Darbepoetin-alfa and intravenous iron administration after autologous hematopoietic stem cell transplantation: A prospective multicenter randomized trial

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We conducted a randomized study analyzing the impact of darbepoetin alfa (DA) administration with or without intravenous (i.v.) iron on erythroid recovery after autologous hematopoietic cell transplantation (HCT). Patients were randomized between no DA (Arm 1), DA 300 μg every 2 weeks starting on Day 28 after HCT (Arm 2), or DA plus i.v. iron 200 mg on Days 28, 42, and 56 (Arm 3). The proportion achieving complete hemoglobin (Hb) response within 18 weeks (primary end point) was 21% in Arm 1 ($n = 24$), 79% in Arm 2 ($n = 25$), and 100% in Arm 3 ($n = 23$; $P < 0.0001$). Erythropoietic response was shown to be significantly higher in Arm 3 ($n = 46$) than in Arm 2 ($n = 50$; $P = 0.008$), resulting in lower DA use, reduced drug costs, and improved quality of life scores, but the effect on transfusions was not significant. In multivariate analysis, DA administration ($P < 0.0001$), i.v. iron administration ($P = 0.0010$), high baseline Hb ($P < 0.0001$), and low baseline creatinine ($P = 0.0458$) were independently associated with faster achievement of complete Hb response. In conclusion, DA is highly effective to ensure full erythroid reconstitution after autologous HCT when started on Day 28 post-transplant. I.v. iron sucrose further improves erythroid recovery. Am. J. Hematol. 00:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

After autologous hematopoietic cell transplantation (HCT), most patients experience prolonged anemia [1]. Given the tight association between hemoglobin (Hb) levels and quality of life (QOL) [2,3], we sought to study ways of improving Hb levels after autologous HCT.

Erythropoietin (Epo) is the critical regulatory factor of erythropoiesis. Elevated serum Epo levels are observed transiently after intensive conditioning regimens without concomitant changes in Hb [1,4,5]. The peak Epo levels are usually observed 7 days after transplantation, at the time of the nadir of erythropoietic activity [1,4,5]. With marrow recovery, Epo levels decrease slowly and remain adequate for the degree of anemia until Day 21 when they become inappropriately low [1,6]. Epo values then return to appropriate levels around Day 100 after the transplant [1,4–6].

Several prospective studies have administered very high doses of intravenous (i.v.) rHuEpo starting on Day 1 and continuing for 1–2 months or until erythroid engraftment. These studies uniformly failed to show any advantage for rHuEpo therapy [7–11]. In contrast, we previously reported the results of a retrospective study suggesting that Epo therapy starting on Day 28 after autologous HCT was highly effective to improve Hb levels [6], and observed that i.v. iron appeared to improve response in some patients with low transferrin saturation [6]. This prompted us to conduct a multicenter prospective randomized study analyzing the impact of darbepoetin alfa (DA) administration with or without i.v. iron on erythroid recovery after autologous HCT.

Patients and Methods

Study design

The study was a 3-arm multicenter randomized study of DA administration after autologous HCT (Supporting Information Fig. 1). In the first part of the study, subjects in Group 1 received neither DA nor i.v. iron, those in Group 2 received DA alone starting on Day 28 after the transplant, whereas those in Group 3 received DA and i.v. iron saccharate (Venofer®) (Venofer® was kindly provided by Fresenius Belgium), both starting at Day 28 after the transplant. The study initially planned to include 25 subjects in each arm (randomization 1:1:1). The comparison between the three groups (Part 1 of the study) is thus based on these 75 patients. The protocol was then amended to randomize (randomization 1:1) 50 additional subjects in Groups 2 and 3. The comparison between Groups 2 and 3 (Part 2 of the study) is thus based on the full cohort in each of these two arms. The study was approved by the Ethics Committee of the University of Liège and of other participating centers. All patients signed a written consent form.

Eligibility criteria

Patients given autologous HCT for multiple myeloma or lymphoma were eligible if they were older than 16 years and younger than 70 years, did not have terminal organ failure, had serum ferritin > 100 μg/L, and had adequate marrow recovery as determined by a neutrophil count $> 1 \times 10^9$/L and achievement of platelet transfusion independence. Those who underwent two autologous HCT for multiple myeloma could be included twice and were considered as "separate subjects" for the clarity of analyses. Exclusion criteria included HIV

Additional Supporting Information may be found in the online version of this article.

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Conflict of interest: YB has been a consultant and has taken part in advisory boards and in speakers' bureau with Amgen and Vifor.

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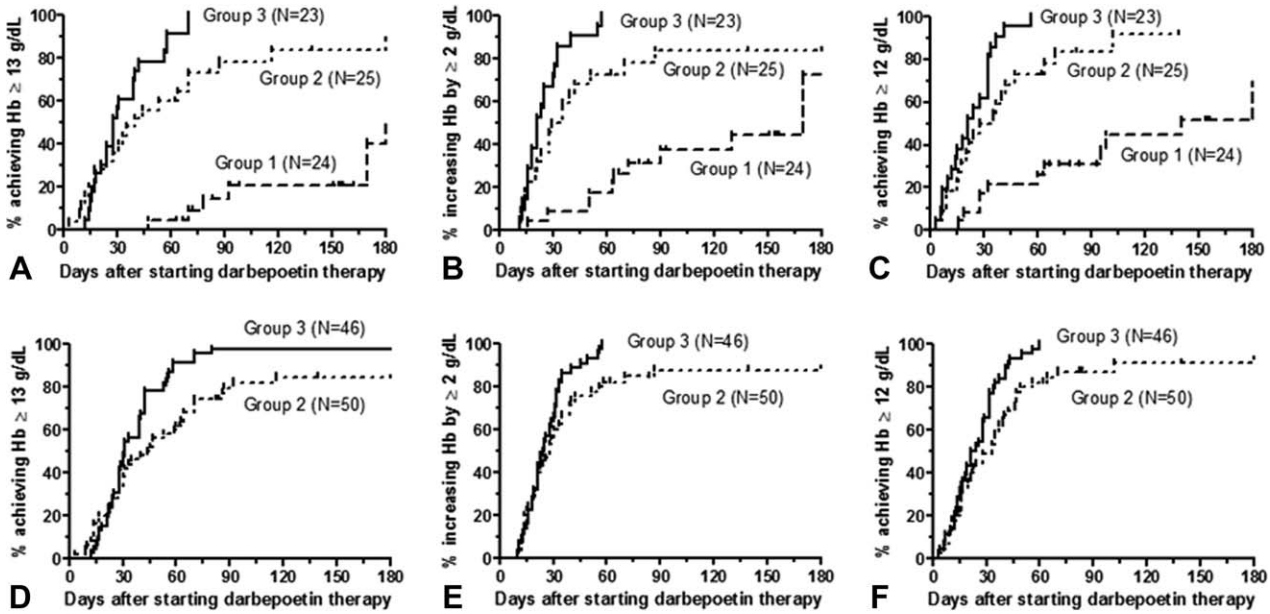

Figure 1. Kaplan-Meier probability of achieving Hb levels ≥13 g/dL (A and D), increasing Hb by ≥2 g/dL (B and E), or achieving Hb levels ≥12 g/dL (C and F) in the three groups (study Part 1, Panels A–C) or in Groups 2 and 3 (study Part 2, Panels D–F) from first day of DA administration (or from Day 28 after HCT for patients in Group 1). Group 1 = no DA, Group 2 = DA, and Group 3 = DA + i.v. iron.

seropositivity; known allergy to erythropoiesis-stimulating agent (ESA) or i.v. iron saccharate; evidence of active hemorrhage, hemolysis, vitamin B12, or folate deficiency (in these case inclusion into the protocol could be delayed up until Day 42 if the problem was resolved); uncontrolled infection, arrhythmia or hypertension (in these cases inclusion into the protocol could be delayed up until Day 42 if the problem was resolved); evidence of severe iron overload (serum ferritin > 2,500 μg/L); or risk of transferrin oversaturation with i.v. iron (transferrin saturation >60%).

Study treatments

DA (Aranesp®) was administered subcutaneously at the dose of 300 μg. The first dose was given on Day 28 and the following doses at 2-week intervals on Days 42, 56, 70, 84, 98, and 112 post-transplant. Once the target Hb (13 g/dL) was attained, the dose of DA was reduced by half to 150 μg. If the Hb increased to >14 g/dL, DA was withheld and resumed at the dose of 150 μg when the Hb decreased < 13 g/dL. If the Hb decreased to < 12 g/dL, the dose of DA was increased to 300 μg again. Iron sucrose (Venofer®) was administered i.v. at the dose of 200 mg on Days 28, 42, and 56 after HCT. No iron supplementation was allowed in Arm 1 nor in Arm 2 before Day 70 after the transplant. I.v. iron (300 mg) was allowed in Arms 2 and 3 if there was evidence of functional iron deficiency (defined as transferrin saturation < 20%) beyond Day 70. One red blood cell (RBC) unit was transfused when the Hb value was between 7.0 and 7.9 g/dL. Two RBC units were transfused if the Hb value was below 7 g/dL. Platelets were transfused if needed, according to the institutional guidelines.

Study end points

The primary end points of the study were the proportion of complete correctors (reaching Hb ≥ 13 g/dL) before Day 126 (2 weeks after last dose of Aranesp®) in each arm, and the median time to achieve a Hb level ≥13 g/dL in each arm. Secondary end points included the median time to increase Hb level by ≥2 g/dL in each arm; the proportion of responders (increasing Hb by ≥2 g/dL) before Day 126 in each arm; the proportion of correctors (reaching Hb ≥ 12 g/dL) before Day 126 in each arm; the proportion requiring RBC transfusions between Days 28 and 126 in each arm; the total number of RBC transfusions between Days 28 and 126 in each arm; the area under the curve (AUC) of mean Hb level between Days 28 and 126 after the transplant in each arm, the mean Hb values on Days 42, 56, 70, 84, 98, 112, and 126 in each arm; and QOL in each arm. QOL was measured by Functional Assessment of Cancer Therapy-Fatigue questionnaires [12].

Laboratory analyses

Complete blood cell counts, percentages of reticulocytes, transferrin saturation, serum ferritin, and serum soluble transferrin receptor (sTfR; a quantitative measure of total erythropoietic activity) were measured as previously reported [13–18].

Statistical analyses

The proportion of patients who achieved targeted Hb levels before Day 126 post-HCT in each arm was displayed in a Kaplan–Meir curve and compared by a log rank test. Day 126 post-HCT was chosen because it corresponded to Day 98 after initiation of DA and iron sucrose therapy, i.e., 2 weeks after the last scheduled dose of DA. Median times to reach 13 g/dL, 12 g/dL, or 2 g/dL Hb increment in each group were compared using the log rank test. For the latter analyses, subjects who died or received a second HCT were censored at the time of death/second HCT. To allow meaningful comparison with DA-treated patients, the time to achieve Hb levels of 13 g/dL or 12 g/dL, as well as the time to achieve a 2 g/dL Hb increment were calculated from Day 28 after HCT for those included in the control arm. Transferrin saturation, numbers of transfusions, QOL scores as well as Hb, reticulocytes, sTfR, and ferritin levels in the three arms were compared using the Mann-Whitney test. AUCs of Hb were calculated in each group from Days 28 to 126 post-HCT. For AUC calculations, patients who relapsed, died or received a second HCT before Day 126 carried forward until Day 126 their last Hb value measured before this event. Potential factors associated with faster achievement of Hb levels ≥13 g/dL were assessed by multivariate Cox regression models. These included DA administration, i.v. iron administration, patient body weight, sex, number of transplanted CD34+ cells, as well as baseline Hb, platelet count, reticulocyte count, serum ferritin, transferrin saturation, creatinine, and C-reactive protein (CRP). Baseline refers to Day 28 after HCT, i.e., just before initiation of DA and/or i.v. iron therapy. Two-way analysis of variance (ANOVA) was used to analyze the impact of treatment group and time-point (Day 28 vs. Day 70 vs. Day 126 post-transplant) on QOL scores. Correlations between QOL and Hb levels were assessed with the Spearman test. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA) and SAS version 9.2 for Windows (SAS Institute, Cary, NC).

Results

Patients

Seventy-five subjects were included between March 2004 and September 2006 in the first part of the study, 25 in each arm (Supporting Information Fig. 1). Three, 1 in Groups 1 and 2 in Group 3, were found to be ineligible due to baseline ferritin > 2,500 μg/L. Therefore, the evaluable population included in Part 1 of the study comprised 72

patients, with 24, 25, and 23 randomized in Arms 1, 2, and 3, respectively. After the amendment, 52 additional subjects were included between September 2006 and January 2008 so as to schedule a total of 100 patients in Groups 2 + 3 combined. Four, two in Group 2 and two in Group 3, were found to be ineligible due to being platelet transfusion dependent, having baseline ferritin > 2,500 μg/L or transferrin saturation > 60%. Therefore, the evaluable population included in Part 2 of the study comprised 96 patients, with 50 and 46 randomized in Arms 2 and 3, respectively. Characteristics of the 120 patients evaluable for efficacy are described in Supporting Information Table I; these characteristics were comparable in the different study arms, in Part 1 as well as in Part 2 of the study. Although subjects randomized to the control arm were transplanted with slightly lower numbers of $CD34^+$ cells than those randomized in the two other arms, the kinetics of hematological engraftment were similar in the three arms (Supporting Information Table I). Baseline Hb was 10.4 ± 1.3 g/dL (10.3, 10.5, and 10.3 in Groups 1, 2, and 3, respectively). All 127 patients included in the study formed the patient population for safety analyses.

TABLE I. Erythropoietic Response in the Three Groups (Study Part 1)

	Group 1 (N = 24)	Group 2 (N = 25)	Group 3 (N = 23)	*P* value
Time to Hb = 13 g/dL				
% achieving (by Day 126 post-HCT)	21	79	100	<0.0001
Median (days) after starting DA	(190)	40	28	
Time to Hb + 2 g/dL				
% achieving (by Day 126 post-HCT)	38	84	100	<0.0001
Median (days) after starting DA	(170)	32	21	
Time to Hb = 12 g/dL				
% achieving (by Day 126 post-HCT)	45	84	100	<0.0001
Median (days) after starting DA	(140)	32	21	
Hb AUC (Days 28–126)	1,049 ± 125	1,219 ± 156	1,282 ± 80	<0.0001
RBC transfusions				
N patients (yes/no)	2/22	4/21	0/23	NS
N units/patient	0.6 ± 2.5	0.6 ± 1.9	0	NS

Hb, hemoglobin; AUC, area under the curve; RBC, red blood cells; HCT, hematopoietic cell transplantation; DA, darbepoetin alfa; NS, not significant.

Treatments

No patient in Group 1 received any DA or i.v. iron. Per protocol, initiation of treatment was delayed beyond Day 28, by 1 week in three (one in Group 2 and two in Group 3) and by 2 weeks in three others (two in Group 2 and one in Group 3), until resolution of infection. DA was administered per protocol in all except two subjects, one of them receiving 300 μg instead of 150 μg as sixth dose, and one receiving 150 μg instead of 300 μg as first and second dose. Hence, the total cumulative dose of DA administered in Groups 2 and 3 was within 1% of the perprotocol dose. I.v. iron was administered per protocol in all except seven subjects who received lower doses than scheduled (two received 200 mg, three received 400 mg, and two received 500 mg in total). As permitted per protocol, three patients in Group 2 received 300, 600, and 900 mg, respectively, of i.v. iron beyond Day 70; three in Group 3 also received additional i.v. iron doses of 200, 900, and 900 mg, respectively. Hence, the total cumulative dose of i.v. iron administered in Group 3 was within 1% of the scheduled dose.

Part 1 of the study

Primary end point. The proportions reaching Hb levels ≥13 g/dL before Day 126 (complete responders) were 21% in Group 1, 79% in Group 2, and 100% in Group 3. Median times (after Day 28) to achieve a Hb level ≥13 g/dL were 190 days in Group 1, 40 days in Group 2, and 28 days in Group 3, respectively ($P < 0.0001$; Table I and Fig. 1A).

Secondary end points. Patients (one in Group 1, three in Group 2, and two in Group 3) who had Hb levels >12.0 g/dL at baseline were not included in the following analyses. The proportions achieving a Hb increment ≥2 g/dL before Day 126 (responders) were 38% in Group 1, 84% in Group 2, and 100% in Group 3. Median times (after Day 28) to achieve a Hb increment ≥2 g/dL were 170 days in Group 1, 32 days in Group 2, and 21 days in Group 3, respectively ($P < 0.0001$; Table I and Fig. 1B). The proportions attaining a Hb level ≥12 g/dL before Day 126 (correctors) were 45% in Group 1, 84% in Group 2, and 100% in Group 3. Median times (after Day 28) to achieve a Hb level ≥12 g/dL were 140 days in Group 1, 32 days in Group 2, and 21 days in Group 3, respectively ($P < 0.0001$; Table I and Fig. 1C). The AUC of Hb between Days 28 and 126 were 1,049 ± 125 in Group 1, 1,219 ± 156 in Group 2, and 1,282 ± 80 in Group 3 ($P < 0.0001$), respectively (Table I). The proportions requiring RBC transfusions or the mean numbers of transfusions received between Days 28 and 126 after HCT were not different between the three groups (Table I). Finally, mean Hb levels were significantly higher in the two DA groups compared to Group 1 from days 42 to 150 after the transplant, whereas values were significantly higher in Group 3 than in Group 2 from Days 70 to 112 after HCT (Fig. 2A).

Other analyses. Figure 2 displays the evolution of reticulocytes (panel B) and sTfR (panel C), whereas Fig. 3 shows the evolution of serum ferritin (panel A) and transferrin saturation (panel B) in the three groups. Although the surge in reticulocytes after initiation of DA ± i.v. iron therapy was very transient, the increase in total erythropoietic activity was quite sustained ($P < 0.0001$). Although transferrin saturation did not differ among groups, ferritin levels decreased faster in Group 2 compared with Group 1, and levels were intermediate in Group 3. After cessation of DA therapy, transferrin saturation and ferritin levels were higher in Group 3 compared to the other two groups.

Part 2 of the study

Primary end point. The proportions attaining Hb levels ≥13 g/dL before Day 126 (complete responders) were 82% in Group 2, and 98% in Group 3. Median times (after Day 28) to achieve a Hb level ≥13 g/dL were 45 days in Group 2, and 31 days in Group 3 ($P = 0.008$; Table II and Fig. 1D).

Secondary end points. Patients (five in Group 2 and two in Group 3) who had Hb levels >12.0 g/dL at baseline were not included in the following analyses. The proportions achieving a Hb increment ≥2 g/dL before Day 126 (responders) were 88% in Group 2, and 100% in Group 3. Median times (after Day 28) to achieve a Hb increment ≥2 g/dL were 28 days in Group 2, and 25 days in Group 3 ($P = 0.0231$; Table II and Fig. 1E). The proportions reaching a Hb level ≥12 g/dL before Day 126 (correctors) were 87% in Group 2 and 100% in Group 3. Median times (after Day 28) to achieve a Hb level ≥12 g/dL were 33 days in Group 2 and 23 days in Group 3, respectively ($P = 0.0059$; Table I and Fig. 1F). The AUC of Hb between Days 28 and 126 were 1,219 ± 134 in Group 2 and 1,272 ± 78 in Group 3 ($P = 0.02$), respectively (Table I). The proportion requiring RBC transfusions was higher in Group 2 compared to Group 3 ($P = 0.0276$) but the mean numbers of transfusions received between Days 28 and 126 after HCT were not different between the two groups (Table I). Finally, mean Hb levels were significantly higher in Group 3 than in Group 2, from days 70 to 112 after HCT (Fig. 2D).

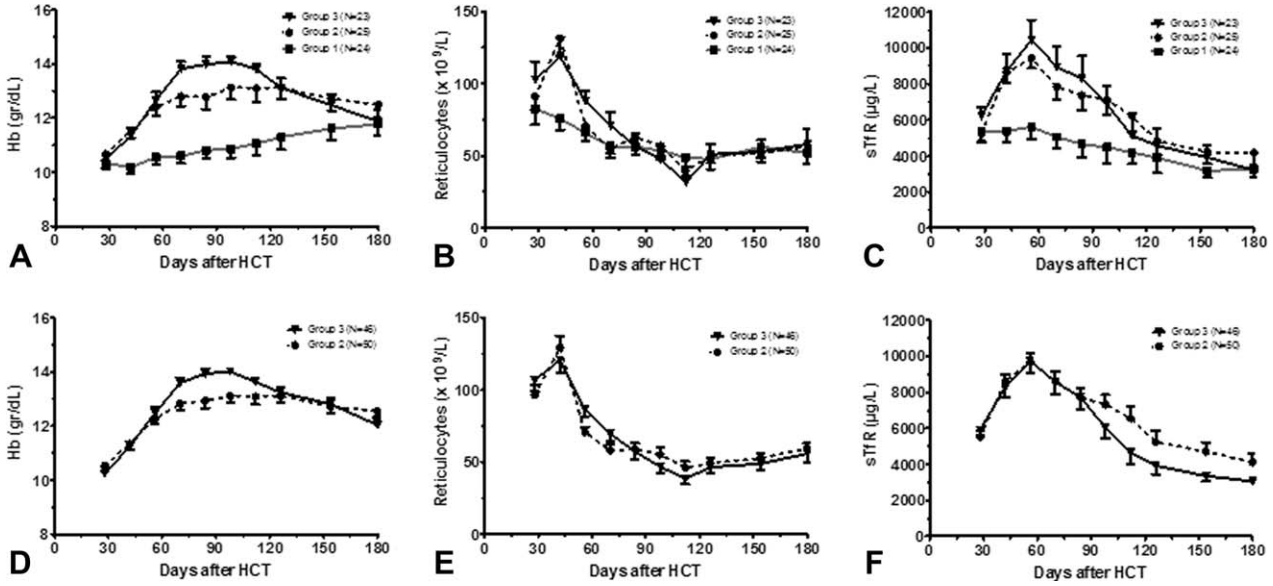

Figure 2. Hb levels (A and D), absolute reticulocyte counts (B and E), and sTfR levels (C and F) in the three groups (study Part 1, Panels A–C) or in Groups 2 and 3 (study Part 2, Panels D–F). Group 1 = no DA, Group 2 = DA, and Group 3 = DA + i.v. iron.

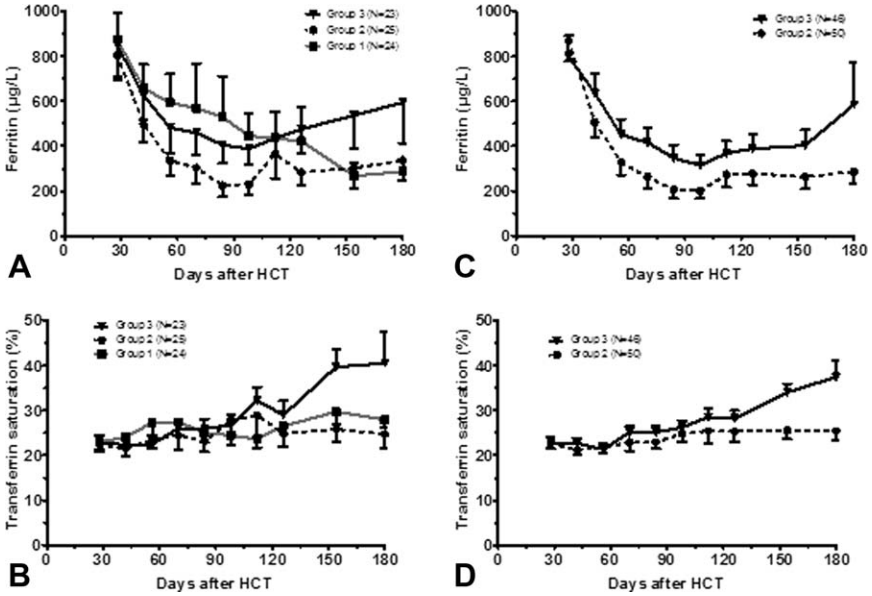

Figure 3. Ferritin levels (A and C) and transferrin saturation (B and D) in the three groups (study Part 1, Panels A–C) or in Groups 2 and 3 (study Part 2, Panels D–F). Group 1 = no DA, Group 2 = DA, and Group 3 = DA + i.v. iron.

Other analyses. Although reticulocyte counts in the two groups were quite superimposable (Fig. 2E), sTfR values diverged after Day 98, with values in Group 2 being higher than those in Group 3 (Fig. 2F). Whereas transferrin saturation (Tsat) remained comparable in the two groups before increasing lately in Group 3 (Fig. 3D), serum ferritin values were constantly elevated in Group 3 compared to Group 2 (Fig. 3C). About 40% (39% in Group 1, 38% in Group 2, 41% in Group 3) of all patients had Tsat $< 20\%$ at baseline. While on DA, about 40–45% in Group 2 and 35–40% in Group 3 had such low Tsat (this was 20–25% in Group 1 during the same time frame). At the end of follow-up, Tsat was still low in 30% in Group 2, but only in 3% in Group 3 (10% in Group 1). We also compared the total dose of DA given in the two DA groups. The total cumulative dose of DA given was $1{,}440 \pm 496$ µg in Group 2 versus $1{,}210 \pm 401$ µg in Group 3 ($P = 0.015$; Table II). We then calculated the difference in Belgian theoretical costs of drug acquisition in the two groups. Purchase of 600 mg Venofer® (100 mg vials) would represent an additional cost of 91.2 €. Based on the actual cumulative doses used, the costs of Aranesp® (300 µg syringes) acquisition would have been 3,346 € in Group 2 vs. 2,811 € in Group 3. Therefore, the overall cost savings in drug acquisition would have been 444 € (3,346 − 2,811 − 91 = 444 €) when i.v. iron was used.

Predictors of complete Hb response

We analyzed all 120 evaluable subjects for predictors of complete Hb response (primary end point). In univariate analysis, DA administration ($P < 0.0001$), i.v. iron administration ($P < 0.0001$), high baseline Hb ($P < 0.0001$) or

TABLE II. Erythropoietic Response in Groups 2 and 3 (Study Part 2)

	Group 2 (N = 50)	Group 3 (N = 46)	*P* value
Time to Hb = 13 g/dL			
% achieving (by Day 126 post-HCT)	82	98	0.008
Median (days) after starting DA	45	31	
Time to Hb + 2 g/dL			
% achieving (by Day 126 post-HCT)	88	100	0.0231
Median (days) after starting DA	28	25	
Time to Hb = 12 g/dL			
% achieving (by Day 126 post-HCT)	87	100	0.0059
Median (days) after starting DA	33	23	
Hb AUC (Days 28–126)	1,219 ± 134	1,272 ± 78	0.0200
DA cumulative dose (μg)	1,440 ± 496	1,210 ± 401	0.0150
RBC transfusions			
N patients (yes/no)	5/45	0/46	0.0276
N units/patient	0.3 ± 1.4	0	NS

Hb, hemoglobin; AUC, area under the curve; RBC, red blood cells; HCT, hematopoietic cell transplantation; DA, darbepoetin alfa; NS, not significant.

reticulocytes ($P = 0.0418$), low baseline creatinine ($P = 0.0454$) or transferrin saturation ($P = 0.0283$), and high number of transplanted CD34+ cells ($P = 0.0357$) were each associated with faster achievement of Hb levels $\geq$13 g/dL. Body weight, sex, as well as baseline platelet count, serum ferritin, and CRP were not predictive. In multivariate analysis, only DA administration ($P < 0.0001$), i.v. iron administration ($P = 0.0010$), high baseline Hb ($P < 0.0001$), and low baseline creatinine ($P = 0.0428$) remained significantly associated with faster achievement of a complete Hb response (Supporting Information Table II).

QOL scores

Unfortunately, a high proportion of patients did not fill in their QOL questionnaires. QOL forms were filled in only by 31 subjects at Day 28, 40 at Day 70, and 29 at Day 126. It was therefore not possible to run meaningful comparisons between the three groups in Part 1 of the study, and QOL data were analyzed altogether. QOL scores increased from Day 28 (115 ± 35) to Day 70 (132 ± 26), and Day 126 (131 ± 33) post-transplant [not significant (NS)]. QOL scores at single time-points were not significantly different between Groups 1 and 2 nor between Groups 2 and 3, but scores tended to be higher in Group 3 compared to Group 1 and this reached statistical significance on Day 70 (143 ± 22 vs. 113 ± 32, $P = 0.026$). Throughout the study, QOL scores were higher in Group 3 (140 ± 28) compared to Groups 1 (107 ± 34, $P < 0.001$), and 2 (123 ± 28, $P < 0.05$) ($P = 0.0007$ overall). To make the distinction between the effects of treatment group and time elapsed since transplantation on QOL scores, we used two-way ANOVA: group ($P = 0.0417$), but not time-point, significantly affected QOL scores. We also examined the relationships between QOL scores and Hb values. QOL scores were higher when Hb was >12 g/dL (137 ± 26) than in anemic patients (115 ± 34; $P = 0.0013$), but did not further improve when Hb increased beyond this threshold. There was a statistically significant correlation between QOL and Hb levels (Spearman $R = 0.43$, $P < 0.0001$); this was clearly demonstrated for Hb values below 12 g/dL (Spearman $R = 0.46$, $P = 0.0017$) but was not observed at higher Hb levels.

Safety

No side effect was reported in the immediate period after injection of either DA or i.v. iron, except in one patient in Group 2 who reported eyelid and lip swelling during 3–4 days, an event not encountered after subsequent doses. Serious adverse events included thrombosis ($n = 3$; two in Group 1 and one in Group 3), fluid retention (two in Group 2), depression (one in Group 1), pericarditis (one in Group 2), severe hyponatremia (one in Group 2), blurred vision (one in Group 3), and Guillain-Barré syndrome (one in Group 3).

The incidence of infection was similar in the three groups. Specifically, 5 of 25 subjects (20%) in Group 1, 7 of 52 (13%) in Group 2 and 10 of 50 (20%) in Group 3 experienced at least one infectious episode between Days 28 and 126 after HCT. The number of infections per patient between Days 28 and 126 was 0.4 ± 0.7, 0.2 ± 0.5, and 0.2 ± 0.5 in Groups 1, 2, and 3, respectively (NS).

While on study, two patients in Group 1 (one received radiotherapy and the other bortezomib), three in Group 2 (one received bortezomib, one chemotherapy, and one underwent a second transplant), and one in Group 3 (received chemotherapy) progressed. No other patient received any form of anticancer therapy after transplantation.

Systolic and diastolic blood pressures were similar in the three arms throughout the study (data not shown).

DA and i.v. iron therapy had no impact on platelet and WBC counts and differential, nor on CRP, creatinine, or liver function tests.

Discussion

As detailed in Introduction section, serum Epo levels after autologous HCT are usually inappropriately low for the degree of anemia between Days 21 and 100 after the transplant [1,5,6,15,19]. This provides a window of opportunity for treatment with ESAs to be potentially more effective than treatment in the early post-transplant period, which has consistently failed in previous trials [7–11]. On the other hand, it is now well demonstrated that adequate delivery of iron to the bone marrow is an important consideration in clinical settings in which ESA are used [20]. Indeed, effective stimulation of erythropoiesis by ESA is often associated with functional iron deficiency, a situation characterized by inadequate storage iron supply for the increased iron 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. In a previous single-arm Phase II study, we observed that ESA therapy was effective in accelerating erythropoiesis when started around 1 month after autologous HCT, but that functional iron deficiency occurred frequently [6]. The aim of the current prospective, multicenter, randomized study was to evaluate the efficacy of DA with or without i.v. iron to promote erythropoiesis after autologous HCT. Several observations have been made.

In the first part of the study, we showed that DA was remarkably efficient to promote erythropoiesis with 79% of patients receiving DA alone versus 21% of controls achieving the primary end point of the study (complete Hb response; Table I and Fig. 1A). All other analyses, including Hb response, Hb correction, and Hb AUC, were very significantly in favor of DA compared to no treatment (Table I; Figs. 1B,C and 2A), all confirming the results of our retrospective study [6]. However, this did not translate into a reduction in RBC transfusion requirements in DA-treated patients because most transfusions occurred in the first 4 weeks after HCT and very few after Day 28. Erythropoietic

activity, as assessed by sTfR levels, expanded almost twice under DA therapy and slowly declined when it was discontinued (Fig. 2C). In contrast, reticulocyte counts (Fig. 2B) did not differ significantly in controls and treated patients, underscoring the poor quantitative value of this parameter in evaluating erythroid response [16,20,21]. Our trial is the first prospective, randomized trial to demonstrate efficacy of ESA therapy after autologous HCT. One previous large [8], as well as several smaller [7,9,11,22–25] prospective ESA trials failed to achieve responses because they administered ESA early after HCT, at a time when endogenous serum Epo levels are already considerably elevated because of severely restricted erythropoietic activity [1,26]. We undertook to provide ESA in a time frame (i.e. after Day 28 post-HCT) when replacement treatment is more likely to be effective because serum Epo levels are usually defective [6].

In the second part of the study, we examined the impact of i.v. iron administration on erythroid response to DA. This is the first study to examine the effect of i.v. iron administration in the setting of autologous HCT. I.v. iron further enhanced erythropoietic response to DA. Compared to those receiving DA alone, patients administered i.v. iron experienced faster Hb responses and Hb correction in a larger proportion (Table II; Figs. 1D–F and 2D). Virtually all patients treated with DA and i.v. iron achieved the primary as well as secondary end points of the study. Such response rates have never been attained before in any ESA trial in cancer patients. The use of i.v. iron significantly decreased the proportion of patients transfused (no transfusion in patients receiving DA + i.v. iron), but not the number of RBC units per patient because overall transfusion rates were very low (Table II). Whereas reticulocyte counts were again superimposable in the two groups (Fig. 2E), sTfR levels remained similar in the first 8 weeks of DA therapy but were 25% higher in Group 2 thereafter (Fig. 2F). This is more likely due to earlier cessation of DA therapy in Group 3 because of faster achievement of response, than to a higher degree of functional iron deficiency in Group 2, because transferrin saturation curves only diverged after DA was stopped in the two groups (Fig. 3D). In addition, we have previously shown in rats that the effect of erythropoietic stimulation largely outweighed the effect of iron deficiency on sTfR levels [27]. The incidence of functional iron deficiency, as identified by Tsat values below 20%, was quite high at baseline (40%) and remained substantial in Groups 2 (40–45%) and 3 (35–40%) throughout DA therapy, being significantly higher than in Group 1. Finally, the cumulative dose of DA used was significantly lower in patients receiving i.v. iron, in whom complete Hb correction required only four instead of almost five 300 μg doses of DA (Table II). Hence, the additional theoretical cost of i.v. iron was largely offset by calculated savings in DA costs: patients receiving DA + i.v. iron had a 16% better response rate (primary end point) for a 13% lower overall drug cost. These observations are in line with recent studies in cancer patients given ESA therapy [28–32]. In these trials and a subsequent meta-analysis [33], except in one study [34] in which iron therapy may not have been optimal [35,36], i.v. iron therapy allowed to achieve faster Hb responses [30–32] and/or higher response rates [28–33,37], with decreased transfusion requirements [32,33] and decreased costs [30,38]. I.v. iron may overcome hepcidin-induced resistance to oral iron in patients with iron deficiency anemia [39], even though responses to i.v. iron of cancer patients receiving ESA therapy may be somewhat lower in those with higher hepcidin levels [36].

QOL has been shown to be improved when anemic cancer patients are treated with ESA up to Hb values of 12 g/dL [40,41]. Therefore, we aimed at investigating QOL changes in our trial, but encountered a low rate of return of QOL questionnaires. Despite this, combining all patients in the analysis allowed us to make some interesting conclusions. QOL scores were higher in nonanemic compared to anemic patients. We also observed a good correlation between QOL and Hb levels when Hb values were below 12 g/dL but not when they were further increased. QOL scores apparently increased with time post-transplant. This may be expected independently of anemia recovery in patients undergoing such intensive, demanding therapy as autologous HCT. However, QOL scores were also higher in those receiving DA + i.v. iron. Two-way ANOVA showed that treatment group, but not time elapsed since transplantation, were significantly affecting QOL scores. Nevertheless, further investigations in larger number of subjects are required before reaching definitive conclusions.

Importantly, DA therapy and i.v. iron administration were not associated with any undue immediate or delayed toxicity. DA and i.v. iron therapy had no impact on the platelet and WBC lineages, on renal or liver function, nor on blood pressure. Meta-analyses have demonstrated a 1.6-fold increase in the risk of thromboembolic events with ESA therapy in cancer patients [41]. In our trial, the rates of thromboembolic events were quite similar in the three groups and did not correlate with high Hb values. I.v. iron as administered in this trial was not associated with any infusional toxicity. This may have been facilitated by the exclusion of patients with high transferrin saturation, in whom i.v. iron may have induced an excess of nontransferrin bound iron. Although ferritin levels were somewhat higher in patients having received i.v. iron together to DA compared to those given DA alone (Fig. 3C), these levels were lower than those observed in controls, except at the very end of the follow-up period (Fig. 3A). The rates of infection or serious adverse events were also similar in the three groups. Meta-analyses have suggested decreased survival of ESA-treated cancer patients, but not in patients receiving concomitant chemotherapy [42]. In the current trial short-term disease progression was not increased by ESA or i.v. iron, but longer follow-up is required before drawing firm conclusions.

Our study has two important limitations. First, the target Hb value of 13 g/dL is no longer recommended by current guidelines because of the concern that this high Hb level may be associated with adverse outcomes [43,44]. When we initiated and run our trial, there were no such recommendations to limit the target Hb at 12 g/dL, this is why we decided to aim at normalizing Hb in the hope of restoring QOL as soon as possible after the transplant. However, our current policy is no longer to aim at achieving normal Hb levels, but to target Hb values around or just below 12 g/dL, as recommended by published guidelines [43,44]. Second, although our previous pilot studies [6,17] indicated higher transfusion risks, transfusion requirements of untreated patients beyond Day 30 post-transplant were limited in this trial. Given this low risk of transfusion and the absence of definitive data on QOL, the value of ESA therapy after autologous HCT remains insufficient to be recommended, except in patients with impaired renal function. As the proportion requiring transfusions was lower in the i.v. iron group ($P = 0.0276$), if ESA therapy is to be administered, it should be accompanied by appropriate i.v. iron supplementation.

In summary, our data indicate that DA, when started on Day 28 post-transplant, is safe and highly effective to ensure full erythroid reconstitution after autologous HCT but should probably be used only if the transfusion risk is significant. I.v. iron sucrose further hastens erythroid recovery, improving

response rates, reducing drug acquisition costs, and apparently decreasing transfusion rates and enhancing QOL.

Author Contributions

F.B. and Y.B. designed the research study. All coauthors performed the study. F.B., A.J., L.S., and Y.B. analyzed the data. F.B. and Y.B. wrote the article. The manuscript was read, edited, and approved by all coauthors.

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