

## Graft-versus-host disease

# *In vivo* T cell depletion with pretransplant anti-thymocyte globulin reduces graft-versus-host disease without increasing relapse in good risk myeloid leukemia patients after stem cell transplantation from matched related donors

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### Summary:

One-hundred and two patients with good risk myeloid leukemia (CML first chronic phase or AML first CR) were transplanted from HLA-related donors after conditioning with ( $n = 45$ ) or without anti-thymocyte globulin (ATG) ( $n = 57$ ). One graft failure was observed in the non-ATG and none in the ATG group. The median time to leukocyte engraftment ( $>1 \times 10^9/l$ ) was 16 (range 12–33) in the ATG group and 17 days (range 11–29) in the non-ATG group (NS) and for platelet engraftment ( $>20 \times 10^9/l$ ) 24 and 19 days ( $P = 0.002$ ), respectively. Acute GVHD grade II–IV was observed in 47% of the non-ATG and in 20% of the ATG group ( $P = 0.004$ ). Grade III/IV GVHD occurred in 7% of the ATG and in 32% of the non-ATG group ( $P = 0.002$ ). Chronic GVHD was seen in 36% and 67% ( $P = 0.005$ ), respectively. After a median follow-up of 48 months (range 2–128), the 5-year estimated OS is 66% (95% KI: 51–81%) for the ATG group and 59% (95% KI: 46–72%) for the non-ATG group (NS). The 5-year estimated DFS is 64% (95% KI: 50–78%) for ATG and 55% (95% KI: 43–67%) for the non-ATG regimen (NS). The 5-year probability of relapse was 5% in the ATG and 15% in the non-ATG group (NS). ATG as part of the conditioning regimen leads to a significant reduction in GVHD without increase of relapse in patients with myeloid leukemia after stem cell transplantation from HLA-related donors.

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Allogeneic stem cell transplantation from HLA-identical siblings for patients suffering from acute or chronic myeloid leukemia has become a curative treatment option. Transplant-related mortality as the cause of death is approximately 30%, and most deaths are related directly or indirectly to acute or chronic graft-versus-host disease (GVHD). Allogeneic stem cell transplantation from HLA-identical siblings with an unmanipulated graft resulted in an incidence of acute GVHD of approximately 30 to 60%.<sup>1</sup> Several investigators have shown that removal of T cells from the graft by *ex vivo* T cell depletion resulted in a dramatic decrease in GVHD. However, it became rapidly apparent that T cell depletion resulted in a high incidence of graft failure and an increased risk of leukemia relapse.<sup>2–6</sup> Partial or selective depletion of different subpopulations of T cells, or T cell depletion with T cell add-back has been investigated and resulted either in enhanced graft failure or increased risk of GVHD after T cell add-back.<sup>7–9</sup> Other forms of T cell depletion including T cell depletion with monoclonal antibodies either *in vivo* or *ex vivo* have been investigated extensively, resulting in a decrease of GVHD, but a relatively high incidence of graft failure has been observed.<sup>10,11</sup> By adding monoclonal anti-52 antibodies to the conditioning regimen to deplete residual host T cells, the problem of graft failure has been partly overcome.<sup>12</sup> Another strategy of *in vivo* T cell depletion is the use of anti-thymocyte globulin as part of the conditioning regimen. We and others have recently shown that ATG as part of the preparative regimen in unrelated stem cell transplantation reduces the risk of severe acute and chronic GVHD and of graft failure without an obvious increase in relapse.<sup>13–15</sup> In HLA-identical sibling transplantation anti-thymocyte globulin (ATG) has only been investigated to enhance engraftment in T cell-depleted grafts,<sup>16,17</sup> but serotherapy with ATG alone as *in vivo* T cell depletion has not been investigated to date in this setting. In the current study we investigated ATG as part of the conditioning regimen in good risk leukemia patients and compared the results with a historical group of patients who were transplanted at our institution without ATG. The primary objective was

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to evaluate toxicity, engraftment and incidence of acute and chronic GVHD. The secondary objective was to determine relapse rate and survival of both groups.

## Materials and methods

### Patients

The study group consisted of 102 patients with myeloid leukemia receiving related donor stem cell transplants between 1990 and 2000 at the University Hospital Eppendorf in Hamburg. Forty-five patients received ATG, while 57 patients were conditioned without ATG. Forty-seven patients suffered from acute myeloid leukemia in first CR and 55 patients suffered from chronic myeloid leukemia in first chronic phase. Patients with AML were mainly classified as standard risk, which means normal cytogenetic or inversion 16. Patients with translocation (8;21) or (15;17) were excluded. Patients with CML were mainly transplanted within 2 years of diagnosis. Written informed consent was received from each patient. Both groups were well balanced with respect to age, HLA-mismatch and gender. Median follow-up in the ATG group was shorter than in the non-ATG group (26 vs 65 months,  $P < 0.001$ ), because ATG was mainly used after 1996. The stem cell source was bone marrow in 88 patients and peripheral blood stem cells in 14 patients. Eleven of them were in the non-ATG and three in the ATG group ( $P = 0.02$ ). Patients' characteristics are shown in Table 1.

### HLA-typing and donor matching

HLA-A and -B antigens were typed by serologic methods; HLA-DRB1 alleles were typed with sequence-specific oligonucleotide probes. Donors were required to match recipients for the serological defined HLA-A and -B antigens, as well as HLA-DRB1 alleles. In all patients the pretransplantation lymphocyte cross matches with patient sera and donor cells were performed in all cases. There were 89 patients completely matched for HLA-A, -B and -DRB1, 13 patients were mismatched either at HLA-A, B or -DRB1. A single locus HLA mismatch was noted in seven patients in the ATG- and in six patients in the non-ATG group.

### Conditioning regimens

Patients with AML received conditioning with busulfan (14–16 mg/kg), etoposide (30–45 mg/kg) and cyclophosphamide (120 mg/kg), and patients with CML received busulfan (14–16 mg/kg) and cyclophosphamide (120 mg/kg). Six CML patients received additional etoposide 30 mg/kg. Phenytoin was given to prevent busulfan-induced seizures. Uroepithelial prophylaxis was achieved with hyperhydration and mesna. Bone marrow or peripheral blood stem cells were infused 24–48 h after the last cyclophosphamide administration (day 0). No manipulation of the graft was performed. For PBSC, related donors were mobilized with  $2 \times 5 \mu\text{g/kg/day}$  G-CSF according to the local protocol. On day 5 leukapheresis was performed in an outpatient setting and continued on days 6 or 7 if necessary, to collect at least  $3 \times 10^6$  CD34<sup>+</sup> cells/kg/bodyweight (BW) of the patient's weight.

### GVHD prophylaxis

GVHD prophylaxis consisted of cyclosporin A (3 mg/kg, given from day -1 to 6 months post transplantation). The dose of cyclosporin A was adjusted to cyclosporin A serum levels. Cyclosporin A was tapered from day 84 and discontinued on day 180. Methotrexate (10 mg/m<sup>2</sup>) was given on days 1, 3 and 6 post transplantation. Anti-thymocyte-globulin of rabbit source (ATG-Fresenius, Bad Homburg, Germany) was given to 45 patients. Sixteen patients received ATG at a dose of 30 mg/kg over 12 h on days -3, -2 and -1, and two patients received ATG 30 mg/kg on days -2 and -1, while 26 patients received ATG 30 mg/kg only on day -1. One patient received a cumulative dose of ATG of 120 mg/kg. Patients received intravenous globulin on days 1, 7, 14, 21, 28, 56, 84 and 120. All patients received additionally metronidazole (Clont) (Bayer, Leverkusen, Germany) at a dose of 400 mg i.v., given three times a day from conditioning until discharge. The standard criteria were used for acute and chronic GVHD.<sup>18</sup> Acute GVHD was treated with high-dose steroids, and extensive chronic GVHD with cyclosporin A and steroids. Chronic GVHD was evaluated in patients who survived at least 80 days with sustained engraftment.

**Table 1** Patients' characteristics ( $n = 102$ )

	ATG group	Non-ATG group	P value
Number of patients	45	57	NS
Diagnosis:			
AML, 1st CR	21	26	NS
CML, 1st CP	24	31	NS
Median age (range)	39 (11–58)	33 (16–57)	NS
Median months of follow-up (range)	26 (7–75)	65 (1–126)	<0.001
HLA-identical	38	51	NS
one HLA mismatch	7	6	NS
Male/Female	21/24	31/26	NS
Stem cell source BM/PBSC	42/3	46/11	0.02

### Regimen-related toxicity

Regimen-related toxicity affecting the hepatic, cardiac, pulmonary, mucous membranes was graded using the Bearman score.<sup>19</sup> The maximum score for each organ system was recorded. Attempts were made to separate toxicities due to GVHD from therapy-related toxicity. Veno-occlusive disease of the liver was graded according to the Seattle criteria.<sup>20</sup>

### Supportive care

All patients were nursed in single rooms with hepa-filtered air. Antibiotic prophylaxis consisted of ofloxacin or ciprofloxacin, and antifungal prophylaxis of fluconazole and, in case of prior mycotic infection, of amphotericin B. Aciclovir was given as herpes prophylaxis from day 1 until day 180. *Pneumocystis carinii* prophylaxis consisted of either of trimethoprim and sulfamethoxazol on 3 days a week or a monthly inhalation of pentamidine. All red blood products and platelets were irradiated before infusion, and patients seronegative for CMV only received blood products from CMV-negative donors. All patients received haematopoietic growth factors (G-CSF, 5 µg/kg) intravenously beginning on day +1 or day +5 and continued until the absolute granulocyte count was >1.0/nl for 3 consecutive days. CMV seropositive patients were monitored weekly for antigenemia. In cases of positive antigenemia ganciclovir therapy (10 mg/kg/daily) was started.

### Statistical analysis

Statistical analysis was performed by using WIN-STAT-software (Kalmia, Cambridge, MA, USA). Survival curves for disease-free survival and overall survival were estimated by the Kaplan–Meier method. The log rank test was performed for statistical analysis for time-dependent analysis of survival, relapse and disease-free survival. The chi-square test was used for analysis of treatment-related mortality. A *P* value of <0.05 was considered significant.

## Results

### Engraftment and graft failure

No graft failure was observed in the ATG group, while one graft failure was seen in the non-ATG group. The median time to leukocyte engraftment ( $>1 \times 10^9/l$ ) was 16 (range, 12–33) in the ATG group and 17 days (range, 11–29) in the non-ATG group (NS). Platelet engraftment ( $>20 \times 10^9/l$ ) was reached for the ATG group after a median of 24 days (range, 14–277) and for the non-ATG group after 19 days (range, 11–34) (*P* = 0.002).

### Graft-versus-host disease

Acute GVHD grade II–IV was observed in 47% of the non-ATG and in 20% of the ATG group (*P* = 0.004). Severe grade III/IV GVHD occurred in 7% of the ATG and in 32% of the non-ATG group (*P* = 0.002). Acute GVHD

grade I was seen in 23% of the patients in the non-ATG and in 16% of the ATG-group (NS). Overall, chronic GVHD was seen in 36% of the ATG and in 67% of the non-ATG group (*P* = 0.005). Extensive cGVHD was observed more frequently in patients conditioned without ATG (33% vs 17%, *P* = 0.08).

### Toxicity and transplant-related mortality (TRM)

Toxicity of the liver grade I to IV according to the Bearman scale was higher in the ATG than in the non-ATG group (89% vs 71% *P* = 0.04). Additionally, a trend to more VOD-caused mortality was observed in the ATG group (3 vs 1 patient). More skin toxicity, mainly erythema, was seen in the ATG group (48% vs 22%, *P* = 0.009). Other toxicities such as mucositis or pulmonary toxicity were observed to the same extent in both groups (Table 2). Treatment-related mortality did not differ in either group. However, in the non-ATG group more GVHD-related deaths were noted, while in the ATG group a trend to higher mortality due to sepsis and fungal infections was seen. In contrast, deaths due to interstitial pneumonia were mainly seen in the non-ATG group (see Table 3).

### Overall survival and disease-free survival

After a median follow-up of 26 months (range, 7–75) in the ATG and of 65 months (range, 1–126) in the non-ATG group, the 5-year estimated overall survival is 66% (95% KI: 51–81%) for the ATG group and 59% (95% KI: 46–72%) for the non-ATG group (NS). The 5-year estimated disease-free survival is 64% (95% KI: 50–78%) for ATG and 55% (95% KI: 43–67%) for the non-ATG regimen (NS) (Figure 1 and 2). The 5-year estimated overall survival for AML patients was 68% (95% KI: 46–90%) for the ATG and 72% (95% KI: 54–90%) for the non-ATG group (NS). The 5-year estimated disease-free survival for AML patients was 63% (95% KI: 39–87%) and 68% (95% KI: 49–87%), respectively (NS) (Figure 3). The 5-year estimated overall survival for CML patients was 63% (95% KI: 44–84%) with ATG and 48% (95% KI: 29–67%) without ATG (NS). Relapse in CML patients was defined as hematological or cytogenetic relapse. The 5-year estimated disease-free survival for CML patients was 63% (95% KI: 44–84%) and 45% (95% KI: 24–62%), respectively (NS) (Figure 4). In the CML group there were 13 patients with a single locus mismatched donor (seven patients in the ATG and six patients in the non-ATG group). In this subgroup, the 5-year estimated disease-free survival for ATG conditioned patients was 83% and for patients conditioned without ATG only 15% (*P* = 0.03) (Figure 5). However, the number of patients is too low to draw any definite conclusions from this observation. During follow-up, relapse was observed in six patients. Median time to relapse after transplantation was 15 months (range, 5–64). Three relapses in AML patients were seen, one in the ATG and two in the non-ATG group. In CML patients no hematologic or cytogenetic relapses have been observed to date in the ATG group, whereas three relapses were seen in the non-ATG group. Overall, the 3-year probability of relapse was 5% in the ATG and 15% in the non-ATG group (NS).

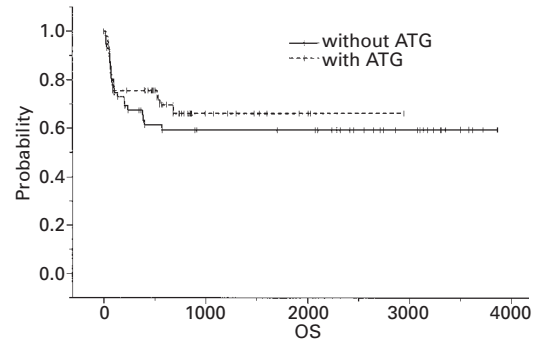
**Table 2** Toxicity

	ATG group	Non-ATG group	P value
Lung (grade I–IV) <sup>a</sup>	9%	9%	NS
Liver (grade I–IV) <sup>a</sup>	89%	71%	0.04
Stomatitis (grade I–III) <sup>a</sup>	100%	95%	NS
Skin	48%	22%	0.009
Treatment-related mortality	31%	33%	NS

<sup>a</sup>According to the Bearman-scale.

**Table 3** Treatment-related mortality

	Non-ATG group (n = 20 33%)	ATG group (n = 14 31%)
GVHD	5	1
Sepsis	1	2
Aspergillosis	3	3
Graft failure	1	0
VOD	1	3
Interstitial pneumonia	4	1
TTP	0	1
Viral infection	4	2
Bleeding	1	1



**Figure 1** Overall survival of all patients conditioned with or without ATG.

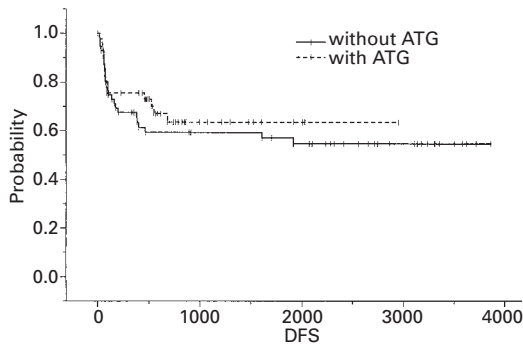
**Table 4** Engraftment, GVHD and survival after allogeneic transplantations

	ATG (Fresenius)	non-ATG	P value
Median leukocyte engraftment >1/nl	16 days (range, 12–33)	17 days (range, 11–29)	NS
Median platelet engraftment >20/nl	24 days (range, 14–277)	19 days (range, 11–34)	0.002
Graft failure	0	1	NS
Acute GVHD grade II–IV	20%	47%	0.004
Acute GVHD grade III/IV	7%	32%	0.002
Chronic GVHD (overall)	36%	67%	0.005
Chronic GVHD extensive	17%	33%	0.08
Probability of relapse (3 years)	4%	15%	NS
Estimated overall survival (5 years) (95% CI)	66% (51–81)	59% (43–67)	NS
Estimated disease-free survival (5 years) (95% CI)	64% (50–78)	55% (43–67)	NS
Estimated overall survival (5 years) (95% CI) for CML	63% (44–84)	48% (29–67)	NS
Estimated disease free survival (5 years) (95% CI) for CML	63% (44–84)	45% (24–62)	NS
Estimated overall survival (5 years) (95% CI) for AML	68% (46–90)	72% (54–90)	NS
Estimated disease-free survival (5 years) (95% CI) for AML	63% (39–87)	68% (49–87)	NS

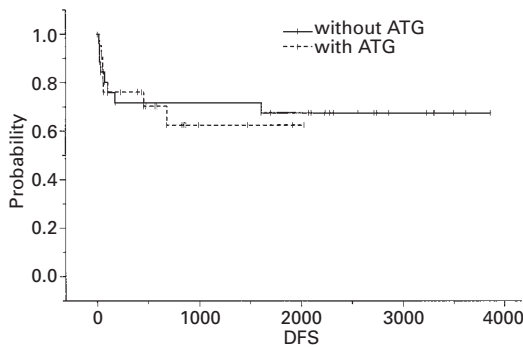
**Discussion**

Severe acute and chronic GVHD are among the leading causes of death after allogeneic stem cell transplantation. A major concern for surviving patients with severe acute and chronic GVHD is a drastic reduction in quality of life. This retrospective comparative study convincingly shows for the first time that the incorporation of anti-thymocyte globulin as part of the conditioning regimen significantly reduces the incidence of acute and chronic GVHD without increasing relapse in allogeneic stem cell transplantation

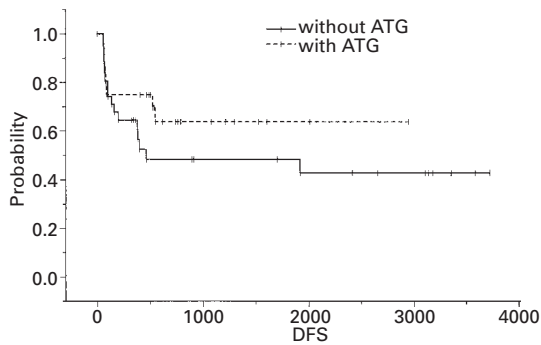
from related donors. ATG has been primarily used as a treatment option for severe steroid-resistant GVHD. Furthermore, ATG as part of the conditioning regimen for allogeneic transplantation in patients with severe aplastic anemia was able to induce rapid and sustained engraftment.<sup>21,22</sup> In these trials, beside ensuring engraftment, a low incidence of GVHD has been observed. Therefore, ATG as part of the conditioning regimen has been used successfully in unrelated stem cell transplantation.<sup>15–17,23,24</sup> In our study with related donors we did not observe any graft failures in the ATG group. This is in contrast with other forms of



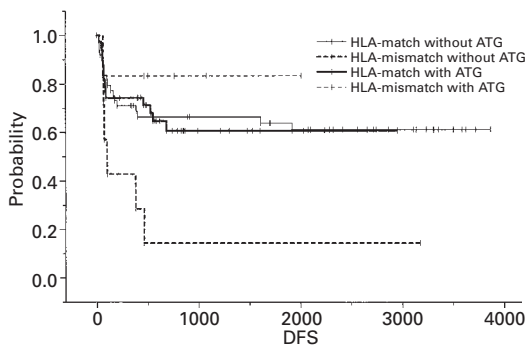
**Figure 2** Disease-free survival of all patients conditioned with or without ATG.



**Figure 3** Disease-free survival of AML patients in first CR conditioned with or without ATG.



**Figure 4** Disease-free survival of CML patients in first CP conditioned with or without ATG.



**Figure 5** Disease-free survival of all patients with HLA-matched or mismatched donors conditioned with or without ATG.

T cell depletion, which result in a low incidence of GVHD, but a high incidence of graft failure.<sup>2,3,4,6</sup> A high incidence of graft failure has also been reported for *in vitro* T cell depletion with Campath 1-M, an antibody recognizing the CD52 antigen. The risk of graft failure with this approach has been only partly overcome with the additional administration of Campath 1-G for *in vivo* T cell depletion of the recipient before graft infusion.<sup>10,12</sup> The benefit of ATG to facilitate engraftment in combination with other forms of T cell depletion in related stem cell transplantation has only been shown for a few patients with acute leukemia.<sup>13,14</sup> We were also able to show that ATG leads to a significant reduction in severe acute and chronic GVHD in comparison to patients transplanted without ATG. This, however, did not result in a reduction in treatment-related mortality in our study, but to a trend to better survival without reaching statistical significance. In contrast to other forms of T cell depletion this trend was more pronounced in patients with CML (5 years OS: 63% vs 48%) than in patients with AML (68% vs 72%), especially in a subgroup of patients with CML transplanted from a mismatched donor. The major concern of any form of T cell depletion is the high incidence of relapse. In smaller studies with T cell depletion a dramatic increase in relapse was seen in CML patients in chronic phase,<sup>3,5</sup> while in AML patient some centers reported no increase in relapse.<sup>11,25</sup> In a large study by the IBMTR, however, it could be clearly shown that T cell depletion caused a higher incidence of relapse in AML and CML, but the risk was lower in patients with AML than in patients with CML.<sup>6</sup> Attempts to overcome this high incidence of relapse by donor lymphocyte infusions were associated with a high incidence of acute and chronic GVHD.<sup>9,26</sup> Despite the fact that our study was not performed as a randomized trial and the follow-up of the ATG group is rather short at 26 months, it is notable that no relapses have been seen to date in CML patients. Besides a trend to better survival, one should further keep in mind that less GVHD especially less chronic GVHD is an important improvement in quality of life for the surviving patients. We observed higher skin toxicity in the ATG group which is related directly to ATG-induced erythema and which completely resolved in all cases within a week of discontinuation of ATG. A critical issue in using pre-transplant ATG might be the kind of ATG preparation. The ATG used in this study was an immunoglobulin derived from rabbits. After immunization with cells from a T lymphoblast cell line (Jurkat T cell line), this highly purified immunoglobulin consists of antibodies exhibiting a direct effect towards lymphoblastic T cells resulting in T cell depletion via opsonization and lysis following complement activation. Pharmacokinetic studies of rabbit ATG using ELISA techniques and an inhibitory effect on phytohemagglutinin-induced blastogenesis showed a dose-dependent effect of ATG. Rabbit IgG was detectable over 4 weeks after administration in the recipient of bone marrow transplantation, and the effect of the phytohemagglutinin-response on normal mononuclear cells lasted up to 4 days post transplantation.<sup>27</sup> This form of anti-thymocyte globulin mainly acts against activated T cells.

Other investigators used a broad-spectrum anti-T lymphocyte-globulin (Thymoglobulin, Merieux; IMTX,

SangStat) as part of the conditioning regimen to reduce severe GVHD in unrelated bone marrow transplantation.<sup>23</sup> In a comparative study, different anti-T cell antibodies during conditioning for unrelated stem cell transplantation were used: either ATG (Fresenius) or Thymoglobulin (IMTX) or anti-CD3 antibody (OKT3) to prevent GVHD in unrelated bone marrow transplantation. In this non-randomized study, the incidence of severe grade III/IV acute GVHD was 12% in the ATG (Fresenius) group and 0% in the Thymoglobulin (IMTX) group. The incidence of chronic GVHD was 46% in the ATG (Fresenius) group and 44% in the Thymoglobulin (IMTX) group. The engraftment data were comparable. Relapse-free survival was 61% in the ATG (Fresenius) group and 40% in the Thymoglobulin (IMTX) group, mainly due to a high relapse rate in the CML group, with 61% in the Thymoglobulin (IMTX) group and only 10% in the ATG (Fresenius) group.<sup>28</sup> However, it can not be excluded that the low relapse rate and the certain engraftment might be due to the intensified conditioning regimen with busulfan, etoposide and cyclophosphamide in AML patients. In our study the incorporation of ATG did not lead to a single case of EBV-associated lymphoma, which is a major problem of T cell depletion and other ATG preparations.<sup>23,29</sup> We conclude that ATG (anti-rabbit, ATG-Fresenius) as part of the conditioning regimen in patients with myeloid leukemia who have undergone allogeneic stem cell transplantation from related donors leads to a significant reduction in severe acute and chronic GVHD without an increase in relapse. The lower incidence of acute and chronic GVHD did not result in a decrease in treatment-related mortality or to a significantly longer DFS or OS, but to an improvement in a quality of life for surviving patients. Longer follow-up is necessary to determine late relapse. This study also provides a rationale for a prospective randomized trial in patients with standard risk myeloid leukemia undergoing allogeneic stem cell transplantation from related donors.

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