The impact of alemtuzumab scheduling on graft versus host disease following unrelated donor fludarabine and melphalan allografts

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Short title

Alemtuzumab scheduling in unrelated donor allografts

Abstract

Alemtuzumab conditioning is highly effective at reducing the incidence of acute and chronic graft versus host disease (GVHD) in reduced intensity fludarabine and melphalan transplantation with ciclosporin monotherapy. Less frequent and lower dose scheduling may be used with sibling donors but an optimal regimen for matched unrelated donors has not been defined. In this retrospective observational study of 313 patients, the incidence and severity of GVHD was compared in patients receiving three different dose schedules: the standard 100mg regimen (20mg on day -7 to -3), 60mg (30mg day -4 and -2) or 50mg (10mg on day -7 to -3). Patients treated with 100mg, 60mg or 50mg developed acute GVHD grade I-IV with an incidence of 74%, 65% and 64%, respectively, while 36%, 32% and 41% developed chronic GHVD. An excess of severe acute grade III/IV GVHD was observed in the 50mg cohort (15% vs. 2-6%; $p = 0.016$). The relative risk of severe acute grade GVHD remained more than three-fold higher in the 50mg cohort, compared with 100mg, after adjustment for differences in HLA match, age, gender mismatch, CMV risk and diagnosis ($p = 0.030$). The findings indicate that the 60mg alemtuzumab schedule was comparable to 100mg but more attenuated schedules may increase the risk of severe grade GVHD.

Keywords

Alemtuzumab
T cell depletion
Graft versus host disease
Conditioning regimens

Introduction

Alemtuzumab (humanized anti-CD52 antibody) is highly effective at reducing the incidence of acute and chronic GVHD in the setting of reduced intensity transplantation with fludarabine and melphalan (1-7). When delivered to recipients during conditioning therapy, it effects *in vivo* depletion of both recipient and donor T cells, NK cells, B cells, monocytes and dendritic cells, owing persistence in the recipient with a half life of 8 days (8-11). Freedom from GVHD is associated with partial chimerism of donor T cells but this may be corrected with donor lymphocyte infusions to deliver good overall survival with minimal long term morbidity (7,12-14).

The original fludarabine, melphalan and alemtuzumab regimen used an empiric alemtuzumab schedule of 100mg comprising five 20mg doses given on consecutive days between day -7 and -3. This regimen is effective at abrogating GVHD in mixed cohorts of matched related and unrelated donor transplants used to treat patients with both myeloid and lymphoid malignancy (1-7). It has also been noted that GVHD is well controlled in PBSC and BM grafts from unrelated donors (15) and that a degree of antigen mismatching is well tolerated (16). Similar schedules of alemtuzumab have also been used with other fludarabine-based reduced intensity protocols with equivalent efficacy (14).

Excessive T cell depletion may be associated with increased relapse and risk of infection (11,17-19) and several groups have shown that dose reduction is possible in unrelated donor transplantation. A number of schedules with doses of between 50-100mg, administered over 2-5 days, have been tested (20-22) and it is reported that as little as 10mg reduces the burden of GVHD (23). A phased dose de-escalation study in sibling transplants concluded that a single dose of 30mg on day -1 was sufficient to reduce GVHD to a similar level as 100mg of alemtuzumab (24) but a comparable study has not been performed using only unrelated donors in a common protocol. Retrospective comparison of 30mg and 60mg dosing in sibling and unrelated donor transplants, respectively, indicated that the unrelated cohort still experienced more GVHD and had higher donor T cell chimerism (25).

Owing to the long in vivo half-life of alemtuzumab, the total dose and scheduling both have the potential to modify GVHD risk substantially (11) but there is no consensus about an optimal regimen in fludarabine-melphalan unrelated donor transplantation. Here we report a retrospective observational study in which we compared three commonly used protocols. Reduction and compression of the alemtuzumab schedule to two 30mg doses on day -4 and -2 was comparable to 100mg between days -7 to -3 but patients receiving 50mg alemtuzumab between day -7 to -3 were at greater risk of severe acute grade III/IV GVHD.

Methods

Patients and donors

Data were collated from three UK transplant centers: University College Hospital/Royal Free Hospital, University College London Hospitals NHS Foundation

Trust, London; Northern Centre for Bone Marrow Transplantation, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne; and, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham. Sequential patients transplanted between January 2007 and December 2011 were included. Patients were over the age of 18 years at transplantation, had a hematological malignancy at any stage and were transplanted with an unrelated donor with at least 8/10 HLA four digit allele matching. The number of transplants with less than 10/10 matching was too small to discern differences between A, B, C, or DQ antigen mismatches. The major stem cell source was PBSC at all centers. All patients gave consent for their clinical data to be reported anonymously according to local ethical approval. Clinical data were collected by transplant center data managers and analyzed by KG, KP and MC.

Conditioning and alemtuzumab dosing

All patients were conditioned with five doses of fludarabine 30mg/m^2 daily days -7 to -3 and melphalan 140mg/m^2 day -2. GVHD prophylaxis consisted of ciclosporin monotherapy starting at 3mg/kg/day adjusting to an initial level of at least 200ng/ml. Each center delivered a different alemtuzumab schedule. London patients received 100mg in five 20mg daily doses given consecutively from day -7 to day -3 Newcastle patients received 60mg as two 30mg doses on day -4 and day -2 and Birmingham patients received 50mg in five 10mg doses given consecutively from day -7 to day -3. Donor lymphocyte infusions (DLI) were given for mixed chimerism in all centers according to three monthly escalating schedules starting at 6 months post transplantation with a dose of 1 x 10^6 CD3+ cells per kg. As the DLI schedule did not start until 6 months, it did not influence the incidence or severity of acute GVHD.

Study endpoints and supportive care

The primary endpoints of the study were the raw incidence and severity of acute GVHD according to Seattle grading and the cumulative incidence of chronic GVHD. Secondary endpoints included donor T cell chimerism, CMV reactivation, non-relapse mortality (NRM), relapse and overall survival. Patients were evaluable for acute GVHD if they survived until engraftment and for chronic GVHD if they lived more than 100 days. CMV reactivation was defined as more than 2 sequential blood PCR results > 2 x10^3 copies/ml or requiring anti-CMV therapy with ganciclovir, valganciclovir or foscarnet. Anti-viral prophylaxis was maintained with acyclovir 200mg BD PO in the 100mg cohort or 200mg TDS PO in the 60mg and 50mg chorts.

Statistical analysis

Analysis was performed according to EBMT guidelines (26-29), on consecutive patients transplanted between January 2007 and December 2011. Chi-square, Mann-Witney tests and Kaplan-Meier survival curves were plotted with GraphPad Prism 6 and cumulative incidence analysis with competing risks was performed with the cmprsk. function of the open-source software R. Kaplan-Meier curves were compared with log-rank (Mantel-Cox) tests while cumulative incidence curves were compared with Gray's test . Patients were censored at last follow up. Multivariate

analysis was performed in SPSS using binary logistic regression (grade III/IV vs other)

Results

Conditioning protocols used in this three-center retrospective observational study are summarized in **Figure 1.** Both the scheduling and total dose of Alemtuzumab was different between each cohort.

Figure 1

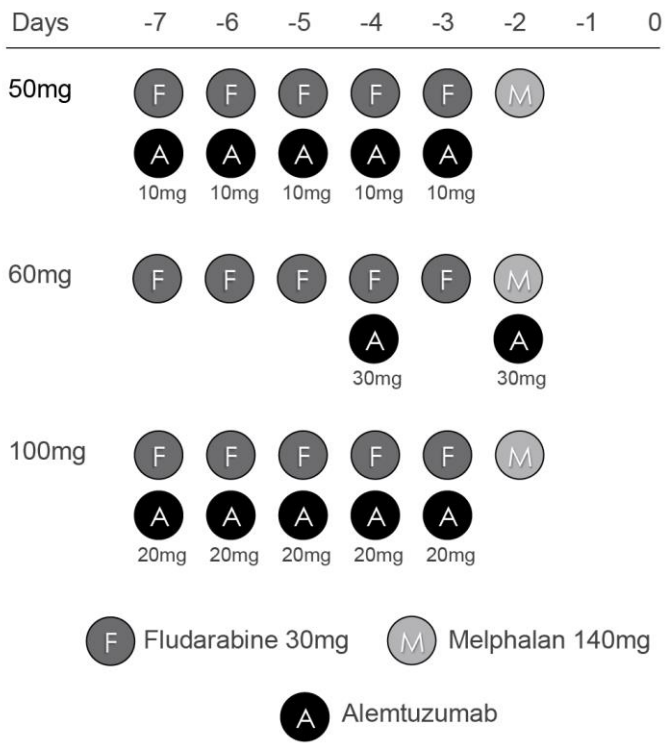


Figure 1. Preparative regimens

Outline of preparative regimens for the three patient cohorts.

Patient characteristics

Consecutive patients recruited at each center were included in the study. Several differences were noted in the patient characteristics reflecting local practice and referral bias, summarized in **Table 1.** Patients in each cohort were matched for donor cell source (90-97% receiving PBSC), HLA matching (69-83% at least 10/10), disease status and follow up. There were however significant differences in age, the proportion of female to male transplants, the frequency of high risk CMV positive recipients and the distribution of disease between acute myeloid leukemia and lymphoproliferative disease. The 50mg cohort had the highest rate of high risk CMV serostatus (negative into positive; 25.23%; p = 0.0058) and patients were more likely

to have a diagnosis of AML or MDS (62.62%; p = 0.0005). Patients receiving 60mg of alemtuzumab were younger (median age 45; p = 0.0051) and had no female to male transplants (p = 0.009). Patients receiving 100mg had the highest rate of female to male transplants (21.82%; p = 0.009)) and the most patients with lymphoproliferative disease (48.51%; p = 0.0005).

	50mg (n =107)	60mg (n =72)	100mg (n =134)	P-value
Age at transplantation				
Median (range)	56 (24-71)	**45 (22-67)**	50 (21-67)	**0.0051**
Graft				
Peripheral blood	97.20%	90.28%	97.47%	0.0551
Bone marrow	2.80%	9.72%	2.53%	
HLA status				
10/10	69.16%	83.33%	70.90%	0.1002
<10/10	30.84%	16.67%	29.10%	
Gender (donor/recipient)				
Female to male	5.61%	**0.00%**	21.82%	**0.009**
CMV (donor/recipient)				
- / positive	**42.99%**	38.89%	40.30%	**0.018**
positive / negative	**25.23%**	12.50%	11.94%	
negative / negative	31.78%	48.61%	47.76%	
Disease indication				
ALL	3.74%	1.39%	3.73%	**0.0005**
AML/MDS	**62.62%**	50.00%	32.84%	
Lymphoproliferative disorders	25.23%	44.44%	48.51%	
Other	8.41%	4.17%	14.93%	
Status at transplantation				
Untreated	5.61%	2.78%	3.73%	0.0909
Partial remission	10.28%	6.94%	17.91%	
Complete remission	74.77%	81.94%	55.22%	
Relapse/progression	5.61%	4.17%	6.72%	
Other	3.74%	4.17%	16.42%	
Follow up period				
Median follow up (months)	18.12	21.63	19.12	0.3339

Table 1. Patient Characteristics

Summary of patient demographics, donor source, HLA matching and gender, CMV serostatus, disease and disease status at transplantation. Differences between cohorts were compared with t-tests or contingency analysis and p values were derived. Significantly outlying values by 2x2 Chi-square tests are indicated in bold in the table.

Acute GVHD

There were no differences in the overall incidence of acute GVHD between the three cohorts of patients with 64-74% of patients experiencing at least grade I GVHD and 32-40% of patients experiencing clinically significant grade II-IV GVHD in all cohorts **(Figure 2A).** However there distribution of acute GVHD grades was skewed (p = 0.0124 for all grades), with an excess of grade III/IV acute GVHD in the 50mg cohort (15% compared with 2-6%; p = 0.0161 for grades III-IV vs. grades 0-II; Chi-square tests). It was not possible to calculate the cumulative incidence of acute GVHD from the available data, so early relapse death remains a potential competing risk. However, the relapse risk within 100 days was consistently low at 5.6%, 1.4% and 6.7% respectively for the 50mg, 60mg and 100mg cohorts. HLA matching was considered an important potential confounding variable by subgroup analysis. The 50mg and 100mg cohorts had a slighter higher percentage of <10/10 HLA-matched donors than the 60mg cohort but this was not a significant baseline difference and <10/10 matched donors did not experience more GVHD, within each cohort. **(Figure 2B)**.

Figure 2

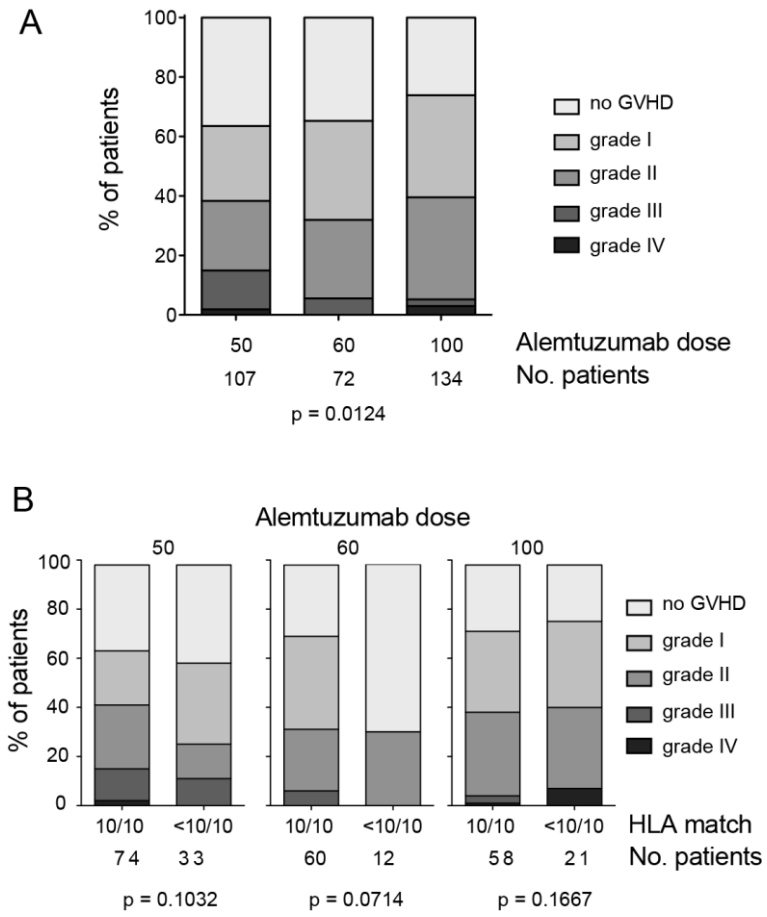


Figure 2. Overall incidence of acute GVHD

A. Comparison of maximal acute GVHD grades between cohorts. No significant difference was detected by chi square tests between cohorts.
B. Comparison of maximal acute GVHD grade between ≥ 10/10 and <10/10 matched unrelated donor transplants within each cohort. There were no significant differences by chi square tests.

The control of severe grade GVHD in the <10/10 patients was not improved by higher dosing of alemtuzumab. In fact, less severe GVHD was observed in the 50mg and 60mg cohorts who received a <10/10 antigen matched donor compared with 10/10 matched donors, presumably due to confounding factors in the selection of lower risk patients or anticipatory adjustment of post-transplant immunosuppression. The number of patients with <10/10 mismatching was not sufficient to stratify the degree of mismatch further.

Multivariate analysis

Although an apparent excess of grade III/IV acute GVHD was observed in the 50mg cohort, several variables that might affect the incidence of GHVD or were non-randomly distributed between the cohorts. A multivariate analysis was therefore performed to analyse the variables that might predict the bivariate outcome grade III/IV GVHD vs all other grades. The covariates considered were Alemtuzumab schedule 100mg, 60mg or 50mg; >10/10 or 10/10 HLA matching; age group <40, 41-60 and >60; female into male or other; CMV negative into positive or other; and, diagnosis MDS/AML or NHL. In a general linear model, older age, female into male, CMV high risk and NHL were associated with increased but non-significant risk of grade III/IV acute GVHD. Only alemtuzumab schedule was significantly predictive of grade III/IV GVHD conferring more than a three-fold increased risk, which remained after adjustment for the other variables **(Table 2).**

Variable	Univariate Odds Ratio of GVHD III/IV	p value	Multivariate Odds Ratio of GVHD III/IV	p value
Alemtuzumab 60mg vs 100mg	1.067	0.920	0.837	0.792
Alemtuzumab 50 mg vs 100mg	**3.190**	**0.014**	**3.024**	**0.030**
<10/10 vs 10/10 match	0.933	0.888	0.881	0.803
Age 41-60 vs <40	0.847	0.783	0.638	0.458
Age >60 vs <40	0.607	0.285	1.144	0.858
F into M vs other	0.340	0.300	0.342	0.318
CMV neg pos vs other	1.491	0.415	1.423	0.499
NHL vs MDS/AML	1.393	0.620	2.086	0.162

Table 2. Mulitvariate analysis of the risk of grade III/IV acute GVHD

Effect of covariables on the incidence of grade III/IV GVHD. Odds ratios are given for the first variable versus the second as baseline.

Chronic GVHD

The cumulative incidence of chronic GVHD was similar in all cohorts ranging from 33% in the 100mg and 32% in the 60mg cohort, to 41% in the 50mg cohort at two years (p = 0.6248) **(Figure 3A).** Although there was a trend for more chronic GVHD in the 50mg cohort, the number of patients with extensive chronic GVHD was not increased between the cohorts (p = 0.1612) **(Figure 3B).**

Figure 3

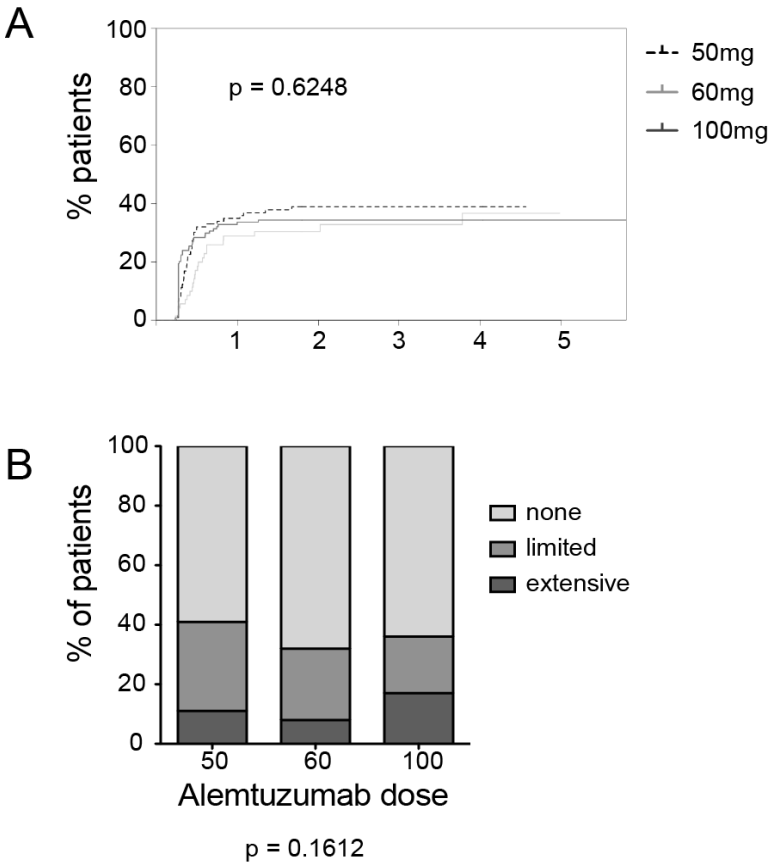


Figure 3. Incidence and severity of chronic GVHD

A. Comparison of cumulative incidence of chronic GVHD analyzed with relapse and death as competing risks. Patients were censored at last follow up. No significant difference was detected between cohorts.

B Comparison of the maximal severity of chronic GVHD between cohorts. No significant difference was detected between cohorts.

Engraftment and donor chimerism

In keeping with previous reports, the rate of engraftment and achievement of complete donor myeloid chimerism was more than 95% in all cohorts (not shown)**.** The level of donor T cell chimerism was however very variable **(Figure 4A).** Analysis was constrained by the reporting donor chimerism in the 100mg cohort in bands of 0-49; 50-94 and ≥95, so data from the other cohorts were transformed to match. Only just over half of all patients achieved more than 95% donor chimerism with the lowest levels of donor T cell chimerism observed in the 60mg cohort (p = 0.0460). This may reflect a lower proportion of mismatched donors or more liberal use of post-transplant immunosuppression in this cohort. Continuously distributed data were available for the 50mg and 60mg cohorts and showed a significant skewing between cohorts with lower median T cell chimerism in the 60mg cohort (85% vs. 96%; p = **(Figure 4B).**

Figure 4

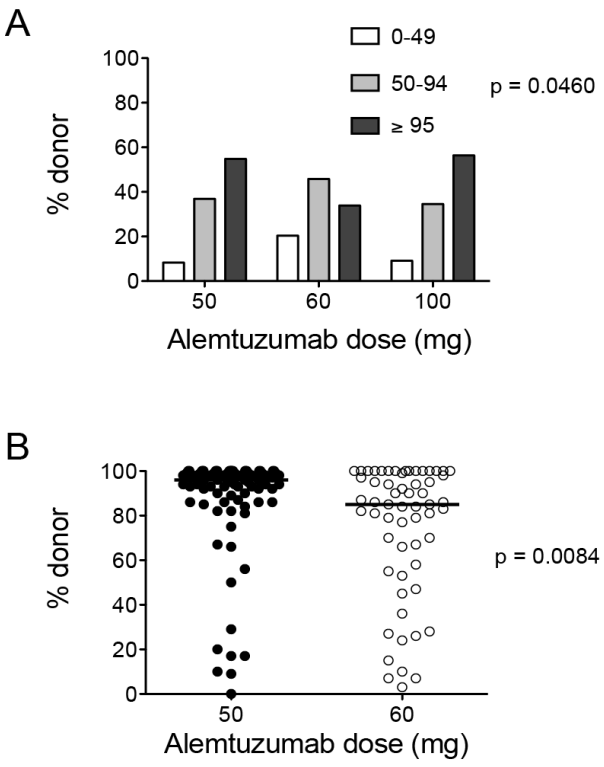


Figure 4. Chimerism

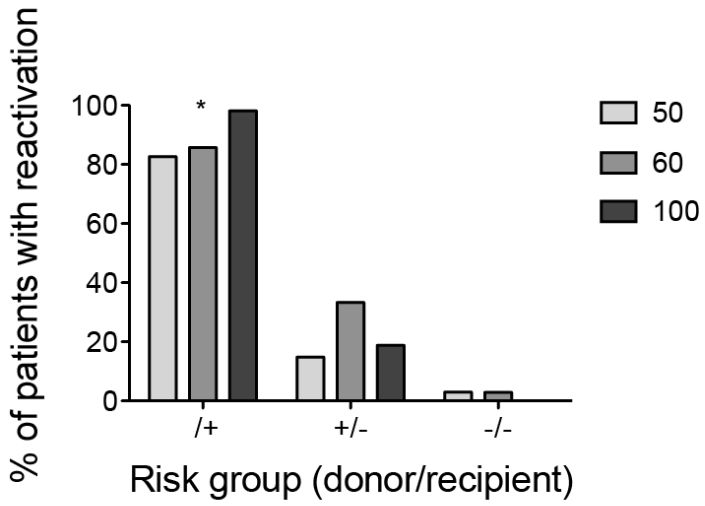
A. Donor T cell chimerism at 100 days. Bins of 0-50; 50-95 and 95-100 were selected based on the discontinuous data available for the 100mg cohort. A significant difference was detected owing to lower T cell chimerism in the 60mg cohort.

B. Comparison of donor T cell chimerism at 100 days for patients in 50mg and 60mg cohorts showing skewing towards lower median T cell chimerism in the 60mg cohort (Mann-Witney test).

CMV reactivation

The risk of CMV reactivation is high with alemtuzumab containing regimens. More than 80% of patients in all cohorts with high risk serostatus (positive recipient) had a positive CMV PCR result after transplant. Intermediate status (positive donor into negative recipient) reactivation was similar in all cohorts. Within the high risk serostatus groups, significantly higher rates of reactivation were observed in the 100mg cohort (p= 0.0267) **(Figure 5).**

Figure 5



* p = 0.0267

Figure 5. CMV reactivation

The proportion of patients in each cohort requiring treatment for CMV reactivation, according to CMV risk status. A higher risk of reactivation was detected for high risk CMV positive recipients in the 100mg cohort (*).

Mortality and survival

No significant differences were observed in the cumulative incidence of non-relapse mortality at 2 years between 100mg, 60mg and 50mg cohorts (16%, 16%, and 24%, respectively; p = 0.3232) **(Figure 6A)** or relapse rates at 2 years (36%, 24%, and 29%, respectively; p = 0.3454) **(Figure 6B)**. The overall survival of each cohort was not significantly different and is shown for the major disease groups AML/MDS and NHL **(Figure 6C).** Trends for lower survival in the 50mg cohort were observed in both relapse and non-relapse mortality. There were no differences within each

cohort when survival was stratified according to diagnosis or disease risk (not shown).

Figure 6

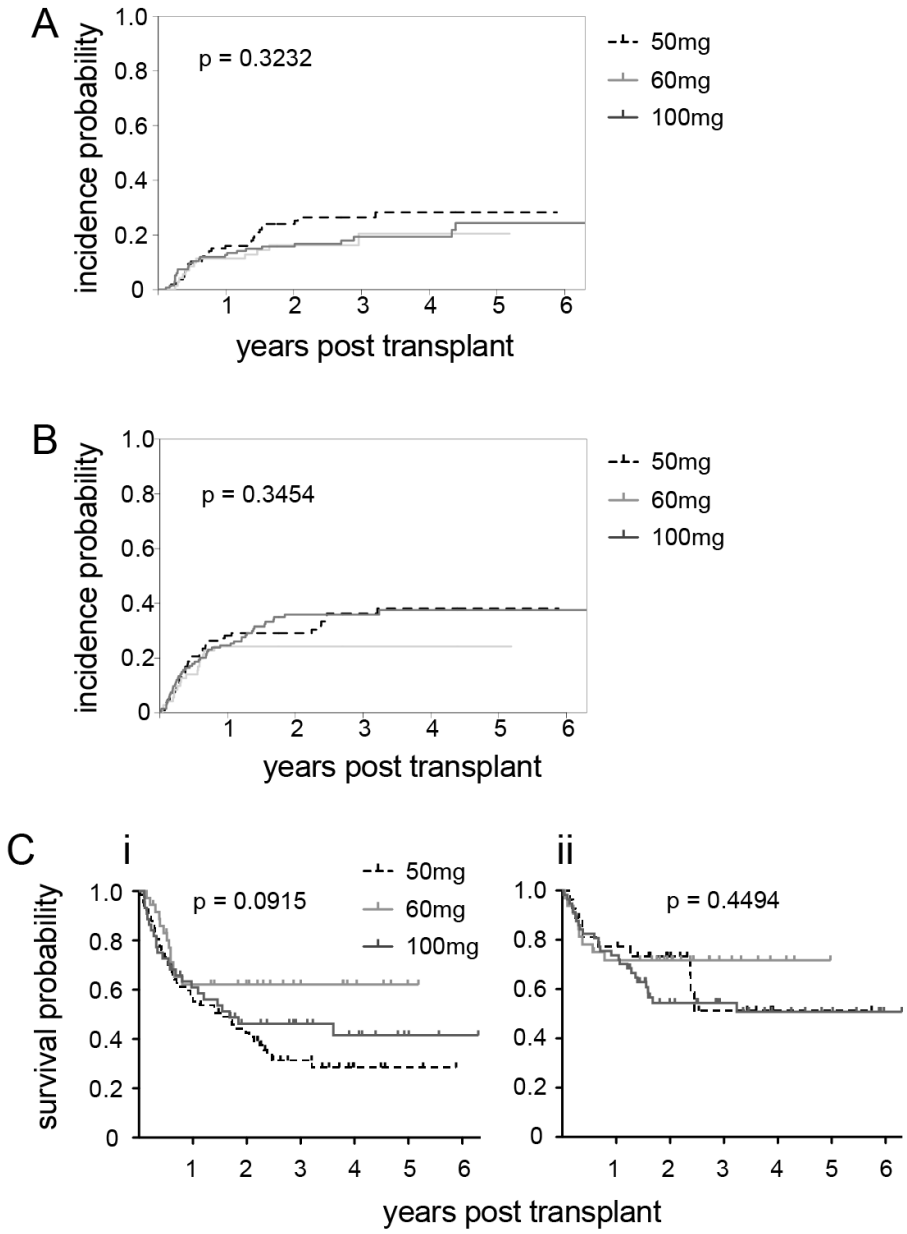


Figure 6. Outcome

A. Cumulative incidence of NRM with relapse as a competing factor showing no significant difference between the cohorts by Gray's test.

B. Cumulative incidence of relapse with NRM as a competing factor showing no significant difference between the cohorts by Gray's test.
C. Overall survival for patients with i) AML/MDS or ii) NHL by cohort. Kaplan-Meier curves were compare by Log Rank (Mantel-Cox) test.

Discussion

The use of T cell depletion remains controversial in all forms of allogeneic bone marrow transplantation but particularly in the setting of reduced intensity conditioning, where it is argued that graft versus tumor effects need to be stronger to compensate for attenuated cytoreduction(19,30). However, even with minimal conditioning, T replete transplants still incur a similar burden of graft versus host disease to full intensity transplants albeit with delayed kinetics(31). The prevalence of GVHD with reduced intensity conditioning is further amplified by the increasing age of eligible patients and widespread use of peripheral blood stem cells. For these reasons, many UK centers have developed reduced intensity protocols using alemtuzumab to effect in vivo T cell depletion and reduce acute GVHD(1-7). This approach is supported by a retrospective comparison of T depletion strategies with reduced intensity regimens showing superior control of acute and chronic GVHD and a trend for greater overall survival with alemtuzumab-containing regimens, despite higher relapse rates (19). These data underscore the need to define optimal dosing regimens that balance the risks of toxicity and relapse.

Several cohort studies have compared protocols with and without alemtuzumab and arrived at the conclusion that overall survival is similar and survivorship quality is enhanced even though there are higher rates of relapse and CMV reactivation in patients given alemtuzumab. In sibling transplants for lymphoproliferative disease, acute and chronic GVHD was reduced by alemtuzumab with no impact on overall survival (32). In a comparison of patients with AML, alemtuzumab reduced the incidence of extensive chronic GVHD from 47% to 4% and was associated with slightly superior survival than those not receiving alemtuzumab (5). This study included sibling and matched unrelated donor transplants given a range of alemtuzumab schedules from 30mg to 100mg. Another more controlled study of sibling and unrelated transplants for AML showed that 100mg alemtuzumab reduced the incidence of acute grade II-IV GVHD from 58.1% to 23.3% and chronic GVHD from 78.4% to 16.0% with no difference in overall survival(33). Increases in the rate of CMV reactivation with alemtuzumab do not appear to impact on survival with modern pre-emptive antiviral therapy (34). Although intervention with DLI may be required to achieve an optimal outcome in alemtuzumab-containing transplants, recipients of DLI appear to enjoy a favorable outcome in both lymphoid and myeloid malignancy (14,35).

The scheduling of alemtuzumab has a significant impact on plasma concentration on the day of transplant(11). We have previously demonstrated that the 60mg regimen results in a similar level of alemtuzumab to the 100mg regimen at 5-6μg/ml on day +1 (25), thus it is not surprising that comparable control of GVHD was observed. It would be erroneous to conclude that 60mg was a safe threshold dose without

reference to the timing of administration. The excess of severe acute GVHD in the 50mg cohort probably reflects earlier scheduling as much as a lower total dose, resulting in a substantially lower in vivo concentration. Although Alemtuzumab levels have not been measured in this regimen, the continuously distributed T cell chimerism at day 100 is consistent with a much lower level of donor T cell depletion in the 50mg cohort, compared with the 60mg cohort. Even later scheduling of two 30mg doses to day -2 to -1 has been proposed and is likely to deliver alemtuzumab concentrations that exceed the original 100mg regimen(36). The 100mg dose of alemtuzumab may be used with <10/10 antigen matched donors(16) and it appeared here that reduction to 60mg or 50mg did not significantly increase GVHD, relative to patients with 10/10 matched donors given the same prophylaxis.

These results indicate that fludarabine-melphalan reduced intensity transplantation is feasible with or without alemtuzumab but the question remains whether it is possible to define an optimal schedule of alemtuzumab that achieves good control of GVHD and survivorship without excessive detriment to immune reconstitution. A phase II study in sibling transplants reported that a significant dose reduction to a single 30mg vial at day -1 almost completely ablated acute grade II-IV GVHD and extensive chronic GVHD (both 4%). Another dose reduction study using a variety of fludarabine-based regimens showed that lower doses were also feasible in mixed sibling and unrelated donors given a variety of conditioning regimens, but there was insufficient power to discriminate between different dose levels (23)

In unrelated donor transplants, there does not appear to be a simple linear relationship between alemtuzumab schedule and GVHD risk. At the highest alemtuzumab dose of 100mg, the rate of grade II-IV GVHD in our study was approximately 10-fold higher (40%) than sibling transplants exposed to 30mg of alemtuzumab (4%), although the majority was grade II, and remained relatively constant with 60mg and 50mg dosing. This is consistent with a previous report of unrelated donors transplants performed with 100mg of alemtuzumab(16). A recent study comparing the 60mg schedule for unrelated donors with 30mg for sibling transplants showed similarly high rates of grade II-IV GVHD (44%) and donor T cell chimerism in the unrelated transplants(25). Reducing alemtuzumab to 40mg or less did not appear to increase the rate of grade II-IV acute GVHD in a series of small cohorts (23). However, below 60mg, there may be a trend towards increasing severe grade III/IV acute GVHD. In our study, patients receiving 50mg experienced 15% grade III/IV acute GVHD compared with 2-6% in the 60mg and 100mg cohorts. This bias was also observed in the 10/10 matched donors. An even higher rate of 23.8% severe acute grade III/IV GVHD was reported in unrelated donor transplants receiving 40mg of alemtuzumab although this did not further increase when alemtuzumab dose was reduced to 20mg and 10mg (23).

The main limitation of this study is the difficulty in controlling center bias. The increase in grade III/IV acute GVHD in the 50mg cohort remained more than three-fold after adjustment for the major covariables that might have influenced GVHD, but

other factors such as disease risk index and performance status could not be completely controlled for. Stratification according to disease risk was not possible given the many different indications that were included in the analysis and Hematopoietic Cell Transplantation Comorbidity Index data were not routinely reported for patients transplanted over this period. Less tangible bias may also have been introduced by variations in clinical practice such as the use of post-transplant immunosuppression. Although centers reported similar operating procedures governing the withdrawal of ciclosporin, ciclosporin levels were not collected as primary data. Similarly, detailed donor lymphocyte infusion records were not available. All three centers reported a common escalating DLI regimen but we could not audit this directly. Similar rates of chronic GVHD were observed, regardless of any institutional variations in practice that may have occurred in relation to the use of DLI.

In conclusion, this study suggests that it is feasible to use two 30mg doses of alemtuzumab in fludarabine-melphalan unrelated donor transplants without significantly increasing the risk of GVHD or impairing outcome compared with the original 100mg dose. Given at day -4 and -2, this results in similar plasma levels compared with five 20mg doses (25) and is cheaper, assuming a 30mg vial size. Further dose reduction may be feasible with later scheduling (day -2 to -1) and ideally, should be studied prospectively with in vivo alemtuzumab levels and accurate T cell chimerism, in addition to clinical outcome data. The current 50mg regimen in use in the UK was associated with an increased risk of severe grade III/IV acute GVHD, although confounding factors may have increased the baseline risk in this cohort. Overall, the use of T cell depletion remains subject to historical precedent and individual center bias regarding the acceptable burden of GVHD in transplant survivors.

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Authorship contributions

Kile Green analyzed data and produced figures
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Graham Jackson provided clinical data
Anne M Dickinson provided clinical data
Kirsty Thomson provided clinical data
Stephen Mackinnon designed the study

Charles Craddock designed the study
Karl S Peggs designed the study and wrote the paper
Matthew Collin designed the study and wrote the paper

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