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Post-transplant T cell chimerism predicts graft versus host disease but not disease relapse in patients undergoing an alemtuzumab based reduced intensity conditioned allogeneic transplant

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ABSTRACT

In this multicentre retrospective study we have studied the impact of T cell chimerism on the outcome of 133 patients undergoing an alemtuzumab based reduced intensity conditioning allograft (RIC). The median age of the patients was 50 years (range 42–55 years). 77 patients were transplanted using an HLA identical sibling donor while 56 patients received a fully matched volunteer unrelated donor graft. 64 patients had a lymphoid malignancy and 69 were transplanted for a myeloid malignancy. 38 patients (29%) relapsed with no significant difference in risk of relapse between patients developing full donor and mixed donor chimerism in the T-cell compartment on D + 90 and D + 180 post transplant. Day 90 full donor T cell chimerism correlated with an increased incidence of acute GVHD according to NIH criteria ($p = 0.0004$) and the subsequent development of chronic GVHD. Consistent with previous observations, our results confirmed a correlation between the establishment of T cell full donor chimerism and acute GVHD in T deplete RIC allografts. However our study failed to identify any correlation between T cell chimerism and relapse risk and challenge the use of pre-emptive donor lymphocyte infusions (DLI) in patients with mixed T cell chimerism transplanted using an alemtuzumab based RIC regimen.

1. Introduction

The advent of reduced intensity conditioning (RIC) regimens has permitted the extension of a potentially curative graft-versus-leukaemia (GVL) effect to large numbers of patients with haematological malignancies in whom allogeneic transplantation was previously contra-indicated either on the grounds of age or co-morbidity [1,2]. The development of full donor lympho-haematopoietic chimerism is generally considered a prerequisite for the genesis of a graft versus malignancy effect on the basis that mixed T cell chimerism reflects directional tolerance [3,4]. Accumulating data has confirmed the establishment of T cell full donor chimerism (FDC) as a predictor of disease relapse and graft-versus-host disease (GvHD) after a T replete RIC allograft. In these reports the presence of mixed chimerism in the T cell population at day 90 correlated with an increased risk of disease relapse providing a rationale for the administration of donor lymphocyte infusions (DLI) with the aim of achieving full donor T cell chimerism [5].

The significant risk of acute and chronic GVHD after T replete RIC allografts led to the incorporation of T cell depleting antibodies such as alemtuzumab or anti-thymocyte globulin into reduced intensity regimens. This has proved a highly effective method of reducing the risk of acute and chronic GVHD but is associated with a higher incidence of mixed T cell chimerism than is observed after a T-replete regimen. However conflicting data exists on the clinical relevance of mixed T cell chimerism after alemtuzumab based RIC allografts [6–8]. We therefore wished to identify which factors predict the establishment of T cell chimerism after an alemtuzumab based allograft and correlate post-transplant $CD3^+$ cell chimerism with disease relapse and GVHD.

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Table 1
Patient characteristics.

Number of patients		All patients 133 % (*n*)
Age		Median 50 IQR 42–55
Sex	Male	58 (77)
	Female	42 (56)
Disease type	Lymphoid	48 (64)
	Myeloid	52 (69)
CR	Yes	62 (82)
	No	38 (51)
Transplant type	Sibling	58 (77)
	Unrelated	42 (56)
Stem cell source	BM	8 (10)
	PB	92 (123)
Conditioning regimen	BEAM/C	24 (32)
	Flu/Bu/C	17 (23)
	Flu/MeI/C	59 (78)
GVHD prophylaxis	CyA	76 (101)
	CyA + MTX	24 (32)

2. Materials and methods

153 patients from 3 transplant centres who underwent reduced intensity alemtuzumab T-deplete allogeneic transplant were included in the study. Of those 13 who died in the first 100 days post transplant and had no chimerism data available were excluded from the study. A further 11 patients who died between D + 90 and D + 180 were also excluded from the analysis. 133 patients were eligible for the analysis of T cell chimerism at D + 90 and D + 180 post transplant (Table 1). The median age was 50 years (range 42–55) and the median follow up was 36 months (range 7–108 months). 82 patients (61%) were in CR at the time of transplant and 51 (39%) were in PR or had stable disease. 77 patients were transplanted using an HLA identical sibling and 56 patients received a volunteer unrelated donor allograft. Unrelated donor selection was performed according to published criteria and involved serological typing for HLA-A and HLA-B antigens and molecular typing for HLA-C, DRB1, and DQB1 or full molecular typing for HLA-A, HLA-B, HLA-C, DRB1, and DQB1.

78 patients (59%) were conditioned with fludarabine 30 mg/m^2 for 5 days, melphalan 140 mg/m^2 for 1 day and alemtuzumab 50 mg over 5 days, 23 patients (17%) had fludarabine 30 mg/m^2 for 5 days, busulphan 3.2 mg/kg IV for 4 days and alemtuzumab 50 mg over 5 days and 32 (24%) received carmustine 300 mg/m^2 for 1day,etoposide 200 mg/m^2 for 4 days, cytarabine 200 mg/m^2 for 4 days, melphalan 110 mg/m^2 for 1 day and alemtuzumab 50 mg over 5 days. All patients received alemtuzumab at a daily dose of 10 mg between days −6 and −2. All patients received post transplant GVHD prophylaxis with ciclosporin A alone or, for those transplanted using a BEAM alemtuzumab regimen, in combination with short course methotrexate. In the absence of active GVHD a ciclosporin taper was commenced on D + 60 for the sibling donor allografts and D + 90 for unrelated donor transplants or earlier at clinician's discretion in patients with high risk disease.

Prophylactic fluconazole or itraconazole and acyclovir was administered according to institutional policies. All patients received prophylaxis against Pneumocystis jiroveci pneumonia, using co-trimoxazole. Patients at risk of cytomegalovirus (CMV) re-activation were monitored weekly by PCR analysis.

2.1. Statistical methods

Assessing predictive factors of chimeric status: A combination of χ^2 tests, Fisher's exact tests (both for categorical data), and Wilcoxon tests (continuous data) were used to compare chimeric status groups in terms of baseline patient characteristics including age, gender, disease type, relapse/progression status at transplant, type of transplant, stem cell source, CD34$^+$ progenitor cell dose, conditioning regimen, and GVHD prophylaxis. These characteristics were then considered for inclusion in logistic regression modelling to determine important factors in predicting chimeric status. The final model was selected using the stepwise procedure in SAS with 10% entry and exit criteria.

Effect of chimeric status on transplant outcome: Patient characteristics at baseline were assessed univariately for their impact on the main time to event (TTE) outcomes, overall survival (OS), disease free survival (DFS), relapse/progression rate (RR) and non-relapse mortality (NRM) and also the occurrence of acute and chronic GVHD. OS was calculated as the time from transplant until death from any cause or last known follow-up. DFS was calculated as the time from transplant until death or relapse/progression, whichever came first, or last known follow-up. RR was calculated as the time from transplant until relapse/progression, patients were censored at death if this occurred before relapse/progression and last known follow-up. NRM was calculated as the time from transplant to death without prior relapse/progression, patients were censored at relapse/progression if occurred prior to death and last known follow-up. Kaplan–Meier curves and log-rank statistics were calculated for overall survival. Cumulative incidence was used to calculate relapse incidence and non-relapse mortality. Cox's proportional hazard modelling techniques were used to assess TTE outcomes. Logistic regression was carried out to assess the impact upon GVHD. The final models were achieved using the stepwise procedure in SAS with 10% entry and exit criteria. Chimeric status was then included in the final model to assess the effect on outcome. Sensitivity analysis was performed using the landmark method to assess the impact of chimeric status on TTE outcomes from 2 specific landmark dates, 90 and 180 days post transplant. The analysis did not consider patients whose event occurred before the landmark dates and which might therefore bias the effect of chimeric status on outcome. Log-rank statistics and Kaplan–Meier curves were produced for whole blood and T cell fractions separately.

2.2. Chimerism analysis

Analysis of haemopoietic and CD3$^+$ T-lymphocyte chimerism was performed at D + 90 and D + 180 within the first year of the allograft. To obtain purified populations of T-lymphocytes, CD3$^+$ cells were separated from density gradient separated peripheral blood mononuclear cells using MACS (Miltenyi Biotec). On FACScan analysis, greater than 95% of cells thus isolated expressed CD3. For sex-matched allografts, DNA was extracted from cell suspensions. The degree of donor/host chimerism was determined by multiplex PCR of microsatellite markers by applying 5 fluorescently labelled primer pairs for the loci MBP (A and B), FGA, D18S391, D18S386 and D13S634. Two microlitres of PCR product was loaded onto a 6% ployacrylamide gel on an ABI-373 gene scanner. Relative heights of donor and host cells in the sample were calculated based on the peak heights and areas of informative alleles (assay sensitivity 1%). Fluorescence in situ hybridization (FISH) was used to monitor chimerism in sex-mismatched allografts. In brief, cell suspensions were fixed using 3:1 ratio methanol:acetic acid fixative and the level of donor/host chimerism was determined by analysis of 250 interphase cells using Vysis CEPXY probe specific for the X centromere and Y heterochromatin (assay sensitivity 1%). Full donor chimerism (FDC) was defined as the presence of ≥95% cells of donor