

Recombinant Human Erythropoietin Therapy Is Very Effective after an Autologous Peripheral Blood Stem Cell Transplant When Started Soon after Engraftment

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ABSTRACT

Purpose: Previous trials of recombinant human erythropoietin (rHuEpo) therapy after autologous hematopoietic stem cell transplantation have administered very high doses of i.v. rHuEpo starting on day 1 and continuing for 1–2 months until erythroid engraftment and have shown no benefit of rHuEpo therapy. We sought to establish a more effective use of rHuEpo in this setting.

Experimental Design: In this report, we show in a first cohort of 45 lymphoma or myeloma patients undergoing peripheral blood stem cell transplant (control group) that endogenous erythropoietin levels are high for the degree of anemia during the first 3 weeks after transplant but become adequate or slightly decreased thereafter. We thus enrolled 41 consecutive similar patients in a trial of rHuEpo therapy at a dose of 500 units/kg/week started on day 30 after the transplant.

Results: The 12-week probability of achieving hemoglobin (Hb) levels of 13 g/dl was 87% in rHuEpo-treated patients versus 14% in controls ($P = 0.0001$). Mean Hb levels were significantly higher in the rHuEpo group than in the control group from day 42 through day 150 after transplant (P s of <0.05 to <0.001). Two of 41 patients in the rHuEpo group versus 12 of 45 patients in the control group had Hb levels of <9 g/dl between day 42 and day 100 after the transplant ($P = 0.0078$).

Conclusions: Anemia after autologous peripheral blood stem cell transplant is exquisitely sensitive to rHuEpo when therapy is started soon after engraftment. This is the first convincing report showing that rHuEpo is effective in this setting. Our data set the stage for a more rational use of

rHuEpo after autologous hematopoietic stem cell transplantation and should renew interest in erythropoietin therapy in this setting. Prospective, randomized trials should investigate the impact of rHuEpo therapy on transfusion requirements and quality of life.

INTRODUCTION

Epo² is the critical regulatory factor of erythropoiesis. In patients with normal kidney function, serum Epo levels increase exponentially when an anemia develops. The adequacy of serum Epo levels is best assessed by the O/P ratio, with a value < 1 indicating that Epo production is lower than expected for the degree of anemia (1). After high-dose chemotherapy, serum Epo levels first rapidly increase to disproportionately high levels for 1–3 weeks, with peak values usually observed in the first week after the conditioning regimen (2, 3). With marrow recovery after an autologous PBSCT, Epo levels progressively return to an appropriate range, and the duration of this correction phase inversely correlates with the speed of engraftment (4).

Epo expands erythropoiesis mainly by preventing apoptosis of late erythroid progenitor cells and proerythroblasts (5). Epo is therefore unlikely to increase red blood production when endogenous Epo is elevated and nearly all available progenitors are already surviving and differentiating to mature red cells. Previous trials of rHuEpo therapy after autologous HSCT have not taken this pathophysiology of erythropoiesis into account. All studies have administered very high doses of i.v. rHuEpo starting on day 1 and continuing for 1–2 months or until erythroid engraftment and have shown no advantage to rHuEpo therapy (6–15). Therefore, soaking patients with huge doses of rHuEpo at a time when the erythroid marrow has not developed enough erythroid precursors to respond and when endogenous Epo production is appropriate or even excessive for the degree of anemia may not be the best way to use rHuEpo after transplantation. In the allogeneic setting, we have recently shown that rHuEpo therapy is very efficient when based on the pathophysiology of Epo production after allogeneic HSCT, *i.e.*, when rHuEpo therapy was started 35–1444 days after the transplant (16). In this study, we demonstrate that Epo production normalizes or becomes slightly deficient after day 28 post-autologous PBSC transplantation and that rHuEpo therapy (500 units/kg/

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² The abbreviations used are: Epo, erythropoietin; rHuEpo, recombinant human erythropoietin; HSCT, hematopoietic stem cell transplantation; PBSCT, peripheral blood stem cell transplant; Hb, hemoglobin; O/P, observed/predicted; PBSC, peripheral blood stem cell; G-CSF, granulocyte colony-stimulating factor; sTfR, serum-soluble transferrin receptor; TS, transferrin saturation; BMT, bone marrow transplant; GM-CSF, granulocyte macrophage colony-stimulating factor; Hct, hemocrit.

week) is very efficient when started around 1 month after the transplant.

MATERIALS AND METHODS

Patients. Eighty-six recipients of an autologous PBSC transplant [51 males and 35 females, aged 15–69 years (median age, 53 years)] were analyzed. They had multiple myeloma ($n = 46$), non-Hodgkin's lymphoma ($n = 35$), or Hodgkin's disease ($n = 5$). Forty-five consecutive patients transplanted in 1994–1998 in whom serum Epo levels were available served as the control group, and 41 consecutive patients transplanted after March 2001 were included in the rHuEpo group (Table 1). Conditioning before transplantation consisted of various combinations of high-dose myeloablative chemotherapy with ($n = 18$) or without ($n = 68$) total body irradiation. A minimum of 2×10^6 CD34+ PBSCs/kg were infused on day 0. Single-donor platelet transfusions were given if platelet counts decreased below 15×10^9 /liter. The trigger for packed RBC transfusions was 8 g/dl for all patients receiving rHuEpo and 9 g/dl for patients included in the control group (general change in transfusion policy between the two study periods). Otherwise, the clinical management of the patients was identical in the two groups. The control and rHuEpo groups were well balanced for sex, diagnosis (although there was a tendency for more lymphomas among controls and more myeloma in the rHuEpo group), conditioning, and burst-forming units-erythroid and CD34+ cell doses, but the rHuEpo group was older ($P = 0.001$). Their pretransplant Hb was identical: 9.9 ± 1.1 g/dl in the control group *versus* 10.1 ± 1.7 in the rHuEpo group ($P = 0.62$). The two groups did not differ for neutrophil and platelet engraftment or for the numbers of days of G-CSF and platelet transfusions.

rHuEpo Treatment. Forty-one patients were scheduled to start rHuEpo on day 30 after PBSCT at a dose of 500 units/kg/week (given s.c.). Initiation of rHuEpo was delayed in some patients until resolution of ongoing infections. rHuEpo was administered (three times per week in the 15 first patients and once weekly in the 26 others) with the aim of achieving and maintaining Hb levels of 13 g/dl. This initial target was chosen to ensure easy maintenance of Hb values above 12 g/dl thereafter. Once the target Hb was achieved, the dose of rHuEpo was reduced by 50% and then further reduced on a biweekly basis so as to use the lowest dose capable of maintaining the Hb above 12 g/dl but no higher than 14 g/dl. In the rHuEpo group, patients received i.v. iron (Venofer; 600 mg in three divided doses over 3–6 weeks) when TS was below 20%, unless the target Hb was already attained. i.v. iron was never given to patients not on rHuEpo therapy, and functional iron deficiency occurred in only five cases among them.

Laboratory Analyses. Complete blood counts, percentages of reticulocytes, sTfR (Ref. 17; a quantitative measure of total erythropoietic activity), and serum Epo levels were measured as reported previously (16, 18). Based on regression equations obtained in appropriate reference subjects between Hct on the one hand and log(Epo) on the other, predicted log(Epo) values were derived for each Hct, and O/P ratios of O/P Epo values were calculated (1, 19). The mean \pm SD Epo O/P ratio in a cohort of 31 normal donors was 1.03 ± 0.08 .

Statistical Methods. Unpaired Student's *t* tests were used to compare biological variables in two groups. Welch's correction was used in case of unequal variance. The number of transfusions in the same or different groups of patients were compared using Wilcoxon matched pair or Mann-Whitney *U* tests, respectively. Times to response to rHuEpo therapy were

Table 1 Patient characteristics

	Control group	rHuEpo group	<i>P</i>
No. of patients	45	41	
Age (mean \pm SD) (yrs)	43 ± 11	56 ± 8	0.001
Sex (male/female)	29/16	22/19	NS
Disease			
Non-Hodgkin's lymphoma	22	13	NS
Multiple myeloma	19	27	
Hodgkin's disease	4	1	
Conditioning regimen			
TBI ^a	11	7	NS
No TBI	34	34	
Graft composition (mean \pm SD)			
CD34+ cells $\times 10^6$ /kg	16.2 ± 29.3	12.5 ± 8.8	NS
BFU-E $\times 10^4$ /kg	223 ± 324	177 ± 172	NS
Days to 1% reticulocytes (median)	12	13	NS
Days to unsupported 27% Hct (median)	16	15	NS
Days to last RBC transfusion (median)	10	7	<0.05
Days to 0.5×10^9 /liter neutrophils (median)	9	9	NS
Days to 1×10^9 /liter neutrophils (median)	10	10	NS
Days to unsupported 20×10^9 /liter platelets (median)	11	9	NS
Days to unsupported 100×10^9 /liter platelets (median)	17	15	NS
Days to last platelet transfusion (median)	9	7	NS
Number of days of G-CSF (mean \pm SD)	10 ± 4	11 ± 2	NS
RBC transfusions before day 30 (mean \pm SD)	3 ± 2	2 ± 2	NS
Platelet transfusions (mean \pm SD)	3 ± 3	2 ± 2	NS

^a TBI, total body irradiation; BFU-E, burst-forming units-erythroid.

studied by life-table analyses, and Wilcoxon rank tests were used for comparisons between groups. To allow meaningful comparison with rHuEpo-treated patients, the time to achieve Hb levels of 13 g/dl and the time to achieve a 2 g/dl Hb increment were calculated from day 30 after HSCT also for patients included in the control group. Areas under the curve were calculated in each group from day 30 to day 180 using 10.1 g/dl (the mean Hb value on day 30 in the control group) as the baseline *Y* value. Because the two groups had a different trigger value for RBC transfusion, we also calculated the number of patients in each group who had a Hb value below 9 g/dl at any time between days 42 and 100 after the transplant. Differences between groups were calculated by Fisher's exact test. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA).

RESULTS

Evolution of O/P Epo Ratio in PBSC Recipients Not Receiving rHuEpo. After PBSCT, serum Epo levels peaked on day 0 with a mean O/P Epo of 1.19 ± 0.16 ($P < 0.0001$ compared with 31 healthy donors; Fig. 1). Thereafter, endogenous Epo levels decreased slowly and remained adequate for the degree of anemia until day 21, when levels became inappropriately low. Epo values returned to appropriate levels around day 100 after the transplant. This pattern was superimposable in multiple myeloma and lymphoma patients.

Erythropoietic Responses to rHuEpo. In the rHuEpo group, mean O/P Epo was 0.87 ± 0.13 ($P < 0.0001$ compared with 31 healthy donors), and O/P Epo was below 1.0 in 85% of the patients when rHuEpo was started. Day 30 serum creatinine was 11.0 ± 3.9 mg/liter in the control group *versus* 11.3 ± 5.0 mg/liter (NS) in the rHuEpo group. Before day 30, the evolution of sTfR, reticulocytes, and Hb was strictly superimposable (Fig. 2), and the speed of erythroid engraftment was similar (Table 1) in the two groups. Whereas erythropoiesis (as assessed with sTfR) remained stable between day 30 and day 150 after HSCT in the control group, it expanded very rapidly above the upper normal limit for sTfR ($7000 \mu\text{g/liter}$) in the rHuEpo group and slowly decreased to baseline values when rHuEpo dosage was reduced (Fig. 2A). This expansion of erythropoietic activity

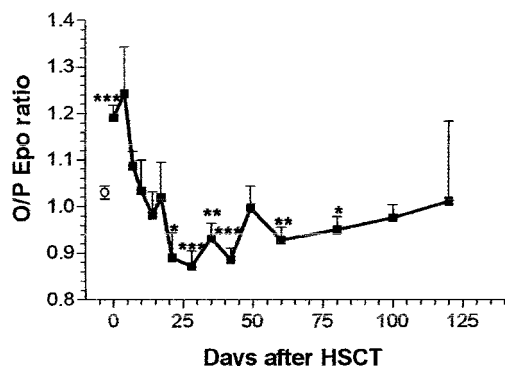


Fig. 1 Endogenous serum Epo levels, as assessed by O/P Epo ratios (mean \pm SE) after autologous PBSCT. Mean \pm SE values of 31 normal donors are also shown (\circ).

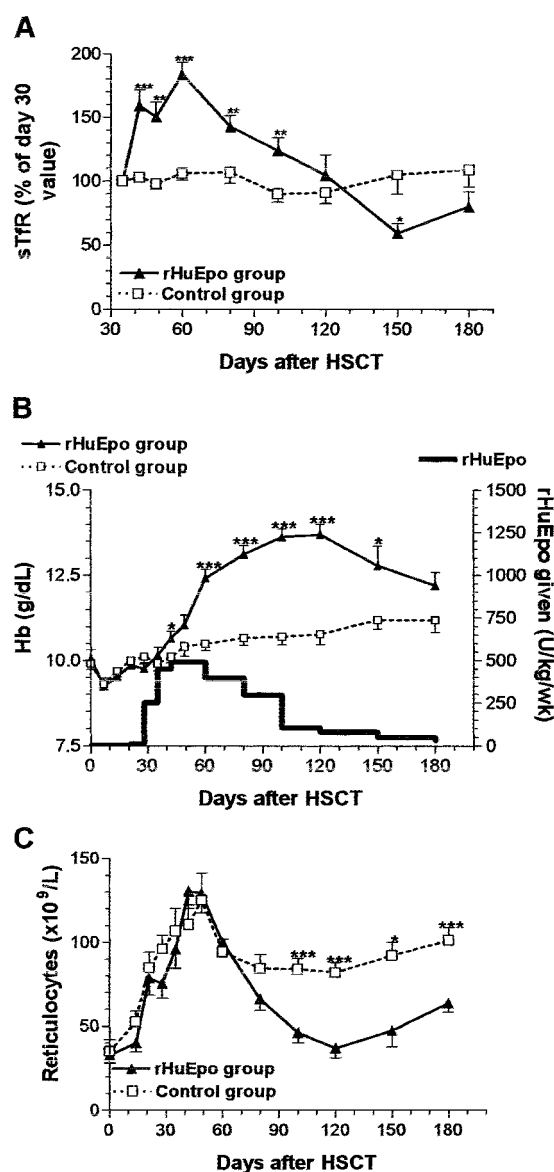


Fig. 2 Evolution of sTfR levels (A), Hb levels (B), and reticulocyte counts (C) after transplantation. *Ps* are given for comparisons between the two groups: *, <0.05 ; **, <0.01 ; ***, <0.001 .

translated into a progressive Hb response over a period of 10 weeks in the rHuEpo group, whereas Hb levels increased much more slowly in the control group (Fig. 2B). The 2-week sTfR increment was highly correlated with the 4-week Hb increment ($r = 0.57$; $P < 0.0001$; Fig. 3). On the other hand, reticulocyte counts did not differ between the two groups (Fig. 2C). The median time to a Hb increment of 2 g/dl was 5 weeks in the rHuEpo group *versus* 16 weeks in the control group ($P < 0.0001$). Hb values of 13 g/dl were achieved after a median of 6 weeks in the rHuEpo group *versus* >16 weeks in the control group ($P < 0.0001$; Fig. 4). There were no differences in response to therapy given weekly or thrice weekly. The median time to a Hb increment of 2 g/dl and the median time to achieve

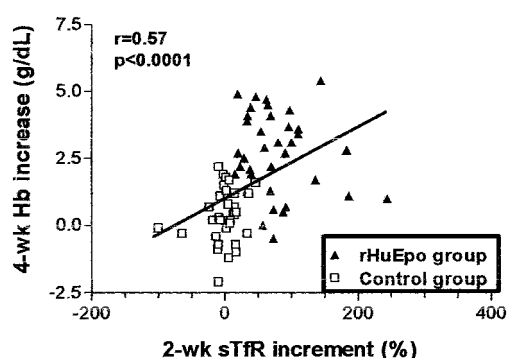


Fig. 3 Correlation between the 2-week sTfR increment and the 4-week Hb increment from day 30 after PBSCT. The two extreme patients showing large sTfR increments but slow Hb responses had cytomegalovirus infection and functional iron deficiency, respectively, soon after initiation of rHuEpo therapy.

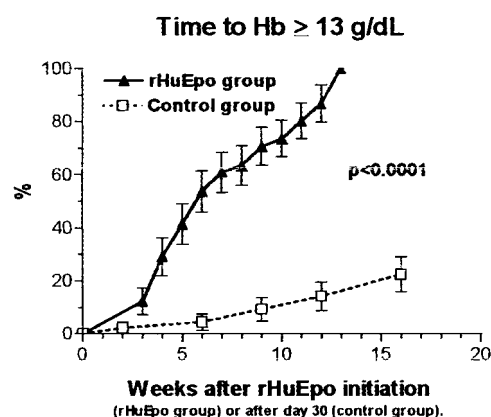


Fig. 4 Kaplan-Meier plots of time to Hb \geq 13 g/dL.

a Hb level of 13 g/dl were 5 and 9 weeks for patients receiving rHuEpo thrice weekly and 4 (NS) and 6 (NS) weeks for patients receiving rHuEpo once weekly. The overall 12- and 15-week probability of achieving a complete response (Hb $>$ 13 g/dl) was 87% and 100% in the rHuEpo group *versus* 14% and 22% ($P < 0.0001$) in the control group. Mean Hb levels were significantly higher in the rHuEpo group compared with the control group from day 42 through day 150 after the transplant (Fig. 2B). The area under the curve of Hb between days 30 and 180 was 279 ± 153 in rHuEpo-treated patients compared with 156 ± 128 in controls ($P = 0.0001$). Platelet counts and neutrophils on rHuEpo treatment did not decrease compared with those of the control group. Day 100 platelet and neutrophil counts were $131 \pm 82 \times 10^9/\text{liter}$ and $2220 \pm 1150 \times 10^9/\text{liter}$ in the control group *versus* $165 \pm 81 \times 10^9/\text{liter}$ and $2610 \pm 990 \times 10^9/\text{liter}$ in the rHuEpo group, respectively.

Transfusions. Because of the higher Hb trigger for transfusion, the number of RBC transfusions before day 30 was slightly greater (3 *versus* 2) in the control group. Eight of 45 patients in the control group *versus* 0 of 41 patients in the rHuEpo group required RBC transfusions after day 30 posttransplant ($P = 0.0059$). Four controls with Hb slightly below 9.0

g/dl were not transfused because the patient or physician refused: if they had been transfused, the difference in transfusion rates between the groups would have been more significant. However, part of this difference was probably due to the higher Hb trigger for RBC transfusion in the control group (9 g/dl) compared with the rHuEpo group (8 g/dl). To circumvent this limitation, we examined the proportion of patients with a Hb value $<$ 9 g/dl at any time between day 42 and 100 because these patients would have been transfused at a transfusion trigger of 9 g/dl. Only 2 of 41 patients in the rHuEpo group compared with 12 of 45 patients in the control group ($P = 0.0078$) experienced such a Hb value below 9 g/dl between day 42 and day 100 after the transplant.

Iron Supplementation by i.v. The evolution of TS is shown in Fig. 5A. TS was increased immediately after the conditioning regimen and normalized with erythropoietic recovery. After day 30, rHuEpo therapy induced a significant reduction in TS that later corrected slowly with iron supplementation and reduced rHuEpo dosage. All but eight patients in the rHuEpo group experienced functional iron deficiency. Twenty-one patients received i.v. iron supplementation, and 12 patients did not because they had already reached the target Hb ($n = 9$),

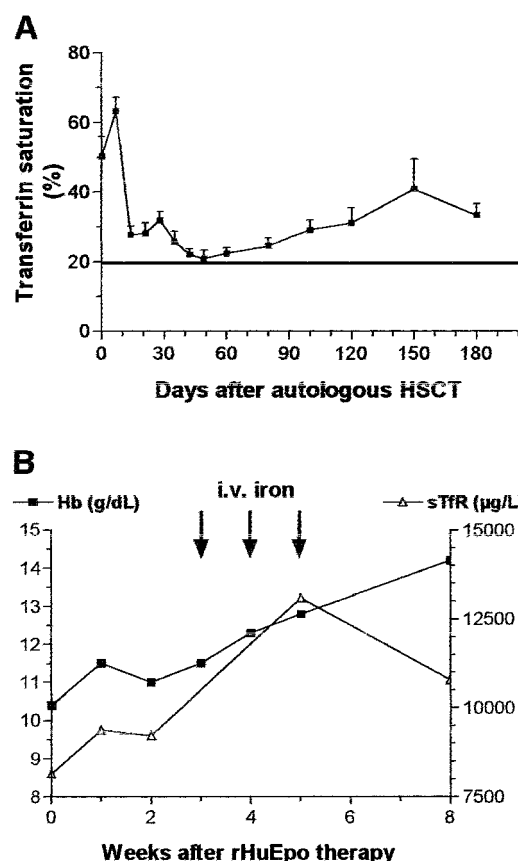


Fig. 5 A, evolution of TS after the transplant in the rHuEpo group. B, evolution of Hb and erythropoiesis (assessed by sTfR) in a patient who developed functional iron deficiency. Whereas response to rHuEpo was poor until then, erythropoiesis and Hb increased rapidly after i.v. iron administration (arrows).

functional iron deficiency was caused by infection ($n = 2$), or the patient refused ($n = 1$). Fig. 5B illustrates the prompt response to i.v. iron supplementation in a patient with functional iron deficiency and poor response to rHuEpo before iron therapy.

DISCUSSION

Elevated serum Epo levels are observed transiently after intensive conditioning regimens without concomitant changes in Hb (2, 3, 18). The peak Epo values are usually observed 7 days after transplantation, at the time of the nadir of erythropoietic activity. With marrow recovery, Epo levels progressively return to an appropriate range, and the duration of this correction phase inversely correlates with the speed of engraftment (4). Thereafter, endogenous Epo remains appropriate for the degree of anemia in autologous BMT recipients (2, 3). This study first shows that after autologous PBSC transplantation, endogenous Epo levels decreased slowly and remained adequate for the degree of anemia until day 21, when levels became inappropriately low. Epo values returned to appropriate levels around day 100 after the transplant. These findings suggested that providing rHuEpo to autologous PBSC recipients around day 30 after the transplant could be an effective physiological approach.

Previous trials of rHuEpo therapy after autologous HSCT have not taken this pathophysiology of erythropoiesis into account. All studies to date have administered very high doses of i.v. rHuEpo starting on day 1 and continuing for 1–2 months or until erythroid engraftment and failed to demonstrate efficacy (6–15). Although only one of them was powered enough to demonstrate an advantage of rHuEpo therapy (9), the absolute differences in mean Hb levels or speed of engraftment between treated and control patients were always minimal. Ayash *et al.* (8) administered 1400 units/kg/week rHuEpo for 4 weeks to 10 patients with solid tumors undergoing auto-BMT. Eight of 10 rHuEpo-treated patients *versus* 20 of 37 historical controls achieved a Hct of 30% within 32 days (NS), but the number of RBC transfusions was the same (9 *versus* 9 units). Locatelli *et al.* (10) gave rHuEpo (725 units/kg/week, days 1–30) to 10 children undergoing purged auto-BMT for acute leukemia. There was no difference with 10 historical controls for reticulocyte output, sTfR increase, or the number of transfusions. Pene *et al.* (7) gave GM-CSF and rHuEpo (1050 units/kg/week) to 18 auto-BMT patients until Hb reached 12 g/dl. As compared with 6 concomitant and 65 historical controls receiving GM-CSF alone, there was no difference for the median time to a Hb ≥ 12 g/dl or the number of RBC transfusions (10 *versus* 12 units). Pedrazzini (6) compared seven controls with six patients receiving rHuEpo (2100 units/kg/week) and GM-CSF from day 10 to the day of neutrophil engraftment and observed no difference for the median time to a Hb of 10 g/dl or the number of transfusions before (10 *versus* 10 units) or after (5 *versus* 8 units) day 35. Vannucchi *et al.* (14) gave rHuEpo (1050 units/kg/week, days 1–21) and G-CSF to 30 patients with lymphoid malignancies undergoing auto-BMT. Ten patients each received G-CSF and rHuEpo, G-CSF alone, or no growth factor. Median time to a Hct of $\geq 30\%$ was not faster, and RBC transfusion needs were not reduced by rHuEpo. Miller and Mills (12) conducted a randomized trial of rHuEpo (1400 units/kg/week for 4 weeks followed by 600 units/kg/week for 3 weeks) *versus*

placebo in 50 patients undergoing auto-BMT with purged marrow for acute myelogenous leukemia or non-Hodgkin's lymphoma. The Hb level at day 50 was higher in the rHuEpo group (10.5 *versus* 9.5 g/dl; $P = 0.02$), but this did not translate into reduced RBC requirements (13 *versus* 15 units). Chao *et al.* (11) randomized 35 lymphoma patients to receive rHuEpo (600 units/kg/week, days 1–30) or placebo in addition to G-CSF. rHuEpo was started 3 weeks before high-dose therapy but was held during conditioning. There was no difference for the number of RBC transfusions before day 30 (8 *versus* 6 units). Link *et al.* (9) conducted a prospective, randomized, placebo-controlled multicenter trial in 114 patients after auto-BMT. Patients received 1050 units/kg/week rHuEpo until day 41 or until independence from transfusions with a stable Hb of >9 g/dl. There was no difference in the regeneration of reticulocytes, time to transfusion independence, or the number of RBC transfusions. Given this negative experience after auto-BMT, there is only one study after PBSC transplantation. Pierelli *et al.* (13) gave rHuEpo (150 units/kg s.c. every 48 hours, days 1–11) and G-CSF to 15 patients with breast or ovarian carcinoma undergoing PBSC T. Compared with eight historical controls, there was no effect on erythroid recovery. The same group extended their observations with the same protocol, adding 15 patients receiving the same schedule of rHuEpo but with GM-CSF. There was no impact on RBC transfusion requirements.

Therefore, we took a more physiological approach by providing rHuEpo to patients 30 days after the transplant. We had previously shown that a similar attitude was very effective in the allogeneic HSCT setting (16). The current study proves that this approach is also very efficient in the autologous PBSC T setting, with complete correction of Hb achieved in 100% of the patients (*versus* 22% in the control group). We elicited to seek a Hb target of 13 g/dl to demonstrate the full potential of rHuEpo therapy in the setting of autologous HSCT and to be easily able to maintain Hb values above 12 g/dl after the target had been reached. The median time to a Hb increment of 2 g/dl was 5 weeks in the rHuEpo group *versus* 16 weeks in the control group. This was obtained with median doses of 500 units/kg/week, 2–3 times lower than the doses used in previous transplant trials but similar to doses used in cancer-associated anemia (20). However, the rate and quality of response were far superior to those achieved in the anemia of cancer, where the proportion of patients increasing Hb by 2 g/dl is 40–60%, and it typically takes 6–16 weeks to increase Hb by 2 g/dl (20). In addition, there was no difference in response to therapy given weekly or thrice weekly. This observation confirms the similar results of rHuEpo therapy given qw or tiw after an allogeneic transplant when rHuEpo is also started 1 month after the transplant (21).

The expansion of erythropoietic activity by rHuEpo therapy was considerable, reaching sTfR values well above the normal range in the majority of the patients (Fig. 2). This expansion reached its maximum even faster than in renal failure patients (who received lower doses of rHuEpo; Ref. 22). When rHuEpo therapy was discontinued, erythropoiesis declined slowly. Hb increases correlated with early signs (sTfR increment) of expansion of erythropoietic activity. On the other hand, reticulocyte counts did not differ significantly in controls and treated patients, underscoring the poor quantitative value of this parameter in evaluating erythroid response (20, 23, 24). Reticu-

locytes after day 80 were lower in the rHuEpo group because therapy was stopped in most patients who then had higher Hb values compared with untreated patients.

It is now well demonstrated that adequate delivery of iron to the bone marrow is an important consideration in clinical settings in which rHuEpo is used (20). Indeed, effective stimulation of erythropoiesis by rHuEpo is often associated with functional iron deficiency, a situation characterized by an inadequate supply of stored iron to provide enough iron for the increased requirements in the erythroid marrow. This can occur even in the presence of increased iron stores if these stores cannot be mobilized fast enough to meet the demand. The experience in iron-replete renal failure patients treated with rHuEpo has clearly indicated that oral iron supplementation is only marginally superior to no iron, whereas i.v. iron substantially improves response to rHuEpo therapy (25). Functional iron deficiency (as evidenced by low TS or increased percentage of hypochromic RBCs) has also been observed in a large proportion of cancer patients treated with rHuEpo (26), and many recent protocols have included a recommendation for i.v. iron supplementation when there is evidence of functional iron deficiency. In our rHuEpo group, functional iron deficiency occurred frequently, and i.v. iron supplementation generally resulted in prompt erythropoietic response to rHuEpo (Fig. 4). Whether systematic early i.v. iron supplementation would further improve the speed and level of response to rHuEpo remains to be determined prospectively.

Our study of course has the limitations of nonrandomization. The rational use of rHuEpo after autologous HSCT as proposed here should renew interest in Epo therapy. However, previous trials that targeted initial erythroid recovery rather than the more physiologically appropriate period that follows engraftment were all negative. Therefore, when we started our trial, there was no basis for a randomized trial but rather a need for a large Phase II trial identifying a new approach with an appropriate dose and timing for rHuEpo therapy after autologous transplantation. Apart from the multicenter trial conducted by Link *et al.* (9), our trial includes by far the largest number of patients treated with rHuEpo in this setting. The results are impressive and suggest that autologous HSCT [similar to allogeneic HSCT (16)] is associated with the best response rate to rHuEpo outside the setting of uremia. This trial now justifies the development of prospective, randomized, placebo-controlled trials that should investigate clinical end points previously shown to be improved by rHuEpo therapy in other settings, such as transfusion requirements and quality of life, as well as cost-effectiveness.

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