

ORIGINAL ARTICLE

Difficult stem cell mobilization despite adequate CD34⁺ cell dose predicts shortened progression free and overall survival after autologous HSCT for lymphoma

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Hematopoietic growth factors alone or in combination with myelosuppressive chemotherapy are used to mobilize peripheral blood stem cells for autologous transplantation. To identify characteristics of successful mobilization with granulocyte colony-stimulating factor (G-CSF) alone and to study the impact of immediate chemotherapy mobilization following G-CSF mobilization, we treated 175 chemotherapy sensitive lymphoma patients with G-CSF (G) mobilization and leukapheresis followed by chemotherapy plus G-CSF (CG) mobilization and leukapheresis and then autologous transplantation. Patients with stage I/II disease at diagnosis and ≤5 years from diagnosis were more likely to mobilize successfully with G-CSF alone (G). CG mobilization led to superior stem cell yields compared to the preceding mobilization with G (median 2.37 vs 1.37 ($\times 10^6$ CD34⁺ cells/kg); $P < 0.0001$). Patients ($n = 58$, 33%) with successful G-CSF mobilization ($\geq 2 \times 10^6$ CD34⁺ cells/kg) had quicker platelet recovery and improved progression free and overall survival compared to patients who had adequate collection only after chemotherapy mobilization or to those who failed to collect an adequate graft with either type of mobilization. The poor clinical outcome of patients with difficult mobilization using either method identifies them as a high-risk group who might benefit from alternative therapies.

Bone Marrow Transplantation (2007) **40**, 111–118; doi:10.1038/sj.bmt.1705708; published online 28 May 2007

Keywords: autologous transplant; lymphoma; stem cell mobilization

(OS) for many patients with relapsed lymphoma.¹ Transplantation is now performed almost exclusively with a peripheral blood stem cell (PBSC) graft, since it shortens the period of neutropenia without the concomitant expense and morbidity associated with bone marrow harvest.^{2,3} PBSC can be collected after mobilization with either chemotherapy plus granulocyte colony-stimulating factor (G-CSF) or with G-CSF alone (G), although mobilization after chemotherapy may increase stem cell yield.⁴ Previous studies have demonstrated that prior radiation therapy, marrow infiltration or fibrosis, and extensive prior chemotherapy hinder PBSC collection.^{5–7} Specific patient characteristics predicting mobilization of an adequate graft with G are uncertain. Furthermore, it is unclear if initial G-CSF mobilization will impair the yield of subsequent chemotherapy mobilization for PBSC collection.

The impact on DFS and OS of multiple mobilization attempts for patients with poor collection is unclear and previous analyses have conflicting results.^{8–10} To further address this, we prospectively studied patients with lymphoma planned for high dose therapy and autologous HSCT undergoing graft mobilization with G followed by chemotherapy plus G-CSF (CG). The primary end point of the study was to identify characteristics of patients who would successfully mobilize with G. Secondary end points were (1) to determine if G mobilization immediately prior impaired collections with CG mobilization and (2) to determine if transplant outcomes of OS, progression-free survival (PFS) and engraftment were different based on mobilization success.

Introduction

Autologous hematopoietic stem cell transplantation (HSCT) improves disease-free (DFS) and overall survival

Patients and methods

Patient eligibility

Patients with chemotherapy sensitive non-Hodgkin's (NHL) or Hodgkin's (HL) lymphoma in partial or complete remission were enrolled between January 1996 and August 2004. All patients were less than 70 years of age with a Karnofsky performance status of at least 80% and had adequate organ function as defined by an ejection fraction of 45% or greater, a diffusing capacity of the lung for carbon monoxide (DLCO) of more than 50% predicted, and a creatinine clearance of more than 60 ml/min. Bone

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Received 29 September 2006; revised 16 March 2007; accepted 10 April 2007; published online 28 May 2007

marrow biopsy at the time of enrollment required a minimum of 20% cellularity with less than 10% morphologic involvement with lymphoma. Patients with refractory disease, HIV-positive patients and those with human T-lymphotrophic virus-1 (HTLV-1) associated lymphomas were excluded. Written informed consent was obtained from all patients with approval from the University of Minnesota Institutional Review Board.

Study design

Mobilization and leukapheresis. All patients underwent two cycles of mobilization and leukapheresis. G-CSF (Filgrastim, AMGEN, Thousand Oaks, CA, USA) was administered at a dose of 250 $\mu\text{g}/\text{m}^2$ daily for 5 days before leukapheresis and daily during leukapheresis. G-CSF was rounded to the nearest vial size with the maximum daily dose administered 480 $\mu\text{g}/\text{day}$. Leukapheresis started on day 6 for G mobilization and was planned daily for 3 days. All patients were then hospitalized for chemotherapy with intravenous cyclophosphamide (4000 $\text{mg}/\text{m}^2 \times 1$ dose), mitoxantrone (8 $\text{mg}/\text{m}^2 \times 2$ doses, 24 h apart), cytarabine (1000 $\text{mg}/\text{m}^2 \times 2$ doses, 12 h apart) and dexamethasone (20 $\text{mg}/\text{m}^2 \times 4$ doses, 12 h apart). G-CSF was started on day 4 at 250 $\mu\text{g}/\text{m}^2$ (maximum 480 $\mu\text{g}/\text{day}$) and continued through the second course of leukapheresis for CG mobilization. Leukapheresis commenced on the first day after the absolute neutrophil count (ANC) was 700/ μl or higher and was planned daily for 3 days. At the discretion of the treating physician, poor collections defined as $<0.1 \times 10^6$ $\text{CD}34^+$ cells/kg on day 1 or a combined total of $<0.3 \times 10^6$ $\text{CD}34^+$ cells/kg by day 2 could result in cessation of collections. No manipulation or graft purging was performed.

Patients who did not achieve a combined PBSC collection of more than 1.5×10^6 $\text{CD}34^+$ cells/kg with both mobilization attempts were evaluated for a bone marrow harvest. Patients were required to have adequate hematologic parameters without growth factor or transfusion support (ANC $>1500/\mu\text{l}$ and platelets $>100\,000/\mu\text{l}$) and a bone marrow cellularity of at least 20% without morphologic evidence of lymphoma. Patients received granulocyte macrophage colony-stimulating factor (GM-CSF; Leukine, Berlex, Montville, NJ, USA) 250 $\mu\text{g}/\text{m}^2/\text{day}$ for 5 days and underwent a 1.51 bone marrow harvest under general anesthesia.

Transplantation. All patients were restaged before transplant and patients without disease progression were transplanted and included in this analysis. Patients received pre-transplant conditioning with intravenous cyclophosphamide (120 mg/kg total in two daily doses) followed by 1320 cGy fractionated total body irradiation given in eight fractions over 4 days ($n=93$; 53%). Patients with HL or with prior extensive radiation received intravenous cyclophosphamide (6000 mg/m^2 total in four daily doses), carmustine 300 mg/m^2 and etoposide (900 mg/m^2 total in six doses over 3 days) ($n=82$; 47%). All cryopreserved PBSC (plus marrow if required, $n=28$) were infused on day 0 and G-CSF 250 $\mu\text{g}/\text{m}^2/\text{day}$ (maximum 480 $\mu\text{g}/\text{day}$) started on day 0 until the ANC was greater than 2500/ μl for 2 days.

Antimicrobial and transfusion support were standardized per institutional protocols.

Statistical analysis

The results are presented as median and range where applicable. Unless otherwise stated, differences with two-sided $P<0.05$ were considered statistically significant. Univariate analyses were performed using a χ^2 test for categorical variables and analysis of variance or, if evidence of non-normality, a Kruskal–Wallis test for continuous variables.

For primary end point and transplant outcome analyses, an adequate graft was defined as 2×10^6 $\text{CD}34^+$ cells/kg actual body weight collected by a single mobilization method. Patient cohorts were determined as follows: **G-CSF Success** if the patient collected $\geq 2 \times 10^6$ $\text{CD}34^+$ cells/kg with G; **Chemotherapy Success** if the patient collected $<2 \times 10^6$ $\text{CD}34^+$ cells/kg with G but $\geq 2 \times 10^6$ $\text{CD}34^+$ cells/kg after CG and **Poor Mobilization** for patients unable to collect $\geq 2 \times 10^6$ $\text{CD}34^+$ cells/kg with either G or CG.

PFS and OS curves were estimated using the method of Kaplan–Meier and compared using log-rank tests.¹¹ PFS was determined from the time of transplant until first relapse or disease progression in patients not achieving complete remission. OS was defined from the time of transplant until death from any cause. The Cox proportional hazards model was used for multivariate analyses of survival outcomes and to determine prognostic factors for G mobilization success and poor mobilization.¹² Variables analyzed included age at transplant, gender, disease type (NHL vs HL), prior radiation, prior marrow involvement, disease stage at diagnosis, presence of B symptoms at diagnosis, international prognostic index (IPI) at diagnosis, number of prior chemotherapy regimens, number of prior chemotherapy cycles, time from diagnosis to harvest, and disease status at the time of transplant. For variables in which data were missing, the case was analyzed with those in which the factor was present for multivariate analysis. Neutrophil engraftment was defined as the first of 3 consecutive days with an ANC $\geq 500/\mu\text{l}$. Platelet engraftment was the first of three consecutive measures of a platelet count $\geq 20\,000/\mu\text{l}$ without transfusion in the prior 7 days. Neutrophil and platelet engraftment were analyzed using cumulative incidence curves with death as a competing risk. Cumulative incidence curves were compared using the method of Gray.¹³

Results

Patients

A total of 175 patients received an autologous HSCT and are included in this analysis. All patients underwent serial mobilization and leukapheresis for PBSC collections – first with G, then with CG. Patient characteristics are shown in Table 1. Based on leukapheresis yields, 58 patients (33%) had G-CSF Success, 53 patients (30%) had Chemotherapy Success and 64 (37%) had Poor Mobilization. For the Poor Mobilization group, the combined collection for both mobilization methods may have exceeded 2×10^6 $\text{CD}34^+$

Table 1 Patient characteristics in cohorts with differing mobilization success

	All patients n = 175 [100%]	G-CSF Success $\geq 2 \times 10^6$ CD34 ⁺ /kg n = 58 [33%]	G-CSF $< 2 \times 10^6$ but Chemo CD34 ⁺ /kg n = 53 [30%]	Poor Mobilization and Chemo $< 2 \times 10^6$ CD34 ⁺ /kg n = 64 [37%]	P-value
Age years (median, range)	46 (11–70)	42 (14–67)	51 (21–65)	44 (11–70)	NS
<i>Gender</i>					
Male	97 [55]	37 [64]	32 [60]	28 [44]	NS
Female	78 [45]	21 [36]	21 [40]	36 [56]	
<i>Disease type</i>					
<i>NHL</i>					<0.05 (*for low grade NHL only)
Low grade	15 [9]	3 [5]	10 [19]*	2 [3]	
Mantle cell	10 [6]	5 [9]	2 [4]	3 [5]	
Intermediate grade	91 [52]	24 [41]	37 [70]	30 [47]	
High grade	12 [7]	3 [5]	3 [6]	6 [9]	
HL	57 [33]	23 [40]	11 [21]	23 [36]	
<i>Previous radiation</i>					
No	116 [66]	42 [72]	35 [66]	39 [61]	NS
Yes	54 [31]	15 [26]	17 [32]	22 [34]	
Unknown	5 [3]	1 [2]	1 [2]	3 [5]	
<i>Previous marrow involvement</i>					
No	125 [71]	42 [72]	35 [66]	48 [75]	NS
Yes	42 [24]	12 [21]	17 [32]	13 [20]	
Unknown	8 [5]	4 [7]	1 [2]	3 [5]	
<i>Stage at diagnosis</i>					
I	14 [8]	5 [9]	4 [8]	5 [8]	NS
II	46 [26]	20 [34]	11 [21]	15 [23]	
III	37 [21]	13 [22]	9 [17]	15 [23]	
IV	78 [45]	20 [34]	29 [55]	29 [45]	
<i>B symptoms at diagnosis</i>					
No	100 [57]	30 [52]	33 [62]	37 [58]	NS
Yes	67 [38]	25 [43]	19 [36]	23 [36]	
Unknown	8 [5]	3 [5]	1 [2]	4 [6]	
<i>IPI at diagnosis^a</i>					
0	31 [27]	12 [35]	8 [21]	11 [28]	NS
1	58 [52]	18 [53]	22 [56]	18 [46]	
2	20 [18]	3 [9]	8 [21]	9 [23]	
3	3 [3]	1 [3]	1 [3]	1 [3]	
Number of prior chemotherapy regimens (median, range)	2 (1–5)	2 (1–4)	2 (1–4)	2 (1–5)	NS
Number of prior chemotherapy cycles (median, range)	8 (3–36)	8 (4–22)	8 (5–36)	9 (3–22)	NS
Time from diagnosis to harvest months (median, range)	18 (4–190)	16 (6–85)	21 (6–190)	18 (4–161)	NS
<i>Disease status at time of transplant</i>					
PR1	27 [15]	9 [16]	8 [15]	10 [16]	NS
CR1	14 [8]	4 [7]	3 [6]	7 [11]	
\geq PR2	88 [50]	29 [50]	29 [55]	30 [47]	
\geq CR2	46 [26]	16 [28]	13 [25]	17 [27]	
<i>Conditioning regimen</i>					
Cy/TBI	93 [53]	29 [50]	33 [62]	31 [48]	NS
CBV	82 [47]	29 [50]	20 [38]	33 [52]	

Abbreviations: CBV = cyclophosphamide, carmustine, etoposide; CR = complete remission; Cy = cyclophosphamide; G-CSF = granulocyte colony-stimulating factor; HL = Hodgkin's lymphoma; IPI = international prognostic index; NHL = non-Hodgkin's lymphoma; PR = partial remission; TBI = total body irradiation.

P-value: compares G-CSF Success vs Chemotherapy Success vs Poor Mobilization.

^aExcludes patients with HL and peripheral T-cell NHL (n = 6).

cells/kg. The cohorts were similar except a greater number of Chemotherapy Success patients had low grade NHL ($P < 0.05$).

Mobilization and leukapheresis

The median number of collections with each mobilization method was three. In the entire study population, CG mobilization yielded nearly twofold greater collections (1.37 vs 2.37×10^6 CD34⁺ cells/kg; $P < 0.0001$) (Table 2). Stem cell yields from CG mobilization were greater than ($n = 97$) or equivalent to ($\pm 0.5 \times 10^6$ CD34⁺ cells/kg, $n = 26$) G mobilization in 70% of patients. Figure 1 depicts the CD34⁺ cells/kg collected by G mobilization vs CG mobilization for all 175 patients and identifies the three distinct groups.

Table 3 details the mobilization results and cell doses infused in the three groups. G mobilization yielded a median 3.5-fold greater CD34⁺ PBSC collection in the G-CSF Success vs the other two groups. However, CG mobilization resulted in similar (1.06-fold more) CD34⁺ cell yields in the G-CSF Success and Chemotherapy Success groups, but 16.2-fold more than the Poor Mobilization group.

One hundred and thirty-four patients (77%) had combined yields of $\geq 2 \times 10^6$ CD34⁺ cells/kg. Thirteen (7%) additional patients had a combined collection totaling 1.5 – 1.99×10^6 CD34⁺ cells/kg. These patients received PBSC as their sole graft source. The remaining 28 patients (16%) required GM-CSF primed bone marrow harvest collecting a median 1.64×10^6 CD34⁺ cells/kg (0.27–6.19). The final median total nucleated cell dose infused was similar among the three groups but the median CD34⁺ cell dose infused differed significantly (Table 3).

Owing to body mass and vial size rounding, the median daily dose of G-CSF ($\mu\text{g}/\text{kg}$) was 5.7 (2.5–8.4) for the entire study population. The Poor Mobilization group received a higher median dose of G-CSF (Table 3). For the entire population, there was a median of 18 days (14–86) between the completion of G harvest and the start of leukapheresis after CG mobilization. The median time from the completion of both mobilization methods and transplant was significantly longer in the Poor Mobilization group owing to the prolonged recovery time for the 28 patients who required a bone marrow harvest (Table 3).

Prognostic factors for G-CSF mobilization success

We analyzed clinical characteristics predicting G-CSF Success and Poor Mobilization (Table 4). Stage I/II disease and a shorter time from diagnosis to harvest (< 60 months) were significantly associated with G-CSF Success. For 60 patients with stage I/II disease at diagnosis, 25 (42%) had

successful G mobilization compared to 30 (28%) of the 107 patients with stage III/IV disease ($P = 0.04$). For the 152 patients collected within 60 months from diagnosis, 55 (36%) had successful mobilization with G compared to only three (14%) of 21 patients collected beyond 60 months of diagnosis ($P = 0.03$). We demonstrated a trend towards successful G mobilization in patients without prior radiation compared to those previously irradiated. Female subjects had a 2.11-fold greater chance of poor mobilization with either mobilization method ($P = 0.02$). Neither the total number of chemotherapy cycles nor number of regimens was associated with G-CSF Success or Poor Mobilization.

Post-transplant engraftment: impact of mobilization success

All patients achieved neutrophil engraftment at a median of 10 days post transplant (1–15 days). G-CSF Success patients had a slightly, but significantly, shorter time to

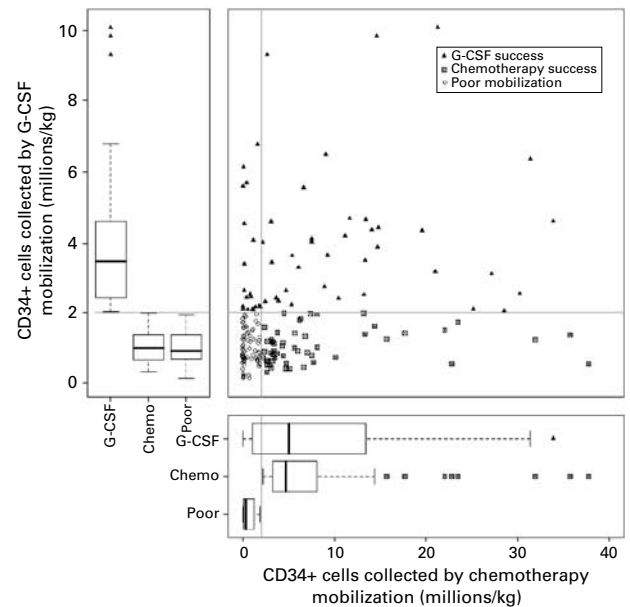


Figure 1 Three groups are defined by their graft CD34⁺ cell content. Each point represents the total G-CSF collection and the total chemotherapy collection for every patient. G-CSF (\blacktriangle) depicts those patients who collected $\geq 2 \times 10^6$ CD34⁺ cells/kg body weight with G-CSF only (G-CSF Success). Chemotherapy (\blacksquare) failed G-CSF mobilization but had successful chemotherapy mobilization (Chemotherapy Success). Poor (\circ) are those patients who did not obtain $\geq 2 \times 10^6$ CD34⁺ cells/kg with either mobilization method (Poor Mobilization). The box plots depict the median, quartiles, and outliers for each patient by cohort and method mobilized. Note the x and y axis scales differ owing to the greater overall yield with chemotherapy mobilization. G-CSF, granulocyte colony-stimulating factor.

Table 2 Comparison of PBSC yields and number of collections with G-CSF mobilization alone vs Chemotherapy + G-CSF

	G-CSF mobilization	Chemotherapy + G-CSF mobilization	P-value
Yield CD34 ⁺ cells $\times 10^6$ per kg median (range)	1.37 (0.12–10.1)	2.37 (0.0–100.7)	< 0.0001
Number of leukaphereses (range)	3 (2–4)	3 (1–8)	NS
Number of patients collecting $\geq 2 \times 10^6$ CD34 ⁺ cells per kg (%)	58 (33%)	90 (51%)	NS

Abbreviations: G-CSF = granulocyte colony-stimulating factor; PBSC = peripheral blood stem cell.

Table 3 Mobilization and total graft cell doses infused based on collection criteria

	<i>G-CSF Success</i> <i>collection</i> $\geq 2 \times 10^6$ <i>CD34⁺/kg</i> n = 58 [33%]	<i>Chemotherapy Success</i> <i>collection</i> $< 2 \times 10^6$ but <i>Chemo</i> $\geq 2 \times 10^6$ <i>CD34⁺/kg</i> n = 53 [30%]	<i>Poor Mobilization</i> <i>G-CSF</i> <i>and Chemo collection</i> $< 2 \times 10^6$ <i>CD34⁺/kg</i> n = 64 [37%]	P-value
Days between G-CSF and chemotherapy mobilization (median, range)	20 (16–88)	20 (16–39)	21 (16–52)	<0.05
Days from completion of leukapheresis and transplant (median, range)	17 (11–31)	17 (11–86)	30 (13–82)	<0.0001
Daily dose of G-CSF ($\mu\text{g}/\text{kg}$) (median, range)	5.5 (2.5–8.3)	5.6 (3.5–8.2)	6.0 (4.4–8.4)	<0.04
Total number of Aphereses (median, range)	6 (4–6)	6 (5–9)	6 (3–12)	NS
Total CD34 ⁺ cell dose collected $\times 10^6/\text{kg}$ with G-CSF alone (median, range)	3.43 (2.02–10.09)	0.99 (0.30–1.98)	0.89 (0.12–1.93)	<0.0001
Total CD34 ⁺ cell dose collected $\times 10^6/\text{kg}$ with chemotherapy mobilization (median, range)	5.03 (0.01–100.68)	4.74 (2.19–37.73)	0.31 (0.0–1.90)	<0.0001
Total CD34 ⁺ cell dose infused $\times 10^6/\text{kg}$ body weight (median, range)	8.69 (2.10–104.4)	5.55 (2.59–38.2)	2.32 (0.83–6.98) ^a	<0.0001
Total nucleated cell dose infused $\times 10^8/\text{kg}$ body weight (median, range)	12.64 (2.04–47.7)	11.14 (4.81–23.4)	11.68 (2.94–43.7) ^a	NS

Abbreviation: G-CSF = granulocyte colony-stimulating factor.
P-value compares G-CSF Success vs Chemotherapy Success vs Poor Mobilization.
^aIncludes bone marrow harvests (n = 28).

Table 4 Prognostic factors predicting graft mobilization

<i>Predicting successful collection with G-CSF mobilization</i>		
	<i>Odds ratio (95% CI)</i>	<i>P-value</i>
<i>stage at diagnosis</i>		
Stage I or II	1.00	
Stage III or IV	0.41 (0.20–0.83)	<0.02
<i>Time from diagnosis to harvest</i>		
≤ 60 months	1.00	0.05
> 60 months	0.28 (0.06–0.89)	
<i>Prior radiation</i>		
No	1.00	0.08
Yes (and unknown)	0.51 (0.23–1.07)	
<i>Predicting poor mobilization with both G-CSF and chemotherapy mobilization</i>		
<i>Sex</i>		
Male	1.00	
Female	2.11 (1.13–3.98)	0.02

Abbreviations: CI = confidence interval; G-CSF = granulocyte colony-stimulating factor.
Shown are odds ratios from multivariate logistic regression analysis considering demographic factors from Table 1.
No other factors were significantly associated with the end points shown.

neutrophil engraftment (G-CSF Success: 9 days (1–13) vs Chemotherapy Success: 10 days (8–14) vs Poor Mobilization: 11 days (8–15); $P < 0.001$). Platelet engraftment occurred in 94% of patients at a median of 20 days (11–118 days). The G-CSF Success cohort had a significantly shorter time to platelet recovery (G-CSF Success: 17 days (11–48) vs Chemotherapy Success: 18 days (13–97) vs Poor Mobilization: 28 days (14–118); $P < 0.001$) (Figure 2).

Overall and progression-free survival

At a median follow-up of 36 months, the median PFS and OS for the entire cohort were 24 months ((95% confidence

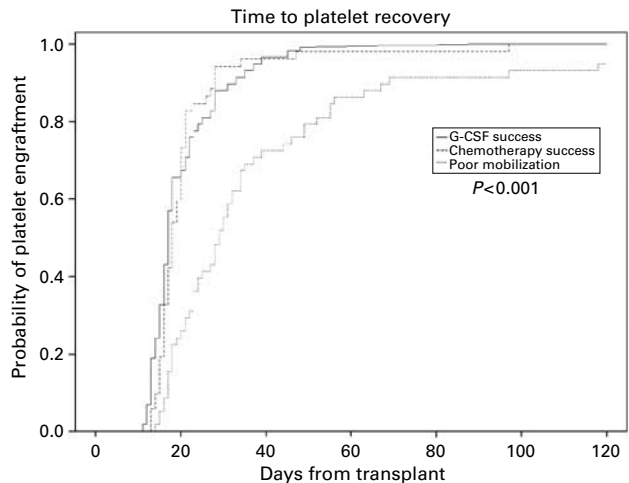


Figure 2 Time to platelet recovery. Time for recovery was significantly longer in patients with Poor Mobilization; median 28 days (14–118 days) compared to the G-CSF Success group (17 days (11–48)) and the Chemotherapy Success group (18 days (13–97)). G-CSF, granulocyte colony-stimulating factor.

interval (CI)) 12–45) and 77 months (42–not reached), respectively. For all patients, PFS at 1 and 5 years was 57% ((95% CI) 50–65) and 38% (30–49), respectively. Median PFS was marginally better for the G-CSF Success patients. For G-CSF Success, the median PFS was 45 months, while for Chemotherapy Success and Poor Mobilization, the median PFS was 19 months and 13 months, respectively ($P < 0.06$). The 5-year PFS was not significantly different (G-CSF Success 49% (36–67) vs Chemotherapy Success 35% (22–54) vs Poor Mobilization 30% (17–53); $P = 0.145$) (Figure 3).

For the entire cohort, OS at 1 and 5 years was 81% (75–87) and 53% (42–67). Mobilization success with G led to improved median OS compared to the Poor Mobilization cohort (G-CSF Success 102 months (48–not reached) vs

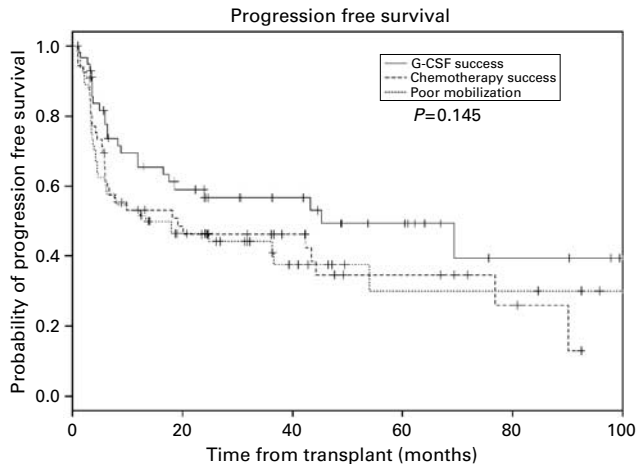


Figure 3 Progression-free survival by graft mobilization cohort. The G-CSF Success cohort demonstrated significantly improved progression-free survival compared to either Chemotherapy Success or Poor Mobilization. There was no difference between the Chemotherapy Success and Poor Mobilization cohorts. G-CSF, granulocyte colony-stimulating factor.

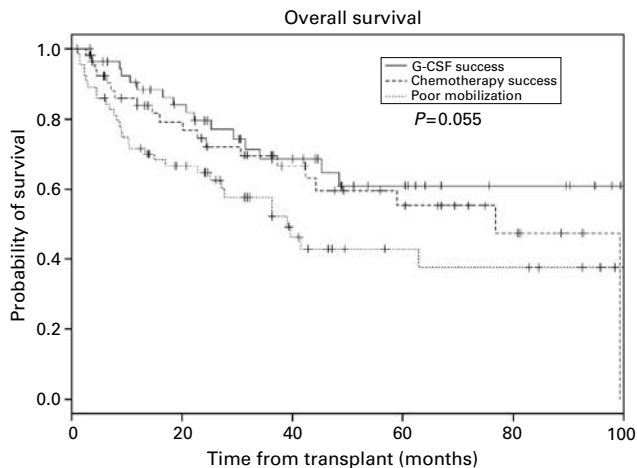


Figure 4 Overall survival by graft mobilization cohort. Overall survival was improved in patients with G-CSF Success compared to the Poor mobilization cohort. There was no difference in OS between the G-CSF Success and Chemotherapy Success cohorts. G-CSF, granulocyte colony-stimulating factor.

Chemotherapy Success 77 months (42–not reached) vs Poor Mobilization 39 months (27–not reached); $P=0.02$). There was no difference between the G-CSF Success and Chemotherapy Success groups ($P=0.37$). OS at 5 years was 61% (47–79), 55% (41–75) and 43% (30–61), for the G-CSF Success, the Chemotherapy Success and the Poor Mobilization groups ($P=0.055$), respectively (Figure 4).

Multivariate analysis for PFS and OS demonstrated significantly worse outcomes for Poor Mobilization patients (Table 5). G-CSF Success was associated with significantly superior PFS compared to Chemotherapy Success and Poor Mobilization patients. Failure to mobilize with either mobilization method was associated with significantly poorer OS. More than two chemotherapy regimens and a shorter time from diagnosis to harvest were

Table 5 Multivariate analysis for progression-free- and overall survival

Progression free survival		
	Relative risk (95% CI)	P-value
<i>Mobilization group</i>		
G-CSF success	1.00	
Chemotherapy success	1.73 (1.01–2.96)	0.044
Poor mobilization	1.86 (1.11–3.13)	0.019
<i>Number of chemotherapy regimens</i>		
≤2	1.00	
>2	1.93 (1.19–3.11)	0.008
<i>Time from diagnosis to harvest</i>		
≤18 months	1.00	0.043
>18 months	0.64 (0.41–0.99)	
<i>Overall survival</i>		
<i>Mobilization group</i>		
G-CSF success	1.00	
Chemotherapy success	1.51 (0.76–3.01)	0.24
Poor mobilization	2.59 (1.37–4.89)	<0.003
<i>Age</i>		
≤45 years	1.00	
>45 years	1.91 (1.13–3.24)	0.016
<i>Number of chemotherapy regimens</i>		
≤2	1.00	
>2	2.85 (1.61–5.03)	<0.001
<i>Time from diagnosis to harvest</i>		
≤18 months	1.00	0.03
>18 months	0.56 (0.33–0.95)	
<i>Year of transplant</i>		
≤2000	1.00	0.06
>2000	0.61 (0.36–1.03)	

Abbreviations: CI = confidence interval; G-CSF = granulocyte colony-stimulating factor.

associated with significantly worse PFS and OS. Age >45 years was associated with significantly poorer OS. There was a trend toward improved OS for those patients transplanted between 2001 and 2004. Neither gender, diagnosis, stage, number of chemotherapy cycles, nor other factors were significantly associated with OS and PFS.

Three patients (1.7%) died before day 100 without evidence of relapse. An additional five patients died before day 100 due to lymphoma relapse or progression. Seven (88%) of the eight patients that died early were in the Poor Mobilization group. For the entire study population, 71 (41%) patients have died. The causes of death in the three mobilization groups were similar.

Discussion

Our study found that patients with early stage disease or mobilization performed within 5 years of diagnosis are more likely to have adequate PBSC yields after G mobilization compared to patients with advanced disease or mobilization further than 5 years from diagnosis. Although some patients have poor mobilization with G,

the attempt does not preclude successful CG mobilization. Additionally, multivariate analysis found that poor mobilization results in inferior PFS and OS compared to patients who successfully mobilize with either G or with CG. Successful mobilization with G predicts improved PFS compared to patients requiring CG to obtain an adequate PBSC graft.

Engraftment after autologous HSCT depends, in part, on the quantity of CD34⁺ hematopoietic progenitor cells infused.¹⁴ Several analyses have looked at the impact of repeated mobilization attempts in patients with poor mobilization.^{15–19} Some suggest that stem cells can be adequately harvested using alternate mobilization techniques such as high-dose cytokines, combination cytokines, chemotherapy mobilization or bone marrow harvest.^{15,17,18} In our study, GM-CSF mobilized bone marrow harvest yielded adequate salvage grafts but the Poor Mobilization cohort still had inferior PFS and OS despite an adequate graft and prompt neutrophil engraftment. This is in accordance with two retrospective analyses strongly suggesting that lymphoma patients with poor mobilization have worse outcomes.^{8,9} A third study showed no difference in event free and OS but a trend towards increased early death in the cohort of poor mobilizers.¹⁰

Chemotherapy mobilization has been observed to yield larger numbers of CD34⁺ progenitors than mobilization with G-CSF alone. Narayanasami *et al.*⁴ reported a randomized study of 47 patients mobilized with G-CSF or cyclophosphamide plus G-CSF and found that chemotherapy plus G-CSF yielded threefold greater numbers of CD34⁺ cells without improvement in engraftment, PFS or OS despite a smaller graft in the G-CSF mobilized group. We demonstrated a nearly twofold greater PBSC yield after CG mobilization compared to G mobilization, suggesting that a prior G-CSF mobilization attempt does not hinder subsequent chemotherapy mobilization if required. Our results are similar to those of Koc *et al.*²⁰ in which a twofold increase in CD34⁺ cells was found in patients who received G-CSF mobilization followed by chemotherapy mobilization.

Our findings differed somewhat from previous reports that prior radiation or extensive prior chemotherapy result in poor stem cell yield.^{5–7} We did find that a longer duration from diagnosis to harvest was predictive of inadequate mobilization with G and hypothesize that this may be a surrogate for more extensive therapy. Different from the reports of Watts *et al.*⁵ and Haas *et al.*⁶ in which extensive prior therapy impaired mobilization with CG, we looked for factors predictive of successful G mobilization. Bensinger *et al.* found that patients with more than six prior cycles of chemotherapy collected a lower number of CD34⁺ cells but their patient population was heterogeneous in both malignancy diagnoses and mobilization methods confounding a direct comparison to our findings. We analyzed the numbers of chemotherapy cycles and regimens both continuously and dichotomized at medians, but neither predicted Poor Mobilization in our study. The only predictor identifying Poor Mobilization patients was female gender, although the clinical relevance of this finding is unclear.

We used a modest dose of G-CSF for both mobilization cycles. Previous reports have documented improved PBSC yield with higher doses of G-CSF.²¹ Nademanee reported that G-CSF 10 µg/kg/day compared to 5 µg/kg/day resulted in 1.8-fold ($P=0.04$) greater yield of CD34⁺ cells/kg. However, since adequate stem cell grafts ($\geq 2 \times 10^6$ CD34⁺ cells/kg) were infused in all cohorts, the observed differences in PFS and OS are not attributable solely to the G-CSF dose used.

All PBSC yields were based on actual body weight. Previous analyses have demonstrated that the CD34⁺ cell dose based on ideal body weight (IBW) is a better predictor of engraftment.^{22,23} Reanalysis of our data using IBW reclassified 17 patients as G-CSF Success, but their PFS and OS remained significantly inferior to those categorized as G-CSF Success by actual body weight (not shown). Consequently, when using mobilization as a predictor of HSCT outcome, classification using actual body weight minimizes false positive mobilization success.

Our study is unique in that patients were segregated into three cohorts based on mobilization outcomes with G followed by CG. We observed delayed engraftment as well as decreased median PFS and OS for the Poor Mobilization cohort. Sugrue *et al.*²⁴ similarly observed delayed platelet engraftment and decreased OS in hard to mobilize patients despite a minimum graft size of 1.8×10^6 CD34⁺ cells/kg. Three other studies have reported similar results, although none have been able to compare outcomes of different mobilization techniques.^{8–10} A study from Stanford included 170 NHL patients mobilized with chemotherapy and G-CSF receiving purged autografts and reported no difference in the 3 year OS and event-free survival (EFS) between patients collecting greater or less than 2×10^6 CD34⁺ cells/kg.¹⁰ Similar to our results, patients with poor mobilization had delayed engraftment and women were more likely to be poor mobilizers. Two other studies have reported a negative impact on survival for patients who are poor mobilizers.^{8,9} Gordan *et al.* reported 90 patients with NHL or HL, initially mobilized with G-CSF and, salvage mobilization with either higher doses of G-CSF, chemotherapy plus G-CSF, or bone marrow harvest.⁸ By univariate but not multivariate analysis, poor mobilization decreased OS. A more recent analysis by Pavone *et al.*⁹ evaluated 262 patients with HL and NHL receiving chemotherapy based mobilization. They demonstrated that more than three prior chemotherapy regimens predicted poor mobilization and, consequently, a decreased EFS and OS for patients with poor mobilization.

Failure to mobilize progenitors using either G-CSF or chemotherapy plus G-CSF may be a powerful predictor of HSCT overall success. Our data suggest that patients who mobilize poorly ($< 2 \times 10^6$ CD34⁺ cells/kg) with either G or with chemotherapy and G-CSF should be considered for other therapies including allogeneic stem cell transplant. Alternatively, additional mobilization attempts with novel agents such as AMD3100 could be considered, although it is unclear if this will improve PFS and OS outcomes.^{25–27} Further studies to confirm that poor PBSC mobilization identifies lymphoma patients with inferior autologous HSCT outcomes are needed.

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