Flow Cytometry for Clinical Estimation of Circulating Hematopoietic Progenitors for Autologous Transplantation in Cancer Patients

Salvatore Siena, Marco Bregni, Bruno Brando, Nadia Belli, Fernando Ravagnani, Lorenza Gandola, Angelica C. Stern, Peter M. Lansdorp, Gianni Bonadonna, and A. Massimo Gianni

Optimum methods of harvesting circulating hematopoietic progenitors for autologous transplantation to support myeloablative cancer therapy are still uncertain, mostly because of the lack of an assay for marrow-repopulating stem cells. The CFU-GM assay, commonly used indirect indicator of the quality of the graft, is poorly standardized and provides results evaluable only retrospectively. Based on the knowledge that hematopoietic progenitors express CD34 and CD33 differentiation antigens, we developed a dual-color direct immunofluorescence flow cytometry assay with the aim of replacing the CFU-GM assay advantageously. For this purpose, we applied both assays to 157 blood samples obtained daily throughout 20 different recoveries from pancytopenia induced by high-dose cyclophosphamide or etoposide chemotherapy with or without recombinant human GM colony-stimulating factor (rhGM-CSF). The appearance of CD34+ cells in the circulation indicated that hematopoietic progenitors had increased to more than 500 CFU-GM/mL, a level clinically adequate for large-scale harvest by leukapheresis. Total CD34+ cells correlated well with CFU-GM (r = .89), and data could be fitted by a linear regression line described by the equation $y = 388.3 + 64.0x$, where $y = \text{CFU-GM/mL}$ and $x = \text{CD34+ cells per microliter}$. Moreover, in a series of six patients treated with myeloablative chemotherapy/early hematopoietic recovery of marrow functions was predicted more accurately by the number of transplanted CD34+/CD33+ cells than by either total nucleated cells, CFU-GM, CD34+/CD33+ cells, or CD34+/CD33+ cells. Data presented in this article favor clinical use of the CD34+/CD33+ flow cytometry assay to guide harvesting of circulating hematopoietic progenitors for autologous transplantation and contribute to better understanding of the role played by circulating hematopoietic progenitor cell subsets in marrow recovery after myeloablative cancer therapy.

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Hematopoietic progenitor cells circulating in peripheral blood (PB) can be harvested, cryopreserved, and then transplanted to reconstitute the hematopoietic system of cancer patients treated with potentially curative, although myeloablative, high doses of radiotherapy, chemotherapy, or both.1,2 In recent years, autologous transplantation of circulating hematopoietic progenitor cells has attracted considerable clinical interest because of several potential advantages in comparison with bone marrow (BM)-derived hematopoietic progenitors. These advantages include the possibility of (a) circumventing the need of general anesthesia; (b) harvesting even if iliac bones are damaged by previous radiotherapy or infiltrated with malignant cells; and (c) most important, achieving restoration of sustained hematopoietic functions more rapidly than with BM-derived progenitor cells.3,4 The latter advantage, if confirmed as reproducible in controlled clinical studies with large series of patients, will probably represent considerable progress in improving the therapeutic index of high-dose radiotherapy, chemotherapy, or both for selected malignancies.

The quantity and quality of circulating hematopoietic progenitor cells required for successful (ie, rapid, complete, and sustained) hematopoietic reconstitution after myeloablative cancer therapy is still uncertain. Because the marrow-repopulating pluripotent stem cell cannot be identified in humans, the committed progenitor CFU-GM is the most commonly considered indirect indicator of marrow-repopulating ability. The threshold dose of circulating CFU-GM for successful transplantation varies widely in different reports, ranging between 15 and $50 \times 10^6$ CFU-GM per kilogram of patient body weight.1,3 Such large quantities of progenitors can be harvested from PB by a practical number of leukaphereses in a reasonable time, ie, two to five daily leukaphereses in 2 to 5 consecutive days, only at the time of marrow recovery from cancer chemotherapy-induced pancytopenia. This timing is motivated by the occurrence of an outburst of hematopoietic progenitor cells in the circulation, especially when intensive chemotherapy is followed by administration of recombinant human GM colony-stimulating factor (rhGM-CSF).3,4 It is unfortunate that the outburst does not occur invariably in all patients, and its magnitude may vary in each individual case depending on a variety of partly explained factors.2 Presently, the CFU-GM colony assay provides some quantitative information on the hematopoietic progenitor cells contained in a given blood sample. This assay is poorly standardized among different laboratories because of its complex nature, involving fastidious pretesting of materials just to maintain reproducibility in each laboratory. Consequently, circulating CFU-GM are not strictly comparable among different institutions and problems inherent in the technique itself partly account for the wide ranges of the CFU-GM threshold doses reported in the literature.1,2 Furthermore, the 2-week wait necessary before results are available also disqualifies the CFU-GM assay from being used clinically.

From the Divisions of Medical Oncology, Immunohematology, and Radiotherapy, Cristina Gandini Transplantation Unit, Istituto Nazionale Tumori, Milan; the Division of Nephrology, Renal Transplantation Unit, Ospedale Niguarda, Cà Granda, Milan; Sandoz Clinical Research, Basel, Switzerland; Terry Fox Laboratory, Cancer Control Agency of British Columbia, Vancouver, BC, Canada; and Institute of Medical Sciences, Università degli Studi, Milan, Italy.

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Address reprint requests to Salvatore Siena, MD, Istituto Nazionale Tumori, Via Venezian 1, Milan 1 20133, Italy.

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as a real-time guide to harvest recovery-phase blood cells when an outburst of circulating hematopoietic progenitor cells is expected to occur.

Circulating hematopoietic progenitor cells can be distinguished by flow cytometry based on expression of surface membrane molecules referred to as CD34 and CD33 antigens similar to their BM counterpart. Cells expressing the CD34 antigen (CD34+ cells) comprise virtually all hematopoietic progenitors forming CFU-GM, BFU-E, megakaryocytic (CFU-Mk), multilineage (CFU-Mix), and blast cell colonies (CFU-Blast). The latter undifferentiated hematopoietic progenitors notably possess many of the features of stem cells in that they are capable of self-renewal and also of commitment to a number of hematopoietic lineages. In accordance with this in vitro finding, transplantation of enriched populations of autologous CD34+ cells completely restores hematopoiesis of lethally irradiated baboons and probably of patients treated with myeloablative cancer therapy. Furthermore, CD34+ cells can be divided into functionally distinct progenitor populations based on their expression of the CD33 antigen. The earliest progenitors, such as CFU-Blast and long-term culture initiating cells, express the CD34 antigen exclusively (CD34+/CD33-), whereas more differentiated committed colony-forming progenitors express both the CD34 and CD33 antigens (CD34+/CD33+). Based on this knowledge, we evaluated the clinical feasibility of using a flow cytometry assay instead of a colony assay to estimate hematopoietic progenitor cells circulating in the PB of cancer patients recovering from pancytopenia induced by intensive chemotherapy with or without rhGM-CSF.

Our first aim was to develop a simple and reproducible assay to be used clinically to decide on a real-time basis (a) which patients experience an overshoot of circulating hematopoietic progenitors of relevant magnitude for considering the large-scale collection of these cells by leukapheresis, (b) when leukaphereses should be performed, and (c) how many consecutive leukapheresis procedures are necessary to harvest quantities of hematopoietic progenitor cells sufficient for successful transplantation. Our second aim was to determine whether information on hematopoietic progenitor cell subsets provided by the CD34 and CD33 immunofluorescence analysis will predict the marrow-repopulating ability of transplanted PB autologous grafts.

**MATERIALS AND METHODS**

**Patients and therapy protocols.** In a series of 16 patients, 20 high-dose chemotherapy courses and relative hematopoietic recoveries were evaluated (Table 1). In all patients, BM status was assessed by morphological analysis of bilateral (Hodgkin’s disease and non-Hodgkin’s lymphoma) or monolateral iliac crest marrow aspirates and biopsies. After patients had given written informed consent, they were treated according to high-dose sequential chemotherapy protocols approved by the institute’s committee for clinical investigation. These protocols implied administration of high-dose cyclophosphamide [HD-CTX 7 g/m² divided into five doses intravenously (IV) for 1 hour every 3 hours] or high-dose etoposide (HD-VP16 2 g/m² IV for 8 hours), both drugs possessing marked anticancer effect and inducing overshoot of circulating hematopoietic progenitors after transient pancytopenia.

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<th>Dose (µg/kg/day)</th>
<th>Schedule*</th>
<th>Route</th>
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**Table 1. Clinical Characteristics of Patients**

Abbreviations: BC, breast cancer; CI, continuous infusion; HD, Hodgkin’s disease; HD-CTX, cyclophosphamide; HD-VP16, etoposide; MM, multiple myeloma; NHL, non-Hodgkin’s lymphoma; SC, subcutaneous. Arrow: Patient was initially treated with rhGM-CSF 11 µg/kg/day for 7 consecutive days and then with 5.5 µg/kg/day for 7 more days.

*Number of days of rhGM-CSF administration after chemotherapy.

†Half of this daily dose was administered subcutaneously every 12 hours.
Beginning on day +1 after HD-CTX and on day +3 after HD-VP16, 16 patients received glycosylated (Sandoz, Basel, Switzerland) or nonglycosylated (Schering-Plough, Milan, Italy) rhGM-CSF for 14 days. Type of rhGM-CSF as well as dose, schedule, and route of administration for each patient and chemotherapy course are shown in Table 1.

During hematopoietic recovery after transient pancytopenia induced by HD-CTX or HD-VP16, PB buffy-coat cells were collected and cryopreserved to be used as the source of hematopoietic stem cells to support subsequent myeloablative cancer treatments (ie, 10 to 12.5 Gy total body irradiation and 120 to 140 mg/m² melphalan for Hodgkin’s disease, non-Hodgkin’s lymphoma, and multiple myeloma or 200 mg/m² melphalan for breast cancer). After therapy with HD-CTX or HD-VP16 with or without rhGM-CSF and starting on the first day leukocyte counts reached ≥1,000/µL and platelet counts reached ≥70,000/µL, patients underwent PB leukaphereses on 3 to 4 consecutive working days with a continuous-flow blood cell separator (IBM-COBÉ 2997, MEDAS, Genoa, Italy). The possibility that nonspecific binding of the FITC-conjugated 8G12 antibody to recovery-phase hematopoietic cells could also account for CD34+ cell counts was ruled out using a PE-conjugated isotype-matched (IgG1) irrelevant CD19 antibody control as described previously.

For CFU-GM colony assay, 5 mL heparinized whole blood was erythrocyte-depleted by gravity sedimentation with 33% Emagel (Behring, Scoppito, Italy) for 30 minutes at 37°C; the leukocyte-enriched fraction was then cultured in triplicate at 20,000 to 400,000 cells/mL in semisolid medium, as described elsewhere. Leukapheresis cell suspensions were cultured at the same cell concentration without erythrocyte depletion because their hematocrit was less than 3% in all cases. CFU-GM were assayed in Iscove’s modified Dulbecco’s medium (IMDM, GIBCO, Paisley, England) containing 0.3% agar, 20% fetal calf serum (Flow, Opera, Italy), and 10% K563 bladder carcinoma cell line conditioned medium as source of CSFs. After 7 and 14 days of incubation at 37°C in 5% CO2/95% humidified air atmosphere, CFU-GM (≥50 cells per aggregate) were scored under an inverted microscope. The count of circulating CFU-GM was determined by multiplying their frequency (number of colonies/number of cultured leukocytes) by the leukocyte count in the same sample.

In our laboratory, using the above technique and materials in untreated patients with solid tumors without BM involvement, the median value of steady-state circulating CFU-GM was 149 (range 39 to 329) colonies per milliliter of PB. The intrinsic accuracy of CFU-GM colony assay, as measured by calculation of the CV of 100 triplicate samples, was 1% to 30% when the plating efficiency corresponded to 50 to 150 colonies per culture dish and more than 30% with lower or higher colony numbers.

Statistical analysis. We estimated the relationship between counts of circulating hematopoietic progenitors evaluated by flow cytometry and colony assay by linear regression and correlation analysis using a commercially available computer program (Statworks, Cricket Software, Philadelphia, PA). We used the same program to calculate the CV of results of the two assays.

RESULTS

Comparison between indirect and direct immunofluorescence to detect circulating hematopoietic progenitors. Direct immunofluorescence with FITC-conjugated 8G12 antibody detected circulating CD34+ cells with the same bright fluorescence of indirect immunofluorescence with anti-HPCA-1 antibody plus FITC-conjugated anti-mouse F(ab’)2 immunoglobulin second antibody. In comparison with the indirect technique that has been used in previous studies, direct immunofluorescence provided the following advan-
ESTIMATION OF CIRCULATING CD34+/CD33- CELLS

For the purpose of this study, we evaluated whole blood samples from patients expected to experience overshoot of circulating hematopoietic progenitors of variable magnitude depending on type of malignancy, presence or absence of BM involvement, chemotherapy, and rhGM-CSF administration. Counts of leukocytes, and PE-CD33) reagents, one of which is not monoclonal and thus is variable with each preparation; and (c) simpler and faster sample processing, implying 50 minutes instead of 150 minutes. Furthermore, using dual-color immunofluorescence as described in this article, CD34+/CD33- and CD34+/CD33+ cell percentages were clearly ascertainable in blood samples despite wide variations of leukocyte counts ranging between 100 and 20,000/μL (Figs 2 through 4). Based on these observations, all subsequent analyses of hematopoietic progenitors were performed with dual-color CD34 and CD33 direct immunofluorescence by flow cytometry in parallel to triplicate CFU-GM colony assay.

Circulation of hematopoietic progenitors was a transient and dynamic phenomenon. As shown in Figs 2 and 3 for the same representative patient, CD34+/CD33- and CD34+/CD33+ cells appeared in the circulation during hematopoietic recovery after chemotherapy-induced pancytopenia. Their number per microliter reached a peak during the third week after chemotherapy and then decreased to undetectable levels after 2 to 4 days in parallel with the increase of mononuclear CD34+/CD33+ cells with bright fluorescence, the latter phenotype representing mostly immature, though nonclonogenic, granulomonoeyctic cells. Any combination of phenotypes, ie, CD34+/CD33- and CD34+/CD33+ (Fig 4, middle panel), or CD34+/CD33- (Fig 4, left panel), or CD34+/CD33+ (Fig 4, right panel) could be found without a clear pattern across the spectrum of patients studied.

Flow cytometry versus colony assay to estimate circulating hematopoietic progenitors. For the purpose of this study, we evaluated whole blood samples from patients expected to experience overshoot of circulating hematopoietic progenitors of variable magnitude depending on type of malignancy, presence or absence of BM involvement, chemotherapy, and rhGM-CSF administration. Counts of leukocytes,
CD34+/CD33- cells, CD34+/CD33+ cells, total CD34- cells, CD34+/CD33- cells, day 7 CFU-GM, and day 14 CFU-GM were estimated in 157 blood samples obtained daily from patients during 20 different hematopoietic recoveries (Table 1).

Because CFU-GM colony assay represents the currently used method of enumerating hematopoietic progenitors, we compared CFU-GM results with those of CD34 and CD33 dual-color immunofluorescence by flow cytometry as well as with leukocyte counts. The latter variable was taken into account to determine whether simple leukocyte counts during hematopoietic recovery after chemotherapy are themselves predictors of increasing numbers of hematopoietic progenitors. Day 7 and day 14 CFU-GM values were plotted against measurements of leukocytes, CD34+/CD33-, CD34+/CD33+, total CD34+, and CD34-/CD33- cells. All variables tended to increase with increasing CFU-GM levels. The strength of these positive correlations was measured by calculating the correlation coefficient (r) (Table 2). Leukocyte counts correlated poorly with CFU-GM (r = .56 and r = .67 with day 7 and day 14 CFU-GM, respectively) and total CD34+ cells (r = .49), demonstrating that increasing leukocyte counts after chemotherapy do not necessarily reflect expansion of the compartment of circulating hematopoietic progenitors. As expected, virtually no correlation was found between

Fig 3. Circulating hematopoietic progenitors evidenced by bivariate scattergrams generated by combining right-angle light-scatter and red fluorescence of CD33+ cells (upper dot cluster in each panel). Cell specimens and processing were the same as Fig 2. CD34 green fluorescence scattergrams of the same blood samples are shown in Fig 2.

Fig 4. Dual-color immunofluorescence analyses of the density distributions of CD34 and CD33 antigens on whole blood leukocytes during hematopoietic recovery after HD-CTX and rhGM-CSF. Representative bivariate contour plots of CD34 and/or CD33 antigen(s)-positive cells were generated as described in the Materials and Methods section. Left, middle, and right panels are relative to patient 5 on day +17, patient 8 on day +14, and patient 9 on day +10 after chemotherapy. Any combination of phenotypes, ie, CD34+/CD33- and CD34+/CD33+ (middle panel), or CD34+/CD33- (left panel), or CD34+/CD33+ (right panel) could be found without a clear pattern across the spectrum of patients studied.
Table 2. Correlations Between CFU-GM, Leukocytes, and Subsets of Hematopoietic Progenitors Detected With CD34 and CD33 Dual-Color Immunofluorescence by Flow Cytometry

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<th>Independent Variable (x)</th>
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<tr>
<td>Day 14 CFU-GM</td>
<td>.67</td>
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Results relative to analyses of 157 PB specimens were plotted on horizontal (x) and vertical (y) axis. All variables tended to increase with increasing CFU-GM levels. The strength of these positive correlations was measured by calculating the correlation coefficient r as indicated.

circulating CFU-GM and the number of CD34+/CD33- cells (r = .11 and r = .18 with day 7 and day 14 CFU-GM, respectively). Determinations of CD34+/CD33+, and total CD34+/CD33+, and total CD34+ cells correlated well with those of CFU-GM. In particular, the best positive correlation was found between circulating day 14 CFU-GM and the total number of CD34+ cells (r = .89).

Data relative to day 14 CFU-GM and total CD34+ cells (Fig 5) might be fitted by a linear regression line described by the equation $y = 388.3 + 64.0x$, in which $y$ equals the count of day 14 CFU-GM and $x$ equals the count of hematopoietic progenitors evaluated as CD34+ cells. The intercept of the regression line corresponded to 388.3 day 14 CFU-GM per milliliter, representing the estimated value of day 14 CFU-GM when CD34+ cells were undetectable ($x = 0$). For clarity, Table 3 shows various levels of total CD34+ cells with the corresponding actual values of CFU-GM measured by colony assay as well as those estimated by linear regression analysis. Together, these data demonstrate that: (a) the threshold of flow cytometry to estimate circulating hematopoietic progenitors as CD34+ cells corresponded to approximately 500 day 14 CFU-GM per milliliter; (b) determinations of CD34+ cells did not imply false-positive results; and (c) when circulating hematopoietic progenitors increased threefold above steady-state values, their increase was measurable indifferently either by CFU-GM colony assay or by CD34 direct immunofluorescence and flow cytometry.

Flow cytometry v colony assay to determine peak counts of circulating hematopoietic progenitors after chemotherapy. Optimum harvesting of hematopoietic progenitor cells by leukaphereses requires the knowledge of their outburst timing in PB. Because of the 2-week wait necessary until colony results are available, the CFU-GM assay provides information usable only retrospectively. To determine if same-day determinations of circulating CD34+ cell numbers by flow cytometry can be used to guide leukaphereses, we evaluated when hematopoietic progenitor cell peak counts were detected by using flow cytometry or CFU-GM colony assay. Table 4 shows that in our series of 20 hematopoietic recoveries after chemotherapy circulating hematopoietic progenitors were detected as CD34+ cells or day 14 CFU-GM in 75% and 100% of patients, respectively. Patients 2, 3, 15, 16, and 19 had undetectable levels of circulating CD34+ cells because the levels of hematopoietic progenitor cells as demonstrated by CFU-GM assay remained below the threshold of detection by flow cytometry. Among the remaining 15 evaluable patients, 11 (73%) were shown to have peak counts of hematopoietic progenitors on the same day by both methods; in the remaining four patients (27%), the peak of CD34+ cells anticipated that of CFU-GM by 1 day.

Characterization of hematopoietic progenitor cells used for autologous transplantation after myeloablative therapy. Six consecutive patients were treated with total body irradiation plus melphalan and then transplanted with PB cells harvested at the time of hematopoietic recovery after HD-CTX or HD-VPl6. Transplanted hematopoietic progenitors were characterized in parallel by CFU-GM colony assay as well as by CD34 and CD33 flow cytometry (Table 5). In five of six patients, the dose of transplanted day 14 CFU-GM was 1.6- to 9.1-fold higher than the maximum proposed threshold dose of $50 \times 10^6$/kg. According to the regression equation shown in Fig 5, the $50 \times 10^6$ CFU-GM/kg threshold dose should correspond to $7.8 \times 10^6$ total CD34+ cells/kg. In practice, in the five patients transplanted with more than $50 \times 10^6$ day 14 CFU-GM/kg, the dose of total CD34+ cells comprised between 21.8 $\times 10^6$ and 77.7 $\times 10^6$/kg, (ie, 2.7- to 9.9-fold higher than the estimated total CD34+ cell threshold dose). In patient 18, the dose of transplanted day 14 CFU-GM as well as total CD34+ cells corresponded to approximately half of both threshold
doses. Table 6 shows that patients who received more than $80 \times 10^8$ day 14 CFU-GM per kilogram or more than $20 \times 10^9$ total CD34$^+$ cells per kilogram experienced extraordinarily rapid and complete hematopoietic recovery. The only patient (patient 18) who received a lower dose of hematopoietic progenitors ($23.3 \times 10^8$ day 14 CFU-GM per kilogram or $4.65 \times 10^9$ total CD34$^+$ cells per kilogram) experienced unsatisfactory platelet recovery and slower recovery of leukocytes and erythrocytes. Thus, these data further indicate that determinations of the number of total CD34$^+$ cells can be used to estimate the number of hematopoietic progenitors in autologous PB grafts.

In this limited and heterogeneous series of patients (with or without BM involvement, with or without rhGM-CSF after transplantation, glycosylated or nonglycosylated rhGM-CSF), among the six parameters that we used to characterize the circulating progenitor cell graft (nucleated cells, CD34$^+$/CD33$^-$ cells, CD34$^+$/CD33$^+$ cells, CD34$^+$/CD33$^+$ cells, day 14 CFU-GM), the best predictor of the rate of early hematopoietic engraftment appeared to be the number of transplanted CD34$^+$/CD33$^+$ cells (Table 6).

**DISCUSSION**

Cancer patients treated with intensive chemotherapy may or may not experience an expansion of the circulating hematopoietic progenitor cell compartment of sufficient magnitude to justify large-scale collection of these cells for subsequent autologous transplantation after further myeloablative cancer therapy. Lack of a clinical assay for real-time determination of the number of circulating hematopoietic progenitors can expose patients with scarce or without overshoot of circulating hematopoietic progenitor cells to insufficient or useless leukapheresis procedures. To overcome this problem, we developed a dual-color CD34/CD33 direct immunofluorescence assay suitable for clinical estimation of circulating hematopoietic progenitor cells. Recent availability of the 8G12 MoAb that, unlike all other CD34 antibodies to date, can be directly labeled with fluorochromes without loss of binding properties provided advantageous direct immunofluorescence.$^{14,15}$

We report that a strong positive correlation exists between circulating CFU-GM colonies and either total CD34$^+$ cells, CD34$^+$/CD33$^-$ cells, or CD34$^+$/CD33$^+$ cells. This observation demonstrates that counts of CFU-GM, the committed progenitor so far used to judge the engraftment capability of autologous PB grafts, are good indirect indicators of the other nongranulomonocytic hematopoietic progenitors existing among CD34$^+$ cells. Furthermore, because of the correlation existing between measurements of circulating CFU-GM and total CD34$^+$ cells, it is appropriate to use detection of total CD34$^+$ cells by flow cytometry assay to estimate the number hematopoietic progenitor cells that can be harvested clinically.

During steady-state hematopoiesis, total CD34$^+$ cells are undetectable in PB because levels of circulating hematopoietic progenitors are below the threshold of detection by flow cytometry. During recovery from pancytopenia induced by intensive HD-CTX or HD-VP16 cancer therapy with or without rhGM-CSF, the appearance of total CD34$^+$ cells in the circulation indicates that hematopoietic progenitor cells have risen to levels corresponding to more than 500 CFU-GM per microliter, ie, at least threefold more
Estimation of circulating CD34+/CD33+ cells

According to our regression equation describing the relationship between circulating CD34+ cells and CFU-GM, this threshold dose corresponds to $7.8 \times 10^6$ total CD34+ cells per kilogram of body weight. Thus, during hematopoietic recovery after intensive chemotherapy-induced pancytopenia, the appearance of CD34+ cells in the circulation indicates that a clinically relevant overshoot of hematopoietic progenitor cells is occurring. As a possible guideline, provided platelet counts are $\geq 70,000/\mu L$, PB leukapheresis should be started as soon as CD34+ cells appear in the circulation and continued until the threshold dose of $7.8 \times 10^6$ CD34+ cells per kilogram of body weight is achieved.

Recovery of marrow functions after myeloablative chemotherapy and transplantation of autologous hematopoietic progenitor cells is a complex and partly understood phenomenon. Experimental studies in rodents and retrospective evaluation of available clinical data in humans indicate that transplantation is followed by two phases of reconstitution of marrow functions in myeloablated patients, with a phenomenon. Experimental studies in rodents indicate that transplantation is followed by two phases of reconstitution of marrow functions in myeloablated patients. According to our regression equation describing the relationship between circulating CD34+ cells and CFU-GM, this threshold dose corresponds to $7.8 \times 10^6$ total CD34+ cells per kilogram of body weight. Thus, during hematopoietic recovery after intensive chemotherapy-induced pancytopenia, the appearance of CD34+ cells in the circulation indicates that a clinically relevant overshoot of hematopoietic progenitor cells is occurring. As a possible guideline, provided platelet counts are $\geq 70,000/\mu L$, PB leukapheresis should be started as soon as CD34+ cells appear in the circulation and continued until the threshold dose of $7.8 \times 10^6$ CD34+ cells per kilogram of body weight is achieved.

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planted committed progenitor cells, and a second sustained engraftment phase is produced by the pluripotent stem cell. According to this multiphase model, rapid and sustained hematopoietic reconstitution in myeloablated patients should be ensured by transplantation of an optimum number or proportion of pluripotent stem cells as well as committed hematopoietic progenitors. In this regard, we preliminarily report that early hematopoietic recovery of marrow function after myeloablative chemoradiotherapy is predicted more accurately by the number of transplanted CD34+/CD33- cells than by either total nucleated cells, CFU-GM, CD34+/CD33- cells, or CD34+/CD33- cells. Furthermore, transplantation of BM depleted of CD33+ cells has resulted in sustained although delayed reconstitution of hematopoiesis. These observations further substantiate the hypothesis that after transplantation CD34+/CD33- cells may include progenitors responsible for early hematopoietic recovery and CD34+/CD33- cells responsible for a second sustained engraftment phase. In humans, prospective determinations of transplanted CD34+/CD33- and CD34+/CD33- cells are expected to define the possible role of these progenitor cell subsets in predicting the marrow-repopulating capability of PB grafts.

Our data favor use of the CD34/CD33 flow cytometry assay to guide harvesting of circulating hematopoietic progenitor cells for transplantation and constitute the basis for better understanding of the role played by hematopoietic progenitor cell subsets in marrow recovery after myeloablation.

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REFERENCES


Table 6. Hematopoietic Recovery After Myeloablative Total Body Irradiation Plus High-Dose Melphalan and Transplantation of Circulating Hematopoietic Progenitor Cells

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Neutrophils</th>
<th>Platelets</th>
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<tbody>
<tr>
<td></td>
<td>≥1,000/µL</td>
<td>≥500/µL</td>
</tr>
<tr>
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</tr>
<tr>
<td>18</td>
<td>+13</td>
<td>+13</td>
</tr>
</tbody>
</table>

*Patient number refers to unique patient number shown in Table 1. For each patient, detailed characterization of transplanted hematopoietic progenitor cells is shown in Table 5.
†Patients 9 and 10 did not require any packed RBC (PRBC) transfusion because lowest levels of hemoglobin were 10.2 and 9.9 g/dL, respectively.
‡Patient 10 did not require any platelet transfusion because lowest platelet count was 30,000 per microliter.
§Highest unsupported platelet count was 30,000 per microliter on day +60.
Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients

S Siena, M Bregni, B Brando, N Belli, F Ravagnani, L Gandola, AC Stern, PM Lansdorp, G Bonadonna and AM Gianni