Factors predicting long-term survival after T-cell depleted reduced intensity allogeneic stem cell transplantation for acute myeloid leukemia

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ABSTRACT

Background
Reduced intensity conditioning regimens permit the delivery of a potentially curative graft-versus-leukemia effect in older patients with acute myeloid leukemia. Although T-cell depletion is increasingly used to reduce the risk of graft-versus-host disease its impact on the graft-versus-leukemia effect and long-term outcome post-transplant is unknown.

Design and Methods
We have characterized pre- and post-transplant factors determining overall survival in 168 patients with acute myeloid leukemia transplanted using an alemtuzumab based reduced intensity conditioning regimen with a median duration of follow-up of 37 months.

Results
The 3-year overall survival for patients transplanted in CR1 or CR2/CR3 was 50% (95% CI, 38% to 62%) and 44% (95% CI, 31% to 56%), respectively compared to 15% (95% CI, 2% to 36%) for patients with relapsed/refractory disease. Multivariate analysis demonstrated that both survival and disease relapse were influenced by status at transplant (P=0.008) and presentation cytogenetics (P=0.01). Increased exposure to cyclosporine A (CsA) in the first 21 days post-transplant was associated with an increased relapse risk (P<0.0001) and decreased overall survival (P<0.0001).

Conclusions
Disease stage, presentation karyotype and post-transplant CsA exposure are important predictors of outcome in patients undergoing a T-cell depleted reduced intensity conditioning allograft for acute myeloid leukemia. These data confirm the presence of a potent graft-versus-leukemia effect after a T-cell depleted reduced intensity conditioning allograft in acute myeloid leukemia and identify CsA exposure as a manipulable determinant of outcome in this setting.

Key words: reduced intensity conditioning, graft-versus-leukemia, acute myeloid leukemia.

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Introduction

Allogeneic stem cell transplantation represents the most effective anti-leukemic treatment modality in patients with acute myeloid leukemia (AML). Donor versus no-donor analyses have confirmed that allogeneic transplantation, when performed using a myeloablative conditioning regimen, improves disease free survival (DFS) and overall survival (OS) compared with conventional chemotherapy in patients in first complete remission (CR).[1] However, this benefit is restricted to younger adults because of the excessive transplant toxicity associated with myeloablative preparative regimens in older patients.

The advent of reduced intensity conditioning (RIC) regimens has permitted the extension of a potentially curative graft-versus-leukemia (GVL) effect to older patients in whom allogeneic transplantation has previously been contra-indicated on the grounds of age or co-morbidity.[2-4] Although it is incontestable that RIC regimens have reduced the immediate toxicity of allografting, there remains a substantial risk of acute and chronic graft-versus-host disease (GVHD) in patients transplanted with a T-cell replete stem cell inoculum. As a result, GVHD now represents the major cause of morbidity and mortality after an RIC transplant limiting both the potential for post-transplant immunotherapy as well as the possibility of transplanting frailer patients.[2,3,5,6] Whilst administration of T-cell depleting antibodies such as alemtuzumab (anti-CD52) or anti-thymocyte globulin (ATG), reduces the risk of both acute and chronic GVHD[7-9] there remain concerns that depletion of T cells from the stem cell inoculum may compromise a GVL effect and abrogate the ability of an RIC allograft to deliver long-term disease free survival. Although a number of studies have demonstrated encouraging disease free survival rates in patients with high-risk AML transplanted using a T-cell depleted RIC regimen,[10-12] little is understood of the magnitude of any GVL effect in this setting or what factors determine long-term survival in these patients. The intensity of post-transplant immunosuppression has previously been shown to be a critical determinant of relapse and outcome in patients with AML transplanted using a myeloablative conditioning regimen confirming the presence of a potent GVL effect in this setting.[13,14] Although the efficacy of RIC allografts is predicated on their ability to deliver a GVL effect, the impact of post-transplant immunosuppression on transplant outcome has never, to our knowledge, been studied in patients transplanted using a reduced intensity regimen.

In order to identify factors determining long-term outcome, we have analyzed the impact of pre- and post-transplant variables on survival in 168 patients with AML transplanted using a T-cell depleted RIC regimen over a ten year period.

Design and Methods

Eligibility criteria

One hundred and sixty-eight patients with AML were transplanted from February 1997 to June 2007 at 10 UK centers. Ethics committee approval was obtained at each center and patients were enrolled after written informed consent had been obtained. Patients over the age of 45 years were eligible as were younger patients with co-morbidities, a history of prior stem cell transplantation or an expressed desire to be transplanted using a reduced intensity regimen.

Patients' characteristics

The characteristics of the patient population are summarized in Table 1. The median age of the patient cohort was 54 years. Data concerning presentation karyotype was available in 166 patients and cytogenetic risk stratification was performed using MRC criteria.[15] Indications to perform a RIC transplant were age either alone or in conjunction with co-morbidities or prior SCT in 80%, prior SCT in 10%, the presence of co-morbidities in 8% and patient preference in 2%.

Transplant details and monitoring

The conditioning regimen incorporated fludarabine (30mg/m^2/day for 5 days) melphalan (140mg/m^2 for 1 day) and alemtuzumab (days -5 to -1) as previously reported.[7,8] Ninety-seven patients received alemtuzumab at a dose of 10 mg/day for five days, 68 patients received alemtuzumab at a dose of 20mg/day for five days. Three patients received alemtuzumab at a dose of 10 mg/day for three days. There was no significant difference in pre-transplant patient characteristics between patients receiving different alemtuzumab doses. CsA was used as GvHD prophylaxis at an initial dose of 5 mg/kg/day with the aim of discontinuing administration at day 90. Intravenous CsA was switched to an oral formulation as soon as the patient could tolerate oral medication. Trough CsA levels were measured two to three times a week during the first 21 days post-transplant and analyzed using an immunoassay technique (Dade Dimension®) during the first 21 days post-transplant. CsA dosing was adjusted to maintain levels of 200-300mcg/L during this period. CsA exposure during the first 21 days post transplantation (CsA_{21}) was determined by plotting CsA trough levels against time post-transplant and calculating the mean CsA trough concentration adjusted for time.

Patients were transplanted using an HLA identical sibling donor (n=76) or volunteer unrelated donor (n=92). Unrelated donor selection was performed using serological or low-resolution molecular typing for Class 1 (HLA-A and HLA-B, HLA-C) alleles and molecular typing for Class II (DRβ1) alleles. Up to one antigen mismatch at Class I or Class II loci was permitted.

Anti-microbial prophylaxis was determined by local protocols but all patients received prophylactic trimethoprim/sulfamethoxazole or nebulized pentamidine as prophylaxis against *Pneumocystis jirovecii* pneumonia. Aciclovir was administered as antiviral prophylaxis. If either patient or donor were seropositive for cytomegalovirus (CMV) pre-transplant, plasma specimens were monitored weekly for evidence of CMV re-activation by PCR analysis until 100 days post-transplant. Patients with evidence of CMV re-activation received pre-emptive therapy with ganciclovir.

Chimerism studies were performed on a T-cell purified subset at three months post-transplant in a proportion of patients using fluorescence in situ hybridization (FISH) or variable tandem repeat polymorphism analysis by polymerase chain reaction (PCR). T-cell chimerism data was available in 78 patients at day +90 post-transplant. Donor lymphocytes were not routinely administered as part of the transplant protocol. Twenty-four patients received DLI either as management of mixed hemopoietic chimerism (n=9) or at disease relapse (n=15).

Outcomes and statistical analysis

Long-term follow-up data are available on all patients. The median duration of follow-up on living patients is 37 months (range 16-114 months) and 112 patients were transplanted three years or more prior to the final data analysis. The results of 51 previously reported patients have been updated with a further 36 months follow-up[11] and data on 117 additional patients have been included. Two patients died before day 28 and were excluded

from analysis of engraftment kinetics. Survival curves were constructed using the Kaplan-Meier method[16] and the log-rank test[17] was used to assess differences between groups. TRM was defined as death in CR or death related to transplantation where it was not possible to assess disease status prior to death. Univariate analyses of the association of these post-transplant outcomes with clinical risk factors were calculated using univariate Cox regression analyses.[18] Clinical risk factors included were gender (M/F), age (≤60 yrs or >60 yrs), cytogenetics (adverse or intermediate), donor type (matched sibling or volunteer unrelated donor), cell dose, patient CMV serostatus and disease status at time of transplant (complete remission or refractory/relapse). Cumulative incidence curves were used in a competing risks setting death being treated as a competing event to calculate probabilities of chronic GVHD, TRM and relapse.[19] Multivariate analyses were performed using backward selection methods for Cox's proportional hazards regression and variables with a P value of <0.1 in the previous univariate analysis were included.

Individualized CsA_{21} values were included as a continuous variable in both univariate and multivariate Cox's regressions analyses. The impact of post-transplant immunosuppression on outcomes as a prognostic factor was assessed by adding CsA_{21} to the previously selected multivariate Cox's regression models to assess its prognostic value above and beyond known clinical risk factors. Hazard ratios and associated 95% confidence intervals are adjusted to express CsA exposure in terms of 500 unit intervals. Tests of significance were two-sided and had a significance level of 0.05 or less. Data were analyzed using SAS statistical software (SAS Institute, SAS Circle, North Carolina, USA).

Results

Engraftment and chimerism

One hundred and sixty-four of the 166 assessable patients engrafted. The median time to acquisition of an absolute neutrophil count greater than 0.5×10^9/L was 14 days (range 7-25 days). The median time to acquisition of a platelet count greater than 50×10^9/L was 16 days (range 7-66 days). Primary graft failure was documented in 2 patients, both recipients of unrelated grafts. Fifty-seven of 78 patients in whom chimerism data was available demonstrated full donor chimerism in the T-cell fraction at day 90.

Overall survival

At the time of analysis, 73 (43%) patients were alive. The 3-year OS for patients transplanted in CR1 or CR2/CR3 was 50% (95% CI, 38% to 62%) and 44% (95% CI, 31% to 56%), respectively compared to 15% (95% CI, 2% to 36%) for patients with relapsed/refractory disease (Figure 1A). The 3-year OS for patients with intermediate risk cytogenetics was 48% (95% CI, 39% to 57%) compared with 32% (95% CI, 18% to 46%) for patients with adverse risk cytogenetics (Figure 1B). There was no impact of patient age on OS. No difference was noted in outcome between patients receiving an allograft from a volunteer unrelated donor and those using a sibling donor (P=0.24). Alemtuzumab dose did not impact on outcome. In univariate analysis, adverse risk cytogenetics and active disease at the time of transplant were associated with a decreased OS. Multivariate analyses showed both relapsed/refractory disease and adverse-risk cytogenetics to be associated with a decreased OS (Table 2A).

Disease-free survival

The 3-year DFS for patients transplanted in CR1 or CR2/CR3 was 49% (95% CI, 39% to 58%) and 42% (95% CI, 28% to 54%), respectively. The 3-year DFS for patients with intermediate risk cytogenetics was 47% (95% CI, 38% to 56%) compared to 32% (95% CI, 19% to 46%) for patients with adverse risk cytogenetics. In univariate analysis, the variables associated with a decreased DFS were relapsed/refractory status at the time of transplant and adverse risk cytogenetics. Multivariate analysis demonstrated that relapsed/refractory disease and adverse-risk cytogenetics were associated with a decreased DFS (Table 2B).

Disease relapse

Disease relapse occurred in 50 (29%) patients and represented the major cause of patient death (49% of all deaths). The median time to relapse was 6.5 months

Table 1. Patient and disease characteristics of 168 patients with acute myeloid leukemia transplanted using an alemtuzumab based reduced intensity conditioning regimen.

Characteristic	N. of patients	%
Age (years)		
Median	54	
Range	18-71	
<60	133	80
≥60	35	20
Sex		
Male	89	53
Female	79	47
Diagnosis		
CR1	86	51
CR2/3	64	38
Relapsed/refractory	18	11
Cytogenetics*		
Intermediate	126	76
Adverse	40	24
Stem cell source		
HLA identical sibling	76	45
Unrelated donor	92	55
Previous stem cell transplant	17	10
Previous autologous SCT	10	6
Previous allogeneic SCT	7	4
CMV Status		
+/+	74	44
-/-	48	29
-/+	21	12
+/-	21	12
Not known	4	2
CD34 infused per kg ($\times10^6$)		
Median	5.0	
Range	0.7-20.1	
Follow-up for survivors (months)		
Median	37	
Range	16-114	
Indication for reduced intensity conditioning		
Age†	135	80
Autologous/allogeneic	17	10
Comorbidity	14	8
Patient choice	2	2

*SCT: stem cell transplant; CR, complete remission; *cytogenetics missing in 2 patients, †with or without co-morbidity or prior SCT).*

(inter-quartile range, 4-11 months). Seventy-eight percent of patients destined to relapse had done so within one year post-transplant. Twenty percent (95% CI, 13% to 29%) of patients with intermediate risk cytogenetics transplanted in remission relapsed compared to 39% (95% CI, 23% to 60%) of patients with adverse risk cytogenetics transplanted in remission (P=0.04) (Figure 2A). The 1-year relapse risk for those transplanted in CR was 24% (95% CI, 17%-32%) compared to 59% (95% CI, 35%-85%) for those with relapsed/refractory status (Figure 2B). There was no difference in relapse risk between recipients of sibling and unrelated donor transplants (P=0.95). Development of acute or chronic GVHD did not influence relapse rates (P=0.12 and P=0.6, respectively). Alemtuzumab dose did not impact on the incidence of disease relapse (P=0.16). Twenty-four patients received DLI as treatment for mixed chimerism (n=9) or disease relapse (n=15). Ten patients who received DLI died of relapsed disease. In univariate analysis, the only factor associated with disease relapse was the presence of relapsed/refractory disease at the time of transplant (HR,3.0;P=0.004).

GVHD and transplant-related mortality

Forty-seven patients (27%) developed Grade II-IV acute GVHD. Twelve (7%) patients developed Grade III-IV acute GVHD. Thirty-eight patients developed chronic GVHD: 27 limited chronic GVHD and 11 extensive chronic GVHD. Alemtuzumab dose did not impact on the incidence of acute or chronic GVHD (P=0.2 and 0.6, respectively).

The day 100 TRM was 10% (95% CI, 7%-16%) and 17% (95% CI, 12%-24%) at one year post-transplant. Univariate analysis showed relapsed/refractory disease to be the only variable associated with higher day-100 mortality (HR, 4.5; P=0.006).

Impact of CsA exposure on transplant outcome

Sixty-three consecutively treated patients from three of the largest contributing centers were included in the subanalysis of the effect of CsA exposure on transplant outcome. The median CsA exposure value in the first 21 days post-transplant (CsA_{21}) was 4032 mcg.day/L (IQR 3456-4947). Univariate analysis of OS found increasing CsA_{21} to be associated with an increased risk of death (HR, 1.36, 95% CI, 1.11-1.50; P<0.0001) (Figure 3). Multivariate analysis to include both disease status at transplant and presentation cytogenetics demonstrated CsA_{21} to be a significant prognostic factor for OS (HR, 1.29; 95% CI, 1.11-1.50; P<0.001) (Table 3). Univariate analysis of disease relapse demonstrated increasing CsA_{21} to be associated with an increased risk of relapse. Multivariate analysis to include disease status at transplant and presentation cytogenetics demonstrated CsA_{21} to be a significant prognostic factor for relapse (HR, 1.83; 95% CI, 1.48-2.27; P<0.001)

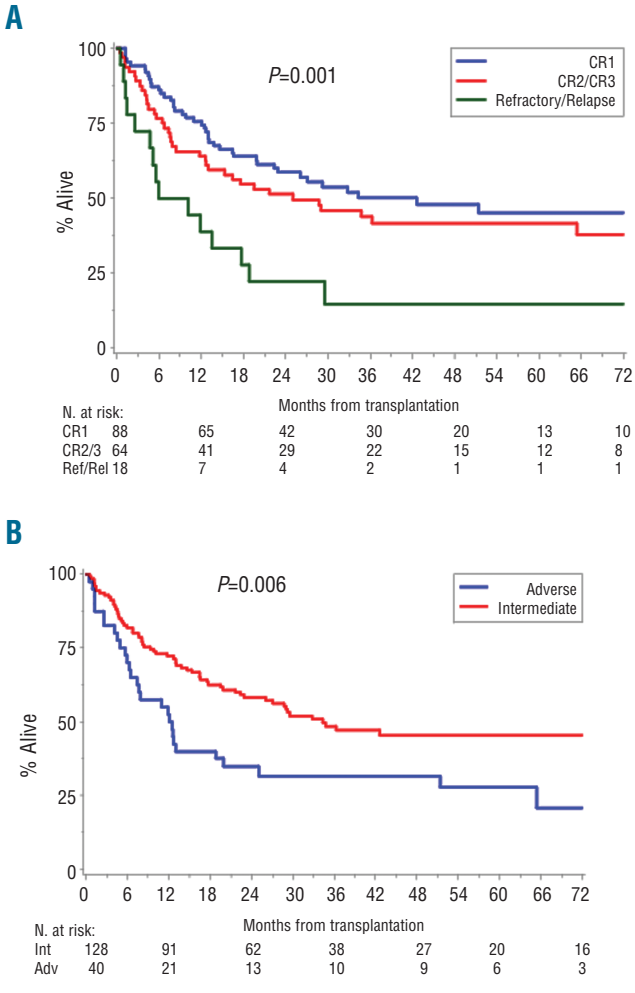

Figure 1. (A) **Overall survival after an alemtuzumab based RIC allograft for acute myeloid leukemia according to disease status at the time of transplant.** (B) **Overall survival according to cytogenetic risk classification at diagnosis.**

Table 2A. Univariate and multivariate analyses of donor and patient factors predicting overall survival.

	Univariate Analysis		Multivariate Analysis		
	HR	P	HR	95% CI	P
Relapsed/refractory disease	2.6	0.001	1.5	1.1-1.9	0.008
Adverse cytogenetics	1.9	0.006	1.7	1.1-2.7	0.01

Table 2B. Univariate and multivariate analyses of donor and patient factors predicting disease free survival.

	Univariate Analysis		Multivariate Analysis		
	HR	P	HR	95% CI	P
Relapsed/refractory disease	2.9	<0.001	2.6	1.5-4.7	0.001
Adverse cytogenetics	1.8	0.002	1.6	1.2-2.9	0.03

There was no association between the incidence of acute GVHD and CsA_{21} (P=0.9). There was a trend towards an increased risk of chronic GVHD in patients with a low CsA_{21} but this did not reach statistical significance (P=0.1).

Discussion

This study, to our knowledge the largest reported series of reduced intensity allografts in AML, defines pre- and post-transplant variables which predict outcome after a T-cell depleted RIC allograft in AML. This allows identification of a sizeable population of older patients with AML in whom encouraging disease free survival rates can be achieved with only a modest risk of acute or chronic GVHD. The delineation of a population of older AML patients who have a favorable outcome after a T-cell depleted RIC allograft is of importance given the fact that T-replete reduced intensity regimens are associated with substantial GVHD related morbidity and mortality, particularly in older recipients of unrelated donor grafts.[5,6] The demonstration in this study that that there is no apparent impact of age or donor stem cell source on outcome is of particular relevance, given the fact that the majority of older patients with AML lack a fit sibling donor, and supports the continued use of antibodies such as ATG or alemtuzumab in a defined population of older patients with AML. It should be noted, however, that only 20% of the patients reported in this paper were over the age of 60 and it will be important to confirm our findings in an older cohort of patients.

Disease relapse was the major cause of treatment failure in this series. Three factors predict for an increased risk of disease relapse: the presence of active disease at the time of transplant, adverse risk cytogenetics at diagnosis and increased intensity of post-transplant immunosuppression. It has previously been demonstrated that remission status is a critical predictor of relapse and survival after reduced intensity allografts for AML[5,20,21] and our results confirm the importance of developing novel conditioning regimens in this population of patients.[22] Whilst cytogenetic status has previously been shown to be an important predictor of outcome in patients transplanted using a myeloablative regimen,[23] this is the first study, to our knowledge, which demonstrates that presentation cytogenetics determine outcome after a T-cell depleted reduced intensity regimen and underlines the potential importance of adjunctive anti-leukemic therapies, such as radioimmunotherapy, in patients with adverse risk cytogenetics.[24] Patients destined to relapse do so early with the great majority having relapsed within the first year post-transplant. Consequently, interventions designed to decrease the risk of disease recurrence must be focused on the immediate post-transplant period. This limits the potential benefit of early DLI, whether administered pre-emptively or prophylactically, given the substantial risk of GVHD associated with the use of DLI in the first 12 months post-transplant.[25]

The extent to which T-cell depletion compromises the ability of an RIC allograft to deliver a GVL effect remains controversial. Thus, although an inverse correlation between disease relapse and the occurrence of chronic GVHD has been convincingly demonstrated in T-cell replete RIC allografts in AML,[26] no such relationship has been demonstrated in alemtuzumab based reduced intensity allografts.[12] Our data, which demonstrates a correlation between the intensity of post-transplant immunosup-

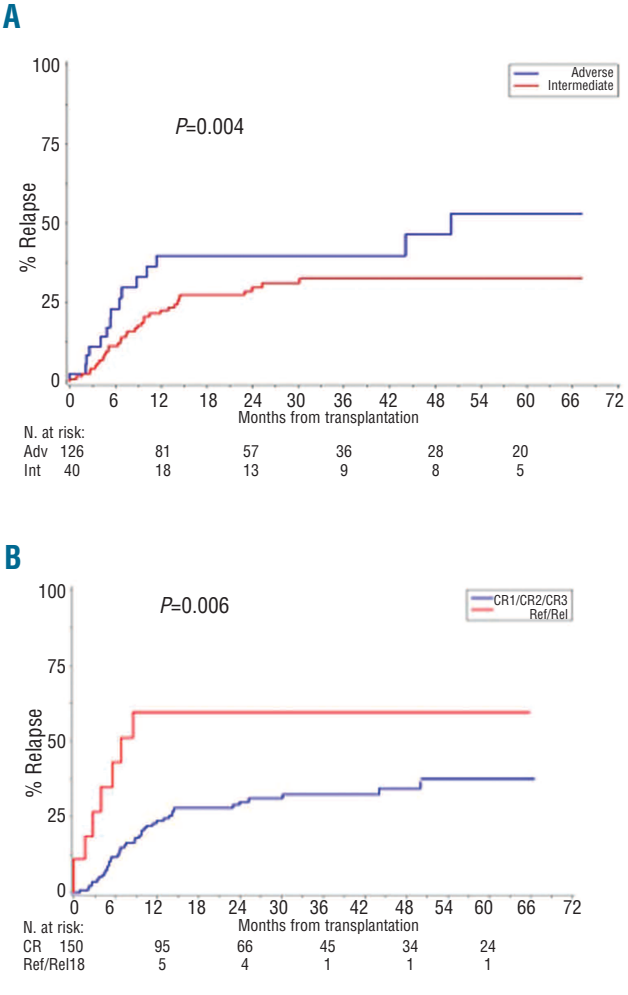

Figure 2. (A) **Impact of cytogenetic status at presentation on relapse incidence and relapse kinetics after an alemtuzumab based RIC allograft for acute myeloid leukemia.** (B) **Impact of disease status at time of transplantation on relapse incidence and relapse kinetics.**

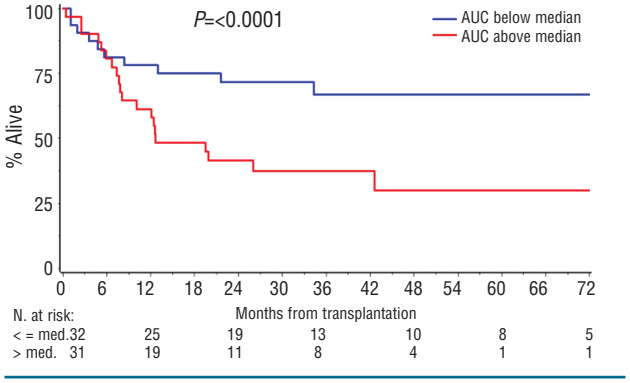

Figure 3. **Overall survival according to CsA_{21} (above or below mean CsA exposure) in 63 patients undergoing RIC allograft for acute myeloid leukemia.**

Table 3. Impact of CsA exposure (CsA_{21}) on transplant outcome in 63 patients with acute myeloid leukemia transplanted using an alemtuzumab based RIC regimen.

	Univariate Analysis		Multivariate Analysis		
	HR	P	HR	95% CI	P
Overall survival	1.36	<0.001	1.29	1.11-1.50	<0.001
Disease free survival	1.41	<0.001	1.35	1.16-1.57	<0.001
Relapse risk	1.80	<0.001	1.83	1.48-2.27	<0.001
100 day TRM	0.92	0.7	0.87	0.55-1.36	0.5
Acute GVHD*	1.018	0.9	-	-	-
Chronic GVHD*	0.74	0.1	-	-	-

*+ Adjusted HR/OR provided for CsA_{21} in units of 500 mcg.day/L. *Odds Ratios for occurrence of GVHD.*

pression and relapse risk, confirms that a potent GVL effect is retained in T-cell depleted RIC allografts for AML providing the degree of post-transplant immunosuppression is not excessive. Bacigalupo has previously demonstrated an important impact of CsA dose on outcome after a T-cell replete myeloablative allograft for AML[13,14] and tacrolimus exposure has been correlated with outcome in patients transplanted for refractory AML.[27] However, the impact of CsA exposure on outcome after a reduced intensity allograft has not, to our knowledge, been studied previously. Further studies are, therefore, now required to define the optimal level and duration of post-transplant immunosuppression in T-depleted and T-cell replete reduced intensity allografts. Furthermore, manipulation of CsA exposure in the immediate post-transplant period appears to be a potentially valuable strategy by which the outcome of AML patients may be improved. There is controversy concerning the cellular mediator of the GVL effect in AML. These data confirm not only that a potent GVL effect can be exerted after a T-cell depleted RIC allograft in AML but also that the cellular effector of GVL is profoundly susceptible to inhibition by CsA. Whilst it has generally been assumed that donor T cells are the principal effectors of GVL, the recent observation that NK cell number and function is influenced by CsA is, therefore, of interest.[28]

In summary, these data identify pre- and post-transplant factors determining outcome after an alemtuzumab based RIC allograft in AML. It is clear that there is a potent and readily manipulable determinant GVL effect after a T-cell depleted RIC allograft in AML. Further studies are required to define the impact of post-transplant immunosuppression on outcome in patients undergoing RIC allografts for other hematologic malignancies and in patients transplanted using other forms of T-cell depletion, such as ATG or T-cell replete regimens. In patients transplanted using an alemtuzumab based regimen, randomized trials addressing CsA dose intensity and duration after RIC allografts are required.

Authorship and Disclosures

CC recruited patients, analyzed data and wrote the paper. SN, EN and ST collected data. AP, JY, EL, PK, JS, DM, GC, TL, KP, PV, FC and MC recruited patients. LB, CB and ND analyzed data. ET recruited patients and collected data. SM and NR designed the study and recruited patients.

The authors reported no potential conflicts of interest.

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