

Antithymocyte globulin for the prevention of graft-versus-host disease after unrelated hematopoietic stem cell transplantation for acute myeloid leukemia: results from the multicenter German cooperative study group

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Summary:

A total of 155 patients with acute myeloid leukemia (AML) received hematopoietic stem cell transplants from unrelated donors after standard conditioning. Clinical outcome after the use of two different antithymocyte globulins for the prevention of graft-versus-host disease (GvHD) was analyzed in a retrospective study as follows: rabbit ATG (Thymoglobulin Sangstat/Genzyme, $n = 49$, median age 42 years, 53% in CR, further ATG-S); rabbit ATG (ATG-Fresenius, $n = 38$, median age 42 years, 58% in CR, further ATG-F) or no ATG ($n = 68$, median age 36 years, 55% in CR). The groups were comparable regarding disease status at transplant, age, CMV status and cytogenetics. Grade III–IV acute GvHD was found in 15% in the ATG and 27% in the no ATG group ($P = 0.44$). The most important independent risk factors for chronic GvHD (cGvHD) were the use of ATG, disease status at transplant and conditioning. cGvHD developed significantly more frequently in no ATG group. With the median follow-up of 34 months, the 5-year survival is 42% for those transplanted in CR. To conclude, these data demonstrate that the transplants performed in CR, with ATG, are associated with a good outcome, low incidence of cGvHD and no increase of relapse.

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Transplantation of HSC from unrelated donors is increasingly used for the treatment of acute myeloid leukemia (UD-

AML).^{1–3} Acute myeloid leukemia (AML) patients lacking a suitable sibling donor have a probability of approximately 80% to find an HLA-compatible unrelated donor. ATG has been initially incorporated into preparative regimens for UD transplants to ensure engraftment. Several studies, however, suggest also a beneficial effect of ATG in preventing acute graft-versus-host disease (aGvHD).^{4–7} The only randomized study, examining the use of ATG for the prophylaxis of GvHD published 3 years ago, suggests that ATG significantly reduces the risk for severe acute GvHD but increases the risk for infections.⁸ In addition, survival was similar even though extensive chronic GvHD (cGvHD) was significantly reduced in patients receiving ATG. The major drawbacks of all these studies is that they were not performed for a specific entity of disease. Since the biology of the underlying disease plays a major role with respect to the susceptibility to a graft versus leukemia (GvL) effect, the use of ATG has to be examined separately for specific diseases.

Here, we report the results of a retrospective study analyzing the effect of two different rabbit ATGs, incorporated into the preparative regimens for UD-transplants in AML patients compared to AML patients receiving no ATG. The purpose of the study was to compare the incidence and severity of acute and chronic GvHD as well as treatment-related mortality and leukemia-free survival (LFS).

Patients and methods

Patients

Transplants were performed between September 1994 and October 2002. Clinical characteristics of the patients are given in Table 1. The interval from the time of diagnosis to treatment was similar in the three groups. More patients had *de novo* AML in the ATG-F as compared to the ATG-S and the no ATG group ($P = 0.03$). Half of the patients were in CR at transplant (53% in ATG-S, 58% in ATG-F, 50% in no ATG). The karyotype was known for 131 of the 155 patients. Cytogenetic abnormalities were grouped according to published criteria adopted by SWOG.^{9–13} Nine patients (7%) had favorable karyotype. Chromosomal abnormalities classified as a good prognostic factor

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Table 1 Patient characteristics

	No ATG	ATG-S	ATG-F	<i>P</i> ^a
No. of patients	68	49	38	
Age (median, range)	36 (16–55)	42 (18–60)	42 (21–54)	0.14
<i>Gender of patient</i>				
Male	33 (49%)	29 (59%)	19 (50%)	0.50
Female	35 (51%)	20 (41%)	19 (50%)	
<i>Disease</i>				
AML de novo	40 (59%)	28 (57%)	31 (82%)	0.03
MDS RAEB-t, secondary AML	26 (38%)	19 (39%)	4 (10%)	
Unspecified AML/biphen. AL	2 (3%)	2 (4%)	3 (8%)	
<i>Cytogenetics</i>				
Favorable	4 (6%)	1 (2%)	4 (10%)	0.41
Intermediate	34 (50%)	31 (63%)	22 (58%)	
Unfavorable	10 (15%)	13 (27%)	12 (32%)	
Unknown	20 (29%)	4 (8%)	—	
<i>Status at transplant</i>				
CR	34 (50%)	26 (53%)	22 (58%)	0.74
Relapse	26 (38%)	9 (19%)	11 (29%)	
Primary induction failure	5 (7.5%)	11 (22%)	4 (10)	
Untreated/unspecified (MDS)	3 (4.5%)	3 (6%)	1 (3%)	

^a χ^2 -test.

included patients with abnormalities of inv(16)/t(16;16)/del(16q) or t(15;17) with any additional abnormalities, or t(8;21) without either a del(9q) or being part of a complex karyotype. A total of 35 patients (27%) had karyotypes indicative of poor prognosis. This was defined by the presence of one or more abnormalities of chromosome 5 and 7, -5/del(5q), -7/del(7q), inv(3q), abnormalities of chromosome 11, 20q or 21q, del(9q), t(6;9), t(9;22), abnormalities of 17p, and complex karyotypes defined as three or more abnormalities. The intermediate risk category included 87 patients (66%) characterized by +8, -y, +6, del(12p) or normal karyotype. Most of the patients had intermediate risk AML according to cytogenetics (63% in ATG-S vs 58% in ATG-F vs 50% in no ATG).

Transplant characteristics of the patients are given in Table 2. The source of HSC was bone marrow in 78% of patients in ATG-S, 45 and 76% in ATG-F and the no ATG group, respectively ($P=0.001$). There was no difference regarding CMV status and female donor to male recipients' type of unrelated donor transplant between the three groups of patients. Total body irradiation (TBI)-based conditioning regimen was performed in 39% of patients in ATG-S, 82% in ATG-F and 48% in the no ATG group ($P<0.0001$). Growth factors were not routinely administered to accelerate engraftment.

HLA typing and donor selection

Donors were matched by serology for HLA-A and -B and by DNA-Typing of the HLA class II antigens. Donor and recipient match was defined as identity at HLA-A, -B, DRB1 and DQB1 loci and was comparable in the three groups. HLA-mismatch transplant was defined as up to one HLA-mismatch in GvH and HvG direction according to

German Consensus on Immunogenetic Donor Search for unrelated transplants.¹⁴ In total, 118 (76%) donor recipients pairs were HLA-identical. One antigen difference was accepted in 37 pairs.

Engraftment and GvHD

Myeloid engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count exceeded $0.5 \times 10^9/l$. Graft failure was defined as the lack of myeloid engraftment in patients surviving in remission for at least 28 days after transplantation. Criteria for diagnosis, grading and managing of acute and chronic GvHD were used as described.^{15–18} For GvHD prophylaxis, 5 mg/kg cyclosporine A was given intravenously daily from day -3 to day 20 adjusted to the blood plasma level, then 6–10 mg/kg daily orally for at least 6 months. Methotrexate was also given at 15 mg/m² on day 1 and 10 mg/m² on days 3 and 6. No additional GvHD prophylaxis has been used for mismatched donors. ATG-S (Thymoglobulin[®], Sangstat, Lyon, France, now Genzyme, Cambridge, MA, USA) was given from day -5 to day -2 at a total dose of 15 mg/kg b.w. ($n=3$) or 10 mg/kg b.w. ($n=28$), and from day -3 to day -1 at a total dose of 7.5 mg/kg b.w. ($n=6$) and 5 mg/kg b.w. ($n=12$). ATG-F (Fresenius, Graefelfing, Germany) was given from day -3 to day -1 at a total dose of 45 mg/kg b.w. ($n=11$) and 60 mg/kg b.w. ($n=27$). Prior to starting the 8 h ATG infusion, patients received 100 mg i.v. prednisolone plus ranitidine and clemastine.

Conditioning regimen and transplantation

Busulfan-based standard conditioning regimen (16 mg/kg b.w.) and cyclophosphamide (120 mg/kg b.w.) was given to

Table 2 Transplant characteristics

	No ATG	ATG-S	ATG-F	P ^a
<i>Gender of donor</i>		(1 unknown)		
Male	36 (53%)	28 (58%)	27 (71%)	0.19
Female	32 (47%)	20 (42%)	11 (29%)	
<i>Stem cell source</i>				
Bone marrow	52 (77%)	38 (78%)	17 (45%)	0.001
Peripheral blood	16 (23%)	11 (22%)	21 (55%)	
<i>Type of unrelated donor</i>				
HLA-matched	54 (79%)	35 (71%)	29 (76%)	0.61
HLA-mismatched	14 (21%)	14 (29%)	9 (24%)	
<i>CMV status (recipient/donor)</i>				
Neg/neg	14 (22%)	19 (39%)	12 (32%)	0.38
Neg/pos	11 (18%)	5 (10%)	7 (19%)	
Pos/neg	21 (34%)	15 (31%)	7 (19%)	
Pos/pos	16 (26%)	10 (20%)	11 (30%)	
<i>Conditioning</i>				
Busulfan-based	35 (52%)	30 (61%)	7 (18%)	<0.001
TBI-based	33 (48%)	19 (39%)	31 (82%)	
<i>GvHD prophylaxis</i>				
CsA/MTX (±predn.)	57 (84%)	40 (82%)	34 (89%)	0.57
CsA/MMF (±predn.)	8 (12%)	9 (18%)	3 (8%)	
CsA/MTX/MMF (±predn.)	2 (3%)	—	1 (3%)	
CsA/prednisolone	1 (1%)	—	—	

^aχ²-test.

61, 18 and 52% of patients in the ATG-S, ATG-F and no ATG group, respectively ($P < 0.001$). The remaining patients had 12 Gy TBI in six fractions (from day -6 to day -4), followed by cyclophosphamide (120 mg/kg bw, day -3 and -2). All patients received prophylactic fluconazole and aciclovir. All patients received unmanipulated HSCT as graft. In total, 107 (69%) patients received BM and 48 (31%) PBSC. The graft was transfused via a tunneled central vein line over 1–2 h. The mean mononuclear cell (MNC) count of the graft was cells/kg b.w. of the recipients.

Statistical methods

Definitions. The primary study objectives were: (i) *transplant-related mortality (TRM)*, defined as all causes of nonleukemic deaths; (ii) *relapse incidence (RI)*, as defined on the basis of morphological evidence of leukemia in bone marrow or extramedullary organs. For evaluating probability of relapse, patients dying either from toxicity or from any other cause not related to leukemia were censored and (iii) *LFS* was defined as time interval from transplant to first event (either relapse or death in complete remission) and (iv) *GvHD*: aGvHD was diagnosed and graded at each transplant center according to Seattle criteria.¹⁵ cGVHD was defined according to standard criteria.¹⁶ Patients surviving without relapse for more than 100 days post transplant with sustained donor engraftment were considered as evaluable for cGVHD.

Statistical analysis. Values reported for quantitative variables were median and range. The following patient or graft characteristics were analyzed for their potential prognostic value on outcome: patients' and donors' characteristics (age, patient and donor sex, CMV serology), disease factors (*de novo* or secondary leukemia) and transplant-related factors (disease status at the time of transplantation, HLA compatibility, GVHD prophylaxis, ATG). For continuous variables, the median was taken as a cut point. To compare the three subgroups of patients receiving one of the two types of ATG or not, we used χ²-test for categorical variables.

Statistical analyses were independently performed for each end point, that is, aGVHD grade III–IV, cGVHD, RI, TRM and LFS. Incidences of event were nonparametrically estimated. Patients were censored at the time of relapse or at last follow-up.¹⁹

Probability of LFS was estimated by the product-limit method.²⁰ The univariate significance was estimated by Log-Rank test (Mantel–Cox). All variables differing between the three groups or potential prognostic factors were included into a Cox proportional hazard model.²¹ Acute or chronic GVHD were events competing with death. Relapse and nonrelapse mortality were events that compete with themselves. Accordingly, estimations of the incidence of these events relied on the nonparametric estimator of cumulative incidence curves, while predictive analyses were based on the proportional hazards model for the subdistribution of competing risks.²² These analyses were performed using the *cmprsk* package (developed by Gray) on Splus and SPSS software.²²

Results

Engraftment

Of the 155 patients analyzed, 20 died within 28 days of transplantation. A total of 135 patients could be evaluated for hematological recovery, and all but 15 (four ATG, 11 no ATG, four of them experiencing early relapse on day 30 ($n=1$), day 31 ($n=2$) and day 49 ($n=1$)) achieved sustained donor engraftment. There was no difference in the rejection rate between the groups ($P=0.14$). Neutrophil engraftment occurred at a median of 18, and 19 days (ATG and no ATG group, respectively) ($P=0.14$) and was comparable in the groups.

Side effects of ATG

ATG was well tolerated provided the patients were premedicated. Chills and fever were common but manageable with antihistamines and by prolonging the infusion time of ATG. All patients received the ATG dose prescribed.

Incidence and severity of aGvHD

The cumulative incidence of severe aGvHD (grade III–IV) was 15 and 27% in the two groups, respectively ($P=0.44$) (Figure 1).

Limited and extensive cGvHD

In total, 91 (59%) of the 155 patients were alive on day 100. Extensive cGvHD was diagnosed in 17 and 40% in ATG and the no ATG group, respectively. The overall incidence of cGvHD was significantly higher in patients not having received ATG (76%) as compared to the ATG group (36%) (Figure 2, $P<0.0001$). Furthermore, the use of ATG-F was associated with a lower incidence of cGvHD as compared to those patients having received ATG-S ($P=0.05$) (Table 3).

Nonrelapse related death and recurrence of leukemia

The cumulative TRM at 3 years was 42 and 40% in the ATG and no ATG group ($P=0.81$), respectively. The

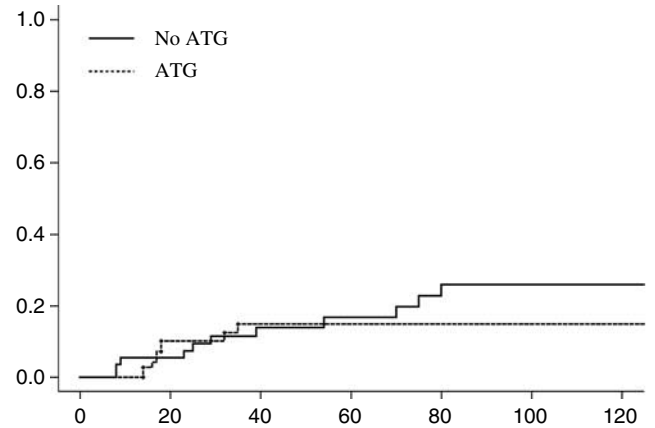


Figure 1 Cumulative incidence of aGvHD grade \geq III.

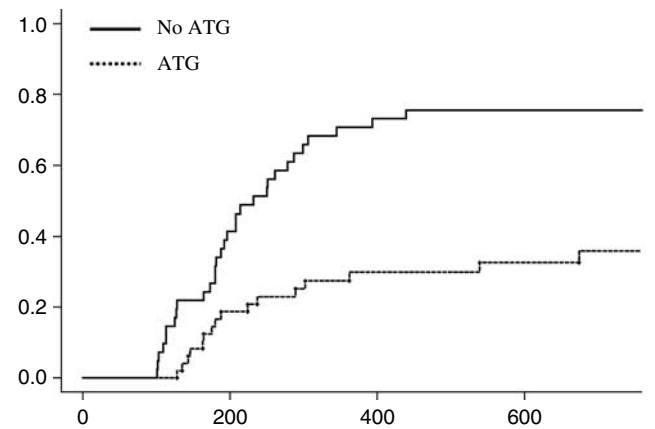


Figure 2 Cumulative incidence of cGvHD.

Table 3 Univariate analysis for LFS, RI, TRM, aGvHD and cGvHD for all patients

Factor	LFS	RI	TRM	aGvHD	cGvHD	cGvHD extensive
Patient age	NS	NS	NS	NS	NS	NS
Disease status	0.0003*	<0.0001*	NS	NS	NS	NS
Recipient–donor sex combination	NS	NS	NS	NS	NS	NS
Graft cell number (MNC)	NS	NS	NS	NS	NS	NS
Graft cell number (CD34+)	NS	NS	NS	NS	NS	NS
Donor matching	NS	NS	NS	NS	NS	NS
Stem cell source	NS	NS	NS	NS	NS	NS
Conditioning regimen	NS	0.002*	0.04*	NS	NS	NS
ATG (yes or no)	NS	NS	NS	NS	0.0001*	NS
ATG (S vs F)	0.003*	0.014*	NS	NS	0.05*	NS
GvHD prophylaxis	NS	NS	0.007*	0.0001*	NS	NS
Cytogenetics risk group	NS	NS	NS	NS	NS	NS
CMV status of donor	NS	NS	NS	NS	NS	NS
CMV status of recipient	NS	NS	NS	NS	NS	NS

*P-value, significant factor.

LES = leukemia-free survival; RI = relapse incidence; TRM = transplant-related mortality; aGvHD = acute graft-versus-host disease; cGvHD = chronic GvHD.

actuarial risk of relapse was 28 and 31% in ATG and no ATG patients at 3 years, respectively (Figure 3). The difference between the ATG-F and ATG-S (41 vs 15%, $P=0.01$) group reached statistical significance (Table 3).

Overall survival by cytogenetic risk analysis

Of the 131 patients with known cytogenetics, 84 (64%) died. Among these patients leukemia-free survival (LFS) at 5 years varied from 51 to 25% according to the cytogenetic risk status, intermediate and poor being similar. There was a trend for an increased RI in patients with poor cytogenetic risk AML ($P=0.06$).

LFS

With a median follow-up of 34 months (1–89), the projected 3-year LFS was 30 and 29% for the ATG and the no ATG group, respectively ($P=0.82$) (Figure 4). The difference between ATG-F and ATG-S (38 vs 21%) reached statistical significance ($P=0.003$, Table 3). As

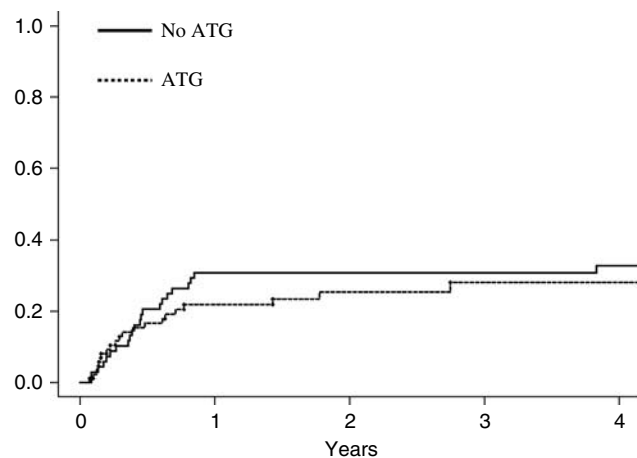


Figure 3 Cumulative incidence of relapse.

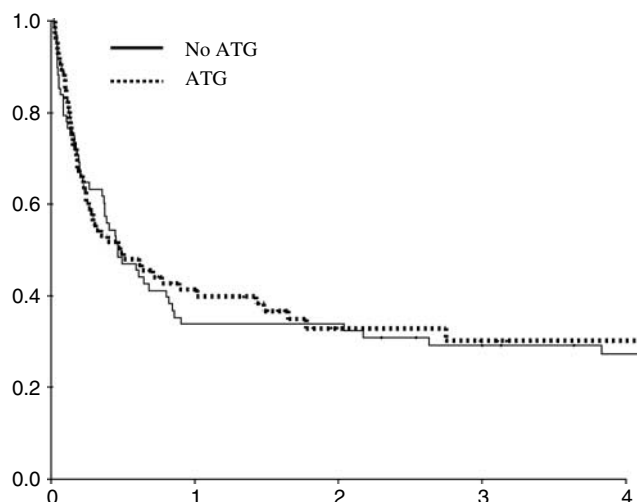


Figure 4 LFS.

expected, patients transplanted in CR had a superior outcome compared to those transplanted in advanced disease (42 vs 16%, $P=0.003$).

Univariate analysis

We looked for variables predictive of LFS, RI, TRM and acute and chronic GvHD in univariate analysis in all 155 patients (Table 3). LFS was significantly better in patients with CR at the time of HSCT and in patients receiving ATG-S (compared to those receiving ATG-F). RI was significantly increased in patients with advanced disease at the time of HSCT and in patients receiving ATG-F (compared to those receiving ATG-S). There was a trend to an increased RI in patients with poor cytogenetics ($P=0.06$). RI was significantly increased in patients receiving TBI. TRM and aGvHD were significantly decreased in patients receiving CsA/MTX for the GvHD prophylaxis. The incidence of aGvHD grade III–IV was 27% and 15% ($P=0.44$) in the recipients without and with ATG, respectively. The incidence of cGvHD was significantly increased in patients receiving no ATG compared to patients receiving ATG (76 vs 36%, $P<0.001$). In addition, extensive cGvHD was increased in the no ATG group. Among the patients receiving ATG, the use of ATG-F was associated with a lower risk of cGvHD, higher RI and lower LFS in comparison to ATG-S in univariate analysis.

Multivariate analysis

Diagnosis (*de novo* vs other), phase of disease (CR vs advanced), patients age (<39 vs >39 years), source of stem cells (PB vs BM), conditioning (TBI vs Bu), HLA matching (mismatched vs identical), CMV status of recipient (CMV-positive vs CMV-negative); CMV status of donor (CMV-positive vs CMV-negative) and GvHD prophylaxis (CsA/MTX vs other) were entered in a multivariate COX analysis with RI, TRM, LFS, aGvHD grade III/IV. Above variables were entered into a multivariate analysis of 91 patients alive and evaluable on day 100, with chronic GvHD (limited and extensive) and extensive GvHD as an end point (yes or no).

The most important independent risk factor for cGvHD of the whole group were the use of ATG, disease status at transplant and conditioning. The use of ATG, disease status at transplant and donor CMV were also significant factors influencing extensive cGvHD. ATG had a protective effect for cGvHD (limited and extensive) when entered as ATG vs no ATG ($P<0.0001$ and $P=0.008$, respectively) (Table 4).

The most important independent risk factor for unfavourable outcome with respect to LFS and relapse was disease status at transplant. Conditioning with TBI was significantly influencing relapse incidence. GvHD prevention with MTX and cyclosporine was significantly better than other. Owing to missing data for the MNC number in 20% of patients and CD34+ cells in 46% of patients the Cox regression analysis was not performed for this factor.

Table 4 Cox regression analysis for RI, TRM, LFS, aGvHD and cGvHD for all patients

Factor	Relapse		TRM		LFS		acute GvHD gr III-IV		chronic GvHD		chronic GvHD (extensive)	
	P	RR (95%CI)	P	RR (95%CI)	P	RR (95%CI)	P	RR (95%CI)	P	RR (95%CI)	P	RR (95%CI)
ATG	—	—	—	—	—	—	—	—	—	—	—	—
Disease status	0.0001	5.3 (2.7-1.037)	—	—	—	—	—	—	0.0001	0.27 (0.15-0.5)	0.008	0.3 (0.12-0.73)
Conditioning regimen	0.008	2.5 (1.26-4.9)	—	—	0.001	0.48 (0.31-0.75)	—	—	0.02	0.39 (0.18-0.87)	0.007	0.2 (0.1-0.64)
GvHD prophylaxis	—	—	—	—	—	—	—	—	0.04	1.91 (1-3.53)	—	—
Donor CMV	—	—	0.011	0.41 (0.21-0.82)	—	—	0.016	0.2 (0.05-0.73)	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	0.03	0.33 (0.12-0.9)

RR = indicates relative risk; CI = confidence interval; GvHD = graft-versus-host disease; TRM = transplant-related mortality; LFS = leukemia-free survival; RI = relapse incidence.

Discussion

We here report on a large and homogeneous cohort of adult patients transplanted for AML from an unrelated donor after standard busulfan- or TBI-based conditioning. The outcome of these transplants was retrospectively analyzed according to the use of two different rabbit ATGs, ATG-S (SangStat/Genzyme) or ATG-F (Fresenius), vs no ATG, as part of the conditioning regimen.

The most important result of this study is a clearcut reduction in cGvHD by the incorporation of ATG into conditioning. In multivariate analysis, use of ATG was a significant protective factor for the occurrence of cGvHD (limited and intensive) as well as for extensive cGvHD alone. Since cGvHD has a major negative impact on quality of life of long-term survivors after allogeneic stem cell transplantation,²³ this is a relevant finding, even in the absence of a potential survival advantage. Our results are similar to those of a randomized study by Bacigalupo *et al*⁸ who showed that ATG-S at a dose of 15 mg/kg b.w. given from day -5 to -2 significantly reduced extensive cGvHD when compared to transplants without ATG. aGvHD in this same study was lowered in a dose-dependent manner, with 7.5 mg/kg ATG-S not having an effect and 15 mg/kg b.w. having the greatest effect, although at the price of an increased infection rate. In our study, we could not find a significant change in incidence of aGvHD at the given doses. However 46 of the 49 patients in the ATG-S group of our study received less or equal to 10 mg ATG/kg b.w. corresponding to the lower dose levels of Bacigalupo *et al*.

Within the patients treated with ATG, we observed a significantly more profound reduction in overall cGvHD after the use of ATG-F than after ATG-S. Although not confirmed by multivariate analysis, this may be a noteworthy observation since it suggests that there may be clinically relevant biological differences between the two products. ATG-S has a much higher concentration of active antibodies as ATG-F and is therefore used at lower doses.²⁴ However, in the setting of hematopoietic stem cell transplantation, no data exist as to what doses may be equivalent. Recent publications suggest that median doses of 6-8 mg/kg b.w. ATG-S may result in lowest TRM and best survival.^{25,26} Schleuning *et al*²⁷ in a large group of chronic myeloid leukemia (CML) patients demonstrated beneficial effects on severe aGvHD and survival only with ATG-F doses of 60 mg/kg b.w. and higher. The median doses of ATG-S and ATG-F given in our study, therefore, may be considered as roughly comparable. Still, the cumulative incidence of cGvHD was much lower in the group having received ATG-F as compared to ATG-S, despite a slight bias in the ATG-F group towards a more frequent use of peripheral stem cells as a graft source. With the use of peripheral blood stem cells, one would have expected more, not less extensive cGvHD.^{28,29}

The second important finding of this study relates to the cumulative relapse incidence in the three patient groups. Patients with ATG-F had the highest relapse risk (actuarial risk of relapse at 3 years 41%), followed by those without ATG (31%). Following ATG-S, the relapse risk was lowest (15%) and significantly lower than after ATG-F. Whereas the results in the ATG-F group support the well-known

and expected inverse correlation between cGvHD and relapse after allogeneic transplants for myeloid leukemia,³⁰ this paradigm cannot explain the results in the patients treated with ATG-S. Not only that with reduction of cGvHD the relapse incidence was not higher than in the group not treated with ATG, it was even half. This finding is difficult to understand but again illustrates that the two ATG compounds used in this study have very different biological activity. ATG-S is manufactured by immunization of rabbits with resting human thymocytes and has a very broad antibody spectrum focusing mainly, but not exclusively, on T cells at all stages of maturation.^{31,32} ATG-F is raised against Jurkat cells, a T-lymphoblastoid cell line, resulting in a relative skewing of the T-cell antibody spectrum towards activated cells.³³ Via these antibodies, different mechanisms of T-cell cytotoxicity are described^{31,33–36} In addition, some evidence exists that ATG-F may block alloreactive T-cell activation through interaction with host antigen-presenting cells via the CTLA-4/B7 costimulatory pathway.³³ By this, ATG-F may interrupt the transduction of a positive signal for cytokine secretion and proliferation of antigen-specific T cells, thus allowing establishment of host tolerance and abrogating GvHD, and also possibly the GvL effect. Recently, Baurmann *et al*³⁷ demonstrated that immune reconstitution of T-cell subsets is significantly slower after ATG-S than after ATG-F at doses comparable to this study. NK cell reconstitution was left largely unaffected. If therefore ATG-S leads to a more profound T-cell depletion of the graft than ATG-F, one might speculate that other cellular players of a clinical GvL effect like NK cells may gain a more important role.³⁸ Researchers in favor of a GvL mechanism related to NK cell function in matched unrelated transplants argue that modulation of T-cell alloreactivity may be necessary to disclose a potentially beneficial effect of NK cell alloreactivity on LFS.³⁹

Relapse risk and LFS in our study by multivariate analysis were also significantly dependent on busulfan- vs TBI-based conditioning, with a higher relapse risk for the latter. Conditioning, therefore, is a confounding variable in this study since there were significantly more patients in the ATG-F group prepared with TBI than in the ATG-S or no ATG group. However, the available randomized studies^{40–42} concluded on a lower, not a higher relapse incidence, and a slightly more favorable LFS in AML patients prepared with TBI as compared to oral busulfan. This discrepancy between the literature and our results again may be related to the use of ATG concomitant to TBI, which via a weaker GvL effect may lead to an increased relapse risk after TBI.

LFS in our study as expected was also linked to status before transplant with a better survival for patients in CR and for patients without unfavorable karyotype. This is in keeping with other reports^{1,13,43} showing that cytogenetics are the most potent predictor for response to post-remission therapy in AML. Within the patient groups of our study, there was a significant difference in the projected 3-year LFS according to ATG-S (38%), ATG-F (21%) and no ATG used (29%) in favor of ATG-S, suggesting that the reduced relapse incidence outweighed the negative effect of somewhat more cGvHD as compared to ATG-F. These

results are in contrast to a recent report of Schleuning *et al*⁴⁴ stating a significantly better overall survival in patients with CML transplanted from unrelated donors after the use of ATG-F as compared to ATG-S. CML, however, is a disease with a well-known, exquisite sensitivity to T-cell mediated GvL.⁴⁵ It may well be that in this setting, the net advantage of less cGvHD with ATG-F is greater than in a less-sensitive, more rapidly proliferating entity like AML.

In conclusion, our data shed an interesting light on a potentially differential, future use of ATG serotherapy as an integral part of unrelated stem cell transplantation in AML and open a new avenue for further clinical examination.

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