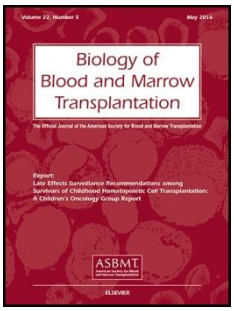
Accepted Manuscript



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

Please cite this article as: 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, Treosulfan, Fludarabine Conditioning for HSCT in Children with Primary Immunodeficiency: UK Experience, *Biology of Blood and Marrow Transplantation* (2017), https://doi.org/10.1016/j.bbmt.2017.11.009.

Treosulfan, fludarabine conditioning for HSCT in children with Primary Immunodeficiency: UK experience

Authors

Mary A.Slatter MD[1], Kanchan Rao MD[2], Intan Juliana Abd Hamid PhD[1,3], Zohreh Nademi PhD[1], Robert Chiesa MD[2], Reem Elfeky MD[2], Mark S. Pearce PhD[4], Persis Amrolia PhD[2], Austen Worth PhD[2], Terence Flood MD[1], Mario Abinun MD[1], Sophie Hambleton DPhil[1], Waseem Qasim PhD[2], Hubert B.Gaspar PhD[2], Andrew J. Cant MD[1], Andrew R. Gennery MD[1], Paul Veys MD

Institutions

Department of Paediatric Immunology, Newcastle upon Tyne Hospital NHS Foundation Trust, Newcastle upon Tyne, UK[1]

Great Ormond Street Hospital NHS Trust, London, UK[2]

Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam Malaysia[3]

Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK[4]

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

22 e-mail: mary.slatter@nuth.nhs.uk

23 Phone: 0191 2823767 Fax: 0191 2820497

24 There was no specific funding for this study.

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

26

27

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[1-4] and non-malignant disorders[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[16] but with less systemic toxicity compared to standard doses of busulfan[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[18]. Less toxic regimens may reduce early and late adverse effects particularly fertility[19, 20]. There are several reduced toxicity regimens that have been utilised by investigators in PID[21-23]. Initial results suggest that specific conditioning regimens may be preferable in certain PID diseases with severe co-morbidities[24], or with donor type and stem cell source, or appear to have enhanced toxicity in children under one year of age[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[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 [26, 27]. 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[28]. Chronic GVHD (cGVHD) was scored according to the National Institutes of Health criteria[29].

Chimerism

Donor chimerism was measured by labelling blood with anti-CD3, -CD19 or -CD15 micro beads 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 perfomed 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)[9]. 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.

GVHD

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/m^2 and 42g/m^2 treosulfan in terms of achieving T or myeloid chimerism > 95% (p=0.34 and 0.22 repsectively).

Immune reconstitution

Data on lymphocyte reconstitution are shown in supplementary Tables EI 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/m^2 (n=8) or 30g/m^2 (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.

Haemophagocytic Lymphohistiocytosis (HLH)

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[24, 25]. 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[30], it is important to delineate the best treatment options for such infants[31]. Good long-term immune reconstitution requires at least some donor myeloid chimerism, which is much more reliably achieved when pre-HSCT conditioning is given.[32, 33] 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[8]. 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[22, 24, 34]. 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[35]. 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[36-39].

Previously, excellent results have been achieved using a low dose targeted busulfan regimen in combination with fludarabine[40]. 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[19]. In addition, further pharmacokinetic studies on

treosulfan are needed to identify whether specific PK targeting is advantageous, as for busulfan[41-43]. Many centres are using additional thiotepa 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[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[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.

359 **References**

360 1. Boztug H, Sykora KW, Slatter M, Zecca M, Veys P, Lankester A, et al. European
361 Society for Blood and Marrow Transplantation Analysis of Treosulfan Conditioning
362 Before Hematopoietic Stem Cell Transplantation in Children and Adolescents With
363 Hematological Malignancies. Pediatr Blood Cancer 2016; 63:139-48.
364 2. Boztug H, Zecca M, Sykora KW, Veys P, Lankester A, Slatter M, et al. Treosulfan-
365 based conditioning regimens for allogeneic HSCT in children with acute
366 lymphoblastic leukaemia. Ann Hematol 2015; 94:297-306.
367 3. Wachowiak J, Sykora KW, Cornish J, Chybicka A, Kowalczyk JR, Gorczynska E, et al.
368 Treosulfan-based preparative regimens for allo-HSCT in childhood hematological
369 malignancies: a retrospective study on behalf of the EBMT pediatric diseases
370 working party. Bone Marrow Transplant 2011; 46:1510-8.
371 4. Beier R, Schulz A, Honig M, Eyrich M, Schlegel PG, Holter W, et al. Long-term follow-
372 up of children conditioned with Treosulfan: German and Austrian experience. Bone
373 Marrow Transplant 2013; 48:491-501.
374 5. Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, et al.
375 Treosulfan based conditioning for allogeneic HSCT in children with chronic
376 granulomatous disease: a multicentre experience. Blood 2016; 128(3):440-448
377 6. Slatter MA, Boztug H, Potschger U, Sykora KW, Lankester A, Yaniv I, et al. Treosulfan-
378 based conditioning regimens for allogeneic haematopoietic stem cell transplantation
379 in children with non-malignant diseases. Bone Marrow Transplant 2015; 50:1536-41.
380 7. Burroughs LM, Nemecek ER, Torgerson TR, Storer BE, Talano JA, Domm J, et al.
381 Treosulfan-based conditioning and hematopoietic cell transplantation for
382 nonmalignant diseases: a prospective multicenter trial. Biol Blood Marrow
383 Transplant 2014; 20:1996-2003.

8. Lehmberg K, Albert MH, Beier R, Beutel K, Gruhn B, Kroger N, et al. Treosulfan-based conditioning regimen for children and adolescents with hemophagocytic lymphohistiocytosis. Haematologica 2014; 99:180-4.

9. Slatter MA, Rao K, Amrolia P, Flood T, Abinun M, Hambleton S, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. Blood 2011; 117:4367-75.

10. Mathews V, George B, Viswabandya A, Abraham A, Ahmed R, Ganapule A, et al. Improved clinical outcomes of high risk beta thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. PLoS One 2013; 8:e61637.

11. Bernardo ME, Piras E, Vacca A, Giorgiani G, Zecca M, Bertaina A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood 2012; 120:473-6.

12. Greystoke B, Bonanomi S, Carr TF, Gharib M, Khalid T, Coussons M, et al. Treosulfan-containing regimens achieve high rates of engraftment associated with low transplant morbidity and mortality in children with non-malignant disease and significant co-morbidities. Br J Haematol 2008; 142:257-62.

13. Choudhary D, Sharma SK, Gupta N, Kharya G, Pavecha P, Handoo A, et al. Treosulfan-thiotepa-fludarabine-based conditioning regimen for allogeneic transplantation in patients with thalassemia major: a single-center experience from north India. Biol Blood Marrow Transplant 2013; 19:492-5.

14. Strocchio L, Zecca M, Comoli P, Mina T, Giorgiani G, Giraldi E, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in children with sickle cell disease. Br J Haematol 2015; 169:726-36.

15. Dinur-Schejter Y, Krauss AC, Erlich O, Gorelik N, Yahel A, Porat I, et al. Bone marrow transplantation for non-malignant diseases using treosulfan-based conditioning. Pediatr Blood Cancer 2014.

16. Sjoo F, Hassan Z, Abedi-Valugerdi M, Griskevicius L, Nilsson C, Remberger M, et al. Myeloablative and immunosuppressive properties of treosulfan in mice. Exp Hematol 2006; 34:115-21.

17. Glowka FK, Romanski M, Wachowiak J. High-dose treosulfan in conditioning prior to hematopoietic stem cell transplantation. Expert Opin Investig Drugs 2010; 19:1275-95.

18. Malar R, Sjoo F, Rentsch K, Hassan M, Gungor T. Therapeutic drug monitoring is essential for intravenous busulfan therapy in pediatric hematopoietic stem cell recipients. Pediatr Transplant 2011; 15:580-8.

19. Panasiuk A, Nussey S, Veys P, Amrolia P, Rao K, Krawczuk-Rybak M, et al. Gonadal function and fertility after stem cell transplantation in childhood: comparison of a reduced intensity conditioning regimen containing melphalan with a myeloablative regimen containing busulfan. Br J Haematol 2015.

20. Lawitschka A, Faraci M, Yaniv I, Veys P, Bader P, Wachowiak J, et al. Paediatric reduced intensity conditioning: analysis of centre strategies on regimens and definitions by the EBMT Paediatric Diseases and Complications and Quality of Life WP. Bone Marrow Transplant 2015; 50:592-7.

21. Chiesa R, Veys P. Reduced-intensity conditioning for allogeneic stem cell transplant in primary immune deficiencies. Expert Rev Clin Immunol 2012; 8:255-66; quiz 67.

22. Rao K, Amrolia PJ, Jones A, Cale CM, Naik P, King D, et al. Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. Blood 2005; 105:879-85.

23. Burroughs LM, Storb R, Leisenring WM, Pulsipher MA, Loken MR, Torgerson TR, et al. Intensive postgrafting immune suppression combined with nonmyeloablative conditioning for transplantation of HLA-identical hematopoietic cell grafts: results of a pilot study for treatment of primary immunodeficiency disorders. Bone Marrow Transplant 2007; 40:633-42.

24. Straathof KC, Rao K, Eyrich M, Hale G, Bird P, Berrie E, et al. Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study. Lancet 2009; 374:912-20.

25. Ritchie DS, Seymour JF, Roberts AW, Szer J, Grigg AP. Acute left ventricular failure following melphalan and fludarabine conditioning. Bone Marrow Transplant 2001; 28:101-3.

26. Chiesa R, Gilmour K, Qasim W, Adams S, Worth AJ, Zhan H, et al. Omission of in vivo T-cell depletion promotes rapid expansion of naive CD4+ cord blood lymphocytes and restores adaptive immunity within 2 months after unrelated cord blood transplant. Br J Haematol 2012; 156:656-66.

27. Lane JP, Evans PT, Nademi Z, Barge D, Jackson A, Hambleton S, et al. Low-dose serotherapy improves early immune reconstitution after cord blood transplantation for primary immunodeficiencies. Biol Blood Marrow Transplant 2014; 20:243-9.

28. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995; 15:825-8.

29. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant 2015; 21:389-401 e1.

30. Brown L, Xu-Bayford J, Allwood Z, Slatter M, Cant A, Davies EG, et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. Blood 2011; 117:3243-6.

31. Dorsey MJ, Dvorak CC, Cowan MJ, Puck JM. Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening. J Allergy Clin Immunol 2017; 139:733-42.

32. Borghans JA, Bredius RG, Hazenberg MD, Roelofs H, Jol-van der Zijde EC, Heidt J, et al. Early determinants of long-term T-cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency. Blood 2006; 108:763-9.

33. Neven B, Leroy S, Decaluwe H, Le Deist F, Picard C, Moshous D, et al. Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. Blood 2009; 113:4114-24.

34. Klaudel-Dreszler MA, Kalwak K, Kurenko-Deptuch M, Wolska-Kusnierz B, Heropolitanska-Pliszka E, Pietrucha B, et al. Treosulfan-based conditioning regimen in a second matched unrelated peripheral blood stem cell transplantation for a pediatric patient with CGD and invasive aspergillosis, who experienced initial graft failure after RIC. Int J Hematol 2009; 90:571-5.

35. Marsh RA, Lane A, Mehta PA, Neumeier L, Jodele S, Davies SM, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. Blood 2016; 127:503-12.

36. Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood 2014; 124:822-6.

37. Balashov D, Shcherbina A, Maschan M, Trakhtman P, Skvortsova Y, Shelikhova L, et al. Single-Center Experience of Unrelated and Haploidentical Stem Cell Transplantation with TCRalphabeta and CD19 Depletion in Children with Primary Immunodeficiency Syndromes. Biol Blood Marrow Transplant 2015; 21:1955-62.

38. Bertaina A, Pitisci A, Sinibaldi M, Algeri M. T Cell-Depleted and T Cell-Replete HLA-Haploidentical Stem Cell Transplantation for Non-malignant Disorders. Curr Hematol Malig Rep 2017; 12:68-78.

39. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, et al. TCRalphabeta+ and CD19+ cell depleted Haploidentical and Mismatched Hematopoietic Stem Cell Transplantation in Primary Immune Deficiency. J Allergy Clin Immunol 2017.In press

40. Gungor T, Teira P, Slatter M, Stussi G, Stepensky P, Moshous D, et al. Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. Lancet 2014; 383:436-48.

41. Ten Brink MH, Ackaert O, Zwaveling J, Bredius RG, Smiers FJ, den Hartigh J, et al. Pharmacokinetics of treosulfan in pediatric patients undergoing hematopoietic stem cell transplantation. Ther Drug Monit 2014; 36:465-72.

42. Ten Brink MH, Zwaveling J, Swen JJ, Bredius RG, Lankester AC, Guchelaar HJ. Personalized busulfan and treosulfan conditioning for pediatric stem cell transplantation: the role of pharmacogenetics and pharmacokinetics. Drug Discov Today 2014; 19:1572-86.

43. Ansari M, Theoret Y, Rezgui MA, Peters C, Mezziani S, Desjean C, et al. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematopoietic stem cell transplantation. Ther Drug Monit 2014; 36:93-9.

44. Cowan MJ, Gennery AR. Radiation-sensitive severe combined immunodeficiency: The arguments for and against conditioning before hematopoietic cell transplantation--what to do? J Allergy Clin Immunol 2015; 136:1178-85.

45. Slack J, Albert MH, Balashov D, Belohradsky BH, Bertaina A, Bleesing J, et al. Outcome of hematopoietic cell transplantation for DNA double-strand break repair disorders. J Allergy Clin Immunol 2017.In press

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

Diagnosis	Number
SCID	39
WAS	20
CGD	17
HLH	18
MHC II	7
Omenn's	5
CHH	4
IPEX	3
CD40L	3

DOCK8	3
Colitis	3
LAD	3
NKT	2
ZAP70	2
PI3K	2
Severe immune dysregulation	9
Combined Immunodeficiency	8
XIAP	1
XLP-like	1
ALPS	1
CTLA4	1
IRF8	1
FADD	1
ITK	1
NEMO	1
Undefined neutrophil disorder	1
Hyper IgE	1
CTP synthase1	1
JIA	1

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 deficienc, 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

553 T-cell kinase deficiency, NEMO NF-kappa-B essential modulator deficiency, JIA Juvenile
554 Idiopathic arthritis.

555

556

557

558

559

560

561 **Table II. Deaths**

Diagnosis	Donor	Time post HSCT	Cause
HLH	MUD BM	Day-2	HLH, toxicity
CGD	MSD BM	Day+1	Severe inflammation, toxicity
HLH	MMUD cord	Day+7	Infection (Parainfluenza 3)
Autoimmune enteropathy	MMUD cord	Day+23	Pulmonary haemorrhage
SCID. Intestinal atresias	MMUD cord	1 month	Infection (Pseudomonas)
HLH	MUD cord	Day+34	Pulmonary haemorrhage
HLH	MMUD cord	1.4 months	Infection (Parainfluenza 3)
HLH	MMUD cord	2 months	Infection
CID	MMUD cord	2 months	Multiorgan failure
Omenn's	MUD cord	2.5 months	GVHD grade IV
CID	MUD PBSC	5 months	GVHD grade IV
Severe Immune dysregulation	MUD PBSC	5 months	Infection (adenovirus)
HLH	MUD PBSC	5 months	Infection (Aspergillus)

			Secondary graft failure.
ALPS	MMFD PBSC	6 months	Infection (CMV) Graft failure
CID	MMUD BM	6 months	CD20 Neg PTLD, EBV
HLH	MFD BM	8 months	GVHD
Autoimmune enteropathy	MSD BM	10 months	Infection (adenovirus) Respiratory failure
HLH	MMUD cord	10 months	GVHD Infection (RSV)
IPEX	MMUD PBSC	11 months	Respiratory failure
Omenn's	MUD BM	11 months	GVHD Cerebral infarcts
CGD	MUD PBSC	23 months	Infection (influenza) GVHD
XIAP	MUD PBSC	24 months	Infection (JC virus Leukoencephalopathy)
CγC SCID Thymectomy due to cardiac surgery	MFD BM	24 months	Respiratory failure post DLI
Omenn's RAG 1	MSD BM	25 months	Pneumonitis, Chronic lung disease
RAG SCID	MSD BM	33 months	Infection whilst being treated for Ph+ pre B cell ALL (absent donor myeloid and B cell chimerism)
HLH	MSD BM	36 months	MDS/AML

SCID	MMUD cord	48 months	Infection

Abbreviations: HSCT Haematopoietic stem cell transplantation, HLH Haemophagocytic lymphohistiocytosis, CGD Chronic granulomatous disease, SCID Severe Combined Immunodeficiency, CID Combined immunodeficiency, ALPS Autoimmune lymphoproliferative syndrome, IPEX Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, XIAP X-linked inhibitor of apoptosis deficiency, CγC Common gamma chain, RAG Recombinatin activating gene, BM Bone marrow, PBSC Peripheral blood stem cells, MUD Matched unrelated donor, MSD Matched sibling donor, MMUD Mismatched unrelated donor, MMFD Mismatched family donor, GVHD Graft versus host disease, CMV Cytomegalovirus, EBV Epstein Barr virus, PTLD Post transplant lymphopoliferative disease, RSV Respiratory syncytial virus, JC John Cunningham, DLI Donor lymphocyte infusion, Ph Philadelphia, ALL Acute lymphocytic leukaemia, MDS Myelodysplasia, AML Acute myeloid leukaemia.

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.

Stem cell source/ Donor	**PBSC**	**BM**	**Cord**	***Total***	**Survival**
MUD	44	15	14	***73***	**64 (88.6%)**
MMUD	13	14	27	***54***	**44 (83.6%)**
MFD	9 (2 MSD)	20 (10 MSD)	0	***29***	**22 (75.9%)**
Total	***66***	***49***	***41***	***156***	**130 (83.3%)**
Survival	**60 (90.9%)**	**39 (79.6%)**	**31 (75.6%)**	**130 (83.3%)**	

610 Abbreviations: PBSC Peripheral blood stem cells, BM Bone marrow, MUD Matched unrelated
611 donor, MSD Matched sibling donor, MMUD Mismatched unrelated donor, MFD Matched
612 family donor, MMFD Mismatched family donor

613

614

615

616

617

618

619

620

621

622

623 **Table IV Second procedures**

Diagnosis	1st HSCT	Indication	Time to/type 2nd procedure	Outcome
Undefined neutrophil disorder	MSD BM	25% myeloid chimerism. Abnormal neutrophils	10m MUD PBSC Bu/flu/alem	Alive and well
CGD	MUD PBSC	Dropped to 0% myeloid chimerism	DLI for slipping chimerism - no effect, then 19m MUD PBSC Bu/flu/alem	Alive and well
ADA	MUD cord	Poor immune reconstitution	12m MUD PBSC Flu/mel/alem	Alive and well
CHH	MMUD cord	Poor immune reconstitution	16m MMUD PBSC Flu/mel/alem	Alive and well
HLH	MUD PBSC	Secondary graft failure	Unconditioned unmanipulated	Died infection (Aspergillus) 5m post 1st

			boost 4m	HSCT
FADD	MFD PBSC	Low level mixed chimerism	Unconditioned unmanipulated boost 10m	Stable low level mixed chimerism Alive
CHH	MMUD BM	Aplasia despite 100% donor chimerism	Unconditioned unmanipulated boost 7m	100% donor Alive and well
CGD	MUD PBSC	GVHD Hypocellular	Unconditioned unmanipulated boost 22m	Died infection (influenza) GVHD 23m post 1st HSCT
XIAP	MUD PBSC	Hypocellular	DLI then unconditioned unmanipulated boost 17m	Died Infection (JC leukoencephalopathy) 2 years post 1st HSCT
SCID Thymectomy	MFD BM	Poor immune reconstitution	DLI 1 year post	Died respiratory failure 2yrs post HSCT
Autoimmune enteropathy	MSD BM	Poor immune reconstitution Adenovirus	DLI 5m post	Died infection (adenovirus) Respiratory failure 10m post HSCT
SCID	MUD BM	Poor immune reconstitution despite 100% donor chimerism	DLI 33m post	Liver acute GVHD grade III post DLI, resolved. Alive and well but ongoing poor immune reconstitution

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, flu fludarabine, mel melphalan, alem alemtuzumab,

Supplementary tables

Table EI 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.

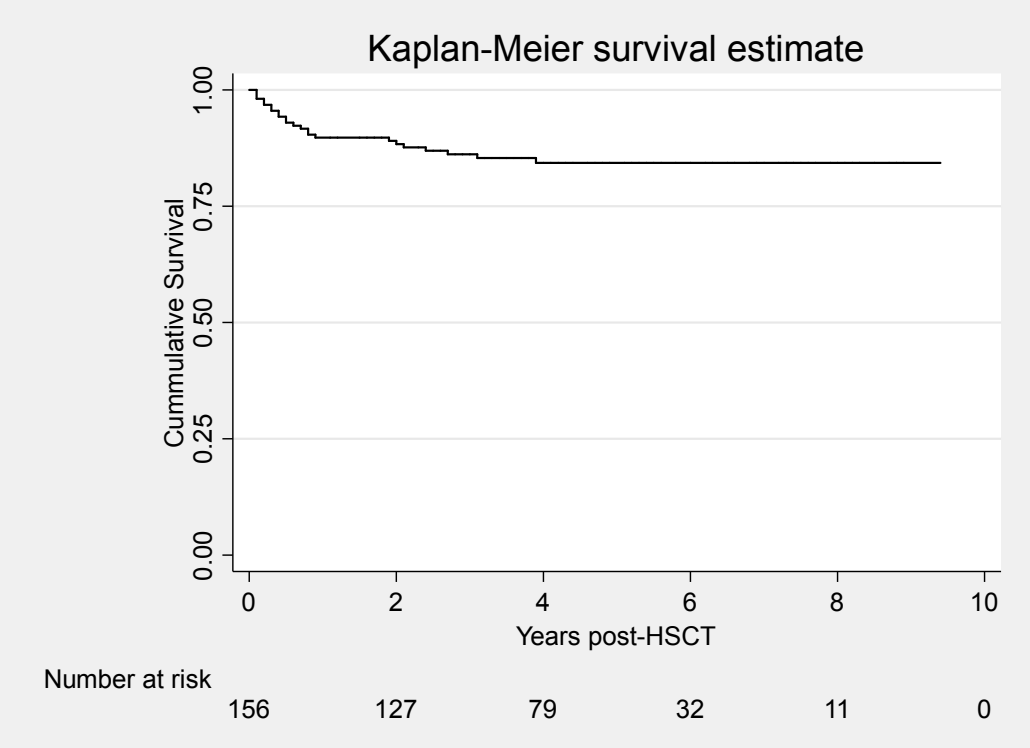
Supplementary Figure S1

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).

660 Figures

661 Figure I



662

663 At 2 years post-HSCT = 88.3% (95% CI 82.1 – 92.5%)

664 At 5 years post-HSCT = 77.5% (95% CI 77.2 – 89.3%)

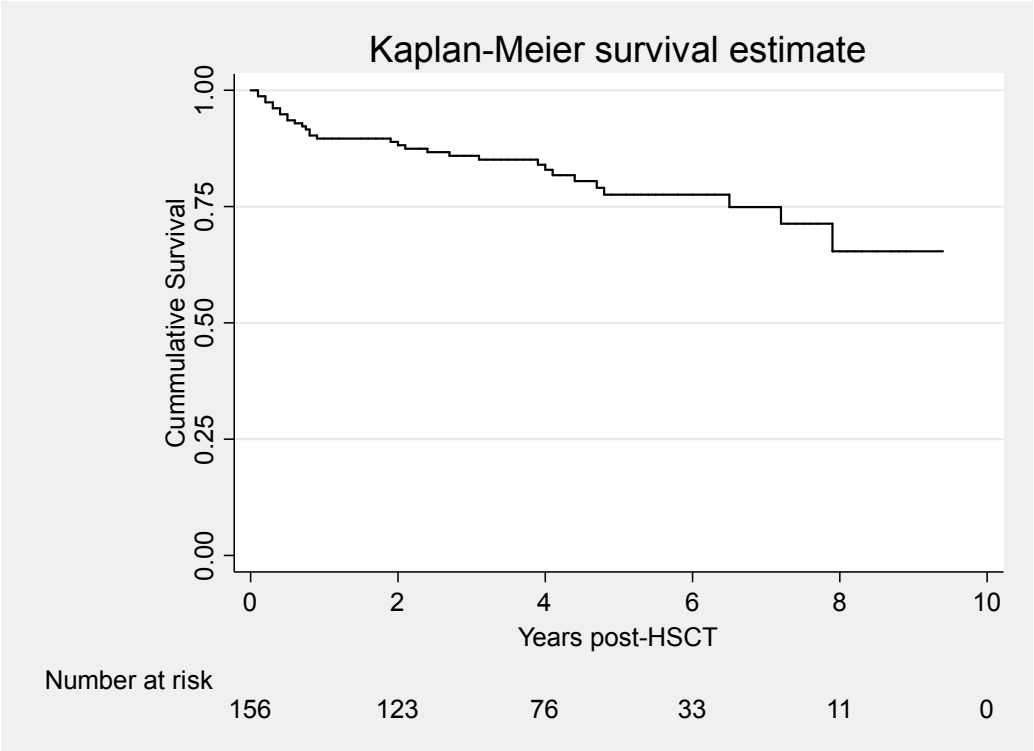
665

666

667

668 Figure II

669 An event was death or additional procedure



670

671

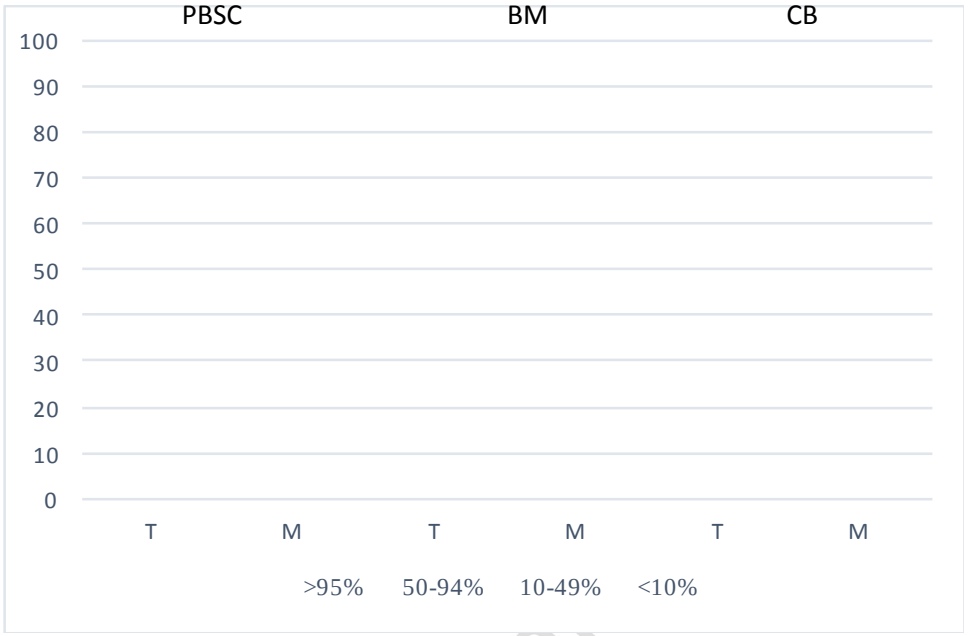
672 At 2 years post-HSCT = 88.1% (95% CI 81.8 – 92.3%)

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

674

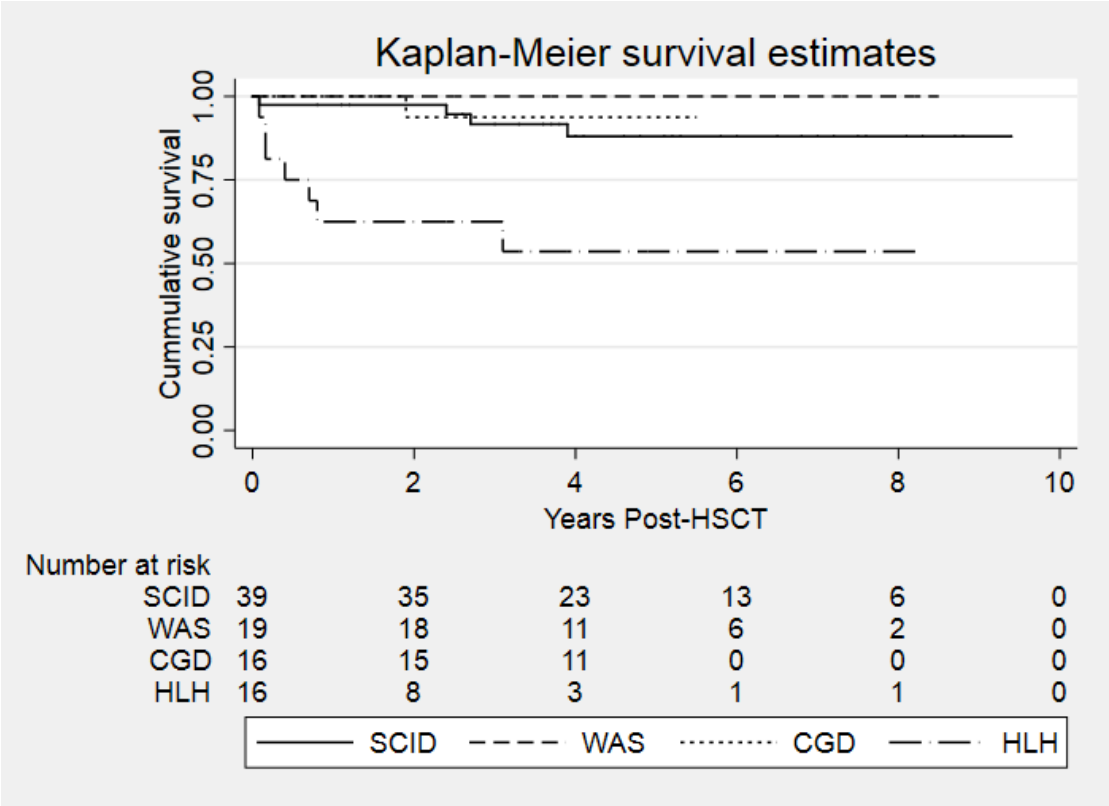
675

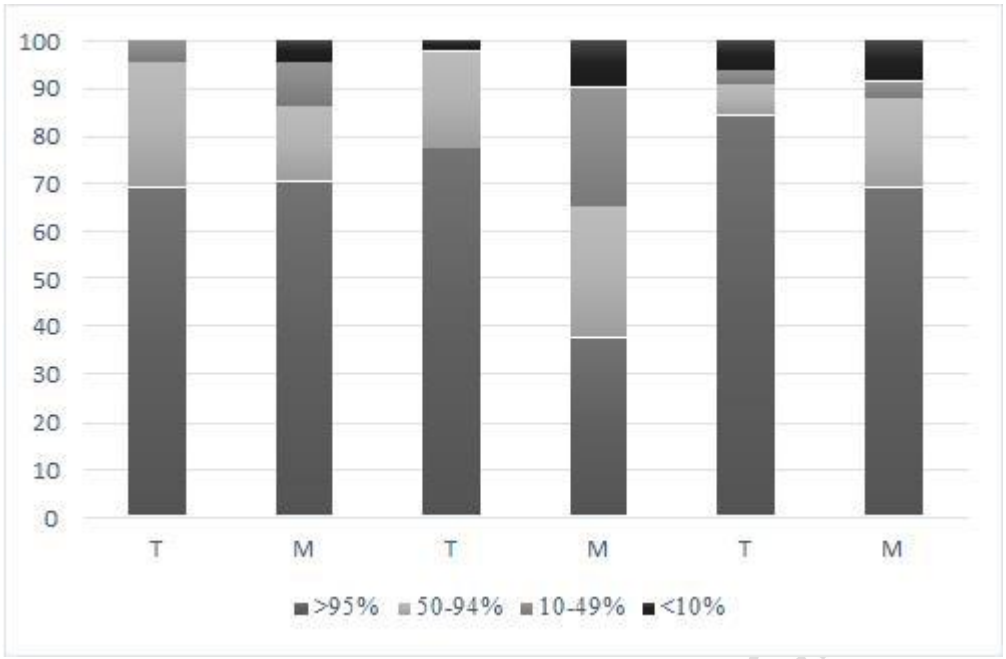
Figure III



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

Figure IV





703

704 Figure 3.jpg