ASBMT American Society for Blood and Marrow Transplantation

Prognostic Factors Affecting Outcome after Allogeneic Transplantation for Hematological Malignancies from Unrelated Donors: Results from a Randomized Trial

Jürgen Finke,[1] Claudia Schmoor,[2] Wolfgang A. Bethge,[3] Hellmut D. Ottinger,[4] Matthias Stelljes,[5] Axel R. Zander,[6] Liisa Volin,[7] Dominik A. Heim,[8] Rainer Schwerdtfeger,[9] Karin Kolbe,[10] Jiri Mayer,[11] Johan A. Maertens,[12] Werner Linkesch,[13] Ernst Holler,[14] Vladimir Koza,[15] Martin Bornhäuser,[16] Hermann Einsele,[17] Hartmut Bertz,[1] Olga Grishina,[2] Gérard Socié,[18] for the ATG-Fresenius Trial Group*

Several prognostic factors for the outcome after allogeneic hematopoietic stem-cell transplant (HSCT) from matched unrelated donors have been postulated from registry data; however, data from randomized trials are lacking. We present analyses on the effects of patient-related, donor-related, and treatment-related prognostic factors on acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, nonrelapse mortality (NRM), disease-free survival (DFS), and overall survival (OS) in a randomized, multicenter, open-label, phase III trial comparing standard graft-versus-host-disease (GVHD) prophylaxis with and without pretransplantation ATG-Fresenius (ATG-F) in 201 adult patients receiving myeloablative conditioning before HSCT from HLA-A, HLA-B antigen, HLA-DRB1, HLA-DQB1 allele matched unrelated donors. High-resolution testing (allele) of HLA-A, HLA-B, and HLA-C were obtained after study closure, and the impact of an HLA 10/10 4-digit mismatch on outcome and on the treatment effect of ATG-F versus control investigated. Advanced disease was a negative factor for relapse, DFS, and OS. Donor age ≥40 adversely affected the risk of aGVHD III-IV, extensive cGVHD, and OS. Younger donors are to be preferred in unrelated donor transplantation. Advanced disease patients need special precautions to improve outcome. The degree of mismatch had no major influence on the positive effect of ATG-F on the reduction of aGVHD and cGVHD.

Biol Blood Marrow Transplant 18: 1716-1726 (2012) © *2012 American Society for Blood and Marrow Transplantation*

KEY WORDS: Allogeneic hematopoietic stem-cell transplantation, Graft-versus-host-disease prophylaxis, HLA-matching

INTRODUCTION

Due to extended availability of donors, allogeneic hematopoietic stem-cell transplant (HSCT) from unrelated donors is now done more frequently than from related donors in Europe [1]. Standards of graft-versus-host disease (GVHD) prophylaxis with

From the [1]Department of Hematology and Oncology, Universitätsklinikum Freiburg, Freiburg, Germany; [2]Clinical Trials Unit Universitätsklinikum Freiburg, Freiburg, Germany; [3]Department of Hematology and Oncology, Universitätsklinikum Tübingen, Tübingen, Germany; [4]Klinik und Poliklinik für KMT, Universitätsklinikum Essen, Essen, Germany; [5]Department of Hematology and Oncology, Universitätsklinik Münster, Münster, Germany; [6]Department of Internal Medicine, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [7]Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; [8]Department of Hematology, Universitätsspital Basel, Basel, Switzerland; [9]Centre of Blood and Bone Marrow Transplantation, Stiftung Deutsche Klinik für Diagnostik, Wiesbaden, Germany; [10]Department of Internal Medicine, Universitätsmedizin der Johannes-Gutenberg-Universität Mainz, Mainz, Germany; [11]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Masaryk University, Brno, Czech Republic; [12]Department of Hematology, I.K. UZ Gasthuisberg, Leuven, Belgium; [13]Department of Hematology, Universitätsklinikum Graz, Graz, Austria; [14]Department of Hematology and Oncology, Klinikum der Universität Regensburg, Regensburg, Germany; [15]Department of Hematology and Oncology, Charles University Hospital, Plzen, Czech Republic; [16]Center for Bone Marrow Transplantation, Universitätsklinikum Carl Gustav Carus, Dresden, Germany; [17]Medizinische Klinik und Poliklinik II, Universitätsklinik Würzburg, Würzburg, Germany; and [18]Service d'Hématologie-Greffe de Moelle, Hôpital Saint Louis, Paris, France.

Financial disclosure: See Acknowledgments on page 1725.

*Members listed at the end of the article.

Correspondence and reprint requests: Jürgen Finke, MD, Allogeneic Stem Cell Transplantation Section, Department of Hematology and Oncology, Freiburg University Medical Center, Hugstetter Str. 49, D-79106 Freiburg, Germany (e-mail: juergen.finke@uniklinik-freiburg.de).

Received March 8, 2012; accepted June 4, 2012

1083-8791/$36.00

http://dx.doi.org/10.1016/j.bbmt.2012.06.001

cyclosporine A (CyA) and methotrexate have been established in matched sibling donor transplantation [2]; however, application in the setting of unrelated donor transplant results in less reliable outcome because of the higher rates of GVHD and transplantation-related mortality [3-5]. The practice of allogeneic HSCT has changed over the years, eg, with the improvement of HLA-matching increasing use of less intensive conditioning and peripheral blood (PB)-derived grafts as well as better supportive care and routine introduction of quality management programs [6].

In order to reduce the risk of GVHD in transplantation from unrelated donors, various methods of in vivo and in vitro T cell depletion have been tested without showing a benefit [7,8]. Recently, we could demonstrate in a randomized trial that the addition of antithymocyte globulin Fresenius (ATG-F) to standard CyA/short course methotrexate for GVHD prophylaxis results in reduced incidence of acute GVHD (aGVHD) and chronic GVHD (cGVHD) without an increase in relapse or nonrelapse mortality (NRM), and without compromising overall survival (OS) [9].

When choosing an unrelated donor, emphasis is put on HLA-matching and many centers require a 10 of 10 allele (HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1) matched donor because mismatching in 1 or more loci results in increased GVHD and worse outcome [10-14]. In our trial, donors and recipients were required to be antigen matched for HLA-A and HLA-B (2-digit) and allele matched for HLA-DRB1 and HLA-DQB1 (4-digit). Matching for HLA-C was not required. High-resolution testing (4-digit allele) results of HLA-A, HLA-B, and HLA-C were obtained retrospectively from all study centers after study closure. In this article, we are able to present, we believe for the first time, results on the impact of an HLA 10/10 4-digit mismatch on outcome and on the treatment effect of ATG-F versus control.

Analyses of phase II or registry data suggest that additional other factors affect outcome after unrelated donor transplantation [15-21]. Because of the retrospective nature of these data, the definite value of each factor is not clear for the general practice in allogeneic HSCT from unrelated donors. A randomized trial is an ideal study design for a reliable analysis of the effects of prognostic factors on outcome. Treatment is standardized and because of the randomized allocation it is independent from prognostic factors and, thus, is not to be regarded as a confounding variable [22].

The focus of a recent analysis of our randomized trial was cGVHD [23]. As one aspect, we looked at the effects of prognostic factors on cGVHD. Our analysis showed that donor age of 40 years or more and type of disease diagnosis affected the risk of extensive cGVHD, and that the type of disease diagnosis and type of conditioning regimen affected the risk of limited or extensive cGVHD. In this report, we present a comprehensive analysis of the effects of prognostic factors on aGVHD and cGVHD, relapse, NRM, disease-free survival (DFS), and OS after an extended follow-up of a median of 3 years of our randomized GVHD prevention trial in unrelated donor transplantation [9].

PATIENTS AND METHODS

Patients and Procedures

As reported previously [9], within a randomized prospective multicenter phase III trial, a standard GVHD prophylaxis with the addition of ATG-F (ATG-F group) was compared to a standard prophylaxis alone (control group) in patients with acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), osteomyelofibrosis (OMF), receiving myeloablative conditioning before HSCT from matched unrelated donors (Table 1) with respect to GVHD, engraftment, relapse, NRM, DFS, OS, and safety. The primary endpoint for the treatment comparison was the occurrence of severe aGVHD grade III to IV or death within 100 days posttransplantation.

Standard GVHD prophylaxis was CyA starting day −1 and a short course of methotrexate 15 mg/m^2, day +1, and 10 mg/m^2 days +3, +6, +11. Patients in the ATG-F group received additional ATG-F at a dose of 20 mg/kg on day −3, day −2, and day −1 (total dose, 60 mg/kg) before transplantation. All patients received myeloablative conditioning regimens (further details [9]).

Donors and recipients were required to be antigen matched for HLA-A and HLA-B (2-digit) and allele matched for HLA-DRB1 and HLA-DQB1 (4-digit). Matching for HLA-C was not required. Since closure of the trial, high-resolution testing (4-digit allele) results of HLA-A, HLA-B, and HLA-C determined locally by certified laboratories were obtained retrospectively (Table 1).

Patients were recruited between May 2003 and February 2007 in 33 European centers including Israel. The study was approved by the appropriate independent ethics committees, and was done in accordance with the good clinical practice guidelines, the ethical principles of the Declaration of Helsinki, the national law, and guidelines of the participating countries. All patients gave written informed consent. Project management, statistical planning and analysis, randomization, data management, and clinical monitoring were conducted by the Clinical Trials Unit, University Medical Center Freiburg, Germany, independently from the sponsor Fresenius Biotech.

Table 1. Baseline Patient, Donor, and Treatment Characteristics

Characteristic	ATG-F (N = 103)	Control (N = 98)
Patient age (median, range)	40 (18-60)	39 (18-60)
<40 years	47	50
≥40 years	56	48
Donor age (median, range)	35 (20-58)	37 (18-56)
<40 years	62	64
≥40 years	32	30
Unknown	9	4
Patient/donor sex		
Patient male/donor female	14	13
Other	87	85
Unknown	2	0
Patient/donor CMV status		
Patient negative/donor negative	23	44
Other	80	54
Diagnosis		
ALL	37	33
AML	55	46
CML	6	11
MDS	5	5
OMF	0	3
Disease status		
Early	64	43
Advanced	39	55
Conditioning regimen		
TBI/Cy	54	48
Busulfan/Cy	26	26
TBI/etoposide/Cy	11	6
TBI/other	7	9
No TBI/other	5	9
Stem-cell source		
BM	21	16
PB	82	82
Number of infused CD34+ cells (median, range)*	7.4 (0.1-28.5)	7.3 (2.4-17.1)
$<7.5 \times 10^6$ cells/kg	43	41
$\geq 7.5 \times 10^6$ cells/kg	38	38
Unknown	1	3
Mean CyA level day −1 to day +30 (median, range)	213 (115-449)	233 (114-437)
<220 ng/mL	53	42
≥220 ng/mL	50	55
Unknown	0	1
HLA 10/10 mismatch (4-digit)		
No mismatch	61	58
Mismatch	31	29
8/8† match, HLA-C mismatch	18	16
8/8 mismatch,‡ HLA-C mismatch	7	5
8/8 mismatch,‡ HLA-C match	4	6
8/8 mismatch,‡ HLA-C unknown	1	2
8/8 unknown, HLA-C mismatch	1	0
Unknown	11	11
8/8 match, HLA-C unknown	4	5
8/8 unknown	7	6
HLA 10/10 mismatch (4-digit)		
No mismatch	61	58
Mismatch on HLA-class I antigen, HLA-class II allele level	23	24
HLA-A 2-digit mismatch	0	1
HLA-B 2-digit mismatch	0	1
HLA-C 2-digit mismatch	22	19
HLA-DQB1 4-digit mismatch	1	2
HLA-DRB1 4-digit mismatch	0	1
Mismatch on HLA-class I allele level	8	5
HLA-A 4-digit mismatch	1	1
HLA-B 4-digit mismatch	2	2
HLA-C 4-digit mismatch	3	2
HLA-A and HLA-B 4-digit mismatch	1	0
HLA-B and HLA-C 4-digit mismatch	1	0
Unknown	11	11
HLA-C mismatch (2-digit/4-digit)		
No mismatch	67	65
Mismatch	26	21
2-digit match/4-digit mismatch	4	2
2-digit mismatch	22	19
Unknown	10	12

ATG-F indicates antithymocyte globulin Fresenius; CMV, cytomegalovirus; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; OMF, osteomyelofibrosis; TBI, total body irradiation; Cy, cyclosporin; BM, bone marrow; PB, peripheral blood; CyA, cyclosporin A.

Data are n or median (range).

*Only for transplantation of PB (n = 164).

†8/8 refers to HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1.

‡Twenty-five patient/donor pairs with 8/8 mismatch: 6 had mismatch on the HLA-A, HLA-B antigen, HLA-DRB1, HLA-DQB1 allele level, thus being protocol deviations (1 ATG-F, 5 control); 19 had mismatch only on the HLA-A, HLA-B allele level (11 ATG-F, 8 control, 2 HLA-A, 13 HLA-B, 4 HLA-A and HLA-B different).

Statistical Analysis

The effects of the following prognostic factors on time to aGVHD grade II to IV, aGVHD III to IV, time to limited/extensive cGVHD, extensive cGVHD, time to relapse, time to NRM, DFS time, and OS time were analyzed: patient age (≥40 years versus <40 years), donor age (≥40 years versus <40 years), patient and donor sex (patient male/donor female versus other), patient and donor cytomegalovirus (CMV) status (patient and donor negative versus other), diagnosis (AML versus MDS versus ALL versus CML/OMF), disease status (advanced versus early), conditioning regimen (total body irradiation [TBI] versus no TBI), stem cell source (PB versus bone marrow [BM]), CD 34+ cells infused (≥7.5 versus $<7.5 \times 10^6$ cells/kg in PB derived grafts only), and CyA exposure (mean level ≥220 versus <220 ng/mL during the initial 4 weeks posttransplantation). The endpoints were defined as described [9].

The probability of event over time in groups defined by the prognostic factors was estimated by the cumulative incidence function. Relapse and death were considered as competing events for aGVHD and cGVHD. Relapse and NRM were considered as competing events, respectively. For patients who did not experience the event in question, time from

transplantation to the last documented follow-up was used as a censored observation.

The prognostic effects of the factors with respect to the time-to-event variables were analyzed with Cox regression models for the event-specific hazard functions using 2-sided Wald tests. The randomized treatment was included for adjustment. To estimate the effect sizes, the hazard ratios (HRs) between groups defined by the prognostic factors were calculated with 95% confidence intervals (CIs). Univariate analyses of the factors were performed, evaluating their effects separately. In multiple regression analyses, those factors that showed a prognostic effect in the univariate analysis with $P \leq .05$ were analyzed simultaneously.

For an investigation to determine if the effects of the prognostic factors were influenced by the randomized treatment, Cox regression models were used, including the prognostic factor, the treatment, and the interactive effect between prognostic factor and treatment. From these models, treatment-specific HRs between groups defined by the prognostic factors were calculated with 95% CIs.

The influence of the degree of HLA-mismatch on the time-to-event variables and on the treatment effect (ATG-F versus control) was analyzed separately using a Cox regression model including HLA-mismatch (4-digit) 10/10, the treatment effect, the interaction between HLA-mismatch and treatment, disease status, and stem-cell source. From this model, the treatment effects (ATG-F versus control) were estimated separately in patients with HLA 10/10 4-digit match, and HLA 10/10 4-digit and tests for heterogeneous treatment effects (interactions) were performed.

All analyses are post-hoc analyses of the randomized trial. No adjustment for multiple testing was performed. Statistical analysis was performed using the Statistical Analysis System, version 9.2. The study is registered with the World Health Organization primary registers, numbers DRKS00000002/NCT00655343.

RESULTS

Study Patients

Two hundred one adult patients with a median age of 40 years (range, 18-60 years) were randomized to ATG-F (n = 103) or control (n = 98) and underwent transplantation. One further randomized patient did not undergo transplantation and was excluded from all analyses. Table 1 shows the baseline patient characteristics in detail. Patients received as graft source either BM (n = 37) or granulocyte colony-stimulating factor (G-CSF) stimulated PB apheresis products (n = 164). Median CD34+ cell counts were 2.91×10^6/kg recipient body weight and 7.39×10^6/kg recipient body weight for marrow and PB grafts, respectively. Median donor age was 36 years (range, 18-58 years).

Since closure of the trial, high-resolution testing (4-digit) results for HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 (8/8) were obtained for 187 of the 201 study patient/donor pairs. Twenty-five of 187 pairs were mismatched (8/8) in HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 (6 of them on the HLA-A, HLA-B antigen, HLA-DRB1, and HLA-DQB1 allele level, thus being protocol deviations, and the other 19 on the HLA-A and HLA-B allele level). Additionally, high-resolution testing (4-digit) results, including HLA-C (10/10), were available for 179 patient/donor pairs. Thirty-four of 153 patient/donor pairs with 8/8 match and available HLA-C testing had HLA-C mismatch (4-digit). Overall, 60 of 179 patient/donor pairs (34%) had a 4-digit difference. For 47 of them, the difference was on the HLA-class I antigen, HLA-class II allele level; for 13 of them, the difference was on the HLA-class I allele level. Details are given in Table 1.

The median follow-up time was 3 years (25% quartile, 2.5 years; 75% quartile, 3.9 years). The aGVHD II to IV and III to IV before relapse occurred in 83 and 36 patients, respectively, and limited/extensive and extensive cGVHD before relapse occurred in 75 and 47 patients, respectively. Relapse was observed in 63 patients, NRM was observed in 52 patients, resulting in 115 events with respect to DFS. Overall, 103 patients have died.

Effects of Prognostic Factors

The results of the univariate analyses of the effects of prognostic factors are shown in Figure 1 and Figure S1 (supplementary material) and Tables S1, S2, S3, and S4 (supplementary material).

In univariate analyses, the following factors showed effects with $P \leq .05$: patient age affected aGVHD II to IV, NRM, and OS; diagnosis affected aGVHD II to IV, aGVHD III to IV, limited/extensive cGVHD, and extensive cGVHD; conditioning regimen affected limited/extensive cGVHD; disease status affected aGVHD III to IV, relapse, NRM, DFS, and OS; and donor age affected aGVHD III to IV, extensive cGVHD, and OS.

These factors were included in multiple regression analyses, in which the tests of the effects of the following factors showed $P \leq .05$ (Table 2). Patients ≥40 years versus <40 years negatively affected NRM (HR = 1.81; 95% CI, 1.03-3.19; $P = .041$) and positively affected aGVHD II to IV (HR = 0.62; 95% CI, 0.40-0.97; $P = .034$). There was no effect of patient age on aGVHD III to IV, cGVHD, and on relapse. Diagnosis had an effect on aGVHD II to IV ($P = .013$), on aGVHD III to IV ($P = .036$), on limited/extensive cGVHD ($P = .017$), and on extensive cGVHD ($P = .032$) with an increased risk for patients with MDS and for patients with CML/OMF. Patients with a TBI-containing conditioning regimen had

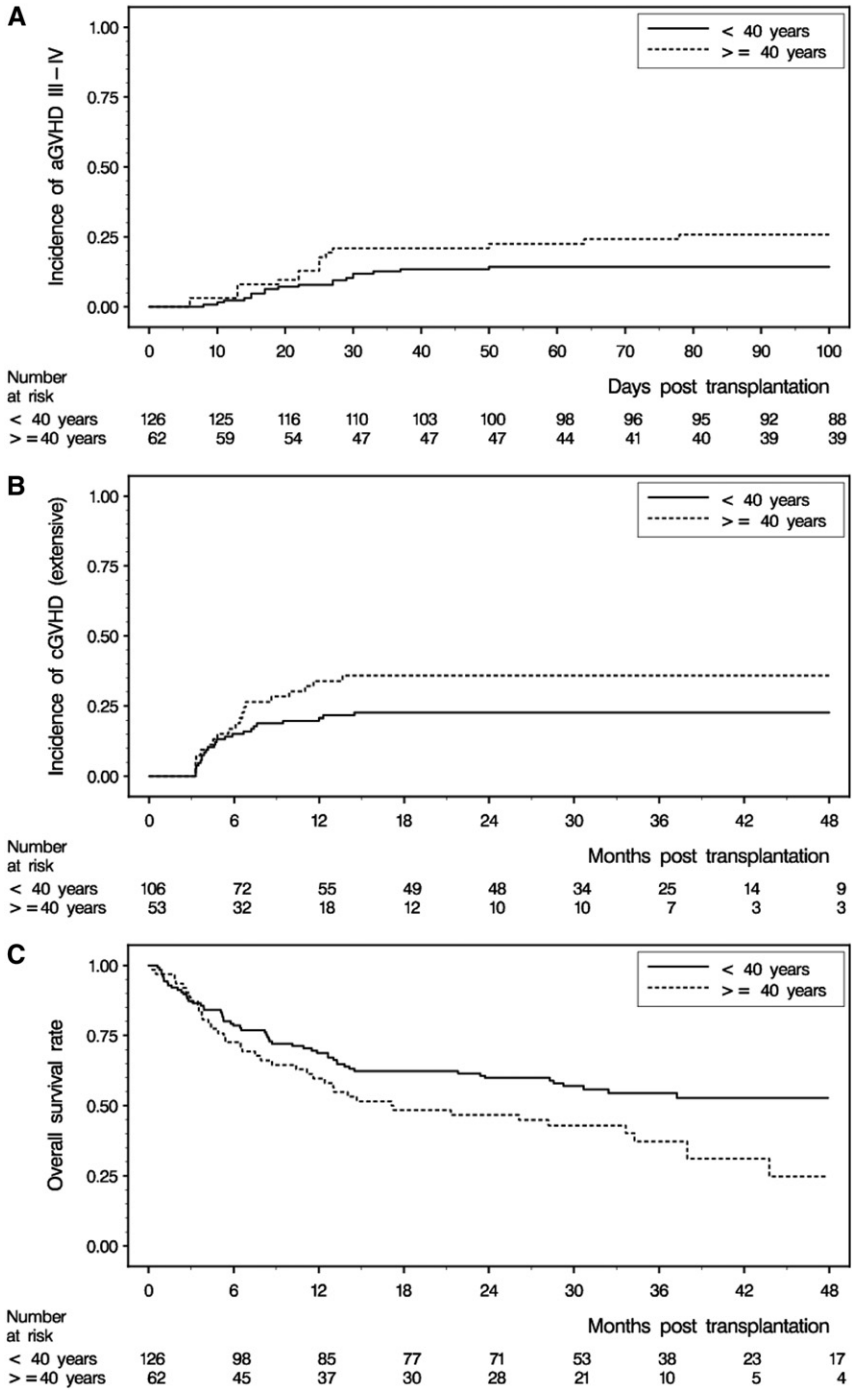

Figure 1. Effect of donor age, estimated from multiple regression model (Table 2). (A) Effect on acute graft-versus-host disease (aGVHD) III-IV, hazard ratio (≥40 versus <40 yr) = 2.57; 95% confidence interval (CI), 1.27-5.21; $P = .009$. (B) Effect on extensive chronic graft-versus-host disease (cGVHD), hazard ratio (≥40 versus <40 yr) = 2.06; 95% CI, 1.12-3.79; $P = .021$. (C) Effect on overall survival, hazard ratio (≥40 versus <40 yr) = 1.66; 95% CI, 1.10-2.51; $P = .016$.

a decreased risk of limited/extensive cGVHD (HR = 0.39; 95% CI, 0.23-0.68; $P = .0007$). Conditioning regimen had no effect on the other outcomes. Advanced disease was a negative factor for relapse (HR = 1.72; 95% CI, 1.03-2.87; $P = .038$), DFS (HR = 1.74; 95% CI, 1.19-2.54; $P = .004$), and OS (HR = 1.94; 95% CI, 1.27-2.97; $P = .002$).

Donor age ≥40 years adversely affected the risk of aGVHD III to IV (HR = 2.57; 95% CI, 1.27-5.21; $P = .009$), extensive cGVHD (HR = 2.06; 95% CI, 1.12-3.79; $P = .021$), and OS (HR = 1.66; 95% CI, 1.10-2.51; $P = .016$). The cumulative incidents of aGVHD III to IV and extensive cGVHD were 26% and 35% for patients with donors of at least 40 years

Table 2. Effects of Prognostic Factors (Multiple Regression Analyses Adjusted for Treatment)

		aGVHD II-IV			aGVHD III-IV		
Factor	Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Patient age	≥40 versus <40 yr	0.62	0.40-0.97	.034	–	–	–
Donor age	≥40 versus <40 yr	–	–	–	2.57	1.27-5.21	.009
Diagnosis	MDS versus AML	3.09	1.28-7.45	.013	5.72	1.74-18.9	.036
	ALL versus AML	1.28	0.77-2.11		1.19	0.52-2.73	
	CML/OMF versus AML	2.26	1.20-4.24		1.59	0.59-4.28	
Disease status	Advanced versus early	–	–	–	2.11	0.95-4.69	.067
		cGVHD Limited/Extensive			cGVHD Extensive		
Factor	Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Donor age	≥40 versus <40 yr	–	–	–	2.06	1.12-3.79	.021
Diagnosis	MDS versus AML	1.95	0.79-4.81	.017	4.03	1.31-12.4	.032
	ALL versus AML	1.60	0.88-2.89		1.59	0.77-3.25	
	CML/OMF versus AML	2.83	1.42-5.65		2.65	1.13-6.22	
Conditioning regimen	TBI versus no TBI	0.39	0.23-0.68	.0007			
		–	–	–			
		Relapse			NRM		
Factor	Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Patient age	≥40 versus <40 yr	–	–	–	1.81	1.03-3.19	.041
Disease status	Advanced versus early	1.72	1.03-2.87	.038	1.69	0.95-2.99	.073
		DFS			OS		
Factor	Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Patient age	≥40 versus <40 yr	–	–	–	1.46	0.97-2.19	.070
Donor age	≥40 versus <40 yr	–	–	–	1.66	1.10-2.51	.016
Disease status	Advanced versus early	1.74	1.19-2.54	.004	1.94	1.27-2.97	.002

aGVHD indicates acute graft-versus-host-disease; HR, hazard ratio; CI, confidence interval; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; OMF, osteomyelofibrosis; cGVHD, chronic graft-versus-host-disease; TBI, total body irradiation; NRM, nonrelapse mortality; DFS, disease-free survival; OS, overall survival.

and 14% and 23% for patients with donors younger than 40 years, respectively. OS rates 3 years after HSCT were 37% and 55% for patients with donors of at least 40 years and <40 years, respectively. When donor age was analyzed as a continuous covariate, the risk of aGVHD III to IV and of extensive cGVHD per donor age decade was increased (HR = 1.75; 95% CI, 1.18-2.58; $P = .005$ and HR = 1.38; 95% CI, 1.00-1.92; $P = .051$). The risk of death per decade of donor age was increased (HR = 1.28; 95% CI, 1.01-1.61; $P = .038$).

Other analyzed factors such as patient and donor sex, patient and donor CMV status, stem cell source, CD 34+ cell number infused (in PB-derived grafts only), and CyA exposure during the initial 4 weeks posttransplantation did not show significant effects in our trial.

Interactions between Prognostic Factors and Treatment

To examine whether the effects of prognostic factors were heterogeneous in the ATG-F group and the control group, interactive effects between treatment and prognostic factors were examined. Figure S2 (supplementary material) shows the effects of the prognostic factors with 95% CIs on the various outcomes separately for the ATG-F group and the control group. There were no strong interactions between treatment and prognostic factors with all *P* values of tests of interactive effects >.05, except for aGVHD II to IV, indicating that the effects of prognostic factors detected equally apply to both treatment groups. The effect of donor age on NRM was more pronounced in the ATG-F group (HR = 3.11; 95% CI, 1.25-7.73) than in the control group (HR = 1.01; 95% CI, 0.47-2.15), test of interaction $P = .067$, but it has to be considered that the 95% CIs of the HRs are large, and that multiple comparisons are performed in these extended subgroup analyses.

Effects of HLA-Mismatch (4-Digit, 10/10) on Outcome and Treatment Effect

To evaluate the influence of HLA-mismatch (4-digit, 10/10) on outcome and on the treatment effect (ATG-F versus control), the effects of treatment and the degree of HLA-mismatch on the various endpoints are shown in Table 3, Table 4, and Figure 2. One hundred nineteen patients underwent transplantation from a 10/10-matched donor (ATG-F, n = 61; control, n = 58), and 60 patients from a donor with a mismatch (ATG-F, n = 31; control, n = 29).

Table 3. Treatment Effect (ATG-F versus Control) (Multiple Regression Analyses, Adjusted for Disease Status, and Stem-Cell Source)

	Treatment Effect ATG-F versus Control				Test of Heterogeneous Treatment Effects (Interaction)
	HLA 10/10 4-Digit Match (N = 119)		HLA 10/10 4-Digit Mismatch (N = 60)		
Outcome	HR	95% CI	HR	95% CI	*P* Value
aGVHD II-IV	0.48	0.27-0.86	0.61	0.26-1.44	.65
aGVHD III-IV	0.39	0.16-0.93	0.54	0.10-2.94	.73
cGVHD limited/extensive	0.22	0.11-0.45	0.58	0.27-1.24	.06
cGVHD extensive	0.08	0.03-0.28	0.32	0.12-0.86	.09
Relapse	1.45	0.69-3.03	1.43	0.63-3.27	.98
NRM	0.68	0.33-1.39	0.53	1.18-1.60	.71
DFS	0.98	0.59-1.62	0.99	0.52-1.88	.98
OS	0.88	0.51-1.50	0.87	0.44-1.71	.99

ATG-F indicates antithymocyte globulin Fresenius; HR, hazard ratio; CI, confidence interval; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NRM, nonrelapse mortality; DFS, disease-free survival; OS, overall survival.

All tests for heterogeneous treatment effects in patients with HLA-matched and HLA-mismatched donors (ie, tests for interactions [Table 3]), showed *P* values >.50, except for limited/extensive cGVHD (*P* = .06) and for extensive cGVHD (*P* = .09). This indicates that for the positive effect of ATG-F on aGVHD and cGVHD shown in previous analyses [9,23], no large differences are seen in patients with 10/10-matched donors and in patients with mismatched donors. The beneficial effect of ATG-F on extensive cGVHD is somewhat stronger with HLA-matched (HR = 0.08; 95% CI, 0.03-0.28) than with HLA-mismatched donors (HR = 0.32; 95% CI, 0.12-0.86), but also present with this degree of mismatch (Figure 2B). Also, for limited/extensive cGVHD, the effect is stronger with HLA-matched donors, but tends to result in the same direction for HLA-mismatched donors (Table 3). No effect of ATG-F versus control on relapse risk, NRM, DFS, and OS was seen in HLA-matched as well as in HLA-mismatched transplantations (Table 3).

Patients with a mismatched donor had a slightly lower aGVHD III to IV rate than patients with a 10/10-matched donor (Figure 2A), but as the analysis of the effect of HLA 10/10 mismatch shows (Table 4), this may still be due to chance (HR = 0.39; 95% CI, 0.13-1.16; *P* = .09). With respect to aGVHD II to IV, there is almost no difference between patients with a 10/10 matched and mismatched donor (HR = 0.74; 95% CI, 0.38-1.41; *P* = .36; Table 4). The cGVHD rates are also similar for patients with 10/10 matched and mismatched donors, and no effect of HLA-mismatch on relapse, NRM, DFS, and OS could be shown (Table 4).

Table 4. Effect of HLA-Mismatch (10/10, 4-Digit) (Multiple Regression Analyses, Adjusted for Treatment, Disease Status, and Stem-Cell Source)

	Effect of HLA 10/10 4-Digit Mismatch		
	Mismatch vs No Mismatch		
Outcome	HR	95% CI	*P* Value
aGvHD II-IV	0.74	0.38-1.41	.36
aGvHD III-IV	0.39	0.13-1.16	.09
cGvHD limited/ extensive	1.27	0.68-2.38	.45
cGvHD extensive	1.18	0.58-2.39	.65
Relapse	1.74	0.77-3.94	.19
NRM	1.02	0.47-2.20	.96
DFS	1.30	0.75-2.26	.36
OS	1.30	0.72-2.33	.38

HR indicates hazard ratio; CI, confidence interval; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NRM, nonrelapse mortality; DFS, disease-free survival; OS, overall survival.

DISCUSSION

Within this prospective randomized trial, 3 important prognostic factors independently influencing the outcome after HSCT from unrelated donors could be identified: patient age, donor age, and disease status. Whereas older patient age and advanced disease are known prognostic factors in allogeneic HSCT from matched sibling donors, the influence of older donor age on severe aGVHD and cGVHD, as well as OS, has only be suggested from registry data analysis [24].

Retrospective analysis on 6978 BM transplantations performed within the national marrow donor program revealed donor age significantly related to GVHD and OS with an OS rate of 33% and 25% in recipients who underwent transplantations from donors ages 18 to 30 and >45 years, respectively [24]. A recent registry analysis in 932 recipients of unrelated donor blood-derived grafts showed a beneficial effect of higher CD34 cell counts in the grafts [25]. This retrospective patient cohort differs from our trial among other aspects with regard to sample size, patient age, including children, as well as the inclusion of ablative and nonablative conditioning; in addition, any influence of patient and donor age was not reported [25].

A large, retrospective single-center analysis on risk factors for aGVHD and cGVHD in 2941 patients who

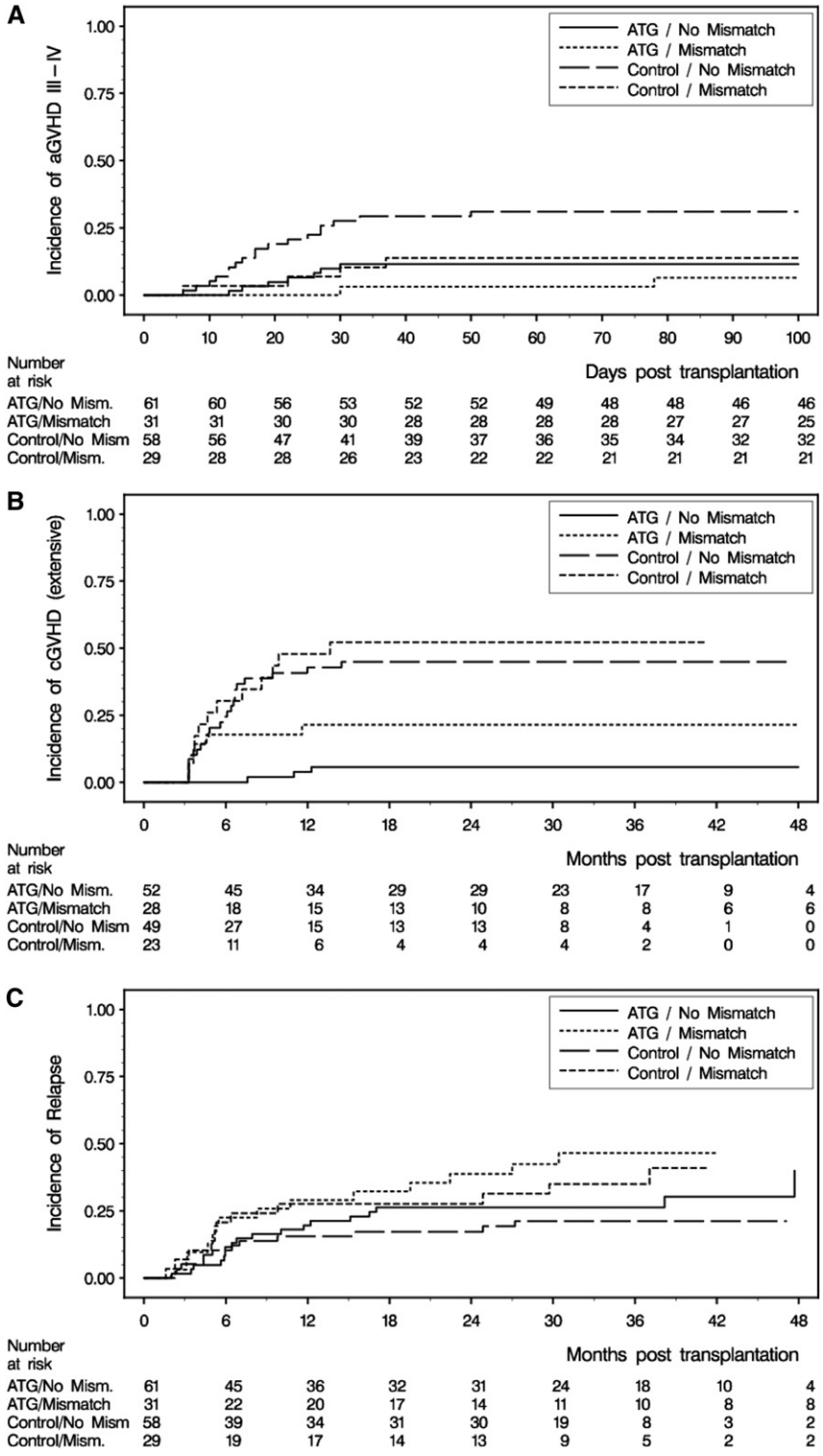

Figure 2. Effect of treatment and HLA-mismatch. (A) Effect on acute graft-versus-host disease (aGVHD) III-IV. (B) Effect on extensive chronic graft-versus-host disease (cGVHD). (C) Effect on relapse.

underwent transplantations from related and unrelated donors in Seattle demonstrated increased risks for aGVHD and, to a lesser extent, for cGVHD by the use of mismatched or matched unrelated donors. Older patient age, female donors to male recipients, and the use of G-CSF-mobilized PB cells were associated with increased risks for cGVHD, whereas the use of TBI was associated with increased risk for aGVHD, suggesting different mechanisms in aGVHD and cGVHD induction, respectively [17].

Because only 20% of the patients in our study received BM transplantations, no extensive analysis

regarding the influence of graft source can be performed. Recently, analysis from the Blood and Marrow Transplant Clinical Trials Network trial 0201, randomly comparing BM with blood-derived grafts in unrelated donor transplantations, showed an increased risk of cGVHD without a negative influence on survival after blood-derived grafts [26]. In this trial, which tested the influence of graft source, the majority of patients received a GVHD prophylaxis with calcineurin inhibitors and methotrexate only. In contrast, our GVHD prophylaxis trial tested the role of additional ATG-F, demonstrating the effective reduction of the risk of cGVHD [9,23]. With different questions addressed in these 2 randomized trials, no direct comparison can be made. However, because 80% of the patients in our GVHD prevention trial received blood-derived grafts, we suggest the additional use of ATG-F to avoid severe aGVHD and cGVHD for this population.

The negative effect of diagnosis of MDS and CML, respectively, in our trial has to be interpreted with caution, because few patients with these diagnoses and all with high risk underwent transplantations.

Several issues have to be kept in mind when interpreting our results and putting these into the general context of unrelated HSCT. In our randomized trial, only adult patients up to the age of 60 with a Karnofsky performance score of at least 60 were included, and they frequently had advanced and active malignant disease. Furthermore, 82% of the patients received G-CSF-primed PB-derived grafts, and all our patients received high-dose myeloablative conditioning. Therefore, our analysis cannot address issues related to immunosuppressive conditioning only, transplantations with reduced intensity conditioning, or severe comorbidities.

Recently, a retrospective study from North America focused on the influence of ATG or alemtuzumab in related and unrelated donor transplantation after reduced intensity conditioning [27]. Because of higher relapse rates, the authors suggested a cautious approach to the general use of these agents. Of note, other types of ATG, different from ATG-F applied in our trial, were used in the American transplantation centers. Furthermore, the low-intensity conditioning in contrast to high-dose myeloablative conditioning in our trial may have contributed to the worse outcome.

When planning the trial in the early 2000s, compatibility with 2-digit typing for class I was generally accepted and the role of HLA-C matching was less clear. When communicating the beneficial effect of additional ATG-F for GVHD prophylaxis on aGVHD and cGVHD [9], the question was raised whether this effect still holds true in 10/10 allele matched transplantations. With our present analysis on 179 patients with complete data on 4-digit typing of 201 study patients, no interactive effects between matching and treatment could be demonstrated. This indicates that for the positive effect of ATG-F on aGVHD and cGVHD shown in previous analyses [9], no large differences are seen in patients with 10/10-matched donors and in patients with mismatched donors.

We did not find a significant influence of 10/10 mismatching on outcome in our trial. The results were similar when we looked at the influence of HLA-C mismatch in otherwise 8/8-matched patient/donor pairs (results not shown in detail). It has to be stressed that this does not mean that allele matching is of minor importance. All patients were required to be matched on the allele 4-digit level in DRB1 and DQB1. In light of new analyses from large patient cohorts, the role of DQB1 can be questioned. The value of HLA-matching has been established from large registries. Our trial with 201 patients is too small to draw definite conclusions regarding the value of specific mismatches. However, even in 10/10 HLA-matched unrelated donor transplantations, the incidence of severe aGVHD and cGVHD was not trivial and the addition of ATG-F can reduce this risk. Mismatches in unrelated donor transplantations are more often accepted in patients with younger age and/or more advanced disease [4]. The use of ATG-F can reduce the risk of GVHD both in completely matched as well as mismatched transplantations, similar to the degree of mismatching that was seen in our trial.

Our data are of relevance for strategies in unrelated donor HSCT. Patient-related and disease-related factors often cannot be influenced: patient age has to be accepted and remission frequently cannot be achieved in high-risk or advanced leukemia; in fact, extensive therapy before allogeneic HSCT is an independent risk factor for OS after HSCT [19]. Novel approaches apply ablative or reduced intensity conditioning in aplasia after antileukemic chemotherapy and result in promising outcomes [28-30].

In contrast, donor and transplantation-related issues can be adapted to the need of the patient (eg, for patients with high-risk malignant disease, a rapid donor search is warranted). A prospective trial in high-risk patients with AML demonstrated the beneficial effect of allogeneic HSCT as compared to no HSCT on OS, with grafts derived from unrelated donors (HR = 0.69; 95% CI, 0.48-0.99; $P = .046$) [31].

By choosing a younger unrelated donor, severe aGVHD and cGVHD, as well as OS after unrelated donor HSCT, can be significantly improved.

ATG-FRESENIUS TRIAL GROUP

Coordinating investigator: J. Finke.

Sponsor: Fresenius Biotech GmbH, Gräfelfing, Germany: H. Schneeberger, G. Börner, H.J. Gamperl, J. Jauch, J. Petan, and A. Kainz.

Clinical Trials Unit, University Medical Center, Freiburg, Germany: C. Schmoor, study statistician; O. Grishina, Ch. Müller, J. Scheele, study physicians; B. Schilling, M. Kaufmann, study data manager; R. Tostmann, A. Kollmer, S. Nuss, and C. Wenig, study coordinators.

Investigators: *Germany* — Freiburg: J. Finke, H. Bertz, and M. Egger. Tübingen: W.A. Bethge, H. Einsele, R.K.F. Müller, and M. Häntschel. Essen: H.D. Ottinger and R. Trenschel. Münster: M. Stelljes. Hamburg: A.R. Zander, N.M. Kröger, F.A. Ayuketang, and C. Wolschke. Wiesbaden: R. Schwerdtfeger, J. Kaltenhäuser, T. Stübig, and R.H. Taube. Mainz: K. Kolbe, S. Thomas, A. Ullmann, and A. Schulze-Bergkamen. Regensburg: E. Holler and J. Hahn. Dresden: M. Bornhäuser. München: H.J. Kolb and R. Reibke. Nürnberg: H. Wandt and K. Schaefer-Eckart; *France* — Paris: G. Socié, E. Gluckman, H. Esperou, and M. Robin. Lyon: M. Michallet and F. Nicolini. Paris: A. Buzyn and M.T. Rubio. Lille: I. Yakoub-Agha, J.P. Jouet, and M.O. Petillon. Nancy: P. Bordigoni, L. Clement, A. Salmon, and P. Feugier; *Finland* — Helsinki: T. Ruutu, L. Volin, E. Juvonen, and A. Nihtinen. Turku: K. Remes, M. Putkonen, M. Itälä, and M. Kauppila; *Czech Republic* — Brno: J. Mayer, M. Krejci, and Y. Brychtová. Plzen: V. Koza and M. Karas. Hradec Kralove: J. Voglova, J. Lebavy, and P. Zak; *Switzerland* — Basel: D. Heim, A. Gratwohl, J. Passweg, C. Arber, and A. Tsakiris; *Austria* — Graz: W. Linkesch, S. Reitter, and P.B. Staber. Linz: D. Lutz and O. Krieger; *Belgium* — Leuven: J.A. Maertens, K. Theunissen, M. Boogaerts, and G. Verhoef; *Israel* — Haifa: J. Rowe and T. Rosenvald-Zuckermann. Jerusalem: M. Shapira, R. Or, and I. Resnik. Tel-Hashomer: A. Nagler, A. Shimoni, and R. Yerushalmi; *Spain* — Barcelona: J. Sierra and D. Valcarcel. Salamanca: J.A. Pérez Simón and M. Astorga; *Italy* — Firenze: A. Bosi and S. Guidi.

ACKNOWLEDGMENTS

Financial disclosure: The study was funded by Fresenius Biotech GmbH, Gräfelfing, Germany. Jürgen Finke has received travel and lecture fees from Fresemius Biotech GmbH; Wolfgang A. Bethge has received consultancy and lecture fees from Fresemius Biotech GmbH; Karin Kolbe and Hartmut Bertz have received travel and lecture fees from Fresemius Biotech GmbH; Matthias Egger has received travel fees from Fresemius Biotech GmbH; the remaining authors declare no conflict of interest.

Authorship Statement: J.F., H.B., and G.S. contributed to design and conception of the study, acquisition of data, and analysis and interpretation of data. C.S. contributed to design and conception of the study, and analysis and interpretation of data; O.G. contributed to analysis and interpretation of data; W.A.B., H.D.O., M.S., A.R.Z., L.V., D.A.H., R.S., K.K., J.M., J.A.M., W.L., E.H., V.K., M.B., and H.E. contributed to acquisition of data, and analysis and interpretation of data. All authors contributed to the writing of the article, and finally approved the manuscrpt.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.06.001

REFERENCES

1. Baldomero H, Gratwohl M, Gratwohl A, et al. The EBMT activity survey 2009: trends over the past 5 years. *Bone Marrow Transplant*. 2011;46:485-501.
2. Sorror ML, Leisenring W, Deeg HJ, Martin PJ, Storb R. Twenty-year follow-up of a controlled trial comparing a combination of methotrexate plus cyclosporine with cyclosporine alone for prophylaxis of graft-versus-host disease in patients administered HLA-identical marrow grafts for leukemia. *Biol Blood Marrow Transplant*. 2005;11:814-815.
3. Arora M, Weisdorf DJ, Spellman SR, et al. HLA-identical sibling compared with 8/8 matched and mismatched unrelated donor bone marrow transplant for chronic phase chronic myeloid leukemia. *J Clin Oncol*. 2009;27:1644-1652.
4. Dehn J, Arora M, Spellman S, et al. Unrelated donor hematopoietic cell transplantation: factors associated with a better HLA match. *Biol Blood Marrow Transplant*. 2008;14:1334-1340.
5. Weisdorf DJ, Nelson G, Lee SJ, et al. Sibling versus unrelated donor allogeneic hematopoietic cell transplantation for chronic myelogenous leukemia: refined HLA matching reveals more graft-versus-host disease but not less relapse. *Biol Blood Marrow Transplant*. 2009;15:1475-1478.
6. Gratwohl A, Brand R, Niederwieser D, et al. Introduction of a quality management system and outcome after hematopoietic stem-cell transplantation. *J Clin Oncol*. 2011;29:1980-1986.
7. Pavletic SZ, Carter SL, Kernan NA, et al. Influence of T-cell depletion on chronic graft-versus-host disease: results of a multicenter randomized trial in unrelated marrow donor transplantation. *Blood*. 2005;106:3308-3313.
8. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98:2942-2947.
9. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10:855-864.
10. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576-4583.
11. Weisdorf D, Spellman S, Haagenson M, et al. Classification of HLA-matching for retrospective analysis of unrelated donor transplantation: revised definitions to predict survival. *Biol Blood Marrow Transplant*. 2008;14:748-758.
12. Petersdorf EW, Malkki M, Gooley TA, Martin PJ, Guo Z. MHC haplotype matching for unrelated hematopoietic cell transplantation. *PLoS Med*. 2007;4:e8.
13. Woolfrey A, Klein JP, Haagenson M, et al. HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:885-892.

14. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood*. 1998;92:3515-3520.
15. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. *Blood*. 2004;104:961-968.
16. Sorror ML, Giralt S, Sandmaier BM, et al. Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: combined FHCRC and MDACC experiences. *Blood*. 2007;110:4606-4613.
17. Flowers ME, Inamoto Y, Carpenter PA, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood*. 2011;117:3214-3219.
18. Eapen M, Logan BR, Confer DL, et al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biol Blood Marrow Transplant*. 2007;13:1461-1468.
19. Craddock C, Labopin M, Pillai S, et al. Factors predicting outcome after unrelated donor stem cell transplantation in primary refractory acute myeloid leukaemia. *Leukemia*. 2011;25:808-813.
20. Craddock C, Nagra S, Peniket A, et al. Factors predicting long-term survival after T-cell depleted reduced intensity allogeneic stem cell transplantation for acute myeloid leukemia. *Haematologica*. 2010;95:989-995.
21. Malard F, Szydlo RM, Brissot E, et al. Impact of cyclosporine-A concentration on the incidence of severe acute graft-versus-host disease after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2010;16:28-34.
22. Simon R, Altman DG. Statistical aspects of prognostic factor studies in oncology. *Br J Cancer*. 1994;69:979-985.
23. Socié G, Schmoor C, Bethge WA, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. *Blood*. 2011;117:6375-6382.
24. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood*. 2001;98:2043-2051.
25. Pulsipher MA, Chitphakdithai P, Logan BR, et al. Donor, recipient, and transplant characteristics as risk factors after unrelated donor PBSC transplantation: beneficial effects of higher CD34+ cell dose. *Blood*. 2009;114:2606-2616.
26. Anasetti C, Logan BR, Lee SJ, et al. Increased incidence of chronic graft-versus-host disease (GVHD) and no survival advantage with Filgrastim-mobilized peripheral blood stem cells (PBSC) compared to bone marrow (BM) transplants from unrelated donors: Results of Blood and Marrow Transplant Clinical Trials Network (BMT CTN) protocol 0201, a phase III, prospective, randomized trial [abstract]. *ASH Annual Meeting Abstracts*. 2011;118:1.
27. 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.
28. Marks R, Potthoff K, Hahn J, et al. Reduced-toxicity conditioning with fludarabine, BCNU, and melphalan in allogeneic hematopoietic cell transplantation: particular activity against advanced hematologic malignancies. *Blood*. 2008;112:415-425.
29. Schmid C, Schleuning M, Schwerdtfeger R, et al. Long-term survival in refractory acute myeloid leukemia after sequential treatment with chemotherapy and reduced-intensity conditioning for allogeneic stem cell transplantation. *Blood*. 2006;108:1092-1099.
30. Platzbecker U, Thiede C, Füssel M, et al. Reduced intensity conditioning allows for up-front allogeneic hematopoietic stem cell transplantation after cytoreductive induction therapy in newly-diagnosed high-risk acute myeloid leukemia. *Leukemia*. 2006;20:707-714.
31. Schlenk RF, Döhner K, Mack S, et al. Prospective evaluation of allogeneic hematopoietic stem-cell transplantation from matched related and matched unrelated donors in younger adults with high-risk acute myeloid leukemia: German-Austrian trial AMLHD98A. *J Clin Oncol*. 2010;28:4642-4648.