ORIGINAL ARTICLE

Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease

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ABSTRACT

BACKGROUND

Chronic graft-versus-host disease (GVHD) is the leading cause of later illness and death after allogeneic hematopoietic stem-cell transplantation. We hypothesized that the inclusion of antihuman T-lymphocyte immune globulin (ATG) in a myeloablative conditioning regimen for patients with acute leukemia would result in a significant reduction in chronic GVHD 2 years after allogeneic peripheral-blood stem-cell transplantation from an HLA-identical sibling.

METHODS

We conducted a prospective, multicenter, open-label, randomized phase 3 study of ATG as part of a conditioning regimen. A total of 168 patients were enrolled at 27 centers. Patients were randomly assigned in a 1:1 ratio to receive ATG or not receive ATG, with stratification according to center and risk of disease.

RESULTS

After a median follow-up of 24 months, the cumulative incidence of chronic GVHD was 32.2% (95% confidence interval [CI], 22.1 to 46.7) in the ATG group and 68.7% (95% CI, 58.4 to 80.7) in the non-ATG group (P<0.001). The rate of 2-year relapse-free survival was similar in the ATG group and the non-ATG group (59.4% [95% CI, 47.8 to 69.2] and 64.6% [95% CI, 50.9 to 75.3], respectively; P=0.21), as was the rate of overall survival (74.1% [95% CI, 62.7 to 82.5] and 77.9% [95% CI, 66.1 to 86.1], respectively; P=0.46). There were no significant between-group differences in the rates of relapse, infectious complications, acute GVHD, or adverse events. The rate of a composite end point of chronic GVHD–free and relapse-free survival at 2 years was significantly higher in the ATG group than in the non-ATG group (36.6% vs. 16.8%, P=0.005).

CONCLUSIONS

The inclusion of ATG resulted in a significantly lower rate of chronic GVHD after allogeneic transplantation than the rate without ATG. The survival rate was similar in the two groups, but the rate of a composite end point of chronic GVHD–free survival and relapse-free survival was higher with ATG. (Funded by the Neovii Biotech and the European Society for Blood and Marrow Transplantation; ClinicalTrials.gov number, NCT00678275.)

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A Quick Take is available at NEJM.org

Chronic graft-versus-host disease (GVHD) is a major complication of allogeneic stem-cell transplantation that results in later illness and death and a reduction in quality of life.[1,2] Risk factors for chronic GVHD are the use of peripheral blood as a source of stem cells, a history of acute GVHD, and the use of donated stem cells with high numbers of T cells.[3-7] In a meta-analysis, the Stem Cell Trialists' Collaborative Group reported an incidence of extensive chronic GVHD of 47% after peripheral-blood stem-cell transplantation from an HLA-identical sibling.[4] In 2012, more than 70% of the stem-cell transplantations performed in Europe used peripheral blood as a source of stem cells.[6] Although the prevention of acute GVHD has improved in the past two decades, no major improvement has been made in the prevention of chronic GVHD, and randomized studies of therapies aimed at reducing the incidence of chronic GVHD have not been able to achieve their goal.[8,9] Only antihuman T-lymphocyte immune globulin (ATG) has been shown to lower the incidence of chronic GVHD after stem-cell transplantation from an unrelated donor[10,11] and, in smaller, retrospective studies, after HLA-identical transplantation.[12,13] Here we report the results of a trial that was designed to study whether including ATG in the myeloablative conditioning regimen would reduce the risk of chronic GVHD among patients in complete remission from acute leukemia who received peripheral-blood stem cells from an HLA-identical sibling.

Methods

Study Design

In this prospective, multicenter, open-label, phase 3 study, patients were randomly assigned to receive ATG or not receive ATG as part of a myeloablative conditioning regimen. The regimen consisted of cyclophosphamide (120 mg per kilogram of body weight) and total body irradiation (12 Gy) or busulfan (16 mg per kilogram orally or 12.8 mg per kilogram intravenously), with or without etoposide (30 to 60 mg per kilogram), as well as ATG at a dose of 10 mg per kilogram for those randomly assigned to the ATG group, on 3, 2, and 1 days before a transplantation of allogeneic peripheral-blood stem cells from an HLA-identical donor. (For more information on patient disposition see Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.) All siblings were matched serologically for HLA-A and HLA-B and by high-resolution DNA matching for HLA-DRB1 and HLA-DQB1 alleles. Patients were eligible for study participation if they were 18 to 65 years of age, had acute myeloid or lymphoblastic leukemia that was in its first or subsequent complete remission, and had an indication for an allogeneic hematopoietic stem-cell transplantation. Other major criteria for inclusion were adequate function of the major organ systems (hepatic, renal [creatinine clearance of more than 30 ml per minute], pulmonary [diffusing capacity of the lung for carbon monoxide of more than 35%], and cardiac [left ejection fraction of at least 30%]) and the use of stem cells from peripheral blood as the graft source. GVHD prophylaxis consisted of the administration of cyclosporine and a short course of methotrexate (15 mg per square meter of body-surface area on day 1 after transplantation and 10 mg per square meter on days 3, 6, and 11 after transplantation).

Patients were randomly assigned in a 1:1 ratio, with stratification according to center and disease risk. The randomization code was generated by nQuery Advisor, version 6.0, with the use of a permuted, block-randomization plan and a block size of 4. Investigators were unaware of the group assignments until the end of the clinical part of the trial. Each participating center received only complete random blocks.

Study End Points

The primary end point of the study was the cumulative incidence of chronic GVHD at 2 years as determined according to the revised Seattle criteria and the National Institutes of Health (NIH) criteria.[14,15] Secondary end points included the incidences of engraftment, acute GVHD, nonrelapse-related death, relapse-free and overall survival at 2 years, and a composite end point of chronic GVHD–free and relapse-free survival at 2 years. Acute GVHD was diagnosed according to Glucksberg's criteria,[16] and chronic GVHD according to the modified Seattle criteria (for categorization of chronic GVHD as clinical limited or clinical extensive)[14] (see Tables S2 and S3, respectively, in the Supplementary Appendix) and according to the NIH criteria for global chronic GVHD scoring (mild, moderate, or severe) and organ involvement (scored as 0, 1, 2 or 3,

with higher scores indicating greater involvement).[15] Toxicity in both groups was graded according to the Bearman score.[17] The study started recruitment in October 2006. At the time, there was confusion among European investigators regarding the need to register the trial before initiation of enrollment; the trial was registered in May 2008.

STUDY OVERSIGHT

The study was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. An independent ethics committee at each study site approved the protocol (available at NEJM.org). All patients provided written informed consent. The study was sponsored by the University Medical Center Hamburg–Eppendorf and was conducted in collaboration with the Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation. The first, second, and last authors designed the study, and Pharmalog (Munich) gathered the data with the use of an electronic case-report form. The third from last author analyzed the data, and that author and the first author vouch for the data and analysis and adherence to the study protocol. The first author wrote the first draft, and all the coauthors made the decision to submit the manuscript for publication. No one who is not an author contributed to the writing of the manuscript. ATG (ATG-Fresenius) was supplied by Neovii Biotech, which also provided additional financial support but did not contribute to the analysis or approve the manuscript for publication.

STATISTICAL ANALYSIS

We used cumulative incidence analysis to assess chronic GVHD–free survival and relapse-free survival and labeled death before the event of interest as a competing risk.[18,19] Death from relapse was used as a competing risk for the cumulative incidence analysis of death without relapse. Overall survival was assessed with the use of Kaplan–Meier analysis. Further treatment-group comparisons were performed with the use of chi-square tests for nominal data, Mann–Whitney U-tests for ordinal data, and analyses of variance for interval or ratio-scale data. Exploratory, post hoc, Cox multiple regression analysis was used for subgroup analyses and for the purpose of determining the effect on the incidence of chronic GVHD and on overall survival of recipient age, first versus second remission, acute lymphoblastic versus acute myeloid leukemia, cytogenetic risk, risk of underlying disease, difference between the sex of the donor and the sex of the recipient, cytomegalovirus positivity in the recipient, type of conditioning, CD34+ cells transplanted, grade of acute GVHD and chronic GVHD (time dependent [i.e., the corresponding event can occur at different points in time after transplantation]) in addition to treatment assignment (ATG vs. non-ATG). Confidence intervals for the differences in risk according to treatment group were determined with the use of Wilson's method.[20]

The sample size was calculated on the basis of expected 2-year rates of chronic GVHD of 25% in the ATG group and 55% in the non-ATG group. We estimated that a sample size of 160 patients (80 patients in each group) would give the study at least 90% power to reject the null hypothesis in a log-rank test model. All patients who received at least one dose of the conditioning regimen were evaluated for efficacy and safety. Confirmatory testing of the prespecified primary outcome measure, 2-year cumulative incidence of chronic GVHD, was performed in the full analysis set at a type I error level of 0.5 (two-sided). All other P values are considered descriptively (including those that are not significant).

Cumulative incidence analyses were performed with the use of NCSS statistical software, version 9, and R statistical software, version 2.10.1 (cmprsk package).[21,22] IBM SPSS Statistics software, version 22.0, was used for all other analyses.

RESULTS

PATIENT CHARACTERISTICS

From December 2006 through February 2012, a total of 168 patients were enrolled at 27 centers; 7 patients were excluded before randomization because they did not meet the inclusion criteria. Of the 161 patients who underwent randomization, 6 were excluded from the analysis because the donor withdrew (2 patients) or progressive disease developed (4 patients). The baseline characteristics of the remaining 155 patients are shown in Table 1. The two groups were well balanced with respect to the Disease Risk Index for

Table 1. Baseline Characteristics of the Participants.*

Characteristic	ATG Group (N=83)	Non-ATG Group (N=72)	P Value
Age — yr			0.04
Median	39.0	43.5	
Range	18.0–64.0	21.0–61.0	
Male sex — no. (%)	53 (63.9)	40 (55.6)	0.29
Time between diagnosis and stem-cell transplantation — mo			0.87
Median	5.2	5.3	
Interquartile range	5.2 (3.9–7.1)	5.3 (4.3–6.7)	
Diagnosis — no. (%)			
Acute myeloid leukemia	55 (66.3)	55 (76.4)	0.17
Acute lymphoid leukemia	28 (33.8)	17 (23.6)	
High cytogenetic risk — no. (%)	31 (37.3)	22 (30.6)	0.19
Disease Risk Index — no./total no. (%)†			0.42
Intermediate	60/79(75.9)	55/67 (82.1)	
High	19/79 (24.1)	12/67 (17.9)	
No. of courses of chemotherapy before transplantation			0.89
Median	2	2	
Range	1–10	1–8	
Disease status at transplantation — no. (%)			0.45
First complete remission	73 (88.0)	66 (91.7)	
Second complete remission	10 (12.0)	6 (8.3)	
Conditioning regimen — no. (%)			0.90
Total-body irradiation plus cyclophosphamide	23 (27.7)	18 (25.0)	
Busulfan plus cyclophosphamide	56 (67.5)	51 (70.8)	
Total-body irradiation plus etoposide	4 (4.8)	3 (4.2)	
Donor's age — yr			0.71
Median	39.1	42.7	
Range	12.2–64.7	18.7–63.8	
Female donor–male recipient pairs — no. (%)	20 (24.1)	16 (22.2)	0.75
Cytomegalovirus IgG-positive recipients — no. (%)	50 (60.2)	42 (58.3)	0.90
Infused CD34+ cells×10^{-6}/kg			0.41
Median	5.4	5.0	
Range	2.0–11.5	2.3–11	

* A total of 168 patients were enrolled in the study; 7 were excluded before randomization and 6 were withdrawn from the analysis since they did not receive the conditioning regimen or undergo stem-cell transplantation. ATG denotes antihuman T-lymphocyte immune globulin.

† The Disease Risk Index includes an assessment of disease type and disease status at the time of transplantation.

patients undergoing allogeneic stem-cell transplantation (see Table S1 in the Supplementary Appendix),[23] which is determined according to disease status and cytogenetic risk; the number of infused CD34+ cells; and other variables. The only baseline characteristic that differed between the groups was the median age, which was higher in the non-ATG group than the ATG group (43.5 years vs. 39.0 years, P=0.04). The median dose of cyclosporine at the time of transplantation and at day 100 was 3 mg per kilogram and did not differ significantly between groups (P=0.63).

GRAFT FAILURE AND ENGRAFTMENT

No graft failure occurred in the ATG group, and one failure occurred in the non-ATG group (1.4% of the patients). The median times to leukocyte engraftment (absolute neutrophil count, $\geq 0.5\times10^9$ per liter) and platelet engraftment (platelet count, $\geq 20\times10^9$ per liter) were significantly longer in the ATG group than in the non-ATG group: 18 days (range, 10 to 31) versus 15 days (range, 11 to 34) (P<0.001) and 20 days (range, 10 to 110) versus 13 days (range, 6 to 29), respectively (P<0.001) (Table 2).

ACUTE AND CHRONIC GVHD

The rate of acute GVHD of grade 2, 3, or 4 was 10.8% in the ATG group and 18.1% in the non-ATG group (P=0.13), and the rate of acute GVHD of grade 3 or 4 was 2.4% in the ATG group and 8.3% in the non-ATG group (P=0.10) Table 2). The median time to the onset of chronic GVHD was 164 days after transplantation in both groups. The cumulative incidence of chronic GVHD at 2 years was 32.2% in the ATG group (95% confidence interval [CI], 22.1 to 46.7) and 68.7% in the non-ATG group (95% CI, 58.4 to 80.7) (P<0.001) (Fig. 1). The cumulative incidence of minimal or modest chronic GVHD at 2 years, classified as clinical limited chronic GVHD according to the revised Seattle criteria (see Table S3 in the Supplementary Appendix), was 28.1% (95% CI, 18.3 to 43.2) in the ATG group and 53.3% (95% CI, 40.6 to 70.1) in the non-ATG group (P=0.005; see Fig. S2 in the Supplementary Appendix). The corresponding cumulative incidence of clinical extensive chronic GVHD was 7.6% (95% CI, 3.0 to 19.6) and 52.4% (95% CI, 39.3 to 69.9) (P<0.001) (Fig. 2A).

The NIH scores for global and organ-specific chronic GVHD were significantly lower in the ATG group than in the non-ATG group (P<0.001); chronic GVHD was classified as mild in 15.6% of the patients in the ATG group versus 16.7% in the non-ATG group, moderate in 8.4% versus 25.0%, and severe in 2.4% versus 22.2% (Table 2). According to NIH scores, in all organs except genital organs, there was less chronic GVHD involvement of grade 2 or higher in the ATG group than in the non-ATG group, with significantly less involvement of the skin, the mouth, and the eyes (Table 3). A subgroup analysis of chronic GVHD shows the effect of ATG in subgroups of the full analysis cohort (Fig. S3 in the Supplementary Appendix).

In multivariate analyses, the use of ATG did not result in a significantly greater rate of relapse than the rate without ATG (hazard ratio, 1.03; 95% CI, 0.57 to 1.88; P=0.91), but the nonsignificant difference in relapse does not justify the conclusion of equivalence. The factors that were associated with the development of chronic GVHD included the presence of previous acute GVHD (hazard ratio, 1.62; 95% CI, 1.29 to 2.05; P<0.001), the combination of female donor and male recipient (hazard ratio, 2.01; 95% CI, 1.11 to 3.65; P=0.02), and the use of ATG (hazard ratio, 0.37; 95% CI, 0.21 to 0.65; P=0.001) (Table S1 in the Supplementary Appendix).

At 1 year, 91% of the patients in the ATG group who could be evaluated had discontinued cyclosporine, but only 39% in the non-ATG group had done so. Complete resolution of GVHD or the absence of chronic GVHD was reported for 83% of patients in the ATG group versus 63% of patients in the non-ATG group at 6 months, 77% versus 44% at 12 months, and 75% versus 40% at 24 months. Ongoing chronic GVHD was noted in 17% of the patients in the ATG group and in 36% of those in the non-ATG group at 6 months, in 23% and 36%, respectively, at 12 months, and in 25% and 60%, respectively, at 24 months.

TOXICITY, NONRELAPSE-RELATED MORTALITY, AND RELAPSE

Infectious complications were observed in 57.8% of patients in the ATG group and in 54.2% of patients in the non-ATG group (P=0.65). No post-transplantation lymphoproliferative disorder was observed during follow-up. The rate of cytomegalovirus reactivation was not significantly higher in the ATG group than in the non-ATG group (21.7% and 25.0%, respectively), nor was the rate of Epstein–Barr virus reactivation (3.6% and 1.4%, respectively) or fungal infection (3.6% and 4.2%, respectively) (Table 2). The rate of nonrelapse-related death at 2 years was 14.0% (95% CI, 8.0 to 24.1) in the ATG group and 12.0% (95% CI, 6.1 to 22.4) in the non-ATG group (P=0.60) (Fig. 2B). Toxic effects to organs as measured by means of Bearman's score did not differ significantly between groups with the exception of gastrointestinal toxic effects, for which the rate was significantly lower in the ATG group than in the non-ATG group (28.9%

Table 2. Rates of Engraftment, Infection, Acute and Chronic GVHD, and Other Complications after Stem-Cell Transplantation.

Variable	ATG Group (N=83)	Non-ATG Group (N=72)	P Value*
Graft failure — no. (%)	0	1 (1.4)	
Days to engraftment — median (range)			
Absolute neutrophil count ≥0.5×10^9/liter	18 (10–31)	15 (11–34)	<0.001
Platelet count ≥20×10^9/liter	20 (10–110)	13 (6–29)	<0.001
Infectious complication — no. (%)	48 (57.8)	39 (54.2)	0.65
Cytomegalovirus reactivation — no. (%)	18 (21.7)	18 (25.0)	0.63
Epstein–Barr virus reactivation — no. (%)	3 (3.6)	1 (1.4)	0.38
Post-transplantation lymphoproliferative disorder — no. (%)	0	0	
Pulmonary infection — no. (%)	6 (7.2)	10 (13.9)	0.18
Fungal infection — no. (%)	3 (3.6)	3 (4.2)	0.86
Acute GVHD within 100 days after transplantation — no. (%)	21 (25.3)	25 (34.7)	0.20
Overall grades of acute GVHD — no. (%)			0.15
0	62 (74.7)	46 (65.2)	
1	12 (14.5)	12 (16.7)	
2	7 (8.4)	7 (9.7)	
3	2 (2.4)	4 (5.6)	
4	0	2 (2.8)	
2–4	9 (10.8)	13 (18.1)	0.13
3 or 4	2 (2.4)	6 (8.3)	0.10
Chronic GVHD	22 (26.5)	46 (63.9)	<0.001
Day of onset			0.80
Median	163.5	164.0	
Interquartile range	91–246	101–220	
De novo — no./total no. (%)†	10/22 (45.5)	23/46 (50.0)	
Quiescent — no./total no. (%)†	8/22 (36.4)	16/46 (34.8)	0.93
Progressive — no./total no. (%)†	4/22 (18.2)	7/46 (15.2)	
Severity according to revised Seattle criteria — no. (%)‡			<0.001
Limited	17 (20.5)	22 (30.6)	
Extensive	5 (6.0)	24 (33.3)	
Severity according to NIH criteria — no. (%)§			<0.001
Mild	13 (15.7)	12 (16.7)	
Moderate	7 (8.4)	18 (25.0)	
Severe	2 (2.4)	16 (22.2)	

* Where no value is shown, the number of events was too low to calculate a P value.
† According to National Health Institutes of Health (NIH) criteria, de novo indicates the onset of chronic GVHD without a history of acute GVHD, quiescent the onset of chronic GVHD after the resolution of acute GVHD, and progressive the onset of chronic GVHD without resolution of acute GVHD.
‡ Chronic, limited, and extensive GVHD are defined according to the Seattle criteria (see Table S3 in the Supplementary Appendix).
§ Chronic, mild, moderate, and severe GVHD are defined according the NHI criteria (see Table S8 in the Supplementary Appendix).

vs. 52.8%, P=0.03) (Tables S5 and S6 in the Supplementary Appendix). No significant between-

group differences in severe or other adverse events related to study drug were found (Table S7 in the Supplementary Appendix). The cumulative incidence of relapse at 2 years was 32.2% (95% CI, 23.4 to 44.2) in the ATG group and 25.5% (95% CI, 16.3 to 44.2) in the non-ATG group (P=0.17) (Fig. 2C).

SURVIVAL

The 2-year rate of relapse-free survival was 59.4% (95% CI, 47.8 to 69.2) in the ATG group and 64.6% (95% CI, 50.9 to 75.3) in the non-ATG group (P=0.21), and the 2-year rate of overall survival was 74.1% (95% CI, 62.7 to 82.5) and 77.9% (95% CI, 66.1 to 86.1), respectively (P=0.46) (Fig. 2D and 2E). In a multivariate analysis, only the diagnosis of acute lymphoblastic leukemia versus acute myeloid leukemia influenced survival (hazard ratio, 2.64; 95% CI, 1.18 to 5.89; P=0.02), whereas the hazard ratio for survival with ATG versus non-ATG was 0.74 (95% CI, 0.34 to 1.65; P=0.47) (Table S4 in the Supplementary Appendix). The rate of the composite end point of 2-year survival free from chronic GVHD and free from relapse was 36.6% (95% CI, 25.2 to 48.0) in the ATG group and 16.8% (95% CI, 9.2 to 26.4) in the non-ATG group (P=0.005) (Fig. 2F).

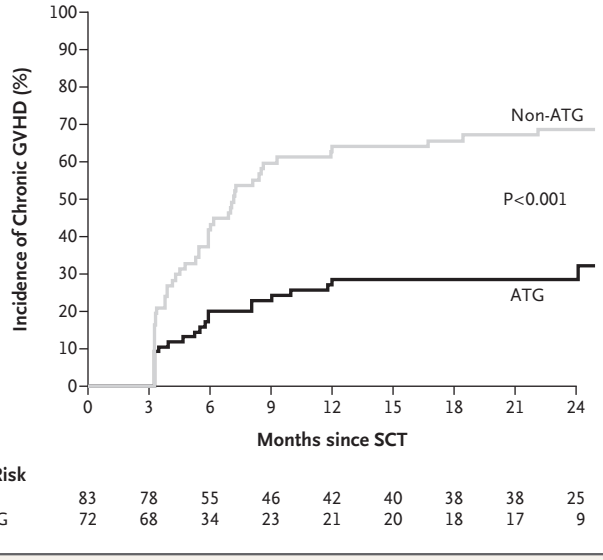


Figure 1. Cumulative Incidence of Chronic GVHD, According to Treatment Group.

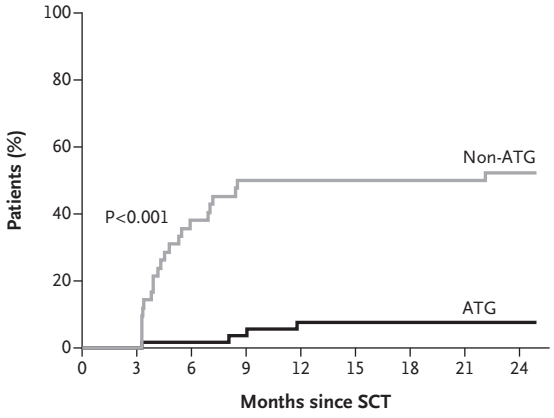
ATG denotes antihuman T-lymphocyte immune globulin, GVHD graft-versus-host disease, and SCT stem-cell transplantation.

DISCUSSION

Chronic GVHD is the leading cause of late illness and death after allogeneic hematopoietic stem-cell transplantation.[1,2] One of the risk factors for the development of chronic GVHD after allogeneic stem-cell transplantation is the use of peripheral-blood stem cells as a graft source,[4] a factor that may have contributed to the increased incidence of chronic GVHD recently observed by Arai et al.,[3] since T-cell levels in grafts are higher than those in the marrow. In our study, the incidence of chronic GVHD at 2 years was 36.5 percentage points lower when ATG was added to the conditioning regimen than when it was not added; the rate of relapse was not significantly higher with ATG, but the nonsignificant difference in relapse rates does not justify the conclusion of equivalence.

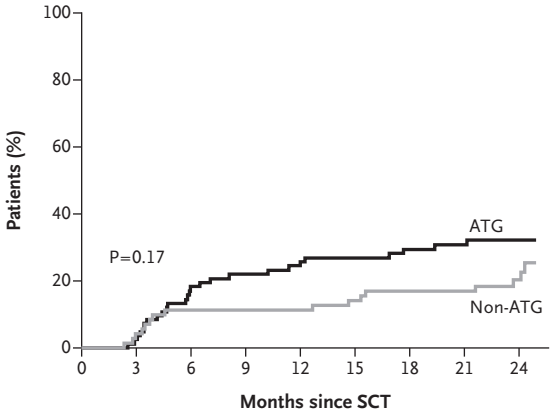
More important, the between-group difference in the rate of chronic GVHD was seen mainly in patients with the clinical extensive form of chronic GVHD, with a rate that was 44.8 percentage points lower in the ATG group than in the non-ATG group (7.6% vs. 52.4%), and a lower rate of chronic GVHD involvement was observed in nearly all patients. Within 1 year after transplantation, 91% of patients in the ATG group but only 39% of those in the non-ATG group had discontinued immunosuppressive medication with cyclosporine. The major source of concern associated with any T-cell depletion is the higher risk of relapse that results from the loss of graft-versus-leukemia effects, a result that has been observed in several studies in which in vivo and ex vivo T-cell–depleted grafts were used.[24] After a follow-up of 2 years, we did not observe a significantly higher rate of relapse in the ATG group than in the non-ATG group, with the result that the rates of relapse-free and overall survival were similar in the two groups. Even if a modest increase in the rate of relapse in the ATG group cannot be ruled out, the significantly lower incidence of chronic GVHD with ATG resulted in a significantly higher rate of 2-year survival free from chronic GVHD among patients who received ATG than

A Incidence of Clinical Extensive Chronic GVHD



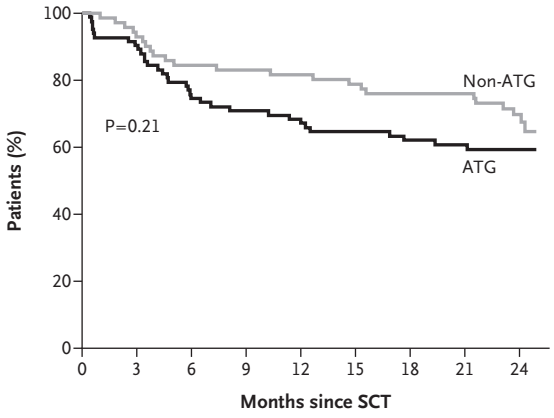
No. at Risk									
ATG	63	58	49	43	41	39	37	37	24
Non-ATG	47	43	23	18	18	18	17	16	9

B Relapse



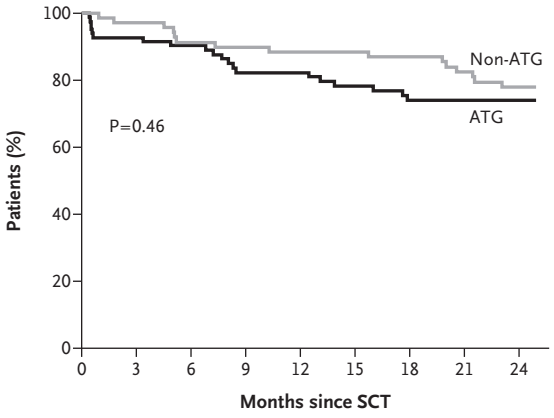
No. at Risk									
ATG	83	78	61	58	55	52	49	47	33
Non-ATG	72	67	61	60	58	56	54	54	35

C Relapse-free Survival



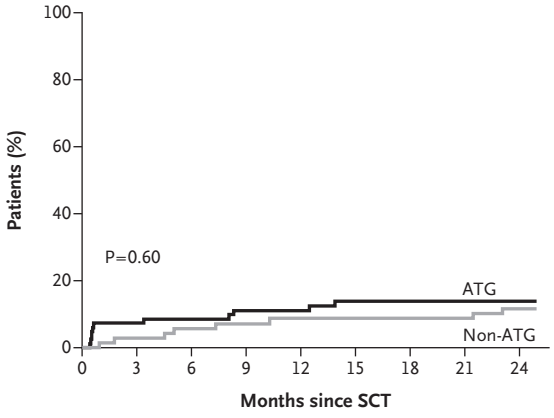
No. at Risk									
ATG	83	76	61	58	55	52	49	47	33
Non-ATG	72	67	61	60	58	56	54	54	35

D Overall Survival



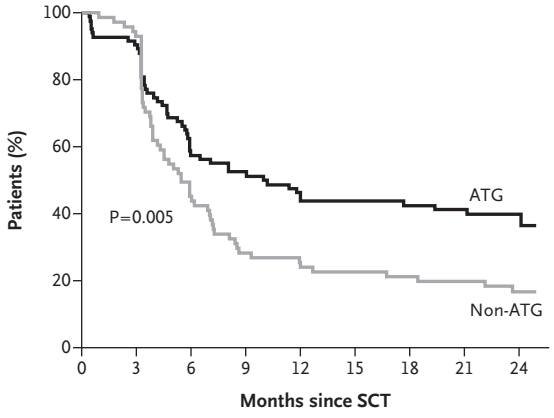
No. at Risk									
ATG	83	78	70	63	62	58	54	53	36
Non-ATG	72	68	64	63	61	60	59	56	35

E Nonrelapse-Related Death



No. at Risk									
ATG	83	78	70	63	62	58	54	53	36
Non-ATG	72	68	64	63	61	60	59	56	36

F Chronic GVHD–free+Relapse-free Survival



No. at Risk									
ATG	83	76	47	42	37	35	34	34	22
Non-ATG	72	67	32	21	19	17	16	15	8

Table 3. Maximum Organ Involvement According to NIH Criteria.*

Organ	ATG Group (N=83)	Non-ATG Group (N=72)	Difference in Risk (95% CI)
	number (percent)		*percentage points*
Skin	0	14 (19.4)	−19.4 (−30 to −10.7)
Oral mucosa	1 (1.2)	7 (9.7)	−8.5 (−17.6 to −1.3)
Eyes	0	12 (16.7)	−16.7 (−26.9 to −8.5)
Liver	5 (6.0)	11 (15.3)	−9.3 (−19.9 to 0.5)
Gastrointestinal tract	1 (1.2)	2 (2.8)	−1.8 (−8.4 to 4.1)
Lungs	1 (1.2)	4 (5.6)	−4.4 (−12.3 to 1.9)
Genitals	1 (1.2)	0	1.2 (−4.0 to 6.5)
Joints and fascia	0	3 (4.2)	−4.2 (−11.5 to 1.0)

* According to the National Institutes of Health (NIH) criteria for organ involvement, which rates the extent and severity of chronic GVHD for each organ or site and takes organ function into account, the maximum involvement of the organs listed was ≥2 on a scale of 0 to 3, with 0 indicating no disease, 1 mild disease, 2 moderate disease, and 3 severe disease.

among those who did not receive ATG (50% vs. 23%). Because the patients in the ATG group were aware of group assignment, the possibility of a certain bias in the grading of chronic GVHD cannot be excluded. The most frequent cause of treatment failure after transplantation is relapse, which in patients with acute leukemia occurs predominantly in the first or second year after transplantation.[25] In the current study, the rate of the composite end point of 2-year survival free from chronic GVHD and free from relapse was significantly higher among those who received ATG than among those who did not (37% vs. 17%).

Chronic GVHD is the second leading cause of death among patients who survive for 2 years after transplantation; extending the follow-up period for these patients would confirm its effect on long-term survival.[1] Unfortunately, our study was terminated at 2 years, and longer-term follow-up data for the patients who participated in this study are not available. We did not observe a higher rate of infectious complications after ATG, as has been reported after the implementation of other T-cell depletion techniques.[10,11,26]

Although some progress has been made in preventing acute GVHD, most attempts to reduce the risk of chronic GVHD have been unsuccessful.[27-29] A reduction in the rate of severe acute GVHD and in the rate of chronic GVHD has been observed with ATG only in the case of matched or mismatched, unrelated allogeneic hematopoietic stem-cell transplantation.[10,11] However, neither of these studies used chronic GVHD as the primary end point.

More recently, post-transplantation cyclophosphamide has been shown in a prospective study to reduce the risk of both acute and chronic GVHD, even after haploidentical stem-cell transplantation, but data from prospective randomized studies are lacking.[30] Other T-cell–depleting strategies (e.g., treatment with alemtuzumab, an anti-CD52 monoclonal antibody, or ex vivo CD34 selection) have been pursued, and low rates of GVHD have been reported. Although such T-cell–depleting strategies resulted in a higher risk of relapse among patients with chronic myeloid leukemia, this increased risk does not seem to apply to patients with acute leukemia[31,32]; however, concern about an increased risk of relapse associated with the use of T-cell depletion remains.

The ATG preparation used in our study is a polyclonal antihuman T-lymphocyte immune globulin derived from rabbits after immunization with the Jurkat human T-cell line. This highly purified immune globulin consists of antibodies exhibiting a direct effect on T cells through opsonization and lysis after complement activation. Because antigens such as CD19 or CD138 are also targeted by ATG, antitumor effects have been observed in B-cell cancers and to a lesser

Figure 2 (facing page). Cumulative Incidence of Extensive Chronic GVHD, Relapse, Relapse-free and Overall Survival, Nonrelapse-Related Death, and Relapse, and the Combined Incidence of Chronic GVHD–free and Relapse-free Survival According to Treatment Group.

extent in myeloid cancers.[33] Taking into account the effect of donor T cells and B cells on the development of chronic GVHD,[34-36] the effect of ATG on antigen-presenting cells such as B cells and dendritic cells[37] and the induction of regulatory T cells[38] may have contributed to the significant reduction in chronic GVHD. Other ATGs derived from rabbits or horses have also been used, but because of their different immunologic properties and the lack of reliable comparative studies, the different brands and doses are not interchangeable and the results may not be generalizable to other ATGs.

In summary, our study shows that incorporation of ATG in the myeloablative conditioning regimen before transplantation of peripheral-blood stem cells from HLA-identical siblings resulted in a significantly lower rate of chronic GVHD than the rate without ATG. The rates of relapse-free and overall survival were similar in the two groups, but the rate of a composite end point of survival free from chronic GVHD and survival free from relapse was significantly higher with ATG.

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APPENDIX

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REFERENCES

1. Wingard JR, Majhail NS, Brazauskas R, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. J Clin Oncol 2011;29:2230-9.
2. Martin PJ, Counts GW Jr, Appelbaum FR, et al. Life expectancy in patients surviving more than 5 years after hematopoietic cell transplantation. J Clin Oncol 2010;28:1011-6.
3. Arai S, Arora M, Wang T, et al. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the Center for International Blood and Marrow Transplant Research. Biol Blood Marrow Transplant 2015;21:266-74.
4. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol 2005;23:5074-87.
5. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med 2012;367:1487-96.
6. Passweg JR, Baldomero H, Peters C, et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. Bone Marrow Transplant 2014;49:744-50.
7. Arora M, Klein JP, Weisdorf DJ, et al. Chronic GVHD risk score: a Center for International Blood and Marrow Transplant Research analysis. Blood 2011;117:6714-20.
8. Deeg HJ, Lin D, Leisenring W, et al. Cyclosporine or cyclosporine plus methylprednisolone for prophylaxis of graft-versus-host disease: a prospective, randomized trial. Blood 1997;89:3880-7.
9. Storb R, Deeg HJ, Pepe M, et al. Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukemia: long-term follow-up of a controlled trial. Blood 1989;73:1729-34.
10. 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;10:855-64.
11. 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;98:2942-7.
12. Wolschke C, Zabelina T, Ayuk F, et al. Effective prevention of GVHD using in vivo T-cell depletion with anti-lymphocyte globulin in HLA-identical or -mismatched sibling peripheral blood stem cell transplantation. Bone Marrow Transplant 2014;49:126-30.
13. Bonifazi F, Bandini G, Arpinati M, et al. Intensification of GVHD prophylaxis with low-dose ATG-F before allogeneic PBSC transplantation from HLA-identical siblings in adult patients with hematological

malignancies: results from a retrospective analysis. Bone Marrow Transplant 2012; 47:1105-11.
14. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant 2003;9:215-33.
15. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945-56.
16. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.
17. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 1988;6:1562-8.
18. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94: 496-509.
19. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1988;16:1141-54.
20. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med 1998;17:873-90.
21. Gray RJ. cmprsk: subdistribution analysis of competing risks. 2011 (http://cran.r-project.org/web/packages/cmprsk/index.html).
22. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transplant 2007;40:381-7.
23. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood 2014;123:3664-71.
24. 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;117:6963-70.
25. Pavletic SZ, Kumar S, Mohty M, et al. NCI first international workshop on the biology, prevention, and treatment of relapse after allogeneic hematopoietic stem cell transplantation: report from the Committee on the Epidemiology and Natural History of Relapse following Allogeneic Cell Transplantation. Biol Blood Marrow Transplant 2010;16:871-90.
26. Storek J, Witherspoon RP, Webb D, Storb R. Lack of B cells precursors in marrow transplant recipients with chronic graft-versus-host disease. Am J Hematol 1996;52:82-9.
27. Bacigalupo A, Van Lint MT, Occhini D, et al. Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. Blood 1991;77:1423-8.
28. Kansu E, Gooley T, Flowers ME, et al. Administration of cyclosporine for 24 months compared with 6 months for prevention of chronic graft versus host disease: a prospective clinical randomized trial. Blood 2001;98:3868-70.
29. Sullivan KM, Storek J, Kopecky KJ, et al. A controlled trial of long-term administration of intravenous immunoglobulin to prevent late infection and chronic graft-vs.-host disease after marrow transplantation: clinical outcome and effect on subsequent immune recovery. Biol Blood Marrow Transplant 1996;2:44-53.
30. Kanakry CG, Tsai HL, Bolaños-Meade J, et al. Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. Blood 2014;124:3817-27.
31. Pasquini MC, Devine S, Mendizabal A, et al. Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. J Clin Oncol 2012;30:3194-201.
32. Chakraverty R, Orti G, Roughton M, et al. Impact of in vivo alemtuzumab dose before reduced intensity conditioning and HLA-identical sibling stem cell transplantation: pharmacokinetics, GVHD, and immune reconstitution. Blood 2010;116: 3080-8.
33. Ayuk FA, Fang L, Fehse B, Zander AR, Kröger N. Antithymocyte globulin induces complement-dependent cell lysis and caspase-dependent apoptosis in myeloma cells. Exp Hematol 2005;33:1531-6.
34. Brüggen MC, Klein I, Greinix H, et al. Diverse T-cell responses characterize the different manifestations of cutaneous graft-versus-host disease. Blood 2014;123:290-9.
35. Sarantopoulos S, Stevenson KE, Kim HT, et al. High levels of B-cell activating factor in patients with active chronic graft-versus-host disease. Clin Cancer Res 2007;13:6107-14.
36. Zorn E, Kim HT, Lee SJ, et al. Reduced frequency of FOXP3+ CD4+CD25+ regulatory T cells in patients with chronic graft-versus-host disease. Blood 2005;106:2903-11.
37. Fang L, Fehse B, Engel M, Zander AR, Kröger N. Antithymocyte globulin induces ex vivo and in vivo depletion of myeloid and plasmacytoid dendritic cells. Transplantation 2005;79:369-71.
38. Shimony O, Nagler A, Gellman YN, et al. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. J Clin Immunol 2012;32:173-88.