**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 GVHD**

Journal:	<i>British Journal of Haematology</i>
Manuscript ID:	BJH-2010-01775.R1
Manuscript Type:	Ordinary Papers
Date Submitted by the Author:	19-Jan-2011
Complete List of Authors:	<p>Shaw, Bronwen; Royal Marsden Hospital, Haemato-Oncology Apperley, Jane; Department of Haematology RUSSELL, NIGEL; Nottingham City Hospital, Haematology Craddock, Charles; Leukaemia Unit, Department of Haematology Liakopoulou, Effie; Christie NHS FT, Haematology and Transplantation Potter, M; royal marsden hospital, haematology Wynn, Robert F; RMCH, Department of Haematology Gibson, Brenda; Royal Hospital for Sick Children, Dept of Haematology Pearce, Rachel; University College London, BSBMT KIRKLAND, KEIREN; BSBMT, HEAD OF DATA REGISTRY Lee, Julia; King's College Hospital, BSBMT Data Registry Madrigal, Alejandro; Anthony Nolan Research Institute, Royal Free Hospital Cook, Gordon; St James' Institute of Oncology, Haematology Byrne, Jennifer L; Nottingham University, Department of Haematology</p>
Key Words:	TRANSPLANTATION, STEM CELLS, T CELL DEPLETION

Title: 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 GVHD

Running title: Stem cell source in unrelated transplantation

Authors: B E Shaw, MD ^{1,2},
J F Apperley, MD ³,
N H Russell, MD ⁴,
C Craddock, MD ⁵,
E Liakopoulou, MD ⁶,
M N Potter, MD ²,
R Wynn, MD ⁷,
B Gibson, MD ⁸,
R M Pearce, Msc ⁹,
K Kirkland, Msc ⁹,
J Lee, Msc ⁹,
J A Madrigal, MD ¹,
G Cook, MD ¹⁰,
J L Byrne, MD ⁴

Affiliations: ¹Anthony Nolan Trust, London,
²Royal Marsden Hospital, London,
³Imperial College, London,
⁴Nottingham University Hospital (City Campus), Nottingham, ⁵Queen Elizabeth Hospital, Birmingham,
⁶Christie Hospital, Manchester,
⁷Manchester Children’s Hospital, Manchester,
⁸Royal Hospital for Sick Children, Glasgow,
⁹BSBMT, London,
¹⁰St James’s University Hospital, Leeds

Corresponding author: Dr Bronwen Shaw
The Anthony Nolan Research Institute
Royal Free Hospital, Pond Street
Hampstead, London
NW3 2QG, UK
Email: bshaw@doctors.org.uk
Telephone: +442072848339
Fax: +442072848331

Key words: unrelated donor, T cell depletion, myeloablative, PBSC, BM

Word count: 3520

Abstract: 196

Figures: 4

Tables: 2

References: 35

Scientific category: Transplantation

Authorship statement:

JLB, NHR and BES contributed to the design and analysis. RP performed the statistical analysis. All authors contributed to the data collection, writing and review of the manuscript.

None of the authors have any financial disclosures or conflicts of interest

Unrelated donor peripheral blood stem cell transplants incorporating pre-transplant in-vivo Alemtuzumab are not associated with an increased risk of significant acute or chronic GVHD

B E Shaw, J F Apperley, N H Russell, C Craddock, E Liakopoulou, M N Potter, R Wynn, B Gibson, R Pearce, K Kirkland, J Lee, J A Madrigal, G Cook, J L Byrne

Abstract

There is little information published on the long-term outcomes in recipients of T-cell depleted (TCD) unrelated donor (UD) transplants comparing Peripheral Blood Stem Cells (PBSC) with Bone Marrow (BM). We present retrospective outcome data on 306 recipients of myeloablative, HLA-matched UD allografts using pre-transplant in-vivo Alemtuzumab. Transplants were performed between 2000–2007 for CML (CP1) and acute leukaemia (CR1/2) and 184 patients received BM and 122 PBSC. The median age was 28.9 years (<1-58years) and the median follow-up was 48 months. Overall survival at 8 years is 53%. The incidence of acute GvHD was significantly higher in PBSC (65%) than BM recipients (49%; p=0.012). This represented only grade 1 GVHD with no difference in grade II-IV aGvHD (BM 23% PBSC 24%). The incidence of chronic GvHD, either overall (BM 47%, PBSC 49%) or extensive (BM 15%, PBSC 13%) was not increased with PBSC. The incidence of relapse, non-relapse mortality and survival were not significantly different. Whilst accepting the limitations of retrospective analyses, we suggest the increased risk of GvHD in recipients of PBSC in T-replete transplants is offset by in-vivo Alemtuzumab, and that either stem cell source can be used with good outcomes in this setting.

Introduction

The use of unrelated donors for allogeneic transplantation has extended the availability of this potentially life-saving therapy to those who lack a sibling donor. In view of the increased genetic disparity between recipient and donor the incidence of some complications, such as graft versus host disease (GvHD), are increased. Recent advances in technology, including those related to HLA-typing, have resulted in the outcome of UD transplants reaching that of transplants using sibling donors (Goldstone and Rowe 2009, Lee, *et al* 2009), in part due to a reduction in the incidence of severe GvHD. One highly successful strategy to reduce GvHD is the use of T cell depletion (TCD) - either *in vivo* or *ex vivo* (Kottaridis, *et al* 2000, Marks, *et al* 2000). A commonly used method of in-vivo TCD is Alemtuzumab, given for several days pre-transplant. Alemtuzumab is an antibody against CD52 and as such depletes not only T-cells, but also other cell subset such as B cells and dendritic cells(Hale, *et al* 1998). Alemtuzumab has been shown to persist in the patient's circulation for up to 30 days post infusion, allowing for depletion of CD52-positive cells from the patient as well as from the donor cells on infusion(Rebello, *et al* 2001). Historically all donors provided stem cells via a bone marrow (BM) harvest. Since the 1990s we have had the ability to collect GCSF -mobilised peripheral blood stem cells (PBSC) and since 2000 their use in UD has increased considerably (Ljungman, *et al* 2009). In view of the low incidence of significant short-term side effects and the ease of donation, approximately 70-80% of unrelated donors opt to donate via this method in preference to BM harvesting (Miller, *et al* 2008, Pulsipher, *et al* 2009). It was clear from early experience that the number of cells (CD34+

and CD3+ cells) harvested via PBSC was greater than that by BM harvesting (Pavletic, *et al* 1998). While this was associated with a significantly shorter engraftment times, there were concerns that the incidence of GvHD would be increased with the use of PBSC. Indeed, a number of studies, predominantly in sibling transplants, showed an increased incidence of both acute and/or chronic GvHD in PBSC recipients (Schmitz, *et al* 2005, Schmitz, *et al* 2006, Storek, *et al* 1997) without, however, an impact on patient outcome (Friedrichs, *et al*, Gallardo, *et al* 2009, Ringden, *et al* 2002, Schmitz, *et al* 2005). More recent studies have considered UD transplants (Blau, *et al* 2001, Eapen, *et al* 2007, Garderet, *et al* 2003, Remberger, *et al* 2005, Remberger, *et al* 2001), again confirming an increase in GvHD with PBSC, but not consistently showing any difference in outcome. Most of these studies, however include recipients of both HLA matched and mismatched grafts and all report series where transplants are predominantly T cell replete. A few studies have suggested a differential effect on survival due to stem cell source, both in sibling (Eapen, *et al* 2004, Group 2005) and UD transplants (Eapen, *et al* 2007, Garderet, *et al* 2003). The large meta-analysis including 1111 adult recipients of HLA-identical sibling grafts (Group 2005), showed that PBSC was associated with increased incidences of both acute and chronic GvHD. However, PBSC was also associated with a decrease in relapse in both early and late stage disease, with a significantly improved survival in recipients of PBSC with late stage disease. Conversely, in a later study (Eapen, *et al* 2004) in children receiving HLA-identical sibling allografts, PBSC was associated with a higher incidence of chronic GvHD, as well as a worse overall survival and a higher transplant related mortality (TRM). Furthermore in a CIBMTR study of UD transplant outcome, Eapen *et al* (Eapen, *et al* 2007) showed a significantly higher incidence of both grade II-IV acute GvHD and of chronic GVHD. Although there was no detrimental effect on overall survival in the whole group, the subgroup of patients with chronic myeloid leukaemia (CML) in first chronic phase (CP1) receiving PBSC did have a significantly worse overall survival, with a trend to an increase in TRM. In the study by Garderet *et al* (Garderet, *et al* 2003), there was a trend to an increase in acute (but not chronic) GvHD in patients with ALL receiving PBSC, which was associated with an inferior survival compared to BM (without an impact on relapse).

We hypothesised that any negative impact caused by increased GvHD using PBSC compared to BM might be abrogated in recipients of UD transplants incorporating in-vivo Alemtuzumab conditioning for T cell depletion. Here we present data on the outcome in 306 UK transplant recipients with leukaemia studying the impact of stem cell source on outcome.

Materials and methods

Data collection

Patients fulfilling the requirements for this study were identified from the database held by the British Society for Blood and Marrow Transplantation (BSBMT) registry in London, UK. Data are reported at fixed time points in the form of the MED A and B forms derived from the European Group for Blood and Marrow Transplant (EBMT)). Data collected by the Anthony Nolan Trust from a previous study were added to the BSBMT database. 306 patients from 19 centres were identified. Supplementary data requests were sent to all centres.

Patients, definitions and inclusion criteria

Criteria for inclusion were: 1. a transplant at a UK centre between 2000 and 2007. 2. myeloablative conditioning 3. in-vivo pre-transplant T cell depletion using alemtuzumab 4. HLA matched (10/10) unrelated donor 5. standard risk leukaemia (acute myeloid or lymphoblastic leukaemia in first or second complete remission (CR), CML in CP1).

HLA typing was performed in the local Histocompatibility and Immunogenetics laboratories by molecular methods to achieve high resolution types.

Alemtuzumab schedule and dose were given according to institutional protocols. Both the overall dosing and the days of infusion differed between institutions. However the dose of Alemtuzumab within an institution did not differ depending on whether the stem cell product was BM or PBSC. CMV was treated pre-emptively. Donor leukocyte infusions (DLI) were not used routinely, but given in some cases according to institutional protocols for mixed chimerism, relapse or viral reactivations.

Primary Graft Failure was defined as a failure to achieve a neutrophil count of $0.5 \times 10^9/l$ by day 28 and was evaluable only in those surviving at least 28 days after the transplant. Relapses were defined as haematological, cytogenetic or molecular. Acute and chronic GvHD were defined using internationally accepted criteria.

The protocols used were approved by the individual institutional review boards of all of the contributing hospitals. Informed consent for the transfer of data to, and the analysis by, the BSBMT was obtained from all patients.

Statistical analyses

Relationships between categorical variables were analysed by Fisher’s exact test. Continuous variables such as age were tested using the k-sample equality of medians test. Overall survival was calculated by Kaplan-Meier analysis. Univariate analysis of OS and PFS was performed using the log-rank test, and multivariate analysis by Cox regression. Cumulative incidence of non-relapse mortality was calculated by competing risks regression, with death from relapse as the competing risk. Relapse rate was calculated as a cumulative incidence by competing risks regression, with transplant related death and chronic GvHD as the competing risks. Cumulative incidence of cGvHD was calculated by competing risks regression with death and relapse as competing risks. All multivariate models were designed to include any factor which had a p value of <0.2 in univariate analysis plus the source of stem cells. In view of the relatively small dataset and number of significant variables, stepwise inclusion / exclusion were not applied.

Statistics tests were performed using Stata (StataCorp, Texas 77845 URL: <http://www.stata.com>), with competing risks calculated using “stcompet” (Enzo Coviello, Italy; May Boggess, StataCorp); and using R (R Development Core Team (2006). R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>), with competing risks calculated using the package “cmprsk” (RJ Gray).

Results

Patient and transplant characteristics

306 patients were included in the study. Patient, disease and transplant characteristics are presented in Table 1. The majority of conditioning regimens included TBI (92%), usually in combination with cyclophosphamide (84%), and additional GvHD prophylaxis with cyclosporine alone or cyclosporine and short-course methotrexate was used. In all patients T cell depletion was performed using pre-transplant in vivo Alemtuzumab. The two groups were similar, except for an increased use of BM in patients with CML ($p=0.007$) and the disease stage differed significantly between the groups ($p=0.001$). 24 patients received donor lymphocyte infusions (DLI) post-transplant, 17 for disease relapse, 2 for viral reactivations and 5 for mixed chimerism.

Engraftment

1% (4/306) of patients had primary graft failure. Of these, 2 received BM and 2 received PBSC (two had a second allograft and are alive, while two succumbed from infection or disease progression, one each in the BM and PBSC groups). A further 4 patients who received BM had secondary graft failure (two of whom remain alive following further cellular therapy) while none of the PBSC recipients developed this complication. There was no statistically significant difference between stem cell sources in the incidence of graft failure overall ($p=0.306$). The median time to neutrophil engraftment was 18 days (range: 9-61 days). This was significantly faster in recipients of PBSC (15 days) than BM (20 days, $p<0.0001$). Likewise, platelet engraftment was significantly faster in recipients of PBSC (17 days) than BM (28 days, $p<0.0001$), with a median of 24 days (5-469).

Graft versus Host Disease (GvHD)

All patients who achieved neutrophil engraftment were considered eligible for analysis. The incidence of acute GvHD (aGvHD) of any grade, grade II-IV and grade III-IV were 56%, 23% and 4% respectively. There was a significantly higher risk of developing aGvHD in the recipients of PBSC (65%) compared to BM recipients (49%, $p=0.012$), but this risk was only apparent in grade 1 disease. Most importantly, there were no significant differences in severe aGvHD between the groups (**grade III/IV: BM 4% PBSC 5%, $p=0.554$**) (Table 2). No other factor had a significant impact on the incidence or severity of aGvHD in either univariate or multivariate analysis.

Overall chronic GvHD (cGvHD) developed in 119/235 patients (48% at 5 years). There was no significant difference in the incidence of cGvHD dependant on stem cell source (5 years: 47% following BMT and 49% following PBSC; $p=0.48$) (figure 1). Strikingly, extensive cGvHD occurred in only 15% of BM recipients and 13% of PBSC recipients ($p=0.831$). Factors associated with cGvHD were CMV seropositivity in the patient (**Hazard ratio (HR) =1.56; Confidence interval (CI) 1.07 – 2.27, $p=0.029$**) and disease in CR1/CP1 (**HR 0.66; CI 0.43 - 1.00, $p=0.054$**). In multivariate analysis, including these two factors and source of stem cells and age, only disease status at transplant showed a trend towards significance (**HR=0.68; CI 0.44 – 1.05, $p=0.081$**).

Non-relapse mortality (NRM)

NRM was 9% and 20% at day 100 and 1 year respectively (figure 2). There were no significant differences in NRM between recipients of BM or PBSC (day 100: 9% and 11%, 1 year: 20% and 20%, and 5 year: 23% and 25% respectively, $p=0.77$). There was no significant impact on NRM

due to the presence or absence of aGvHD. Factors associated with NRM in univariate analysis were: patient CMV (HR 2.15; CI 1.34 – 3.47, p=0.001) and donor (HR 1.54; CI 0.93 – 2.53, p=0.090) CMV seropositivity. In multivariate analysis (including these factors, patient age and stem cell source) the only factor to remain significantly associated with a higher mortality was CMV seropositivity in the patient (HR=1.98; CI 1.16 – 3.38, p=0.012). The causes of NRM were: GvHD (22%), infection (62%) and other (16%), which did not differ significantly depending on stem cell source (p=0.358).

Relapse

The cumulative incidences of relapse were 36% and 41% at 2 and 5 years respectively (figure 3). Relapse was predominantly haematological in patients with acute leukaemia (86%), with 6% defined as cytogenetic and 8% defined as molecular. In contrast, only 41% of relapses in CML were haematological with 26% defined as cytogenetic and 33% defined as molecular. There was a trend to a difference in relapse rate according to whether the patient received BM or PBSC (5 years: 45% and 33% respectively, p=0.096). The only factor to impact significantly on disease relapse was disease type, with a relapse risk of 58% at 5 years in CML patients compared to 33% in AL patients (p<0.001). There was a trend to an increase in relapse in CMV seronegative patients (HR=0.69; CI 0.46-1.04, p=0.075). There were no differences in relapse risk between patients with AL transplanted in CR1 or CR2 (both 33%). In multivariate analysis, including disease, patient and donor CMV and stem cell source, the only significant factor was the underlying disease (HR=2.03; CI 1.38-2.98, p<0.001). There was no significant impact on relapse due to the presence or absence of aGvHD.

Survival

The median follow up is 48 months (3-110 months). This was significantly longer in recipients of BM than PBSC (59 vs. 36 months, p=0.001) (figure 4). The overall survival in the whole group was 53% at 8 years, with progression free survival of 36% at the same time point. There were no significant differences in survival between patient who received BM or PBSC (8 years: 54% and 52% respectively, p=0.571). Likewise progression free survival was similar (8 years: 32% and 42% respectively, p=0.225). There was no significant impact on OS due to the presence or absence of aGvHD. Two factors were significantly associated with an improved survival in univariate analysis: CML compared to AL (p=0.005) and CMV seronegativity in patients (p=0.025). Both of these factors remained significant in multivariate analysis (HR 0.57; CI 0.37-0.86, p=0.010 and HR 0.70; CI 0.49-0.99, p=0.046 respectively), while PBSC vs. BM was not significant (HR 1.03; CI 0.73 – 1.49, p=0.826).

Discussion

This study is the first to consider the impact of stem cell source in a cohort of in-vivo T cell depleted HLA matched UD allograft recipients. In contrast to previous studies of T-replete UD transplants, we show no increase in the risk of grade II-IV acute GVHD or of chronic GVHD in recipients of PBSC. The only significant difference between BM and PBSC recipients in this study was a higher incidence of grade I aGvHD in the PBSC group.

In 617 adult recipients of T cell replete UD transplants, Eapen et al (Eapen, *et al* 2007) reported an increase in the incidence of overall, but not grade III/IV, aGvHD with PBSC. In addition,

unlike our report, the incidence of cGvHD was also found to be significantly higher in the patients receiving PBSC. Two smaller studies by Gardaret et al (Gardaret, *et al* 2003) (n=213) and Blau et al (Blau, *et al* 2001) (n=74) reported significant increases in aGvHD (only in HLA mismatched patients in the latter study), but no increase in cGvHD. In two studies by Remberger et al, reporting on both short (Remberger, *et al* 2001) and long term (Remberger, *et al* 2005) follow-up in 214 adult transplant recipients, there was no increase in aGvHD while the incidence of chronic extensive GvHD was significantly higher when PBSC was used compared to BM.

There are a number of clear differences between these studies, the most important being the use or not of T-cell depletion, in particular, Alemtuzumab. A possible additional factor explaining the lack of impact of stem cell source on cGvHD is the differing length of follow-up in each study. The median follow-up in the Remberger study is over 4 years (which is the same as in our current study), while that in the other studies ranges between 1.5 and 3 years and, in some, differs between the PBSC and BM groups. The pattern of GvHD may also be of interest. All of the studies to date report outcomes using the 'old' classification of GvHD i.e. using a simply time cut off between acute and chronic. It may be that using the reclassification of GvHD in future studies, would provide further insights (Filipovich 2008). In our study we noticed that the time to onset of cGVHD was shorter in the PBSC than the BM group (data not shown), suggesting that a proportion of the 'chronic' GvHD would now be reclassified as late onset acute or persistent rather than de novo chronic GvHD. It is well recognised that chronic GvHD is more likely in patients who have suffered acute GvHD (especially of higher grades) which may explain the increase in cGvHD in the Eapen study (where the incidence of acute GvHD was higher than in our study), while this may be less discernable in the Blau and Gardaret study where the overall and grade II/IV aGvHD incidence was less.

T cell depletion using Alemtuzumab was universal in our study. This method of GvHD prophylaxis was either absent or used in a very small minority of patients in the other studies except for the Remberger study where one third of patients received ATG. Direct comparisons between ATG and Alemtuzumab cannot be made given that Alemtuzumab depletes all CD52-positive cells and not just T cells. The use of ATG in only a proportion of patients and the likelihood of 'hidden' HLA mismatches in that study, especially for HLA-C (low resolution HLA-A and -B and high resolution -DRB1 matching criteria were used) may account for the high incidence of chronic GvHD that they observed. All of the recipients included in our study had high resolution typing performed.

The significant reduction in overall incidence of GvHD following TCD strategies (in particular grade III-IV acute and extensive chronic) compared to that following T cell replete transplantation has been reported in both randomised (Bacigalupo, *et al* 2006, Bacigalupo, *et al* 2001, Finke, *et al* 2009, Wagner, *et al* 2005) as well as non-randomised studies (Chakraverty, *et al* 2002, Craddock, *et al* 2009, Kennedy-Nasser, *et al* 2008, Kottaridis, *et al* 2000, Malladi, *et al* 2009, Mohty, *et al* 2003). Conversely, both infection (Bacigalupo, *et al* 2001, Malladi, *et al* 2009) and relapse (Wagner, *et al* 2005) may be increased in the TCD setting, due to delayed immune reconstitution and a reduced graft versus leukaemia effect. In this study CMV seropositivity in the recipient was associated with an inferior survival. It is well recognised that CMV reactivation is common following TCD (Finke, *et al* 2009, Wagner, *et al* 2005). Although the direct impact of CMV reactivation on mortality is minimal due to pre-emptive strategies, it is

known that CMV seropositivity is associated with a predisposition to other infections and may impact on survival in other less well defined ways (Craddock, *et al* 2001, Nichols, *et al* 2002). Although the relapse risk in the AL patients in this study was acceptable, the relapse risk in CML was disappointingly high (although not significantly different based on stem cell source). This may be due to the inclusion of TCD in the conditioning as previously reported (Wagner, *et al* 2005).

Neither our or other UD studies have reported any significant differences due to stem cell source in TRM, relapse or OS in the overall group studied. In the Eapen study there was a trend to a higher TRM, with a significantly lower OS, in recipients with CML in CP1, although the reason for this is not clear. In the Gardaret study patients with ALL receiving PBSC had a significantly lower DFS and OS. Besides the obvious differences with regards to TCD, other differences between the studies are noted, such as the inclusion of both paediatric and adult patients, the degree of HLA matching, the diseases and stage of disease included, medical GvHD prophylaxis and the year of transplantation. None of the studies in UDs are randomised and the results of the BMT CTN 0201 randomised trial (Peripheral Blood versus Bone Marrow Grafts from Unrelated Donors) (http://www.cibmtr.org/Studies/ClinicalTrials/BMT_CTN/Protocols/0201/index.html) are keenly awaited.

It is recognised that the current study has a number of limitations. In common with most retrospective studies there is some heterogeneity in the study group. Although TCD using Alemtuzumab was universal, the schedule and dosage varied between centres. Importantly, however, the dose of Alemtuzumab within an institution did not differ depending on whether the stem cell product was BM or PBSC. In addition the use of cyclosporin (with or without methotrexate) differed by centre. DLI were given for various reasons, but none as part of a planned immunotherapy program. Any impact of DLI on outcomes should be negligible, as most were given for relapse, and this was taken into account as a competing risk for chronic GVHD. Although the number of CD34+ cells is known, the data on CD3+ and other cell subset content of the graft were not available.

In conclusion, in this study we confirm the low incidence of severe GvHD following TCD myeloablative transplantation from UD for patients with standard risk leukaemia, with excellent long-term survival rates. The use of either BM or PBSC was associated with equally good outcomes. The only significant difference between the groups was an increase in grade 1 acute GvHD in the PBSC recipients. The risk of extensive cGVHD was low and did not differ with stem cell source. These data suggest that either stem cell source can be used with equivalent results and that there is no deleterious effect from the use of PBSC in Alemtuzumab containing unrelated donor transplants.

Acknowledgements

We would like to thank all of the UK transplant teams who have contributed patients to this study.

Contributors

CIC: Hospital: Transplant lead: data manager

205: Hammersmith Hospital: Prof Jane Apperley: Priscilla Plocki
 717: Nottingham University Hospital (City Campus): Prof Nigel Russell: Pam Nelson
 387: Queen Elizabeth Hospital Birmingham: Prof Charles Craddock: Janice Ward
 780: Christie's Manchester: Dr Effie Liakopoulou: Thomas Dalton
 218: Royal Marsden Hospital: Dr Mike Potter: Helena Woods
 521: Manchester Children's Hospital: Dr Robert Wynn: Mary Coussons
 707: Royal Hospital for Sick Children, Glasgow: Dr Brenda Gibson: Graham Stewart
 501: Royal Hospital Liverpool: Dr Richard Clark: Lynne Laing
 254: St James's University Hospital Leeds: Dr Gordon Cook: Rachel Goodall/Karen Benn
 778: Royal Hallamshire Hospital, Sheffield: Dr John Snowden: Barbara Holt
 303: Cardiff: Dr Keith Wilson: Sandra Nicholas
 276: Newcastle University Hospital: Dr Graham Jackson: Linda McNally
 284: Heartlands, Birmingham: Dr Don Milligan: James Whitehouse
 713: Leicester Royal Infirmary: Dr Ann Hunter: Rik Lewin
 539: St George's Hospital, London: Prof Edward Gordon-Smith: Preeti Datta-Nemdharry
 566: Addenbrooke's Hospital, Cambridge: Dr Charles Crawley: Debra Tournant
 243: Great Ormond Street Hospital: Dr Paul Veys: Kamil Sanaullah
 263: The London Clinic: Dr Mike Potter: Anjaya Tailor
 866: St Mary's Hospital: Dr Josu De La Fuente: Farah O'boyle

Authorship contributions

JLB, NHR and BES contributed to the design and analysis. RP performed the statistical analysis. All authors contributed to the data collection, writing and review of the manuscript.

Conflict of Interest

There are no conflicts of interest to disclose for any of the authors.

References

- Bacigalupo, A., Lamparelli, T., Barisione, G., Bruzzi, P., Guidi, S., Alessandrino, P.E., di Bartolomeo, P., Oneto, R., Bruno, B., Sacchi, N., van Lint, M.T. & Bosi, A. (2006) Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. *Biology of Blood and Marrow Transplantation*, **12**, 560-565.
- Bacigalupo, A., Lamparelli, T., Bruzzi, P., Guidi, S., Alessandrino, P.E., di Bartolomeo, P., Oneto, R., Bruno, B., Barbanti, M., Sacchi, N., Van Lint, M.T. & Bosi, A. (2001) Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*, **98**, 2942-2947.
- Blau, I.W., Basara, N., Lentini, G., Guenzelmann, S., Kirsten, D., Schmetzer, B., Bischoff, M., Roemer, E., Kiehl, M.G. & Fauser, A.A. (2001) Feasibility and safety of peripheral blood stem cell transplantation from unrelated donors: results of a single-center study. *Bone Marrow Transplantation*, **27**, 27-33.

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

Craddock, C., Nagra, S., Peniket, A., Brookes, C., Buckley, L., Nikolousis, E., Duncan, N., Tauro, S., Yin, J., Liakopoulou, E., Kottaridis, P., Snowden, J., Milligan, D., Cook, G., Tholouli, E., Littlewood, T., Peggs, K., Vyas, P., Clark, F., Cook, M., Mackinnon, S. & Russell, N. (2010) Factors Predicting Long Term Survival After T Cell Depleted Reduced Intensity Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia. *Haematologica*, **95**, 989-9.

Craddock, C., Szydlo, R.M., Dazzi, F., Olavarria, E., Cwynarski, K., Yong, A., Brookes, P., de la Fuente, J., Kanfer, E., Apperley, J.F. & Goldman, J.M. (2001) Cytomegalovirus seropositivity adversely influences outcome after T-depleted unrelated donor transplant in patients with chronic myeloid leukaemia: the case for tailored graft-versus-host disease prophylaxis. *British Journal of Haematology*, **112**, 228-236.

Eapen, M., Horowitz, M.M., Klein, J.P., Champlin, R.E., Loberiza, F.R., Jr., Ringden, O. & Wagner, J.E. (2004) Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: the Histocompatibility and Alternate Stem Cell Source Working Committee of the International Bone Marrow Transplant Registry. *Journal of Clinical Oncology*, **22**, 4872-4880.

Eapen, M., Logan, B.R., Confer, D.L., Haagenson, M., Wagner, J.E., Weisdorf, D.J., Wingard, J.R., Rowley, S.D., Stroncek, D., Gee, A.P., Horowitz, M.M. & Anasetti, C. (2007) Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biology of Blood Marrow Transplantation*, **13**, 1461-1468.

Filipovich, A.H. (2008) Diagnosis and manifestations of chronic graft-versus-host disease. *Best Practical Research in Clinical Haematology*, **21**, 251-257.

Finke, J., Bethge, W.A., Schmoor, C., Ottinger, H.D., Stelljes, M., Zander, A.R., Volin, L., Ruutu, T., Heim, D.A., Schwerdtfeger, R., Kolbe, K., Mayer, J., Maertens, J.A., Linkesch, W., Holler, E., Koza, V., Bornhauser, M., Einsele, H., Kolb, H.J., Bertz, H., Egger, M., Grishina, O. & Socie, G. (2009) Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncology*, **10**, 855-864.

Friedrichs, B., Tichelli, A., Bacigalupo, A., Russell, N.H., Ruutu, T., Shapira, M.Y., Beksac, M., Hasenclever, D., Socie, G. & Schmitz, N. Long-term outcome and late effects in patients transplanted with mobilised blood or bone marrow: a randomised trial. *Lancet Oncology*, **11**, 331-338.

Gallardo, D., de la Camara, R., Nieto, J.B., Espigado, I., Iriondo, A., Jimenez-Velasco, A., Vallejo, C., Martin, C., Caballero, D., Brunet, S., Serrano, D., Solano, C., Ribera, J.M., de la Rubia, J. & Carreras, E. (2009) Is mobilized peripheral blood comparable with bone marrow as a source of hematopoietic stem cells for allogeneic transplantation from HLA-identical sibling donors? A case-control study. *Haematologica*, **94**, 1282-1288.

Garderet, L., Labopin, M., Gorin, N.C., Polge, E., Fouillard, L., Ehninger, G.E., Ringden, O.,

- Finke, J., Tura, S. & Frassoni, F. (2003) Patients with acute lymphoblastic leukaemia allografted with a matched unrelated donor may have a lower survival with a peripheral blood stem cell graft compared to bone marrow. *Bone Marrow Transplantation*, **31**, 23-29.
- Goldstone, A.H. & Rowe, J.M. (2009) Transplantation in adult ALL. *Hematology American Society Hematology Educational Program*, 593-601.
- Group, S.C.T.C. (2005) Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *Journal of Clinical Oncology*, **23**, 5074-5087.
- Hale, G., Zhang, M.J., Bunjes, D., Prentice, H.G., Spence, D., Horowitz, M.M., Barrett, A.J. & Waldmann, H. (1998) Improving the outcome of bone marrow transplantation by using CD52 monoclonal antibodies to prevent graft-versus-host disease and graft rejection. *Blood*, **92**, 4581-4590.
- Kennedy-Nasser, A.A., Bollard, C.M., Myers, G.D., Leung, K.S., Gottschalk, S., Zhang, Y., Liu, H., Heslop, H.E., Brenner, M.K. & Krance, R.A. (2008) Comparable outcome of alternative donor and matched sibling donor hematopoietic stem cell transplant for children with acute lymphoblastic leukemia in first or second remission using alemtuzumab in a myeloablative conditioning regimen. *Biology of Blood and Marrow Transplantation*, **14**, 1245-1252.
- Kottaridis, P.D., Milligan, D.W., Chopra, R., Chakraverty, R.K., Chakrabarti, S., Robinson, S., Peggs, K., Verfuether, S., Pettengell, R., Marsh, J.C., Schey, S., Mahendra, P., Morgan, G.J., Hale, G., Waldmann, H., de Elvira, M.C., Williams, C.D., Devereux, S., Linch, D.C., Goldstone, A.H. & Mackinnon, S. (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood*, **96**, 2419-2425.
- Lee, D.H., Kwon, Y.J., Lim, J., Kim, Y., Han, K., Chung, N.G., Jeong, D.C., Cho, B. & Kim, H.K. (2009) Comparable outcomes of HLA-matched unrelated and HLA-identical sibling donor bone marrow transplantation for childhood acute myeloid leukemia in first remission. *Pediatric Transplantation*, **13**, 210-216.
- Ljungman, P., Bregni, M., Brune, M., Cornelissen, J., Witte, T.D., Dini, G., Einsele, H., Gaspar, H.B., Gratwohl, A., Passweg, J., Peters, C., Rocha, V., Saccardi, R., Schouten, H., Sureda, A., Tichelli, A., Velardi, A. & Niederwieser, D. (2009) Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. *Bone Marrow Transplantation*, **45**, 219-3.
- Malladi, R.K., Peniket, A.J., Littlewood, T.J., Towlson, K.E., Pearce, R., Yin, J., Cavenagh, J.D., Craddock, C., Orchard, K.H., Olavarria, E., McQuaker, G., Collin, M. & Marks, D.I. (2009) Alemtuzumab markedly reduces chronic GVHD without affecting overall survival in reduced-intensity conditioning sibling allo-SCT for adults with AML. *Bone Marrow Transplantation*, **43**, 709-715.
- Marks, D.I., Bird, J.M., Vettenranta, K., Hunt, L., Green, A., Cornish, J.M., Goulden, N., Pamphilon, D.H., Steward, C.G. & Oakhill, A. (2000) T cell-depleted unrelated donor bone marrow transplantation for acute myeloid leukemia. *Biology of Blood and Marrow Transplantation*, **6**, 646-653.
- Miller, J.P., Perry, E.H., Price, T.H., Bolan, C.D., Jr., Karanes, C., Boyd, T.M., Chitphakdithai, P. & King, R.J. (2008) Recovery and safety profiles of marrow and PBSC donors:

experience of the National Marrow Donor Program. *Biology of Blood and Marrow Transplantation*, **14**, 29-36.

Mohty, M., Bay, J.O., Faucher, C., Choufi, B., Bilger, K., Tournilhac, O., Vey, N., Stoppa, A.M., Coso, D., Chabannon, C., Viens, P., Maraninchi, D. & Blaise, D. (2003) Graft-versus-host disease following allogeneic transplantation from HLA-identical sibling with antithymocyte globulin-based reduced-intensity preparative regimen. *Blood*, **102**, 470-476.

Nichols, W.G., Corey, L., Gooley, T., Davis, C. & Boeckh, M. (2002) High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *Journal of Infectious Disease*, **185**, 273-282.

Pavletic, Z.S., Joshi, S.S., Pirruccello, S.J., Tarantolo, S.R., Kollath, J., Reed, E.C., Bierman, P.J., Vose, J.M., Warkentin, P.I., Gross, T.G., Nasrati, K., Armitage, J.O., Kessinger, A. & Bishop, M.R. (1998) Lymphocyte reconstitution after allogeneic blood stem cell transplantation for hematologic malignancies. *Bone Marrow Transplantation*, **21**, 33-41.

Pulsipher, M.A., Chitphakdithai, P., Miller, J.P., Logan, B.R., King, R.J., Rizzo, J.D., Leitman, S.F., Anderlini, P., Haagenson, M.D., Kurian, S., Klein, J.P., Horowitz, M.M. & Confer, D.L. (2009) Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the National Marrow Donor Program. *Blood*, **113**, 3604-3611.

Rebello, P., Cwynarski, K., Varughese, M., Eades, A., Apperley, J.F. & Hale, G. (2001) Pharmacokinetics of CAMPATH-1H in BMT patients. *Cytotherapy*, **3**, 261-267.

Remberger, M., Beelen, D.W., Fauser, A., Basara, N., Basu, O. & Ringden, O. (2005) Increased risk of extensive chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplantation using unrelated donors. *Blood*, **105**, 548-551.

Remberger, M., Ringden, O., Blau, I.W., Ottinger, H., Kremens, B., Kiehl, M.G., Aschan, J., Beelen, D.W., Basara, N., Kumlien, G., Fauser, A.A. & Runde, V. (2001) No difference in graft-versus-host disease, relapse, and survival comparing peripheral stem cells to bone marrow using unrelated donors. *Blood*, **98**, 1739-1745.

Ringden, O., Labopin, M., Bacigalupo, A., Arcese, W., Schaefer, U.W., Willemze, R., Koc, H., Bunjes, D., Gluckman, E., Rocha, V., Schattenberg, A. & Frassoni, F. (2002) Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. *Journal of Clinical Oncology*, **20**, 4655-4664.

Schmitz, N., Beksac, M., Bacigalupo, A., Ruutu, T., Nagler, A., Gluckman, E., Russell, N., Apperley, J., Szerm, J., Bradstock, K., Buzyn, A., Schlegelberger, B., Matcham, J. & Gratwohl, A. (2005) Filgrastim-mobilized peripheral blood progenitor cells versus bone marrow transplantation for treating leukemia: 3-year results from the EBMT randomized trial. *Haematologica*, **90**, 643-648.

Schmitz, N., Eapen, M., Horowitz, M.M., Zhang, M.J., Klein, J.P., Rizzo, J.D., Loberiza, F.R., Gratwohl, A. & Champlin, R.E. (2006) Long-term outcome of patients given transplants of mobilized blood or bone marrow: A report from the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation. *Blood*, **108**, 4288-4290.

Storek, J., Gooley, T., Siadak, M., Bensinger, W.I., Maloney, D.G., Chauncey, T.R., Flowers, M., Sullivan, K.M., Witherspoon, R.P., Rowley, S.D., Hansen, J.A., Storb, R. & Appelbaum,

F.R. (1997) Allogeneic peripheral blood stem cell transplantation may be associated with a high risk of chronic graft-versus-host disease. *Blood*, **90**, 4705-4709.

Wagner, J.E., Thompson, J.S., Carter, S.L. & Kernan, N.A. (2005) Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial. *Lancet*, **366**, 733-741.

For Peer Review

Table 1: Patient and donor demographics

Variable	BM (n=184)	PBSC (n=122)	P value
Year of transplant (median)	September 2002	February 2005	<0.001
Patient age (median, range)	28 (1-54)	30 (<1-58)	0.426
Patient gender			
Male	114 (62%)	73 (60%)	0.721
Female	70 (38%)	49 (40%)	
Donor/patient gender			
Donor M, recipient M	83 (49%)	57 (48%)	0.497
Donor M, recipient F	48 (28%)	30 (25%)	
Donor F, recipient M	22 (13%)	13 (11%)	
Donor F, recipient F	18 (11%)	18 (16%)	
Unknown	13	4	
Conditioning regimen			
TBI/Cy	158 (86%)	99 (83%)	0.439
TBI/other agent	11 (7%)	12 (9%)	
BuCy	14 (8%)	6 (5%)	
Other	1 (<1%)	5 (3%)	
Donor/patient CMV status			
Donor pos, recipient neg	23 (13%)	8 (7%)	0.273
Donor pos, recipient pos	29 (17%)	18 (16%)	
Donor neg, recipient neg	93 (53%)	63 (55%)	
Donor neg, recipient pos	29 (17%)	26 (23%)	
Unknown	10	7	
Diagnosis			
ALL	65 (35%)	46 (38%)	0.007
AML	53 (29%)	52 (43%)	
CML	66 (36%)	24 (20%)	
Stage			
AL: CR1	62 (34%)	64 (52%)	0.001
AL: CR2	55 (30%)	34 (28%)	
CML: CP1	67 (36%)	24 (20%)	
CD34+ cell dose (median, range)	2.91 (0.24 – 21.6)	5.83 (0.77 – 27.4)	<0.001

M – male, F - female, TBI – total body irradiation, Cy – cyclophosphamide, Bu – busulphan, AML – acute myeloid leukaemia, ALL - acute lymphoblastic leukaemia, CML – chronic myeloid leukaemia, AL – acute leukaemia, CR – complete remission, CP – chronic phase

Table 2: Incidence of acute GvHD comparing PBSC to BM.

Acute GvHD	BM	PBSC	P value
Present	87 (49%)	77 (65%)	0.012
Grade I	46 (26%)	48 (40%)	0.554*
Grade II	33 (19%)	23 (19%)	
Grade III	5 (3%)	4 (3%)	
Grade IV	1 (1%)	2 (2%)	
Unknown grade	2	0	

*Refers to the lack of statistical significance between those with grade III/IV acute GvHD in the BM and PBSC group (i.e grade III/IV vs 0-II).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure legends:

Figure 1: The incidence of cGvHD using either PBSC or BM. This did not differ significantly dependent on stem cell source.

Figure 2: Non-relapse mortality using either PBSC or BM.

Figure 3: The incidence of relapse using either PBSC or BM

Figure 4: Overall survival in the study cohort dependent on the use of PBSC or BM. No significant difference was seen.

For Peer Review

Figure 1

Figure 2

Figure 3

Figure 4