Leukemia (2006) 20, 2081–2086

www.nature.com/leu

SPOTLIGHT

KEYNOTE ADDRESS

Transfusions after nonmyeloablative or reduced-intensity conditioning regimens

F Baron, G Vanstraelen and Y Beguin

Department of Medicine, Division of Hematology, University of Liège, Liège, Belgium

Leukemia (2006) **20,** 2081–2086. doi:10.1038/sj.leu.2404431; published online 12 October 2006

Introduction

Allogeneic hematopoietic cell transplantation (HCT) following reduced-intensity conditioning (RIC) or nonmyeloablative conditioning has been an effective treatment for many patients with hematological malignancies, as well as for selected patients with solid cancers or inherited blood disorders.

A number of different RIC or nonmyeloablative conditioning regimens have been explored. The separation of what constitutes a nonmyeloablative versus a RIC regimen is somewhat arbitrary (Table 1), although Giralt proposed some criteria for nonmyeloablative conditioning that included (1) no eradication of host hematopoiesis, (2) prompt hematologic recovery (<4 weeks) without transplant and (3) presence of mixed chimerism upon engraftment.[1] Similarly, the distinction between RIC and myeloablative conditioning has been discussed; the criteria proposed by Giralt[1] included ⩽5 Gy total body irradiation (TBI), ⩽9 mg/kg busulfan dose, ⩽140 mg/m^2 melphalan dose and ⩽10 mg/kg thiothepa dose.[1] RIC regimens have combined modest doses of fludarabine (used mainly for its immunosuppressive activity) with consequent (but nonmyeloablative) doses of alkylating agents such as melphalan (140 mg/m^2)[2] or busulfan (4–8 mg/kg),[3] given to produce major anti-tumor effects with the hope of controlling the malignancy before the occurrence of the immune-mediated graft-versus-tumor effects. In contrast, nonmyeloablative conditioning has used potent immunosuppressive regimens to overcome host-versus-graft reactions (graft rejection),[4,5] allowing engraftment of donor hematopoietic and immune cells. Thus, following nonmyeloablative conditioning, eradication of both host hematopoiesis and tumor cells is mediated nearly exclusively by immune-mediated graft-versus-host effects.[6,7]

The degree of myelosuppression induced by RIC or nonmyeloablative regimens has varied from one regimen to another, those including intermediate doses of either busulfan (8 mg/kg) or melphalan (140 mg/m^2) being the most myelosuppressive, whereas the one using low-dose TBI (2 Gy) with or without fludarabine being the least myelosuppressive (Figure 1 and Table 1).[16]

We compared erythropoietic activity in patients given allogeneic grafts after either myeloablative ($n=57$) or nonmyeloablative ($n=54$) conditioning (Figure 2).[17] Nonmyeloablative conditioning consisted of 2 Gy TBI ($n=20$), 2 Gy TBI plus fludarabine (90 mg/m^2, $n=29$), or cyclophosphamide (3 g/m^2) plus fludarabine (90 mg/m^2, $n=5$). Erythropoietic activity was quantified by assessing the levels of the serum soluble transferrin receptor (sTfR, a quantitative marker of total erythropoietic activity). After myeloablative conditioning, erythropoietic activity decreased sharply and remained abnormally low the first month after HCT. In contrast, erythropoietic activity remained consistently above the lower limit of normal values after nonmyeloablative conditioning, demonstrating that the regimens were indeed mildly myelosuppressive.

In this article, we first describe what is currently known about red blood cell (RBC) and platelet transfusion requirements after RIC or nonmyeloablative conditioning, and then review which factors are associated with increased transfusion requirements. In the second part of the review, we discuss the potential role of hematopoietic growth factors such as erythropoietin (Epo) or granulocyte colony-stimulating factor (G-CSF) after nonmyeloablative or RIC HCT.

RBC transfusions

RBC transfusion requirements

Given that some degree of erythropoietic activity persists after nonmyeloablative conditioning,[17] it was expected that patients receiving nonmyeloablative conditioning would require less RBC transfusions than those given myeloablative regimens. Indeed, several studies found decreased RBC transfusion requirements after nonmyeloablative conditioning or RIC in comparison to what is seen after myeloablative conditioning.[18–20]

Weissinger *et al.*[18] compared RBC transfusions in patients given allogeneic peripheral blood stem cells (PBSC) from human leukocyte antigen (HLA)-identical siblings after nonmyeloablative ($n=40$) versus myeloablative ($n=67$) conditioning. Nonmyeloablative conditioning consisted of 2 Gy TBI with ($n=10$) or without ($n=30$) added fludarabine (90 mg/m^2). Sixty-three percent of the nonmyeloablative recipients required RBC transfusions compared to 96% of those given myeloablative conditioning ($P=0.0001$). Furthermore, the number of RBC units transfused was reduced in nonmyeloablative recipients with a median of 2 (range, 0–50) units transfused versus 6 (range, 0–34) in those receiving myeloablative conditioning ($P=0.0001$). High transfusion requirements before HCT ($P=0.0005$) and donor–recipient ABO incompatibility (major mismatch, $P=0.0001$) were each associated with increased RBC transfusion requirements in both patient groups. Similar findings were found by Sorror *et al.*[19] comparing data from patients given HLA-matched unrelated grafts after nonmyeloablative (consisting of 2 Gy TBI plus fludarabine, $n=60$) versus myeloablative conditioning ($n=74$). Specifically, 88% of nonmyeloablative recipients received a median of three RBC transfusions compared to 100% myeloablative recipients, who required a median of five RBC transfusions ($P=0.005$).

Correspondence: Dr Y Beguin, Division of Hematology, University of Liège, CHU Sart-Tilman, 4000 Liège, Belgium.
E-mail: yves.beguin@chu.ulg.ac.be
Received 13 June 2006; revised 22 July 2006; accepted 22 August 2006; published online 12 October 2006

Table 1 Commonly used nonmyeloablative or RIC regimens in relation to their myelosuppressive properties[8]

Regimen (reference)
Nonmyeloablative
Low-dose TBI (2 Gy)[5,9]
Low-dose TBI (2 Gy)+fludarabine[10]
Fludarabine+cyclophosphamide[4,11]
RIC
Cyclophosphamide 200 mg/kg+ATG[12]
Fludarabine+melphalan 140 mg/m^2 ± alemtuzumab[2,13]
Fludarabine+oral busulfan (8 mg/kg)+ATG[3,14,15]

Abbreviations: ATG, anti-thymocyte globulin; RIC, reduced-intensity conditioning; TBI, total body irradiation. [2]Regimens are classified from less to more myelosuppressive. Note that this classification is not based on direct experimentation, and is thus hypothetical.
Criteria for RIC included ⩽5 Gy TBI, ⩽9 mg/kg busulfan, ⩽140 mg/m^2 melphalan and ⩽10 mg/kg thiothepa.[1]

Ivanov *et al*.[20] analyzed RBC transfusion requirements in 110 consecutive patients given grafts from HLA-identical siblings after RIC ($n=64$; consisting of fludarabine 180 mg/m^2, oral busulfan 8 mg/kg and various doses of anti-thymocyte globulins (ATG)) or myeloablative conditioning ($n=46$). Postgrafting immunosuppression included cyclosporine (CSP) given alone in all RIC recipients and in 18 of 46 myeloablative recipients, or combined with short methotrexate (MTX) in 28 myeloablative recipients. Eighty-nine percent of the patients given RIC required RBC transfusions compared to 100% of myeloablative recipients. In addition, the number of RBC units transfused was significantly reduced in RIC recipients, with a median of 4 (range, 0–28) units transfused versus 12 (range, 2–56) in those given myeloablative conditioning ($P<0.0001$). In multivariate analysis, high RBC transfusion requirements were independently predicted by myeloablative conditioning versus RIC ($P=0.0005$), and marrow versus PBSC as stem cell source ($P<0.0001$). Further, among RIC recipients, low hemoglobin (Hb) levels before HCT ($P<0.0001$) and high ATG doses ($P=0.009$) were each independently associated with high RBC transfusion requirements.

Le Blanc *et al*.[21] analyzed data from 58 patients given grafts after nonmyeloablative conditioning ($n=24$; consisting of fludarabine plus 2 Gy TBI) versus RIC ($n=34$; consisting mainly of fludarabine 180 mg/m^2, oral busulfan 8 mg/kg and ATG) conditioning. Postgrafting immunosuppression combined CSP with either mycophenolate mofetil (MMF) or MTX. The number of RBC units transfused was significantly reduced in nonmyeloablative recipients, with a median of 0 (range, 0–12) units transfused versus 4 (range, 0–23) in those given RIC ($P=0.02$).

Finally, Canals *et al*.[22] studied data from 77 patients given PBSC after RIC consisting of fludarabine (150 mg/m^2) plus either melphalan (140 mg/m^2) or busulfan (10 mg/kg). Postgrafting immunosuppression combined CSP and short MTX. The main factor increasing RBC transfusion requirements was the occurrence of severe acute graft-versus-host disease (GVHD). Specifically, patients with grade III–IV acute GVHD ($n=12$) required a median of 15 (range, 8–40) RBC units the first 100 days after HCT, whereas patients without grade III–IV acute GVHD ($n=65$) received a median of 2 (range, 0–14) RBC units ($P<0.01$). In patients without grade III–IV acute GVHD, RBC transfusion requirements until day 100 after HCT were higher in patients with major ABO mismatch than in those given ABO-compatible grafts ($P=0.02$).

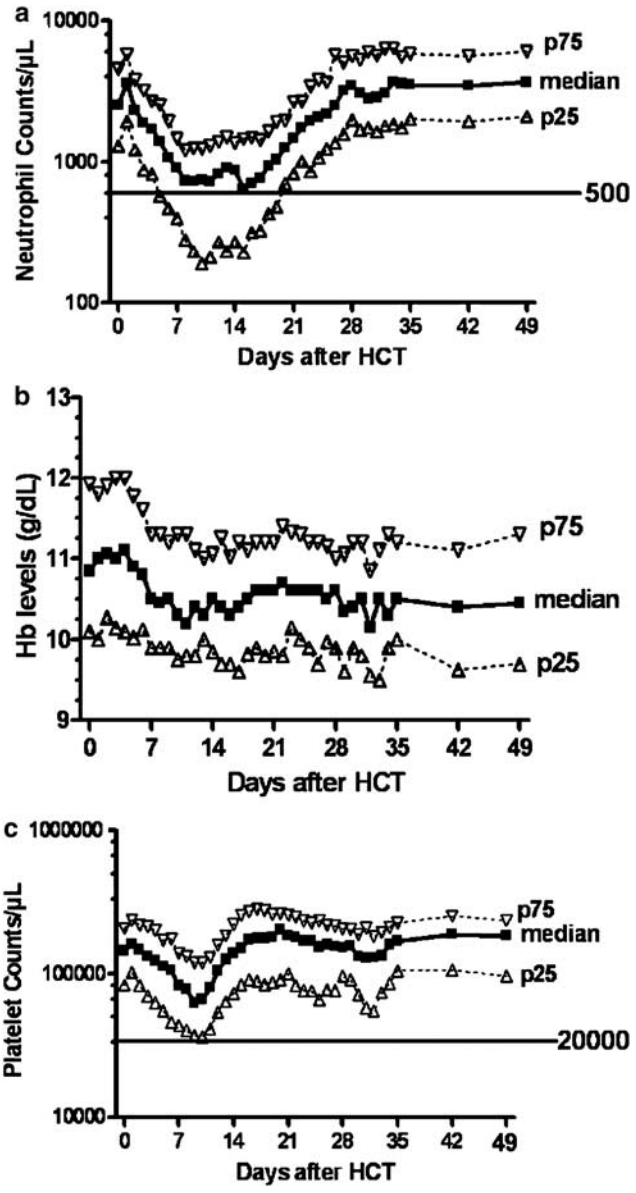

Figure 1 Neutrophil, Hb and platelet count changes after HLA-matched related ($n=85$) or unrelated ($n=35$) HCT following conditioning with 2 Gy TBI with or without fludarabine (90 mg/m^2) ($n=120$).[25] The graph shows the medians (black squares), 25th percentile (open triangles) and 75th percentile (inverted open triangles). Neutrophil counts stayed above 500/μl and platelet counts remained above 40 000/μl in the majority of patients, demonstrating that the conditioning is truly nonmyeloablative. This figure was originally published in *Blood* (Baron *et al*. Kinetics of engraftment in patients with hematologic malignancies given allogeneic HCT after nonmyeloablative conditioning. *Blood* **104**: 2254–2262). ©The American Society of Hematology.

Taken together, these studies suggested that intensity of the conditioning regimen, Hb levels before HCT, stem cell source, acute GVHD and major ABO mismatch affected RBC transfusion requirements after nonmyeloablative or RIC HCT (Table 2).

The case of ABO incompatibility

ABO antigens are potent immunogens expressed on the surface of RBC, RBC progenitors/precursors and also on the surface of a number of nonhematopoietic cells. Although the ABO group

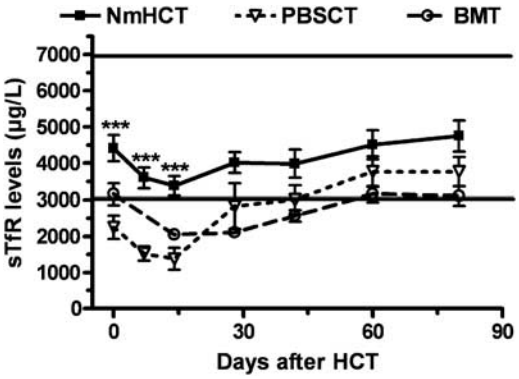

Figure 2 Erythropoietic activity, as assessed by sTfR levels (mean±s.e.m.) after nonmyeloablative (PBSC, $n=54$) HCT versus myeloablative (marrow (BMT, $n=47$) or PBSC (PBSCT, $n=10$)) HCT. Horizontal lines show the upper and lower limits of normal values. *P*-values are given for comparisons between the nonmyeloablative PBSC and the myeloablative PBSC groups: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Table 2 Factors associated with high transfusion requirements after nonmyeloablative or RIC HCT

Factor	*Transfusions (reference)*	
	RBC	*Platelets*
Poor marrow function before HCT[a]	Yes[18,20]	Yes[18]
Intensity of the conditioning regimen	Yes[18–21]	Yes[18,19,21]
Stem cell source	Yes[20]	Not known
Major ABO mismatch	Yes[18,22]	Not known
Severe acute GVHD	Yes[22]	Yes[22]

Abbreviations: GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; RBC, red blood cell; RIC, reduced-intensity conditioning.
[a]Assessed by hemoglobin values and/or pre-HCT RBC transfusion requirements for RBC transfusions, or by platelet levels and/or pre-HCT platelet transfusion requirements for platelet transfusions.

system has been of critical importance in transfusion medicine, it has had little impact on HCT outcomes after myeloablative conditioning.[23] Indeed, although the presence of a major ABO mismatch between donor and recipient was associated with delayed recovery of erythropoietic activity and occurrence of pure red cell aplasia, it did not have any impact on the incidence of grade II–IV acute GVHD or on overall survival.[23] The mechanisms of erythropoietic impairment in case of ABO mismatch after myeloablative conditioning have not been completely elucidated, but include direct inhibition of donor erythropoiesis by residual host-derived anti-ABO isohemagglutinins (HA),[24] even if virtually all host-derived hematopoiesis (including plasma cells producing anti-donor HA) has been eradicated by the conditioning regimen.

As an important characteristic of many nonmyeloablative regimens has been the persistence of a certain amount of host-derived hematopoiesis (including B-cell lymphopoiesis) for up to 6–12 months after HCT,[4,6,25] it has been hypothesized that delayed disappearance of host plasma cells producing anti-donor HA might lead to particularly delayed donor erythropoiesis, and a high frequency of pure red cell aplasia, after ABO-incompatible nonmyeloablative HCT.

Supporting this hypothesis, Bolan *et al*.[26] found that donor RBC chimerism (defined as the initial detection of donor RBCs in peripheral blood) was markedly delayed following nonmyeloablative ($n=14$) versus myeloablative ($n=12$) conditioning (median, 114 versus 40 days; $P<0.0001$) in patients with major ABO incompatibility with their donor. Further, four out of 14 nonmyeloablative versus zero out of 12 myeloablative recipients experienced pure red cell aplasia ($P=0.10$). Interestingly, donor RBC chimerism closely correlated with decreasing host anti-donor HA levels. The latter disappeared more slowly after nonmyeloablative than after myeloablative conditioning (median, 83 versus 44 days; $P=0.03$). In addition, CSP discontinuation induced potent graft-versus-plasma cell effects, and allowed resolution of pure red cell aplasia in all four patients. The existence of a graft-versus-plasma cell effect was further supported by the observation that severe acute GVHD was associated with a rapid decrease in anti-donor HA titers in two patients, in agreement with a previous study by Mielcarek *et al*.[27] showing faster decrease in anti-donor HA in patients developing acute GVHD than in those without acute GVHD, after myeloablative conditioning. However, a recent study described the persistence of significant percentages of recipient-derived plasma cells in patients with pure red cell aplasia after nonmyeloablative conditioning, despite full donor T-cell and granulocyte chimerisms.[28] This suggests that host-derived plasma cells might be less susceptible than host-derived T cells or myeloid cells to donor-derived alloreactive T cells.[29]

In agreement with the study of Bolan *et al*.,[26] Zaucha *et al*.[30] found that the median time to reach HA titers <1:1 was at least 133 days in patients given ABO-incompatible grafts after nonmyeloablative conditioning, approximately twice longer than after myeloablative conditioning. Further, donor erythropoiesis was effective only when low or absent anti-donor HA titers were achieved.

Finally, it should be emphasized that some RIC regimens, particularly those containing alemtuzumab, are likely to eradicate most of the host-derived anti-donor HA-producing plasma cells, and thus might not be associated with delayed donor RBC chimerism in comparison to what is seen with myeloablative conditioning.[31]

Platelet transfusions

Several studies have found lower platelet transfusion requirements in patients given nonmyeloablative conditioning or RIC than in those receiving a myeloablative regimen.[18,19]

Weissinger *et al*.[18] studied platelet transfusion requirements in patients given allogeneic PBSC from HLA-identical siblings after nonmyeloablative ($n=40$) versus myeloablative ($n=67$) conditioning. Nonmyeloablative conditioning consisted of 2 Gy TBI with ($n=10$) or without ($n=30$) added fludarabine (90 mg/m^2). Twenty-three percent of nonmyeloablative recipients required platelet transfusions compared to 100% of those given myeloablative conditioning ($P<0.0001$). Further, the number of platelet units given was reduced after nonmyeloablative conditioning, with a median of 0 (range, 0–214) units transfused versus 24 (range, 4–358) after myeloablative conditioning ($P<0.0001$). High platelet transfusion requirements before HCT were also associated with increased transfusion requirements in both patient groups ($P=0.01$).

Sorror *et al*.[19] analyzed transplantation-related toxicities including transfusion requirements after HLA-matched unrelated HCT in 134 concurrent patients given either nonmyeloablative ($n=60$) or myeloablative ($n=74$) conditioning. Sixty-three percent of nonmyeloablative patients received a median of

SPOTLIGHT

one platelet transfusion compared to all myeloablative patients given a median of seven transfusions ($P<0.0001$).

Le Blanc *et al*.[21] compared platelet transfusion requirements in 58 patients given grafts after nonmyeloablative ($n=24$; consisting of fludarabine plus 2 Gy TBI) versus RIC ($n=34$; consisting mainly of fludarabine 180 mg/m^2, oral busulfan 8 mg/kg and ATG) conditioning. Postgrafting immunosuppression combined CSP with either MMF or MTX. The number of platelet units transfused was significantly reduced in nonmyeloablative recipients with a median of 0 (range, 0–6) units transfused versus 2 (range, 0–24) in those given RIC ($P<0.001$).

Canals *et al*.[22] studied data from 77 patients given PBSC after RIC consisting of fludarabine (150 mg/m^2) plus either melphalan (140 mg/m^2) or busulfan (10 mg/kg). Postgrafting immunosuppression combined CSP and short MTX. As observed for RBC transfusion requirements, the main factor increasing platelet transfusion requirements was the occurrence of severe acute GVHD. Specifically, patients with grade III–IV acute GVHD ($n=12$) required a median of 15 (range, 4–20) platelet transfusions the first 100 days after HCT, whereas patients without grade III–IV acute GVHD ($n=65$) received a median of 2 (range, 0–20) platelet transfusions ($P<0.01$).

Finally, Mohty *et al.* analyzed platelet recovery and transfusion needs in 90 patients receiving grafts from HLA-identical siblings after a RIC regimen consisting mainly of fludarabine, busulfan and ATG (Mohty M *et al*. *Blood* 2005; **106** (Part 1): abstract #961). Low platelet counts before RIC ($P=0.07$) and occurrence of grade III–IV acute GVHD ($P=0.0001$) were associated with increased requirements for platelet transfusions.

Potential role for hematopoietic growth factors

Epo

Epo is the key regulatory factor of erythropoiesis, and acts mainly by preventing apoptosis of late erythroid progenitors. In normal patients, serum Epo levels increase exponentially with decreasing Hb levels. After myeloablative allogeneic HCT, serum Epo levels first rapidly increased to disproportionately high levels for 1–3 weeks and then became inappropriately low for the degree of anemia, resulting in low erythropoietic activity and prolonged anemia.[32] As predicted by these observations, Epo therapy offered minimal benefit when started immediately after HCT,[33] but was quite efficient when started more than 4 weeks after HCT,[34] that is, when serum Epo production became impaired.

There are two important differences in the physiology of erythropoiesis following nonmyeloablative versus myeloablative conditioning. First, a certain amount of host erythropoietic activity persists after nonmyeloablative conditioning (Figure 2), suggesting that Epo therapy might be efficient even when started on day 0 after nonmyeloablative HCT. Secondly, contrary to endogeneous Epo deficiency observed after myeloablative

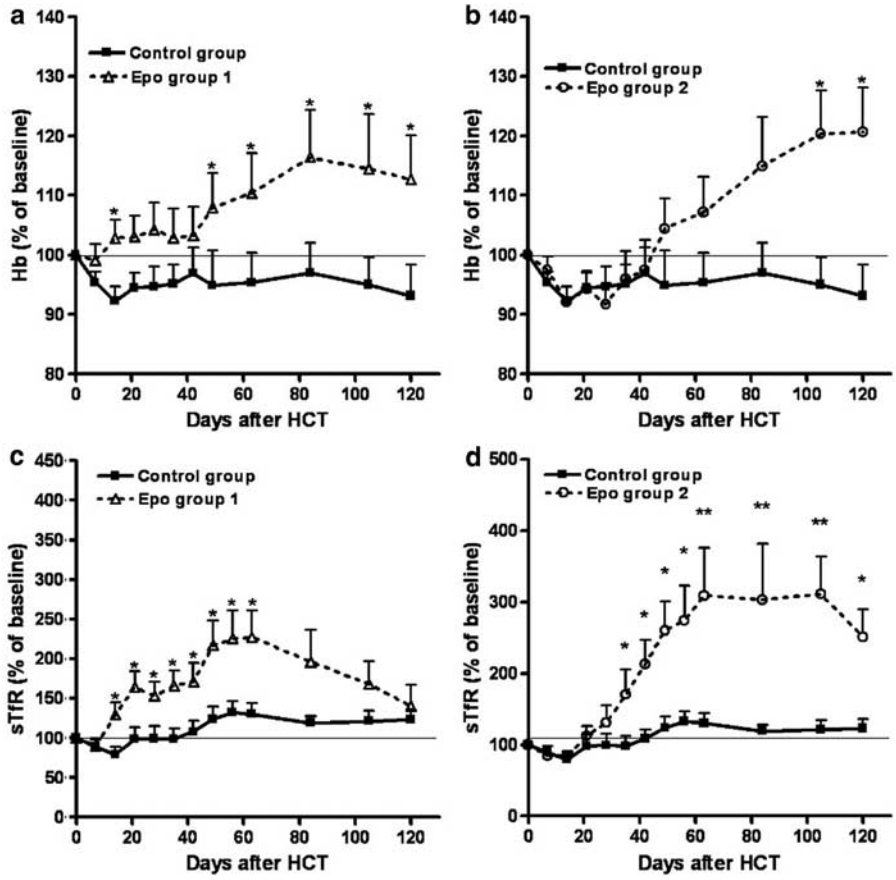

Figure 3 Hb values and sTfR levels after nonmyeloablative HCT in patients given ($n=46$) or not given (control group, $n=14$) rHuEpo. Values are normalized relative to their value on the day of transplantation. The right panels provide the evolution in Epo group 1 (rHuEpo started on day 0; $n=19$) and left panels the evolution in Epo group 2 (rHuEpo started on day 30; $n=27$). *P*-values are given for comparisons with the control group at any particular time point by Student's *t*-tests (*<0.05,**<0.01, ***<0.001). Reproduced with permission from Vanstraelen *et al*.[36]

conditioning, Epo production remains adequate for the degree of anemia throughout the post-transplant period after nonmyeloablative conditioning, suggesting that Epo therapy might be less efficient after nonmyeloablative than after myeloablative conditioning, when started after 4 weeks following transplantation.[17]

Two recent studies have investigated the use of Epo after nonmyeloablative conditioning or RIC.[35,36] Ivanov *et al*.[35] gave Epo (either 30 000 IU epoetin-beta or 150 mg darbepoetine-alpha per week, started on day 1) to 20 consecutive patients following RIC allogeneic HCT (Epo group). Conditioning combined fludarabine, busulfan and ATG. Epo therapy was continued until the Hb level reached 14 g/dl or until day 60. Twenty-seven matched patients not receiving recombinant human erythropoietin (rHuEpo) served as controls (control group). Median Hb levels on days 30 and 60 after HCT were 11.6 and 12.8 g/dl in the Epo group versus 10.0 g/dl ($P=0.0001$) and 9.9 g/dl ($P<0.0001$) in the control group, respectively. In addition, there was a trend for lower RBC transfusion requirements in the Epo group (median 2 units (range, 2–4 units)) compared to the control group (median 4 units (range, 2–12 units); $P=0.07$).

We analyzed erythropoietic activity (assessed by sTfR levels) and Hb levels in 60 patients given allogeneic grafts after nonmyeloablative conditioning.[36] Fourteen patients did not receive Epo (control group), 19 were given Epo from day 0 after HCT (Epo group 1), whereas 27 were scheduled to start Epo on day 28 after HCT (Epo group 2). Recombinant human Epo was administered subcutaneously at a dose of 500 U/kg/week, with the aim of achieving Hb levels of 13 g/dl. During the first month as well as between days 30 and 180, sTfr levels and Hb values were significantly higher in patients receiving rHuEpo compared to those not receiving it (Figure 3). However, transfusion requirements were significantly decreased only during the first month in patients given Epo from day 0 (Epo group 1, $P=0.0169$). Interestingly, donor T-cell chimerism levels above 60% on day 42 was the best predictor of achieving Hb values >13 g/dl, suggesting possible inhibition of donor erythropoiesis by residual recipient lymphocytes, in patients with low donor T-cell chimerism levels.

Taken together, these studies indicated that giving Epo (either 30 000 IU epoetin or 150 mg darbepoetin-alpha per week) early after nonmyeloablative or RIC HCT could reduce RBC transfusion needs. A prospective randomized trial is ongoing at our center, with Epo starting around day 0, to assess its clinical benefit in terms of transfusion requirements and quality of life. However, until the clinical benefits of Epo therapy in this setting are clearly demonstrated, its use should be restricted to patients included in clinical trials.

G-CSF

G-CSf has been administered to hasten neutrophil recovery after myeloablative allogeneic HCT, and its potential impact on immune recovery and GVHD incidence has been extensively debated (reviewed by Mohty *et al*.[37]). However, no study thus far analyzed the impact of G-CSF use after nonmyeloablative conditioning or RIC. Some groups of investigators gave G-CSF in most patients,[2,38] whereas others either did not use it or gave it only in case of granulocytopenia.[5,39,10] In one study, in which RIC consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2) and alemtuzumab (100 mg) and postgrafting immunosuppression of CSP alone, there was a suggestion that time to neutrophil recovery could be 1 day shorter in patients given G-CSF at 5 μg/kg/day (13 ± 3 days, $n=32$) compared to those not given G-CSF (14 ± 4 days, $n=6$) (NS). Prospective randomized studies are therefore needed to clarify the impact of G-CSF administration on HCT outcome after nonmyeloablative or RIC regimens.

Conclusion

Despite the fact that nonmyeloablative and RIC regimens are associated with lower transfusion requirements than myeloablative ones, most patients given nonmyeloablative conditioning or RIC required RBC and/or platelet transfusions. Predictive factors for high transfusion requirements include low Hb or platelet values before HCT, intensity of the conditioning regimen, use of marrow instead of PBSC as stem cell source, occurrence of severe acute GVHD and major ABO mismatch (for RBC transfusions). In case of major ABO incompatibility, donor erythroid engraftment is further delayed after nonmyeloablative compared to myeloablative conditioning, because of the persistence of anti-donor HA-secreting host-derived plasma cells for up to several months after nonmyeloablative conditioning. The potential role of hematopoietic growth factors such as Epo, G-CSF, thrombopoietin or thrombopoietin receptor ligands (such as the AMG 531, which has shown promising activity in patients with immune thrombocytopenic purpura)[40] warrants further investigations. Their rational use might allow performing nonmyeloablative or RIC HCT without RBC and platelet transfusions in most patients.

References

1 Giralt S. Reduced-intensity conditioning regimens for hematologic malignancies: what have we learned over the last 10 years? *Hematology* 2005; **2005**: 384–389.
2 Kottaridis PD, Milligan DW, Chopra R, Chakraverty RK, Chakrabarti S, Robinson S *et al*. *In vivo* CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood* 2000; **96**: 2419–2425.
3 Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G *et al*. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
4 Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S *et al*. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood* 1999; **94**: 3234–3241.
5 McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG *et al*. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
6 Baron F, Sandmaier BM. Chimerism and outcomes after hematopoietic cell transplantation following nonmyeloablative conditioning. *Leukemia* 2006, July 27 [E-pub ahead of print].
7 Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R *et al*. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 2005; **23**: 1993–2003.
8 Baron F, Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders (Review). *Mol Ther* 2006; **13**: 26–41.
9 Kerbauy FR, Storb R, Hegenbart U, Gooley T, Shizuru J, Al-Ali HK *et al*. Hematopoietic cell transplantation from HLA-identical sibling donors after low-dose radiation-based conditioning for treatment of CML. *Leukemia* 2005; **19**: 990–997.
10 Maris MB, Sandmaier BM, Storer BE, Maloney DG, Shizuru JA, Agura E *et al*. Unrelated donor granulocyte colony-stimulating

factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. *Biol Blood Marrow Transplant* 2006; **12**: 454–465.
11 Khouri IF, Saliba RM, Giralt SA, Lee M-S, Okoroji G-J, Hagemeister FB *et al.* Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. *Blood* 2001; **98**: 3595–3599.
12 Sorror ML, Leisenring W, Deeg HJ, Martin PJ, Storb R. Twenty-year follow-up in patients with aplastic anemia given marrow grafts from HLA-identical siblings and randomized to receive methotrexate/cyclosporine or methotrexate alone for prevention of graft-versus-host disease (Letter to the Editor). *Biol Blood Marrow Transplant* 2005; **11**: 567–568.
13 Giralt S, Thall PF, Khouri I, Wang X, Braunschweig I, Ippolitti C *et al.* Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood* 2001; **97**: 631–637.
14 Mohty M, de Lavallade H, Ladaique P, Faucher C, Vey N, Coso D *et al.* The role of reduced intensity conditioning allogeneic stem cell transplantation in patients with acute myeloid leukemia: a donor vs no donor comparison. *Leukemia* 2005; **19**: 916–920.
15 Shimoni A, Kröger N, Zabelina T, Ayuk F, Hardan I, Yeshurun M *et al.* Hematopoietic stem-cell transplantation from unrelated donors in elderly patients (age >55 years) with hematologic malignancies: older age is no longer a contraindication when using reduced intensity conditioning. *Leukemia* 2005; **19**: 7–12.
16 Appelbaum FR. Dose intensity and the toxicity and efficacy of allogeneic hematopoietic cell transplantation (Keynote Address). *Leukemia* 2005; **19**: 171–175.
17 Baron F, Fillet G, Beguin Y. Erythropoiesis after nonmyeloablative stem-cell transplantation is not impaired by inadequate erythropoietin production as observed after conventional allogeneic transplantation. *Transplantation* 2002; **74**: 1692–1696.
18 Weissinger F, Sandmaier BM, Maloney DG, Bensinger WI, Gooley T, Storb R. Decreased transfusion requirements for patients receiving nonmyeloablative compared with conventional peripheral blood stem cell transplants from HLA-identical siblings. *Blood* 2001; **98**: 3584–3588.
19 Sorror ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C *et al.* Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. *Blood* 2004; **104**: 961–968.
20 Ivanov V, Faucher C, Mohty M, Bilger K, Ladaique P, Sainty D *et al.* Decreased RBCTs after reduced intensity conditioning allogeneic stem cell transplantation: predictive value of prior Hb level. *Transfusion* 2004; **44**: 501–508.
21 Le Blanc K, Remberger M, Uzunel M, Mattsson J, Barkholt L, Ringden O. A comparison of nonmyeloablative and reduced-intensity conditioning for allogeneic stem-cell transplantation. *Transplantation* 2004; **78**: 1014–1020.
22 Canals C, Muniz-Diaz E, Martinez C, Martino R, Moreno I, Ramos A *et al.* Impact of ABO incompatibility on allogeneic peripheral blood progenitor cell transplantation after reduced intensity conditioning. *Transfusion* 2004; **44**: 1603–1611.
23 Seebach JD, Stussi G, Passweg JR, Loberiza Jr FR, Gajewski JL, Keating A *et al.* ABO blood group barrier in allogeneic bone marrow transplantation revisited. *Biol Blood Marrow Transplant* 2005; **11**: 1006–1013.
24 Barge AJ, Johnson G, Witherspoon R, Torok-Storb B. Antibody-mediated marrow failure after allogenic bone marrow transplantation. *Blood* 1989; **74**: 1477–1480.
25 Baron F, Baker JE, Storb R, Gooley TA, Sandmaier BM, Maris MB *et al.* Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood* 2004; **104**: 2254–2262.
26 Bolan CD, Leitman SF, Griffith LM, Wesley RA, Procter JL, Stroncek DF *et al.* Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation. *Blood* 2001; **98**: 1687–1694.
27 Mielcarek M, Leisenring W, Torok-Storb B, Storb R. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. *Blood* 2000; **96**: 1150–1156.
28 Griffith LM, McCoy Jr JP, Bolan CD, Stroncek DF, Pickett AC, Linton GF *et al.* Persistence of recipient plasma cells and anti-donor isohaemagglutinins in patients with delayed donor erythropoiesis after major ABO incompatible non-myeloablative haematopoietic cell transplantation. *Br J Haematol* 2005; **128**: 668–675.
29 Kroger N, Zagrivnaja M, Schwartz S, Badbaran A, Zabelina T, Lioznov M *et al.* Kinetics of plasma-cell chimerism after allogeneic stem cell transplantation by highly sensitive real-time PCR based on sequence polymorphism and its value to quantify minimal residual disease in patients with multiple myeloma. *Exp Hematol* 2006; **34**: 688–694.
30 Zaucha JM, Mielcarek M, Takatu A, Little M-T, Gooley T, Baker J *et al.* Engraftment of early erythroid progenitors is not delayed after non-myeloablative major ABO-incompatible haematopoietic stem cell transplantation. *Br J Haematol* 2002; **119**: 740–750.
31 Peggs KS, Morris EC, Kottaridis PD, Geary J, Goldstone AH, Linch DC *et al.* Outcome of major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation may be influenced by conditioning regimen. *Blood* 2002; **99**: 4642–4643.
32 Beguin Y, Clemons GK, Oris R, Fillet G. Circulating erythropoietin levels after bone marrow transplantation: inappropriate response to anemia in allogeneic transplants. *Blood* 1991; **77**: 868–873.
33 Link H, Boogaerts MA, Fauser AA, Slavin S, Reiffers J, Gorin NC *et al.* A controlled trial of recombinant human erythropoietin after bone marrow transplantation. *Blood* 1994; **84**: 3327–3335.
34 Baron F, Sautois B, Baudoux E, Matus G, Fillet G, Beguin Y. Optimization of recombinant human erythropoietin therapy after allogeneic hematopoietic stem cell transplantation. *Exp Hematol* 2002; **30**: 546–554.
35 Ivanov V, Faucher C, Mohty M, Bilger K, Ladaique P, Sainty D *et al.* Early administration of recombinant erythropoietin improves hemoglobin recovery after reduced intensity conditioned allogeneic stem cell transplantation. *Bone Marrow Transplant* 2005; **36**: 901–906.
36 Vanstraelen G, Baron F, Willems E, Bonnet C, Hafraoui K, Frère P *et al.* Recombinant human erythropoietin therapy after allogeneic hematopoietic cell transplantation with a nonmyeloablative conditioning regimen: low donor chimerism predicts for poor response. *Exp Hematol* 2006; **34**: 841–850.
37 Mohty M, Faucher C, Blaise D. Graft-versus-host-disease and granulocyte colony-stimulating factor administration after allogeneic stem cell transplantation. *Leukemia* 2005; **19**: 500–503.
38 Wong R, Giralt SA, Martin T, Couriel DR, Anagnostopoulos A, Hosing C *et al.* Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. *Blood* 2003; **102**: 3052–3059.
39 Baron F, Maris MB, Storer BE, Sandmaier BM, Panse JP, Chauncey TR *et al.* High doses of transplanted $CD34^+$ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. *Leukemia* 2005; **19**: 822–828.
40 Wang B, Nichol JL, Sullivan JT. Pharmacodynamics and pharmacokinetics of AMG 531, a novel thrombopoietin receptor ligand. *Clin Pharmacol Ther* 2004; **76**: 628–638.