Clinical impact of human Jurkat T-cell-line-derived antithymocyte globulin in multiple myeloma patients undergoing allogeneic stem cell transplantation

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ABSTRACT

Background

Antithymocyte globulin or human Jurkat T-cell-line-derived antilymphocyte globulin is used in allogeneic stem cell transplantation to induce *in vivo* T-cell depletion to facilitate engraftment and lower graft-versus-host disease. *In vitro* studies suggest that antithymocyte globulin, besides causing T-cell depletion, has strong anti-myeloma activity.

Design and Methods

We evaluated the anti-myeloma activity of antilymphocyte globulin in a melphalan/fludarabine-based reduced intensity conditioning regimen as well as the incidence of graft-versus-host disease in 138 multiple myeloma patients who underwent allogeneic stem cell transplantation with (n=79) or without (n=59) antilymphocyte globulin.

Results

Leukocyte and platelet engraftment were faster in the group not receiving antilymphocyte globulin (13 *vs.* 16 days, $p<0.001$ and 11 *vs.* 19 days, $p< 0.001$, respectively). Inclusion of antithymocyte globulin led to a better day 100 overall response rate (93% *vs.* 78%, $p=0.03$) and complete response rate (59% *vs.* 39%, $p=0.04$), to a lower incidence of both acute grade III/IV graft-versus-host-disease (11% *vs.* 22%, $p=0.10$) and chronic graft-versus-host disease (23% *vs.* 65%, $p<0.001$) and to a trend to improved event-free survival at 3 years (39% *vs.* 27%, $p=0.5$). There was no difference in the estimated cumulative incidence of treatment-related mortality at 1 year between the groups receiving or not antilymphocyte globulin (25% *vs.* 26%). In a multivariate analysis treatment with antilymphocyte globulin was the only significant factor for achievement of a complete remission (RR:2.57, $p=0.02$).

Conclusions

Inclusion of antithymocyte globulin in allogeneic stem cell transplantation protocols for patients with multiple myeloma may increase remission rates and at the same time prevent graft-versus-host disease with no increase of relapses.

Key words: multiple myeloma, antithymocyte globulin, antilymphocyte globulin, allogeneic stem cell transplantation, reduced intensity conditioning.

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Introduction

Multiple myeloma is a malignant B-cell disease characterized by the clonal expansion of malignant plasma cells. The introduction of novel agents over the last decade has widened treatment options for myeloma patients.[1] Autologous stem cell transplantataion has been shown to improve remission and survival rates.[2-4] Allogeneic stem cell transplantation for multiple myeloma is associated with lower relapse rates compared to autologous transplantation and may lead to long-term disease-free survival in some patients,[5] but the results of studies comparing allogeneic and autologous stem cell transplantation remain controversial.[6,7] Novel strategies are required to combat this disease and further improve the outcome of myeloma patients. A possible approach would be to target cell surface antigens with well tolerated humanized monoclonal antibodies. Candidate antigens for monoclonal serotherapy in myeloma have been CD20, CD40, CD52, CD138, CD74 and interleukin 6-receptor.[8-12] The limited success of monoclonal serotherapy is most probably due to the very heterogeneous phenotype of myeloma cells which may vary in the same patient depending on the maturation stage of the malignant cells and vary even more from patient to patient. It may, therefore, be worthwhile targeting various surface antigens with a combination of monoclonal antibodies or with polyclonal antibody preparations.

Polyclonal antithymocyte (antilymphocyte) globulin (ATG) preparations are extensively used in organ transplantation and in allogeneic stem cell transplantion mainly due to their T-cell-depleting potential. As part of reduced intensity conditioning regimens, ATG preparations have been shown to improve engraftment and reduce the incidence of severe graft-versus-host disease (GvHD).[13,14] ATG preparations are produced by immunizing rabbits or horses with human thymocytes or the Jurkat T-lymphoblastic cell line and contain antibodies targeting a broad spectrum of epitopes expressed on various hematopoietic cells. Irrespective of their mode of preparation, various ATG preparations have been shown to possess potent anti-B-cell activity.[15] We and others have recently shown *in vitro* anti-myeloma cytotoxicity of two commercially available ATG preparations, namely ATG-Fresenius® and Thymoglobulin®.[16,17] These findings are further supported by the results of a xenograft model.[18] The anti-myeloma effect of ATG is due to complement-mediated cytotoxicity as well as caspase-dependent apoptosis.[16,17] ATG therefore not only targets a variety of epitopes, but may also induce several pathways for cell death.

To evaluate this anti-myeloma effect in a clinical setting, we analyzed 138 patients with multiple myeloma, who underwent melphalan/fludarabine based reduced intensity conditioning allogeneic stem cell transplantation with (n=79) or without (n=59) ATG-Fresenius®. We considered remission rates, incidence of acute and chronic GvHD as well as event-free and overall survival.

Design and Methods

We analyzed a total of 138 patients who underwent melphalan/fludarabine-based allogeneic stem cell transplantation for multiple myeloma. Seventy-nine patients (57%) received ATG, 59 patients (43%) no ATG. The conditioning regimen consisted of melphalan (median total dose, 140 mg/m^2; range, 100-150 mg/m^2) administered intravenously on days –3 and –2 and fludarabine (median total dose, 120 mg/m^2; range, 90-180 mg/m^2) given between days –7 to –3. Major inclusion criteria were ineligibility for a standard myeloablative allogeneic stem cell transplant due to age (>45 years), severe organ dysfunction, active fungal infection or reduced Performance Status. Major exclusion criteria were cardiac insufficiency with an ejection fraction of < 30%, liver transaminase more than three times the baseline value or creatinine clearance <30 mL/min. Patients were treated either using an autologous/allogeneic tandem approach for mainly newly diagnosed disease, as recently reported,[13,19] or received reduced intensity conditioning as salvage treatment after failure of prior autografting.[20,21] Patients were treated within Spanish,[19] German[13] or Israeli treatment protocols. The Spanish protocol included only sibling donors without the use of ATG, while the German and Israeli protocols included both sibling and unrelated donors. In the German and Israeli studies, ATG was given to all recipients of unrelated grafts, while its use was facultative in recipients of grafts from related donors. The studies were approved by the local ethics committees and all patients gave written informed consent. Donors were selected on the basis of serological typing for class I antigens (HLA-A and HLA-B) and molecular typing for HLA-DRB1 and HLA-DQB1.

Patients' characteristics

The patients' characteristics are summarized in Table 1. Most patients had advanced disease. All patients had undergone at least one autotransplant. Both groups were balanced in the following characteristics: patients' sex, presence of deletion 13 chromosomal abnormality, cycles of prior chemotherapy, and remission status prior to allotransplant. Fewer patients in the ATG group had experienced relapse after a prior autotransplant (47% *vs.* 63%, p=0.08). More patients in the ATG group than in the no-ATG group had received a graft from a matched unrelated donor (70% *vs.* 2%, p=0.001). Deletion 13 data were only available for 53 of 79 patients in the ATG group and 13 of 59 patients in the no-ATG group, however, in this cohort no difference was seen between the two groups (p=0.1).

Graft-versus-host disease prophylaxis

Patients in the ATG group received ATG (rabbit) at a median dose of 30 mg/kg (range, 10-90 mg/kg) on days –3 to –1. Thirty-one patients received ATG at a dose >30 mg/kg, while 48 patients received ATG at a dose ≤30 mg/kg. All patients received cyclosporine A starting on day –1 as a 3 mg/kg intravenous infusion and then switched to an equivalent oral dose as soon as possible.

Table 1. Patients' characteristics.

	No-ATG	*ATG*	*p value*
Number of patients	59	79	
Age (median)	54 years (range, 34-65)	52 years (range, 32-64)	
ATG-dose			
0	59	--	
≤30 mg/kg body weight		48	
>30 mg/kg body weight		31	
Patients' sex			
Male	35	52	0.5
Female	24	27	
Deletion 13 (FISH) (n=66)			
Positive	3 (23 %)	25 (47 %)	0.1
Negative	10 (77 %)	28 (53 %)	
Prior chemotherapy			
≤10 cycles	50 (85 %)	61 (81 %)	0.6
>11 cycles	9 (15 %)	14 (19 %)	
Prior autotransplant			
n=1	51 (86 %)	58 (74 %)	0.2
n=2	7 (12 %)	19 (24 %)	
n=3	1 (2 %)	2 (2 %)	
Relapse after prior autotransplant			
yes	37 (63 %)	37 (47 %)	0.08
no	22 (37 %)	42 (53 %)	
Status prior to allotransplant			
Complete remission	5 (9 %)	6 (8 %)	
Partial remission	24 (41 %)	44 (56 %)	0.1
Minimal residual disease	8 (13 %)	2 (3 %)	
Stable disease	6 (10 %)	9 (11 %)	
Progressive disease	16 (27 %)	18 (22 %)	
Donor			
Related (HLA-identical sibling)	58 (98 %)	24 (30 %)	<0.001
Matched unrelated	1 (2 %)	55 (70%)	
Graft source			
Peripheral blood stem cells	59 (100 %)	70 (89 %)	0.01
Bone marrow	0	9 (11 %)	
Median CD 34$^+$ cells/ kg body weight	5.6×10^6 (range, 2.4-23)	4.7×10^6 (range, 0.4-15.8)	0.08

Cyclosporine A was tapered off as from day +100 and discontinued between days +140 and +180 if no GvHD occurred. Methotrexate (10 mg/m^2) was administered on days +1, +3 and +6. Mycophenolate mofetil (1 g twice daily) was given from day +1 to day +28. Fifty-eight patients (98%) in the no-ATG group and 74 patients (94%) in the ATG group received cyclosporine A and metrotrexate. Five patients (6%) in the ATG group received cyclosporine A and mycophenolate mofetil. One patient in the no-ATG group (2%) received cyclosporine A alone. Acute and chronic GvHD were graded according to standard criteria.[22,23] Apart from the administration of ATG, there was no difference in GvHD prophylaxis between the two groups.

Supportive care

All patients were nursed in reverse isolation in conventional or laminar air flow rooms. Acyclovir and fluconazole or itraconazole were routinely administed to all patients. Prophylaxis against *Pneumocystis carinii* consisted of cotrimoxazole or pentamidine inhalation. All blood products were irradiated with 25 Gy. Cytomegalovirus-negative patients received only cytomegalovirus-negative blood products. Cytomegalovirus-positive patients were monitored at least weekly for cytomegalovirus infection by polymerase chain reaction and/or antigenemia assay. Pre-emptive therapy was started with 10 mg/kg gancyclovir per day after two consecutive positive polymerase chain reaction results or one positive antigenemia assay. Neutropenic fever was treated with broad-spectrum antibiotics according to each center's policy.

Statistical methods

The χ^2 test or Fisher's exact test was applied for categorical variables and the Mann-Whitney test for continuous variables. Cumulative rates of overall survival, event-free survival, transplant-related mortality, and relapse were calculated using the Kaplan-Meier method, and groups were compared with the log-rank test. Multivariate analysis was performed by forward stepwise logistic regression. All analyses were performed with the SPSS version 10/11 [SPSS Inc., Chicago, USA]. End-points were rates of complete and partial remissions on day 100 after allografting, transplant-related mortality at 1 year, incidence and severity of acute GvHD, incidence and severity of chronic GvHD, incidence of relapse, event-free survival and overall survival.

Definitions

The criteria for complete remission were the absence of detectable monoclonal immunoglobulin in serum and no light chains in urine, negative immunofixation and less than 5% plasma cells in a marrow aspirate. Partial remission was defined as at least a 75% reduction in monoclonal immunglobulin in serum or 90% light chains in a 24-hour urine collection. Progressive disease was defined as an increase of at least 25% in serum monoclonal immunoglobulin or in light chains in urine. Refractory disease after chemotherapy or autografting was defined as the failure to achieve complete, partial or minor remission. Stable disease after allografting was defined as the failure to achieve complete, partial or minor remission but no progression after allografting.[24]

Results

The results are summarized in Tables 2A and 2B.

Engraftment

No primary graft failure was observed. Sustained leukocyte counts >1×10^9/L were achieved more rapidly in the no-ATG group (13 days; range, 6-21) than in the ATG group; (16 days; range, 9-23), (p<0.001). Similar

results were observed for sustained platelet counts >20×10^9/L without platelet transfusion (11 *vs.* 19 days, p<0.001) (Table 2).

Graft-versus-host disease

All 138 patients were evaluable for acute GvHD, while 113 patients were evaluable for chronic GvHD. The incidences of grade II-IV and grade III-IV acute GvHD were lower in the ATG group than in the no-ATG group but the differences did not reach statistical significance, (32% *vs.* 42%, p=0.21 and 11% *vs.* 22%; p=0.10, respectively). The incidences of overall and extensive chronic GvHD were significantly lower in the ATG group than in the no-ATG group (23% *vs.* 65% and 3% *vs.* 37% respectively; p<0.001). In a separate analysis of patients who received a graft from a sibling, there was a trend to less grade II-IV acute GvHD in the ATG group compared to in the no-ATG group (21% *vs.* 43%; p=0.08). The incidence of chronic GvHD was significantly lower in the ATG group than in the no-ATG group (p<0.001) (Table 2B and Figure 1).

Response

To minimize the impact of a graft-versus-myeloma effect on response, we evaluated response to treatment on day 100 post-allografting. In all, 93% of the patients in the ATG group and 78% in the no-ATG group showed an objective response (complete or partial remission) after allografting (p=0.03). The rate of complete remission was also significantly higher in the ATG group than in the no-ATG group (59% *vs.* 39%; p=0.04), (Table 2A). It is worth noting that administration of ATG led to an increase in the complete remission rate after allografting from 39% in the no-ATG group to 57% in those who received ATG ≤30 mg/kg body weight and 63% in those who received ATG >30 mg/kg body weight (p=0.03). Likewise the rate of objective responses (partial and complete remissions) increased from 78% to 91% and 96%, respectively (p=0.02). Among the subgroup of patients who received a graft from a sibling, there were trends to better response in terms of overall responses (86% *vs.* 78%) and complete remission rates (55% *vs.* 39%) for patients who received ATG compared to those who did not (Table 2B); however, given the low number of patients, these differences did not attain statistical significance. When the best response after allogeneic stem cell transplantation was analyzed, the rate of objective responses (complete

Table 2A. Results of all patients.

	No-ATG	*ATG*	*p value*
Response (n=51 and n=71)			
- Complete remission, n. (%)	20 (39 %)	42 (59 %)	0.04
- Complete/partial remission, n. (%)	40 (78 %)	66 (93 %)	0.03
Acute GvHD (n=59 and n=79)			
Grade II–IV	42%	32%	0.21
Grade III/IV	22%	11%	0.10
Chronic GvHD (n=49 and n=64)			
Overall	65%	23%	<0.001
Limited	29%	20%	
Extensive	37%	3%	
Relapse (at 3 years)	64 %	47 %	0.41
Treatment-related mortality (at 1 year)	26 %	25 %	0.60
Leukocyte engraftment (range)	13 days (6-21)	16 days (9-23)	<0.001
Platelet engraftment (range)	11 (4-42)	19 days (4-63)	<0.001
Median follow-up (range)	32 months (3-80)	27 months (3-65)	0.28

Table 2B. Results of patients who received a graft from a sibling (n=82).

	No-ATG	*ATG*	*p value*
Response (n=51 and n=22)			
- Complete remission, n. (%)	20 (39%)	12 (54%)	0.30
- Complete/partial remission, n. (%)	40 (78%)	19 (86%)	0.53
Acute GvHD (n=58 and n=24)			
Grade II–IV	43%	21%	0.08
Chronic GvHD (n=48 and n=21)			
None	35%	86%	<0.001
Limited	27%	14%	
Extensive	38%	0%	

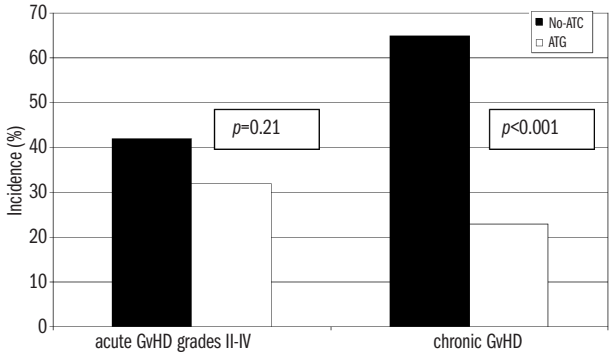

Figure 1. Effect of antithymocyte globulin on the incidence of acute and chronic graft-versus-host disease.

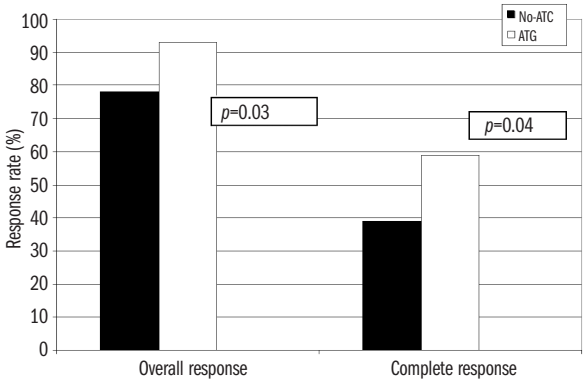

Figure 2. Effect of antithymocyte globulin on response in patients with multiple myeloma.

Table 3. Dose-dependent effect of antithymocyte globulin.

	No-ATG (n=59)	*ATG ≤30 mg/kg body weight (n=48)*	*ATG >30 mg/kg body weight (n=31)*	*p value*
Acute GvHD (n=138)				
Grade II–IV	42%	29%	36%	0.40
Grade III/IV	22%	13%	10%	0.10
Chronic GvHD (n=113)				
Limited	28%	15%	28%	<0.001
Extensive	37%	3%	4%	
None	35%	82%	68%	
Complete remission post-allograft	39 %	57 %	63 %	0.03
Complete/partial remission post-allograft	78 %	91 %	96 %	0.02
Relapse (at 3 years)	64%	52%	37%	0.39
Leukocyte engraftment; median (range)	13 days (6-21)	16 (12-23)	16 (9-22)	<0.001
Platelet engraftment; median (range)	11 days (4-42)	20 (4-39)	19 (12-63)	<0.001

and partial remissions) remained 93% in the ATG group and 78% in the no-ATG group, while the rate of complete remission increased from 39 to 43% in the no-ATG group and from 59 to 60% in the ATG group. Since response can only be measured in patients not in complete remission at the time of allografting, we performed a separate analysis for these patients. In the univariate analysis only partial remission prior to allografting, unrelated donor and ATG were prognostic factors for achieving complete remission. In the multivariate analysis ATG was the only significant prognostic factor for achieving complete remission (RR: 2.57; 95% CI: 1.17-5.64; p=0.02).

Relapse

After a median follow-up of 27 months (range, 3-65 months) for the ATG group and 32 months (range, 3-80 months) for the no-ATG group, the incidence of relapse was 33% *vs.* 42%, respectively (p=0.1), with estimated 3-year relapse rates of 47% (95% CI: 33-61%) *vs.* 64% (95% CI: 47-81%), respectively (p=0.41). A separate analysis of patients who had not relapsed after a prior autograft (n=64) revealed estimated 3-year relapse rates of 31% (95% CI: 14-48%) for the ATG group and 43% (95% CI: 18-68%) for the no-ATG group (p=0.77). Estimated 3-year relapse rates for patients who had relapsed after a prior autotransplant (n=74) were 68% (95% CI: 48-88%) for the ATG group and 83% (95% CI: 63-100%) for the no-ATG group (p=0.81), indicating that ATG did not lead to an increase in relapse rate. Indeed, there was a trend to lower estimated 3-year relapse rates with increasing doses of ATG: 64% (95% CI: 47-81%) in the no-ATG group, 52% (95% CI: 35-69%) in those who received ATG ≤30 mg/kg body weight and 37% (95% CI: 14-60%) in those who received ATG > 30 mg/kg body weight (p=0.39).

Treatment-related mortality

There was no difference in the estimated cumulative incidence of trasplant-related mortality at 1 year between the ATG group (25%; 95% CI: 15-35%) and the no-ATG group (26%; 95% CI: 14-38%). Analysis of patients who had not relapsed prior to allografting (n=64) also showed similar estimated cumulative transplant-related mortality for the ATG group (18%; 95% CI: 6-30%) and the no-ATG group (19%; 95% CI: 2-36%). The same was true for patients who had relapsed after a prior autotransplant (n=74), since the ATG group had an estimated cumulative transplant-related mortality of 34% (95% CI: 17-51%) while that of the no-ATG group was 31% (95% CI: 15-47%). There was no difference in infection- and GvHD-related deaths between the two groups.

Overall survival

The estimated 3-year overall survival was 53% (95% CI: 40-66%) for the ATG group and 43% (95% CI: 29-57%) for the no-ATG group (p=0.46). For patients with-

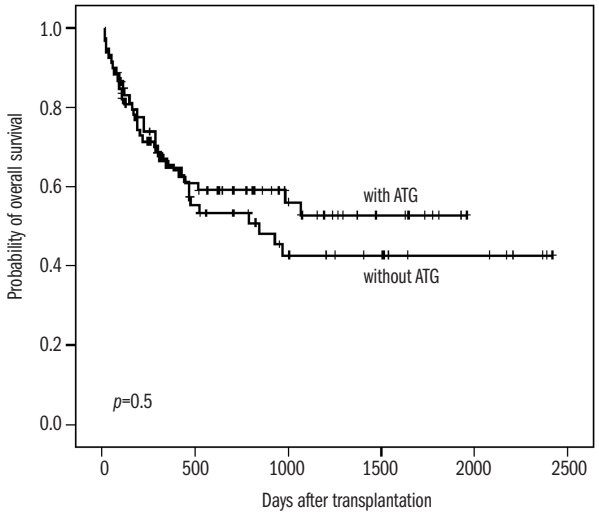

Figure 3. Overall survival in the groups of patients given or not given antithymocyte globulin.

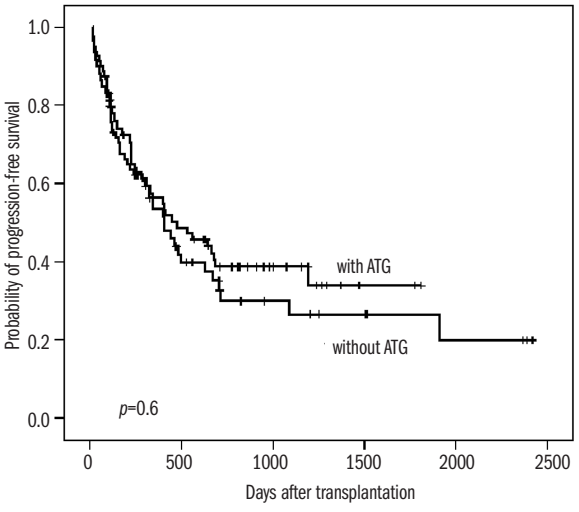

Figure 4. Progression-free survival in the groups of patients given or not given antithymocyte globulin.

out relapse after an autograft conducted prior to allografting (n=64) the estimated 3-year overall survival for the ATG group was 74% (95% CI: 60-88%) compared to 56% (95% CI: 34-78%) for the no-ATG group (p=0.27). For patients who had relapsed after an autotransplant (n=74) overall survival was estimated at 30% (95% CI: 10-50%) for the ATG group and 32% (95% CI: 14-50%) for the no-ATG group.

Event-free survival

The estimated 3-year event-free survival was 39% (95% CI: 27-51%) for the ATG group and 27% (95% CI: 14-40%) for the no-ATG group (p=0.55). For patients without relapse prior to allografting (n=64), the estimated 3-year event-free survival for the ATG group was 55% (95% CI: 39-71%) compared to 46% (95% CI: 24-68%) for the no-ATG group (p=0.90). For patients who had relapsed after a prior autotransplant (n = 74) the event-free survival was estimated to be 21% (95% CI: 7-35%) for the ATG group and 12% (95% CI: 0-26%) for the no-ATG group.

Discussion

Monoclonal humanized antibodies, though well tolerated, have been of limited success in the treatment of multiple myeloma because targeted antigens are often expressed only on a subset of myeloma cells.[8-12] Polyclonal ATG as well as the monoclonal alemtuzumab (anti-CD52) enhance engraftment and reduce the incidence of severe GvHD after standard as well as reduced intensity conditioning allogeneic stem cell transplantation from matched and mismatched unrelated donors.[13,14,25,26] The potential of these serotherapies to enhance engraftment and prevent severe GvHD is at least in part due to the *in vivo* T-cell depletion associated with their use.

By virtue of their methods of preparation ATG contain antibodies recognizing a wide range of surface antigens expressed on T-, B-, plasma, NK-, and dendritic cells.[15,27] Anti-myeloma cytotoxicity of ATG has been reported before *in vitro*, and in a xenograft model.[16-18] In two recent reports, ATG as part of a reduced intensity conditioning regimen for patients with multiple myeloma was found to be associated with a higher rate of complete remission and lower probability of relapse compared to the effects of alemtuzumab.[28,29] Since the incidence of acute GvHD was lower in patients who received alemtuzumab, the lower remission and higher relapse rates could at least in part be explained by the abrogation of the graft-versus-myeloma effect.

Within two prospective trials that used the same melphalan/fludarabine-based conditioning regimens, we compared the effect of incorporation of ATG-Fresenius®. Response to treatment was evaluated on day 100 to minimize the impact of the graft-versus-myeloma effect. Our results show higher rates of overall response (p=0.03) and complete response (p=0.04) in the patients treated with ATG. Furthermore our data indicate an ATG-dose-dependent effect with respect to both overall response (p=0.02) and complete response (p=0.08). At the same time, the incidences of acute grade II-IV GvHD and chronic GvHD in the ATG group were much lower than would otherwise be expected following allografting from unrelated donors[30] as well as in comparison with the incidences in the no-ATG group, thus confirming the expected potent immunosuppressive effect. An analysis of the subgroup of patients who were grafted from a sibling donor expectedly confirmed the reduction in the incidence of chronic GvHD but also showed a trend to better response in those patients who received ATG, even though these patients all received ATG ≤ 30 mg/kg. We are conscious of the retrospective nature of this study and the fact that prognostic factors such as β_2-microglobulin and cytogenetics are not well accounted for. These factors, as much as the fact that the no-ATG group contained more patients who had relapsed after prior autografting, as has been reported before,[20,21] would be expected to affect progression-free survival, overall survival and relapse but not response rates.

In *in vitro* studies the cytotoxic effect of both ATG-Fresenius® and Thymoglobulin® were observed at concentrations above 100 μg/mL, with IC_{50} at about 250 μg/mL and above.[16,17] Such concentrations of ATG can only be routinely expected *in vivo* when doses higher than 20 mg/kg body weight are administered.[31,32] The doses of ATG-Fresenius® are generally of this magnitude while doses of Thymoglobulin® are usually much lower (4-12.5 mg/kg). Our results are not, therefore, in contrast to those of the EBMT study which reported a lower response and lower event-free survival among recipients of ATG, because Thymoglobulin® is the most commonly used ATG preparation and the EBMT study did not distinguish between the two ATG preparations.[32] Although it may not be possible to assess the quantity of functional ATG *in vivo* directly, the concentration of ATG in serum has been shown to be dose-dependent and correlates with functional effects. [33] Doses of ATG that only produce concentrations up to 100 μg/mL may thus induce potent T-cell-depletion and a consequent weakening of the graft-versus-myeloma effect without any anti-myeloma effect. Doses ensuring ATG concentrations above 250 μg/mL would, on the other hand, be expected to induce both T-cell-depletion and potent anti-myeloma cytotoxicity, which would compensate for a weakened graft-versus-myeloma effect. It should be noted that the results of this study are restricted to antilymphocyte globulin (ATG-Fresenius®) and not to other available ATG preparations, which might have different effects on myeloma cells as well as on T-cell-depletion at the concentrations used in stem cell transplantation. It is possible that other ATG preparations that are given at lower doses, such as Thymoglobulin®, have less *in vivo* anti-myeloma activity, but still a strong T-cell-depleting effect, which could weaken the graft-versus-myeloma effect. This would explain the low response rate and the high relapse rate observed after dose-reduced allografting from HLA-identical siblings in the IFM 99 trial in which 12.5 mg/kg thymoglobulin was included in the preparative regimen.[6]

Administration of ATG shortly before allogeneic

transplantation ensures T-cell-depletion in the recipient (to facilitate engraftment) and, given its long *in vivo* half-life, T-cell-depletion of the graft to reduce the incidence of severe GvHD.[25,31,34-36] The current results suggest beneficial effects of ATG in terms of myeloma cytotoxicity as well as reduction of the incidence of chronic GvHD, without obvious abrogation of the graft-versus-myeloma effect. This is of importance since previous studies suggested a negative correlation between the occurrence of chronic GvHD and the risk of relapse.[20,21] In conclusion, our results may help development of future treatment protocols for patients with myeloma.

Authorship and Disclosures

FA: wrote the manuscript, analyzed the data and gave final approval of the manuscript; JAP-S, AS, AS, TZ, RS, RM, HGS, AA, J-JL, DA, CW, AN, ARZ: analyzed the data, approved the final version of the manuscript, acquires of data; JFSM: analyzed and interpreted the data, approved the final version of the manuscript, acquired data; NK: designed the study, wrote the study, analyzed and interpreted the data.

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