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Comparison between antithymocyte globulin and alemtuzumab and the possible impact of KIR-ligand mismatch after dose-reduced conditioning and unrelated stem cell transplantation in patients with multiple myeloma

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Summary

We compared antithymocyte globulin (ATG) with alemtuzumab in 73 patients with multiple myeloma, who underwent reduced conditioning with melphalan/fludarabine, followed by allogeneic stem cell transplantation from human leucocyte antigen-matched or -mismatched unrelated donors. The ATG group had more prior high-dose chemotherapies ($P < 0{\cdot}001$), while bone marrow was used more as the stem cell source in the alemtuzumab group ($P < 0{\cdot}001$). Alemtuzumab resulted in faster engraftment of leucocytes ($P = 0{\cdot}03$) and platelets ($P = 0{\cdot}02$) and in a lower incidence of acute graft *versus* host disease (GvHD) grades II–IV (24% vs. 47%, $P = 0{\cdot}06$). More cytomegalovirus (CMV) seropositive patients in the alemtuzumab group experienced CMV reactivation (100% vs. 47%, $P = 0{\cdot}001$). The cumulative incidence of treatment-related mortality at 2 years was 26% [95% confidence interval (CI) = 12–37%] for ATG vs. 28% (95% CI = 15–55%) for alemtuzumab, $P = 0{\cdot}7$. There was no significant difference in the estimated 2-year overall and progression-free survival between ATG and alemtuzumab: 54% (95% CI: 39–75%) vs. 45% (95% CI: 28–73%) and 30% (95% CI: 16–55%) vs. 36% (95% CI: 20–62%) respectively. In multivariate analysis, treatment with alemtuzumab had a higher risk for relapse (hazard ratio: 2·37; $P = 0{\cdot}05$) while killer immunoglobulin-like receptor (KIR)-ligand mismatch was protective for relapse ($P < 0{\cdot}0001$). We conclude that alemtuzumab produced less acute GvHD, but higher probability of relapse. The data implicated a major role of KIR-ligand mismatched transplantation in multiple myeloma.

Keywords: allogeneic stem cell transplantation, alemtuzumab, antithymocyte globulin, multiple myeloma, killer immunoglobulin-like receptor ligand.

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Non-myeloablative or dose-reduced stem cell transplantation is a promising treatment approach in patients with haematological malignancies, who are not eligible for a standard conditioning procedure, because of age, performance status or active infection. Several reduced-toxicity regimens have been investigated for haematological malignancies and solid tumours, whose antitumour effect relies on a graft *versus* malignancy effect rather than on chemotherapy effects (Slavin *et al*, 1998; Childs *et al*, 2000; Giralt *et al*, 2001; McSweeney *et al*, 2001). The use of unrelated donors in standard myeloablative transplantation is associated with a higher rate of graft failure, higher incidence of graft *versus* host disease (GvHD) and more toxicity in comparison with sibling transplantation, limiting this approach to younger patients with a good performance status (McGlave *et al*, 1993). Several dose-reduced regimens, including low-dose total body irradiation (Maris *et al*, 2003), fludarabine and melphalan (Giralt *et al*, 2001) or busulphan (Nagler *et al*, 2001) have been used with unrelated donors and minimal regimen-related extramedullary toxicity has been observed. However, one trial using the melphalan/fludarabine regimen, reported an incidence of severe acute grade III/IV GvHD of 39% and a high treatment-related mortality (TRM) because of GvHD (Giralt *et al*, 2001). Therefore, strategies to prevent high TRM by reducing acute GvHD without abrogating the graft *versus* malignancy effect are needed. A high rate of graft-failure was observed when T cells were depleted from the graft by $CD34^+$ cell selection (Bornhauser *et al*, 2002); the use of *in vivo* T-cell depletion strategies as part of the conditioning regimen may enhance engraftment by T-cell depletion of the recipient. As a result of the long half-life of depleting antibodies, T-cell depletion of the graft will occur '*in vivo*', resulting in a lower rate of severe GvHD. Antithymocyte globulin (ATG) as well as alemtuzumab (Campath-1H) have been shown to ensure allogeneic engraftment with a low rate of severe GvHD after dose-reduced conditioning followed by matched and mismatched unrelated stem cell transplantation (Nagler *et al*, 2001; Chakraverty *et al*, 2002; Kroger *et al*, 2002a). Because allogeneic stem cell transplantation seems to be a curative treatment option in patients with multiple myeloma, reduced-intensity conditioning followed by allogeneic stem cell transplantation has become a commonly used treatment approach in these patients (Badros *et al*, 2001; Kroger *et al*, 2002b; Maloney *et al*, 2003; Peggs *et al*, 2003). However, the experience of allogeneic stem cell transplantation from unrelated donors in multiple myeloma is still limited (Kroger *et al*, 2002a; Peggs *et al*, 2003; Shaw *et al*, 2003) and further studies are necessary to determine the optimal conditioning regimen and GvHD prophylaxis. In the current retrospective analysis, we compared the results in patients with multiple myeloma who were transplanted from human leucocyte antigen (HLA)-matched or -mismatched unrelated donors in two prospective studies, which were carried out in the UK and in Germany or Israel respectively. Both studies were based on the melphalan/fludarabine regimen, but the UK study used alemtuzumab plus ciclosporin A (CSA) for GvHD prophylaxis, whilst the German study used ATG (Anti-rabbit, Fresenius, Bad Homburg, Germany) plus CSA and methotrexate. Furthermore, we investigated the potential influence of killer immunoglobulin-like receptor (KIR)-ligand mismatch in GvH-direction on outcome.

Patients and methods

We analysed a total of 73 patients who received ATG ($n = 48$) or Campath 1-H ($n = 25$). The major inclusion criteria for both protocols were ineligibility for a standard unrelated stem cell transplantation because of age (>45 years), prior high-dose regimen, severe organ dysfunction, active fungal infection or reduced performance status. Major exclusion criteria were cardiac insufficiency with an ejection fraction of <30%, liver transaminases greater than three times the upper limit of normal, creatinine clearance <30 ml/min. Both studies were approved by the local ethics committees and all patients gave written informed consent. Unrelated donor selection was performed using serological typing for class I antigen (HLA-A and HLA-B) and molecular typing for HLA-DRB1 and HLA-DQB1. To determine KIR-ligand mismatch, molecular typing for HLA-A, B and HLA-C was also performed. KIR-ligand mismatch was defined as described by Ruggeri *et al* (2002). KIR-ligand incombatibility was determined by the absence of one donor KIR-ligand class I allele in the recipient. Because KIR2DL1 recognizes HLA-Cw4-related alleles, KIR2DL2/3 HLA-Cw3-related alleles and KIR3DL1 HLA-B alleles sharing the Bw4 specificity, these receptors were taken into consideration. Eight patients received stem cell transplantation from a KIR-incompatible donor with KIR-ligand mismatch in GvH direction. All KIR-ligand incompatibility involved the HLA-C locus.

Chimaerism studies were performed by means of fluorescence *in situ* hybridization for sex-mismatched transplantation or by microsatellite polymerase chain reaction (PCR) or PCR to amplify DNA sequences that are specific for the Y-chromosome as described recently (Kottaridis *et al*, 2000; Fehse *et al*, 2001). Patients with mixed haematopoietic chimaerism and residual disease 6 months after transplantation were candidates for a donor-lymphocyte infusion in both protocols. Unrelated donors gave written informed consent according to the national standards and procedures of the relevant registries.

Patients characteristics

The patients' major clinical characteristics are listed in Table I. Most of the patients had advanced disease, and 50% of the ATG group and 52% of the alemtuzumab group had experienced relapse after a prior autograft. More patients in the ATG group had prior autologous stem cell transplantations ($P < 0{\cdot}001$). More patients in the ATG group than in the alemtuzumab group received peripheral blood stem cells as a

Table I. Patients' characteristics.

	Antithymocyte globulin	Alemtzumab	*P*-value
Number of patients	48	25	
Median age (years)	50 (range, 32–62)	47 (range, 33–60)	0·5
Patient sex			
Male [*n* (%)]	31 (65)	21 (84)	0·08
Female [*n* (%)]	17 (35)	4 (16)	
Type			
IgG [*n* (%)]	21 (44)	15 (60)	0·5
IgA [*n* (%)]	10 (20)	5 (20)	
Light chain [*n* (%)]	13 (27)	5 (20)	
Non-secretory [*n* (%)]	3 (6)	–	
Plasmacell-leukaemia [*n* (%)]	1 (2)	–	
Median prior chemotherapy regimens	3 (range, 1–12)	2 (range, 1–4)	0·03
Prior autologous transplants			
None [*n* (%)]	–	10 (40)	<0·001
One [*n* (%)]	36 (75)	15 (60)	
Two [*n* (%)]	12 (25)	–	
Relapse to prior autologous high-dose therapy [*n* (%)]	24 (50)	13 (52)	0·8
No relapse to standard or high-dose chemotherapy [*n* (%)]	18 (37)	9 (36)	0·8
Recipient			
CMV-positive [*n* (%)]	35 (73)	14 (56)	0·2
CMV-negative [*n* (%)]	13 (28)	11 (44)	
Graft source			
PBSC [*n* (%)]	39 (81)	7 (28)	<0·001
BM [*n* (%)]	9 (19)	18 (72)	
Status prior to allo-SCT			
CR/PR [*n* (%)]	29 (60)	17 (68)	0·5
NC/PD [*n* (%)]	19 (40)	8 (31)	
Time from diagnosis to SCT >24 months [*n* (%)]	22 (48)	12 (48)	0·9
HLA-mismatch [*n* (%)]	5 (10)	5 (20)	0·3
KIR-ligand-mismatch GvH-direction [*n* (%)]	7 (15)	1 (5)	0·4
Median follow-up: days (range)	554 (range, 381–727)	945 (range, 484–1406)	0·18

CMV, cytomegalovirus; PBSC, peripheral blood stem cells; BM, bone marrow; allo-SCT, allogeneic stem cell transplantation; CR, complete remission, PR, partial remission; NC, no change; PD, progressive disease.

graft-source (81% vs. 28%, $P < 0{\cdot}001$). No difference between donor sex, HLA-mismatch, remission status prior to allogeneic stem cell transplantation, cytomegalovirus (CMV) serostatus of the recipient or age was observed between the two groups. KIR-ligand mismatch in GvH-direction was observed in seven patients of the ATG group and in one patient of the alemtuzumab group ($P = 0{\cdot}42$). The median follow-up was slightly shorter in the ATG group (median: 554 vs. 945 d, $P = 0{\cdot}18$).

Conditioning regimen

In both trials, the conditioning regimen consisted of melphalan and fludarabine. In the UK study melphalan (140 mg/m^2) was given intravenously on day −2, while fludarabine (150 mg/m^2) was given intravenously divided over 5 d, from day −7 to −3. In the German study, a median melphalan dose of 140 mg/m^2 (range 100–150 mg/m^2) was given intravenously divided on day −3 and −2, while a median fludarabine dose of 120 mg/m^2 (range, 90–180 mg/m^2) was given intravenously between day −7 to −3 and −5 to −3.

GvHD prophylaxis

In the UK study, GvHD prophylaxis was with alemtuzumab, 100 mg in total, given intravenously as 20 mg/d for 5 d (days −8 to −4). Further GvHD prophylaxis consisted of CSA in both groups, starting on day −1 at 3 mg/kg as intravenous infusion and switched to an equivalent oral dose as soon as possible. CSA was reduced on day +100 and discontinued

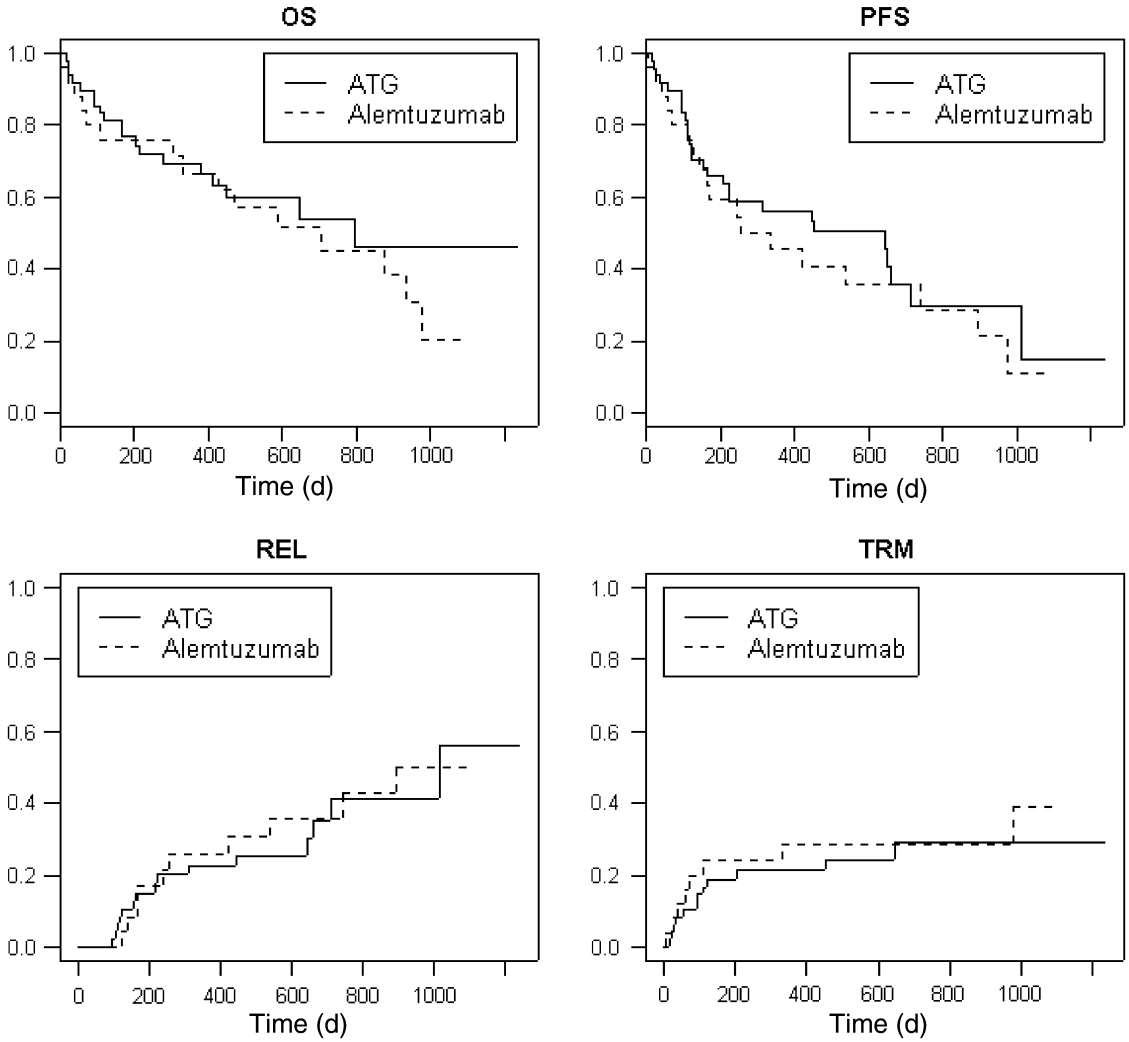

Fig 1. Overall survival, progression-free survival, relapse-incidence and treatment-related mortality of all patients according to the treatment with antithymocyte globulin ($n = 48$) or alemtuzumab ($n = 25$).

between days +140 and +180, if no GvHD had occurred. In the German study, ATG (Anti-rabbit) was used at a median dose of 60 mg/kg (range, 30–60 mg/kg), divided intravenously over 12 h on day −3 to −1. Furthermore, all patients received methotrexate, (10 mg/m^2) intravenously on days +1, +3 and +6. Acute and chronic GvHD was graded according to the international standard criteria (Shulman *et al*, 1980; Przepiorka *et al*, 1995).

Supportive care

All patients were nursed in reverse isolation in conventional or laminar airflow rooms. Prophylaxis against *pneumocystis carinii* was carried out with cotrimoxazole or pentamidine inhalation. Acyclovir and fluconazole or itraconazole prophylaxis was routinely used in all patients. CMV-negative patients received only CMV-negative blood products. All blood products were irradiated to 25 Gy. Febrile neutropenic fever was treated with broad-spectrum antibiotics according to the centres' policy for treatment of neutropenic fever. CMV-positive recipients were monitored at least weekly for CMV infection by PCR within the UK study and by PCR or/and antigenaemia-assay within the German study. Pre-emptive therapy was started with 10 mg/kg gancyclovir per day after two consecutive positive PCR results or one positive antigenaemia assay.

Statistical methods

Differences in the distributions of the risk factors in different groups was evaluated by the chi-squared test on the appropriate cross-tabulation for discrete variables and by the Mann–Whitney test for continuous variables. Probabilities of overall survival (OS) and progression-free survival (PFS) were estimated according to the Kaplan–Meier product limit method and differences among groups were tested by the

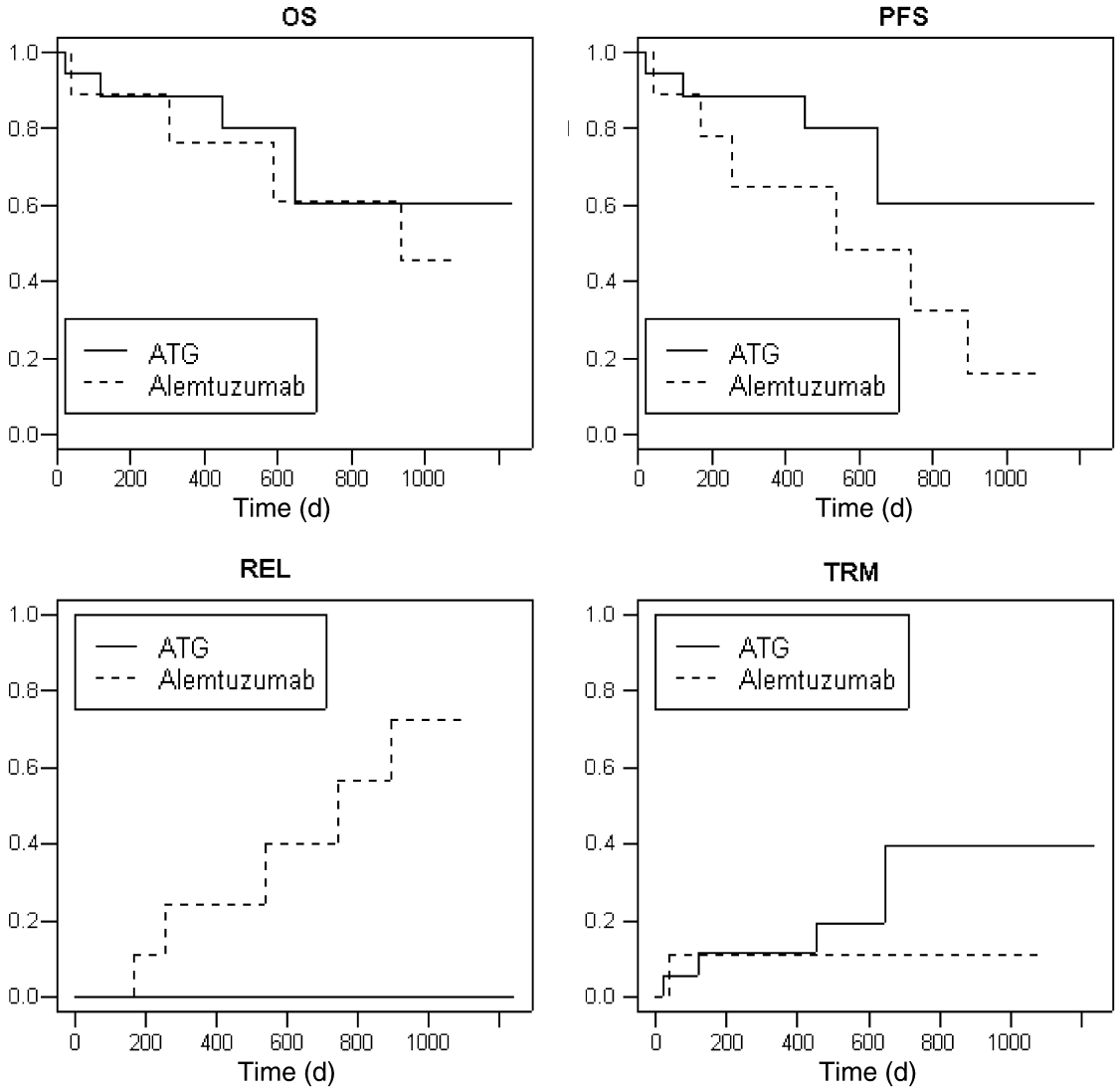

Fig 2. Overall survival, progression-free survival, relapse-incidence and treatment-related mortality according to the treatment with antithymocyte globulin ($n = 18$) or alemtuzumab ($n = 9$) in patients without any relapse prior to allogeneic stem cell transplantation.

log-rank test. The multivariate analysis for OS and PFS was carried out using the Cox proportional hazards model. Estimation of relapse and TRM incidence was performed using the proper estimator of cumulative incidence curves (Gooley *et al*, 1999) and the comparison was made by the Gray test (Gray, 1988). The Fine and Gray model for cumulative incidence was used to assess adjusted effects in multivariate analysis (Fine & Gray, 1999). The same methodology was used to compare engraftment, acute GvHD and chronic GvHD, taking into account their timing and the occurrence of death as a competing risk, in addition to the more traditional comparison of total percentages only. For the multivariate analysis, all variables with *P*-values from the univariate analysis below 0·2 were considered as potential prognostic factors, and the selection was carried out by applying a backward procedure.

The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) versions 11·0·1 (2001) and R 1·6·2 (2003); in particular, the analyses in the framework of competing risks were carried out using the additional R package 'CMPRSK' developed by Gray, version 2 1–2 (2000).

Results

Engraftment

No primary graft failure occurred in either group. Two patients in the ATG group died prior to engraftment. Achievement of a sustained leucocyte count $>1 \times 10^9$/l for three consecutive days was faster in the alemtuzumab than in the ATG group (median: 13 vs. 15 d, $P = 0{\cdot}003$) and the same was true for sustained platelet engraftment $>20 \times 10^9$/l with-

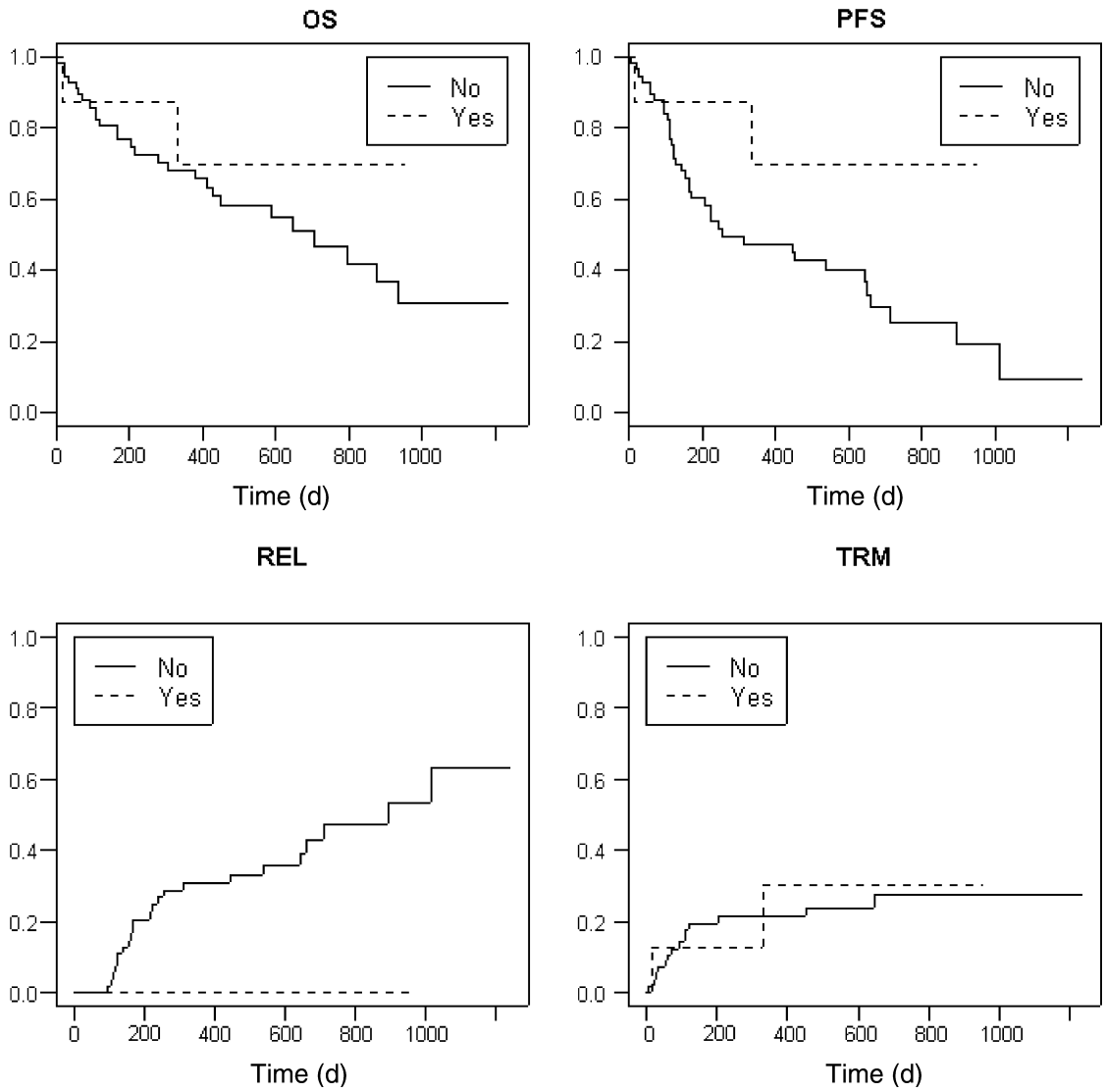

Fig 3. Overall survival, progression-free survival, relapse-incidence and treatment-related mortality according to the killer immunoglobulin-like receptor ligand mismatch in graft *versus* host-direction (yes: $n = 8$; no: $n = 57$).

out platelet transfusion (median: 13 vs. 19 d, $P = 0{\cdot}002$) (Table II)

Response and donor-lymphocyte infusion

A total of 94% of the patients in the ATG group and 90% of the patients of the alemtuzumab group showed an objective response (complete or partial remission) after allograft ($P = 0{\cdot}4$). No change or minor response was observed in two patients in each treatment group. However, the rate of complete remission after allograft was higher in the ATG group than in the alemtuzumab group (51% vs. 19%, $P = 0{\cdot}01$). Full chimaerism at day 100 was observed in all but one patient in the ATG group, while five patients of the alemtuzumab group achieved only mixed chimaerism. Patients with residual disease or/and mixed chimaerism were eligible for donor lymphocyte infusion (DLI): seven patients of the ATG group and seven patients of the alemtuzumab group received pre-emptive DLIs, usually starting with 1×10^6 $CD3^+$ cells/kg with dose escalations at 10–12-week intervals (Table II).

Graft versus host disease

Patients receiving alemtuzumab had a slightly lower incidence of acute GvHD grades II–IV (24% vs. 47%, $P = 0{\cdot}06$) than patients who received ATG. Using death within day 100 as a competing risk, the difference was statistically significant ($P = 0{\cdot}05$). For severe grade III/IV acute GvHD, the cumulative incidence for ATG at day 100 was 13% and for alemtuzumab 0% ($P = 0{\cdot}05$). The incidence of chronic GVHD after alemtuzumab was 25% in comparison with 36% after ATG ($P = 0{\cdot}4$). After additional treatment with DLIs because

Table II. Results comparing antithymocyte globulin and alemtuzumab.

	Antithymocyte globulin	Alemtuzumab	*P*-value
Number of patients	48	25	
Leucocyte count $>1 \times 10^9$/l, median day (range)	15 (10–23)	13 (3–22)	0·003
Platelet count $<20 \times 10^9$/l median day (range)	19 (9–43)	13 (9–23)	0·002
PR/CR after allograft (%)	95	90	0·4
Complete remission after allograft (%)	51	19	0·01
Acute GvHD grades II–IV [*n* (%)]	22 (47)	6 (24)	0·05
Acute GvHD grades III–IV [*n* (%)]	6 (13)	0	0·05
Acute GvHD grades II–IV after DLI [*n* (%)]	22 (47)	8 (32)	0·2
Chronic GvHD [*n* (%)]	13 (36)	5 (25)	0·4
Chronic GvHD after DLI [*n* (%)]*	13 (33)	9 (45)	0·4
CMV-reactivation [only CMV-positive recipients (ATG: $n = 34$; alemtuzumab: $n = 12$)] [*n* (%)]	16 (47)	12 (100)	0·001
Cumulative incidence of treatment-related mortality at 1 year (%)	26 (95% CI: 12–37)	28 (95% CI: 15–55)	0·7
Estimated overall survival at 2 years (%)	54 (95% CI: 39–75)	45 (95% CI: 28–73)	0·5
Estimated progression-free survival at 2 years (%)	30 (95% CI: 16–55)	36 (95% CI: 20–62)	0·5
Cumulative incidence of relapse at 2 years (%)	41 (95% CI: 26–66)	29 (95% CI:19–63)	0·9

*Only for mixed chimaerism and residual disease.
PR, partial remission; CR, complete remission; CMV, cytomegalovirus; GvHD, graft *versus* host disease.

of persistent disease or mixed haematopoietic chimaerism ($n = 7$ in each group) the incidence of acute GvHD grades II–IV between alemtuzumab- and ATG-treated patients was similar (32% vs. 47%, $P = 0{\cdot}2$) (Table II). The difference of acute GvHD between ATG and alemtuzumab was mainly because of the patients transplanted with bone marrow showing a higher incidence after ATG-treatment, while those patients who received peripheral blood stem cells showed no clear difference in acute GvHD between the two treatment groups. In the multivariate analysis, alemtuzumab was protective for acute GvHD [hazard ratio (HR): 0·5, 95% confidence interval (CI) = 0·21–1·20], but was not statistically significant ($P = 0{\cdot}12$). The only significant factor for developing acute GvHD was female gender of the recipient (HR:2·88, 95% CI = 1·33–6·26; $P = 0{\cdot}007$).

CMV infection

Among the CMV-positive patients the incidence of CMV reactivation was higher in patients treated with alemtuzumab than with ATG (100% vs. 47%, $P = 0{\cdot}001$). However, no direct CMV-related mortality was observed.

Treatment-related mortality

No difference in the cumulative incidence of TRM at 1 year between the alemtuzumab and the ATG group (28% vs. 26%, $P = 0{\cdot}7$) was found. While four patients in the ATG group died from acute GvHD and related complications, only one patient in the alemtuzumab group expired because of acute GvHD after DLI. In contrast, a higher proportion of patients in the alemtuzumab group died of infectious complications ($5/8 = 62\%$) in comparison with ATG ($4/12 = 33\%$; $P = 0{\cdot}3$). In the univariate analysis, transplantation from a female donor to a male recipient *versus* others ($P = 0{\cdot}001$) and stem cell transplantation for >2 years from diagnosis ($P = 0{\cdot}04$) were significant factors for a higher TRM (Table III). In the multivariate analysis, female donor to male recipient (HR: 4·42, $P = 0{\cdot}006$) and interval of >2 years between diagnosis and stem cell transplantation (HR: 2·75, $P = 0{\cdot}04$) were significant risk factors for a higher TRM (Table III).

Overall survival

The estimated 2-year OS was 54% (95% CI:39–75%) for the ATG group and 45% (95% CI: 28–73%) for the alemtuzumab group ($P = 0{\cdot}5$). In a univariate analysis including all patients, a worse survival was seen for patients with relapse after autograft (HR: 2·87, $P = 0{\cdot}004$), relapse after any prior therapy (HR: 2·48, $P = 0{\cdot}02$) and for male patients with female donors (HR: 2·3, $P = 0{\cdot}02$). OS was better in patients who were in partial or complete remission prior to allogeneic stem cell transplantation (HR: 0·47, $P = 0{\cdot}03$) (Table IV). In a multivariate analysis (Table V) relapse after prior high-dose chemotherapy (HR: 2·89, $P = 0{\cdot}005$) and male patient with female donor (HR: 2·34, $P = 0{\cdot}03$) were significant factors for worse survival after allogeneic stem cell transplantation. However, the gender mismatch interacted with ATG/alemtuzumab treatment: male patients with female donors was only significant for ATG treatment (HR: 4·11, $P = 0{\cdot}004$), but not in those treated with alemtuzumab (HR: 1·9, $P = 0{\cdot}3$).

Progression-free survival

The estimated 2-year PFS was 30% (95% CI: 16–55%) for the ATG group and 36% (95% CI: 20–62%) for the alemtuzumab group ($P = 0{\cdot}5$). If only patients without relapse to any

Table III. Cumulative incidence of relapse and treatment-related mortality (univariate).

	Relapse-rate (2 years) (%)	*P*-value	TRM (1 year) (%)	*P*-value
Treatment				
ATG	41	0·9	26	0·7
Alemtuzumab	29		28	
Relapsed to autograft				
No	29	0·05	14	0·1
Yes	47		33	
Relapse to any therapy				
No	15	0·04	11	0·2
Yes	50		31	
No. of prior high-dose chemotherapies				
One	22	0·8	30	0·9
Two	43		20	
Three	46		33	
HLA-status				
Matched	43	0·09	21	0·3
Mismatched	0		44	
Status at transplantation				
CR/PR	33	0·7	18	0·2
MR/NC/PD	45		34	
Recipient gender				
Female	58	0·003	10	0·1
Male	29		29	
Gender-mismatch				
Female to male	6	0·005	57	0·0006
Others	48		13	
Recipient's CMV-status				
Positive	28	0·1	26	0·4
Negative	56		21	
Graft source				
Peripheral blood stem cells	44	0·7	26	0·7
Bone marrow	36		23	
KIR-ligand mismatch				
No	47	0·05	22	0·9
Yes	0		30	
Interval between diagnosis and SCT				
<2 years	35	0·6	14	0·04
>2 years	41		35	
Age				
<50 years	45	0·5	18	0·2
<50 years	29		30	
No. of prior chemotherapy regimens				
One	20	0·3	22	0·4
Two	38		6	
Three	50		32	

TRM, treatment-related mortality; CR, complete remission, PR, partial remission; MR, minimal response; NC, no change; PD, progressive disease; SCT, stem cell transplantation; CMV, cytomegalovirus.

Table IV. Univariate analysis for overall and progression-free survival.

	OS (2 years) (%)	*P*-value	PFS (2 years) (%)	*P*-value
Relapsed to autograft				
Yes	31	0·004	20	0·001
No	68		48	
No Relapse to any therapy				
No	64	0·02	59	0·002
Yes	41		19	
HLA-mismatched				
Yes	56	0·8	56	0·6
No	49		30	
KIR-ligand mismatch (GvH-direction)				
Yes	70	0·3	70	0·12
No	47		25	
Status at transplantation				
CR/PR vs.	60	0·03	40	0·08
Others	33		21	
Recipient: donor				
Recipient male/donor: female vs.	37	0·02	33	0·4
Others	54		37	
Recipient				
Male vs.	49	0·6	37	0·4
Female	54		26	
Recipient's CMV-status				
Negative vs.	48	0·8	23	0·5
Positive	51		38	
Graft source				
BM vs.	44	0·4	35	0·4
PBSC	53		27	
Treatment				
ATG vs.	54	0·5	30	0·5
Alemtuzumab	45		36	
Age				
<50 years	57	0·4	34	0·5
>50 years	38		32	
Interval between diagnosis and SCT				
<24 months	51	0·3	45	0·08
>24 months	47		21	
No. of prior chemotherapy regimens				
One	52	0·06	49	0·01
Two	67		45	
Three or more	42		18	
No. of prior high-dose chemotherapies				
One	60	0·6	48	0·7
Two	48		29	
Three	40		21	

OS, overall survival; PFS, progression-free survival; CMV, cytomegalovirus; BM, bone marrow; PBSC, peripheral blood stem cells; SCT, stem cell transplantation

therapy prior to allograft are included, the risk of progression/relapse or death to any cause was higher in the alemtuzumab group than in the ATG group, but was not statistically significant (HR: 2·5, $P = 0·16$). In a univariate analysis including all patients, a worse PFS was observed for patients with relapse after a prior autograft (HR: 2·86, $P = 0·001$) or relapse after any therapy prior allogeneic stem cell transplantation (HR: 3·07, $P = 0·002$) or three or more chemotherapy

Table V. Multivariate analysis of overall and progression-free survival, relapse and treatment-related mortality (besides treatment effect of alemtuzumab only factors with *P*-value <0·1).

	OS		PFS		Relapse		TRM	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Relapse to prior high-dose therapy	HR: 2·89 (95% CI:1·39–6·03)	0·005	HR: 2·84 (95% CI: 1·52–5·31)	0·001	HR: 2·07 (95% CI: 0·90–4·79)	0·09	–	–
Gender mismatch (female to male)	HR: 2·34 (95% CI: 1·1–4·96)	0·03	–	–	–	–	HR: 4·42 (95% CI: 1·90–10·28)	0·0006
HLA-mismatch	–	–	–	–	HR: 0·17 (95% CI: 0·04–0·65)	0·01	–	–
KIR-ligand mismatch	–	–	–	–	HR: 0	<0·0001	–	–
Female gender	–	–	–	–	HR: 5·52 (95% CI: 2·23–13·66)	0·0002	–	–
Time from diagnosis to SCT >2 years	–	–	–	–	–	–	HR: 2·75 (95% CI: 1·03–7·36)	0·04
Treatment with alemtuzumab	HR: 1·05 (95% CI: 0·52–2·12	0·9	HR:1·15 (95% CI: 0·63–2·12)	0·6	HR: 2·37 (95% CI: 0·99–5·65)	0·05	HR: 1·13 (95% CI: 0·47–2·70)	0·8

OS, overall survival; PFS, progression-free survival; TRM, treatment-related mortality; SCT, stem cell transplantation.

regimens (HR: 1·65, $P = 0·01$) (Table IV). In the multivariate analysis, only relapse after prior high-dose chemotherapy (HR: 2·9, $P = 0·001$) was a significant factor for worse PFS after allogeneic stem cell transplantation (Table V).

Relapse

No significant difference was observed in the cumulative relapse incidence at 2 years between the ATG group and the alemtuzumab group [41% (95% CI: 26–66%) vs. 36% (95% CI: 19–63%), $P = 0·9$]. If only patients without any relapse prior to allografting were analysed the risk of progression/relapse was higher in the alemtuzumab group than in the ATG group (40% vs. 0%, $P = 0·004$). In a univariate analysis of all patients, male patients ($P = 0·003$), gender mismatch (female to male, $P = 0·005$), KIR-ligand mismatch in GvH-direction ($P = 0·048$) were protective against relapse, while relapse after any chemotherapy ($P = 0·035$) or high-dose chemotherapy prior to allografting ($P = 0·05$) were associated with a higher incidence of relapse. In a multivariate analysis using the Fine and Gray model, treatment with alemtuzumab (HR: 2·37, 95% CI: 0·99–5·65, $P = 0·05$) and female gender (HR: 5·52, 95% CI: 2·23–13·66, $P = 0·0002$) were risk factors for relapse, while HLA-mismatch (HR: 0·17, 95% CI: 0·04–0·65:, $P = 0·01$) and KIR-ligand mismatch (HR: 0, $P < 0·0001$) were associated with reduced relapse incidence.

Discussion

T-cell depletion is the most effective strategy for prevention of GvHD, but it is associated with an increased incidence of graft failure and relapse after stem cell transplantation (Apperley *et al*, 1988; Marmont *et al*, 1991). Non-myeloablative or dose-reduced conditioning regimens followed by HLA-identical sibling allogeneic stem cell transplantation have become an attractive treatment approach with low TRM for patients with haematological malignancies who are not eligible for a standard conditioning, but when unrelated donors are used a high treatment-associated morbidity and mortality are found. Recently, the MD Anderson Cancer Centre reported a TRM of 55% after dose-reduced conditioning with melphalan and purine analog followed by unmanipulated stem cell transplantation from unrelated donors. The incidence of severe grade III/IV acute GvHD was 39% and was responsible for death in 27% of the patients (Giralt *et al*, 2001). The use of *ex vivo* T-cell depletion by $CD34^+$ cell selection to reduce the incidence of severe GvHD was associated with an unacceptably high rate of primary graft failure (Kreiter *et al*, 2001; Bornhauser *et al*, 2002). Therefore, antibody-therapy within the conditioning regimen is an attractive approach to ensure engraftment and to lower the GvHD-associated mortality after dose-reduced conditioning and unrelated stem cell transplantation. In the current retrospective study, we compared the efficacy and outcome of two *in vivo* T-cell depletion strategies, which were used in two prospective dose-reduced conditioning studies in patients with multiple myeloma who were transplanted from an HLA-matched or -mismatched unrelated donor. Both agents, alemtuzumab (Campath-1H) and ATG, induced marked T-cell depletion in the recipient and ensured rapid engraftment and, because of the long half-life, an *in vivo* T-cell depletion of the graft reduced the incidence of severe GvHD (Eiermann *et al*, 1999; Nagler *et al*, 2001; Bacigalupo *et al*, 2002; Chakraverty *et al*, 2002; Kroger *et al*, 2002a; Finke *et al*, 2003; Morris *et al*, 2003a). After 100 mg alemtuzumab relevant serum levels were detectable for approximately 56 d after allogeneic stem cell transplantation, and after 90 mg/kg ATG (Anti-rabbit, Fresenius), detectable anti-Jurkat antibodies persisted up to 3 weeks after allogeneic stem cell transplanta-

tion(Eiermann *et al*, 2001; Morris *et al*, 2003b). Alemtuzumab is a humanized monoclonal antibody against human CD52, an antigen which is expressed on T, B and natural killer (NK) cells, but not on haematopoietic cells (Hale *et al*, 2001). In contrast, ATG consists of polyclonal rabbit immunoglobulins. After immunization with cells from a T lymphoblast cell line (Jurkat T-cell line), this highly purified immunoglobulin consists of antibodies exerting a direct effect towards lymphoblastic T cells, resulting in T-cell depletion via opsonization and lysis following complement activation.

In both treatment arms, the incidence of acute and chronic GvHD was rather low but there was a significantly lower incidence of acute GvHD after alemtuzumab in comparison with ATG. This could be due to the broader *in vivo* T-cell depletion as ATG-Fresenius is only active against activated T cells, but could be also caused by the depletion of host antigen-presenting cells, such as B cells or dendritic cells, by alemtuzumab, as these cells have been shown to be relevant for incidence of acute GvHD(Buggins *et al*, 2002; Klangsinsirikul *et al*, 2002). There seems to be a dose-dependent effect for preventing acute GvHD, but appropriate dose-finding studies are lacking for both drugs. However, after DLI given for mixed chimaerism or to control disease after allografting, the rate of acute GvHD increased in the alemtuzumab group to 32% and no further significant difference in acute GvHD was observed between the two groups. This was in contrast to previous reports, which indicated only a modest incidence of GvHD after delayed DLI in mainly sibling donor transplants (Mackinnon *et al*, 1995; Naparstek *et al*, 1995). It has been recently shown that DLI from unrelated donors after dose-reduced conditioning induced a higher rate of acute GvHD despite the fact that the T-cell dose from unrelated donors was half-a-log less than that from related donors (Ayuk *et al*, 2004; Peggs *et al*, 2004). Although mismatched unrelated donors were included in both studies, no primary graft failure was observed in either group. The leucocyte and platelet engraftment was faster in the alemtuzumab group and was probably related to the additional methotrexate that the ATG arm received. A major difference in outcome between ATG and alemtuzumab was the rate of complete remission and the incidence of CMV reactivation. Both might be due to the more marked immunosuppressive effect of alemtuzumab, given at a dose of 100 mg, in comparison with ATG. These strong immunosuppressive effects may abrogate the graft *versus* myeloma effect and impair immune reconstitution after allogeneic stem cell transplantation. Unfortunately, no data regarding immune reconstitution were available for either group, but *in vitro* studies have shown impairment of NK-cell cytotoxicity by alemtuzumab, which could be a factor in the high incidence of CMV reactivation (Condiotti & Nagler, 1996). A recent retrospective study of HLA-identical sibling transplants comparing alemtuzumab to no *in vivo* T-cell depletion after melphalan/fludarabine based conditioning in lymphoid malignancies similar results were obtained with a lower rate of complete remission and a higher incidence of CMV reactivation observed for the patients of the alemtuzumab group (Perez-Simon *et al*, 2002). The strong immunosuppressive effect of alemtuzumab can also be found in the differing causes of TRM. While in the ATG group more GvHD and GvHD-related deaths were noted, only one patient in the alemtuzumab group expired from GvHD. In contrast, more of the patients in the alemtuzumab group died of infections.

A major concern for both strategies in stem cell transplantation of multiple myeloma remains the high relapse rate (>40% at 2 years) for the whole study population. The high relapse rate might be partly because of the heavily pretreated study population, with more than 66% of the patients in relapse. Therefore the study confirmed previous results, that relapse postautograft is a significant factor for a higher relapse rate after dose-reduced allograft (Kroger *et al*, 2004) and performing allogeneic stem cell transplantation earlier in the course of the disease, such as an auto-allo tandem approach, seems to be associated with a lower risk of relapse (Kroger *et al*, 2002b; Maloney *et al*, 2003). Overall, there was no difference regarding relapse rate between the two treatment groups, but patients in the ATG group had received more high-dose chemotherapy. In the univariate analysis no difference in relapse was found between ATG and alemtuzumab, but, because of the higher pretreatment in the ATG group, treatment with alemtuzumab had more than twofold higher risk for relapse (HR: 2·37) in the multivariate analysis ($P = 0{\cdot}05$).

A new and potentially important observation in allogeneic stem cell transplantation for multiple myeloma was the lower relapse rate for those patients transplanted with a stem cell graft that had KIR-ligand incompatibility in GvH direction, which has been so far only observed for patients with myeloid leukaemias (Ruggeri *et al*, 2002; Giebel *et al*, 2003). While no difference in TRM, OS and PFS was seen for patients with KIR-ligand incompatibility, a significant lower incidence of relapse was observed ($P = 0{\cdot}05$). Furthermore, no severe grade III/IV acute GvHD was observed in the KIR-ligand group in comparison with 10% of the non-KIR-ligand group, but, because of the low number of patients, this difference was not statistically significant (data not shown). Overall, because of the low number ($n = 8$) of patients with KIR-ligand incompatibility in the GvH direction, no definitive conclusion can be drawn so far, but these preliminary results may point to a role of NK-cells in multiple myeloma. Co-incubated NK cells with myeloma cell lines or bone marrow samples obtained from patients with multiple myeloma resulted in a strong cytotoxic effect even without exogenous interleukin 2 (Frohn *et al*, 2002). More recently, a strong anti-myeloma effect could be induced by NK cells in bone marrow-derived myeloma cells, while extramedullary disease in pleural effusion was resistant to NK cells in a major histocompatibility complex class I-dependent fashion (Carbone *et al*, 2004).

In conclusion, both strategies, incorporating either alemtuzumab or ATG within a dose-reduced conditioning to lower GvHD and TRM after unrelated stem cell transplantation in patients with multiple myeloma, were effective and

ensured engraftment, even in mismatched donors. The immunosuppressive effect of alemtuzumab, given at a dose of 100 mg, was stronger than ATG given at a median dose of 60 mg/kg, resulting in a significantly lower incidence of acute GvHD, but also in a lower rate of complete remissions. Alemtuzumab was associated with a higher incidence of CMV infection and virus-related mortality and had a higher risk of relapse in the multivariate analysis. The preliminary observation that KIR-ligand-mismatch resulted in a lower incidence of relapse, especially in those receiving ATG as part of their conditioning regimen, emphasizes the need for further study of the role of NK cells in multiple myeloma

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References

Apperley, J.F., Mauro, F.R., Goldman, J.M., Gregory, W., Arthur, C.K., Hows, J., Arcese, W., Papa, G., Mandelli, F. & Wardle, D. (1988) Bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: importance of a graft-versus-leukaemia effect. *British Journal of Haematology*, **69,** 239–245.

Ayuk, F., Shimoni, A., Nagler, A., Schwerdtfeger, R., Kiehl, M., Sayer, H.G., Zabelina, T., Zander, A.R. & Kroger, N. (2004) Efficacy and toxicity of low-dose escalating donor lymphocyte infusion given after reduced intensity conditioning allograft for multiple myeloma. *Leukemia*, **18,** 659–662.

Bacigalupo, A., Lamparelli, T., Gualandi, F., Bregante, S., Raiola, A.M., Di Grazia, C., Dominietto, A., Bruno, B., Galbusera, V., Frassoni, F., Podesta, M., Tedone, E., Occhini, D. & Van Lint, M.T. (2002) Prophylactic antithymocyte globulin reduces the risk of chronic graft-versus-host disease in alternative-donor bone marrow transplants. *Biology of Blood and Marrow Transplantation*, **8,** 656–661.

Badros, A., Barlogie, B., Morris, C., Desikan, R., Martin, S.R., Munshi, N., Zangari, M., Mehta, J., Toor, A., Cottler-Fox, M., Fassas, A., Anaissie, E., Schichman, S., Tricot, G. & Aniassie, E. (2001) High response rate in refractory and poor-risk multiple myeloma after allotransplantation using a nonmyeloablative conditioning regimen and donor lymphocyte infusions. *Blood*, **97,** 2574–2579.

Bornhauser, M., Platzbecker, U., Theuser, C., Holig, K. & Ehninger, G. (2002) CD34+-enriched peripheral blood progenitor cells from unrelated donors for allografting of adult patients: high risk of graft failure, infection and relapse despite donor lymphocyte add-back. *British Journal of Haematology*, **118,** 1095–1103.

Buggins, A.G., Mufti, G.J., Salisbury, J., Codd, J., Westwood, N., Arno, M., Fishlock, K., Pagliuca, A. & Devereux, S. (2002) Peripheral blood but not tissue dendritic cells express CD52 and are depleted by treatment with alemtuzumab. *Blood*, **100,** 1715–1720.

Carbone, E., Neri, P., Mesuraca, M., Fulciniti, M., Otsuki, T., Pende, D., Groh, V., Spies, T., Pollio, G., Cosman, D., Catalano, L., Tassone, P., Rotoli, B. & Venuta, S. (2004) HLA class I, NKG2D and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells. *Blood*, **105,** 251–258.

Chakraverty, R., Peggs, K., Chopra, R., Milligan, D.W., Kottaridis, P.D., Verfuerth, S., Geary, J., Thuraisundaram, D., Branson, K., Chakrabarti, S., Mahendra, P., Craddock, C., Parker, A., Hunter, A., Hale, G., Waldmann, H., Williams, C.D., Yong, K., Linch, D.C., Goldstone, A.H. & Mackinnon, S. (2002) Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood*, **99,** 1071–1078.

Childs, R., Chernoff, A., Contentin, N., Bahceci, E., Schrump, D., Leitman, S., Read, E.J., Tisdale, J., Dunbar, C., Linehan, W.M., Young, N.S. & Barrett, A.J. (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *New England Journal of Medicine*, **343,** 750–758.

Condiotti, R. & Nagler, A. (1996) Campath-1G impairs human natural killer (NK) cell-mediated cytotoxicity. *Bone Marrow Transplantation*, **18,** 713–720.

Eiermann, T.H., Lambrecht, P. & Zander, A.R. (1999) Monitoring anti-thymocyte globulin (ATG) in bone marrow recipients. *Bone Marrow Transplantation*, **23,** 779–781.

Eiermann, T.H., Freitag, S., Cortes-Dericks, L., Sahm, H. & Zander, A.R. (2001) Jurkat cell-reactive anti-thymocyte globulin assessed ex vivo by flow cytometry persists three weeks in circulation. *Journal of Hematotherapy and Stem Cell Research*, **10,** 385–390.

Fehse, B., Chukhlovin, A., Kuhlcke, K., Marinetz, O., Vorwig, O., Renges, H., Kruger, W., Zabelina, T., Dudina, O., Finckenstein, F.G., Kroger, N., Kabisch, H., Hochhaus, A. & Zander, A.R. (2001) Real-time quantitative Y chromosome-specific PCR (QYCS-PCR) for monitoring hematopoietic chimerism after sex-mismatched allogeneic stem cell transplantation. *Journal of Hematotherapy and Stem Cell Research*, **10,** 419–425.

Fine, J.P. & Gray, RJ. (1999) A proportional hazard model for the subdistribution of a competing risk. *Journal of American Statistical Association*, **94,** 496–509.

Finke, J., Schmoor, C., Lang, H., Potthoff, K. & Bertz, H. (2003) Matched and mismatched allogeneic stem-cell transplantation from unrelated donors using combined graft-versus-host disease prophylaxis including rabbit anti-T lymphocyte globulin. *Journal of Clinical Oncology*, **21,** 506–513.

Frohn, C., Hoppner, M., Schlenke, P., Kirchner, H., Koritke, P. & Luhm, J. (2002) Anti-myeloma activity of natural killer lymphocytes. *British Journal of Haematology*, **119,** 660–664.

Giebel, S., Locatelli, F., Lamparelli, T., Velardi, A., Davies, S., Frumento, G., Maccario, R., Bonetti, F., Wojnar, J., Martinetti, M., Frassoni, F., Giorgiani, G., Bacigalupo, A. & Holowiecki, J. (2003) Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood*, **102,** 814–819.

Giralt, S., Thall, P.F., Khouri, I., Wang, X., Braunschweig, I., Ippolitti, C., Claxton, D., Donato, M., Bruton, J., Cohen, A., Davis, M., Andersson, B.S., Anderlini, P., Gajewski, J., Kornblau, S., Andreeff, M., Przepiorka, D., Ueno, N.T., Molldrem, J. & Champlin, R. (2001) Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*, **97,** 631–637.

Gooley, T.A., Leisenring, W., Crowley, J. & Storer, B.E. (1999) Estimation of failure probabilities in the presence of competing risks:

new representations of old estimators. *Statistics in Medicine*, **18,** 695–706.

Gray, R (1988) A class of k-sample tests for comparing the cumulative incidence of competing risk. *Annals of Statistics*, **16,** 1141–1154.

Hale, G., Cobbold, S., Novitzky, N., Bunjes, D., Willemze, R., Prentice, H.G., Milligan, D., Mackinnon, S. & Waldmann, H. (2001) CAMPATH-1 antibodies in stem-cell transplantation. *Cytotherapy*, **3,** 145–164.

Klangsinsirikul, P., Carter, G.I., Byrne, J.L., Hale, G. & Russell, N.H. (2002) Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. *Blood*, **99,** 2586–2591.

Kottaridis, P.D., Milligan, D.W., Chopra, R., Chakraverty, R.K., Chakrabarti, S., Robinson, S., Peggs, K., Verfuerth, S., Pettengell, R., Marsh, J.C., Schey, S., Mahendra, P., Morgan, G.J., Hale, G., Waldmann, H., de Elvira, M.C., Williams, C.D., Devereux, S., Linch, D.C., Goldstone, A.H. & Mackinnon, S. (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood*, **96,** 2419–2425.

Kreiter, S., Winkelmann, N., Schneider, P.M., Schuler, M., Fischer, T., Ullmann, A.J., Huber, C., Derigs, H.G. & Kolbe, K. (2001) Failure of sustained engraftment after non-myeloablative conditioning with low-dose TBI and T cell-reduced allogeneic peripheral stem cell transplantation. *Bone Marrow Transplantation*, **28,** 157–161.

Kroger, N., Sayer, H.G., Schwerdtfeger, R., Kiehl, M., Nagler, A., Renges, H., Zabelina, T., Fehse, B., Ayuk, F., Wittkowsky, G., Schmitz, N. & Zander, A.R. (2002a) Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. *Blood*, **100,** 3919–3924.

Kroger, N., Schwerdtfeger, R., Kiehl, M., Sayer, H.G., Renges, H., Zabelina, T., Fehse, B., Togel, F., Wittkowsky, G., Kuse, R. & Zander, A.R. (2002b) Autologous stem cell transplantation followed by a dose-reduced allograft induces high complete remission rate in multiple myeloma. *Blood*, **100,** 755–760.

Kroger, N., Perez-Simon, J.A., Myint, H., Klingemann, H., Shimoni, A., Nagler, A., Martino, R., Alegre, A., Tomas, J.F., Schwerdtfeger, R., Kiehl, M., Fauser, A., Sayer, H.G., Leon, A., Beyer, J., Zabelina, T., Ayuk, F., San Miguel, J.F., Brand, R. & Zander, A.R. (2004) Relapse to prior autograft and chronic graft-versus-host disease are the strongest prognostic factors for outcome of melphalan/fludarabine-based dose-reduced allogeneic stem cell transplantation in patients with multiple myeloma. *Biology of Blood and Marrow Transplantation*, **10,** 698–708.

Mackinnon, S., Papadopoulos, E.B., Carabasi, M.H., Reich, L., Collins, N.H., Boulad, F., Castro-Malaspina, H., Childs, B.H., Gillio, A.P. & Kernan, N.A. (1995) Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood*, **86,** 1261–1268.

Maloney, D.G., Molina, A.J., Sahebi, F., Stockerl-Goldstein, K.E., Sandmaier, B.M., Bensinger, W., Storer, B., Hegenbart, U., Somlo, G., Chauncey, T., Bruno, B., Appelbaum, F.R., Blume, K.G., Forman, S.J., McSweeney, P. & Storb, R. (2003) Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. *Blood*, **102,** 3447–3454.

Maris, M.B., Niederwieser, D., Sandmaier, B.M., Storer, B., Stuart, M., Maloney, D., Petersdorf, E., McSweeney, P., Pulsipher, M., Woolfrey, A., Chauncey, T., Agura, E., Heimfeld, S., Slattery, J., Hegenbart, U., Anasetti, C., Blume, K. & Storb, R. (2003) HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood*, **102,** 1021–2030.

Marmont, A.M., Horowitz, M.M., Gale, R.P., Sobocinski, K., Ash, R.C., van Bekkum, D.W., Champlin, R.E., Dicke, K.A., Goldman, J.M., Good, R.A., Herzig, R.H., Hong, R., Masaoka, T., Rimm, A.A., Ringden, O., Speck, B., Weiner, R.S. & Bortin, M.M. (1991) T-cell depletion of HLA-identical transplants in leukemia. *Blood*, **78,** 2120–2130.

McGlave, P., Bartsch, G., Anasetti, C., Ash, R., Beatty, P., Gajewski, J. & Kernan, N.A. (1993) Unrelated donor marrow transplantation therapy for chronic myelogenous leukemia: initial experience of the National Marrow Donor Program. *Blood*, **81,** 543–550.

McSweeney, P.A., Niederwieser, D., Shizuru, J.A., Sandmaier, B.M., Molina, A.J., Maloney, D.G., Chauncey, T.R., Gooley, T.A., Hegenbart, U., Nash, R.A., Radich, J., Wagner, J.L., Minor, S., Appelbaum, F.R., Bensinger, W.I., Bryant, E., Flowers, M.E., Georges, G.E., Grumet, F.C., Kiem, H.P., Torok-Storb, B., Yu, C., Blume, K.G. & Storb, R.F. (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*, **97,** 3390–3400.

Morris, E.C., Rebello, P., Thomson, K.J., Peggs, K.S., Kyriakou, C., Goldstone, A.H., Mackinnon, S. & Hale, G. (2003a) Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. *Blood*, **102,** 404–406.

Morris, E.C., Rebello, P., Thomson, K.J., Peggs, K.S., Kyriakou, C., Goldstone, A.H., Mackinnon, S. & Hale, G. (2003b) Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. *Blood*, **102,** 404–406.

Nagler, A., Aker, M., Or, R., Naparstek, E., Varadi, G., Brautbar, C. & Slavin, S. (2001) Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. *Experimental Hematology*, **29,** 362–370.

Naparstek, E., Or, R., Nagler, A., Cividalli, G., Engelhard, D., Aker, M., Gimon, Z., Manny, N., Sacks, T., Tochner, Z. & Slavin, S. (1995) T-cell-depleted allogeneic bone marrow transplantation for acute leukaemia using Campath-1 antibodies and post-transplant administration of donor's peripheral blood lymphocytes for prevention of relapse. *British Journal of Haematology*, **89,** 506–515.

Peggs, K.S., Mackinnon, S., Williams, C.D., D'Sa, S., Thuraisundaram, D., Kyriakou, C., Morris, E.C., Hale, G., Waldmann, H., Linch, D.C., Goldstone, A.H. & Yong, K. (2003) Reduced-intensity transplantation with in vivo T-cell depletion and adjuvant dose-escalating donor lymphocyte infusions for chemotherapy-sensitive myeloma: limited efficacy of graft-versus-tumor activity. *Biology of Blood and Marrow Transplantation*, **9,** 257–265.

Peggs, K.S., Thomson, K., Hart, D.P., Geary, J., Morris, E.C., Yong, K., Goldstone, A.H., Linch, D.C. & Mackinnon, S. (2004) Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. *Blood*, **103,** 1548–1556.

Perez-Simon, J.A., Kottaridis, P.D., Martino, R., Craddock, C., Caballero, D., Chopra, R., Garcia-Conde, J., Milligan, D.W., Schey, S., Urbano-Ispizua, A., Parker, A., Leon, A., Yong, K., Sureda, A., Hunter, A., Sierra, J., Goldstone, A.H., Linch, D.C., San Miguel, J.F. & Mackinnon, S. (2002) Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood*, **100,** 3121–3127.

Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H.G., Beatty, P., Hows, J. & Thomas, E.D. (1995) 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplantation*, **15,** 825–828.

Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W.D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M.F. & Velardi, A. (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*, **295,** 2097–2100.

Shaw, B.E., Peggs, K., Bird, J.M., Cavenagh, J., Hunter, A., Alejandro, M.J., Russell, N.H., Sirohi, B., Towlson, K., Williams, C.D. & Marks, D.I. (2003) The outcome of unrelated donor stem cell transplantation for patients with multiple myeloma. *British Journal of Haematology*, **123,** 886–895.

Shulman, H.M., Sullivan, K.M., Weiden, P.L., McDonald, G.B., Striker, G.E., Sale, G.E., Hackman, R., Tsoi, M.S., Storb, R. & Thomas, E.D. (1980) Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *American Journal of Medicine*, **69,** 204–217.

Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben Tal, O., Eldor, A. & Or, R. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*, **91,** 756–763.