Accepted Manuscript

Title: Treosulfan, Fludarabine Conditioning for HSCT in Children with Primary Immunodeficiency: UK Experience

Author: Mary A. Slatter, Kanchan Rao, Intan Juliana Abd Hamid, Zohreh Nademi, Robert Chiesa, Reem Elfeky, Mark S. Pearce, Persis Amrolia, Austen Worth, Terence Flood, Mario Abinun, Sophie Hambleton, Waseem Qasim, Hubert B. Gaspar, Andrew J. Cant, Andrew R. Gennery, Paul Veys

PII: S1083-8791(17)30825-X
DOI: https://doi.org/10.1016/j.bbmt.2017.11.009
Reference: YBBMT 54865

To appear in: Biology of Blood and Marrow Transplantation

Received date: 5-9-2017
Accepted date: 8-11-2017


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Treosulfan, fludarabine conditioning for HSCT in children with Primary Immunodeficiency: UK experience

Authors

Mary A. Slatter MD, Kanchan Rao MD, Intan Juliana Abd Hamid PhD, Zohreh Nademi PhD, Robert Chiesa MD, Reem Elfeky MD, Mark S. Pearce PhD, Persis Amrolia PhD, Austen Worth PhD, Terence Flood MD, Mario Abinun MD, Sophie Hambleton DPhil, Waseem Qasim PhD, Hubert B. Gaspar PhD, Andrew J. Cant MD, Andrew R. Gennery MD, Paul Veys MD

Institutions

Department of Paediatric Immunology, Newcastle upon Tyne Hospital NHS Foundation Trust, Newcastle upon Tyne, UK

Great Ormond Street Hospital NHS Trust, London, UK

Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam Malaysia

Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK

Corresponding author

Dr M.A. Slatter,

Paediatric Immunology, Infectious Diseases & Allergy Department

Clinical Resource Building, Block 2, Level 4

Royal Victoria Infirmary, Queen Victoria Road,

Newcastle upon Tyne, NE1 4LP, UK
There was no specific funding for this study.

Conflict-of-interest disclosure: The authors declare no competing financial interests.
Highlights

- Excellent outcome in children post HSCT with treosulfan, fludarabine, for PID.
- Better myeloid chimerism with PBSC. No increased acute GVHD grade III/IV or chronic GVHD.
- 11 with SCID diagnosed at birth alive with up to 8.7 years follow up.
- There was no veno-occlusive disease.

Abstract

We previously published results of 70 children who received treosulfan with cyclophosphamide (30) or fludarabine (40) before haematopoietic stem cell transplantation (HSCT) for Primary Immunodeficiency (PID). Toxicity was lower and T cell chimerism better in those receiving fludarabine, but numbers were relatively small and follow-up short. We now report outcome of 160 children who received homogeneous conditioning with treosulfan, fludarabine mostly with alemtuzumab (n=124).

Median age at transplant was 1.36 years (0.09-18.25). Donors were: matched unrelated, 73; 1-3 antigen mismatched unrelated, 54; matched sibling, 12; other matched family, 17; haploidentical, 4. Stem cell source was: peripheral blood stem cells (PBSCs), 70; Bone marrow, 49; Cord Blood, 41. Median follow up was 4.3 years (0.8-9.4).

Overall survival was 83%. There was no veno-occlusive disease. Seventy-four (46%) had acute GVHD, but only 14(9%) greater than grade II. Four patients were successfully
retransplanted for graft loss or poor immune reconstitution. One further patient who rejected the graft, died.

There was no association between T cell chimerism > 95% and stem cell source, but a significant association with myeloid chimerism > 95% and use of PBSC without an increased risk of significant GVHD compared to other sources. All 11 patients with severe combined immunodeficiency diagnosed at birth are alive with up to 8.7 years follow up.

Long-term studies are required to determine late gonadotoxic effects and pharmacokinetic studies are needed to identify whether specific targeting is advantageous. The combination of treosulfan, fludarabine and alemtuzumab gives excellent results in HSCT for PID.

Key messages

- Excellent outcome in children undergoing HSCT following treosulfan, fludarabine and alemtuzumab for Primary Immunodeficiency.

- Better myeloid chimerism achieved using peripheral blood stem cells compared to bone marrow or cord blood without an increased risk of significant graft versus host disease.

Capsule summary

We report the largest series to date of children with PID undergoing HSCT following homogeneous conditioning with treosulfan and fludarabine. Probability of 2 year survival was 88.3%. Use of PBSC led to better myeloid chimerism.

Key words
Primary Immunodeficiency; Haematopoietic stem cell transplantation; Treosulfan;
Fludarabine; Chimerism

Abbreviations
HSCT Haematopoietic stem cell transplantation, PID Primary Immunodeficiency, aGVHD
Acute graft versus host disease, cGVHD Chronic graft versus host disease, HLA Human
leucocyte antigen, BM Bone marrow, PBSC Peripheral blood stem cells, CB Cord blood, MSD
Matched sibling donor, ATG Anti thymocyte globulin, PCR Polymerase chain reaction, EBV
Epstein-Barr virus, CMV Cytomegalovirus, SCID Severe Combined Immune deficiency, MUD
Matched unrelated donor, MMUD Mismatched unrelated donor, MFD Matched family
donor, MMFD Mismatched family donor, OS Overall survival, CGD Chronic granulomatous
disease, RAG Recombinant activating gene, ALL Acute lymphocytic leukaemia, ZAP 70 Zeta
associated protein, HLH Haemophagocytic lymphohistiocytosis, LAD Leukocyte adhesion
deficiency, WAS Wiskott Aldrich syndrome, PK Pharmacokinetic
Introduction

The use of treosulfan as part of conditioning for haematopoietic stem cell transplant (HSCT) in paediatric practice is increasing for malignant\textsuperscript{1-4} and non-malignant disorders\textsuperscript{5-15}. Treosulfan (L-treitol-1,4-bis-methanesulfonate) is the pro-drug of L-epoxybutane, a water soluble bi-functional alkylating agent with myeloablative and immunosuppressive properties\textsuperscript{16} but with less systemic toxicity compared to standard doses of busulfan\textsuperscript{17}. The use of reduced toxicity conditioning is preferred for patients with primary immune deficiency (PID) as there is no malignant disease to eradicate, stable mixed chimerism achieves cure for most patients and many enter HSCT with chronic infection and end-organ co-morbidities. Additionally, many patients are infants at the time of transplant and may be more susceptible to toxicity\textsuperscript{18}. Less toxic regimens may reduce early and late adverse effects particularly fertility\textsuperscript{19,20}. There are several reduced toxicity regimens that have been utilised by investigators in PID\textsuperscript{21-23}. Initial results suggest that specific conditioning regimens may be preferable in certain PID diseases with severe co-morbidities\textsuperscript{24}, or with donor type and stem cell source, or appear to have enhanced toxicity in children under one year of age\textsuperscript{25}. We previously published results of 70 children with PID who received treosulfan in combination with either cyclophosphamide ($n=30$) or fludarabine ($n=40$) with an overall survival of 81\% (median follow up 19 months) equivalent in those aged less or greater than one year at time of transplant. Toxicity was low but worse after cyclophosphamide, and T cell chimerism was significantly better after fludarabine\textsuperscript{9}. The numbers involved in this study were relatively small and follow-up fairly short. We now report 160 consecutive patients with prolonged follow-up who have received homogeneous conditioning with treosulfan and fludarabine without additional agents such as thiotepa, for a wide variety of PID diagnoses using different types of donor and stem cell source.
Methods

Patients

We performed a retrospective study of 160 consecutive patients with PID who underwent HSCT at the two UK supra-regional referral centres for PID; Great North Children’s Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust (n=90) and Great Ormond Street Hospital NHS Foundation Trust (n=70) between February 2006 and July 2013. Information was collected regarding patient demographics, diagnosis, donor match and stem cell source, conditioning regimen, transplant related complications, graft-versus-host-disease (GVHD), chimerism, immune reconstitution, outcome and length of follow up. Patients were not randomised to receive a specific conditioning regimen and the choice of conditioning was made by the treating medical team. Informed consent was taken from all parents according to the local centre and European Blood and Marrow Transplantation and the Declaration of Helsinki guidelines.

HLA typing was performed by molecular typing for HLA class I and II loci. The unrelated donors were all 7-10/10 HLA matched. Bone marrow (BM n=49), peripheral blood stem cells (PBSC n=70) and cord blood (CB n=41) were used as a stem cell source. Peripheral blood was used for the 4 haploidentical transplants, using the Clinimacs (Miltenyi Biotech Ltd, Surrey, UK) systems for CD3/CD19 depletion.

Treosulfan was given at a dose of 42g/m² (n=102), 36g/m² (n=54) or 30g/m² (n=4) in divided doses on 3 consecutive days. The lower dose of 36g/m² was given to infants less than 1 year of age and 30g/m² to Severe combined immunodeficiency (SCID) patients diagnosed at birth and transplanted very early. Fludarabine 150mg/m² was given to all in 5 divided doses on consecutive days. Alemtuzumab 0.3 – 1.0mg/kg total dose was given to all the patients except those who received a matched sibling donor (MSD) graft (n=6), 1 recipient of
haploidentical CD3/CD19 depleted PBSCs and 30 recipients of CB, 3 of whom received ATG, but 27 no serotherapy. This reflects a different approach to the use of cord blood between the 2 centres. GVHD prophylaxis in the majority of patients consisted of cyclosporine with mycophenolate mofetil which was weaned from day plus 28 in the absence of GVHD. Patients had weekly polymerase chain reaction (PCR) testing of blood for adenovirus, Epstein-Barr virus (EBV), and cytomegalovirus (CMV). Acute GVHD (aGVHD) was assessed using the modified Seattle Glucksberg criteria. Chronic GVHD (cGVHD) was scored according to the National Institutes of Health criteria.

**Chimerism**

Donor chimerism was measured by labelling blood with anti-CD3, -CD19 or -CD15 microbeads and cell lines were separated using an autoMACS® automated bench-top magnetic cell sorter (Miltenyi Biotec Ltd, Surrey, UK). Separated cells were assayed using variable number of tandem repeat (VNTR) or XY fluorescence in situ hybridization analysis for sex mismatched donor-recipient transplants.

**Statistics**

Statistical analysis was performed using STATA version 15. Descriptive analyses were performed using frequency, median, mean and range. Data were analysed using Pearson chi square and Kruskall Wallis tests. Survival outcome was evaluated with Kaplan-Meier estimates and log-rank test. Censoring of patients was defined at time of death or last follow up or second procedure for event free survival. Multivariable logistic regression analysis was performed for evaluation for factors influencing aGVHD and chimerism at last follow up.
Results

There were 39 patients with SCID, 11 of whom were diagnosed at birth due to previous family history, and 121 patients with other forms of combined immunodeficiency, phagocytic disorders, innate defects and disorders of immune regulation as detailed in table I. The median age at transplant was 1.36 years (range 0.09-18.25). Seventy-six patients were transplanted at 12 months of age or less. There was no significant difference in their survival compared with children transplanted over the age of 12 months (p=0.30).

Patients received HSCT from a 10/10 HLA matched unrelated donor (MUD) \(n=73\), HLA MUD (1 to 3 mismatched unrelated donor MMUD) \(n=54\), MSD \(n=12\), other matched family donor (MFD) \(n=17\) or haploidentical mismatched family donor (MMFD) \(n=4\) using treosulfan in combination with fludarabine 150mg/m².

Survival

Median follow up was 4.3 years (0.8-9.4). Overall survival (OS) is shown in figure I. Twenty-seven children died giving an OS of 83%. Only 10 died in the first 100 days (100 day survival of 94%), probability of 2 year survival was 88.3% (95% CI 82.1-92.5%).

Most deaths were associated with infection and/or GVHD and are detailed in table II. One patient with CGD died on day +1 from multiorgan failure. He had previous Aspergillus and mycobacterial infection with severe multisystem inflammation and capillary leak despite high dose steroids and tumour necrosis factor – alpha inhibitor (infliximab) prior to transplant.

Event free survival is shown in figure II. An event was defined as death or additional procedure. Four patients were successfully re-transplanted for graft loss or poor immune reconstitution. In addition, 1 patient with Autoimmune lymphoproliferative syndrome
rejected a haploidentical graft associated with CMV reactivation and died before re-
transplantation. An additional 5 patients received a boost without conditioning from the
original donor. A further 3 patients received donor lymphocyte infusions. Details are shown
in table IV.

Donor and stem cell source

Survival according to type of donor and stem cell source is shown in table III.

There was no significant difference in survival according to type of donor (p=0.5) or stem cell
source (p=0.23).

There has been an increase in the use of PBSC compared to BM (44% and 30.5%
respectively) compared to our previous published series (17% and 57% respectively). The
use of CB has remained the same at 26%.

There was a significant difference in median CD34+ stem cell dose according to stem cell
source (p<0.0001): Median dose in CB was 0.4 x 10^6/kg (0.05-6.3), BM 5.8 x 10^6/kg (1.1-19.5)
and PBSC 13.7 x 10^6/kg (2.0-63.8).

Toxicity

Formal grading using the National Cancer Institute toxicity criteria was not carried out as it
was not standard practice at the time in our centres. Mild skin toxicity was common
including perianal ulceration, pigment changes and occasional peeling. Practice now includes
frequent bathing and the avoidance of barrier creams to the skin on the days that treosulfan
is given. Mucositis was mild. Three children had seizures after completing their 3 doses of
treosulfan: all were already on cyclosporine at the time of seizures and all were under 4
months of age. No veno-occlusive disease (VOD) occurred.
Seventy-four (46%) patients had aGVHD, but only 14 (9%) had grade III/IV aGVHD. There were 6 deaths associated with GVHD and its therapy. Twenty-four patients had cGVHD. GVHD according to stem cell source is shown in Figure S1. There was no significant association between acute or chronic GVHD and stem cell source (p=0.37). Twenty-seven of 41 who received CB stem cells did not receive serotherapy and experienced a particularly high rate of both aGVHD (22 = 82%, although only 2 (7)% with Grade III/IV) and cGVHD (9 of 27, 33%). There was a significantly higher incidence of cGVHD in MMUD compared to MUD (p= 0.04) but no significant difference in aGVHD either grade I/II or III/IV between MMUD and MUD.

**Viral reactivation**

Fifty-six patients had evidence of 1 or more of CMV, EBV and adenovirus replication (35%) detected by PCR in blood post transplant. CMV was detected in 30 patients (27 of whom received treatment with foscarnet, ganciclovir or cidofovir), EBV in 21 (6 received treatment with rituximab, 1 ofatumumab, 1 EBV CTLs), and adenovirus in 24 children (19 of whom received treatment with cidofovir). In 4 cases these viral infections contributed to the death of the child.

**Chimerism**

There was no association between latest T chimerism being > 95% and stem cell source (p=0.20). However there was a significant overall association with myeloid chimerism (p=0.005): the odds of having myeloid chimerism > 95% being highest in the PBSC recipients, followed by cord then bone marrow. (Figure III)
There was no significant difference between unrelated donor and matched family donor recipients in donor T (OR 0.9, 95% CI 0.26, 3.21, p=0.90) or myeloid cell chimerism (OR 1.52, 95% CI 0.52, 4.46, p=0.43).

There was no significant difference between those who received 36g/m² and 42g/m² treosulfan in terms of achieving T or myeloid chimerism > 95% (p=0.34 and 0.22 respectively).

**Immune reconstitution**

Data on lymphocyte reconstitution are shown in supplementary Tables E1 to EIII.

There was no association between stem cell source or serotherapy dose and the kinetics of T lymphocyte reconstitution (at 3 months, 6 months and 12 months post-HSCT).

There were significantly more patients with low age-related B cell numbers at 3 months post HSCT in the group that received PBSC, but this ceased to be significant by 6 months. Receipt of high dose Alemtuzumab (1mg/kg) was also associated with delayed B cell reconstitution, which ceased to be significant by 6 months post-HSCT.

Seven survivors remain on immunoglobulin replacement due to ongoing immunosuppression in 5, recipient myeloid chimerism with absent B cells in 1 Omenn’s syndrome patient and poor immune reconstitution despite 100% donor chimerism in a SCID patient.

**Newborn SCIDs**

Eleven patients with SCID diagnosed at birth due to positive family history were transplanted using treosulfan 36g/m² (n=8) or 30g/m² (n=3) at less than 5 months of age. All are alive with 15-104 months follow up (median 55 months). All patients are off immunoglobulin
prophylaxis except 1 who was given rituximab for autoimmune haemolytic anaemia and has not recovered B cell function. Of 10 patients 6 have 100% and the other 4 have between 74% and 97% donor B cell chimerism.

A further 13 patients who were not diagnosed at birth but presented early were also transplanted at the age of 4 months or less. Their diagnoses were: SCID (n=6), Omenn’s syndrome (n=2), ZAP 70 (n=2), HLH (n=1), LAD (n=1), severe immune dysregulation (n=1), Eight are alive and well with a median follow up of 76 months (40 – 107). The 5 deaths are detailed in table II.

Wiskott Aldrich Syndrome (WAS)

Twenty patients have been transplanted for WAS all with unrelated donors: 14 MUD and 6 MMUD, 10 PBSC, 7 BM and 3 cords. All are alive and well with a median follow up of 52 months (20 - 102). Eighteen have 100% donor T chimerism, 1 has 82% and another 92%. Thirteen have > 95% donor myeloid chimerism - the other 7 patients have between 12 and 92% donor myeloid chimerism. All have normal platelet counts, the patient with 12% myeloid chimerism having had a splenectomy post HSCT.

Chronic Granulomatous Disease

Seventeen patients have been transplanted for CGD: 1 MSD, 12 MUD, 4 MMUD, 13 PBSC and 4 BM. Six had fungal disease prior to transplant, 9 had colitis and 4 were second transplants. Two patients died: one on day + 1 post transplant with multiorgan failure and the other from grade III GVHD 23 months post transplant. Fifteen are alive and well with a median follow up of 53 months (24 - 66). Ten have >95% donor myeloid and T cell chimerism, 4 have > 40% T cell and > 70% myeloid cell chimerism and the remaining patient lost the graft and was successfully re-transplanted.
Sixteen patients have been transplanted for HLH with only 7 survivors (OS 44%). Six received CB with no serotherapy, 5 of whom died. An additional MSD BM recipient who did not receive serotherapy also died. Numbers are small but 6 of 9 who did receive serotherapy are alive (69%), 1 died D-1 from uncontrolled HLH, 1 had secondary graft failure and died of Aspergillus pneumonia and 1 had cGVHD and ongoing HLH.

Survival curves for SCID, WAS, CGD and HLH are shown in figure IV. Survival at 2 years post-HSCT for SCID was 94.6% (80.2 – 98.6%), WAS 100%, CGD 93.7% (63.2 – 99.1%) and HLH 62.5% (34.8 – 81.0%)(Log rank test, p = 0.0001).
Discussion

HSCT following conditioning with treosulfan and fludarabine achieved a probability of 2 year survival of 87.1% in 160 children with PID with a high level of complete or stable mixed chimerism in the diseased lineage, sufficient to cure disease. As in our previous published series there was a high survival rate in children transplanted under 1 year of age in whom toxicity can be a problem with conventional and other reduced intensity conditioning regimens. A 100 day survival of 94% demonstrates the low toxicity of this regimen making it suitable for patients with PID who often have infection and organ damage prior to HSCT. In particular in this series we have demonstrated a higher level of myeloid chimerism in recipients of PBSC compared to CB and BM, without an increased risk of grade III/IV acute or chronic GVHD. There was no significant difference in survival according to type of donor or stem cell source although it would be interesting to evaluate this on a larger number of patients.

With the advent of newborn screening for SCID and knowing that the outcome of HSCT is better for those transplanted before the acquisition of infection and organ damage, it is important to delineate the best treatment options for such infants. Good long-term immune reconstitution requires at least some donor myeloid chimerism, which is much more reliably achieved when pre-HSCT conditioning is given. This report provides evidence of the safety of using treosulfan in very young infants. Eleven SCID patients diagnosed at birth due to previous family history and transplanted aged 4 months or under are alive, 10 with good immune reconstitution.

The outcome for patients with HLH was poor in contrast to Lehmberg’s report of 19 patients with HLH following HSCT with treosulfan, fludarabine, alemtuzumab, with or without thiotepa, who achieved 100% survival. Of note in Lehmberg’s report all patients including
MSD recipients were given alemtuzumab which is likely important due to the hyperinflammatory nature of the disease. In particular in our series the combination of cord blood without serotherapy had a poor outcome and we strongly recommend the inclusion of serotherapy in future for all patients with HLH. Patients with HLH are unusual in terms of those with PID in that they receive etoposide to attain remission before HSCT, and survival is dictated not only by co-morbidities leading to transplant related mortality, but also by failure to attain complete remission at time of HSCT.

Whilst good results in terms of survival have been achieved using reduced intensity regimens such as the combination of fludarabine and melphalan, secure engraftment can be an issue particularly in PID disorders where high levels of donor myeloid chimerism are required to achieve cure. In this study we show that the use of PBSCs is associated with significantly higher myeloid chimerism without any increase in severe GVHD. The relatively high incidence of grade I/II GVHD may reflect the low threshold for making a clinical diagnosis of skin GVHD without biopsy, which in other centres may have been labelled as an engraftment rash. Further work is required to determine optimal timing and dosing of serotherapy to minimise the risks of GvHD and viral reactivation. Whilst there was no significant difference in the incidence of acute GVHD between MUD and MMUD donors there was a significantly greater risk of chronic GVHD with MMUD. Newer techniques of T cell depletion such as CD3+TCR alpha/beta together with CD19+ depletion are enabling a wider spectrum of non SCID PID patients to receive successful haploidentical grafts and will lead to fewer MMUD being used.

Previously, excellent results have been achieved using a low dose targeted busulfan regimen in combination with fludarabine. Prospective studies are needed to compare this to treosulfan and fludarabine. Data on the longterm effects of treosulfan on fertility are lacking and need to be compared to other agents. In addition, further pharmacokinetic studies on
treosulfan are needed to identify whether specific PK targeting is advantageous, as for
busulfan\textsuperscript{41-43}. Many centres are using additional thiotapec in combination with treosulfan and
fludarabine, but in a recent multicentre study of patients with CGD this did not give superior
results in terms of overall survival, graft survival or higher myeloid chimerism\textsuperscript{5}, and may
result in additional toxicities. However numbers were small and further studies are
warranted.

This study shows that the combination of treosulfan and fludarabine is suitable for
conditioning a diverse range of PID diseases, regardless of age, and with all types of donor
and stem cell source, providing a uniformly applicable conditioning strategy in PID. One
caveat to this may be children with DNA repair disorders where there are few data\textsuperscript{44,45}.

Acknowledgments

Supported by NIHR Great Ormond Street Hospital Biomedical Research Centre. The views
expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the
Department of Health.

We would like to thank Patricia Tierney and Zoe Allwood for their contribution to data
management.

Authorship

Contribution: M.A.S., K.R., A.R.G., and P.V. designed the study and wrote the paper; I.J.A.H.,
M.S.P., and M.A.S. analyzed data; and Z.N., R.E., R.C., P.A., A.W., T.F., M.A., S.H., W.Q.,
H.B.G. and A.C. contributed to writing the paper.
References


Legends for tables and figures

Figure I  Overall survival

Figure II  Event free survival. An event was death or additional procedure

Figure III  T and myeloid cell chimerism according to stem cell source. All patients who survived more than 1 year post HSCT were included. Four patients were excluded for whom there was no split cell lineage chimerism available.

Figure IV  Overall survival by diagnosis

Survival at 2 years post-HSCT: SCID = 94.6% (80.2 – 98.6%), WAS = 100%, CGD = 93.7% (63.2 – 99.1%) and HLH = 62.5% (34.8 – 81.0%)(Log rank test, p = 0.0001).

Tables

Table I. Patient diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>39</td>
</tr>
<tr>
<td>WAS</td>
<td>20</td>
</tr>
<tr>
<td>CGD</td>
<td>17</td>
</tr>
<tr>
<td>HLH</td>
<td>18</td>
</tr>
<tr>
<td>MHC II</td>
<td>7</td>
</tr>
<tr>
<td>Omenn's</td>
<td>5</td>
</tr>
<tr>
<td>CHH</td>
<td>4</td>
</tr>
<tr>
<td>IPEX</td>
<td>3</td>
</tr>
<tr>
<td>CD40L</td>
<td>3</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Frequency</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>DOCK8</td>
<td>3</td>
</tr>
<tr>
<td>Colitis</td>
<td>3</td>
</tr>
<tr>
<td>LAD</td>
<td>3</td>
</tr>
<tr>
<td>NKT</td>
<td>2</td>
</tr>
<tr>
<td>ZAP70</td>
<td>2</td>
</tr>
<tr>
<td>PI3K</td>
<td>2</td>
</tr>
<tr>
<td>Severe immune dysregulation</td>
<td>9</td>
</tr>
<tr>
<td>Combined Immunodeficiency</td>
<td>8</td>
</tr>
<tr>
<td>XIAP</td>
<td>1</td>
</tr>
<tr>
<td>XLP-like</td>
<td>1</td>
</tr>
<tr>
<td>ALPS</td>
<td>1</td>
</tr>
<tr>
<td>CTLA4</td>
<td>1</td>
</tr>
<tr>
<td>IRF8</td>
<td>1</td>
</tr>
<tr>
<td>FADD</td>
<td>1</td>
</tr>
<tr>
<td>ITK</td>
<td>1</td>
</tr>
<tr>
<td>NEMO</td>
<td>1</td>
</tr>
<tr>
<td>Undefined neutrophil disorder</td>
<td>1</td>
</tr>
<tr>
<td>Hyper IgE</td>
<td>1</td>
</tr>
<tr>
<td>CTP synthase1</td>
<td>1</td>
</tr>
<tr>
<td>JIA</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: SCID Severe Combined Immunodeficiency, WAS Wiskott Aldrich syndrome, CGD Chronic granulomatous disease, HLH Haemophagocytic lymphohistiocytosis, SID Severe Immune dysregulation, CID Combined Immunodeficiency, MHC II Major Histocompatibility Class II deficiency, LAD Leukocyte adhesion deficiency, CHH Cartilage hair hypoplasia, IPEX Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, CD40L CD40Ligand deficiency, DOCK 8 Dedicator of cytokinesis 8 deficiency, NKT Natural Killer T cell deficiency, ZAP 70 Zeta-chain-associated protein kinase 70 deficiency, PI3K Phosphatidylinositide 3-kinase deficiency, XLP-like X Lymphoproliferative-like syndrome, XIAP X-linked inhibitor of apoptosis deficiency, ALPS Autoimmune lymphoproliferative syndrome, CTLA 4 Cytotoxic T lymphocyte antigen 4 deficiency, IRF 8 Interferon regulatory factor 8 deficiency, FADD Fas-associated death domain protein deficiency, ITK IL-2-inducible
T-cell kinase deficiency, NEMO NF-kappa-B essential modulator deficiency, JIA Juvenile Idiopathic arthritis.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Donor</th>
<th>Time post HSCT</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLH</td>
<td>MUD BM</td>
<td>Day-2</td>
<td>HLH, toxicity</td>
</tr>
<tr>
<td>CGD</td>
<td>MSD BM</td>
<td>Day+1</td>
<td>Severe inflammation, toxicity</td>
</tr>
<tr>
<td>HLH</td>
<td>MMUD cord</td>
<td>Day+7</td>
<td>Infection (Parainfluenza 3)</td>
</tr>
<tr>
<td>Autoimmune enteropathy</td>
<td>MMUD cord</td>
<td>Day+23</td>
<td>Pulmonary haemorrhage</td>
</tr>
<tr>
<td>SCID. Intestinal atresias</td>
<td>MMUD cord</td>
<td>1 month</td>
<td>Infection (Pseudomonas)</td>
</tr>
<tr>
<td>HLH</td>
<td>MUD cord</td>
<td>Day+34</td>
<td>Pulmonary haemorrhage</td>
</tr>
<tr>
<td>HLH</td>
<td>MMUD cord</td>
<td>1.4 months</td>
<td>Infection (Parainfluenza 3)</td>
</tr>
<tr>
<td>HLH</td>
<td>MMUD cord</td>
<td>2 months</td>
<td>Infection</td>
</tr>
<tr>
<td>CID</td>
<td>MMUD cord</td>
<td>2 months</td>
<td>Multiorgan failure</td>
</tr>
<tr>
<td>Omenn’s</td>
<td>MUD cord</td>
<td>2.5 months</td>
<td>GVHD grade IV</td>
</tr>
<tr>
<td>CID</td>
<td>MUD PBSC</td>
<td>5 months</td>
<td>GVHD grade IV</td>
</tr>
<tr>
<td>Severe Immune dysregulation</td>
<td>MUD PBSC</td>
<td>5 months</td>
<td>Infection (adenovirus)</td>
</tr>
<tr>
<td>HLH</td>
<td>MUD PBSC</td>
<td>5 months</td>
<td>Infection (Aspergillus)</td>
</tr>
<tr>
<td>Condition</td>
<td>Donor Type</td>
<td>Time</td>
<td>Cause</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------</td>
<td>-------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Secondary graft failure</td>
<td>ALPS</td>
<td>6</td>
<td>Infection (CMV)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Graft failure</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>CID</td>
<td>6</td>
<td>CD20 Neg PTLD, EBV</td>
</tr>
<tr>
<td>GVHD</td>
<td>HLH</td>
<td>8</td>
<td>CD20 Neg PTLD, EBV</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>Autoimmune enteropathy</td>
<td>10</td>
<td>Infection (adenovirus)</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>HLH</td>
<td>10</td>
<td>Infection (adenovirus)</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>IPEX</td>
<td>11</td>
<td>Infection (adenovirus)</td>
</tr>
<tr>
<td>GVHD</td>
<td>Omenn’s</td>
<td>11</td>
<td>Cerebral infarcts</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>CGD</td>
<td>23</td>
<td>Infection (influenza)</td>
</tr>
<tr>
<td>GVHD</td>
<td>XIAP</td>
<td>24</td>
<td>Infection (JC virus Leukoencephalopathy)</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>CyC SCID Thymectomy due to cardiac surgery</td>
<td>24</td>
<td>Respiratory failure post DLI</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>Omenn’s RAG 1</td>
<td>25</td>
<td>Pneumonitis, Chronic lung disease</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>RAG SCID</td>
<td>33</td>
<td>Infection whilst being treated for Ph+ pre B cell ALL (absent donor myeloid and B cell chimerism)</td>
</tr>
<tr>
<td>CD20 Neg PTLD, EBV</td>
<td>HLH</td>
<td>36</td>
<td>MDS/AML</td>
</tr>
<tr>
<td>SCID</td>
<td>MMUD cord</td>
<td>48 months</td>
<td>Infection</td>
</tr>
<tr>
<td>------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
</tbody>
</table>

Table III. Survival according to donor type and stem cell source

There was no significant difference in survival according to type of donor (p=0.50) or stem cell source (p=0.23).

4 mismatched family donor recipients were excluded due to the small number.

<table>
<thead>
<tr>
<th>Stem cell source/Donor</th>
<th>PBSC</th>
<th>BM</th>
<th>Cord</th>
<th>Total</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUD</td>
<td>44</td>
<td>15</td>
<td>14</td>
<td>73</td>
<td>64 (88.6%)</td>
</tr>
<tr>
<td>MMUD</td>
<td>13</td>
<td>14</td>
<td>27</td>
<td>54</td>
<td>44 (83.6%)</td>
</tr>
<tr>
<td>MFD</td>
<td>9</td>
<td>20</td>
<td>0</td>
<td>29</td>
<td>22 (75.9%)</td>
</tr>
<tr>
<td></td>
<td>(2 MSD)</td>
<td>(10 MSD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>66</strong></td>
<td><strong>49</strong></td>
<td><strong>41</strong></td>
<td><strong>156</strong></td>
<td><strong>130 (83.3%)</strong></td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td><strong>60 (90.9%)</strong></td>
<td><strong>39 (79.6%)</strong></td>
<td><strong>31 (75.6%)</strong></td>
<td><strong>130 (83.3%)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table IV  Second procedures

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>1st HSCT</th>
<th>Indication</th>
<th>Time to/type 2nd procedure</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undefined neutrophil disorder</td>
<td>MSD BM</td>
<td>25% myeloid chimerism. Abnormal neutrophils</td>
<td>10m MUD PBSC Bu/flu/alem</td>
<td>Alive and well</td>
</tr>
<tr>
<td>CGD</td>
<td>MUD PBSC</td>
<td>Dropped to 0% myeloid chimerism</td>
<td>DLI for slipping chimerism - no effect, then 19m MUD PBSC Bu/flu/alem</td>
<td>Alive and well</td>
</tr>
<tr>
<td>ADA</td>
<td>MUD cord</td>
<td>Poor immune reconstitution</td>
<td>12m MUD PBSC Flu/mel/alem</td>
<td>Alive and well</td>
</tr>
<tr>
<td>CHH</td>
<td>MMUD cord</td>
<td>Poor immune reconstitution</td>
<td>16m MMUD PBSC Flu/mel/alem</td>
<td>Alive and well</td>
</tr>
<tr>
<td>HLH</td>
<td>MUD PBSC</td>
<td>Secondary graft failure unmanipulated</td>
<td>Unconditioned unmanipulated</td>
<td>Died infection (Aspergillus) 5m post 1st</td>
</tr>
<tr>
<td>Condition</td>
<td>Procedure</td>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------</td>
<td>----------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FADD</td>
<td>MFD BM boost 4m HSCT</td>
<td>Stable low level mixed chimerism Alive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHH</td>
<td>MMUD BM boost 7m HSCT</td>
<td>100% donor Alive and well</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGD</td>
<td>MUD PBSC boost 22m HSCT</td>
<td>Died infection (influenza) GVHD 23m post 1st HSCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XIAP</td>
<td>MUD PBSC boost 17m HSCT</td>
<td>Died Infection (JC leukoencephalopathy) 2 years post 1st HSCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCID Thymectomy</td>
<td>MFD BM DLI 1 year post</td>
<td>Died respiratory failure 2yrs post HSCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune enteropathy</td>
<td>MSD BM DLI 5m post</td>
<td>Died infection (adenovirus) Respiratory failure 10m post HSCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCID</td>
<td>MUD BM DLI 33m post</td>
<td>Liver acute GVHD grade III post DLI, resolved. Alive and well but ongoing poor immune reconstitution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CGD Chronic granulomatous disease, ADA Adenosine deaminase, CHH Cartilage hair hypoplasia, HLH Haemophagocytic lymphohistiocytosis, FADD Fas-associated death domain protein deficiency, XIAP X-linked inhibitor of apoptosis deficiency, PBSC Peripheral blood stem cells, BM Bone marrow, MUD Matched unrelated donor, MSD Matched sibling donor, MMUD Mismatched unrelated donor, MFD Matched family donor, DLI Donor lymphocyte infusion, m months, GVHD Graft versus host disease, Bu Busulfan, fludarabine, mel melphalan, alem alemtuzumab.
Table E1 Immune reconstitution T cells according to stem cell source

There was no association between the kinetics of T cell reconstitution and different stem cell sources at 3 months, 6 months and 12 months post-HSCT.

Table E2 Immune reconstitution B cells according to stem cell source

There were significantly more patients with low B cells at 3 months post HSCT in the group that received PBSC. This ceased to be significant by 6 months.

Table E3 Immune reconstitution T cells according to serotherapy

There was no association between the kinetics of T cell reconstitution and different serotherapy doses at 3 months, 6 months and 12 months post-HSCT.

Table E4 Immune reconstitution B cells according to serotherapy

There were significantly more patients with low B cells at 3 months post HSCT in the group that received Alemtuzumab 1mg/kg. This ceased to be significant by 6 months.

Supplementary Figure S1

Graft versus host disease according to stem cell source

There was no significant association between acute or chronic GVHD and stem cell source (p=0.37).
At 2 years post-HSCT = 88.3% (95% CI 82.1 – 92.5%)

At 5 years post-HSCT = 77.5% (95% CI 77.2 – 89.3%)
An event was death or additional procedure

At 2 years post-HSCT = 88.1% (95% CI 81.8 – 92.3%)

At 5 years post-HSCT = 77.5% (95% CI 68.5 – 84.3%)
Figure III

Abbreviations: PBSC peripheral blood stem cells, BM bone marrow, CB cord blood, T T lymphocyte cells, M myeloid CD15+ cells
Figure IV

Kaplan-Meier survival estimates

Cumulative survival

Years Post-HSCT

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>SCID</th>
<th>WAS</th>
<th>CGD</th>
<th>HLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39</td>
<td>19</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>18</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>11</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend:
- SCID
- WAS
- CGD
- HLH
Figure 3.jpg