Impact of CD34$^+$ cell dose on the outcome of patients undergoing reduced-intensity-conditioning allogeneic peripheral blood stem cell transplantation

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We analyzed the impact of CD34$^+$ cell dose on the outcome of 86 patients undergoing reduced-intensity conditioning (RIC) allogeneic peripheral blood stem cell transplantation. The RIC was based on fludarabine 150 mg/m^2 and melphalan 140 mg/m^2 or busulphan 10 mg/kg. A median of 5.68 × 10^6 CD34$^+$ cells/kg and 2.86 × 10^8 CD3$^+$ cells/kg were infused. All patients receiving more than percentile 75 (p75) of CD34$^+$ cells reached complete chimerism in T lymphocytes by days 21 to 28, compared with 44% among those receiving p75 or fewer cells ($P = .046$). Overall, 30.3% patients developed grade 2 to 4 acute graft-versus-host disease (aGVHD). Among 83 evaluable patients, 55.8% developed chronic GVHD (cGVHD). The dose of CD34$^+$ cells infused did influence the development of cGVHD, with a cumulative incidence of extensive cGVHD of 74% vs 47% ($P = .02$) among patients receiving more than p75 CD34$^+$ cells vs those receiving p75 or fewer. Projected overall survival (OS) and event-free survival (EFS) at 43 months were 60% and 46%, respectively. Neither the dose of CD34$^+$ cells nor the dose of CD3$^+$ cells infused significantly influenced OS and EFS, although among patients categorized as high-risk, 36% of those receiving p75 or fewer CD34$^+$ cells relapsed or progressed, compared with only 9% among those receiving more than p75 CD34$^+$ cells ($P = .07$). Among patients receiving p75 or fewer CD34$^+$ cells, 36% of high-risk patients relapsed, compared with 10% of low- and intermediate-risk patients ($P = .004$), while relapse rates were not significantly different between both subgroups when we infused more than p75 CD34$^+$ cells, thus indicating that infusing high doses of CD34$^+$ cells ameliorates the negative effect of advanced disease status at transplantation. cGVHD was associated with better EFS (63% vs 16% at 43 months for patients with and without cGVHD; $P < .0001$) and better OS (78% vs 28% for patients with and without cGHVD; $P < .001$). The number of CD34$^+$ cells infused should be tailored to prevent extensive cGVHD among patients categorized as low-risk, while high-risk patients, in whom the graft-versus-leukemia effect may determine disease outcome, should receive high doses of CD34$^+$ cells. (Blood. 2003;102:1108-1113)

Introduction

While in autologous transplantation the studies published so far have shown that infusion of high doses of CD34$^+$ cells leads to a faster hematopoietic engraftment and decreased transplantation-related morbidity,[1-5] within the allogeneic setting information regarding the optimal dose of hematopoietic progenitor cells remains controversial. While some studies have reported a positive impact on outcome in terms of faster engraftment and fewer infectious episodes by infusing high numbers of CD34$^+$ cells in patients undergoing bone marrow transplantation,[6-8] other authors have shown an increased risk of acute or chronic graft-versus-host disease (GVHD) in patients receiving high doses of unmanipulated peripheral blood stem cells (PBSCs).[9,10] Among recipients of CD34$^+$-selected allogeneic transplants, the influence of the number of CD34$^+$ cells on survival remains contradictory, since in CD34$^+$-selected marrow transplantation higher cell doses lead to improved survival,[3] while the opposite occurs with CD34$^+$-selected PBSC transplants.[11] Nevertheless, to the best of our knowledge, the impact of the CD34$^+$ cell dose infused in patients undergoing reduced-intensity-conditioning (RIC) allogeneic transplantation has not been analyzed. Interestingly, in this subset of patients chemotherapy-induced toxicity is significantly reduced,[12,13] thus allowing a better estimation of the impact of the CD34$^+$ cell dose on GVHD and transplantation-related complications without the possible confounding effects of inflammatory cytokine production related to the use of high doses of chemotherapy during the conditioning regimen.[14,15]

The present study analyzes the impact of CD34$^+$ cell dose on the outcome of patients receiving RIC allogeneic PBSC transplants.[12,13]

Patients and methods

Patients

Patients eligible for entry into this study had myeloid or lymphoid malignancies that were potentially treatable with an allogeneic transplantation. Eligible patients were 45 years old or older and/or were at high risk of

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transplantation-related mortality (TRM) considering their pretransplantation evaluation. Between May 1998 and January 2002, 86 patients underwent RIC PBSC transplantation from a human leukocyte antigen (HLA)–identical sibling at 2 transplantation centers in Spain. Patients gave written informed consent for inclusion in the protocol, which was approved by all local ethical review boards and the Spanish Drug Agency (protocol 99-0151). Patient characteristics are shown in Table 1. Disease phase at transplantation was categorized as early (low risk) in 21 cases (acute leukemia or poor-risk myelodysplasia in first complete remission, untreated standard-risk myelodysplasia, first-chronic-phase chronic myelogenous leukemia [CML], lymphoid malignancy in first remission), intermediate in 26 patients (acute leukemia or myelodysplasia in second or higher complete remission, second-chronic-phase or accelerated-phase CML, lymphoid malignancy in second or higher remission) and advanced (high risk) in 39 cases (refractory or relapsed acute leukemia or myelodysplasia, blastic-phase CML, refractory or relapsed lymphoid malignancy, and all second transplantations).

Conditioning regimens and graft-versus-host disease prophylaxis

Two RIC regimens were used, one recommended for lymphoid malignancies and one for myeloid malignancies.[12] The lymphoid RIC regimen consisted of fludarabine 30 mg/m^2 administered intravenously on days −9 to −5, followed by melphalan 70 mg/m^2 intravenously on days −3 and −2. The myeloid regimen consisted of the same doses of fludarabine together with busulphan 1 mg/kg for 10 doses (days −6 to −4, total 10 mg/kg), with phenytoin given as anticonvulsant prophylaxis. PBSCs were infused on day 0.

GVHD prophylaxis consisted of cyclosporine A (CsA) plus short-course methotrexate (MTX). CsA was given at a dose of 1 mg/kg per day intravenously from days −9 to −2, and then 3 mg/kg/d intravenously or orally from day −1. Levels were maintained in the therapeutic range until tapering. MTX was given at a dose of 15 mg/m^2 intravenously on day 1 and a dose of 10 mg/m^2 intravenously on days 3 and 6, followed by folinic acid rescue at the same dose per square meter intravenously every 6 hours for 4 doses starting 24 hours after each dose of MTX. Acute and chronic GVHD were graded by established criteria.[16,17]

Mobilization and harvesting of donor PBSCs

Donors received 10 μg/kg granulocyte colony-stimulating factor (G-CSF) daily for 5 to 6 days and mobilized PBSCs were collected from day 5 until an ideal target dose of at least 4×10^6 CD34$^+$ cells per kilogram of recipient weight was collected. Stem cell apheresis was performed using a continuous flow cell separator, CS 3000 Plus, with a small-volume (50-mL) collection chamber (Baxter, Deerfield, IL).

Table 1. Characteristics of patients (n = 86) at time of transplantation

Characteristics	No.	%
Diagnosis and disease status		
Acute myelogenous leukemia	18	21
Acute lymphocytic leukemia	7	8
Chronic lymphocytic leukemia	7	8
Chronic myelogenous leukemia	6	7
Non-Hodgkin lymphoma	16	19
Multiple myeloma	17	20
Myelodysplastic syndrome	9	10
Hodkgin disease	6	7
Disease status at transplantation		
Low risk	21	24
Intermediate risk	26	30
High risk	39	46
Cytomegalovirus serology		
Patient positive	70	81
Patient negative/donor positive	7	8
Patient and donor negative	9	11
Sex		
Matched	51	59
Mismatched	35	41

Patients' median age at time of transplantation was 52 years (range, 22-66 years).

Immunofluorescence analysis

The absolute number of CD34$^+$ cells was measured in erythrocyte-lysed peripheral blood samples prior to initiating each apheresis as well as in the leukapheresis product. Cells were stained with an anti-CD34 monoclonal antibody (anti–HPCA-2) conjugated with phycoerythrin (PE) and analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, CA) as previously described.[1,2] In order to increase the sensitivity and specificity of the measurement, a 2-step acquisition procedure was performed. In the first step, 20 000 events were acquired; in the forward scatter/side scatter (FSC/SSC) dot plot, granulocytes, monocytes, and lymphocytes were selected, eliminating debris and red blood cells (RBCs) in order to calculate the percentage of nucleated cells. In the second step, 300 000 events were acquired. A side-scatter/CD34-PE live gate was performed in which only cells with high intensity of PE fluorescence were selected; the cluster of CD34$^+$ cells was then selected among these events around the low-intermediate side scatter. The proportion of CD34$^+$ cells was then calculated as follows: number of CD34$^+$ events selected in the second acquisition step × 100/300 000 events × percentage of nucleated cells obtained in the first acquisition step. Then the absolute count of peripheral blood (PB) CD34$^+$ cells/μL was calculated by multiplying the proportion of CD34$^+$ cells by the white blood cell (WBC) count.

In center B, this CD34$^+$ cell assay was slightly modified according to the ISHAGE protocol.[18]

Chimerism studies

After transplantation, serial samples of whole PB, bone marrow (BM), and CD3$^+$- and CD15$^+$-selected cells, obtained using magnetic microbeads according to the manufacturer's protocols (MACS cell separation; Miltenyi Biotec, Bergisch Gladbach, Germany), were analyzed for degrees of donor-recipient chimerism using polymerase chain reaction (PCR) analysis of informative minisatellite regions. Samples were routinely analyzed soon after hematologic recovery (days 21 to 56), on days 90 to 100 and on days 180 to 200, and then every 3 months or in case of disease recurrence or suspicion of graft failure. Chimerism studies were performed with a commercially available automated kit (AmpFlSTR, SGM Plus; Applied Biosystems, Norwalk, CT) as previously reported.[13,19]

Statistical analysis

Events analyzed were calculated from the time of transplantation using Kaplan-Meier product-limit estimates. Patients who experienced TRM were censored at last follow-up. Event-free survival (EFS) was calculated from transplantation until disease progression or death, and those patients who did not reach disease response (complete or partial) any time after transplantation were considered to have experienced an event. Overall survival (OS) was calculated from transplantation until death from any cause, and surviving patients were censored at last follow-up. Univariate Cox regression was used to analyze the impact of time-dependent covariates on EFS and OS. Differences in hematologic recovery (days to reach an absolute neutrophil count [ANC] $> 0.5 \times 10^9$/L, days to reach a platelet count $> 20 \times 10^9$/L) between groups according to the different covariates were analyzed by Kaplan-Meier and log-rank tests. Significant factors were included in a multivariate analysis using a forward stepwise Cox proportional hazards regression model.

Since methods for CD34$^+$ and CD3$^+$ quantification by flow cytometry were not identical in the 2 centers, the data on the number of CD34$^+$ and CD3$^+$ cells infused were separately categorized according to each center's quartile values. Cases within the first quartile from each center were included under the same category (≤ percentile 25 [p25]), as were the cases within the second (p25-50), third (p50-75), and fourth (> p75) quartiles. Therefore 4 groups of CD34$^+$ and CD3$^+$ cells were established. Qualitative parameters and binary variables were analyzed using the analysis of variance (ANOVA) test and the multiple logistic regression model. Significance levels were set at .05.

Results

Characteristics of the product infused and impact on hematopoietic recovery

A median of 5.68 (range, 2.58-15.6) $\times 10^6$ $CD34^+$ cells/kg and 2.86 (range, 0.25-8.41) $\times 10^8$ $CD3^+$ cells/kg were infused. These data were 4.55 (range, 2.75-13.2) $\times 10^6$ $CD34^+$ cells/kg and 2.36 (range, 0.25-6.75) $\times 10^8$ $CD3^+$ cells/kg in center A and 7 (range, 2.58-15.6) $\times 10^6$ $CD34^+$ cells/kg and 3.41 (range, 1.9-8.4) $\times 10^8$ $CD3^+$ cells/kg in center B ($P = .003$ for $CD34^+$ cells and $P = .001$ for $CD3^+$ cells infused). Owing to these differences, both variables were grouped separately according to the quartile values for each center and then fused as previously specified.

No significant differences were observed in terms of $CD3^+$ cells infused depending on the amount of $CD34^+$ cells infused.

All evaluable patients reached levels of more than 500 granulocytes/mm^3 at a median of 15 days (range, 6-23 days) and more than 20 000 platelets/mm^3 at a median of 13 days (range, 0-23 days) after PBSC infusion. Neither the number of $CD3^+$ cells infused nor the $CD34^+$ cell dose infused significantly influenced the speed of hematopoietic recovery. The median (mean) time to neutrophil recovery ($> 500/mm^3$) by quartile of $CD34^+$ cells infused was 16 (15), 16 (16), 14 (15), and 15 (16) days, respectively, while the median (mean) time to platelet recovery ($> 20\,000/mm^3$) was 13 (12), 13 (13), 13 (14), and 11 (11) days, respectively. No differences in the speed of hematopoietic recovery were observed between the 2 centers. Regarding extrahematologic toxicity, overall the regimen was well tolerated: 7% of patients developed nausea of grade 3 or higher, 21% developed mucositis of grade 3 or higher, 7% developed liver toxicity of grade 3 or higher, and 11% developed diarrhea of grade 3 or higher.

Chimerism analysis in bone marrow, peripheral blood, T lymphocytes, and granulocytes was performed on days 21 to 28, 56, 100, 180, 270, and 365 after transplantation. Results on the kinetics of chimerism are shown in Table 2. The number of $CD34^+$ cells infused did not influence the rate of complete chimerism in bone marrow and granulocytes. By contrast, 100% of patients receiving p75 or more of $CD34^+$ cells reached complete chimerism in T lymphocytes by days 21 to 28, as compared with 44% among those receiving p75 or fewer cells ($P = .046$). A trend toward a faster rate of complete chimerism in unfractionated peripheral blood was also observed among those patients receiving higher doses of $CD34^+$ cells (93% of complete chimerism in PB for those receiving p75 or more cells, vs 77% for those receiving p75 or fewer $CD34^+$ cells; $P = .4$). Finally, we did not observe any impact from the $CD3^+$ cell dose infused on the kinetics of chimerism.

Table 2. Percentage of cases with complete chimerism after infusion of $CD34^+$ and $CD3^+$ cells

Source of sample analyzed	Days after transplantation					
	21-28	56	100	180	270	365
Bone marrow	73	81	83	88	86	88
Unseparated peripheral blood	88	82	91	100	100	100
T lymphocytes	57*	58	73	88	100	100
Granulocytes	92	88	92	94	100	100

*100% of patients receiving more than p75 vs 44% of patients receiving p75 or fewer $CD34^+$ cells reached complete chimerism ($P = .046$); in all other instances, cell dose had no significant influence on the kinetics of complete chimerism.

Impact of the product infused on clinical outcome

Overall, 37 patients (43%) developed acute graft-versus-host disease (aGVHD) at a median of 32 days (range, 10-125 days) and 26 (30%) of them developed grade 2 to 4 aGVHD. Among 83 evaluable patients, 48 (57.8%) developed chronic GVHD (cGVHD) at a median of 137 days after transplantation (range, 148 to 196 days). Twenty-two (26.5%) developed limited cGVHD and 26 (31.3%) extensive cGVHD.

Neither $CD34^+$ nor $CD3^+$ cell dose significantly affected the incidence of aGVHD. By contrast, the dose of $CD34^+$ cells infused had a significant influence on the development of cGVHD, with a cumulative incidence of extensive cGVHD of 74% vs 47% ($P = .02$) among patients receiving the higher dose ($>$ p75) vs those receiving the lower dose ($\leq$ p75) of $CD34^+$ cells (Figure 1). Again, the dose of $CD3^+$ cells infused did not influence the incidence of cGVHD. These results were confirmed when we analyzed the results from the 2 centers separately. Thus, in center A 83% of patients receiving more than p75 $CD34^+$ cells developed extensive cGVHD, as compared with 56% among those receiving p75 or fewer $CD34^+$ cells ($P = .004$), and the same trend was observed in center B: incidence of extensive cGVHD of 56% vs 39% for patients receiving high ($>$ p75) vs low ($\leq$ p75) doses of $CD34^+$ cells, respectively.

No other variables were significantly associated with the occurrence of aGVHD. In terms of cGVHD, patients older than 51 years (percentile 50) had a significantly higher incidence of overall and extensive cGHVD than did younger patients (cumulative incidence for overall cGHVD, 100% vs 63%, log-rank $P = .04$; cumulative incidence for extensive cGVHD, 100% vs 43%, log rank $P = .01$). In addition, those patients receiving melphalan had a significantly lower incidence of overall and extensive cGVHD than did those receiving busulphan (cumulative incidence for overall cGVHD, 70% vs 87%, log-rank $P = .008$; cumulative incidence for extensive cGHVD, 42% vs 75%, log rank $P = .01$).

After a median follow-up of 385 days (range, 28-1312 days), 25 patients died, 17 (19.8% of the total) owing to TRM (8 patients owing to aGVHD, 2 owing to cGVHD, 4 owing to fungal or bacterial infection, and 3 owing to other toxicities) and 8 owing to disease progression. Neither the dose of $CD34^+$ cells nor the dose of $CD3^+$ cells infused significantly influenced TRM. We confirmed by both univariate and multivariate analysis that there were no significant differences between the 2 centers in terms of TRM, acute or chronic GVHD, or relapse.

Projected OS and EFS at 43 months were 60% and 46%, respectively (Figure 2). Again, neither the dose of $CD34^+$ cells nor the dose of $CD3^+$ cells infused significantly influenced OS and EFS. Nevertheless, among patients categorized as high-risk according to disease status at transplantation, 36% of those receiving p75 or fewer $CD34^+$ cells relapsed or progressed, compared with only 9% among those receiving more than p75 $CD34^+$ cells ($P = .07$). Moreover, among patients receiving p75 or fewer $CD34^+$ cells, 36% of high-risk patients relapsed, compared with 10% of low- and intermediate-risk patients ($P = .004$), while relapse rates were not significantly different between high-risk and low- or intermediate-risk patients when we infused more than p75 $CD34^+$ cells (9% vs 30%, respectively; $P = .22$).

Regarding EFS, as expected, those patients who developed grade 3 to 4 aGVHD had significantly worse outcomes (15% vs 50% projected EFS at 43 months, $P = .006$). By contrast, cGVHD was associated with better EFS (63% vs 16% projected EFS at 43 months for those patients with and without cGHVD, respectively; $P < .0001$; Figure 3). No other variables affected EFS after RIC.

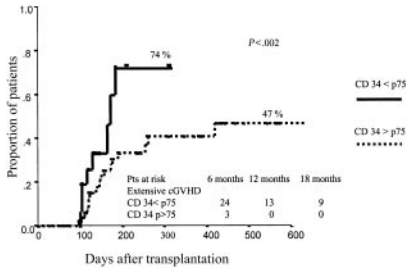

Figure 1. Influence of CD34$^+$ cell dose on the development of extensive cGVHD.

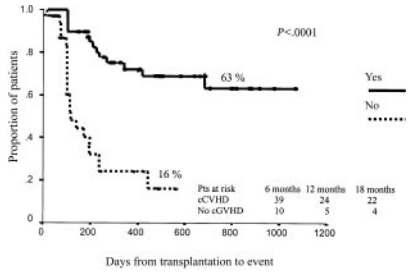

Figure 3. Influence of overall cGVHD on event free survival.

In terms of OS, those patients who developed aGVHD had a significantly worse prognosis than those without aGVHD (51% vs 67% projected OS at 43 months, respectively; $P = .01$; Figure 4). By contrast, those patients who developed cGVHD had significantly better outcomes than those who did not develop cGVHD (78% vs 28% projected OS at 43 months, respectively; $P < .001$), with a hazard rate (HR) of 6.41 (95% confidence interval [CI], 2.38-17.25; $P = .0002$; Figure 5). To further assess the effect of cGVHD on outcome, we grouped patients into 3 categories according to cGVHD (absent, limited, or extensive). Relapse rate was significantly higher among patients who did not develop cGVHD as compared with the other 2 categories: 50% vs 11% and 9%, respectively ($P = .001$). By contrast, no significant differences were observed in terms of TRM among these subgroups of patients, although a trend toward lower TRM was observed among those who developed limited cGVHD (5% TRM) as compared with those who developed extensive cGVHD (15% TRM) or those who did not develop cGVHD (20% TRM; $P = .27$). Finally, in terms of overall survival, patients who developed limited or extensive cGVHD had 87% and 76% projected OS at 43 months, while OS was only 28% for those patients who did not develop cGVHD ($P = .004$), with a hazard rate for patients who developed cGVHD of 5.78 (95% CI, 20.22-27.35; $P = .02$; Figure 6).

No other variables significantly influenced OS after RIC, although the dose of CD34$^+$ cells infused showed a trend to influence outcome. Thus, among patients receiving p75 or fewer CD34$^+$ cells, projected OS at 43 months was 75% versus 48% for low- or intermediate-risk and high-risk patients, respectively ($P = .2$), while among patients receiving more than p75 CD34$^+$ cells, projected OS at 43 months was 60% versus 60%, respectively ($P = .79$; Figures 7 and 8).

Discussion

Recent studies have been reported showing the impact of the CD34$^+$ cell dose on the incidence of acute or chronic GVHD.[9,10,11] Interestingly, this effect has been observed only after allogeneic PBSC transplantation (allo-PBSCT), while trials using bone marrow stem cells have found a positive influence on outcome without increasing the risk of GVHD after infusing high numbers of progenitor cells.[6-8,20] In order to explain these differences, it should be noted that the range of CD34$^+$ cells infused in allogeneic bone marrow transplantation (allo-BMT) is narrower than that used in allo-PBSCT, where the number of stem cells infused is usually higher.[21-25] Moreover, the threshold level of CD34$^+$ cells associated with an increased incidence of GVHD after allo-PBSCT has been high in all studies,[9,10,11] and these levels have rarely been reached after allo-BMT.[6-8,21-25] In addition, functional differences between CD34$^+$ and CD3$^+$ cells infused after treatment with G-CSF and bone marrow–harvested stem cells could also play a role,[26-29] since mesenchymal stem cells, which may have an immunoregulatory effect,[30] are infused after bone marrow but not after PBSC transplantation.[31] Furthermore, Ryncarz and Anasetti[32] have also reported on the capability of CD34$^+$ hematopoietic progenitor cells to present certain antigens to autologous T lymphocytes, which could also explain the relationship between GVHD and CD34$^+$ cells infused.

The present study has been performed in patients who received RIC, which decreases cell lysis and release of cytokines and inflammatory mediators induced by high doses of chemotherapy, thereby avoiding the confounding factor of these mediators of GVHD reactions.[33-35] In this sense, in contrast to previous reports that analyzed the impact of CD34$^+$ cell dose on GVHD after conventional conditioning regimens,[9] we did not find an increase of aGVHD after infusing high doses of CD34$^+$ cells, although we did find an increased risk of extensive cGVHD, in accordance with other studies.[10] It is worth mentioning that while Urbano-Ispizua et al[11] found that the infusion of high doses of CD34$^+$ cells had a negative impact on survival, other reports did not find any impact of CD34$^+$ cell dose on survival.[7,9,10,36] This could be explained by the poor tolerance to GVHD in patients undergoing CD34$^+$-selected allo-PBSCT.[37] Interestingly, in our study the relapse rate among high-risk patients significantly decreased after infusion of high doses of CD34$^+$ cells, thus favorably affecting outcome in this

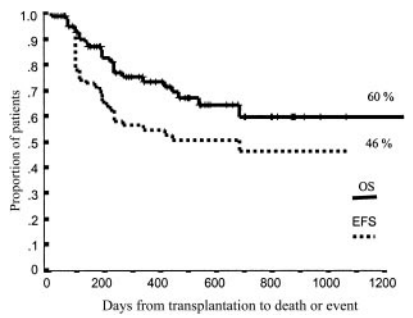

Figure 2. Overall and event-free survival.

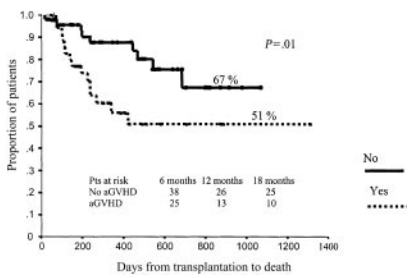

Figure 4. Influence of aGVHD on overall survival.

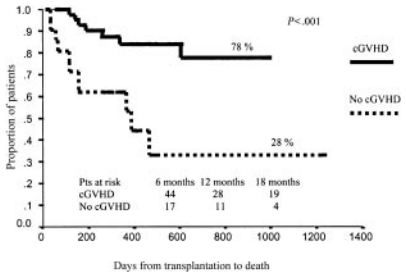

Figure 5. Influence of cGVHD on overall survival.

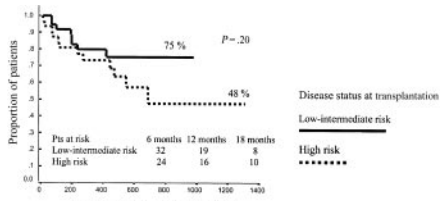

Figure 7. Overall survival among patients receiving p75 or lower CD34$^+$ cell dose.

subgroup of patients. In addition, we found that, after a RIC regimen, cGVHD favorably affects event-free and overall survival, which has already been reported in previous studies performed on patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) after RIC allogeneic transplantation.[12,13] The positive impact of cGVHD on relapse rate has also been reported in some studies after conventional conditioning regimens,[21,38,39] although it has not been found in other series.[10,40,41] Since the therapeutic effect of RIC allogeneic transplantation relies mainly on graft-versus-leukemia (GVL) reactions, it is reasonable to find a link between cGVHD and event-free and overall survival in these type of transplantations.[12,13] Moreover, the ability of allogeneic stem cell transplantation to eradicate leukemia is determined by both the intensity of the conditioning regimen and the GVL effect and, according to this and other studies based on RIC or truly nonmyeloablative conditioning regimens,[42] the lower the conditioning regimen intensity, the more evident the GVL reaction. With our RIC regimen, chemotherapy could initially contribute to disease control while the immune system induces an adequate response, and at the same time a low extrahematologic toxicity and low TRM could be maintained. Nevertheless, it should be noted that since cGVHD is a time-dependent variable, studies showing any impact of cGVHD on survival must be interpreted cautiously, since this relationship could be explained by the fact that patients with longer survival have a higher risk of developing cGVHD and not the contrary. We performed a Cox regression analysis, which confirmed the favorable impact of cGVHD on event-free and overall survival in our study.

As previously reported,[9,10,19-24,43,44] we did not find a relationship between the number of CD3$^+$ cells infused and the incidence of acute or chronic GVHD. This finding could be attributed to the high doses of CD3$^+$ cells infused in all patients, since, according to Kernan and colleagues,[45,46] once the initial threshold of T cells has been exceeded, a further increase in T-cell numbers does not translate into more GVHD. Accordingly, Urbano-Ispizua et al[47] and Wagner et al[48] have reported that T-cell doses of more than 0.2×10^6 and more than 0.5×10^6 cells/kg are associated with an increased incidence of GVHD in both T cell–depleted allo-PBSCT and unmanipulated allo-BMT, respectively, without any differences reported for higher cutoff values. In the present study, all patients received doses much higher than those mentioned.

Finally, in terms of engraftment, we did not find a clear correlation between the number of CD34$^+$ cells infused and the speed of hematopoietic recovery. As we have previously reported with regard to autologous transplantation,[1,2] the higher the number of CD34$^+$ cells infused the faster the engraftment, up to a dose ranging between 2.2 and 2.4×10^6 CD34$^+$ cells/kg. In our experience, doses higher than that do not increase the speed of hematopoietic recovery, although the exact threshold varies among centers depending on the CD34$^+$ quantification procedures. This observation concurs with the results reported by Zaucha et al[10] and Gianni et al,[49] who found that increasing the progenitor cells above a certain threshold had a limited potential to further accelerate neutrophil recovery. In addition, in a previous study including a small number of patients, we reported that after a RIC regimen the number of overall and myelomonocytic committed CD34$^+$ cells does influence the speed of hematopoietic recovery, although only the myelomonocytic committed cell dose retained its influence in multivariate analysis,[44] thereby suggesting that the number of the different CD34$^+$ cell subpopulations is more relevant than the overall number of CD34$^+$ cells infused for predicting the speed of hematopoietic recovery. In the present study, using a RIC regimen, we observed that stable engraftment can be ensured by infusing a CD34$^+$ cell dose greater than or equal to 2.58 CD34$^+$ cells/kg but that higher doses do not increase the speed of hematopoietic recovery. By contrast, the CD34$^+$ cell dose may affect the kinetics of chimerism, since patients receiving higher doses reached complete chimerism in T lymphocytes more rapidly. This could also explain the higher incidence of extensive cGVHD among patients receiving the higher doses of CD34$^+$ cells.

Since the CD34$^+$ cell dose is the only pretransplantation variable that can be manipulated to affect cGVHD incidence, the number of CD34$^+$ cells infused should be tailored cautiously to prevent extensive cGVHD among patients categorized as low-risk according to their disease status before transplantation, while high-risk patients, in which the benefit of GVL may determine disease outcome, should receive high doses of CD34$^+$ cells. In conclusion, in the RIC allogeneic transplantation setting, the CD34$^+$ cell dose, in addition to its impact on engraftment, may have a relevant influence on graft-versus-host disease and graft-versus-tumor effect.

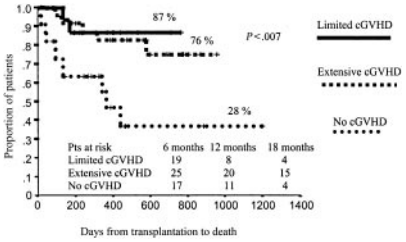

Figure 6. Influence of limited and extensive cGVHD on overall survival.

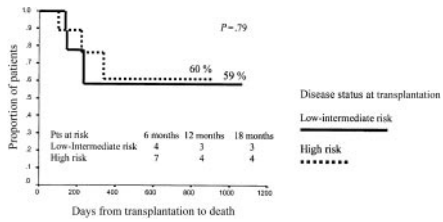

Figure 8. Overall survival among patients receiving more than p75 CD34$^+$ cell dose.

References

1. Pérez-Simón JA, Caballero MD, Corral M, et al. Minimal number of circulating CD34+ cells to assure successful leukapheresis and engraftment in peripheral blood progenitor cell transplantation. Transfusion. 1998;38:385-391.
2. Pérez-Simón JA, Martín A, Caballero D, et al. Clinical significance of CD34+ cell dose in long-term engraftment following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 1999;24:1279-1283.
3. Mavroudis D, Read E, Cottler-Fox M, et al. CD34+ cell dose predicts survival, posttransplantation morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. Blood. 1996;88:3223-3229.
4. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. J Clin Oncol. 1995;13:2547-2555.
5. Kiss JE, Rybka WB, Winkelstein A, et al. Relationship of CD34+ cell dose to early and late hematopoiesis following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 1997;19:303-310.
6. Bittencourt H, Rocha V, Chevret S, et al. Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. Blood. 2002;99:2726-2733.
7. Dominietto A, Lamparelli T, Raiola AM, et al. Transplant-related mortality and long-term graft function are significantly influenced by cell dose in patients undergoing allogeneic marrow transplantation. Blood. 2002;100:3930-3934.
8. Rocha V, Labopin M, Gluckman E, et al. Relevance of bone marrow cell dose on allogeneic transplantation outcomes for patients with acute myeloid leukemia in first complete remission: results of a European survey. J Clin Oncol. 2002; 20:4324-4330.
9. Przepiorka D, Smith TL, Folloder J, et al. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. Blood. 1999;94:1465-1470.
10. Zaucha JM, Gooley T, Bensinger WI, et al. CD 34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transplantation. Blood. 2001;98:3221-3227.
11. Urbano-Ispizua A, Carreras E, Marín P, et al. Allogeneic transplantation of CD 34(+) selected cells from peripheral blood from human leukocyte antigen-identical siblings: detrimental effect of a high number of donor CD 34(+) cells? Blood. 2001; 98:2352-2357.
12. Martino R, Caballero MD, Canals C, et al. Allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning: results of a prospective multicenter study. Br J Haematol. 2001;115:653-659.
13. Pérez-Simón JA, Caballero D, Díez-Campelo M, et al. Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation. Leukemia. 2002; 16:1423-1431.
14. Sullivan KM. Graft-versus-host-disease. In: Thomas ED, Forman SJ, Blume KG, eds. Hematopoietic Cell Transplantation. Oxford, England: Blackwell Science Inc; 1998:515-536.
15. Clift RA, Buckner CD, Appelbaum FR, et al. Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood. 1990;76:1867-1871.
16. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15:825-828.
17. Sullivan KM, Shulman HM, Storb R, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood. 1981; 57:267-276.
18. Sutherland DR, Anderson L, Keeney M, Najar R, Chin-Yee J. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. J Hematother. 1996;5:213-226.
19. Valcárcel D, Martino R, Caballero D, et al. Chimerism analysis following allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning. Bone Marrow Transplant. 2003;31:387-392.
20. Morariu-Zamfir R, Rocha V, Devergie A, et al. Influence of CD 34(+) marrow cell dose on outcome of HLA-identical sibling allogeneic bone marrow transplants in patients with chronic myeloid leukaemia. Bone Marrow Transplant. 2001; 27:575-580.
21. Blaise D, Kuentz M, Fortanier C, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Société Française de Greffe de Moelle. J Clin Oncol. 2000;18:537-546.
22. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med. 2001;344:175-181.
23. Mohty M, Kuentz M, Michallet M, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. Blood. 2002;100:3128-3134.
24. Powles R, Mehta J, Kulkarni S, et al. Allogeneic blood and bone marrow stem cell transplantation in haematological malignant diseases: a randomised trial. Lancet. 2000;355:1231-1237.
25. Flowers ME, Parker PM, Johnston LJ, et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. Blood. 2002; 100:415-419.
26. Pan L, Delmonte J Jr, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood. 1995;86:4422-4429.
27. Talmadge JE, Reed EC, Kessinger A, et al. Immunologic attributes of cytokine mobilized peripheral blood stem cells and recovery following transplantation. Bone Marrow Transplant. 1996; 17:101-109.
28. Mielcarek M, Roecklein BA, Torok-Storb B. CD 14+ cells in granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells induce secretion of interleukin-6 and G-CSF by marrow stroma. Blood. 1996;87:574-580.
29. Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T-helper 2-inducing dendritic cells. Blood. 2000;95:2484-2490.
30. Lazarus HM, Curtin P, Devine S, et al. Role of mesenchymal stem cells (MSC) in allogeneic transplantation: early phase I clinical results [abstract]. Blood. 2000;96:1691.
31. Bacigalupo A, Piaggio G, Podesta M, et al. Influence of marrow CFU-GM content on engraftment and survival after allogeneic bone marrow transplantation. Bone Marrow Transplant. 1995;15: 221-226.
32. Ryncarz RE, Anasetti C. Expression of CD86 on human marrow CD34(+) cells identifies immunocompetent committed precursors of macrophages and dendritic cells. Blood. 1998;91:3892-3900.
33. Jacobsohn DA, Vogelsang GB. Novel pharmacotherapeutic approaches to prevention and treatment of GVHD. Drugs. 2002;62:879-889.
34. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanism of acute graft-versus-host-disease. Biol Blood Marrow Transplant. 1999;5:347-356.
35. Dickinson AM, Sviland L, Dunn J, Carey P, Proctor SJ. Demonstration of direct involvement of cytokines in graft-versus-host reactions using an in vitro human skin explant model. Bone Marrow Transplant. 1991;7:209-216.
36. Sierra J, Storer B, Hansen JA, et al. Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching, and marrow cell dose. Blood. 1997;89:4226-4235.
37. Urbano-Ispizua A, Rozman C, Martinez C, et al. Rapid engraftment without significant GVHD after allogeneic transplantation of CD 34+ selected cells from peripheral blood. Blood. 1997;89:3967-3973.
38. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304:1529-1533.
39. Horowitz MM, Gale RP, Sondel PM, et al. Graft versus leukemia reactions after bone marrow transplantation. Blood. 1990;75:555-562.
40. Zecca M, Petre A, Rondelli R, et al. Chronic graft-versus-host-disease in children: incidence, risk factors, and impact on outcome. Blood. 2002; 100:1192-1200.
41. Schmitz N, Beksac M, Hasenclever D, et al. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood. 2002;100:761-767.
42. McSweeney P, Niederwieser D, Shizuru J, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effect. Blood. 2001;97:3390-3400.
43. Przepiorka D, Anderlini P, Saliba R, et al. Chronic graft-versus-host-disease after allogeneic blood stem cell transplantation. Blood. 2001;98:1695-1700.
44. Menéndez P, Pérez-Simón JA, Mateos MV, et al. Influence of the different CD34+ and CD34− cell subsets infused on the clinical outcome of non-myeloablative allogeneic peripheral blood transplantation from human leucocyte antigen-identical sibling donors. Br J Haematol. 2002;119:135-143.
45. Kernan NA, Bordignon C, Heller G, et al. Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia, I: analysis of risk factors and results of secondary transplants. Blood. 1989;74:2227-2236.
46. Kernan NA, Collins NM, Juliano L, Cartagena T, Dupont B, O'Reilly RJ. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of GVHD. Blood. 1986;68:770-773.
47. Urbano-Ispizua A, Rozman C, Pimentel P, et al. The number of donor CD3(+) cells is the most important factor for graft failure after allogeneic transplantation of CD34(+) selected cells from peripheral blood from HLA-identical siblings. Blood. 2001;97:383-387.
48. Wagner JE, Santos GW, Noga SJ, et al. Bone marrow graft engineering by counterflow centrifugal elutriation: results of a phase I-II clinical trial. Blood. 1990;75:1370-1377.
49. Gianni AM, Bregni M, Siena S, et al. Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells: a changing role for high dose chemo-radiotherapy? Hematol Oncol. 1989; 7:139-148.