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ORIGINAL ARTICLE

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Factors for rapid and sustained hematopoietic reconstitution by circulating progenitor-cell transplantation in non-Hodgkin's lymphoma

Abstract Circulating progenitor cells (CPCs) mobilized from bone marrow will replace the use of bone marrow transplantation because hematopoietic reconstitution is more rapid using the former technique. We report on early and late recovery of hematopoiesis after CPC transplantation in patients with non-Hodgkin's lymphoma (NHL) and analyze the role of variables possibly influencing engraftment. From December 1992 through September 1995, 57 consecutive NHL patients were enrolled in this study. Patients could be divided into 2 groups: the first comprised 32 patients with untreated diffuse large-cell lymphoma and unfavorable prognostic factors; the second comprised 25 patients with resistant or relapsing NHL of low- and high-grade histology. All patients received high-dose chemotherapy (carmustine, cytarabine, etoposide, and melphalan; BEAM) followed by CPC transplantation. In all, 25 patients were treated with granulocyte colony-stimulating factor (G-CSF) after CPC administration. The time to short- and long-term hematologic engraftment and variables correlating with multilineage long-term reconstitution were examined. The time to bilineage (neutrophils and platelets) hematopoietic reconstitution did not differ in G-CSF-treated and -untreated patients. In contrast, the time taken to reach a neutrophil count of 0.5×10^9/l and a WBC of 1×10^9/l was significantly shorter in G-CSF-treated patients. Overall, 33 patients achieved long-term, complete trilineage engraftment after a median of 117 days from CPC transplantation. The leukocyte count was the first parameter to reach full engraftment and hemoglobin was the last. According to Kaplan-Meier analysis, 80% of the patients are projected to reconstitute fully at 12 months after transplantation. Univariate and multivariate analyses showed that sustained, long-term hematopoiesis was significantly related to a younger age, an early bilineage reconstitution, and the quantity of $CD34^+$ cells infused.

Key words Short-term hematologic reconstitution · Long-term sustained hematologic reconstitution · G-CSF · $CD34^+$ cells · Non-Hodgkin's lymphoma

Introduction

Infusion of autologous stem cells harvested from bone marrow or peripheral blood is being applied increasingly in a variety of hematologic malignancies and in some solid tumors [2, 6]. Circulating progenitor cells (CPCs) mobilized from bone marrow using cytotoxic chemotherapy and cytokines will replace the use of autologous, and possibly, allogeneic bone marrow transplantation. The reason CPCs should be preferred to bone marrow transplantation is that hematopoietic reconstitution is more rapid using this technique and, consequently, there is less infectious and hemorrhagic morbidity. Among the many questions remaining unresolved is the issue of whether CPCs mobilized after chemotherapy and granulocyte colony-stimulating factor (G-CSF) administration are capable of maintaining sustained, long-term hematopoiesis. This was addressed in part by the present study, which considered only patients with non-Hodgkin's lymphoma (NHL).

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Patients and methods

Patients and CPC harvest

From December 1992 to September 1995, 57 NHL patients were enrolled. The criteria for entry were an age of 20–65 years, an Eastern Cooperative Oncology Group performance status of >2, and advanced-stage disease. Two groups of patients were examined. The first group comprised 32 untreated patients with diffuse large-cell lymphoma (DLCL; G and H according to the Working Formulation) and with unfavorable prognostic factors at presentation (stages III and IV with a high tumor burden and elevated serum lactate dehydrogenase levels). The therapeutic scheme in these cases included three phases: induction using the MACOP-B [3] regimen for 8 weeks; intensification with a 3-day course of mitoxantrone given at 8 mg/m^2 plus high-dose cytarabine (Ara-C) given at 2 g/m^2 every 12 h and dexamethasone given at 4 mg/m^2 every 12 h (MAD); and administration of recombinant human G-CSF (Filgrastim) at 5 m g/kg per day from day 4 to CPC harvest. CPC harvest was performed in all patients after MAD course 1. To reduce the likelihood of tumor-cell contamination, a second harvest was carried out only in those patients in whom a complete remission (CR) after eight MACOP-B courses was not proven. High-dose chemotherapy (BEAM regimen: carmustine [BCNU], etoposide, Ara-C, melphalan) was commenced after one or two courses of MAD, depending on CR achievement after MACOP-B therapy. All patients received CPC transplantation; none received concomitant autologous bone marrow infusion.

The second group (25 individuals) comprised patients with resistant or relapsing NHL. In those with DLCL histology, a high-dose sequential chemotherapy regimen [4] was used, with cyclophosphamide being given at 7 g/m^2 on day 1; methotrexate, at 8 g/m^2 plus vincristine, at 1.4 mg/m^2 on approximately day 21; and etoposide, at 2 g/m^2 on approximately day 28; G-CSF at 5 mg/kg per day was given from day 2, after cyclophosphamide, to facilitate CPC harvest. In patients with resistant or relapsing disease and low-grade histology, cisplatin, high-dose Ara-C, and dexamethasone combination chemotherapy was used [14]. At least three courses were given, each being followed by administration of G-CSF at 5 mg/kg per day, and CPCs were collected after the first or second course. Details of CD34$^+$ cell counting and colony assay and of the leukapheresis procedure have been reported previously [3].

Transplantation protocol and G-CSF administration

All patients (untreated and relapsed/resistant groups) received the BEAM conditioning regimen: BCNU given at 300 mg/m^2 on day –6; Ara-C, at 200 mg/m^2 i.v. twice per day on days –5 to –2; etoposide, at 100 mg/m^2 twice per day on days –5 to –2; and melphalan, at 140 mg/m^2 on day –1. CPCs were infused on day 0.

The effect of G-CSF after CPC transplantation was examined as follows. It was planned that the first 25 patients would receive G-CSF at 5 mg/kg per day s.c. starting on day 1 and continuing until the neutrophil count was $>5\times10^9$/l. Since the time to platelet and leukocyte recovery was short (<15 days) and the standard deviation from the mean was small, the subsequent 32 consecutive patients were not given G-CSF.

Supportive therapy

Following completion of the chemotherapy, all patients received prophylactic and therapeutic antimicrobial agents as previously reported [3].

Definition of short- and long-term engraftment

Short-term hematologic reconstitution was defined as the time required to achieve a WBC of $\geq1.0\times10^9$/l, an absolute neutrophil count (ANC) of $\geq0.5\times10^9$/l, and an untransfused platelet count of $\geq20\times10^9$/l.

Table 1 Characteristics of 57 NHL patients at transplantation

Characteristic	
Median age (range)	45 (18–65) years
F/M	31/26
First line	32 (56%)
Relapse/resistant	25 (44%)
Disease status:	
Remission/sensitive relapse	30/20
Resistant	7
Median number of prior chemotherapy courses (range)	10 (5–29)
Prior radiation therapy	5 (9%)
Bone marrow involvement	12 (21%)

Table 2 Number of CD34$^+$ cells infused

Category	Number of patients	Median number of CD34$^+$ cells $\times10^6$/kg (range)	*P*
First line	32	15.2 (5.8–65.5)	
Relapse/resistant	25	11.1 (1.7–32.2)	0.018
Total	57	12.2 (1.7–65.5)	

Table 3 Effect of G-CSF on short-term reconstitution after CPC transplantation[a] (*NS* Not significant)

Recovery to ($\times10^{-9}$/l)	G-CSF (n = 25)	No G-CSF (n = 32)	P (Mann-Whitney U-test)
Bilineage	11 (8–55)	13 (10–47)	NS
WBC ≥1.0	10 (8–22)	12 (8–22)	0.0067
ANC ≥0.5	10 (7–17)	12 (7–23)	0.001
Platelets ≥20	10 (7–55)	11 (8–47)	NS

[a] Data represent median values (range)

Table 4 Transfusions, duration of hospitalization, documented infections, and antibiotic requirement[a]

Variable	G-CSF (n = 25)	No G-CSF (n = 32)
Hospitalization (days)	21 (15–60)	21 (18–33)
Erythrocyte transfusion	3 (0–25)	2 (0– 6)
Platelet transfusion	2 (1– 5)	1.5 (1– 4)
Antibiotics (days)	7 (2–15)	7 (4–14)
Febrile (days)	4 (2–16)	3.5 (2– 8)
Documented infections	6	9

[a] Data represent median values (range)

Long-term hematologic reconstitution was defined as the time needed to reach a hemoglobin level of ≥12.0 g/dl, a WBC of $\geq4.0\times10^9$/l, an ANC of $\geq1.5\times10^9$/l, and a platelet count of $\geq150\times10^9$/l.

Statistical analysis

Continuous variables are shown as median values with the range unless otherwise indicated. Selected baseline characteristics and clinical outcomes were compared between groups using the chi-square test for discrete variables and either nonparametric analysis of variance or the Mann-Whitney U-test for continuous variables. Associations between continuous variables were assessed using the Pearson correlation coefficient or the Spearman rank correlation test. Risk ratios and 95% confidence intervals were used to compare groups with regard to major clinical outcomes. Long-term reconstitution during the 30-month follow-up period was assessed using Kaplan-Meier probability

Table 5 Long-term hematologic reconstitution after CPC transplantation

Parameter	Number of patients (%)	Median number of days (range)
Trilineage	33 (58)	117 (18–383)
Hemoglobin ≥12.0 g/dl	48 (84)	84.5 (15–284)
WBC ≥4.0×10^9/l	55 (96)	18 (11–135)
ANC ≥1.5×10^9/l	56 (98)	17 (11–138)
Platelets ≥150×10^9/l	36 (63)	33 (18–383)

curves. Two-sided nominal P values derived from unweighted log-rank statistics are given. The prognostic value of different variables for long-term probability of reconstitution was assessed by multivariate analysis using the Cox multiple regression model. All analyses were done using the SAS statistical package (SAS Institute, Cary, N.C., USA).

Results

Patients

The clinical characteristics of the 57 NHL patients who were treated using the BEAM myeloablative regimen and autografted with CPCs only are presented in Table l. In 32 patients (56%), transplantation was part of the first-line regimen, whereas in 25 (44%), CPC autografts were given as salvage therapy.

Short-term engraftment

Table 2 shows the number of CPCs, identified as $CD34^+$ cells, infused in the two groups of patients. A significantly lower recovery of CPCs was observed in relapsed/resistant NHL. The time to bilineage (neutrophils and platelets) hematopoietic reconstitution and to single-line platelet recovery did not differ in G-CSF-treated and -untreated patients. In contrast, the time taken to reach a neutrophil count of 0.5×10^9/l and a WBC of 1.0×10^9/l was significantly shorter in G-CSF-treated patients (Table 3). Despite this more rapid neutrophil and WBC reconstitution, the days spent in hospital, transfusion needs, and use of antibiotics were not different in G-CSF-treated and -untreated patients (Table 4).

Long-term engraftment

A total of 33 patients (58%) achieved complete trilineage engraftment after a median of 117 days from CPC transplantation (Table 5). The leukocyte count was the first parameter to reach full engraftment and hemoglobin was the last.

In all 24 patients (42%) did not reach the trilineage levels used to define full engraftment. Table 6 shows their peripheral blood count values. It should be noted that the time from transplantation of this patient group was shorter than that of patients who reached full reconstitution, although this difference was not statistically significant (Mann-Whitney U-test P = 0.106).

According to Kaplan-Meier analysis, 80% of patients were projected to reconstitute fully 12 months after transplantation (Fig. 1). No graft failure was registered and no patient lost the engraftment. Table 7 shows the results of univariate and multivariate analyses of trilineage engraftment. A younger age, the number of $CD34^+$ cells per kilogram, and the time to early bilineage reconstitution correlated with achievement of full engraftment.

Discussion

Hematopoietic engraftment using CPCs mobilized by chemotherapy and G-CSF was successful in all 57 NHL patients treated with the same conditioning regimen. G-CSF was used with chemotherapy to prime CPCs prior

Table 6 Blood counts obtained in the 24 patients not achieving long-term trilineage reconstitution[a]

Parameter	Patients in Remission (*n* = 16; 67%)		Relapse (*n* = 8; 33%)		Total (*n* = 24)	
Hemoglobin (g/dl)	11.85	(9.2–14.8)	11.2	(10.2–15.3)	11.45	(9.2–15.3)
ANC ($\times10^9$/l)	2.15	(1.4–5.1)	2.75	(1.3–3.9)	2.25	(1.3–5.1)
Platelets ($\times10^9$/l)	111	(31–216)	101	(44–214)	101	(31–216)

[a] Data represent median values (range)

Table 7 Variables correlating with long-term multilineage reconstitution according to the Cox model

Variable	Risk ratio[a] (range)	Univariate	Multivariate
Age >40 years	0.284 (0.118–0.685)	<0.001	<0.005
Early reconstitution >14 days	0.856 (0.748–0.979)	0.023	0.006
$CD34^+$ >8×10^6/kg	3.105 (0.94 –10.23)	0.051	0.062

[a] Values <1.0 indicate a low probability of reconstitution; values >1.0 indicate a higher probability of reconstitution

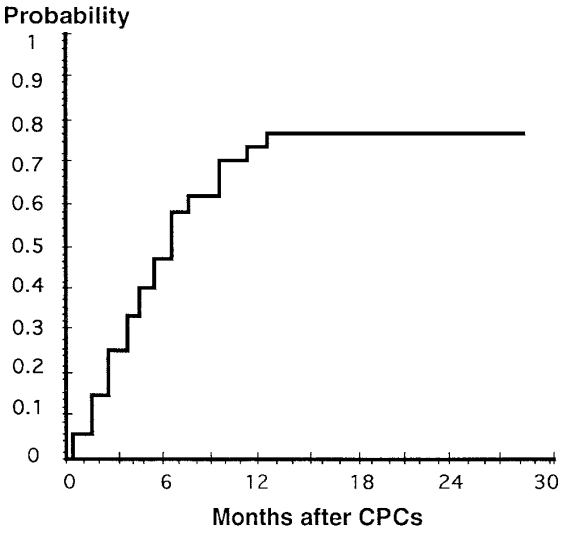

Fig. 1 Probability of trilineage reconstitution after CPC transplantation in 57 NHL patients

to collection, and this led to dramatic expansion of circulating precursors, identified as $CD34^+$ cells [l3]. Thus, following high-dose BEAM chemotherapy a median of 12.2×10^6 $CD34^+$ cells/kg were infused and, in those patients given G-CSF after infusion, neutrophil count recovery was 2 days faster than in untreated patients. This difference, although statistically significant, did not translate into a clinical benefit because no difference in the number of febrile days, antibiotic requirement, documented infections, or days of hospitalization was noted.

We are aware that these results were obtained in a nonrandomized study; however, they are in general accordance with those reported by other investigators [7, 12] and indicate that G-CSF given as an adjunct to CPCs might confer a modest clinical benefit, provided an optimal dose of circulating progenitors is infused. Siena et al. [10] have reported that autografting 8×10^6 CD34+ cells/kg is essential to ensure optimal short-term reconstitution in all patients, regardless of the use of growth factors. No effect on platelet recovery was noted in the two groups (G-CSF-treated and -untreated) in our study, and if we consider the time required to reach bilineage engraftment (ANC $>0.5\times10^9$/l and platelets $>20\times10^9$/l), the results obtained in G-CSF-treated patients did not differ from those seen in G-CSF-untreated patients.

The question of the ability of CPCs to reconstitute long-term hematopoiesis has been addressed in only a few studies [5, 9, 11]. According to Haas et al. [5], long-term reconstitution is achieved as soon as patients have a hemoglobin level of >12 g/dL, a WBC count of $>4.0\times10^9$/l, an ANC of $>1.5\times10^9$/l, and a platelet count of $>150\times10^9$/l. In all 33 patients in our series (58%) met these criteria and it is likely that the reason why the other 24 (42%) did not was the shorter follow-up period after CPC transplantation and the occurrence of early relapses of lymphoma (33%). However, in this group of patients, no graft was lost. According to Kaplan-Meier analysis, 80% of our patients were projected to reconstitute fully at 12 months after CPC transplantation.

Among the variables analyzed for effect on trilineage reconstitution, we found by multivariate analysis a significant predictive role for the time to early bilineage recovery and a younger age. In this respect, variation in hematopoietic stem-cell self-renewal capacity and reduction in marrow myeloid and erythroid precursors have long been recognized as being age-dependent in experimental models [1] and in humans [8]. That decreased early reconstitution time correlates with long-term full reconstitution is confirmed by the results of Haas et al. [5] and should be considered in the planning of further cytotoxic therapy, such as radiotherapy to the previous bulky tumor regions, in the weeks following CPC transplantation. This factor might delay full long-term reconstitution in patients who take longer to recover after transplantation [11].

We also confirmed that the threshold number of $CD34^+$ cells required to predict the likelihood of early and late sustained hematopoietic reconstitution is 8×10^6/kg. This figure contrasts with those of other authors [5, 9] who reported that 2.5×10^6 cells/kg is the threshold for these end points. Although this discrepancy remains largely unexplained, we should point out that in the two studies cited above, $CD34^+$ cells were mobilized by chemotherapy only in the majority of patients reported by Haas et al. [5] and in all patients analyzed by Schwartzberg et al. [9]. This could lead to different mobilization of committed CPCs and primitive uncommitted CPCs with the characteristics of hematopoietic stem cells and could tentatively explain the observed differences.

In conclusion, autografting using G-CSF and chemotherapy-mobilized CPCs is a safe procedure in NHL patients treated with myeloablative chemotherapy. Sustained long-term hematopoiesis is achieved in the majority of patients and depends on a younger age, the time taken to achieve short-term reconstitution, and the number of $CD34^+$ cells infused. Further work should be done to address the issue of whether G-CSF given as an adjuvant to CPC transplantation might play a role not only in short-term reconstitution but also in the duration of sustained hematopoiesis.

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