Antithymocyte Globulin in Reduced-Intensity Conditioning Regimen Allows a High Disease-Free Survival Exempt of Long-Term Chronic Graft-versus-Host Disease

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Raynier Devillier [1,2,3], Sabine Fürst [1], Jean El-Cheikh [1], Luca Castagna [1,4], Samia Harbi [1,2], Angela Granata [1], Roberto Crocchiolo [1,4], Claire Oudin [1,2], Bilal Mohty [1], Reda Bouabdallah [1], Christian Chabannon [2,3,5,6], Anne-Marie Stoppa [1], Aude Charbonnier [1], Florence Broussais-Guillaumot [1], Boris Calmels [3,5,6], Claude Lemarie [5,6], Jèrôme Rey [1], Norbert Vey [1,2,3], Didier Blaise [1,2,3,*]

[1] Hematology Department, Transplantation Program, Institut Paoli Calmettes, Marseille, France
[2] Aix-Marseille Université, Marseille, France
[3] Centre de Recherche en Cancérologie de Marseille (CRCM), Marseille, France
[4] Hematology Unit, Humanitas Cancer Center, Istituto Clinico Humanitas, Rozzano, Milano, Italy
[5] Cell Therapy Facility, Institut Paoli Calmettes, Marseille, France
[6] Inserm CBT-510, Centre d'Investigations Cliniques en Biothérapie, Institut Paoli Calmettes, Marseille, France

ABSTRACT

Nonmyeloablative (NMA) regimens allow the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients considered unfit for standard myeloablative conditioning (MAC) regimens using high-dose alkylating agents with or without total body irradiation (TBI). Reduced-intensity conditioning (RIC) regimens, based on fludarabine (Flu), busulfan (Bu), and rabbit antithymocyte globulin (r-ATG), represent an intermediate alternative between NMA and MAC regimens. This platform was subsequently optimized by the introduction of i.v. Bu and the use of 5 mg/kg r-ATG, based on the hypothesis that these modifications would improve the safety of RIC allo-HSCT. Here we report a study conducted at our institution on 206 patients, median age 59 years, who underwent allo-HSCT after conditioning with Flu, 2 days of i.v. Bu, and 5 mg/kg r-ATG (FBx-ATG) between 2005 and 2012. The prevalence of grade III-IV acute graft-versus-host disease (GVHD) was 9%, and that of extensive chronic GVHD was 22%. Four-year nonrelapse mortality (NRM), relapse, and overall survival (OS) rates were 22%, 36%, and 54%, respectively. NRM tended to be influenced by comorbidities (hematopoietic cell transplantation–specific comorbidity index [HCT-CI] <3 versus HCT-CI ≥3: 18% versus 27%; $P = .075$), but not by age (<60 years, 20% versus ≥60 years, 25%; $P = .142$). Disease risk significantly influenced relapse (2 years: low, 8%, intermediate, 28%, high, 34%; very high, 63%; $P = .017$). Both disease risk (hazard ratio [95% confidence interval]: intermediate, 2.1 [0.8 to 5.2], $P = .127$; high, 3.4 [1.3 to 9.1], $P = .013$; very high, 4.0 [1.1 to 14], $P = .029$) and HCT-CI (hazard ratio [95% confidence interval]: HCT-CI ≥3, 1.7 (1.1 to 2.8), $P = .018$) influenced OS, but age and donor type did not. The FBx-ATG RIC regimen reported here is associated with low mortality and high long-term disease-free survival without persistent GVHD in both young and old patients. It represents a valuable platform for developing further post-transplantation strategies aimed at reducing the incidence of relapse, particularly in the setting of high-risk disease.

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INTRODUCTION

Nonmyeloablative (NMA) regimens allow the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients unfit for standard myeloablative conditioning (MAC) regimens, such as cyclophosphamide (Cy) and full-dose total body irradiation (TBI; 12 Gy) or Cy and busulfan (Bu). NMA regimens are associated with reduced nonrelapse mortality (NRM) and exert disease control relying solely on the allogeneic graft-versus-tumor immune reaction [1,2].

Reduced-intensity conditioning (RIC) regimens deliver a higher degree of myeloablation than NMA regimens but a lower level than MAC regimens. RIC regimens usually include an intermediate dose of alkylating agents, and thus retain a direct antitumor effect, with the risk of higher NRM [3,4]. We previously reported that an RIC regimen composed of an intermediate dose of oral Bu and a low dose of r-ATG resulted in higher disease control, but also in higher NRM compared with a 2-Gy TBI NMA conditioning regimen, with similar overall outcomes [5,6]. We found that with such a conditioning regimen, both graft-versus-host disease (GVHD) and NRM could be satisfactorily controlled without loss of disease control by only a marginal increase in r-ATG dose and a switch from oral to i.v. Bu [7,8].

Here we report the outcome of the first 206 consecutive patients who were treated with this protocol before undergoing allo-HSCT from an HLA-identical related donor or an unrelated donor. With a minimal follow-up of 7 months and a median follow-up of 28 months, our results strongly suggest that although the population is characterized by high-risk features, this protocol allows for an encouragingly high survival rate without disease recurrence or persistent debilitating chronic GVHD.

PATIENTS AND METHODS

Selection Criteria

Patients with the following criteria were included in our analyses: (1) allo-HSCT performed between 2005 and 2012; (2) RIC based on Flu, i.v. Bu

* Correspondence and reprint requests: Didier Blaise, MD. Institut Paoli Calmettes, 232 boulevard Sainte Marguerite, 13009 Marseille, France
E-mail address: blaised@ipc.unicancer.fr (D. Blaise).

(Bx), and r-ATG (FBx-ATG); (3) allo-HSCT from a matched related donor (MRD) or 10/10 HLA-matched unrelated donor (MUD); and (4) peripheral blood stem cells (PBSCs) as the graft source. Our Institutional Review Board approved this study, and all patients provided informed consent in accordance with the Declaration of Helsinki.

Conditioning Regimen and GVHD Prophylaxis

The FBx-ATG conditioning regimen was started on day -6 and included Flu (Fludara; Bayer, Puteaux, France) 30 mg/m^2 daily from day -5 to day -1, Bx (Busilvex, Pierre Fabre, Boulogne-Billancourt, France) 130 mg/m^2 once daily on days -4 and -3, and r-ATG (Thymoglobuline, Genzyme, St. Germain-en-Lay, France) 2.5 mg/kg once daily on days −2 and −1 or on days −3 and −2, as reported previously [9]. Cyclosporine A (Sandimmun; Novartis, Bâle, Switzerland), started on day −1, was used for postgraft immunosuppression. Stem cell harvesting and supportive care were performed as described previously [5].

Stratification of Risk of Relapse and NRM

The risk of relapse in our cohort of patients with different hematologic diseases was characterized using the disease risk index (DRI) as described by Armand et al. [10]. Comorbidities were assessed using the hematopoietic cell transplantation−specific comorbidity index (HCT-CI) [11].

Study Endpoints and Statistical Analysis

The cumulative incidence of GVHD was calculated as described previously [12,13]. NRM and relapse were determined using the Prentice estimation and the Gray test, which allow consideration of competing events [14,15]. Progression-free survival (PFS) and OS were calculated with the Kaplan-Meier method and the log-rank test [16]. In patients who survived without disease recurrence, the prevalence of immunosuppressive treatments served as a surrogate marker of quality of life. Time to events was calculated from the date of allo-SCT. Cox regression was used to analyze the impact of pretransplantation covariates in multivariate analyses of PFS and OS [17]. All survival analyses were performed using R version 2.13.1 (http://www.R-project.org).

RESULTS
Patient and Transplantation Characteristics

A total of 206 consecutive patients were included in our analyses. Baseline patient and transplantation characteristics are described in Table 1. The median patient age was 59 years (range, 19 to 71 years), and 32 patients were age ≥65 years. One hundred and twenty-four patients (60%) underwent transplantation from an MRD. Only 25 patients (12%) presented with a low disease risk index, and 90 patients (46%) had an HCT-CI ≥3. Seventy-six patients (37%) were not in complete remission at the time of transplantation. The median duration of post-transplantation follow-up was 28 months (range, 7 to 76 months).

GVHD, NRM, and Relapse

Post-transplantation events and outcomes of the 206 patients are presented in Table 2. All but 1 patient engrafted. The cumulative incidence of grade III-IV acute GVHD was 9%, and that of extensive chronic GVHD was 22%. The incidence of grade III-IV acute GVHD was higher in patients age ≥60 years (14% versus 5% in those age <60 years; P = .021), that of extensive chronic GVHD was similar in the 2 age groups (25% in those age <60 years versus 19% in those aged ≥60 years; P = .367). Forty-three patients died of nonrelapse-related causes at a median of 6 months (range, 0.4 to 30 months) after allo-HSCT. NRM was estimated at 5% (95% confidence interval [CI], 2% to 8%) at day +100, 16% (95% CI, 12% to 22%) at 1 year, and 22% (95% CI, 16% to 29%) at 4 years (Figure 1A). NRM was only marginally influenced by comorbidities (18% in patients with HCT-CI <3 versus 27% in those with HCT-CI ≥3; P = .075), and was not influenced by age (20% in patients age <60 years versus 25% in those age ≥60 years; P = .142). Sixty-three patients experienced disease recurrence, at a median time of 7 months (range, 0.3 to 68 months) after allo-HSCT, for a 2-year cumulative incidence of relapse of 28% (95% CI, 22% to 34%) (Figure 1A). DRI had a significant correlation with the incidence of relapse at 2 years (low, 8%; intermediate, 28%; high, 34%; very high, 63%; P = .017).

PFS and OS

One-year PFS was 63% (95% CI, 56% to 70%), and 1-year OS was 73% (95% CI, 67% to 79%) (Figure 1B). The causes of death are listed in Table 3. Multivariate analyses showed that age (<60 versus ≥60 years) or the donor type (MRD versus MUD) did not influence PFS and OS (Table 4). For PFS, DRI was the most significant predictive factor, and the predictive value of HCT-CI was close to significance (Table 4). HCT-CI (2-year OS, 73% for <3 versus 54% for ≥3; P = .020; Figure 2A) and DRI (2-year OS, 84% for low versus 68% for intermediate versus 47% for high versus 25% for very high; P = .008; Figure 2B) had a significant influence on OS (Table 4).

Immunosuppressive Treatment and GVHD in 1-Year Progression-Free Survivors

At 1 year after allo-HSCT, 122 patients were alive and progression-free. Among these survivors, 96 (79%) were surviving without GVHD without immunosuppressive treatment (IST) (n = 89; 73%) or with tapering IST (n = 7; 6%). Seven patients (6%) were surviving with IST for persistent extensive chronic GVHD.

Table 1
Patient, Disease, and Transplantation Characteristics (n = 206)

Characteristic	Value
Age, yr, median (range)	59 (19-71)
Donor, n (%)	
MRD	123 (60)
MUD	83 (40)
Diagnosis, n (%)	
Acute myelogenous leukemia	70 (34)
Myelodysplastic syndrome	19 (9)
Acute lymphoblastic leukemia	9 (4)
Non-Hodgkin lymphoma	41 (20)
Hodgkin lymphoma	14 (7)
Chronic lymphoblastic leukemia	14 (7)
Multiple myeloma	31 (15)
Myeloproliferative neoplasm	6 (3)
Chronic myelogenous leukemia	2 (1)
Disease risk index, n (%)	
Low	25 (12)
Intermediate	125 (61)
High	48 (23)
Very high	8 (4)
HCT-CI, n (%)	
0-2	107 (54)
≥3	90 (46)
Unknown	9

Table 2
Outcomes after Allo-SCT (n = 206)

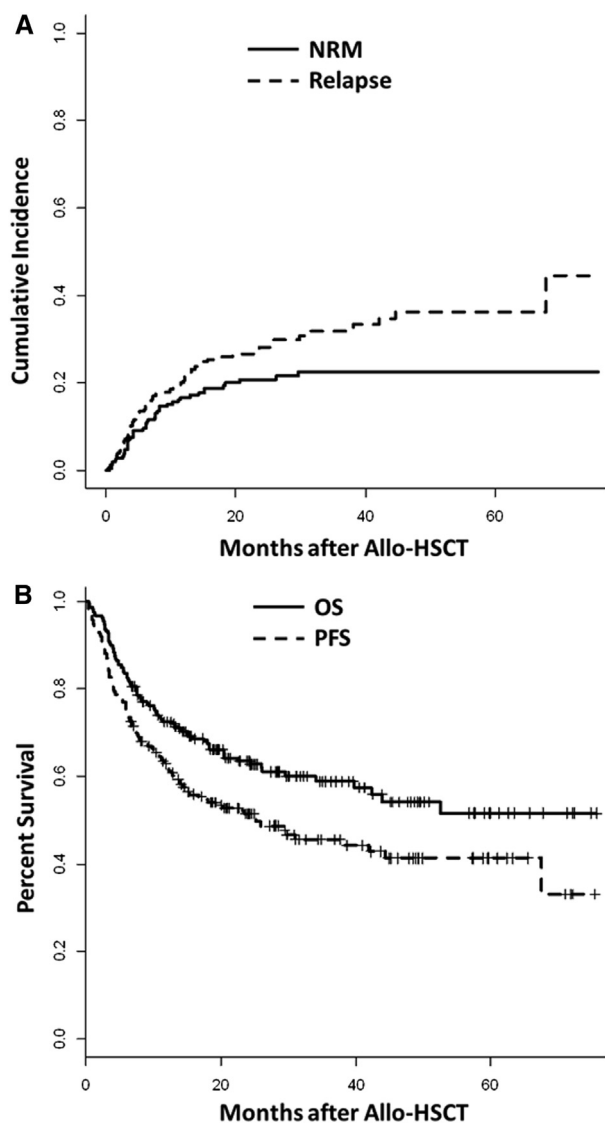| Outcome	Value
Acute GVHD, n (%)	
Grade II-IV	23 (17-29)
Grade III-IV	9 (5-13)
Chronic GVHD, n (%)	
Overall	37 (30-44)
Extensive	22 (17-28)
NRM at 4 yr, n (%)	22 (16-29)
Relapse at 4 yr, n (%)	36 (28-44)
PFS at 4 yr, n (%)	41 (34-50)
OS at 4 yr, n (%)	54 (46-64)
Follow-up, mo, median (range)	28 (7-76)

Figure 1. Outcomes after allo-HSCT. Cumulative incidence of NRM and Relapse (A). Kaplan Meier estimation of OS and PFS (B).

DISCUSSION

This large single-center cohort analysis suggests that our FBx-ATG RIC regimen results in low NRM. We estimated an early NRM at day +100 of only 5%, even though our patient population had a high median age (59 years), a high rate of comorbidities (46% with HCT-CI ≥3), and a high prevalence of measurable disease (37%), supporting the low early toxicity

Table 3
Causes of Death (n = 80)

Cause	n
Nonrelapse death	43
Infection and acute GVHD	16
Infection and chronic GVHD	12
Infection without GVHD	6
Cardiac dysfunction	3
Sinusoidal obstruction syndrome	2
Secondary cancer	2
Graft failure	1
Cerebral hemorrhage	1
Relapse-related death	37

Table 4
Multivariate Analyses of PFS and OS

Variable	HR	95% CI	P Value
PFS			
Age			
<60 yr	1		
≥60 yr	1.1	0.8-1.7	.576
Donor type			
MRD	1		
MUD	0.9	0.6-1.4	.778
Disease risk index			
Low	1		
Intermediate	2.3	1.1-5.1	.036
High	3.0	1.3-7.0	.011
Very high	4.0	1.3-13	.019
HCT-CI			
0-2	1		
≥3	1.5	1.0-2.2	.066
OS			
Age			
<60 yr	1		
≥60 yr	1.2	0.8-2.0	.346
Donor type			
MRD	1		
MUD	1.2	0.7-1.9	.513
Disease risk index			
Low	1		
Intermediate	2.1	0.8-5.2	.127
High	3.4	1.3-9.1	.013
Very high	4.0	1.1-14	.029
HCT-CI			
0-2	1		
≥3	1.7	1.1-2.8	.018

of this conditioning platform. The low incidence of grade III-IV acute GVHD (9%), likely related to the use of r-ATG, contributed to this result. Overall, the NRM in our study cohort compares satisfactorily with that reported by Storb et al. [18] after an NMA regimen (day +100 and 1-year NRM of 4% and 15%, respectively) in patients with a median age of 56 years (range, 17 to 74 years). It is important to observe that HCT-CI, but not age, was the determinant of OS, suggesting that age alone is not a sufficient parameter for allocating patients to less-intensive approaches, as reported previously [19].

The incidence of extensive chronic GVHD in our cohort was low at 22%, and 79% of patients free of disease at 1 year after allo-HSCT were also living without GVHD. This could be considered a surrogate marker for preserved quality of life in the absence of a prospective assessment. We found that this conditioning platform allows a low prevalence of persistent extensive chronic GVHD, contributing to the overall safety profile. This finding supports the important contribution of r-ATG in this setting.

The use of r-ATG remains controversial, especially in the setting of MRD allo-HSCT. Soiffer et al. [20] reported that in vivo T cell depletion with r-ATG at a median dose of 7 mg/kg resulted in a higher incidence of relapse compared with T cell–replete allo-HSCT. Conversely, we and other groups have demonstrated that an r-ATG dose of approximately 5 mg/kg produces effective GVHD prophylaxis without increasing the incidence of relapse [7,8,21,22]. These discordant results underscore the impact of r-ATG dose modulation. Although the optimal dose has not been established, we propose the hypothesis that 5 mg/kg could approach an acceptable compromise, with lower doses associated with insufficient GVHD prophylaxis [5,6] and higher doses associated with increased risk of relapse [20].
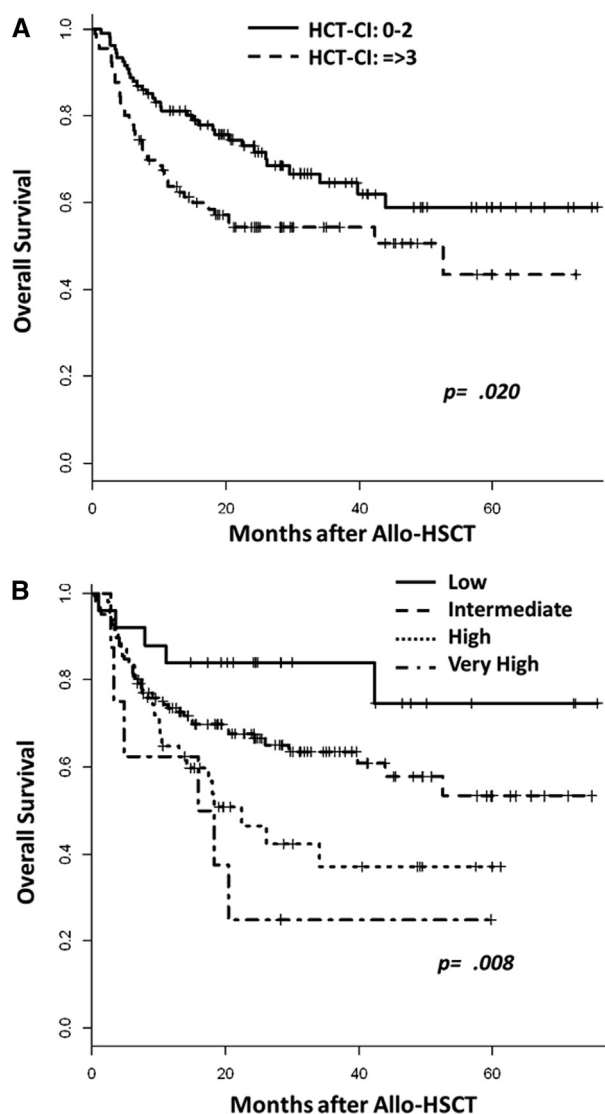
Figure 2. Impacts of HCT-CI (A) and DRI (B) on OS.

Another issue with the use of r-ATG is the risk of infectious complications. Although we did not evaluate such complications in the present study, we recently reported a large cohort of patients who presented with cytomegalovirus (CMV) reactivation after allo-HSCT and showed that in vivo T cell depletion was not associated with an increased CMV reactivation or CMV-related disease [23].

Regarding the efficacy of our proposed approach, the incidence of relapse was 36% at 4 years, confirming that the antitumor effect of the FBx-ATG regimen compares favorably with that of true NMA regimens. Several previous studies reported a cumulative incidence of relapse approaching 50% after allo-HSCT with a Flu plus 2-Gy TBI conditioning regimen [6,18,24]. In our series of patients undergoing allo-HSCT from an MRD or a 10/10-matched MUD, this antitumor effect, in combination with a low NRM, resulted in promising OS (73% at 1 year and 54% at 4 years), particularly when considering the poor prognostic features of the cohort (ie, few patients with a low DRI, a median age of 59 years, and significant comorbidities in approximately 50%). The promising low-toxicity profile found in the present study was also

recently reported by our group in the setting of transplantation from HLA-mismatched unrelated donors [9].

In summary, all of the changes that have occurred over the years, including r-ATG dose modulation and Bu administration [25-27], have led to the development of our FBx-ATG platform with low toxicity but an active antitumor effect. These results were achieved and maintained with a minimum follow-up of 7 months and a median follow-up of 28 months, with some patients undergoing allo-HSCT more than 6 years earlier. This finding supports the evidence of a persistent antitumor effect long after allo-HSCT. Multivariate analysis identified the DRI as the strongest marker for poor survival, demonstrating that post-transplantation relapse in patients with high-risk features after RIC allo-HSCT remains a major concern. With its high predictive value in our series, the DRI is an interesting tool for stratifying patients based on the risk of relapse rather than on disease type, helping address the issue of series with various diagnoses. Thus, we suppose that the DRI could be useful for personalizing the choice of either conditioning regimen or post–allo-HSCT treatments in a risk-adapted strategy. Indeed, our FBx-ATG regimen could be reserved for patients at low and intermediate risk, with more-intensive approaches provided for high-risk and very-high-risk patients, who achieved a 2-year OS of <50% with the FBx-ATG regimen. From this perspective, the increased intensity afforded by higher Bu doses could represent an interesting improvement in the FBx-ATG platform to address the issue of high-risk diseases. Some groups have previously showed that myeloablative doses of i.v. Bu in the FBx-ATG regimen provide both the low NRM observed with RIC regimens and the high disease control observed with standard MAC regimens, even in patients of advanced age and/or with comorbidities [28-30]. This evolution, from the RIC versus MAC paradigm to a myeloablative regimen with reduced toxicity (MA/RTC), highlights the critical role of conditioning intensity and opens up the possibility of myeloablative approaches in a population historically excluded from standard MAC regimens [31].

We conclude that the FBx-ATG RIC platform reported here approaches an acceptable balance between toxicity and efficacy, resulting in low mortality and long-term disease-free survival with preserved quality of life. However, high-risk disease remains a concern, dictating the need for disease control strategies through MA/RTC and/or post-transplantation treatments.

REFERENCES
1. Lowsky R, Takahashi T, Liu YP, et al. Protective conditioning for acute graft-versus-host disease. *N Engl J Med*. 2005;353:1321-1331.
2. McSweeney PA, Niederwieser D, Shizuru JA, 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.
3. Chakraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood*. 2002;99:1071-1078.

4. Slavin S, Nagler A, Naparstek E, 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.

5. Blaise D, Farnault L, Faucher C, et al. Reduced-intensity conditioning with Fludarabin, oral busulfan, and thymoglobulin allows long-term disease control and low transplant-related mortality in patients with hematological malignancies. *Exp Hematol*. 2010;38:1241-1250.

6. Blaise D, Tabrizi R, Boher JM, et al. Randomized study of 2 reduced-intensity conditioning strategies for human leukocyte antigen-matched, related allogeneic peripheral blood stem cell transplantation: prospective clinical and socioeconomic evaluation. *Cancer*. 2013;119:602-611.

7. Crocchiolo R, Esterni B, Castagna L, et al. Two days of antithymocyte globulin are associated with a reduced incidence of acute and chronic graft-versus-host disease in reduced-intensity conditioning transplantation for hematologic diseases. *Cancer*. 2013;119:986-992.

8. Devillier R, Crocchiolo R, Castagna L, et al. The increase from 2.5 to 5 mg/kg of rabbit anti-thymocyte globulin dose in reduced intensity conditioning reduces acute and chronic GVHD for patients with myeloid malignancies undergoing allo-SCT. *Bone Marrow Transplant*. 2012;47:639-645.

9. Devillier R, Furst S, Crocchiolo R, et al. A conditioning platform based on fludarabine, busulfan and two days of rabbit antithymocyte globulin results in promising results in patients undergoing allogeneic transplantation from both matched and mismatched unrelated donor. *Am J Hematol*. 2013. http://dx.doi.org/10.1002/ajh.23592.

10. Armand P, Gibson CJ, Cutler C, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. *Blood*. 2012;120: 905-913.

11. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912-2919.

12. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18:295-304.

13. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.

14. Fine J. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496-509.

15. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.

16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.

17. Cox DR. Regression models and life tables. *J R Stat Soc B*. 1972;34: 187-220.

18. Storb R, Gyurkocza B, Storer BE, et al. Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2013;31:1530-1538.

19. El Kourashy S, Williamson T, Chaudhry MA, et al. Influence of comorbidities on transplant outcomes in patients aged 50 years or more after myeloablative conditioning incorporating fludarabine, BU and ATG. *Bone Marrow Transplant*. 2011;46:1077-1083.

20. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti–T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood*. 2011;117:6963-6970.

21. Russell JA, Turner AR, Larratt L, et al. Adult recipients of matched related donor blood cell transplants given myeloablative regimens including pretransplant antithymocyte globulin have lower mortality related to graft-versus-host disease: a matched pair analysis. *Biol Blood Marrow Transplant*. 2007;13:299-306.

22. Duggan P, Booth K, Chaudhry A, et al. Unrelated donor BMT recipients given pretransplant low-dose antithymocyte globulin have outcomes equivalent to matched sibling BMT: a matched pair analysis. *Bone Marrow Transplant*. 2002;30:681-686.

23. Venton G, Crocchiolo R, Furst S, et al. Risk factors of ganciclovir-related neutropenia after allogeneic stem cell transplantation: a retrospective monocentre study on 547 patients. *Clin Microbiol Infect*. 2013. http://dx.doi.org/10.1111/1469-0691.12222.

24. Gyurkocza B, Storb R, Storer BE, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation in patients with acute myeloid leukemia. *J Clin Oncol*. 2010;28:2859-2867.

25. Andersson BS, Madden T, Tran HT, et al. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant*. 2000;6:548-554.

26. Andersson BS, Kashyap A, Gian V, et al. Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. *Biol Blood Marrow Transplant*. 2002;8:145-154.

27. Thall PF, Champlin RE, Andersson BS. Comparison of 100-day mortality rates associated with i.v. busulfan and cyclophosphamide vs other preparative regimens in allogeneic bone marrow transplantation for chronic myelogenous leukemia: Bayesian sensitivity analyses of confounded treatment and center effects. *Bone Marrow Transplant*. 2004;33:1191-1199.

28. Alatrash G, de Lima M, Hamerschlak N, et al. Myeloablative reduced-toxicity i.v. busulfan-fludarabine and allogeneic hematopoietic stem cell transplant for patients with acute myeloid leukemia or myelodysplastic syndrome in the sixth through eighth decades of life. *Biol Blood Marrow Transplant*. 2011;17:1490-1496.

29. Andersson BS, de Lima M, Thall PF, et al. Once-daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. *Biol Blood Marrow Transplant*. 2008;14:672-684.

30. Liu H, Zhai X, Song Z, et al. Busulfan plus fludarabine as a myeloablative conditioning regimen compared with busulfan plus cyclophosphamide for acute myeloid leukemia in first complete remission undergoing allogeneic hematopoietic stem cell transplantation: a prospective and multicenter study. *J Hematol Oncol*. 2013;6:15.

31. Blaise D, Castagna L. Do different conditioning regimens really make a difference? *Hematology Am Soc Hematol Educ Program*. 2012;2012: 237-245.