. Platelet engraftment was delayed in 2 patients: 1 died of recurrent disease and the other remained platelet-dependent even after her backup marrow was infused. All of the grafts were permanent (median follow-up 9 months, longest follow-up 24 months), and no acute infusional toxicity was observed in any of the study participants.

A prospective, randomized, multicenter phase III study was recently completed using the CEPRATE SC Stem Cell Concentration System in patients with advanced breast cancer (64). After marrow harvest, 94 eligible patients were randomized to receive either an infusion of CD34+ cells selected from marrow or a conventional buffy-coated marrow. All patients received 10 μg/kg/day of G-CSF posttransplant. Engraftment, defined as an absolute neutrophil count (ANC) greater than or equal to 500/mm^3 by day 20 posttransplant, was equivalent in both groups of patients. Toxicity, measured by specific cardiovascular endpoints, was significantly decreased in patients receiving CD34+ selected cells.

The question of tumor contamination in patients with breast cancer was studied in some of the patients enrolled in the studies. Wilbur Franklin and colleagues at the University of Colorado devised an immunocytochemical method of detection for breast cancer cells using four monoclonal antibodies and alkaline phosphatase staining. This slide-based assay, which will be published in more detail elsewhere, is sensitive to approximately one tumor cell in one million cells. Using this assay, Franklin evaluated bone marrow samples and apheresis samples for the presence of tumor cells. Briefly, tumor cells were found in 30% (15/50) of bone marrow specimens, and 27% (9/34) of apheresis specimens were positive by this method. Among patients with tumor detectable by this assay in their marrow or peripheral blood, the tumor burden was approximately a log greater in marrow than in the apheresis product. These data are similar to those published by others (34).

Selection of CD34+ cells can have the ancillary effect of depleting CD34- tumor cells from the marrow or PBPC of women with breast cancer. In patients with tumor contamination demonstrable by immunocytochemistry, Franklin found that CD34+ selection depleted tumor cells to less than the assay's limit of detection in 83% (10/12) of PBPC products and 19% (4/21) of marrow products.

CLINICAL STUDIES WITH CD34+ SELECTED PBPC

The CEPRATE SC has been used in investigational studies at a number of sites to select CD34+ progenitor cells from mobilized peripheral blood. At the present time data are available for more than 100 patients treated at six different clinical sites using four different mobilization regimens. Median days to ANC > 500 cells/μl ranged from 10 to 13 days, while median days to platelets >20,000 cells/μl ranged from 10 to 15 days (Table 1). These data suggest that CD34+ PBPC engraft at least as well as marrow-derived CD34+ progenitors or unselected PBPC; however, a randomized, prospective trial will be necessary to assign statistical significance to these observations.

Several interesting observations can be made from the data in Table 1. First, the study by Brugger and Kanz demonstrates it is possible to obtain an engrafting dose of CD34+ PBPC from a single apheresis using a cytotoxic agent and G-CSF to mobilize the patients. Second, there is a wide range in the number of CD34+ cells collected between site and within site. It is unclear at this time whether this is due to underlying differences in patient populations or to the mobilization kinetics.

Table 1

CD34+ Peripheral Blood Progenitor Cell Transplant Trials

Investigator (site)	Disease	Mobilization (# apheresis)	CD34+ cells ($\times 10^6$/kg)[a]	Days to neutrophils >500/μl[a]	Days to platelet recovery[a]
Shpall (Colorado) $n = 55$	Breast	G-CSF (3)	1.6 (0.4–3.9)	12 (10–14)	15 (10–156+)
Spitzer (St. Louis) $n = 6$	Breast	G-CSF (3)	1.3 (0.9–9.8)	10 (9–12)	11 (10–15)
Somlo (City of Hope) $n = 10$	Breast	G-CSF (3)	1.1 (0.3–3.9)	11 (8–17)	14 (6–20)
Brugger, Kanz (Freiburg) $n = 15$	Breast, lung, lymphoma	VIP+ G-CSF (1)	2.2 (0.3–9.5)	12 (8–16)	15 (10–20)
Schiller, Berenson (UCLA) $n = 15$	Multiple Myeloma	CY+ Steroids+ G-CSF (2)	5.2 (1.6–25.5)	13 (11–15)	12 (9–52+)
Watts, Linch (London) $n = 4$	Lymphoma	CY+G-CSF (1)	>1.0	13 (12–22)	14 (9–21)

[a]Median (range).

CLINICAL STUDIES WITH ALLOGENEIC CD34+ SELECTED PROGENITOR CELLS

Allogeneic BMT is generally regarded as the treatment of choice for most serious hematological malignancies. However, the inability to transplant across a major histocompatibility (MHC) barrier limits the application of allogeneic transplants to those patients for whom an HLA-matched or mismatched related donor can be found. There is a significant risk of graft failure (65) and of severe graft-versus-host disease (GVHD; 66–69) when matched unrelated donors or mismatched related donors are used.

Depletion of T cells from the graft has been shown to reduce the risk of severe GVHD (70–72). Since positive selection of CD34+ progenitor cells from marrow results in about three logs of T-cell depletion, we have begun to evaluate the use of CD34+ selection in allogeneic patients. Support for this approach is found in a study by Andrews et al. (73) in which five baboons received CD34+ allogeneic cells selected using the CellPro system together with cyclosporine as prophylaxis for GVHD. All five animals showed cytogenetic evidence of engraftment, while none of the animals developed serious GVHD.

Three clinical sites have started investigational protocols selecting CD34+ cells from allogeneic bone marrow

Table 2

Allogeneic Transplantation:
CD34+ Cell and T-Cell Content After CD34+ Selection of Donor Cells

	CD34+[a]		CD3+[a]		
CD34+ selected cell fraction	%	$\times 10^6$/kg	%	$\times 10^5$/kg	T-cell log depletion[a]
G-CSF mobilized apheresis (total of 2) $n = 7$	78 (68–79)	3.1 (1.6–5.6)	6 (4–13)	2.1 (1.2–7.2)	2.8 (2.5–3.2)
Bone marrow $n = 15$	83 (77–88)	3.5 (1.0–6.5)	2 (1–2)	0.7 (0.2–0.8)	2.8 (2.5–3.5)

[a]Median (range).

or peripheral blood. As can be seen in Table 2, preliminary data are available for 10 donors. A median of 2.8 logs of T-cell depletion was obtained, regardless of the source (marrow or blood) of progenitor cells. Thus, the number of T cells infused into the patient is quite small, probably in the range of 2–7 × 10^5 cells/kg. It is too early yet to determine the clinical effect of infusing CD34+ selected cells on the incidence and severity of GVHD in these patients. Longer follow-up will be required before conclusions can be drawn regarding other clinical endpoints believed to be related to the T-cell content of the graft including engraftment, graft failure, and disease relapse.

Another possible method of T-cell depletion is elutriation (74,75). In elutriation, the marrow is separated into two major fractions containing either large or small cells. Unfortunately, the majority of CD34+ progenitor cells are eluted in lymphocyte-enriched small cell fractions. This fraction is normally discarded because it contains the T cells that cause GVHD. As a result, fewer CD34+ cells are infused in patients receiving marrow transplants that have been elutriated. As a consequence, there have been reports of delayed engraftment and graft failures in these patients.

In an attempt to overcome this problem, the CEPRATE SC system was used by Steven Noga and Richard Jones at the Johns Hopkins University to recover CD34+ cells from the small-cell fractions obtained by marrow elutriation. The CD34+ selected cells are infused into the patient with the large-cell (T-depleted) fraction. Median time to hematological recovery was shorter in patients receiving elutriated and CD34+ selected marrow than in patients receiving unmanipulated or elutriated marrow. In addition, 5-month survival was also better for the group receiving elutriated and CD34 selected marrow. These data are preliminary and additional studies are necessary before any definite conclusions are possible.

SUMMARY

We have developed an avidin-biotin immunoadsorption technique in conjunction with a monoclonal anti-CD34 antibody that is capable of selecting CD34+ progenitor cells from marrow and mobilized peripheral blood. Clinical studies with these CD34+ selected cells have shown that the cells are capable of rapid and durable engraftment. In addition, there is significantly less infusional toxicity to the patient because the volume in which the CD34+ selected cells are contained is much less than that of a typical marrow or apheresis buffy coat.

Selection of CD34+ progenitor cells also offers other potential advantages, including T-cell depletion of allografts and tumor cell depletion of autografts. CD34+ selection can also be used to facilitate other manipulations of marrow and peripheral blood, including gene transfection, ex vivo stem cell expansion, tumor purging, and progenitor cell banking. Future graft engineering studies are expected to clarify these relationships and enable refinement of the graft to the point at which GVHD can be minimized, graft survival maximized, and relapse-free survival prolonged.

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