Published in final edited form as:
Biol Blood Marrow Transplant. 2017 May 1; 23(5): 805–812. doi:10.1016/j.bbmt.2017.02.007.

Comparison of graft versus host disease with three different alemtuzumab schedules in unrelated donor fludarabine and melphalan allografts

Kile Green[1], **Kim Pearce**[1], **Rob S Sellar**[2,3], **Laura Jardine**[1,4], **Phillip LR Nicolson**[5], **Sandeep Nagra**[6], **Venetia Bigley**[1,4], **Graham Jackson**[4,7], **Anne M Dickinson**[1], **Kirsty Thomson**[2,3], **Stephen Mackinnon**[2,3], **Charles Craddock**[5,6], **Karl S Peggs**[2,3], and **Matthew Collin**[1,4]

[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

[2]Cancer Institute, University College London, London, United Kingdom

[3]Department of Haematology, University College London Hospitals NHS Foundation Trust, London, United Kingdom

[4]Northern Centre for Bone Marrow Transplantation, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom

[5]School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom

[6]Centre for Clinical Haematology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

[7]Northern Institute for Cancer Research, Newcastle University, Newcastle Upon Tyne, United Kingdom

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

Authorship contributions
Kile Green analyzed data and produced figures
Kim Pearce performed statistical analysis
Rob S Sellar provided clinical data
Laura Jardine analyzed data and produced figures
Phillip LR Nicolson provided clinical data
Sandeep Nagra provided clinical data
Venetia Bigley provided clinical data
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

Conflict of interest disclosures
The authors have no conflict of interest to declare.

retrospective observational study of 313 patients, the incidence and severity of GVHD was compared in patients receiving 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 age, gender mismatch, CMV risk and diagnosis (p = 0.030). The findings indicate that 60mg doses of alemtuzumab is comparable to 100mg but lower dosing may increase the risk of severe grade GVHD.

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 dose 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 doses 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 deescalation 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.

Europe PMC Funders Author Manuscripts

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.

Study endpoints

The primary endpoints of the study were the raw incidence and severity of acute GVHD 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.

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

Europe PMC Funders Author Manuscripts

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 and at the time of receiving DLI for the analysis of chronic GVHD. Multivariate analysis was performed in SPSS using binary logistic regression (grade II/IV vs other)

Results

In this three-center retrospective observational study, we compared the incidence and severity of acute GVHD in patients receiving fludarabine 150mg/m2 and melphalan 140mg/m2 combined with the original 100mg alemtuzumab regimen (n=134) (1), or one of two alternative schedules: 60mg given as two doses of 30mg, day -4 and day -2 (n=72) (25); or 50mg administered as five doses of 10mg, days -7 to -3 (n=107) (20,21). Conditioning protocols are summarized in Figure 1.

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%; $p = 0.0005$).

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). 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.

Although the proportion of <10/10 matched donors not reach significance between the cohorts, the relationship between alemtuzumab dose and HLA matching was explored by dividing each cohort into 10/10 and <10/10 antigen matched subgroups (Figure 2B). There were no significant differences in any cohort in the rate of GVHD between the two subgroups nor was the control of severe grade GVHD in the <10/10 patients improved by higher dosing of alemtuzumab. Paradoxically, less severe GVHD was observed in the 60mg cohort who received a <10/10 antigen matched donor, presumably due to confounding factors in the selection of lower risk patients or anticipatory adjustment of post-transplant immunosuppression.

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 were non-randomly distributed between the cohorts. These included: patient age, frequency of female into male and CMV negative into positive transplants and indication for transplantation. 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 dose 100mg, 60mg or 50mg; 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 dose 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).

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).

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).

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)

Europe PMC Funders Author Manuscripts

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).

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). There were no differences within each cohort when 10/10 or <10/10 matched donors were compared or when disease subgroups were analyzed (not shown).

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 doses 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).

Europe PMC Funders Author Manuscripts

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 dose 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 dose 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 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.

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 after adjustment for the major

Europe PMC Funders Author Manuscripts

covariables that might have influenced GVHD, but other factors such as performance status and disease status could not be completely controlled for. Other less tangible bias may 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. Donor lymphocyte infusion data were not studied because the intention was to analyze acute and chronic GVHD arising directly from the allograft procedure. All centers reported using a similar escalating regimen and administration of DLI was a censoring event.

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.

Acknowledgements

The authors thank patients who participated and the transplant teams who provided their clinical care. The research was supported by Bright Red and the British Society for Haematology.

References

1. Kottaridis PD, Milligan DW, Chopra R, et al. In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood. 2000; 96(7):2419–2425. [PubMed: 11001893]
2. Chakraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood. 2002; 99(3):1071–1078. [PubMed: 11807015]
3. Tauro S, Craddock C, Peggs K, et al. Allogeneic stem-cell transplantation using a reduced-intensity conditioning regimen has the capacity to produce durable remissions and long-term disease-free survival in patients with high-risk acute myeloid leukemia and myelodysplasia. J Clin Oncol. 2005; 23(36):9387–9393. [PubMed: 16314618]
4. Delgado J, Thomson K, Russell N, et al. Results of alemtuzumab-based reduced-intensity allogeneic transplantation for chronic lymphocytic leukemia: a British Society of Blood and Marrow Transplantation Study. Blood. 2006; 107(4):1724–1730. [PubMed: 16239425]
5. Malladi RK, Peniket AJ, Littlewood TJ, et al. Alemtuzumab markedly reduces chronic GVHD without affecting overall survival in reduced-intensity conditioning sibling allo-SCT for adults with AML. Bone Marrow Transplant. 2009; 43(9):709–715. [PubMed: 19029965]
6. Thomson KJ, Morris EC, Bloor A, et al. Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2009; 27(3):426–432. [PubMed: 19064981]
7. Thomson KJ, Morris EC, Milligan D, et al. T-cell-depleted reduced-intensity transplantation followed by donor leukocyte infusions to promote graft-versus-lymphoma activity results in excellent long-term survival in patients with multiply relapsed follicular lymphoma. J Clin Oncol. 2010; 28(23):3695–3700. [PubMed: 20606089]

Europe PMC Funders Author Manuscripts

8. Klangsinsirikul P, Carter GI, Byrne JL, Hale G, Russell NH. Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. Blood. 2002; 99(7):2586–2591. [PubMed: 11895797]
9. Buggins AG, Mufti GJ, Salisbury J, et al. Peripheral blood but not tissue dendritic cells express CD52 and are depleted by treatment with alemtuzumab. Blood. 2002; 100(5):1715–1720. [PubMed: 12176892]
10. Ratzinger G, Reagan JL, Heller G, Busam KJ, Young JW. Differential CD52 expression by distinct myeloid dendritic cell subsets: implications for alemtuzumab activity at the level of antigen presentation in allogeneic graft-host interactions in transplantation. Blood. 2003; 101(4):1422–1429. [PubMed: 12393688]
11. Morris EC, Rebello P, Thomson KJ, et al. 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. 2003; 102(1):404–406. [PubMed: 12623851]
12. Peggs KS, Thomson K, Hart DP, et al. Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. Blood. 2004; 103(4):1548–1556. [PubMed: 14576063]
13. Bloor AJ, Thomson K, Chowdhry N, et al. High response rate to donor lymphocyte infusion after allogeneic stem cell transplantation for indolent non-Hodgkin lymphoma. Biol Blood Marrow Transplant. 2008; 14(1):50–58. [PubMed: 18158961]
14. Potter VT, Krishnamurthy P, Barber LD, et al. Long-term outcomes of alemtuzumab-based reduced-intensity conditioned hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myelogenous leukemia secondary to myelodysplastic syndrome. Biol Blood Marrow Transplant. 2014; 20(1):111–117. [PubMed: 24216184]
15. Shaw BE, Apperley JF, Russell NH, et al. Unrelated donor peripheral blood stem cell transplants incorporating pre-transplant in-vivo alemtuzumab are not associated with any increased risk of significant acute or chronic graft-versus-host disease. Br J Haematol. 2011; 153(2):244–252. [PubMed: 21382020]
16. Mead AJ, Thomson KJ, Morris EC, et al. HLA-mismatched unrelated donors are a viable alternate graft source for allogeneic transplantation following alemtuzumab-based reduced-intensity conditioning. Blood. 2010; 115(25):5147–5153. [PubMed: 20371745]
17. Chakrabarti S, Avivi I, Mackinnon S, et al. Respiratory virus infections in transplant recipients after reduced-intensity conditioning with Campath-1H: high incidence but low mortality. Br J Haematol. 2002; 119(4):1125–1132. [PubMed: 12472597]
18. Dodero A, Carrabba M, Milani R, et al. Reduced-intensity conditioning containing low-dose alemtuzumab before allogeneic peripheral blood stem cell transplantation: graft-versus-host disease is decreased but T-cell reconstitution is delayed. Exp Hematol. 2005; 33(8):920–927. [PubMed: 16038785]
19. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood. 2011; 117(25):6963–6970. [PubMed: 21464372]
20. Faulkner RD, Craddock C, Byrne JL, et al. BEAM-alemtuzumab reduced-intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. Blood. 2004; 103(2):428–434. [PubMed: 12969983]
21. Tholouli E, Liakopoulou E, Greenfield HM, et al. Outcomes following 50 mg versus 100 mg alemtuzumab in reduced-intensity conditioning stem cell transplants for acute myeloid leukaemia and poor risk myelodysplasia. Br J Haematol. 2008; 142(2):318–320. [PubMed: 18492100]
22. Ingram W, Devereux S, Das-Gupta EP, et al. Outcome of BEAM-autologous and BEAM-alemtuzumab allogeneic transplantation in relapsed advanced stage follicular lymphoma. Br J Haematol. 2008; 141(2):235–243. [PubMed: 18318762]
23. Bertz H, Spyridonidis A, Wasch R, Grullich C, Egger M, Finke J. A novel GVHD-prophylaxis with low-dose alemtuzumab in allogeneic sibling or unrelated donor hematopoetic cell transplantation: the feasibility of deescalation. Biol Blood Marrow Transplant. 2009; 15(12):1563–1570. [PubMed: 19896080]

24. Chakraverty R, Orti G, Roughton M, et al. Impact of in vivo alemtuzumab dose before reduced intensity conditioning and HLA-identical sibling stem cell transplantation: pharmacokinetics, GVHD, and immune reconstitution. Blood. 2010; 116(16):3080–3088. [PubMed: 20587785]
25. Jardine L, Publicover A, Bigley V, et al. A comparative study of reduced dose alemtuzumab in matched unrelated donor and related donor reduced intensity transplants. Br J Haematol. 2015; 168(6):874–881. [PubMed: 25640315]
26. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part l: unadjusted analysis. Bone Marrow Transplant. 2001; 28(10):909–915. [PubMed: 11753543]
27. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: Regression modeling. Bone Marrow Transplant. 2001; 28(11):1001–1011. [PubMed: 11781608]
28. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999; 94(446):496–509.
29. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. Bone Marrow Transplant. 2010; 45(9):1388–1395. [PubMed: 20062101]
30. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001; 97(11):3390–3400. [PubMed: 11369628]
31. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood. 2003; 102(2):756–762. [PubMed: 12663454]
32. Perez-Simon JA, Kottaridis PD, Martino R, et al. Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. Blood. 2002; 100(9):3121–3127. [PubMed: 12384408]
33. van Besien K, Kunavakkam R, Rondon G, et al. Fludarabine-melphalan conditioning for AML and MDS: alemtuzumab reduces acute and chronic GVHD without affecting long-term outcomes. Biol Blood Marrow Transplant. 2009; 15(5):610–617. [PubMed: 19361753]
34. Peggs KS, Preiser W, Kottaridis PD, et al. Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation. Br J Haematol. 2000; 111(3):782–790. [PubMed: 11122138]
35. Peggs KS, Sureda A, Qian W, et al. Reduced-intensity conditioning for allogeneic haematopoietic stem cell transplantation in relapsed and refractory Hodgkin lymphoma: impact of alemtuzumab and donor lymphocyte infusions on long-term outcomes. Br J Haematol. 2007; 139(1):70–80. [PubMed: 17854309]
36. Okasha D, Kirkwood A, Copland M, et al. Fludarabine, Melphalan and Alemtuzumab Conditioned Reduced Intensity (RIC) Allogeneic Hematopoietic Cell Transplantation for Adults Aged >40 Years with De Novo Acute Lymphoblastic Leukemia: A Prospective Study from the UKALL14 Trial (ISRCTN 66541317). Blood. 2015

Europe PMC Funders Author Manuscripts

Highlights

- Alemtuzumab dosing in unrelated reduced intensity transplantation is undefined.
- 60mg Alemtuzumab provides equivalent control of GVHD compared with 100mg
- 50mg Alemtuzumab is associated with threefold increased risk of severe acute GVHD.

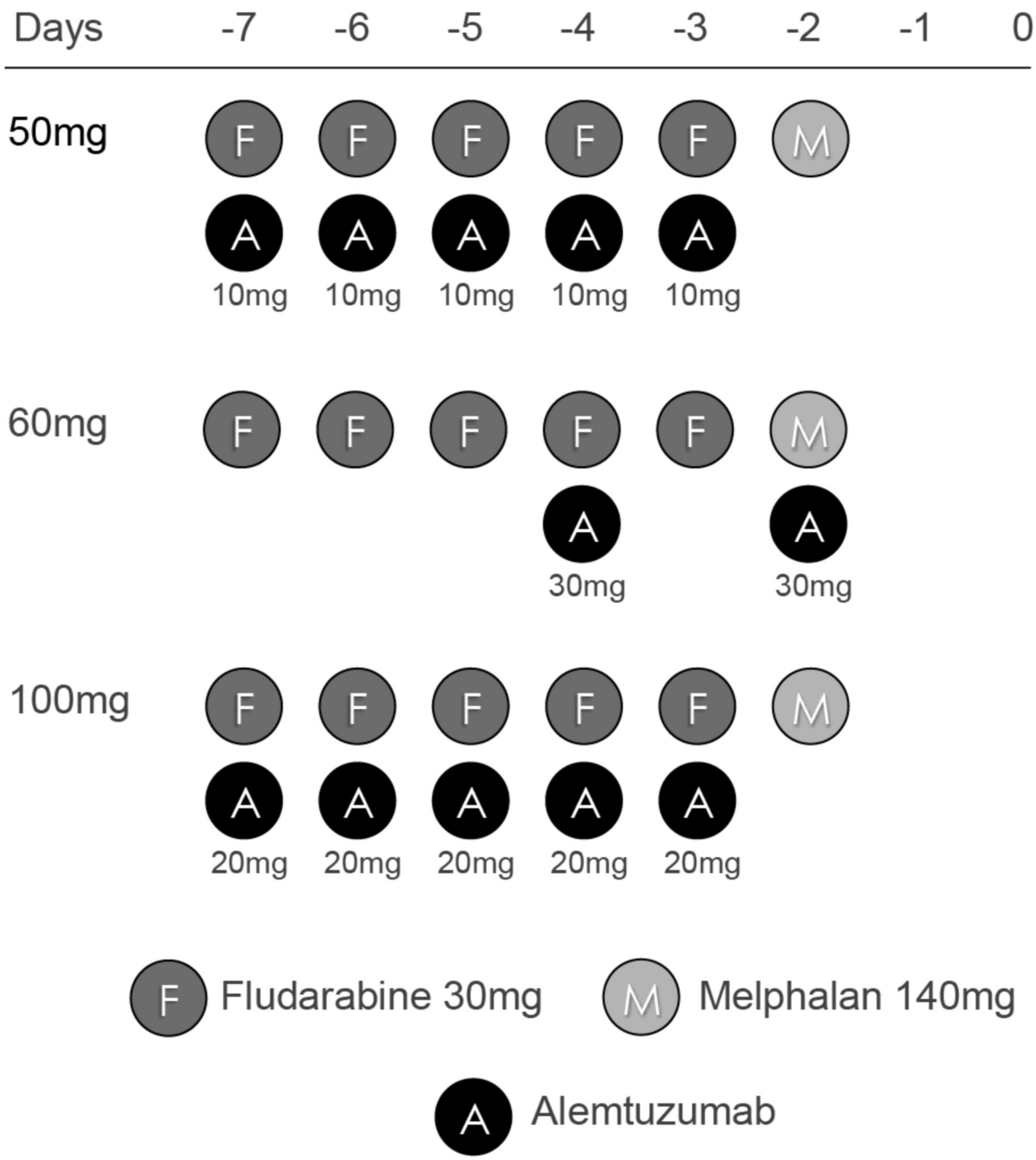

Figure 1. Preparative regimens

Outline of preparative regimens for the three patient cohorts.

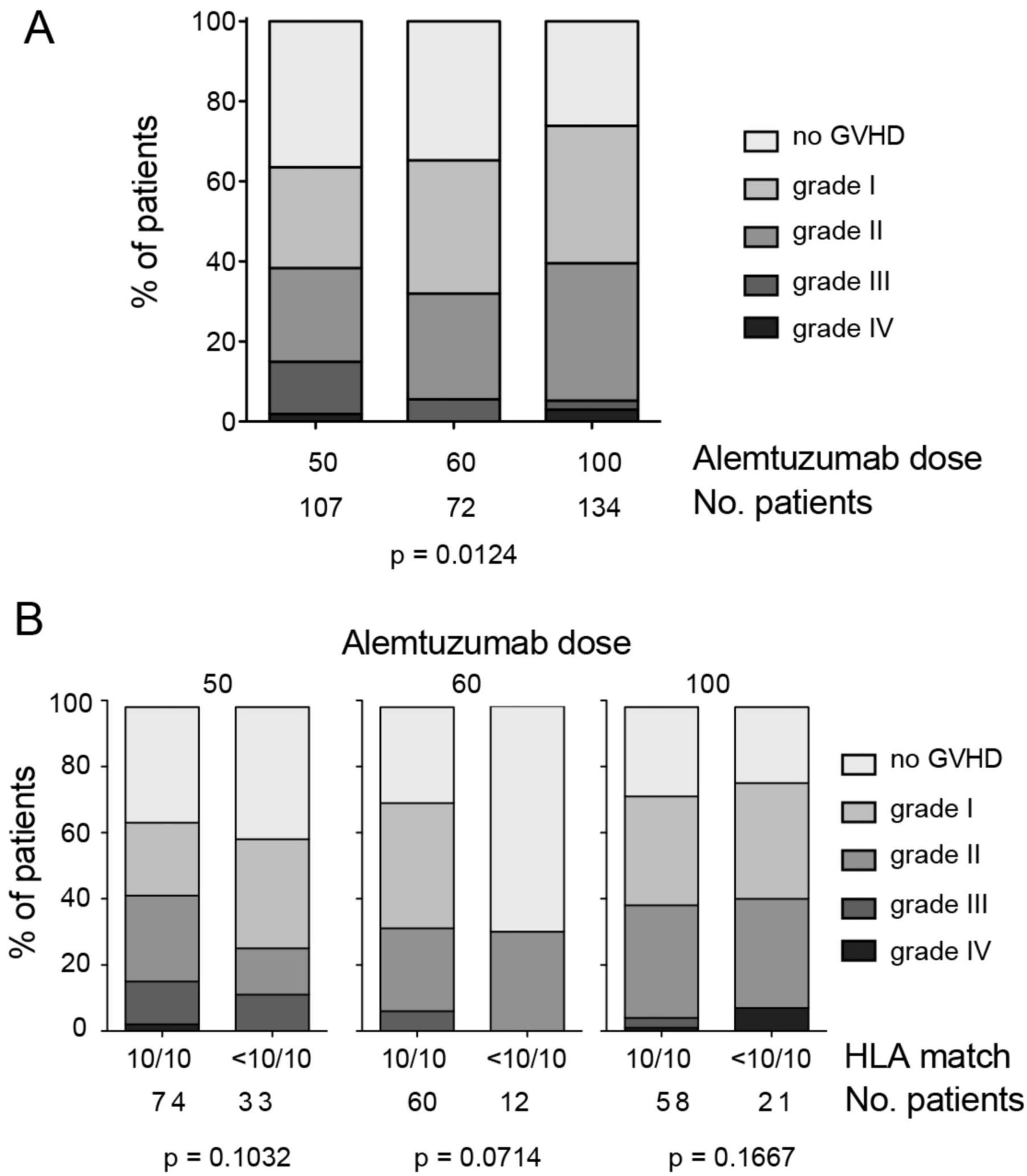

Figure 2. Overall incidence of acute GVHD

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.

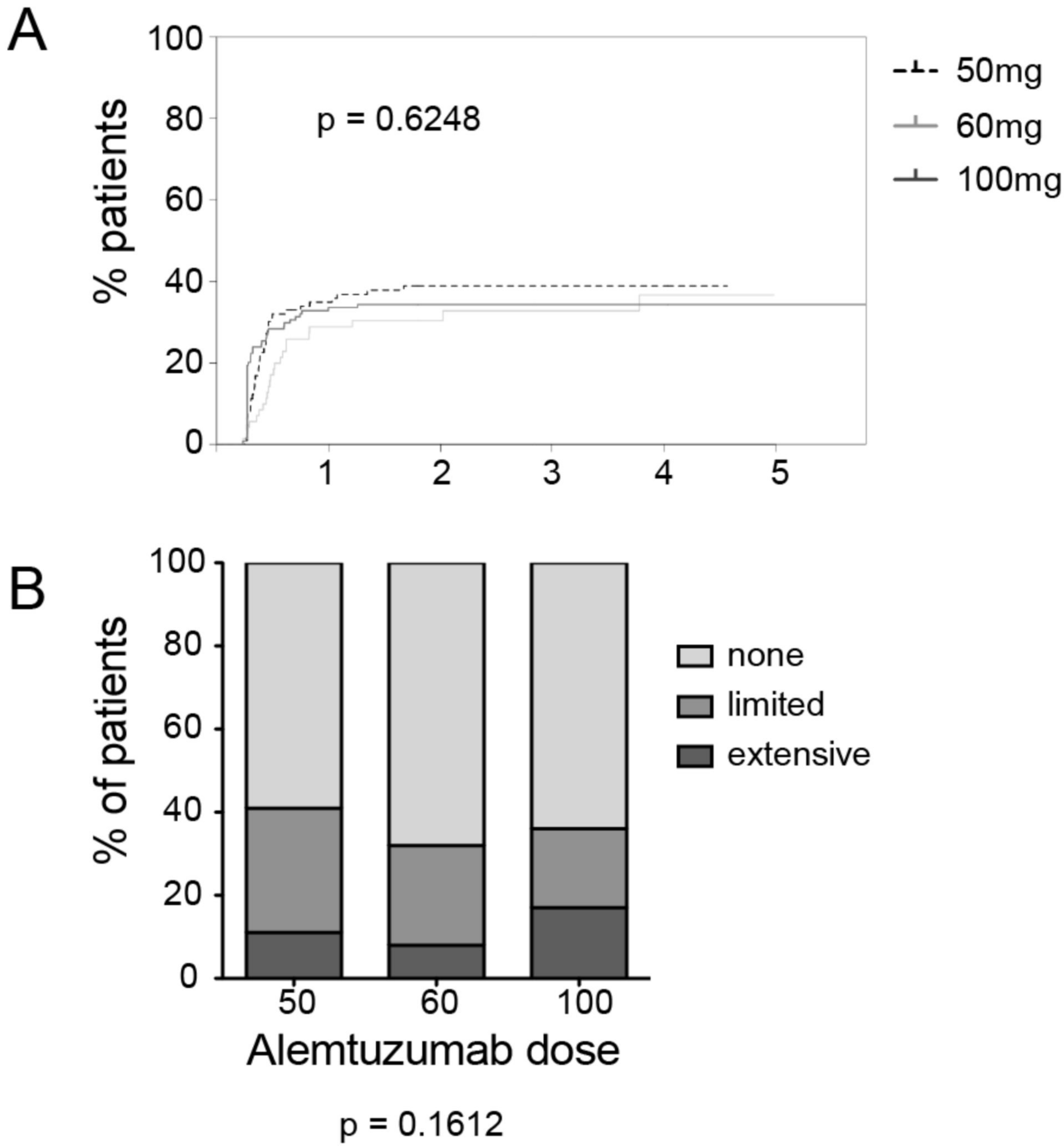

Figure 3. Incidence and severity of chronic GVHD

A. Comparison of cumulative incidence of chronic GVHD analyzed with relapse and death as competing risks. Patients were censored at last follow up or at the point of receiving donor lymphocyte infusion. 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.

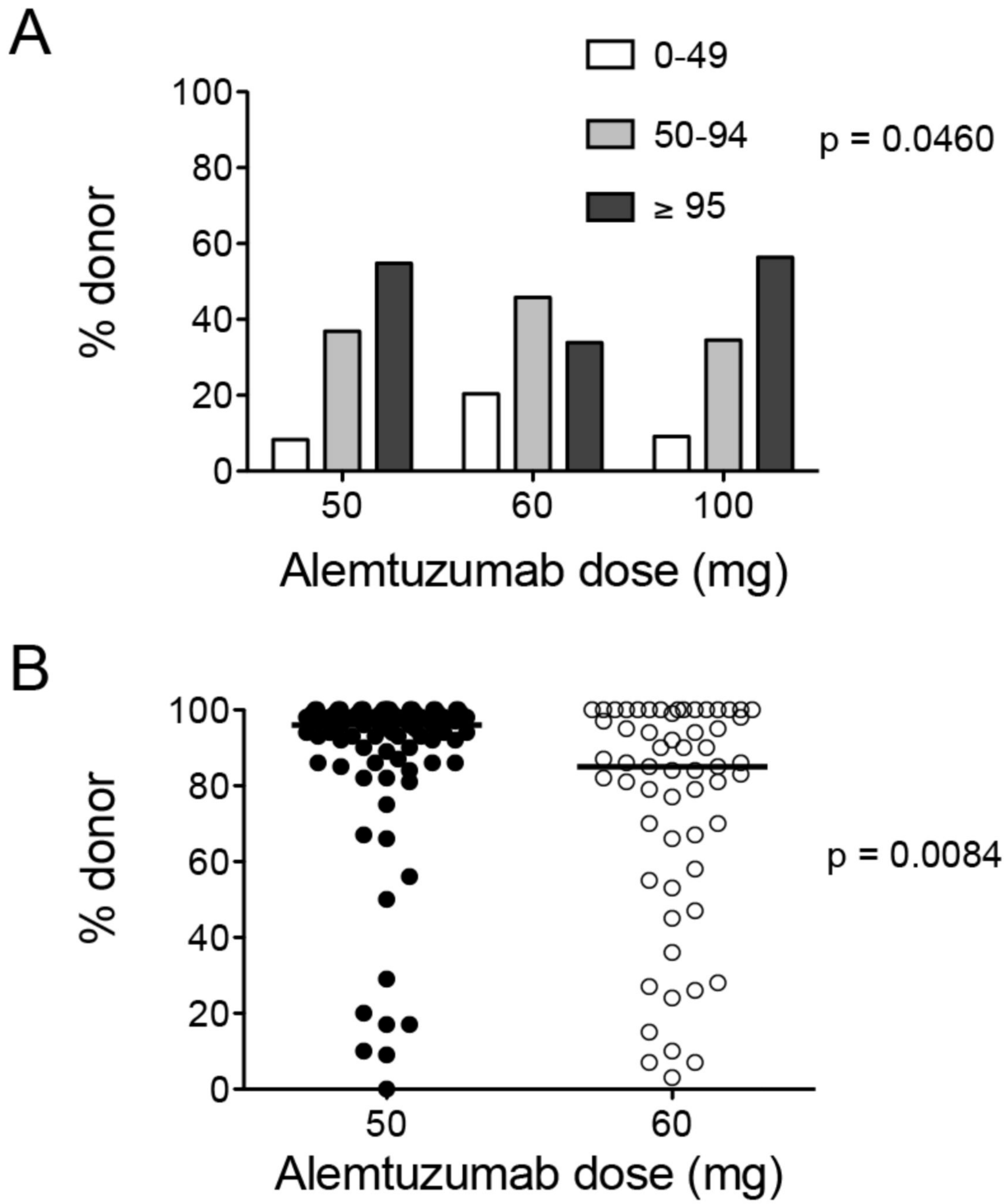

Figure 4. Chimerism

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).

Europe PMC Funders Author Manuscripts

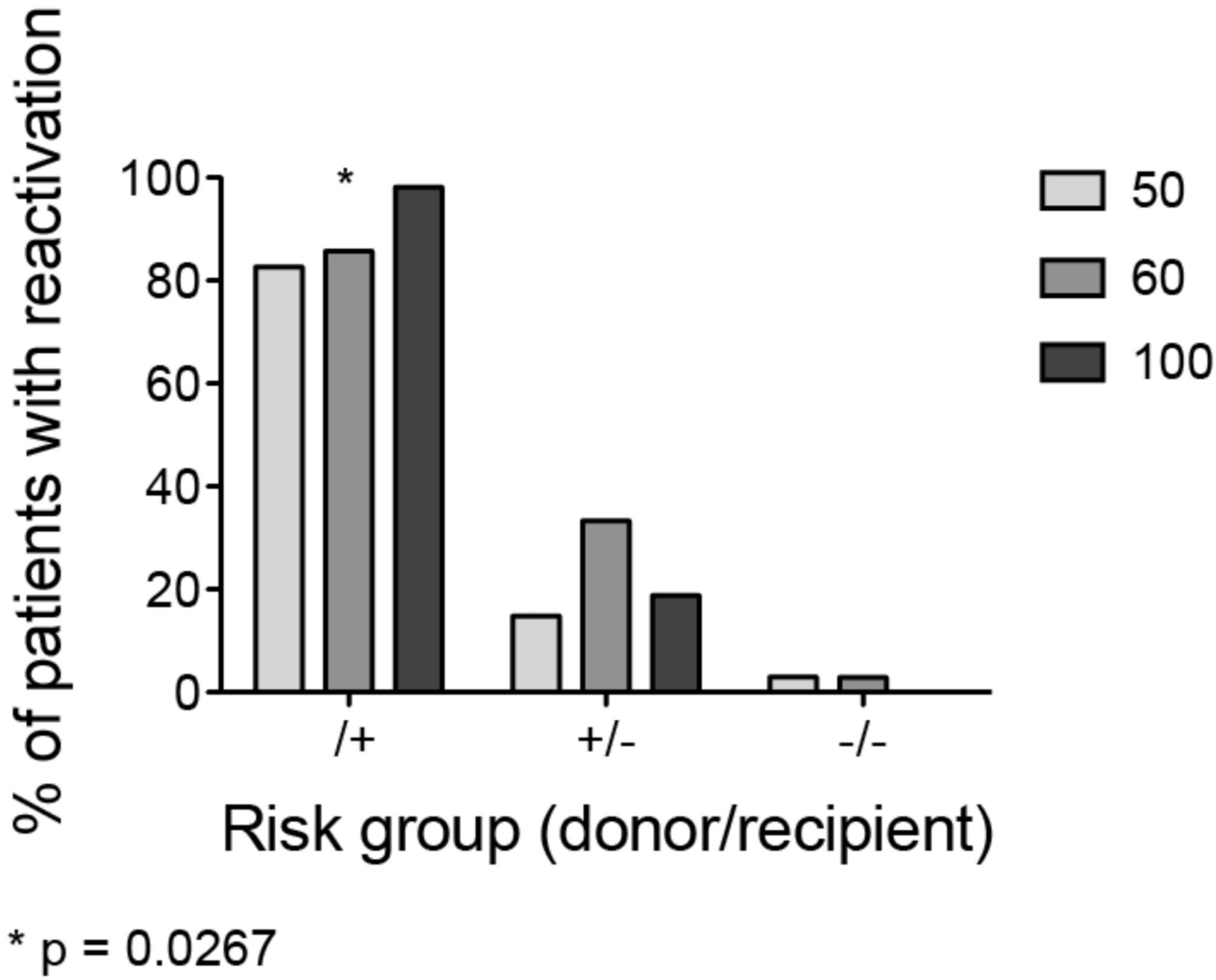

Figure 5. CMV reactivation

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 (*).

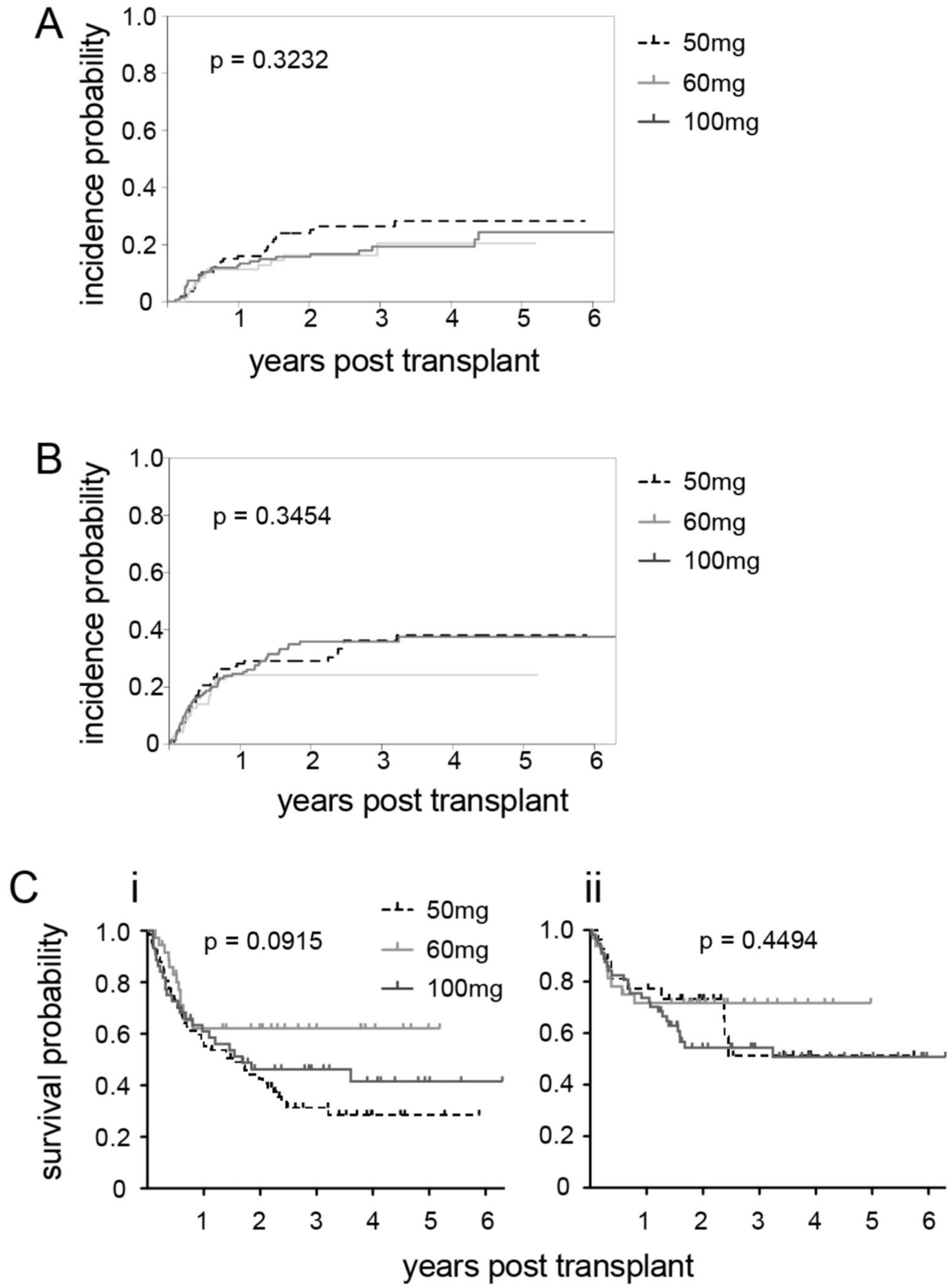

Figure 6. Outcome

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

A. 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.

Europe PMC Funders Author Manuscripts

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.

	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

Europe PMC Funders Author Manuscripts

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

Effect of covariables on the incidence of grade III/IV GVHD. Only Alemtuzumab dose has a significant effect which remains after accounting for other variables that are non-randomly distributed between the groups and could have influenced GVHD.

Variable	**Univariate Relative Risk of GVHD III/IV**	**p value**	**Multivariate Relative risk of GVHD III/IV**	**p value**
Alemtuzumab 60mg vs 100mg	1.067	0.920	0.852	0.811
Alemtuzumab 50 mg vs 100mg	**3.190**	**0.014**	**3.031**	**0.030**
Age 41-60 vs <40	0.847	0.783	0.653	0.477
Age >60 vs <40	0.607	0.285	1.172	0.832
F into M vs other	2.934	0.300	2.959	0.338
CMV neg pos vs other	1.491	0.415	1.409	0.510
NHL vs MDS/AML	1.808	0.376	2.083	0.162