TITLE

Improved Survival after Unrelated Donor Bone Marrow Transplant in Children with Primary Immunodeficiency using a Reduced Intensity Conditioning Regimen.

SHORT TITLE

Improved survival after PID transplants using RIC.

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ABSTRACT

The optimal approach to stem cell transplantation in children with immunodeficiency who lack a matched family donor is controversial. Unrelated donor stem cell transplant gives equivalent outcome to mismatched family donor stem cell transplant in Severe combined immunodeficiency whereas unrelated donors may be preferable in non-Severe combined immunodeficiency children. However, unrelated donor stem cell transplant with conventional conditioning regimens has been associated with significant treatment related toxicity, particularly in non-severe combined immunodeficiency patients with pre-existing organ dysfunction. We report the outcome of a series of 33 consecutive unrelated donor transplants performed at our centre in children with primary immunodeficiency using a reduced intensity conditioning regimen between 1998 and 2001. We have compared these outcomes with a retrospective control cohort of 19 patients transplanted with myeloablative conditioning between 1994 and 1998. All children in both groups had primary engraftment. There was no statistical difference in the speed of immune reconstitution or incidence of graft versus host disease between the 2 groups. Overall survival was significantly better in the reduced intensity conditioning group-31/33 (94%) compared with 10/19 (53%) in the myeloablative conditioning group (p = 0.014). We conclude that the reduced intensity conditioning regimen results in improved survival and reduced transplant related mortality compared to myeloablative conditioning in high risk patients undergoing unrelated donor transplant.
INTRODUCTION

Allogenic stem cell transplant (SCT) is curative for congenital immunodeficiencies but unfortunately only about 10% of these children will have a matched family donor (MFD) \(^1\). The optimal approach for children who do not have a MFD is unclear. Related HLA non-identical transplants have been performed since the early 1980s, when better T-cell depletion strategies became available \(^2,3,4\) and the obvious advantage of a haploidentical transplant is that the donor is readily available. However, the intense T-cell depletion required for these procedures, has posed many problems including graft failure \(^5\) poor immune reconstitution necessitating ‘boost’ transplants \(^6\) and susceptibility to viral infections \(^7\). Another approach is to use an unrelated donor (UD) and there have been successful long term engraftments and immune reconstitution after the use of T replete UD SCT \(^8\) but this has to be counterbalanced against the risk of graft versus host disease (GvHD). For patients with severe combined immunodeficiency (SCID), this problem has been largely overcome, as over the years, the results of matched unrelated donor (MUD) and mismatched family donors have improved significantly and are comparable to the results after HLA identical transplants \(^6,9\). By contrast, this has not been the case with the non-SCID transplants where there has been no improvement in survival since 1985, whatever the donor origin or HLA compatibility \(^9\). Antoine et al \(^9\) report a 59% 3 year survival after MUD SCT for non-SCID patients using myeloablative conditioning (MAT) and Fischer et al \(^7\) report a 45.5% survival in 22 patients with non-SCID primary immunodeficiency (PID) from closely matched donors (phenotypically identical related, matched unrelated donors and 1Ag mismatched related). In both series, the major causes of death were infections and toxicity.

Because of the concerns over toxicity associated with the MAT regimens, some groups have tried and reported successes using reduced intensity conditioning (RIC) regimens for hematologic malignancies \(^10,11,12\). With many of these regimens, reliable engraftment can be achieved without myeloablation and with reduced regimen related toxicity. The intense pre-transplant immunosuppression and T cell alloreactivity are important for cure of haematological malignancies using RIC. In PIDs, with RIC, the aim is to create an immunological platform of host and donor tolerance using pre and post transplant immunosuppression. T cell alloreactivity is less important for cure in these diseases. We have previously reported 8 patients with PID and significant organ dysfunction transplanted with a fludarabine/melphalan based RIC regimen. All children survived the procedure with minimal toxicity and GvHD and with good engraftment and immune reconstitution \(^13\). Following this, we have now used the RIC regimen in over 50 patients. Here we report our results on 33 consecutive UD transplants and have compared it with a retrospective control cohort of 19 patients transplanted with MAT.
PATIENTS AND METHODS

Between April 1994 and January 2002, 52 children with PID underwent SCT from an UD at Great Ormond Street Hospital, London. This study cohort is divided into 2 groups based on the conditioning regimen received. Between April 1994 and December 1998, 19 children received a MAT transplant and between October 1998 and January 2002, 33 children received a RIC transplant. Patient characteristics before SCT are outlined in table 1. The two groups were matched for the major prognostic factors defined by Antoine et al (lancet 2003) in particular, the number of children with B negative SCID and the presence of pulmonary complications pre-transplant. The only variable that differed significantly between the groups was age with the RIC group being considerably older at transplant compared to the MAT group. Informed consent was gained in writing from patients/parents prior to the transplant procedure in all cases and the Reduced Intensity Continuing protocol was registered with the local Research and Development Office protocol no: 99MH11.

In the RIC group, 6 children (18%) had SCID and 27 children (82%) had non-SCID immunodeficiencies. In the MAT group, 7 children (37%) had SCID and 12 children (63%) had non-SCID immunodeficiencies. The molecular diagnoses of the patients with SCID are outlined in table 2a and characterisation of the immunodeficiency phenotype in the non-SCID T cell deficiencies is provided in table 2b. The median age at transplant in the RIC group was 5.9 years (range 0.19- 18 years) and in the MAT group it was 1.9 years (range 0.39-13.08). 73% of children in the MAT group and 74% of children in the RIC group were older than 1 year at the time of transplant. In the RIC group, 21/33 (64%) of the donors were fully matched serologically for class I and by molecular techniques for Class II antigens, 11/33 (33%) were 1 antigen mismatches and there was 1 child who underwent a 2 antigen mismatched transplant. 11/19 (58%) of donors in the MAT group were fully HLA compatible. Of the mismatches 8/19 (42%), 6 were 1 antigen mismatches and 2 were 2 antigen mismatches.

Preparative regimen, transplantation and supportive care

Marrow was used as the source of haemopoietic stem cells in all transplants in both groups. The RIC group was conditioned with Fludarabine (150mg/m²), Melphalan (140mg/m²) and Campath 1H (0.2mg/kg day-8 to –4, n=14) or ATG (2.5mg/kg day-2 to +2, n=19). All children received T replete grafts. The MAT group was conditioned with Busulphan (16mg/kg) and Cyclophosphamide (200mg/kg). T cell depletion (TCD) was used in all but 2 of the MAT transplants. TCD was performed with Campath 1M with donor serum as a source of complement in vitro, together with in vivo Campath 1G (0.2mg/kg day –12 to –8); an add-back of 5x10⁴ CD3 cells/kg was given with the graft. Cyclosporin was used as GvHD prophylaxis for the RIC group and in the MAT group Cyclosporin and Methotrexate (10mg /m² on days 3, 6,11 and 18) were used for GvHD prophylaxis.
Supportive care was identical in both groups. All children received G-CSF 5mcg/kg from day 8 until neutrophil recovery to greater than 1x10⁹/L. Antimicrobial prophylaxis during the transplantation period consisted of aciclovir 750 to 1500 mg/m² from day –1 to –3 and then 1200 to 2400 mg/m² per day orally to 6 months, itraconazole 5mg/kg per day to count recovery, co-trimoxazole adjusted to body surface area from neutrophil recovery to 6 months or normalisation of PHA stimulation index and Penicillin V 250 to 500mg/day from discharge to 2 years post transplant. Patients with sclerosing cholangitis received anti-cryptosporidial prophylaxis with paramomycin 30mg/kg/day orally or azithromycin 10mg/kg/day orally or both. All patients received intravenous Immunoglobulin (IVIg) 0.5g/kg every 3 weeks during the transplantation period and this was continued until CD4 count was more than 300 per microL and serum Immunoglobulin A and M levels had normalised. Blood and urine samples of all patients were screened weekly by DNA PCR and detection of early antigen fluorescent (DEAFF) test respectively to check for CMV. In the MAT group, adenovirus and EBV screening were performed when symptoms such as unresolving pyrexia, lymphadenopathy or unexplained hepatitis developed. In the RIC group, additional prospective monitoring by weekly DNA PCR for EBV and adenovirus on the peripheral blood was performed. About 40% of children in both groups developed adenovirus in the stools. This was not routinely treated and the majority of children with adenovirus in stools did not develop viremia. Only reactivations in the blood will be further detailed. CMV reactivation was treated with ganciclovir. Adenoviremia was treated with withdrawal of immunosuppression where possible and intravenous Ribavirin or Cidofovir. Early EBV reactivation was treated with withdrawal of immunosuppression where possible and Rituximab. Semi quantitative analysis of EBV viral loads was performed every week. Based on these, children received between 1 and 4 doses of Rituximab at weekly intervals. Late EBV reactivations were either treated with Rituximab or a “wait and watch” policy with careful monitoring of viral loads was adopted.

**Engraftment and Immune reconstitution studies**

Engraftment studies were performed in patients alive 1 month after transplantation who had neutrophil recovery (defined as neutrophils > 0.5 x10⁹/L on 2 consecutive days) Engraftment was assayed on the peripheral blood using XY FISH (sex mismatched patient-donor) or VNTR analysis (sex matched). Lineage specific chimerism analysis was possible after 1998. Mixed chimerism (MC) is defined as the presence of more than 5% host derived cells on more than one occasion in the whole blood. This is further categorised into high level MC (95-50% donor chimerism), low level MC (49-10% donor chimerism) or very low level MC (less than 10% donor chimerism). Acute GvHD was assessed using the Seattle criteria. Chronic GvHD is defined as GvHD occurring 100 days or more after BMT and is graded as none, extensive or limited. Immune reconstitution was studied at 1,2,3,6,9,12 and 15 months by FACS analysis of peripheral blood mononuclear cells using fluorescein isothiocyanate-phcycoerythrin-labelled antibodies against CD3, CD4, and CD19, immunoglobulin A and M levels and assay of PHA stimulation index (defined as the ratio of baseline: maximal stimulated levels of ^3H-thymidine uptake in a 3 day culture of peripheral blood mononuclear cells stimulated with a range of concentrations of PHA). Age specific normal ranges for CD3, CD4 and CD19 were used to ascertain times to normalisation of the above. Children alive and with evidence of engraftment
6 months after transplant were included in the analysis of immune reconstitution. Children were immunised at 18 months post transplant if they were off immunoglobulin replacement therapy and did not have GvHD. Vaccine responses were checked 4 weeks after vaccination.

The median duration of follow up for the RIC group is 40 months and for the MAT group it is 104 months.

TRM is defined as death occurring within 100 days of transplant, due to transplant related causes.

TREC analysis

CD4+ and CD8+ cells were isolated from Ficolled blood using anti-CD4 or anti-CD8 antibody-coated magnetic beads (Miltenyi, Harrow, UK). TREC levels were analysed by real-time quantitative polymerase chain reaction (PCR) assay. Cells were lysed using a solution of Proteinase K (Roche), NP40 and Tween 20 and the lysate used in duplicate as the template for real time PCR. These were run on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) under standard reaction conditions and cycling parameters. The primers and probe are previously published. For each run, a standard curve was generated from duplicate samples of 5-fold serially diluted known copies of plasmid DNA, obtained by inserting a human signal joint TREC fragment in the pCR-Blunt Vector (Invitrogen; Life Technologies). The threshold cycle (Ct) value for each duplicate was determined at the point where the fluorescence exceeded the manually set threshold limit. This value was then compared to the standard curve to determine the starting copy number. To normalize for cell equivalents, the β actin gene was quantified under identical real-time PCR conditions used for TREC quantification, primers and probe previously published. As per the TREC assay a standard curve was generated per plate using serial dilutions of a fragment of the β actin gene inserted into the pCR-Blunt Vector.

STATISTICAL ANALYSIS

The MAT and RIC groups were compared with respect to age, pre-transplant diagnosis, presence of pre-transplant co-morbidities and type of donors. These variables were analysed using an exact method to account for the sample sizes and the different sized groups and chi-square was used where appropriate. The end points of survival were determined using the Kaplan-Meier product limits methods and comparisons of survival distribution were performed using the log rank test.
RESULTS

Engraftment and chimerism

All children in both groups had primary engraftment.

RIC group

The median time to neutrophil recovery was 13 days (range 8-34 days) and to an unsupported platelet count of more than $20 \times 10^9$/L was 16 days (range 9-100 days). One child continued to have an unsupported platelet count of $>20 \times 10^9$/L throughout and 1 child was platelet dependent at discharge after a protracted BK virus induced hemorrhagic cystitis. As shown in figure 1, at 1 month post transplant, 32/33 children were alive and engrafted. Of these 82% ($n=27$) of children had 100% donor chimerism and 15% ($n=6$) had high level MC. At 1 year post transplant, 31/33 children were alive. Of these 55% ($n=17$), 32% ($n=10$), 6.5% ($n=2$) and 6.5% ($n=2$) had 100% donor chimerism, high level MC, low level MC and very low level MC respectively. These figures were not significantly different at last follow-up where 55% ($n=17$), 26% ($n=8$), 13% ($n=4$) and 6% ($n=2$) of children have 100% donor chimerism, high level MC, low level MC and very low level MC respectively. Both children with very low level MC were following a 1 antigen mismatched transplant. They have been re-started on prophylactic medications.

MAT group

The median time to neutrophil recovery was 15 days (range 10-23 days) and to an unsupported platelet count of more than $20 \times 10^9$/L was 22.5 days (range 16-40 days). At 1 month post transplant, 14/19 children were alive. All these children had engrafted with 100% donor chimerism in whole blood. At one year post transplant, 11/19 children were alive. Of these 64% ($n=7$) retained 100% donor chimerism and 36% ($n=4$) had high level MC. At last follow-up, 10/19 children are alive. Of these 50% ($n=5$) still retained 100% donor chimerism; the other 50% had high level MC. There were no children with low level or very low level MC.

There was no statistical difference in the speed of neutrophil or platelet recovery between the 2 groups.

Viral reactivations

There were more viral reactivations in the RIC group compared to the MAT group ($p=0.02$). 3 children had CMV reactivation, 5 adeno viremia and 10 EBV reactivations. The CMV and adenovirus reactivations were picked up on prospective monitoring and none of these children had evidence of disease. 6 children with EBV reactivation were classified as having EBV disease defined as the presence of the virus together with the appropriate symptoms in the absence of any other cause. All of them were successfully treated and there were no deaths due to EBV. 3 children with EBV reactivation also had simultaneous reactivation of CMV or adenovirus.
In the MAT group, 3 children had CMV reactivation. None of them had evidence of CMV disease. One child died of EBV lymphoproliferative disease on day 28.

Graft versus host disease

As shown in table 3, the incidence of acute GvHD > grade 2 was equal and low in both groups (9% in the RIC group and 10.5% in the MAT group). No child in the RIC group had limited chronic GvHD compared to 3/19 of children in the MAT group. 1 child in each group had extensive chronic GVHD and both these children died.

Immune reconstitution

Fig 2. shows the recovery of CD3+ and CD4+ T cells and CD19+ B cell counts to normal age related counts post transplant and return to normal T cell function as assessed by PHA stimulation index. From 6 months to two years post BMT an increasing number of children in both groups developed normal T and B cell function. At 12 months post transplant 73%, 64%, 54% and 73% of the MAT group had age related normal levels of CD3, CD4, PHA and CD19 compared to 66%, 65%, 41% and 59% in the RIC group. There was no statistically significant difference in the percentage of children achieving normal age related values at 12 months for any of these parameters. 100% of surviving children in the MAT group have normal production of immunoglobulins and are off IVIg. 5 patients in the RIC group remain on IVIg for incomplete B cell reconstitution. 2 of these children received Rituximab for EBV disease during transplant. Table 4. shows the vaccine responses to Hemophilus influenza and Tetanus. Nearly all children in both groups achieved good antibody titres post vaccination indicating good functional B cell recovery.

Thymic activity post transplant was assessed by measurement of T cell receptor excision circle (TRECs) numbers in separated CD4 and CD8 populations using a quantitative PCR assay (Fig 3). Samples were obtained from 15 patients at a mean of 1076 days post transplant. In 12 patients (Patients A-L) TREC numbers in both CD4 and CD8 populations were determined but in a few patients, values in only CD4+ (Patient M) or CD8+ (Patients N and O) cells were obtained. In the large majority of patients TREC numbers in both CD4+ and CD8+ populations were >5,000 copies/10⁶ cells for both CD4+ (mean 25,443 TREC/10⁶ CD4+ cells) and CD8+ cells (mean (17,179 TREC/10⁶ CD8+ cells) respectively. These values are comparable with TREC values in normal healthy young donors suggesting that in the majority of cases thymic output post transplant is within normal limits. In one individual, Patient K, TREC numbers in both CD4+ (228 TREC/10⁶ CD4+ cells) and CD8+ (462 cells TREC/10⁶ CD8+ cells) were low and in another, Patient G, levels in CD8+ cells were low although CD4 levels were normal. In one other individual, TRECs were not detectable i.e they were beyond the sensitivity of the assay (data not shown). Interestingly all 3 of these patients show poor immune recovery in terms of quantitative lymphocyte recovery and all three remain on immunoglobulin replacement therapy.
Survival

RIC group

SCT with RIC conditioning was extremely well tolerated. 31/33 (94%) children are alive and well at the time of writing. One child died of RSV pneumonitis during conditioning and one child died of chronic GvHD.

As is evident in the survival curve in fig.5a, there was a significantly better overall survival (OS) in the RIC group compared to the MAT group (p=0.014). As there were only 2 deaths in the RIC group, it was not possible to analyse the influence of different variables on mortality. However in the MAT group, there were more deaths in the non-SCID patients (54%) compared to SCID patients (30%). This is shown in fig.5b. and 5c.
**MAT group**

10/19 children are alive and well in this group. Overall mortality was 47% (9/19). TRM was 26% (5/19) (3 regimen related toxicity, 2 infection) at a median of 38 days post transplant. 1 child died of disseminated Cryptosporidiosis at day 130 and 2 children of chronic GvHD at days 217 and 255 post transplant respectively. One child died 4.6 years after transplant due to ongoing respiratory problems after transplant for dyskeratosis congenita.

**Quality of life**

The mean Lansky score for survivors in the RIC group is 97 compared to 94 in the MAT group.
DISCUSSION

In this paper, we report the outcome of a large series of unrelated donor transplants in patients with PID utilising a RIC regimen and have compared these outcomes with a retrospective control cohort transplanted with a MAT regimen.

The most striking aspect of our study is the difference in survival between the 2 groups. 47% of children in the MAT group died, largely due to toxicity and infection. Although it maybe argued that the MAT transplants were done between 1994 and 1998 and there has been significant improvement in supportive care, early detection of viral problems and GvHD treatment since then, these factors alone are unlikely to account for the superior survival of the RIC group. Hitherto, many groups have shown an improvement in survival over time for the SCID patients transplanted from family or unrelated donors 6,9. This has not been the case with the non-SCID patients. In this group, there was an initial improvement in survival between 1973 and 1985, but since then there has been no further improvement18,9. Amongst the non-SCID immunodeficiencies, the T-cell deficiencies had a particularly poor prognosis with a 43% survival at 3 years 9. We too noted a particular poor outcome for the non-SCID patients in the MAT group who had a mortality of 58% (7/12) compared to 28% (2/7) in the SCID group- p=0.0006 (fig.5c). In this respect, it is noteworthy that 27/33 patients (82%) in the RIC group were non-SCID and half of these, 14/27 were T-cell immunodeficiencies making them an extremely high risk group of children. They were also significantly older at transplant than the MAT group. It has been shown in multivariate analysis that age > 12months was the single most important factor determining poor outcome after transplant in patients with SCID 9,19,18. These data imply that the RIC group would be predicted to have a higher mortality from SCT.

Chimerism data on the RIC patients show that initially all children engrafted with donor haematopoiesis and subsequently there was a significant incidence of MC approximating that previously reported after RIC conditioning 11,13. However, it is well established that MC is sufficient to cure the disease phenotype in PIDs. In our study, all children with high and low level MC remain well and free of disease. All patients with phagocytic disorders in the RIC cohort achieved full donor chimerism in the myeloid lineage and would therefore be expected to have normal neutrophil function. In the children with low level MC who have had a longer follow up, it has been possible to demonstrate that this has been maintained and many children have moved from being low level MC to high level or full chimeras on withdrawal of immunosuppression (fig 4). The two children with very low level MC had early onset of falling chimerism following a 1 antigen mismatched transplant procedure, that did not respond to withdrawal of immunosuppression and are likely to need a second transplant procedure. But the RIC procedure was extremely well tolerated even in these high risk children without a fully matched donor and will allow us to consider a second transplant procedure at a later date. Since this study closed, 6 patients with one antigen mismatched UDs, have received the same conditioning regimen with Mycophenolate mofetil in addition to Cyclosporin and peripheral blood (4/6) as the source of stem cells. 5/6 children survive and are 100% engrafted at a median follow-up of 12.4 months (personal communication P.Veys).
We do not as yet have much experience with RIC using umbilical cord blood (UCB) as the source of stem cells. It is possible that the use of UCB could broaden access to an acceptable allogeneic donor and perhaps further reduce the incidence of acute GvHD. The limiting factor for older children may be the cell dose. Trials are being planned in paediatric haematological malignancies to use one or two unrelated UCB units using RIC. Similar trials for PID would be very useful.

Patients receiving highly immunosuppressive RIC transplant regimens may be prone to an increased incidence of viral infections including CMV and EBV. In our study, we likewise observed a high rate of viral reactivations in the RIC group compared to the MAT group; but with early detection and treatment, there were no deaths due to viral reactivations. The fact that many children had reactivation of more than one virus simultaneously emphasises the extremely immunosuppressive nature of this protocol. Initially, we speculated that the use of Campath may cause fewer problems with EBV reactivation but we did not find a significant difference in EBV incidence with ATG or Campath. It is very likely that the children who received Rituximab for the treatment of EBV disease will have poor B cell numbers and need IVIg replacement post transplant for longer. Vigilant prospective monitoring and early treatment of infections are crucial to this protocol.

There is no published data comparing immune reconstitution in children with PID using different conditioning regimens. Overall, both groups of patients seem to have an apparently slower immune reconstitution than reported previously after MAT conditioning. This may be due to the fact that in our study, we have used age specific normal ranges for T and B cell numbers which maybe a more accurate way of assessing immune recovery. At 2 years post transplant, the percentage of children achieving T and B cell reconstitution is comparable to previous reports. Although data on the kinetics of immune reconstitution in immunodeficiency patients is lacking, the pace of T cell reconstitution observed in our RIC patients was similar to that observed by Eyrich et al in a cohort of paediatric patients with non-immunodeficiency disorders undergoing haploidentical transplantation. Immune reconstitution in the RIC group was comparable to that in the MAT group although Campath 1H was used as serotherapy in the RIC group which may be more immunosuppressive than Campath 1G used in the MAT group. More children in the RIC group remain on IVIg and this maybe, as discussed earlier, due to the use of Rituximab in this group. However in those children in the RIC group who achieved normal B cell numbers and normal immunoglobulin production; B cell function as assessed by specific antibody responses to vaccinations, were comparable to the MAT group. Vaccine responses after RIC conditioning have not been previously looked at. All vaccinated children in this group had good responses to Haemophilus influenza and to Tetanus as did the children in the MAT group.

The longevity of immune recovery post RIC transplants is dependent on successful engraftment of multipotent stem cell/progenitor HSC populations and can only be accurately determined by long term analysis of immune function. Analysis of donor chimerism shows that the majority of patients have donor engraftment in multiple lineages; data which suggests that multipotent cells have been engrafted. Further, TREC frequency analysis on a subgroup of patients also shows that the majority have values comparable with normal healthy young donors which demonstrates that pre-
thymic lymphoid progenitors have been successfully engrafted in these patients. In 3 individuals, low TREC values correlates with poor immune recovery. Over time, these individuals are at risk of T cell exhaustion and may require re-transplantation or stem cell boosts.

Acute GvHD >grade 2 has been noted to be an adverse prognostic factor for survival following PID transplants ⁹. Previous groups have reported an incidence of acute GVHD in the RIC setting similar to that following conventional transplant ¹¹. Our patients had a low incidence of GvHD reflecting our use of in vivo T-cell depletion using Campath/ATG ²⁷. Similarly there was a low incidence of chronic GVHD, but extensive chronic GVHD was associated with poor prognosis as is well known.

In summary, despite the limitations of a retrospective, non-randomised study, our study suggests strongly that RIC results in superior survival in children with PID undergoing SCT. Our regimen is well tolerated even in high risk patients with pre-existing organ dysfunction. GvHD and immune reconstitution are comparable to MAT transplants. The highly immunosuppressive nature of the regimen results in a high incidence of viral reactivations. MC is frequently seen but is not associated with disease relapse. On the basis of these data, we recommend the use of RIC in patients with PID undergoing MUD SCT, in non-SCID immunodeficiencies.


(19) Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. Blood. 2002;99:872-878.


Table 1A – Pre-transplant patient characteristics

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<th>RIC</th>
<th>P value</th>
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<td>SCID</td>
<td>7/19 (37%)</td>
<td>6/33 (18%)</td>
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<td>B+/B-</td>
<td>1/5</td>
<td>1/3</td>
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<tr>
<td>Non-SCID</td>
<td>12/19 (63%)</td>
<td>27/33 (82%)</td>
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<td>WAS</td>
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<td>Phagocytic disorders</td>
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<td>Median age at transplant</td>
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<td>MUD Vs mMUD donors</td>
<td>58% Vs 42%</td>
<td>67% Vs 33%</td>
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<td>% children with pulmonary complications</td>
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<td>% children with hepatic complications</td>
<td>26</td>
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**Table 2 A- Molecular classification of SCIDs**

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<td>Undefined (T low, absent PHA)</td>
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**Table 2 B- T cell deficiencies**

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<th>NK</th>
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<th>RIC (n=14)</th>
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<td>CD4 low</td>
<td>Positive</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Positive</td>
<td>2*</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>Negative</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Positive</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CD4 low</td>
<td>positive</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Not assessed</td>
<td>Normal</td>
<td>positive</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* other T cell functional defects
Table 3 – Complications

<table>
<thead>
<tr>
<th></th>
<th>MAT</th>
<th>RIC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute GvHD &lt; grade 2</td>
<td>9/19 (47%)</td>
<td>12/33 (36%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Acute GvHD &gt; grade 2</td>
<td>2/19 (10%)</td>
<td>3/33 (9%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Limited chronic GvHD</td>
<td>3/19 (16%)</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Extensive chronic GvHD</td>
<td>1/19 (5%)</td>
<td>1/33 (39%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Viral reactivations</td>
<td>4/19 (21%)</td>
<td>13/33 (39%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mortality</td>
<td>9/19 (47%)</td>
<td>2/33 (6%)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Table 4- Vaccine responses

<table>
<thead>
<tr>
<th></th>
<th>MAT</th>
<th>RIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. vaccinated</td>
<td>10/10 (100%)</td>
<td>16/31 (52%)</td>
</tr>
<tr>
<td>No. with normal HiB</td>
<td>10/10 (100%)</td>
<td>15/16 (94%)</td>
</tr>
<tr>
<td>antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. with normal tetanus</td>
<td>10/10 (100%)</td>
<td>16/16 (100%)</td>
</tr>
</tbody>
</table>
Figure 1 - Chimerism at engraftment, 1 year and last follow-up.
Fig. 2 Immune reconstitution post BMT

- **a** CD3 recovery post BMT
- **b** CD4 recovery post BMT
- **c** CD19 recovery post BMT
- **d** PHA recovery post BMT
Fig 3a  TREC values in CD4+ cells

Fig 3b  TREC values in CD8+ cells
Fig 4 - Effect of Ciclosporin withdrawal on chimerism- RIC group

![Graph showing the effect of Ciclosporin withdrawal on chimerism in RIC group. The x-axis represents days post BMT, ranging from 30 to 365, and the y-axis represents % donor chimerism, ranging from 0 to 100. The graph includes lines for patients 1 to 6, with markers indicating CyA weaned and stopped.](image-url)
Fig 5. Kaplan-Meier Survival Curves

Fig. 5a  Overall Survival: SCID + Non-SCID

Fig. 5b  SCID Survival

Fig. 5c  Non-SCID Survival

RIC
MAT

p=0.014
p=0.49
p=0.0006
FIGURE LEGENDS

Fig.1  Shows chimerism in the 2 groups at engraftment, at 1 year post transplant and at last follow-up. At 1 year post transplant, 45% of children in the RIC group and 36% of children in the MAT group developed MC. The low level MCs and very low level MCs were found only in the RIC group. However, at last follow-up this remains stable.

Fig.2  panels a-d – Show recovery of CD3, CD4, CD19 and PHA to age related normal levels post transplant. From 6 months to 2 years post BMT, an increasing number of children in both groups developed normal T and B cell function. There was no statistical difference in speed of immune reconstitution between the 2 groups.

Fig.3  panels a and b – TREC numbers in selected T cell populations.
   a) TREC numbers in CD4+ cells and b) in CD8+ cells

Fig.4  Chimerism stabilising or improving after Ciclosporin withdrawal in 6 children with low level MC in the RIC group.

Fig.5  panels a-c- Kaplan- Meier analysis showing the overall survival in the 2 groups. OS was significantly better in the RIC group at 94% compared to 53% in the MAT group. Non-SCID patients in the MAT group did worse with an overall survival of 46% compared to 96% in the RIC group.
Improved survival after unrelated donor bone marrow transplant in children with primary immunodeficiency using a reduced intensity conditioning regimen

Kanchan Rao, Persis J Amrolia, Alison Jones, Catherine M Cale, Paru Naik, Doug King, Graham E Davies, H B Gaspar and Paul A Veys