TITLE – Impact of stem cell source on long term chimerism and event free survival in children with primary immunodeficiency disorders following Fludarabine and Melphalan conditioning regimen

SHORT TITLE – BM vs PBSC following Fludarabine/Melphalan HSCT in children with PID

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ABSTRACT

Background- Reduced intensity conditioning regimens are increasingly being used in the transplantation of primary immunodeficiency disorders but there are no large studies looking at long term lineage specific chimerism.

Objectives- To analyse long term chimerism and event free survival in children transplanted for primary immunodeficiency disorders using reduced intensity conditioning with Fludarabine and Melphalan and to study the impact of donor type and stem cell source.

Methods- 142 children were transplanted with reduced intensity conditioning (RIC) using Fludarabine, Melphalan (Flu/Melph) and for primary immunodeficiency disorders using bone marrow (BM) (*n*=93) or peripheral blood stem cells (PBSC) (*n*=49). Donors were matched unrelated donor (MUD) (*n*=72), mismatched unrelated donor (MMUD) (*n*=37), matched sibling donor (MSD) (*n*=14), matched family donors (*n*=12) and mismatched family donors (*n*=7)

Results- Overall survival at a median follow-up of 7.5 years was 78% irrespective of stem cell source or donor type. When BM was used as stem cell source, 26% of patients ended up with very low levels of donor chimerism (<10% donor), especially in the myeloid lineage. Event free survival (EFS) in this group was significantly lower compared to the rest of the group (25% vs

70%, p< 0.001). With the use of PBSC, over 90% of patients achieved complete donor chimerism or high level mixed chimerism (> 50% donor chimerism) in all lineages.

Conclusions- Based on our experience, we would suggest that PBSC should be the stem cell source of choice in children with PID transplanted using Flu/Melph RIC from matched donors. This is most likely to ensure sustained high level donor chimerism.

Key messages

- Long term myeloid chimerism can be inadequate in a significant number of children transplanted for primary immunodeficiency disorders using reduced intensity conditioning with Flu/Melph when bone marrow is used as stem cell source
- This results in inferior event free survival
- Using peripheral blood as the stem cell source in fully matched donors can abrogate this problem associated with Flu/Melph conditioning regimen

Capsule Summary

This study in 142 children transplanted for PID conditions showed that use of PBSC as stem cell source in matched donors conditioned with Flu/Melph is most likely to ensure adequate long term chimerism and superior event free survival.

Key words

PID, HSCT, chimerism, lineage specific, reduced intensity

Abbreviations

RIC – Reduced intensity conditioning

BM- Bone marrow

PBSC- Peripheral blood stem cells

MUD- Matched unrelated donor

mMUD- Mismatched unrelated donor

MSD- Matched sibling donor

MFD- Matched family donor

mMFD- Mismatched family donor

EFS- Event free survival

HSCT- Haemopoietic stem cell transplantation

PID- Primary immunodeficiency disorders

MC- Mixed chimerism

CC- Complete chimerism

GVHD- Graft versus host disease

cGVHD- Chronic graft versus host disease

MMF- Mycophenolate mofetil

ATG- Antithymocyte globulin

G-CSF- Granulocyte colony stimulating factor

CGD- Chronic granulomatous disease

IVIg- Intravenous immunoglobulin

DLI- Donor lymphocyte infusion

WAS- Wiskott-Aldrich syndrome

HLH- Haemophagocytic lymphohistiocytosis

SCID- Severe combined immunodeficiency

INTRODUCTION

The use of reduced intensity conditioning (RIC) has enabled haemopoietic stem cell transplantation (HSCT) in patients with pre-existing co-morbidities that would preclude HSCT using conventional approaches. Following several reports of superior short and long term survival after RIC for primary immunodeficiency disorders (PID) (1) ; the use of RIC for PID is now the treatment of choice in many institutions, especially in the presence of organ toxicities. RIC regimens frequently combine Fludarabine with another agent such as Melphalan, low dose Busulfan, low dose thiotepa or low dose TBI (2). Flu/Melph is perhaps the most frequently used RIC regimen in adults and in children. Mixed chimerism (MC) is frequently seen with RIC regimens but is often sufficient to cure many immunodeficiency disorders, although in some non-SCID immunodeficiencies, very low levels of mixed chimerism (<10% donor) may be insufficient for cure. Analysis of lineage specific chimerism may be more informative than whole blood chimerism in predicting secondary graft loss following RIC transplantation (3).

To overcome the problems of MC and relapse, most RIC regimens in adults use peripheral blood stem cells (PBSC) wherein increased T cell and stem cell numbers enhance the alloreactivity of the graft and competitively occupy stem cell niches to ensure complete/high levels of donor chimerism (4). In contrast, bone marrow (BM) has hitherto been the stem cell source of choice in paediatric HSCT because of concerns about high rates of chronic graft versus host disease (GVHD) with PBSC and lack of demonstration of any survival advantage with PBSC in the myeloablative setting (5) . No large studies have been published to date addressing the issue of what constitutes the optimal stem cell source in the RIC setting in paediatrics. We present our long term follow up of 142 children transplanted at a single institution using the same RIC regimen (Fludarabine and Melphalan+Campath/ATG) for immunodeficiency conditions. This is the largest series of paediatric RIC

HSCT looking at lineage specific chimerism and outcomes by donor type and stem cell source.

METHODS

All patients transplanted at Great Ormond Street Hospital for primary immunodeficiency disorders between October 1998 and August 2012 and receiving identical reduced intensity conditioning (*n*= 142) with Fludarabine, Melphalan and Campath/ATG are included in this study. The median age at HSCT was 3.29 years (range, 0.19-17.7 years). Written informed consent was obtained from patients or parents prior to the transplantation procedure in all cases, and the reduced-intensity continuing protocol was registered with the local IRB, protocol no: 99MH11.

Donors for the 142 transplants were 10/10 matched unrelated donors (MUD, *n*=72), mismatched unrelated donors (mMUD, *n*=37), matched sibling donors (MSD, *n*=14), matched family donors (MFD, *n*=12) and mismatched family donors (mMFD, *n*=7). Of the 37 mismatched unrelated donors, 35/37 were mismatched at one antigenic locus (HLA A mismatch, *n*=15, HLA C mismatch *n*=15, HLA DQ mismatch *n*=4, HLA DR mismatch *n*=1) and 2/37 were mismatched at 2 antigenic loci (HLA A and B mismatch *n*=1, HLA B and C mismatch *n*=1). All 7 mismatched family donors were mismatched at a single antigen locus. From 1998 to the end of 2001, donors were typed serologically for class I antigens and by molecular techniques for class II antigens. From 2002 onwards, all donors were typed by molecular techniques for Class I and class II antigens.

Bone marrow was used as the stem cell source in 93 transplants and PBSC was used in 49 transplants.

Patient characteristics are detailed in Table 1. The median duration of follow up is 7.5 years (2.7-12 years). Median follow up for the BM and PBSC groups are 11.2 years and 5.2 years respectively.

Conditioning Regimen

All patients received uniform conditioning with Fludarabine 30mg/m^2 from days -7 to -3 and Melphalan 140 mg/m^2 on day -2 and serotherapy with either Alemtuzumab 0.2mg/kg from days -8 to -4 (*n=119*) or ATG 2.5mg/kg (Rabbit, Genzyme Ltd) days -2 to +2 (*n=23*). ATG was used in transplants performed prior to 2001; Alemtuzumab was used in subsequent transplants. GVHD prophylaxis was with Ciclosporin (*n*=86) or Ciclosporin +Mycophenolate mofetil (MMF) (*n*=60). MMF was used in all PBSC transplants and in 11 patients who received BM transplants.

Engraftment and chimerism

Lineage-specific chimerism was assayed from CD3+ T cells and CD15+ granulocytes isolated from peripheral blood using magnetic bead technology on the autoMACS Pro Separator (Miltenyi Biotec Ltd). Cell fraction purities were routinely above 95%. Alternatively peripheral blood mononuclear cells and granulocytes were isolated using Lymphoprep (Robbins Scientific). The Powerplex 16 system (Promega UK Ltd) was used to PCR-amplify 16 fluorescence-labelled short tandem repeat loci in these patient samples. These PCR products were run on an AB3130 Genetic Analyser and analysed using GeneMapper v4.0 software.

Complete chimerism (CC) is described as > 95% donor cells. Mixed chimerism is defined as the presence of more than 5% host-derived cells on more than one occasion. This is further categorized into high-level MC (95%-50% donor chimerism), low-level MC (49%-10% donor chimerism), or very low-level MC (< 10% donor chimerism). Acute GVHD was graded with the method of Przepiorka *et al* and co-workers (6;6) and chronic GVHD (cGVHD) was graded as none, limited, or extensive.

Withdrawal of immunosuppression

In the absence of GVHD, Ciclosporin was tapered from 3 months post HSCT and stopped by 6 months. MMF when used, was weaned from day 28 after HSCT and stopped over 3 weeks in the absence of GVHD. On detection of

MC, CSA weaning was started immediately and stopped over 2-4 weeks depending on the occurrence of GVHD.

Statistics

Groups were compared using Fishers exact test with a two-tailed P value, except where numbers were small, when the Chi-square test with Yates correction was used (GraphPad Prism 5, GraphPad Software Inc, California). P values equal to or less than 0.05 were considered statistically significant. Kaplan-Meier curves were compared using the Mantel-Cox log-rank test. Logistic regression was performed, using SPSS, to identify determinants of very low level chimerism at one year post transplant.

RESULTS

Engraftment and Chimerism according to stem cell source

Lineage specific chimerism was analysed in the BM and PBSC groups at 1month, 3 months, 6 months and 1 year post HSCT and yearly thereafter as shown in Figure 1 and in Table 2a

93 HSCTs were performed using BM as stem cell source and 49 HSCTs were performed using PBSC as stem cell source. The mean CD34 and CD3 doses for the BM and PBSC groups were $9.8x10^6$/kg, $1.8\ x10^8$/kg and $20\ x10^6$/kg, $7.5\ x10^8$/kg respectively.

1 month post HSCT

BM group

90/93 (97%) of patients were alive at 1 month post HSCT and lineage specific chimerism data was available in 88 patients. 98% of patients engrafted with full donor chimerism in the T-cell and myeloid lineages

PBSC group

48/49(98%) of patients were alive at 1 month post HSCT. Only one patient had very low level MC, others had CC in both lineages.

6 months post HSCT

BM group

80/93 (86%) of patients were alive and lineage specific chimerism was available in 79 patients. By 6 months post HSCT, mixed chimerism was more frequent. Whilst over 75% of patients maintained CC or high level MC in both lineages; chimerism in the myeloid lineage dropped significantly with 12/79 (15%) of patients developing very low level MC ($p<0.0001$).

PBSC group

45/49 (92%) of patients were alive. Over 90% of patients maintained CC or high level MC in both lineages.

1 year post HSCT

BM group

75/93 (81%) were alive and lineage specific chimerism was available in 72 patients. T-cell chimerism remained stable in the majority of patients but 14/72 (19%) patients had very low level chimerism in the myeloid lineage.

PBSC group

44/49 (90%) were alive and lineage specific chimerism was available in 41 patients. Once again, the majority of patients maintained stable CC or high level MC with only 2/41 (5%) and 3/41(7%) of patients developing very low level T-cell and myeloid chimerism respectively.

Last follow-up

For the purposes of chimerism studies, last follow-up is defined as the time the patient was last seen and chimerism analysed at our institution. This was at an average of 6.9 years post-transplant (range, 0.4-13.1 years) in the BM group and 3.5 years (range, 0.3-9.7 years) in the PBSC group.

BM Group

At last follow-up, 71/93 (76%) were alive and data were available in 66 patients. T-cell chimerism remained stable between 1 year post-transplant and last follow-up. There was a further increase in the proportion of patients 17/66 (26%) developing very low level MC in the myeloid lineage. This decline in myeloid chimerism between 1 year and last follow-up was not however statistically significant (*p= 0.4)*

Seven patients in the BM group with very low MC eventually had graft loss with return of disease phenotype and 6 of them proceeded to a second transplant procedure. One died without a second procedure. One patient received a donor lymphocyte infusion (DLI), in an attempt to improve chimerism. In patients who underwent a second transplant procedure, chimerism just prior to the second procedure is depicted in Figure 1 and in Table 2a.

PBSC group

41/49 (83%) patients were alive at last follow-up and data was available in 35 patients. In the PBSC group, there was very little change in T-cell or myeloid chimerisms between 1 year and last follow-up. There was one second transplant procedure in the PBSC group and 3 DLIs.

At last follow up, there was a higher incidence of very low level MC in the myeloid series of the BM group (26%) compared to the PBSC group (8%) but this difference was not statistically significant (p=0.41)

Although donors were typed serologically for class I antigens and by molecular techniques for class II antigens from 1998-2001 and by molecular techniques for Class I and class II antigens from 2002 onwards; there was no difference in the incidence of rejection or very low level mixed chimerism in these 2 time periods in either the BM or PBSC groups . In the BM group, 8/40 (20%) rejected or had very low level mixed chimerism prior to 2002 compared to 7/53 (*13%)* after 2002 (*p*=0.4). Forty seven of 49 PBSC transplants were performed after 2002 and here the incidence of very low level mixed chimerism was 2/47 (4%). This was not statistically different compared to the

incidence of very low level mixed chimerism in the BM group post 2002 (*p*=0.16). This analysis excluded sibling donors in both time periods.

Chimerism according to donor type at last follow-up

Lineage specific chimerism was further analysed according to donor type at last follow-up as shown in Figures 2a and 2b and in Table 2b. Numbers in brackets indicate surviving patients with complete data available.

Matched donors

BM group

The majority of patients with MUD (*n*=35) and MFD *(n*=6) had CC or high level MC in both lineages at last follow-up with 17% of patients in both these groups achieving very low level myeloid chimerism.

Matched sibling donors (*n*=10) had a high incidence of very low level myeloid chimerism, 30% (3/10)

PBSC group

Similarly, the majority of patients with MUD (*n*=17) and MFD (*n*=2) had CC or high level MC in all lineages. The incidence of very low level myeloid mixed chimerism was 18% in the MUD group.

Only one patient each was transplanted using a MFD or MSD and both these have CC or high level MC in all lineages

Mismatched donors

BM group

Mismatched donors (mMUD *n*=12, mMFD *n*=4) had a 33% (4/12) and 75% (3/4) incidence respectively of very low level myeloid chimerism. T-cell chimerism was in the high chimerism ranges.

PBSC group

In contrast to the BM group; all the mMUD (*n*=17) have CC in all lineages. This is significant compared to the 33% incidence of very low level myeloid MC in mismatched donors transplanted using BM (p=0.03)

Graft versus host disease

In patients transplanted with BM as the stem cell source, the incidence of acute GVHD ≥ grade II was 25%. The incidence of grades III and IV acute GVHD was low at 9%. 15% developed chronic GVHD of which 4% was classified as extensive.

As shown in Figure 3, the incidence of significant acute GVHD (≥ grade II) was somewhat higher in the PBSC group at 31% but this was not statistically significant compared to the BM group (31% vs 25%, p= 0.42). The overall incidence of grades III and IV acute GVHD was also not significantly higher in the PBSC group (12% vs 9%, p=0.5). Amongst the matched donors only 5/28 (18%) developed ≥ grade II acute GVHD, only one patient developed grades III and IV GVHD (1/28 =4%). However, patients who received mismatched donor PBSCT had a 48% (10/21) incidence of GVHD ≥ grade II. This was significantly higher than the 18% (5/28) incidence of GVHD ≥ grade II in matched donor PBSC transplants (p=0.03). The incidence of severe (grades III and IV) aGVHD was 24% (5/21) in mismatched donors. This was higher than the 4% (1/28) incidence of grades III and IV aGVHD in matched PBSC transplants but this did not reach statistical significance probably due to small numbers.

The incidence of chronic GVHD (cGVHD) in the PBSC group was 24% of which 16% was extensive chronic. This was significantly higher in the PBSC compared to the BM group (p=0.02). This increased incidence of chronic GVHD was also seen exclusively in mismatched donors where 10/21 (48%) developed chronic GVHD, this being extensive in 7/21 (33%). One patient developed limited cGVHD and one patient developed extensive cGVHD in the matched PBSC group. All evaluable patients are off therapy for cGVHD with resolution of symptoms. One patient (PBSC group) has some joint restriction following resolution of sclerodermatous cGVHD.

Survival

Overall survival for the entire group at a median follow-up of 7.5 years was 78%

Bone marrow group

As shown in Figure 4a, 71/93 patients (76%) are alive at a median follow up of 11.2 years. Causes of death in the 22 deceased patients were infection (*n*=12), toxicity (*n*=4), disease progression (*n*=1), GVHD (*n*=3) and others (*n*=2).

Peripheral blood stem cell group

Of 49 patients, 41 are alive (84%) at a median follow- up of 5.2 years. The causes of death in 8 deceased patients were infection (*n*=2), toxicity (*n*=2), and GVHD (*n*=4).

There was no statistical difference in survival according to stem cell source (BM 76% vs PBSC 84%), nor was there any significant difference in survival according to donor type (Figure 4b). The MUD, MMUD, MSD, MFD and mismatched family donor groups had survivals of 81%, 75%, 85%, 75% and 71% respectively.

Second procedures

Seven conditioned second transplant procedures were performed for autologous reconstitution and return of disease at a median of 18 months following the first transplant. Six patients had received BM as stem cell source for their first transplant. Four out of 6 of these patients are alive and cured of their disease at last follow up (one developed limited chronic GVHD). Two patients died of infectious complications during their second transplant procedure.

Only 1 patient receiving PBSC as stem cell source required a second transplant procedure. This patient with chronic granulomatous disease (CGD) underwent an unsuccessful gene therapy procedure and then underwent a successful second Flu/Melph RIC transplant procedure with 100% donor chimerism and is currently well and cured of his disease.

Five donor lymphocyte infusions (DLI) were performed (BM group *n*=2, PBSC group *n*=3). In 4 patients this resulted in stabilisation/improvement of chimerism.

Three patients, all following BM transplants, received CD34 selected boost transplants without conditioning to improve immune reconstitution. All these patients are alive but 2 have on-going sub-optimal immune reconstitution.

Three patients underwent splenectomy (Wiskott-Aldrich syndrome with very low myeloid MC and thrombocytopenia, *n*=1, idiopathic thrombocytopenic purpura, *n*=2) Platelet counts normalised after splenectomy in all 3 patients.

Outcomes in patients with very low level MC within 1 year of HSCT

As shown in Table 3, 21/142 (15%) patients experienced very low level MC at some point in the first year post transplant. 18/21 (86%) of these patients had been transplanted with BM as stem cell source. As shown in Figure 4c, event free survival in this group was significantly worse compared to the rest of the group, 25% vs 70% ($p<0.0001$). In addition to death, second procedures, splenectomy, cellular therapies and return of disease manifestations were all considered as events. Intravenous immunoglobulin (IVIg) replacement therapy alone was not considered an event. Seven of these 21 patients (33%) needed a second transplant procedure. Four were cured after the second procedure, 2 died and one patient had an unsuccessful gene therapy procedure followed by a second curative HSCT. 4/21 (19%) have return of

some disease manifestations and may need a second transplant procedure in the future, 2/21 (10%) had DLI to improve chimerism (improved *n*=1, no improvement *n*=1). One patient with CD40 ligand deficiency died due to progressive liver disease, one has partial disease correction and remains on IVIg replacement, 1 patient with Wiskott- Aldrich syndrome underwent splenectomy which normalised his platelet count but continues to have very low level MC and may be prone to autoimmune manifestations in the future, 1 patient with Haemophagocytic lymphohistiocytosis is clinically stable but with no evidence of donor engraftment so that his long term prognosis remains guarded. 3/21 patients (all severe combined immunodeficiency-SCID) are well and off immunoglobulin replacement and 1 patient is lost to follow-up.

On multivariate analysis, perhaps due to small sample size, none of the predictors analysed for very low level MC (age at transplant, diagnosis, source of stem cells, type of donor or year of transplant) were significant variables.

DISCUSSION

The level of engraftment that is curative following HSCT depends on the disease type and lineages affected. In diseases like SCID, T cell engraftment is crucial while in other PIDs like CGD and leucocyte adhesion deficiency, myeloid engraftment is important for disease cure. In PID it has been shown that long term well-being and durable immune reconstitution requires adequate levels of true stem cell engraftment as evidenced by continuing donor myeloid chimerism (7),(8).Hence, an ideal RIC HSCT regimen should not only ensure low levels of procedure related toxicity but also secure sustained levels of stem cell engraftment.

In our study, patients transplanted using BM as stem cell source had a higher incidence of very low level MC in the myeloid lineage. These patients had much worse event free survival compared to the rest of the group with only 3/21 patients being free of disease after their primary transplant procedure. In addition, in the long term, they are at an increased risk of graft exhaustion and

return of disease manifestations. In contrast, long term donor chimerism was improved in the PBSC group with only 7% developing very low level MC.

PBSC grafts typically contain 1 log more CD34+ stem cells and 1 log more T-cells than BM. The higher levels of donor engraftment observed with PBSC is therefore likely to reflect a combination of both an increased alloreactive 'graft versus marrow' effect mediated by T-cells and greater donor stem cell competition for niches in the BM. The relative contribution of these 2 factors is not known but together they appear to reduce the risk of autologous reconstitution. In one of the few studies comparing stem cell sources in the non-myeloablative setting, Dey *et al* compared PBSC to BM as stem cell source in 54 adults with haematological malignancies. Consistent with our findings, they also observed higher levels of donor chimerism in the PBSC group (83% vs 38%). Similarly, rates of graft loss were also significantly lower in the PBSC group (8% vs 37%) (9)

Lineage specific chimerism analysis of our group led us to identify two 'problem groups' of patients: those transplanted using mMUDs with BM as stem cell source and those transplanted using MSDs (all but one sibling transplant was done using BM as stem cell source). Although survival in these groups was comparable to the rest of the group, the incidence of very low levels of MC was significantly higher in both these cohorts.

The number of patients transplanted using a MSD in our study was small (14 patients, 13 had BM as stem cell source), however, we observed that 30% of these patients had very low myeloid engraftment and one additional patient died due to return of HLH. All patients with very low level MC have had to undergo second transplant procedures. The relatively small stem cell dose acquired from paediatric sibling donors together with insufficient T cell alloreactivity in this predominantly chemo naïve group of patients may have contributed to this increased incidence of graft loss and poor myeloid engraftment in the RIC setting in children. Although there are data on the safety and efficacy of obtaining PBSC from paediatric sibling donors (10), this is not routine practice in the UK and in some other countries. One option for improving engraftment in this group of patients may be to omit/reduce the dose of Alemtuzumab or administer it earlier in the conditioning thereby causing less T cell depletion of the graft and enabling greater graft vs marrow

alloreactivity. Alternatively other RIC protocols may be preferable for PID patients transplanted from MSDs such as recently reported by Gungor *et al* who observed excellent outcomes and high levels of engraftment using a combination of sub-myeloablative doses of Busulfan and Fludarabine in a cohort of 56 patients transplanted for chronic granulomatous disease. This cohort included 21 MSD transplants and their outcome and engraftment results were comparable to the rest of the group (11).

Patients transplanted from mismatched donors using BM as the stem cell source also had a high incidence of very low level myeloid MC (33%). This is consistent with data from adult studies where graft rejection has been a significant problem in the RIC setting using mismatched donors (12), (13),(14). The effect of the mismatch can be overcome by increasing the CD34 dose and the alloreactivity of the graft; both these goals are met by using PBSC and the majority of adult RIC protocols now use PBSC as the preferred stem cell source. In children, there has been a gradual but similar shift in practice but there is a paucity of published literature on stem cell source in the RIC setting in paediatrics.

Between 1998 and 2002, BM was the predominant stem cell source used for HSCT in our cohort of mismatched donors. In view of the high incidence of rejection and very low level MC in the mismatched donor group, from 2002 onwards, based on adult experience, we made two changes to our approach in transplantation using mismatched donors. Firstly, we switched to using PBSC as our preferred stem cell source and based on the experience of the Seattle group, we changed our GVHD prophylaxis to include MMF as a pro-engraftment agent (15). Following this change in practice, we have had no rejections in the MMUD group and 100% of patients achieved complete donor chimerism in all lineages.

This improvement however came at the cost of excessive acute and chronic GVHD which is of no beneficial value to this patient group. In our study, this high incidence of severe acute and chronic GVHD was restricted to PBSC transplants from MMUDs. With matched donors, GVHD was low and equivalent in the BM and PBSC groups. The persistence of host antigen presenting cells following RIC may contribute to the pathogenesis of GVHD (16), (17) and this is likely to be compounded in the presence of an antigenic

mismatch. Our findings are consistent with those of other groups reporting high rates of chronic GVHD with mismatched donors in the RIC setting (18), (19).

Although there are multiple factors contributing to the pathogenesis of GVHD following PBSC transplants (20), one option to reduce GVHD might be to limit the number of T cells in the PBSC graft. This could be achieved by enriching the stem cell collection using CD34+ cell selection and adding back the CD34- cell population to contain a fixed T-cell dose. We are currently studying this approach in our unit and preliminary results are encouraging. Another option for reducing GVHD in this patient group might be to increase the dose of Alemtuzumab; a study by Mead *et al* (21) in adults with haematological malignancies using an identical RIC protocol but giving a total dose of 100 mg of Alemtuzumab (approximately twice the amount in our study) found no difference in the incidence of GVHD between HLA matched and mismatched donors. However the slow immune reconstitution after this dose of Alemtuzumab might be problematic in our cohort of patients, many with ongoing viral infections at the time of HSCT. A further option for this group of patients might be to use G-CSF primed BM allografts. This approach may combine the benefits of PBSC transplant (low rejection, fast cell recovery) with those of BMT (low incidence of cGVHD). Morton *et al* in their prospective randomised study comparing G-CSF primed bone marrow allografts to PBSC transplants in matched donors report comparable engraftment in both arms but with a significant reduction in the incidence of cGVHD in the GCSF-BM arm. The study was closed after the interim analysis at 6 months because the study's end point of significant cGVHD had been reached (22). Larger studies with longer follow-up evaluating the benefit of this approach and documenting donor safety are necessary before it can be recommended for routine use. It is possible that other reduced toxicity protocols such as that reported by Gungor et al (11) may provide adequate engraftment with acceptable GVHD rates in mismatched donors.

In summary, our RIC regimen of Fludarabine and Melphalan resulted in durable engraftment in the majority of patients and comparable overall survival in BM and PBSC groups. However, when BM was used as stem cell source, higher rates of very low level mixed chimerism, particularly in the

myeloid lineage were observed than with PBSC and this was associated with poor event free survival. Patients with matched donors had a low incidence of GVHD and achieved excellent long term engraftment in all lineages using PBSC and this would be our preferred stem cell source for matched donors. Patients with mismatched donors remain a difficult group of patients to transplant, suffering from poor engraftment (with BM) and high levels of GVHD (with PBSC) and for this group we have proposed some potential strategies. Patients transplanted from MSDs also do not achieve good levels of engraftment with our Flu/Melph RIC regimen and we are currently trialling alternative RIC protocols for this group.

Our study has the limitations of a heterogeneous patient population and small sample size and hence we could not conclusively demonstrate a relationship between chimerism and stem cell source in multivariate analysis. It could be argued that the better chimerism results seen in the PBSC group were partly due to the introduction of molecular methods of tissue typing from 2002 onwards; however the fact that the incidence of autologous reconstitution and very low level mixed chimerism did not change in the two time periods suggests that this was possibly not a major confounding factor. Larger prospective studies are needed to further validate our findings, to study the impact of Fludarabine and Melphalan pharmacokinetics on chimerism and to study the disease specific implications of mixed chimerism.

Table 1

Patient characteristics (*n*=142)

Diagnosis		BM	PBSC
PID	**142**	**93**	**49**
CID	37	27	10
SCID	32	25	7
HLH	25	14	11
Phagocytic cell disorders	15	8	7
T- cell immunodeficiency	18	11	7
WAS	8	7	1
XLP	7	1	6
Donors and stem cell source		BM 93(65%)	PBSC 49(35%)
MUD	72 (50%)	49	23
mMUD	37 (26%)	17	20
MSD	14 (10%)	13	1
MFD	12 (8%)	8	4
mMFD	7(5%)	6	1
Median age at transplant (years)	3.29	2.6	5.3
Median year of transplant		2002	2008
Male	89	57	32
Female	53	36	17

PID- primary immunodeficiency, CID- combined immunodeficiency, SCID- Severe combined immunodeficiency, HLH- haemophagocytic lymphohistiocytosis, WAS – Wiskott Aldrich syndrome, XLP- X-linked lymphoproliferative disorder, MUD- matched unrelated donor, mMUD- mismatched unrelated donor, MSD- matched sibling donor, MFD- matched family donor, mMFD- mismatched family donor.

Table 2a

Chimerism according to stem cell source

		BM Group		PBSC Group	
Months post-transplant	Level of Chimerism	T-cell chimerism (%)	Myeloid chimerism(%)	T-cell chimerism (%)	Myeloid chimerism (%)
1month BM *n*=88 PBSC *n*=48	CC High level MC Low level MC Very low level MC	80 (91) 6 (7) 1 (1) 1 (1)	82 (93) 4 (5) 1 (1) 1(1)	47(98) 0 1(2) 0	47(98) 0 0 1(2)
6 months BM *n*=79 PBSC *n*=45	CC High level MC Low level MC Very low level MC	46 (58) 17 (22) 12 (15) 4(5)	48 (61) 11(14) 8(10) 12(15)	35(78) 7(16) 2(4) 1(2)	37(82) 5(11) 1(2) 2(4)
1 year BM *n*=72 PBSC *n*=41	CC High level MC Low level MC Very low level MC	41(72) 17(24) 10(14) 4(5)	41(57) 6(8) 11(15) 14(19)	30(73) 9(22) 0 2(5)	30(73) 6(15) 2(5) 3(7)
Last follow-up BM *n*=66 PBSC *n*=35	CC High level MC Low level MC Very low level MC	42(64) 16(24) 4(6) 4(6)	35(53) 7(11) 7(11) 17(26)	25(71) 7(20) 1(3) 2(6)	24(69) 7(20) 1(3) 3(8)

Table 2b

Chimerism according to donor source at last follow-up

		BM Group		PBSC Group	
Donor	Level of Chimerism	T-cell chimerism (%)	Myeloid chimerism(%)	T-cell chimerism(%)	Myeloid chimerism(%)
MUD BM *n*=35 PBSC *n*=17	CC High level MC Low level MC Very low level MC	23 (66%) 9 (26%) 2 (6%) 1 (3%)	19 (54) 5 (14) 4(12) 6(17)	7(41) 6(35) 2(12) 2(12)	7(41) 7(41) 0 3(18)
mMUD BM *n*=12 PBSC *n*=17	CC High level MC Low level MC Very low level MC	9 (75%) 2 (17%) 0 1(8%)	8 (66%) 0 0 **4(33%)***	17(100) 0 0 0	17(100) 0 0 0
MSD BM *n*=10 PBSC *n*=1	CC High level MC Low level MC Very low level MC	3(30%) 4(40%) 1(10%) 2(20%)	4(40%) 0 3(30%) 3(30%)	0 1(100) 0 0	0 1(100) 0 0
MFD BM *n*=6 PBSC *n*=2	CC High level MC Low level MC Very low level MC	4(67%) 2(33%) 0 0	3(50%) 2(33%) 0 1(17%)	2(100%) 0 0 0	2(100%) 0 0 0
mMFD BM n=4 PBSC n=1	CC High level MC Low level MC Very low level MC	3 (75) 0 1 (25) 0	1 (25) 0 0 3 (75)	1(100%)	1(100%)

CC- complete chimerism, MC- mixed chimerism, BM- bone marrow, PBSC- peripheral blood stem cells, MUD-matched unrelated donor, mMUD- mismatched unrelated donor, MSD-matched sibling donor, MFD-matched family donor,mMFD-mismatched family donor. * statistically significant compared to mMUD in the PBSC group, p=0.03

Table 3

Characteristics of patients with very low level MC within one year of transplant

UPN	Diagnosis	Age at transplant(years)	Donor	Stem cell source	Outcome
GOS006	SCID	0.77	MUD	BM	Well, off Ig replacement
GOS014	T-cell immunodeficiency	0.77	mMUD	BM	Well, off Ig replacement
GOS016	CID	0.32	MUD	BM	Successful 2nd transplant
GOS018	SCID	0.55	mMUD	BM	Chronic lung disease. Remains on Ig replacement
GOS020	SCID	1.46	mMUD	BM	Ig replacement therapy
GOS028	SCID	0.46	mMFD	BM	Chronic lung disease. Remains on Ig replacement
GOS029	CID	10.6	MSD	BM/PBSC*	Ongoing disease manifestations, severe warts, lymphoedema.
GOS033	CD40 ligand deficiency	15.9	mMUD	BM	Died of progressive liver disease
GOS037	SCID	6.3	mMUD	BM	Died following 2nd transplant
GOS044	SCID	0.9	MUD	BM	Well, off Ig replacement
GOS048	SCID	0.3	MUD	BM	Successful 2nd transplant
GOS068	CD40 ligand deficiency	1.3	MSD	BM	Successful 2nd transplant
GOS076	WAS	2.1	MUD	BM	Splenectomy with normalisation of platelet count
GOS087	SCID	1.3	MUD	BM	Lost to follow-up
GOS099	Phagocytic disorder	5.2	MFD	BM	Ongoing skin infections
GOS088	Phagocytic disorder	4.3	MUD	PBSC	Failed gene therapy.Successful 2nd transplant
GOS108	HLH	1.3	MUD	PBSC	DLI with stabilisation of chimerism
GOS111	HLH	0.76	MUD	BM	DLI with improvement in chimerism
GOS005	XLP	3.6	MSD	BM	Died following 2nd transplant
GOS031	WAS	2.5	MSD	BM	Successful 2nd transplant
GOS113	HLH	1.6	mMUD	BM	Well

BM-bone marrow, PBSC-peripheral blood stem cells, MSD- matched sibling donor, MUD- matched unrelated donor, mMUD- mismatched unrelated donor, MFD- matched family donor, SCID- severe combined immunodeficiency, Was-Wiskott Aldrich syndrome, HLH- Haemophagocytic lymphohistiocytosis, XLP- X-linked lymphoproliferative disorder, CID- combined immunodeficiency, DLI- donor lymphocyte infusion, Ig- immunoglobulin.

* This patient received BM which was then topped up with PBSC due to low BM stem cell numbers. For analysis purposes, he is included in the PBSC group.

Figure legends

Figure 1

Lineage specific chimerism in the bone marrow and PBSC groups. Chimerism in the T-cell (T) and myeloid lineage (M) at 1, 6months, 1year post HSCT and at last follow-up in the BM and PBSC groups is shown. At all time points, the incidence of mixed chimerism was higher in the BM group than in the PBSC group especially in the myeloid lineage. At last follow-up, the incidence of very low level MC in the myeloid lineage of the BM group was 26% compared to 8% in the PBSC group.

* In case of second transplants or DLI; chimerism immediately prior to the second procedure is represented here.

Figure 2

Chimerism according to donor source at last follow-up. With BM as stem cell source (2a), MMUDs had a 40% incidence of very low MC, most evident in the myeloid lineage. With PBSC as stem cell source (2b), 100% of mismatched donors achieved complete donor chimerism in all lineages. MSDs also had a 30% incidence of very low level MC in the myeloid lineage of the BM group.

MUD- matched unrelated donor, mMUD- mismatched unrelated donor, MSD- matched sibling donor, MFD- matched family donor, mMFD- matched family donor, T-T cell engraftment, M-Myeloid engraftment

Figure 3

Graft versus host disease following BM and PBSC transplants. Incidence of significant (≥ grade II), severe acute GVHD (grade III and IV) and chronic GVHD was low with BM transplants. There was a significantly higher incidence of acute and chronic GVHD with PBSC transplants from mismatched donors. Incidence of GVHD in PBSC transplants from matched donors was low and similar to that in the BM group. The incidence of severe GVHD was only 4% in the matched PBSC setting.

Figure 4

Overall survival in BM and PBSC groups, event free survival in patients with less than 10% donor chimerism compared to patients with >10% donor chimerism and survival according to donor type is shown. Survival was very good in BM and PBSC groups at 76% and 84% respectively. There was no statistical difference in survival according to donor type. Patients with less than 10% donor chimerism had significantly poorer EFS at only 25% compared to 70% in patients with higher levels of chimerism.

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