The use of Treosulfan in conditioning prior to Haematopoietic Stem Cell Transplantation for children with Primary Immunodeficiency

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DECLARATION

I declare that this thesis is my own work. I have correctly acknowledged the work of others, in accordance with University and Institute guidance on good academic conduct. No part of the material offered has been previously submitted for a degree or other qualification in this or any other university. For all published manuscripts, my independent contributions have been outlined in the appropriate co-authorship forms.

Signature: M. A. Slatter

Date: **3rd October 2020**

ABSTRACT

Primary Immunodeficiencies (PIDs) are inherited disorders that lead to defects in the development and/or function of the immune system. The number of disorders that can be treated by haematopoietic stem cell transplantation (HSCT) has increased rapidly with the advent of next generation sequencing. The methods used to transplant children with PID have improved dramatically over the last 20 years. The introduction of reduced toxicity conditioning is an important factor in the improved outcome of HSCT. Treosulfan has myeloablative and immunosuppressive properties, enabling engraftment with less toxicity than traditionally used doses of busulfan. The use of treosulfan in conditioning prior to HSCT for children with PID is reported in this thesis.

Six published works are presented. The first 2 provide background with up to date information on HSCT and conditioning regimens in children with PID. The increased use of low toxicity treosulfan-based combinations is demonstrated in published paper PP3 which is the largest published series to date of patients with non-malignant disorders who received treosulfan-based conditioning across Europe. The place of treosulfan in conditioning patients specifically with Chronic Granulomatous Disease from centres worldwide is presented in PP4. Close collaboration with Great Ormond Street Hospital, London has led to rigorous monitoring and step by step improvements in the approach to transplant using treosulfan, published in Supplementary paper 1, followed by PP5 and culminating in a prospective pharmacokinetic study presented in PP6, which is the first study to demonstrate an association with high area under the concentration curve (AUC) and increased mortality, and low AUC and poor engraftment.

For each manuscript I present an overview of the study, what was known before, and what the study added to the literature, my contribution to the work and a short discussion of the strengths and limitations.

Treosulfan has been established as a safe and effective agent for conditioning children with PID prior to HSCT. It is firmly incorporated into the conditioning guidelines of the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation. The works presented in this thesis demonstrate the contribution that I have made to the field, and pave the way for future research. It is likely that individualized dosing, not just of treosulfan, but of all agents used in conditioning regimens, will be developed and implemented. This will lead to a reduction in unwanted variability in drug exposure, leading to more predictable and adjustable exposure, and improved outcome of HSCT, with fewer late adverse effects and improved quality of life. Such conditioning regimens can be used as the basis to study the need for additional agents in certain disorders, the dosing of individual cellular components within grafts and effects of adjuvant cellular or immunotherapy post-transplant.

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ABBREVIATIONS

ADA	Adenosine Deaminase
ALL	Acute Lymphoblastic Leukaemia
AML	Acute Myeloid Leukaemia
AUC	Area Under the concentration Curve
BM	Bone Marrow
BMF	Bone Marrow Failure
BSA	Body Surface Area
CB	Cord Blood
CD40L	CD40 Ligand
CGD	Chronic Granulomatous Disease
Cmax	Maximum Concentration
CR	Chronic Remission
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CSA	Cyclosporine A
DBA	Diamond Blackfan Anaemia
DLI	Donor Lymphocyte Infusion
DOCK 8	Dedicator of Cytokinesis
EBMT	European Society for Blood and Marrow Transplantation
EMA	European Medicines Agency
ES	Event Free Survival
GATA 2	GATA binding protein 2 deficiency
GVHD	Graft Versus Host Disease, aGVHD, acute. cGVHD, chronic
HLA	Human Leukocyte Antigen

HLH	Haemophagocytic Lymphohistiocytosis
HSCT	Haematopoietic Stem Cell Transplantation
IEWP	Inborn Errors Working Party
JMML	Juvenile Myelomonocytic Leukaemia
LAD	Leukocyte Adhesion Deficiency
LRBA	Lipopolysaccharide-responsive and beige-like anchor protein
MAC	Myeloablative Conditioning
MDS	Myelodysplasia
MFD	Matched Family Donor
MMF	Mycophenolate mofetil
MPS1H	Mucopolysaccharidosis type I Hurler syndrome
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
OS	Overall Survival
PBSC	Peripheral Blood Stem Cells
PD	Pharmacodynamics
PID/s	Primary Immunodeficiency/Immunodeficiencies
PK	Pharmacokinetics
PNH	Paroxysmal Nocturnal Haemoglobinuria
RAG	Recombinant Activating Gene
SCETIDE	Stem Cell Transplantation for Immunodeficiencies in Europe
SCID	Severe Combined Immunodeficiency
SCN	Severe Congenital Neutropenia
SDS	Shwachman-Diamond Syndrome
SOS	Sinusoidal Obstruction Syndrome

S,S-DEB	(2S,3S)-1,2:3,4-diepoxybutane
S,S-EBDM	(2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 3
TBI	Total Body Irradiation
TDM	Therapeutic Drug Monitoring
TMA	Thrombotic Microangiopathy
TRM	Transplant Related Mortality
VOD	Veno-Occlusive Disease
WAS	Wiskott-Aldrich syndrome
XLP	X-linked lymphoproliferative syndrome

CHAPTER 1: INTRODUCTION

1.1 Primary Immunodeficiencies

Primary Immunodeficiencies (PIDs) are inherited disorders that lead to defects in the development and/or function of the immune system. More than 450 single gene defects have now been identified (Bousfiha et al., 2020). Severe combined immunodeficiency (SCID) is the most profound PID characterised by impaired T- and B-lymphocyte function. It presents in infancy with recurrent opportunistic infections and failure to thrive and left untreated, infants usually die within the first year of life (Fischer et al., 2015). The first successful bone marrow transplant reported by Gatti et al in 1968 achieved immunological reconstitution in a patient with X-linked SCID (Gatti et al., 1968) followed by a second case published in 1969 (De Koning et al., 1969). Other T-lymphocyte immunodeficiencies may present later. Another early bone marrow transplant was performed in 1968 for a patient with Wiskott-Aldrich syndrome (WAS) (Bach et al., 1968). Although prophylactic treatment with antimicrobials and immunoglobulin may improve short-term outlook for such patients, long-term outcome with conservative management is poor with patients dying from infectious, inflammatory or malignant complications (Imai et al., 2004, Aydin et al., 2015, Winkelstein et al., 2003). Innate immune defects may also present in infancy, but with prophylaxis patients may survive until adulthood. However adults, for example with Chronic granulomatous disease (CGD), often suffer recurrent infections, colitis and other inflammatory manifestations, requiring frequent hospital admissions, surgery and leading to early death (Jones et al., 2008). Quality of life has been shown to be superior in transplanted patients compared to those managed conservatively (Cole et al., 2013).

Historically, haematopoietic stem cell transplantation (HSCT) for SCID has led to a higher survival than for non-SCID PIDs. In Europe, data from participating transplant centres are collected in the Stem Cell Transplantation for Immunodeficiencies in Europe (SCETIDE) registry, which reported in 1986, 1990, 1994, 2003 and 2010 (Fischer et al., 1986, Fischer et al., 1990, Fischer et al., 1994, Antoine et al., 2003, Gennery et al., 2010). Survival has improved over time. Importantly patients with SCID diagnosed and transplanted before the development of infection have a better outcome which has led to the introduction of newborn screening in many areas of the world (Kane et al., 2001, Myers et al., 2002, Brown et al., 2011, Pai et al., 2014). Similarly in disease-specific series, outcome is better for younger patients without pre-existing organ damage and infection (Lum et al., 2020, Gennery et al., 2004, Filipovich et al., 2001, Lum et al., 2019a). Awareness of PIDs has grown leading to earlier diagnosis and treatment enabling timely referral for HSCT. Simple precautions such as the use of protective isolation and co-trimoxazole prophylaxis have been followed by incremental changes in methods of tissue typing leading to better human leucocyte antigen (HLA)-matched donors, use of alternative donors such as cord blood and haploidentical donors facilitated by improved methods of T-lymphocyte depletion, reduced toxicity of conditioning regimens, surveillance for viral infections by PCR enabling pre-emptive treatment, better therapies for infections, and improved preventative strategies and treatments for complications such as Graft versus host disease (GVHD).

In the past patients without a defined genetic defect had a poor outcome, maybe because they were only offered transplant as a last resort (Gennery et al., 2010). The advent of the genomic revolution has enabled precise molecular diagnosis to be made at an early stage, which helps to inform optimal treatment.

Gene therapy has been developed for a number of PIDs as an alternative to HSCT. Using autologous haematopoietic stem cells avoids the risks of GVHD. Originally gamma-retroviral vectors were used but these carry a risk of insertional mutagenesis and a number of patients developed leukaemia in the original trial of patients treated for X-linked SCID (Hacein-Bey-Abina et al., 2008). Safer lentiviral vectors are now in use for patients with X-linked SCID, Adenosine deaminase (ADA) SCID, CGD and WAS. Pre-clinical studies are underway for a number of other disorders including X-linked lymphoproliferative (XLP) syndrome, perforin deficiency, autosomal recessive CGD and Recombinant-activating gene (RAG) SCIDs (Booth et al., 2019). In 2016, the European Medicines Agency (EMA) approved the first stem cell gene therapy product for ADA-SCID, called Strimvelis™, which was licensed by GSK (Aiuti et al., 2017). Despite using a gamma-retroviral vector no cases of insertional mutagenesis have occurred to date and worldwide more than 150 cases of ADA SCID have now been treated in various trials (Zhang et al., 2020). Successful gene therapy requires chemotherapy conditioning, but this tends to be a much lower dose than is required prior to HSCT. In some disorders such as CGD it may be difficult to achieve a sufficient level of gene transduction to achieve cure and gene silencing may occur meaning that any benefit is short-lived (Stein et al., 2010). Gene editing is emerging as an important new tool for more precise genetic manipulation of haematopoietic stem cells.

It takes time for culture to change amongst clinicians, patients and their families in terms of who should be offered a transplant, when and how it should be done. Whilst it has been obvious for many years that infants with SCID should be offered curative therapy despite the risks of such a treatment, there was, and still is in some areas, a perception that patients with non-SCID PID need to "earn the right to transplantation by presenting with significant complications or infections"(Gennery et al., 2010). This is likely to have contributed to poorer outcome in these

patients who were transplanted with organ damage and infections already established, thus compounding the problem. Once it became acceptable e.g. to offer transplant to a young patient with CGD, at first the transplant community were understandably cautious to use any donor that was not an HLA identical matched sibling. In Newcastle we have been at the forefront of pushing the boundaries. We demonstrated that a well matched unrelated donor provides as good an outcome as a matched sibling for CGD (Soncini et al., 2009) and led the recent Inborn Errors Working Party (IEWP) Study of 712 patients transplanted for CGD from 101 different centres (Chiesa et al., 2020b). We have shown that with the new technique of CD3+ TCR/CD19+ depletion for mismatched donors, a successful outcome can be achieved in patients without an HLA identical donor, not just for patients with CGD, but for a wide range of PIDs (Lum et al., 2019a, Shah et al., 2018, Elfeky et al., 2019). Now we do not just talk about survival, but about how to ensure good quality of life and long-term immunoreconstitution (Cole et al., 2013, Abd Hamid et al., 2018, Abd Hamid et al., 2017).

Chapter 2, published paper 1 reviews recently published literature on the approach to, and outcome of, HSCT for conventional and emerging PIDs.

1.2 Conditioning in PID

Originally conditioning therapy for any disease was based on the combination of 2 alkylating agents, usually: busulfan and cyclophosphamide, or of cyclophosphamide and total body irradiation (TBI). The goals were: to predictably cause host haematopoietic stem cell toxicity to induce myeloablation, immunosuppression to enable engraftment and prevent rejection, together with antineoplastic activity in the setting of malignancy. Busulfan is widely distributed in organs

such as the liver, lungs, brain and kidneys and is associated with complications of veno-occlusive disease of the liver (VOD), interstitial pneumonia, convulsions and mucositis. Busulfan is highly lipophilic with moderate plasma binding and so it achieves high levels in the liver where it undergoes enzymatic conversion. Tremendous progress has been made to improve the use of busulfan with the introduction of an intravenous preparation and many studies on carefully pharmacokinetically-guided dosing to achieve an area under the curve within a targeted range (Bartelink et al., 2012, Bartelink et al., 2016). However pharmacokinetic variability is still substantial particularly in young children (Malar et al., 2011). Cyclophosphamide is also associated with hepatotoxicity together with haemorrhagic cystitis and acute cardiotoxicity (McDonald et al., 2003).

With time this perception of the role of conditioning prior to HSCT has changed. Now it is seen as providing a platform for the development of donor chimerism and in the setting of some conditions such as myeloid malignancy, a graft-versus-malignancy effect, which is generally more important than the antineoplastic activity of the conditioning itself. In patients with PID there is no advantage in having GVHD because no graft-versus-malignancy effect is required and GVHD can have a significant effect on thymic reconstitution leading to poor immune function.

Myeloablative conditioning (MAC) refers to conditioning that results in irreversible cytopenia and the requirement for stem cell rescue. Truly non-myeloablative regimens cause minimal cytopenia and can in theory be given without stem cell support. Reduced intensity conditioning (RIC) regimens cause profound cytopenia and should be given with stem cells, to reduce the duration of cytopenia, although in some cases cytopenia may be reversible without such support.

The introduction of RIC regimens such as fludarabine and melphalan led to reduced treatment related toxicity but there have been specific cardiac toxicities associated with melphalan in infants (Ritchie et al., 2001). This therefore makes it difficult to use in infants with SCID. Minimal intensity conditioning such as that reported by Straathof et al using an anti-CD45 antibody together with fludarabine and low dose cyclophosphamide may also result in successful engraftment, but availability of such antibodies is limited (Straathof et al., 2009). RIC regimens lead to a higher rate of mixed chimerism and efforts to improve chimerism such as using donor lymphocyte infusions (DLI) risk causing GVHD, leading to immunosuppressive therapy and more infections.

Treosulfan has increased lymphotoxic and myelosuppressive properties compared to melphalan, and leads to fewer, and less severe adverse effects than myeloablative busulfan. In combination with fludarabine, treosulfan leads to prompt engraftment and high levels of donor chimerism associated with MAC, whilst only incurring the more limited non-haematological toxicity associated with RIC regimens. It can be defined as a reduced toxicity myeloablative regimen (Danylesko et al., 2012).

Conditioning regimens for children with PID have broadly followed those of the wider transplant community, but with an emphasis on avoiding the toxicity associated with radiotherapy and highly intensive regimens needed to combat malignancy. Use of chemotherapy in patients with SCID remains a source of international debate. Successful T-lymphocyte engraftment is possible particularly for common gamma chain and Janus kinase 3 deficient SCID without conditioning, but an increasing number of studies indicate that more durable thymopoiesis is obtained after the administration of chemotherapy (Abd Hamid et al., 2017). Patients with DNA repair disorders are

at risk of toxicity from alkylating agents and a RIC regimen with low dose cyclophosphamide and fludarabine is generally recommended (Slack et al., 2018).

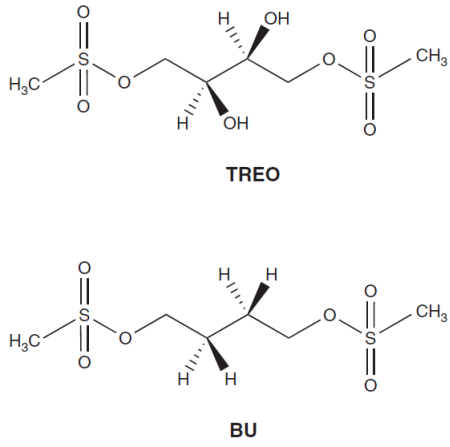
Chapter 2, published paper 2 reviews the latest developments in conditioning regimens for PID.

1.3 What is treosulfan?

Treosulfan (L-treitol-1, 4-bis-methanesulphonate) is a water-soluble bifunctional alkylating agent. It was first made by Peter W. Feit in 1961 (Feit et al., 1970). It is a dihydroxy derivative of busulfan. This introduction of 2 hydroxyl components into the carbon chain gives it different properties to busulfan. It is a pro-drug that undergoes nonenzymatic conversion to a monoepoxide ((2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate (S,S-EBDM)) and a diepoxide ((2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB)). This conversion is pH and temperature dependent. At pH values below 6 and a temperature of 20 degrees in vitro almost no transformation of treosulfan occurs. But under physiological conditions spontaneous conversion into the derivatives occurs. The monoepoxide intermediate and L-diepoxybutane alkylate DNA at guanine residues and produce DNA interstrand crosslinks, resulting in DNA fragmentation and apoptosis. Because it is water-soluble it is easily given intravenously. Because it is hydrophilic and undergoes less distribution in the liver than busulfan the severity and incidence of hepatic complications, particularly VOD are much reduced compared to busulfan. The lack of hepatic metabolism is a great advantage because it decreases the risk of interaction with other concomitant medications such as glutathione level reducers (e.g. cyclophosphamide, paracetamol), hepatic enzyme inducers (e.g. itraconazole) and substrates such as methylprednisolone. Treosulfan also causes significantly less neurotoxicity than busulfan, induces no seizures and consequently does not require

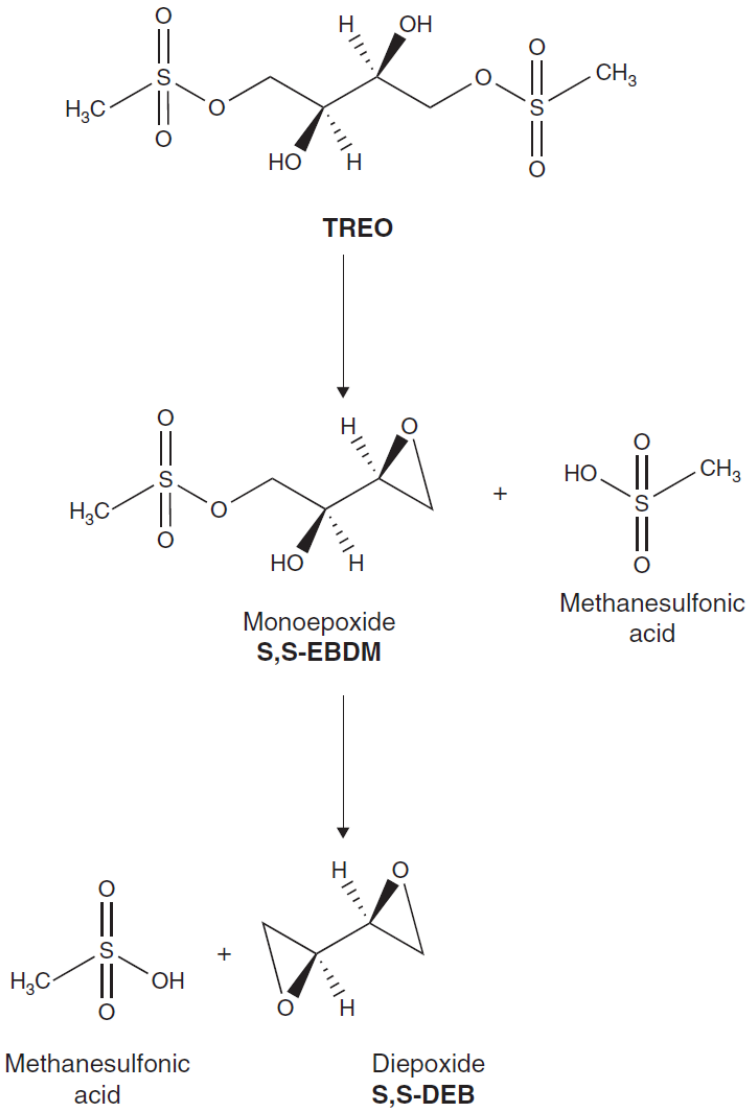
prophylactic anticonvulsant therapy, which is mandatory for busulfan. Treosulfan and its active monoepoxide have been shown to have poor penetration into the central nervous system. However, the brain exposure was greater in juvenile rats, so it is possible that very young infants with an immature blood brain barrier might be more susceptible to neurotoxicity (Romanski et al., 2015).

Figure 1: Structural formulas of treosulfan and busulfan



TREO, treosulfan; Bu, busulfan

Figure 2: Transformation of treosulfan to pharmacologically active epoxides



TREO, treosulfan; S,S-EBDM, (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate; S,S-DEB, (2S,3S)-1,2:3,4-diepoxybutane

With permission from: Franciszek K Główka, Michał Romański & Jacek Wachowiak (2010) High-dose treosulfan in conditioning prior to hematopoietic stem cell transplantation, Expert Opinion on Investigational Drugs, 19:10, 1275-1295, DOI: 10.1517/13543784.2010.517744.

Treosulfan was designated an 'orphan medicine' (a medicine used in rare diseases) in 2004 and given marketing authorisation by the EMA in June 2019 for adult patients with malignant and non-malignant diseases, and in paediatric patients older than one month with malignant diseases, for conditioning prior to allogeneic HSCT, following completion of trials in these patient groups. It is marketed under the name "Trecondi®". (https://ec.europa.eu/health/documents/community-register/htlm/h1351.htm, 2019).

1.4 Introduction to the use of treosulfan in conditioning

Treosulfan has been used in the treatment of ovarian cancer since the 1990s (Masding et al., 1990, Gropp et al., 1998). It has also been shown to have a broad spectrum of anti-tumour activity for example in metastatic melanoma (Neuber et al., 1999), breast carcinoma (Kopf-Maier and Sass, 1992) and Acute Lymphoblastic Leukaemia (ALL) (Fichtner et al., 2003). When used as chemotherapy against malignancy, haematotoxicity limits its use above doses of $10g/m^2$. Two phase I studies with autologous blood stem cell rescue demonstrated that it was possible to escalate the dose to almost 5 times conventional therapy before mucositis, stomatitis, diarrhoea, skin toxicity and acidosis became dose limiting (Scheulen et al., 2000). Studies in mice revealed the pronounced effect of treosulfan on haematopoietic stem cells (Westerhof et al., 2000) and showed that reliable donor-cell engraftment could be achieved using repeated dosing which was at least as effective as busulfan or TBI (van Pel et al., 2003, Ploemacher et al., 2004). It led to limited non-haematopoietic organ toxicity and therefore was a promising alternative to traditionally used conditioning agents. In mice treosulfan was found to cause equivalent myeloablation to busulfan

and cyclophosphamide, but in addition caused much stronger splenic B- and T-cell depletion than busulfan or cyclophosphamide. This immunosuppressive effect is beneficial in terms of using it as a conditioning agent to suppress the host and enable successful engraftment (Sjoo et al., 2006). Casper et al combined treosulfan with fludarabine in 30 adult patients with haematological malignancies in a Phase I/II trial between 1999 and 2002 from 3 German centres. Patients were considered to be at high risk for conventional conditioning. The total dose of treosulfan was $30mg/m^2$ with $150mg/m^2$ fludarabine and ATG was given to recipients of unrelated donors. Toxicity was low and no VOD occurred. Eight patients died : 6 (20%) of non-relapse causes and 2 (6.7%) of relapse giving an estimated overall (OS) of 73% and event free survival (EFS) of 49% after a median follow up of 22 months (range 7.4-33.4 months). Reduction of immunosuppression or DLI cured an additional three patients who relapsed leading to an estimated 63% probability of continuing chronic remission (CR) after a median follow up of 22 months (Casper et al., 2004). This was a landmark study, which began the use of treosulfan in conditioning for HSCT.

Beelen et al reported a low rate of organ toxicities and favourable one year non-relapse mortality combining treosulfan with cyclophosphamide in 18 adult patients with haematological malignancies who were considered high risk for conventional myeloablative conditioning. Cyclophosphamide was chosen to allow a more direct comparison with conventional regimens at the time of TBI and cyclophosphamide, or busulphan and cyclophosphamide. Pharmacokinetic studies were incorporated which showed predictable maximum concentration (Cmax) and area under the concentration curve (AUC) values with low interpatient and interday variability (Beelen et al., 2005).

Use in adults continued. Schmidt-Hieber et al reported using treosulfan and fludarabine for patients with myeloma before allogeneic HSCT leading to an estimated overall survival (OS) of 58% at 2 years (Schmidt-Hieber et al., 2007). In 2006 Giebel et al reported 6 patients with severe aplastic anaemia and a median age of 21 years (range 14-25) transplanted using treosulfan, cyclophosphamide and ATG who all engrafted with a good outcome (Giebel et al., 2006).

The first reported use exclusively in children comes from Sauer et al in 2007. Three patients with Shwachman-Diamond syndrome (SDS) were conditioned with fludarabine, treosulfan and melphalan to replace historically used cyclophosphamide and busulfan or TBI. All engrafted, but 1 patient died post cord blood HSCT with idiopathic pneumonitis syndrome. Therefore they could not conclude that this regimen had the potential to decrease the typical treatment-related toxicities seen in SDS patients undergoing HSCT such as cardiac and pulmonary toxicities (Sauer et al., 2007).

In 2008 Bernardo et al reported a phase I-II prospective trial of 20 patients transplanted for Thalassemia major in 2 Italian centres. The use of treosulfan is attractive in this disease because of the substantial risk of VOD in iron-overloaded patients. HSCT for thalassemia is also associated with a substantial risk of graft failure. The median age at HSCT was 13 years (range 1-28 years). All received thiotepa, treosulfan and fludarabine with ATG in the 17 unrelated donor recipients. This was well tolerated with no cases of VOD. Two patients experienced secondary graft failure. One patient died of grade IV acute GVHD (aGVHD). The 2-year estimates of OS and transfusion-free survival were 95% (CI, 85-100%) and 85% (CI, 66-100%) respectively (Bernardo et al., 2008). This experience has been confirmed in a subsequent publication of a total of 60 patients (Bernardo et al., 2012).

Boztug et al. reported the outcome of treosulfan-based conditioning in 71 children with ALL showing low treatment-related mortality (14%) with an OS at 3 years of 51%. EFS of infants was significantly better compared to older children (Boztug et al., 2015). Kalwak et al. performed a multicentre open label, non-controlled prospective Phase II study in children with haematological malignancies. Seventy children with ALL, acute myeloid leukaemia (AML), Juvenile myelomonocytic leukaemia (JMML) or Myelodysplasia (MDS) were enrolled from 18 centres in 5 European countries. Sixty-five received thiotepa in addition to treosulfan and fludarabine and were included in the analysis. Body surface area (BSA) adapted dosing for treosulfan was used:

BSA $\leq$ 0.5 m^2 received a total dose of 30g/m^2

BSA > 0.5-1.0 m^2 received a total dose of 36g/m^2

BSA > 1.0 m^2 received a total dose of 42g/m^2.

The median follow up was 41.8 months (range 24.2-57.5 months). The 36-month Kaplan-Meier estimates of non-relapse mortality and OS were 3.1% and 83% respectively with a relapse/progression free survival of 73.6%, which compare favourably with other conditioning regimens. There were no primary graft failures, one patient developed secondary poor graft function and received a stem cell boost. There was only one case of grade II hepatic sinusoidal obstruction syndrome (SOS), which resolved. They concluded that treosulfan/fludarabine/thiotepa should be recommended as a suitable myeloablative preparative treatment in children with malignant disorders (Kalwak et al., 2020).

This study together with other data led to the European commission approving treosulfan use in paediatric patients older than 1 month of age with malignant disease.
(https://ec.europa.eu/health/documents/community-register/htlm/h1351.htm, 2019)

1.5 Use of treosulfan in children with PID

Table 1 summarises the reports of treosulfan used for Primary Immunodeficiencies including HLH, Osteopetrosis and GATA 2 deficiency.

Table 1: Use of treosulfan in patients with PID

Author Year	Number of patients Diagnoses *Number*/%	Donor Stem cell source *Number*/%	Treo dose Additional agents *Number*/%	GVHD aGVHD grade *Number*/%	2nd procedures	Survival
Greystoke 2008 Retrospective	**32** PID *13* 41% HLH *5* 5.5% Metabolic *9* 28% OP *4* 12.5% Thal *1* 3%	MRD *10* 31% MMRD *1* 3% (9/10) MUD *11* 35% MMUD *10* 31% BM *17* 53% PBSC *9* 28% CB *5* 16% BM +CB *1* 3%	Tr 42 *26* 81% Tr 36 *6* 19% Flu 150 *28* 91% (+Cyclo120 *1*) or Cyclo200 *3* 9% Alem *23* 72% ATG *5* 16% None *4* 12%	I-II *6* 19% III-IV *2* 6% cGVHD *4* 12%	4 patients 5 HSCT	84% at median FU 417 days Day 100 TRM 3%
Cutting 2008 Retrospective	**23** ALL *11* 48% Biphenotypic leukaemia *1* 4% AML *2* 9% Thal *2* 9% DBA *1* 4% OP *1* 4% HLH *2* 9% JMML *1* 4% MDS *2* 9%	MRD *9* 39% MUD *5* 22% MMUD *9* 39% BM *10* 44% PBSC *2* 8% CB *11* 48%	Tr 42 *1* 4% Tr 36 *22* 96% Cyclo120 *20* 87% Cyclo200 *3* 13% + Mel 140 *1* 4% Flu 180 *1* 4% Etop30 *2* 8% ATG *14* 61% Alem *3* 13% None *6* 26%	I-II *15* 65% II-IV *4* 17% cGVHD NA	2 HSCT post relapse	83%
Slatter 2011 Retrospective	**70 PID** SCID *26* 37% WAS *7* 10% Omenn *7* 10% HLH *4* 6% CID *4* 6% LAD *4* 6% CGD *3* 4% SID *3* 4% CHH *2* 3% IPEX *2* 3% MHC II *2* 3% Other *6* 8%	MRD *21* 30% MMRD *4* 6% MUD *24* 34% MMUD *21* *30%* BM *40* 57% PBSC *9* 13% CB *17* 24%	Tr 42 *43* 61% Tr 36 *27* 39% Flu 150 *40* 57% Cyclo200 *30* 43% Alem *50* 71% ATG *3* 5% OKT3 *1* 1% None *16* 23%	I-II *11* 16% III-IV *7* 10% cGVHD *4* 6%	HSCT 2 Top-up 3	OS 81% Median FU 19 months(range 1-47months)
Burroughs 2014 Prospective	**31** PID *13* 42% HLH *6* 19% BMF *6* 19%	MRD *4* 13% MUD *26* 84% MMUD *1* 3%	Tr 42 *31* 100% Flu 150 *31* 100%	I NA II *16* 52% III-IV *3* 10%	2 HSCT	2 yr 90%

	RBC *6* 19%	BM *29* 94% PBSC *2* 6%	ATG *22* 71% None *9* 29%	cGVHD 2 yr cumulative incidence 21%		Day 100 TRM 0%
Beier 2013 Retrospective	**109** **Non-malignant 51** PID *29* 57% HLH *2* 4% Metabolic *1* 2% OP *3* 6% BMF *7* 14% Thal *8* 15% SCC *1* 2%	MRD *16* 31% MMRD *10* 20% MUD *24* 47% MMUD *1* 2% BM *35* 69% PBSC *11* 21% CB *1* 2% CB + PBSC *2* 4% Unknown *2* 4%	Tr 42 *36* 71% Tr 36 *14* 27% Tr 21 *1* 2% Flu *49* 96% 150-180 7.2mg/kg *2* 4% TT 8-10 *30* 59% Mel *6* 12% ATG *22* 43% Y-RIT *1* 2% Alem *17* 33% OKT3 *8* 16% None *3* 6%	I-II *13* 26% III-IV *5* 10% cGVHD *3* 6%	3 HSCT 2 thal 1 SCN	Non-malignant 88% at 3 years
Lehmberg 2014 Retrospective	**19** HLH *19* 100%	MRD *5* 26% MMRD *2* 11% MUD *6* 31.5% MMUD *6* 31.5% BM *17* 89% PBSC *2* 11%	Tr 42 *13* 68% Tr 36 *6* 32% Flu 150 *16* 84% Flu other *3* 16% TT 7- 10 *14* 74% Alem *19* 100%	I-II *4* 21% III-IV *1* 5% (after DLI) cGVHD *0*	2 HSCT DLI 6	100% at median FU 16 months
Dinur-Schejter 2014 Retrospective	**44 (45 HSCT)** SCID *12* 27% SCN *5* 11% WAS *2* 5% CGD *2* 5% LAD *2* 5% MSMD *1* 2% CID *2* 5% HLH *1* 2% OP *5* 11% Thal *5* 11% RBC *1* 2% SDS *1* 2% CAMT % *1* 2 Hypereosinophilic syndrome *1* 2% Hurler *2* 5% Niemann Pick *1* 2%	MRD *16* 35.5% MMFD *4* 9% MUD *9* 20% MMUD *16* 35.5% BM *25* 56% PBSC *5* 11% CB *9* 20% Unknown *6* 13%	Tr 42 *30* 67% Tr 36 *15* 33% Flu 150 *39* 87% (+TT *20* 44%*)* Cyclo *6* 13% ATG *26* 58% Alem *8* 18% OKT3 *1* 2% None *10* 22%	I-II *8* 18% III-IV *12* 27% cGVHD *7* 16%	6 graft failures. OP 2 HSCT WAS 1 HSCT Niemann-Pick 1 HSCT 2 deaths	70.5%
Burroughs	**14**					

2017 Prospective	SDS *3* 21% DBA *4* 29% GATA2 *2* 14% PNH *4* 29% Undefined BMF *1* 7%	MFD *2* 14% MUD *11* 79% MMUD *1* 7% BM *11* 79% PBSC *3* 21%	Tr 42 *14* 100% Flu 150 *14* 100% ATG *11* 79% None *3* 21%	I NA II *8* 57% III-IV *1* 7% cGVHD *2* 14%	None	93% at median FU 3 years
Haskologlu 2018 Retrospective	**15 PID** DOCK 8 *5* 33% MHC II *3* 20% SCID *3* 20% LAD *1* 7% ITK *1* 7% CD40L *1* 7% CD3 Zeta *1* 7%	MFD *10* 67% MMRD *2* 13% MUD *2* 13% MMUD *1* 7% BM *13* 87% PBSC *2* 13%	Tr 42 *7* 47% Tr 36 *8* 53% Flu 150 *13* 87% (+TT *1* 7%) Cyclo200 *2* 13% ATG *2* 13% Alem *1* 7% None *12* 80%	I-II *7* 47% III-IV *1* 7% cGVHD *2* 13%	2 HSCT	86.7% at median FU 32 months
Shadur 2018 Retrospective	**31 OP** TCIRG1 *21* 69% SNX *10* 19% CA2 *2* 6% CLCN7 *1* 3% RANK *1* 3%	MFD *15* 48% MMFD *1* 3% MUD *11* 36% MMUD *4* 13% BM *26* 84% PBSC *5* 16%	Tr 42 *21* 68% Tr 36 *10* 32% Flu 150 *31* 100% TT 10 *31* 100% ATG *30* 97% Alem *1* 3%	I-II *8* 26% III-IV *4* 13% cGVHD *1* 3%	None	100% OS at median FU 363 days

HLH, Haemophagocytic lymphohistiocytosis; OP, Osteopetrosis; Thal, Thalassemia; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; DBA, Diamond-Blackfan anaemia; JMML, Juvenile myelomonocytic leukaemia; MDS, Myelodysplasia; SCID, Severe Combined Immunodeficiency; WAS, Wiskott Aldrich syndrome; CID, Combined Immunodeficiency; LAD, Leukocyte adhesion deficiency; CGD, Chronic granulomatous disease; SID, Severe immune dysregulation; CHH, Cartilage hair hypoplasia; IPEX, Immunodeficiency polyendocrinopathy X-linked; MHC II, Major histocompatibility type II; BMF, Bone marrow failure; RBC, Red blood cell disorder; SCC, Sickle cell anaemia; SCN, Severe congenital neutropenia; MSMD, Mendelian susceptibility mycobacterial disease; CAMT, Congenital amegakaryocytic thrombocytopenia; SDS, Schwachman Diamond Syndrome; GATA 2, GATA binding protein 2 deficiency; PNH, Paroxysmal nocturnal haemoglobinuria; DOCK 8, Dedicator of cytokinesis 8 deficiency; ITK, Interleukin-2 inducible T-cell kinase deficiency; CD40L, CD40 Ligand deficiency; MRD, Matched related donor; MMRD, Mismatched related donor; MUD, Matched unrelated donor; MMUD, Mismatched unrelated donor; BM, Bone marrow; PBSC, Peripheral blood stem cell; CB, Cord blood; Tr, treosulfan; 30,36 and 42 – all total dose in g/m^2 ; Flu, fludarabine mg/m^2; TT, Thiotepa mg/kg; Cy, cyclophosphamide mg/kg; Alem, Alemtuzumab; ATG, Anti-thymocyte globulin; Etop30, Etoposide 30mg/kg, Mel, Melphalan mg/m^2; Y-RIT, Yttrium coupled CD66 antibody radioimmunotherapy; DLI, Donor lymphocyte infusion.

In 2008 Greystoke et al reported 32 children who received treosulfan prior to HSCT for a variety of non-malignant diseases in Royal Manchester Children's and Great Ormond Street Hospitals. They were selected to receive treosulfan rather than busulfan due to being at risk of toxicity. Diagnoses included PID (18), metabolic disorders (9), Osteopetrosis (4) and beta thalassaemia major (1). Skin toxicity was noted usually with nappy rash but with severe perineal ulceration in some cases. One patient with Wolman syndrome died at day +4 with VOD and there were 4 late deaths – 1 from continuing neurodegeneration from Osteopetrosis, 1 Haemophagocytic lymphohistiocytosis (HLH) with chronic GVHD (cGVHD), rotavirus and HLH, 1 with Mucopolysaccharidosis type 1 Hurler syndrome (MPS1H) who required 2 additional transplants died from GVHD and another child with MPS1H lost donor T cells post cord blood transplant and died from adenovirus more than a year post HSCT. One patient had primary graft failure and was successfully re-transplanted, 3 had secondary graft failure, 2 of whom were successfully re-transplanted. Two further patients with < 50% donor chimerism were being considered for second transplant at the time of publication.

This report set the scene for the use of treosulfan in children requiring HSCT for non-malignant diseases. There was little regimen-related toxicity and the children with PID did particularly well with only 1 death in a patient with HLH and only 2 with low level chimerism needing consideration of a second procedure (Greystoke et al., 2008).

Also in 2008 Cutting et al in Sheffield, reported 23 children in whom they had substituted treosulfan for conventional busulfan. The median age at transplant was 4.5 years (range 5 months to 15 years) and they had a variety of malignant and non-malignant diseases. All patients received treosulfan with cyclophosphamide but dose of cyclophosphamide varied according to disease and

4 patients received additional chemotherapeutic agents (see Table 1). All received cyclosporin as GVHD prophylaxis and 7 received additional short course methotrexate. A patient with ALL failed to engraft, had autologous reconstitution, but remained minimal residual disease negative 13 months post-transplant. A patient with thalassaemia major had stable mixed chimerism 2 years post-transplant but remained transfusion independent. Five patients relapsed (4 ALL, 1 JMML) 3 of whom died, 2 achieved remission post second transplant using TBI-based conditioning and a different unrelated donor. The patient with Osteopetrosis died of aGVHD and adenovirus infection. OS was 83%. Despite a heterogenous cohort of diseases with 9 mismatched donors including 3 4/6 mismatched cords, there was a high rate of engraftment and minimal toxicity. No patients had VOD and mucositis ≥ grade II only occurred in 3 patients all of whom had either additional melphalan or methotrexate as GVHD prophylaxis. Incidence of aGVHD grade III-IV was only 17%. The authors concluded that further improvements were needed for older children with high risk ALL (3 relapses in 7 patients) and planned to increase the dose of treosulfan from $36g/m^2$ to $42g/m^2$ (Cutting et al., 2008).

We began using treosulfan in children with PID in Newcastle in 2007 following discussion with Paul Veys at Great Ormond Street Hospital. Our first patient was 3 months of age with leukocyte adhesion deficiency (LAD) type 1. Due to her young age we were concerned about using busulfan due to the risk of VOD. She received bone marrow from an HLA identical relative following conditioning with treosulfan, fludarabine and alemtuzumab. Eleven years later she is alive and well, off all medication, with stable mixed chimerism of 88% donor T-lymphocyte, 45% B-lymphocyte and 49% CD15+ myeloid cells. Over the next 2 years we tended to combine treosulfan with fludarabine in infants, but combined it with cyclophosphamide 200mg/kg in older children

or those with disorders that were known to be difficult to engraft or require donor myeloid chimerism for cure of the underlying disorder. We postulated that cyclophosphamide would be more myeloablative than fludarabine.

We reported our first 70 children with PID who had received treosulfan-based conditioning in 2011 (Slatter et al., 2011), Supplementary paper 1. This retrospective study reported 40 patients from Newcastle upon Tyne and 30 from Great Ormond Hospital who were transplanted between 2006 and 2009. Patients were not randomised and the choice of conditioning was up to the clinicians. The median age at transplant was 8.5 months (range 1.2-175 months) which is very young compared to most transplant cohorts reflecting the large number of infants who present with PID in infancy and require curative therapy. Sixty-six percent were 12 months or younger at the time of HSCT. Fludarabine total dose 150mg/m^2 was used in 40 children and cyclophosphamide 200mg/kg in 30. There were 13 deaths shown in Table 2, giving an OS of 81%. There was no significant difference in survival between those who received fludarabine and those who received cyclophosphamide.

Table 2: Characteristics of patients who died following treosulfan/fludarabine or treosulfan/cyclophosphamide conditioning

Diagnosis	Age at HSCT months	Condi-tioning	Donor	Stem cell source	Toxicity	aGVHD grade	Last chimerism %	Time to death	Cause
1.HLH	8	Tr 42 Flu Alem	MFD	BM	Rash	Liver III	100	16m	Adenovirus cGVHD
2.HLH	8	Tr 42 Flu Alem	MUD	BM planned	No	NA	NA	D-2	Ongoing HLH
3.ALPS	8	Tr 42 Flu Alem	Haplo CD3/ CD19 depleted	PBSC	No	No	Graft rejection	6m	CMV Graft failure
4.Griscelli	8	Tr 42 Flu	MSD	BM	No	No	Falling	3y	Monosomy 7 MDS/AML
5.SCID Intestinal atresias	8	Tr 36 Flu ATG	MMUD 9/10	CB	No	No	100	1m	Pseudomonal sepsis
6.Omenn's	4	Tr 36 Flu Alem	MUD	BM	seizures	Liver, gut IV	100	11m	GVHD Cerebral infarcts
7.MHC II	17	Tr 42 Cy	MSD	BM	No	No	100	5m	HHV6 pneumonitis
8.CID	96	Tr 42 Cy	MSD	BM	No	No	NA	D+7	CVA
9.CID	81	Tr 42 Cy Alem	MUD	BM	No	No	100	D+34	Pneumonitis Renal failure HHV6
10.SCID	6	Tr 42 Cy Alem	MFD	BM	VOD	No	NA	D+13	VOD Liver failure
11.SCID	1.2	Tr 36 Cy	MSD	CB	seizures	No	100	D+64	Pneumonitis Pulmonary hypertension
12.CHH	42	Tr 36 Cy Alem	MMUD 9/10	BM	Rash	No	100	D+32	CMV Adenovirus Pulmonary haemorrhage
13.SID	134	Tr 36 Cy Alem	MUD	BM	No	Gut Skin IV	100	D+139	Adenovirus GVHD Candida

HLH, Haemophagocytic lymphohistiocytosis; ALPS, Autoimmune lymphoproliferative syndrome; SCID, Severe Combined Immunodeficiency; MHC II, Major Histocompatability type II deficiency; CID, Combined Immunodeficiency; CHH, Cartilage hair hypoplasia; SID, Severe immune dysregulation; Tr treosulfan; 36 and 42 – all total dose in g/m^2 ; Flu, fludarabine 150 mg/m^2 ; Alem, Alemtuzumab; ATG, Anti-thymocyte globulin; Cy, Cyclophosphamide 200mg/kg; MFD, Matched family donor; MSD,

Matched sibling donor; MUD, Matched unrelated donor; MMUD, Mismatched unrelated donor; BM, Bone marrow; PBSC, Peripheral blood stem cell; CB, Cord blood; VOD, veno-occlusive disease; aGVHD, acute graft versus host disease; NA, not available; m, months; D+, day post HSCT; y, years; cGVHD, chronic graft versus host disease; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; CMV, cytomegalovirus; CVA, cerebrovascular attack; HHV6, human herpes virus 6.

Toxicity was not formally graded in this retrospective study but skin toxicity was common, including perineal ulceration, and mucositis was mild. It could be argued that the deaths in patients 8-11 in the Table were possibly related to the conditioning drugs. In the whole cohort 2 patients had severe VOD, both of whom had received treosulfan 42g/m^2 with cyclophosphamide. They both had enterovirus in faeces. One with CD40Ligand (CD40L) deficiency recovered after ventilation and dialysis. This was the last patient in Newcastle to receive treosulfan in combination with cyclophosphamide. The other child with VOD who had had SCID died. It has been shown that there is a strong correlation between blood levels of cyclophosphamide metabolites and VOD. McDonald et al. analysed plasma for cyclophosphamide and its major metabolites in 147 patients who received cyclophosphamide in combination with TBI. Twenty-three (16%) developed moderate or severe VOD. Metabolism of cyclophosphamide was highly variable, particularly for the metabolite *o*-carboxyethyl-phosphoramide mustard, whose area under the curve varied 16-fold. Exposure to this metabolite was statistically significantly related to VOD, bilirubin elevation, nonrelapse mortality, and survival, after adjusting for age and irradiation dose (McDonald et al., 2003). In our study 18 patients (26%) had aGVHD, but only 7 (10%) greater than grade II. Four patients had limited chronic skin GVHD. There were 3 deaths associated with GVHD. Of 42 who were more than 1 year post- transplant, 24 (57%) had 100% donor chimerism in all cell lineages. The rate of viral reactivation was 26%, which is similar to 33% reported by Rao et al in 33 children with PID conditioned with melphalan, fludarabine and alemtuzumab (Rao et al., 2005).

The main findings of the study were:

- There was no significant difference in survival in those transplanted at age 12 months or below, and those over 12 months of age.
- There was no significant difference in survival in terms of stem cell source.
- Toxicity was low but 2 patients who received cyclophosphamide had VOD.
- There was significantly higher T-cell chimerism in those who received treosulfan compared to those who received cyclophosphamide ($p=0.038$).
- There was no significant difference in chimerism between matched family donor (MFD) and unrelated donor recipients.
- Numbers of unrelated donor recipients with sufficient follow up were low but there was a trend for higher chimerism in all cell lines following peripheral blood stem cells (PBSC) compared to bone marrow (BM) and cord blood (CB) without a significant increase in GVHD.

This demonstrated the safety of using treosulfan in infants, toxicity was lower using fludarabine compared to cyclophosphamide and there was no difference in chimerism. Use of PBSCs may lead to better chimerism without the risk of severe GVHD in the context of using Alemtuzumab (Slatter et al., 2011).

This has been the basis for our practice since then:

- We no longer use cyclophosphamide but always combine treosulfan with fludarabine
- With a 10 out of 10 HLA matched donor our first choice is PBSC in preference to BM.

This manuscript has been cited more than 100 times since publication.

In 2013 Beier et al published results of 109 transplants from 9 centres in Germany and Austria with a variety of diseases and conditioning regimens, all of which included treosulfan. All patients transplanted between 2003 and 2009 were included. Fifty-three had non-malignant diseases shown in Table 1. Seventy-one percent of the non-malignant group received thiotepa or melphalan in addition to treosulfan and fludarabine. Toxicity was difficult to evaluate due to the retrospective nature of the study but VOD was seen in only 3 patients with myeloid malignancy who had received either thiotepa or melphalan in addition to treosulfan. Thirteen patients died of whom 7 had non-malignant diseases: 3 with SCID from graft failure with respiratory failure, hepatic failure, and infection respectively, 1 with Hyper-IgM from adenovirus, 1 with SDS from interstitial pneumonia, 1 with thalassaemia from graft failure and infection and 1 with dyskeratosis congenita from late pneumococcal sepsis. Survival in the malignant group was 49%: these patients predominantly had high risk leukaemias, HSCT with treosulfan was used as a last resort and many relapsed. Therefore at this point it was unclear whether the anti-leukaemic properties of treosulfan were similar to busulfan or TBI-based conditioning. Good long-term survival with few late events was seen in non-malignant disease. In particular out of 31 patients with PID including 2 with HLH, 27 survived (87%) (Beier et al., 2013).

In 2014 Lehmberg et al reported 100% overall and disease free survival of 19 patients with HLH from 9 German centres who received treosulfan, fludarabine, alemtuzumab with additional thiotepa in 14. The median age at transplant was 3.9 years (range 0.3 to 22 years) with 6 infants under 12 months and 2 young adults. Two patients required a second transplant, 1 for early graft rejection post CD34+ selected haploidentical HSCT and 1 at day 125 for secondary graft failure.

DLI was given to 5 patients with low level chimerism together with a stem cell boost in 1. A further patient was scheduled for DLI. On multivariate analysis use of a mismatched donor was a risk factor for requiring additional cellular therapy. Sustained engraftment in patients who had received thiotepa was superior on univariate, but not multivariate, analysis. Sixteen patients received defibrotide prophylaxis due to the known risk of VOD in HLH. One patient developed VOD despite prophylaxis. Thirty-seven percent of patients were not in remission of their HLH at time of HSCT and 32% had had CNS involvement. These results are impressive. The use of alemtuzumab in this disease is likely to be important due to the hyperinflammatory nature of HLH. It may suppress remaining HLH activity due to the widespread distribution of CD52 in T cells and antigen presenting cells (Marsh et al., 2010). It almost certainly led to the low rates of GVHD. It may be that optimisation of the dose and timing of alemtuzumab, and the addition of thiotepa, might lead to an improved rate of primary complete donor chimerism particularly for recipients of HLA-mismatched grafts (Lehmberg et al., 2014).

Burroughs et al performed a prospective, multicentre, open-label trial to evaluate the safety and engraftment efficacy of treosulfan combined with fludarabine as a conditioning regimen for patients with non-malignant diseases published in 2014. Diagnoses of the 31 patients are shown in table 1. Thirteen patients had PID and 6 HLH (total 64%) – all except 2 had BM. Tacrolimus and 4 doses of methotrexate were used as post-transplant GVHD prophylaxis. Because of a high incidence of grade III-IV aGVHD in the first 9 patients thymoglobulin (rabbit ATG) was added to the regimen 2mg/kg on days -4 to day -2. This led to a significantly reduced incidence of grade III-IV aGVHD but no difference in the 2 year cumulative incidence of cGVHD. The study was not confined to paediatric patients the median age being 10.7 years (range 0.4 to 30.5 years). Primary engraftment was achieved in all patients and only 1, with sickle cell disease post matched

sibling donor (MSD) BM with a low cell dose of 0.7 x 10^8/kg nucleated cells, had secondary graft failure. This patient received a second HSCT as did a patient with recurrent HLH who developed recurrence post MSD BM when chimerism in CD3+ cells was 36% and CD33+ was 3% donor, which therefore could also be classed as secondary graft failure. Both patients survived in remission. No details were given as to whether the donors were carriers or not. Grade 3 mucositis and grade 3 skin rash occurred in 10% of patients and no patients developed liver toxicity. Two patients aged 5.6 and 23 months had a single focal seizure on D+8 and D+25 respectively. Two patients had grade 4 toxicity: 1 with a diaphragmatic hemiparesis which preceded HSCT, required continuous positive pressure airway support for 8 days and another required ventilatory support for 17 days with herpes stomatitis, mucosal bleeding and pulmonary infiltrates. Day 100 transplant related mortality (TRM) was 0% showing the low toxicity of this regimen. Of 3 deaths, 1 died of CNS HLH on D+233 but with high level mixed chimerism, 1 with paroxysmal nocturnal haemoglobinuria (PNH) who did not receive ATG died of GVHD at D+158 and another patient with HLH in remission died of a surgical complication unrelated to HSCT at D+129. Two of 6 patients with HLH experienced relapse in the setting of mixed chimerism although the one who died with CNS HLH had 75% donor CD3+ and 100% donor CD33+ so this seems surprising. The low incidence of regimen related toxicity and mortality compared with historical results observed with busulfan and cyclophosphamide based regimens for these diseases was highlighted, especially because more than 50% of the patients had risk factors for poor outcome according to scoring using a comorbidity index. Whilst the diseases treated in this study were quite diverse, of note all patients with PID or bone marrow failure (BMF) survived with disease resolution. Long-term stability of engraftment and late effects need to be studied (Burroughs et al., 2014).

In 2014 Dinur-Schejter reported 44 patients, from 3 Israeli centres, who received treosulfan-based conditioning for non-malignant diseases. The median age at transplant was 18 months (range 1 to 181 months) with 37.8% under the age of 1 year. A patient with Niemann-Pick disease had primary graft failure and had a second transplant but died following secondary graft failure with progression of her disease. A patient with Osteopetrosis failed to engraft after a haploidentical transplant and underwent a second transplant, which also failed, but a third led to engraftment but extensive chronic skin GVHD and pulmonary disease. A patient with WAS required a second transplant and a patient with thalassemia rejected his graft and was not re-transplanted. A patient with severe congenital neutropenia (SCN) rejected the graft and died and 2 patients died before engraftment. Pulmonary complications were common (52%) but often occurred in those with pre-transplant respiratory problems. One patient developed VOD. A comparison was made between those who received treosulfan and fludarabine, treosulfan and cyclophosphamide and treosulfan, fludarabine, thiotepa. The combination of treosulfan, fludarabine and thiotepa resulted in higher rates of complete engraftment compared to the other 2 regimens (94.7% compared to 16.7% with treosulfan, cyclophosphamide and 66.7% with treosulfan and fludarabine alone). This did not translate into any difference in overall or disease free survival. It is well known that stable mixed chimerism is sufficient to achieve cure in some non-malignant disorders, but for others high level chimerism is required and so this finding is important (Dinur-Schejter et al., 2015).

In 2017 Burroughs et al published another prospective study of 14 patients with marrow failure disorders conditioned with treosulfan, fludarabine and ATG in 11. Bone marrow failure disorders are challenging to transplant as they often have pre-existing organ dysfunction, which increase the risk of mortality with conventional conditioning agents, but myeloablative regimens are required to eliminate the abnormal marrow and enable sustained donor engraftment. Therefore a

reduced toxicity but still myeloablative regimen is attractive. Two of the patients had GATA 2 deficiency: one had pancytopenia and mild immune dysfunction with T and B cell lymphopenia and trisomy 8 in 1.6% marrow cells, the other also had pancytopenia and T and B cell lymphopenia. Both had a successful outcome with resolution of trisomy 8 post HSCT in the first patient. Eight of the patients had been previously published in the 2014 paper. Median age at HSCT was 15 years (range 2-22 years). All patients engrafted and 13 patients had full donor chimerism, only 1 with Diamond Blackfan anaemia (DBA) had mixed chimerism but cure of disease. There were few clinically significant toxicities and no liver toxicity despite 6 patients having pre-transplant iron overload. Eight patients developed aGVHD grade II and 1 with PNH who had not received ATG had grade IV gut GVHD and died. Two developed cGVHD. This report established that full donor chimerism with minimal toxicity can be achieved using treosulfan, fludarabine and ATG in bone marrow failure disorders (Burroughs et al., 2017).

Haskologlu et al published a single centre retrospective series in 2018 of 15 children with PID conditioned with treosulfan and fludarabine or cyclophosphamide. Median age at transplant was 12 months (range 3 to 180 months). They had an impressive survival of 13 children (86.7%) despite 9 patients having documented hepatic problems and 6 with bronchiectasis pre transplant. Three patients had needed intensive care support pre-transplant and survived. One patient with pre-existing bronchiectasis died of pulmonary failure 13 months post-transplant for CD3 zeta chain deficiency. A second patient with RAG1 deficiency died of sepsis and thrombotic microangiopathy (TMA) 7 months post HSCT. Fourteen engrafted, 1 had primary graft failure following a CD34+ selected haploidentical transplant for T-B-NK+ SCID but was successfully re-transplanted using a matched family donor (MFD) and another required a stem cell boost for secondary graft failure associated with BCGitis which was successful. Skin toxicity was common

including severe perianal dermatitis and ulcers in 3 patients. Three patients with liver dysfunction developed VOD, which responded to defibrotide; 1 of these with Dedicator of cytokinesis 8 (DOCK 8) deficiency had pre-existing cirrhosis and underwent successful liver transplantation 12 months post HSCT due to chronic liver failure. Rates of GVHD were quite high (53.3% aGVHD) but mainly aGVHD grade I-II, and cGVHD (20%). Only 3 patients received serotherapy so a more comprehensive use of serotherapy even for matched family donor transplants, as we do in our centre, may have reduced this rate. The authors concluded that treosulfan-based conditioning is associated with low toxicity, is safe, with high rates of engraftment and full donor chimerism in patients with PID regardless of specific genetic diagnosis and donor type (Haskologlu et al., 2018).

Shadur et al in 2018 reported a retrospective survey of 31 patients transplanted for Osteopetrosis with treosulfan, thiotepa, fludarabine and ATG from the Hadassah Medical Centre in Jerusalem. All patients had autosomal recessive disease termed infantile malignant osteopetrosis. A very impressive 100% OS was achieved. In this disorder transplant outcomes have historically been poor with common complications being VOD, pulmonary hypertension, hypercalcaemia and graft rejection (Orchard et al., 2015, Driessen et al., 2003). Median age at transplant was 1.49 years (range 0.37 to 28.06 years). No patients developed VOD and all patients engrafted. Eleven patients had mixed donor chimerism of whom 8 (out of 31) received MSD or MFD grafts. Small numbers made it impossible to determine whether this was statistically significant but it is an interesting finding. Donor chimerism was only available to day + 120 so longer follow up is required to determine if mixed chimerism is stable and sufficient to cure the underlying disease (Shadur et al., 2018).

1.6 Pharmacokinetics

Pharmacokinetics (PK) refers to the movement of drugs through the body whereas pharmacodynamics (PD) refers to the body's biological response to drugs. PK therefore refers to the rate and extent of distribution of a drug in different tissues, and the rate of elimination of the drug. It describes a drug's exposure by characterising absorption, distribution, bioavailability, metabolism, and excretion as a function of time. PK can be reduced to mathematical equations, which describe the transit of the drug throughout the body, a net balance sheet from absorption and distribution to metabolism and excretion.

The PK two-compartment model divides the body into central and peripheral compartments. The central compartment consists of the plasma and tissues where the distribution of the drug is practically instantaneous. The peripheral compartment consists of tissues where the distribution of the drug is slower.

First order elimination refers to a process in which the amount or concentration of drug in the body diminishes logarithmically over time (Beringer, 2018).
PK studies for treosulfan are scarce. The PK profile of treosulfan is best fitted by a 2-compartment model with first-order elimination. The main PK studies are summarised in Table 3.

In 1998 Hilger RA et al. reported the clinical PK of treosulfan after a single-dose of 8 or 10g/m^2 in 18 adults with advanced or resistant ovarian or small cell lung cancer. Their method was based on separation by reverse-phase high-performance liquid chromatography (HPLC) with

refractometric detection. This enabled detection of treosulfan in plasma and urine. The terminal half-life of treosulfan was in the range of 1.8 hours and the AUC and plasma Cmax values were significantly higher in the 10g/m^2 compared to the 8g/m^2 recipients. Urinary excretion of the parent compound was nearly 30% of the total dose delivered over 48 hours with about 25% being excreted in the first 6 hours after administration (Hilger et al., 1998). Scheulen et al demonstrated a linear increase in AUC up to 56g/m^2 in adult patients. Half-life, volume of distribution and renal elimination were independent of dose (Scheulen et al., 2000). Beelen et al 2005 and Glowka et al 2008 also demonstrated that AUC increased linearly with treosulfan dose (Beelen et al., 2005, Glowka et al., 2008). Glowka et al reported results of PK studies in 7 patients aged 2 to 15 years, 5 of whom received 36g/m^2, 1 30g/m^2 and 1 42g/m^2. They demonstrated a dose dependent increase of AUC and Cmax, but there was high variability (70%) in these parameters suggesting that it may be necessary to do PK evaluation in paediatric patients undergoing treosulfan-based conditioning (Glowka et al., 2008). There was no correlation with outcome and the numbers in the study were very small.

Ten Brink et al. successfully developed and validated a bioanalytical method to quantify treosulfan concentrations in serum and a PK model to describe the concentration-time profile for treosulfan in children. They studied PK in 20 children with a variety of malignant and non-malignant diseases and a median age of 6.2 years at transplantation. All received 42 g/m^2 treosulfan. Limited interpatient variability (14%) was found in contrast to Glowka's results and there was no correlation with outcomes (Ten Brink et al., 2014)

In 2015 Glowka et al. published PK results of treosulfan and it's monoepoxide S,S-EBDM in 16 children aged 0.4 to 18 years with a variety of malignant and non-malignant haematological

disorders. There was a linear correlation between the AUC of S,S-EBDM and treosulfan suggesting that the active epoxy-transformer of treosulfan will not accumulate in the body beyond the parent drug which is important for clinical application and timing of infusion of a transplant (Glowka et al., 2015).

In 2017 Van der Stoep et al. reported PK studies in 77 paediatric patients from Leiden and Rome transplanted for haemoglobinopathies (40.3%), haematological malignancy (15.6%), PID (28.5%) and bone marrow failure or metabolic disease (15.6%). All received treosulfan and fludarabine. Additional thiotepa was given to 67.5%. The median age at transplantation was 4.8 years (interquartile range 1.6-11.4 years). Twelve infants < 1 year of age received 30g/m^2, and 65 patients of 1 year of age or more received 42g/m^2 treosulfan. Donors were MSD (35%), ≥ 9/10 allelic matched unrelated donors (46.8%) and haploidentical related donors (18.2%) following in vitro T cell depletion with either CD34+ selection or TCR αβ+ and CD19+ depletion. Mean day 1 treosulfan exposure was 1744 +/- 795 mg h/l (10g/m^2) and 1561 +/- 511 mg h/l (14g/m^2). There was inter-individual variability of 56 and 33% in the respective groups, showing a large difference in exposure particularly in young children. Because of dose adjustment in young children the mean exposure did not differ significantly but the mean clearance was significantly lower and the mean central volume of distribution was also lower. The immature renal function in infants may contribute to lower treosulfan clearance. The first 19 patients had PK testing on day 1 and day 3 of treosulfan. The mean intra-patient variability was 13.9%. Patients with an AUC > 1650 mg h/l had a statistically higher incidence of mucosal and skin toxicity than those with an AUC under 1350 mg h/l. The risk of experiencing 2 or more toxicities was higher when AUC exceeded 1650

mg h/l compared to AUC under 1350 mg h/l. No relationship between treosulfan exposure and chimerism, aGVHD, treatment related mortality or OS was found (van der Stoep et al., 2017).

This was a landmark study being the largest paediatric cohort reported and the first to show that treosulfan exposure was associated with toxicity. Limitations of the study included large heterogeneity in the primary diseases treated, and the conditioning was not uniform as 67.5% received additional thiotepa. Further studies were deemed necessary to reveal whether treosulfan exposure is related to long-term disease outcome and late treatment-related toxicity such as gonadal toxicity.

Danielak et al in 2018 reported results of PK studies in 14 children looking at both treosulfan and its mono-epoxytransformer S,S-EBDM. They found that the vast majority of treosulfan (approximately 68% of total treosulfan clearance) is transformed to S,S-EBDM and this takes place in the blood circulation so the PK of S,S-EBDM is best described with a linear one-compartmental model. PK of S,S-EBDM was highly variable. In contrast to Glowka's study in 2015, a weak correlation between exposure to treosulfan and S,S-EBDM was reported suggesting the need for monitoring of this active epoxide, apart from the parent compound (Danielak et al., 2018).

In 2018 Mohanan et al from Vellore, India, published PK results of 87 patients who received treosulfan based conditioning prior to transplant for thalassemia major. The cohort of patients was uniform and all received 14g/m^2 for 3 days plus fludarabine and thiotepa. Treosulfan clearance was significantly associated with OS and EFS. The median age was 9 years (range 1.5 to 25 years). Donors were MSD 77%, MFD 13% and matched unrelated donor (MUD) 10%. Of note 93% of

the patients had liver fibrosis. Hepatic SOS was documented in 16 patients and 7 had pulmonary SOS. Seven patients died before day + (D+) 28. Out of 80 remaining patients 2 had primary graft failure and 3 secondary graft failure. A further 8 patients died before D+100. Overall 68 (78%) were alive at last follow up and EFS was 76%. Median follow up was 31.77 months (0.37-55.33 months). Causes of death were: GVHD, SOS, multiorgan failure and infections. Despite a wide inter-individual variation (64% in AUC and 68% in treosulfan clearance), rejection, regimen-related toxicity or TRM were not influenced by PK. However there was a higher risk for low treosulfan clearance towards poor overall and EFS with a trend towards better OS with high treosulfan clearance and low AUC. Therefore they postulated that instead of a maximum tolerated dose of treosulfan, a minimal beneficial dose should be identified with personalised dose monitoring to improve outcome and reduce costs (Mohanan et al., 2018).

In the recent study by Kalwak et al (2020) there was limited variability in interindividual PKs. The BSA - based dosing achieved equivalent treosulfan exposure in all dose groups. However, treosulfan plasma clearance and volume of distribution increased with increasing dose likely due to increasing age and BSA of the patients within the different dose groups. OS decreased with increasing dose of treosulfan but the authors postulate that this was not due to a higher treosulfan exposure but may be related to specific patient-, graft- and/or underlying disease characteristics (Kalwak et al., 2020).

Our joint study with Great Ormond Street Hospital (published paper 6) is the only study to show that high treosulfan AUC is strongly associated with mortality and to a lesser extent low AUC with poor engraftment. All but 2 of 87 patients were affected by PIDs and all received uniform conditioning with treosulfan and fludarabine without additional thiotepa (Chiesa et al., 2019).

In summary PK studies to date show variable results. Dose adjustment in young children is important to limit exposure due to lower clearance and lower central volume of distribution. More recent studies suggest that high exposure is associated with increased toxicity and mortality, but only our study shows an association with low AUC and poor chimerism. Therapeutic drug monitoring guided dosing should be explored particularly for infants and young children undergoing HSCT for non-malignant conditions.

Table 3: PK studies of treosulfan

Author Year ***Number of patients***	**Diagnoses**	**Median age (years)**	**Method**	**Regimen**	**AUC, mg h/l (mean ± SD)**	**Comments**
Beelen 2005 ***18***	AML *6* 33% ALL *7* 39% CML *2* 11% T-NHL *1* 6% MDS *2* 11%	40 51	RP-HPLC +RID	Tr 36 *8* 44% Tr 42 *10* 56% Cy *18* 100%	898 ± 104 1104 ±173	AUC was dose dependent. Low interpatient and interday variation.
Glowka 2008 ***7***	AML *1* 14% AML/ALL *1* 14% HL *2* 30% ALD *1* 14% WAS *1* 14% SAA *1* 14%	14	RP-HPLC + RID	Tr 30 *1* 14% Tr 36 *5* 72% Tr 42 *1* 14% Other NA	735 1309 ±921 1960	Linear increase in AUC with dose. High interpatient variability
Nemecek 2011 ***16***	AML *NA* ALL *NA* MDS *NA*	34	RP-HPLC + RID	Tr 36 *4* 25% Tr 42 *12* 75% Flu *16* 100%	1365 ±293 1309 ±262	No difference in AUC with increasing dose
Ten Brink 2014 ***20***	Haemoglobinopathies *12* 60% Malignancy *4* 20% PID *4* 20%	6.2	RP-HPLC + UV	Tr 42 *20* 100% Flu *19* 95% TAI *1* 5% TT *14* 70%	1639 ±237	AUC is total of Tr + metabolite Low interpatient variability
Glowka 2015 ***16***	NBL *2* 13% ALL *5* 31% ES *2* 13% DBA *1* 6% SCN *1* 6% ALD *2* 13% CML *1* 6% AML *1* 6% WAS *1* 6%	7.5	LC-MS/MS	Tr 30 *1* 6% Tr 36 *8* 50% Tr 42 *7* 44% Other NA	1560 1478 ± 552 2400 ± 1267	Metabolite is eliminated in a short time and is comparable to Tr elimination
Koyyalamudi 2016 ***6***	Malignancy *6* 100%	1 4	RP-HPLC + UV	Tr 36 *3* 50% Tr 42 *3* 50%	1486 ±235 1412 ± 215	AUC is total of Tr + metabolite No difference in AUC with increasing dose

Van der Stoep 2017 *77*	Haemoglobinopathies *31* 40% Malignancy *12* 16% PID *22* 29% BMF *11* 14% Other *1* 1%	4.8	RP-HPLC + UV	Tr 30 *12* 16% Tr 42 *65* 84% Flu *77* 100% TT *52* 68%	1744 ±795 1561 ± 511	High exposure associated with more severe mucositis and skin toxicity.
Danielak 2018 *14*	ALL *4* *30*% AML *1* 7% CML *1* 7% ES *2* 14% NBL *2* 14% SCN *1* 7% WAS *1* 7% ALD *2* 14%	7.7	HPLC-MS/MS	Tr 42 *6* 43% Tr 36 *7* 50% Tr 30 *1* 7%	NA	Weak correlation between Tr exposure and S,S-EBDM suggesting monitoring of active epoxide may be necessary.
Mohanan 2018 *87*	Thalassemia *87* 100%	9	RP-HPLC + RID	Tr 42 *87* 100% Flu *87* 100% TT *87*100%	1396 ± 715	Trend towards better OS with high Tr clearance and low AUC.
Chiesa 2019 *87*	PID *79* 91% IBD *5* 6% JMML *2* 2% IEM *1* 1%	1.6	RP-HPLC + RID	Tr 30 *4* 5% Tr 36 *23* 26% Tr 42 *60* 69% Flu *87* 100%	1530±54 1735±516 1507±835	Association of high AUC with mortality and low AUC with poor engraftment.
Kalwak 2020 *54*	Malignancy *54* 100%	11	RP-HPLC + RID	Tr 30 *5* 9% Tr 36 *23* 43% Tr 42 *26* 48% Flu *54* 100% TT *54* 100%	1700±351 1627±344 1900±296	AUC comparable between 3 dose groups. BSA-based dosing is valid.

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; NHL, Non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome; HL, Hodgkin's lymphoma; ALD, adrenoleukodystrophy; WAS, Wiskott-Aldrich syndrome; SAA, severe aplastic anaemia; NA, not available; PID, Primary Immunodeficiency; NBL, neuroblastoma; ES, Ewing's sarcoma; DBA, Diamond-Blackfan anaemia; SCN, severe congenital neutropenia; BMF, bone marrow failure; IBD, Inflammatory bowel disorder; JMML, juvenile myelomonocytic leukaemia; IEM, Inborn error of metabolism; RP-HPLC, reverse phase high performance liquid chromatography; RID, refractive index detector; UV, ultraviolet detector; LC-MS/MS, liquid chromatography tandem mass spectrometry; Tr, treosulfan; 30,36 and 42 – all total dose in g/m^2 ; Cy, cyclophosphamide; Flu, fludarabine; TAI, total abdominal irradiation; TT, thiotepa; AUC, area under the curve; OS, overall survival; S,S-EBDM, (2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate; BSA, body surface area.

1.7 Summary

The number of PIDs recognised that are amenable to cure, or amelioration of disease, by HSCT has increased rapidly in the last 20 years. The use of reduced toxicity conditioning is attractive because it leads to fewer and less severe short-term toxicities. Treosulfan has myeloablative and immunosuppressive effects and has been proven to enable engraftment with less toxicity, in particular VOD, than conventionally used doses of busulfan. Following the use of treosulfan for conditioning prior to HSCT in adults with haematological malignancies, small multi- or single centre studies have confirmed its safety and efficacy in children with non-malignant disease. Almost all of these studies include a heterogenous group of disorders, not just PID, and heterogenous conditioning with a variety of additional agents combined with treosulfan. Pharmacokinetic studies of treosulfan are limited particularly in patients with PID.

In chapter 2 published papers 1 and 2 elaborate on the latest advances in HSCT for PID and conditioning (Slatter and Gennery, 2018, Lum et al., 2019b). Published paper 3 presents the largest number of children with non-malignant disease who have received treosulfan as part of conditioning for HSCT (Slatter et al., 2015). Published paper 4 demonstrates the successful outcome for patients with CGD, following treosulfan-based conditioning, in a world-wide study (Morillo-Gutierrez et al., 2016). Published paper 5 reports results of 160 patients with PID from Newcastle and Great Ormond Street with homogenous conditioning with treosulfan and fludarabine (Slatter et al., 2018). Finally published paper 6 reports the prospective PK study conducted in 87 patients from Newcastle and Great Ormond Street showing an association of high AUC with mortality and low AUC with poor engraftment (Chiesa et al., 2020a).

CHAPTER 2: SUBMITTED PUBLISHED PAPERS

2.1 PP1.

Mary A. Slatter and Andrew R. Gennery. 2018 (Hematopoietic cell transplantation in primary immunodeficiency – conventional and emerging indications)

Title: Hematopoietic cell transplantation in primary immunodeficiency – conventional and emerging indications

Authors: **Mary A. Slatter** and Andrew R. Gennery

Journal: Expert Review of Clinical Immunology Volume 14, Issue 2, Pages 103-114.

Impact factor: 3.792

Date of publication: 16 January 2018

2.1.1 Overview

We were invited to write this review which focuses on recently published literature on the treatment of PID by HSCT. Together with a review of diseases such as SCID, WAS and CGD which have been transplanted for many years, we presented information on newly emerging PIDs such as gain-of-function mutations in pathways involving T-lymphocyte activation. We aimed to give a broad picture of the approach to, and methods of, transplant including the latest developments for certain complications such as GVHD and endothelial cell activation disorders.

2.1.2 What was known

- HSCT is an established curative treatment for many PIDs.
- Advances in donor selection, graft manipulation, conditioning and treatment of complications have improved outcomes, such that survival for some children is expected to be 90%. With this improvement in survival, attention is now turning to long-term outcome and adverse effects.
- Studies particularly in SCID have demonstrated that early transplantation before infection and organ damage occur, lead to superior survival and outcome. This has led to the introduction of newborn screening for SCID in some areas of the world, particularly the USA.
- An increasing number of reports show that transplant at an early age for non-SCID PID, such as WAS, CGD and CD40L deficiency also lead to better outcomes.
- Transplant-related mortality is mainly due to GVHD, overwhelming infection and to a lesser extent endothelial activation syndromes such as VOD.
- Viral infections pose a significant risk to patients with PID. Viral surveillance particularly by PCR, improves outcome by enabling pre-emptive therapy before disease is established. But, current anti-viral therapy is at best virostatic, requiring functional T-lymphocytes to clear virus, and the drugs have significant side effects.
- VOD is a well recognised complication of chemotherapy, particularly following busulfan. Defibrotide treatment has been shown to improve the outcome from VOD.
- Next generation sequencing is leading to the identification of many new primary immunodeficiencies which may be amenable to HSCT.

2.1.3 What this study added

- A review of recently published literature detailing advances, particularly focused on conditioning regimens and new methods of T-lymphocyte depletion for patients with conventional PIDs.
- Reduced toxicity conditioning leads to fewer short-term toxicities than conventional myeloablative regimens. More data is needed on the long-term effects, particularly on fertility.
- New methods of T-lymphocyte depletion such as CD3+ TCR αβ/CD19+ depletion enable patients with non-SCID PID as well as SCID to be successfully transplanted in the absence of a suitably fully matched donor.
- Methods for improving outcomes for GVHD include second-line therapy with extracorporeal photopheresis, mesenchymal stem cells and JAK-STAT tyrosine kinase receptor inhibitors.
- The use of T lymphocytes specifically directed at viral epitopes and the exploration of genetically modified or selectively depleted add-back T lymphocytes is changing the approach to the treatment of viral infections through transplant.
- An increased understanding of endothelial cell activation disorders which may be triggered by chemotherapy, other drugs, infection and GVHD and lead to widespread organ involvement not limited to VOD of the liver. Treatment with defibrotide and/or complement blockade is emerging as an option to improve outcome.
- A review of the latest approaches and outcomes from HSCT for SCID, CGD, WAS, DOCK 8, Common Variable Immunodeficiency and monogenic autoimmunity.

- A review of the literature regarding transplant outcomes for newly described PIDs, particularly those associated with gain-of-function mutations in pathways involving T-lymphocyte activation.
- An expert commentary on the hot topics facing the field in the next 5 years. These include:
    - Management of patients with SCID detected by newborn screening.
    - A personalised medicine approach to conditioning with PK measurements and serotherapy levels.
    - Choice of donors.
    - Dilemma of whether to transplant patients with newly described PID diseases and when.
    - Challenges of transplanting newly emerging diseases with increased risks of graft rejection and GVHD.

2.1.4 Contribution of the candidate to this work

This invited review was jointly written with ARG. A copy of the Newcastle University co-authorship form can be found in the Appendix.

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REVIEW

Hematopoietic cell transplantation in primary immunodeficiency – conventional and emerging indications

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ABSTRACT

Introduction: Hematopoietic stem cell transplantation (HSCT) is an established curative treatment for many primary immunodeficiencies. Advances in donor selection, graft manipulation, conditioning and treatment of complications, mean that survival for many conditions is now around 90%. Next generation sequencing is identifying new immunodeficiencies, many of which are treatable with HSCT. Challenges remain however with short and long-term sequalae. This article reviews latest developments in HSCT for conventional primary immunodeficiencies and presents data on outcome for emerging diseases,

Areas covered: This article reviews recently published literature detailing advances, particularly in conditioning regimens and new methods of T-lymphocyte depletion, as well as new information regarding approach and out come of transplanting patients with conventional primary immunodeficiencies. The article reviews data regarding transplant outcomes for newly described primary immunodeficiencies, particularly those associated with gain-of-function mutations.

Expert commentary: New methods of graft manipulation have had significant impact on HSCT outcomes, with the range of PIDs treated using T-lymphocyte depletion significantly expanded. Outcomes for newly described diseases with variable phenotypes and clinical features, transplanted when the diagnosis was unknown are beginning to be described, and will improve as patients are identified earlier, and targeted therapies such as JAK inhibitors are used as a bridge to transplantation.

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Hematopoietic stem cell transplantation; TCRαβ depletion; virus-specific cytotoxic T-lymphocytes; severe combined immunodeficiency; Wiskott-Aldrich syndrome; chronic granulomatous disease; DOCK8 deficiency; IPEX syndrome; cytotoxic T lymphocyte antigen-4 deficiency; activated PI3K-δ syndrome; signal transducer and activator of transcription 1 gain of function; Lipopolysaccharide-responsive and beige-like anchor protein deficiency

1. Introduction

Since the first allogeneic hematopoietic stem cell transplants were performed for primary immunodeficiency (PID) in 1968, the number of patients and range of conditions for which transplant is indicated or is the treatment of choice has expanded exponentially. With earlier recognition of disease, increased use of targeted sub-myeloablative or low toxicity myeloablative conditioning regimens, improved methods of graft manipulation, and improving strategies to ameliorate posttransplant complications, survival for transplant of conventional PID is now reaching 90% or more, and attention is turning to long-term outcomes and adverse effects. Autoimmunity and immune dysregulation have long been recognized as a feature of conventional PID [1], but the advent of next-generation sequencing is uncovering many new primary immunodeficiencies where these symptoms, rather than infectious complications, form a characteristic aspect of presentation. This article will review some of the new treatment and supportive care modalities that are being applied to transplant of patients with conventional PID as well as report outcomes of treatment for emerging diseases.

2. Early recognition of disease

Data collected for an increasing number of diseases indicates that transplantation early in the course of disease, before significant infectious and inflammatory complications occur leading to end-organ damage, leads to less severe and significant posttransplant complications with better survival outcomes. This is best demonstrated in severe combined immunodeficiency (SCID), where two landmark studies demonstrated significantly better survival outcome in probands of index cases [2,3]. The negative effect of persisting infection in infants with SCID was convincingly demonstrated in a recent study of patients transplanted since 2000, where survival was shown to be most directly related to presence or absence of infection, rather than age at transplant, use of conditioning chemotherapy or donor stem cell source [4].

Similar data are available for patients with chronic granulomatous disease [5], Wiskott-Aldrich Syndrome [6], and CD40 Ligand deficiency [7]. For these combined immunodeficiencies or innate defects, patients and families should be referred early in the disease course to a transplant center, so that appropriate discussions can take place and donor searches can be initiated. Some families may opt to adopt a 'watch

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and see' policy, whilst others may prefer a preemptive transplant approach, but early referral will enable families to be fully informed as they make their decision.

3. Conditioning regimens

For many PIDs, preparative chemotherapy is necessary to achieve durable donor stem cell engraftment [8]. Although the courses of chemotherapy are short, as complete or partial myelo-ablation is the only goal, nevertheless short- and long-term toxicities are associated with the administration of cytotoxic agents [9]. Particular concerns focus on the effect of chemotherapy given to very young infants in the first couple of months of life who have SCID – such concerns have given rise to the debate concerning use of infused donor stem cells over preparative conditioning regimens [10]. Particular short-term concerns include the development of pneumonitis [11] and veno-occlusive disease [12], whilst long-term issues mainly concern fertility. There are now limited data available regarding fertility in survivors of PID transplants who received busulfan-based regimens [13]. Less-toxic regimens are now in use, with excellent reports for patients with PID, including adult, and otherwise high-risk patients [14,15], but data regarding fertility in these group, particularly those who were infants at time of transplant, are awaited. However, short-term toxicity issues can be overcome by the use of these regimens.

4. Graft manipulation

An HLA-matched sibling is the donor of choice for most allogeneic transplants. However, for many patients, a matched sibling is not possible, and for others, health issues or being affected by the same genetic disease preclude using them as donors.

Matched unrelated donors from the registry are an alternative source of stem cells, but even so, particularly for ethnic minorities, it is not possible to find a match, and the risk of aGvHD, not encouraged in non-malignant disease, rises with increasing HLA mismatch.

An alternative is to use a haplo-identical related donor, and deplete the graft of T lymphocytes prior to infusion. This increases the risk of rejection, however, and leaves the patient at increased risk of viral infection until immune reconstitution occurs with thymopoiesis, approximately 4 months after transplantation [16]. Historical results of using T lymphocyte-depleted haplo-identical donors for PIDs are very poor [6,17–19], but recent adaptations in T lymphocyte depletion have dramatically improved results (Table 1). The most widely reported method uses a magnetic column to attract an organic iron bead bound to an antibody to selectively remove B lymphocytes (to avoid EBV-driven post-transplant lymphoproliferative disease) and T lymphocytes bearing the αβ T lymphocyte receptor, which are associated with graft-versus-host disease, and retain the γδ T lymphocyte receptor-bearing cells which confer antiviral and graft-versus-leukemia affects, as well as Natural Killer cells, and lymphocyte precursors. Three studies are published reporting results in patients with nonmalignant disease, including PID, with survivals of around 90% [20–22]. Rejection has been a complication, and some viral reactivation has occurred, and is a cause of mortality in some cases. Mild aGvHD was also reported. However, these results compare favorably to historic haplo-identical transplant survival data for PID. Modifications to the technique now include re-infusion of genetically modified αβ T lymphocyte receptor bearing cells with an added caspase suicide gene. The addition of these cells may confer additional anti-viral properties, but if aGvHD occurs, the cells can be removed by administration of BPX-501, an inert investigational compound which activates the suicide gene. A clinical trial using this modification is currently in progress.

Table 1. Current modern T lymphocyte depletion methods.

Method	Advantages	Disadvantages
$CD3^+$ TCRαβ/CD19 depletion	Precise dosing of T lymphocyte infusion Less risk of GvHD	Expensive Expertise in cell manipulation required Risk of severe viral disease
$CD3^+$ TCRαβ/CD19 depletion with iCasp9 suicide gene TCRαβ add-back	Greater chance of viral protection	Long-term effect of persistent gene-manipulated cells Expensive Increased risk of GvHD (although can be turned off by activation of iCasp9)
Posttransplant cyclophosphamide	Very cheap No cell manipulation required	Greater risk of GvHD No targeted dosing of T-lymphocytes
$CD3^+CD45RA^+$ depletion	Precise dosing of T lymphocyte infusion increased antiviral effect	Expensive Expertise in cell manipulation required Risk of GvHD

Two alternative T lymphocyte depletion strategies are also reported, although there are few published data for patients with PID. The first also uses magnetic column/organic iron bead technology to remove naive T lymphocytes bearing the CD45RA marker, most likely to cause aGvHD, and retaining the CD45RO-bearing T lymphocytes which are more likely to confer antiviral activity. Only one small study has published results in 5 patients with PID and chronic viral infection – 4 patients engrafted and cleared virus within 2 months of HSCT, mild skin aGvHD was seen in only one patient [23].

A less costly approach is to infuse HLA-haplo-identical stem cells, replete with all other cellular elements and administer two doses of cyclophosphamide within the first week of transplantation. Rapidly proliferating cells are preferentially targeted by cyclophosphamide, and so alloreactive donor T lymphocytes infused with the graft and proliferating upon exposure to allo-antigen are selectively deleted, leaving viral-specific T lymphocytes and lymphocyte precursor cells. Whilst there is a growing experience in malignancy, there are few reports of this method in nonmalignant disease, particularly PID [24–27]. However, aGvHD may be more severe than in the other T lymphocyte depletion methods described.

5. Posttransplant complications

The mortality from HSCT for nonmalignant disease in general, and PID in particular is now extremely low, with most diseases

expecting around 90% survival and cure [28]. The remaining transplant-related mortality mainly occurs from graft-versus-host disease (GvHD), overwhelming viral infection, and to a lesser extent, endothelial cell activation syndromes such as veno-occlusive disease [28,29].

5.1. Acute graft *versus* host disease

Steroid-resistant, or -dependent acute (a) GvHD confers a significantly higher mortality than steroid-responsive disease. Second-line treatment is more controversial: a number of agents are advocated, but none have achieved acceptance as second-line standard of care [30]. Most induce further immunosuppression, making the patient more susceptible to infection, particularly due to viral or fungal pathogens, or secondary malignancies. Two modalities work through immunomodulation, and preserve anti-infective T lymphocyte function. Mesenchymal stromal cells, usually cultured from bone marrow or umbilical cord blood are given as an infusion. Initial dramatic results [31] have subsequently been more tempered [32], but nevertheless can have a significant impact on disease.

Extracorporeal photopheresis is an apheresis procedure, which separates collected peripheral blood mononuclear cells, exposes them to the photoactive drug 8-methoxypsoralen (8-MOP), and UVA radiation, and re-infuses photo-activated cells back into the patient [33]. Although more labor intensive to administer than mesenchymal stromal cells, as several cycles of treatment are generally required, and require the patient to be in hospital, and technically difficult to administer to patients <40 kg, as a blood prime of the apheresis equipment is required, nevertheless, successful results can be achieved [34,35], and extracorporeal photopheresis is currently a recommended second line treatment for acute GvHD in the UK [30]. The mechanism of action is not well understood in either of these treatment modalities.

A new group of agents that are gaining traction in the treatment of acute or chronic GvHD are the tyrosine kinase receptor inhibitors, which block signaling through the JAK-STAT signaling pathway. One example of these agents is ruxolitinib, an orally administered pharmacotherapy, developed for the treatment of myelofibrosis [36]. Several studies now report series of patients with corticosteroid-resistant aGvHD, many who had failed alternative additional immunosuppressive therapies, who, in many cases gain complete or partial remission of aGvHD [37–39]. Further studies are required in all of these new treatment approaches, and their role, and hierarchy in treatment of acute GvHD has yet to be firmly established.

5.2. Viral infections

For any patient undergoing HSCT, viral infections pose a significant risk [4]. For patients with PID, this risk may be enhanced because of the significant preexisting infectious burden that they bring to transplantation. Antiviral therapies are well established, and the principal of viral surveillance, particularly by PCR, to detect emerging virus before it causes disease is widely practiced [40]. The range of antiviral chemotherapeutics against common transplant-related viruses is limited, however, and the emergence of new agents into the armamentarium is slow. Furthermore, existing agents at best are virostatic, requiring functional T lymphocytes to clear the virus, and they have significant side effects, causing cumulative organ dysfunction over time and when used in conjunction with many of the other drugs required to treat a patient through HSCT [41]. The use of T lymphocytes specifically directed at viral epitopes is changing the approach to treatment of virus infections through transplantation [42]. At present, cells are only available against specific viruses and donor banks of virus-specific or multi-virus specific T lymphocytes are not widespread, limiting the frequent use of this effective treatment at present.

5.3. Endothelial cell activation disorders

Injury to endothelial cells can result in a number of clinical syndromes that share the same initial pathogenesis [43]. A number of triggers are recognized that may induce endothelial cell activation, including administration of chemotherapy, given as part of the conditioning regimen, pre-existing infection, and drug-induced damage [43]. Pathophysiological changes include leukocyte-mediated endothelial cell injury, particularly of capillaries, resulting in leakage of large plasma proteins such as fibrinogen, and microvascular thrombosis. Clinical symptoms of this damage range from capillary leak or engraftment syndrome, sinusoidal obstruction syndrome, thrombotic microangiopathy and respiratory complications including intra-alveolar pulmonary hemorrhage and idiopathic pneumonitis syndrome [43]. Whilst a range of treatments has been advocated to treat these complications, two agents in particular show promising therapeutic efficacy. Defibrotide, a polydisperse oligonucleotide has protective effects on the endothelium. Whilst the precise mechanism of action is unclear, it appears to protect endothelial cells from inflammatory damage and restore the thrombotic–fibrinolytic balance. Clinical trials appear to demonstrate superiority of defibrotide in the treatment or prevention of hepatic sinusoidal obstruction syndrome [44], and possibly an effect on the prevention of acute GvHD [45]. Case reports also suggest that there may be efficacy when used to treat thrombotic microangiopathy (TMA) [46]. An alternative possible treatment for TMA is eculizumab [47,48], a humanized monoclonal antibody that selectively inhibits the terminal Complement component, C5, preventing the cleavage of C5 to C5a and C5b thus preventing generation of the terminal complement complex C5b-9, which shares pro-thrombotic and proinflammatory effects with C5a. Further studies are required to determine whether one treatment is superior to another, or whether in an individual patient there are genetic influences, which might favor one treatment over another.

These incremental changes in transplant approach and procedure have resulted in significant improvement in survival and cure post-HSCT for PID, particularly for non-sibling donor transplants.

6. Conventional primary immunodeficiencies

6.1. Severe combined immunodeficiencies

The most profound immunodeficiencies, due to genetic defects which interrupt development of T lymphocytes (±B

lymphocytes and Natural Killer cells), present in infancy with opportunistic infection and persistent viral respiratory and gastrointestinal infection and are generally fatal in the first few months of life without definitive treatment [49]. Transplant in early infancy is more successful than in those transplanted later [2,3], which has led to the introduction of newborn screening programs in many states in the USA [50], by detection of DNA remnants present in lymphocytes indicative of successful T lymphocyte receptor re-arrangement and thus thymopoiesis. However, the determinant of successful HSCT is not age at transplant, but rather the presence of infection at time of transplant, with those free of infection having the best outcomes, regardless of age [4]. Outcome in the presence of infection is also determined by use or omission of preparative chemotherapy prior to transplant [4]. However, the durability of T lymphocyte engraftment, and likelihood of B lymphocyte function is also dependent on use of preparative chemotherapy, depending on the permissiveness of the SCID genotype to engraftment following stem cell infusion, with permissiveness determined by the stage of developmental arrest conferred by the genetic defect [51]. There is some evidence to suggest, that even in permissive SCID genotypes, more durable thymopoiesis is obtained after the administration of chemotherapy [52]. The risk of aGvHD is also increased if HLA-matched unrelated donor stem cells are infused [53]. The use of alkylating-containing conditioning regimens may have significant long-term sequalae in radio-sensitive artemis-deficient SCID [54], and are not recommended in other SCID conditions due to DNA repair disorders [55].

Solutions to the short- and long-term sequalae of administering chemotherapy will become more imperative when most infants are diagnosed in the newborn period through newborn screening programs. Concerns about the administration of toxic chemotherapy to newborns are driving the search for alternative strategies. Minimally intensive regimens utilizing monoclonal antibodies have been successfully used in treating SCID, even with significant co-morbidities, but these still employ low-dose chemotherapeutic agents [56]. Chemotherapy-free conditioning regimens may enable durable engraftment without short- and long-term adverse effects. Unfortunately, neither alemtuzumab monotherapy [57] nor plerixafor [58] in conjunction with granulocyte-colony-stimulating factor appears to facilitate donor stem engraftment in patients. *In utero* murine models have demonstrated beneficial effect of administering an anti-c-Kit receptor antibody, which interrupts an important signaling pathway in homing, adhesion, maintenance, and survival of HSCs in the hematopoietic niche [59]. Some gain in donor stem cell engraftment was observed in transplanting pretreated animals on the first day of life [59]. Clinical trials using therapeutic-grade anti-c-Kit antibodies are in process.

6.2. Other immunodeficiencies

6.2.1. Wiskott–Aldrich syndrome

One of the very first HSCTs performed for PID was a patient with Wiskott–Aldrich syndrome using a matched sibling in 1968 [60]. Early studies demonstrated the superiority of matched sibling HSCT over splenectomy [61]. Subsequently, survival using matched unrelated donors was shown to be equivalent to that of matched sibling donors if the transplant was performed whilst the patient was still young (<5 years of age) [6,62]. Poor outcome continued to be associated with the use of mismatched (T lymphocyte depleted) grafts and poor clinical condition pretransplant [63–65]. Since 2000, overall outcome has improved, including for those patients transplanted using a mismatched family donor, or transplanted when older than 5 years of age [66]. Successful outcomes have been reported using the new T lymphocyte depletion techniques [24,67]. An important observation in a significant subset of long-term survivors was the development of chronic GvHD-independent autoimmunity, strongly associated with partial or mixed donor lymphocyte chimerism, but not full donor chimerism [66,68]. Myeloid donor cell chimerism of <50% was associated with persistent thrombocytopenia [66].

The improving results in HSCT for classical Wiskott–Aldrich syndrome have led to the development of HSCT for a milder phenotype, X-linked thrombocytopenia. Whilst HSCT at an early age is the treatment of choice for patients with classical Wiskott–Aldrich syndrome, treatment choices for patients with X-linked thrombocytopenia are less clear. A large retrospective survey of 173 patients older than 2 years with X-linked thrombocytopenia, characterized by mild-to-moderate eczema or mild, infrequent infections, examined the probability of severe disease-related complications. Significant hemorrhagic episodes, life-threatening infections, autoimmunity and malignancy occurred in 13.9, 6.9, 12.1, and 5.2% of patients, respectively, demonstrating the non-benign nature of the disease and poor event-free survival [69]. A recent retrospective survey of 24 patients with X-linked thrombocytopenia who received HSCT following myelo-ablative conditioning, demonstrated a 100% engraftment rate with overall survival of 83.3% and resolution of pretransplant complications. The four deaths were associated with sepsis related to splenectomy prior to HSCT and severe GVHD-associated aspergillus infections [70].

6.2.2. Chronic granulomatous disease

Early attempts to correct chronic granulomatous disease by HSCT using non-myelo-ablative conditioning regimens and T lymphocyte-depleted matched sibling donor stem cell sources were only partially successful, with an increased requirement for donor lymphocyte infusions to correct poor donor chimerism leading to acute and chronic GvHD, and 30% mortality [71]. A multicenter European study reported on 27 patients who received myeloablative conditioning followed by unmodified HLA-identical stem cells, predominantly from matched sibling donors, in three groups of patients with:

(i) no overt infection or inflammation
(ii) active inflammation or inflammatory sequalae
(iii) treatment-refractory infection [72].

Almost 90% engrafted with full donor chimerism. Acute GvHD was limited to those with active inflammation or treatment-refractory infection, with deaths confined to those with treatment-refractory infection. Overall survival was 85%. Subsequently, outcome using well-matched unrelated donors was shown to be equivalent to using matched sibling donors [5]. More recently, low-toxicity regimens using

targeted busulfan doses or low-toxicity myelo-ablative regimens using treosulfan in combination with fludarabine have demonstrated excellent engraftment, and survival of 90%, even in older patients with significant preexisting comorbidities [73,74]. Recent studies using the latest methods of T lymphocyte depletion have also demonstrated excellent survival [20,21], indicating that a suitable donor should be available for any patient. This is particularly important as new evidence suggests that many female carriers of X-linked chronic granulomatous disease are not asymptomatic, but experience autoimmune phenomena, unrelated to degree of lyonization, and therefore they may not be ideal as stem cell donors [75,76].

6.2.3. DOCK8 deficiency

Defects in dedicator of cytokinesis 8 (DOCK8) cause abnormal cytoskeletal rearrangement, leading to abnormal cell structure, and defective migration and adhesion [77]. Previously described as an autosomal recessive Hyper-IgE syndrome, it is best now considered as a combined immunodeficiency. The majority of patients display a distinctive clinical phenotype characterized by severe eczema, recurrent bacterial skin and lung infections including opportunistic infections, chronic viral skin infections, and in particular severe human papilloma virus infection or molluscum contagiosum, autoimmunity and severe allergies combined with a cellular immunodeficiency and increased risk for malignancy [77,78]. Overall and event-free survival probabilities at 10, 20, and 30 years are 87, 50, 30% and 46, 21, and 4%, respectively [79].

Given the poor overall and event-free survival on conventional treatment, a number of case reports and small series report on the outcome of HSCT [25,80–87]. A mix of donors, stem cell sources and conditioning regimens are reported – accepting potential publication bias, the procedure appears successful with 18/19 (95%) patients surviving. Food allergies may be very slow to resolve post-HSCT [88], although in experience of the authors of this article, they do usually resolve over an extended period of time.

6.2.4. Common variable immunodeficiency

Common variable immunodeficiency (CVID) describes an immunological and genetically heterogeneous group of conditions characterized by hypogammaglobulinemia of at least 2 immunoglobulin isotypes. Two subtypes can be broadly described on clinical grounds. Patients with only a history of infections typically have a normal life expectancy. In contrast, patients with splenomegaly, granuloma, autoimmunity, enteropathy, liver, interstitial lung disease, or neoplasia have a compromised life expectancy and may have evidence of T lymphocyte deficiency [89–91]. More usually treated with antibiotics, replacement immunoglobulin, and immunosuppression, one multi-institutional retrospective study collected data on 25 patients who had undergone HSCT, specifically because of a diagnosis of CVID [92]. Overall survival was surprisingly poor at 48%, although better in the subgroup of patients who were transplanted because they had lymphoma. The leading cause of death was treatment refractory GvHD, associated with infection. Additionally, only half of the survivors were able to discontinue immunoglobulin substitution, and most of those who were unable to discontinue exhibited 100% donor chimerism of blood cells. All but one of the survivors had resolution of the condition constituting the primary indication of transplantation based on results of clinical evaluation and diagnostic tests. Despite these unexpectedly poor results, the study concluded that because HSCT was beneficial in all surviving patients, it could be an effective curative therapy for patients with CVID and secondary complications. However, improved clinical and laboratory risk stratification with targeted GvHD prophylaxis and RIC conditioning strategies are required to guide patient selection and timing of HSCT because of the high treatment-related mortality. Additionally, a major focus on functional and genetic characterization of patients with signs of immune dysregulation is required early during the disease to identify patients with hypomorphic forms of genetically defined PIDs for which the indication for HSCT may be established.

7. HSCT for monogenic autoimmunity

Autoimmunity may be organ specific or systemic. Tissue damage results from self-antigen reactive T- and/or B lymphocytes. The discovery of monogenic immunological disorders that lead to loss of self-tolerance has improved our understanding of how the immune system is regulated and the genomic revolution has led to the discovery of new disorders [93]. The future challenge is to evaluate patients with these disorders and decide on the best treatment options. A greater understanding of the pathways involved has led to application of specific treatments for specific affected pathways. However, definitive cure is HSCT if the gene defect is in the hematopoietic stem cell. The latest update on the classification of PID lists the disorders causing autoimmunity [94], some of which will be covered in this review.

7.1. Immunodysregulation, polyendocrinopathy, enteropathy x-linked (IPEX) syndrome

The best known and first described monogenic autoimmune disorder is IPEX (Immunodysregulation, polyendocrinopathy, enteropathy X-linked) syndrome in which mutations in FOXP3 lead to the absence of CD4+CD25+Foxp3+ regulatory T lymphocytes [95]. This leads to unchecked effector T lymphocyte activation and organ specific autoimmunity, characterized by early-onset enteropathy, eczema, neonatal insulin-dependent diabetes mellitus, and autoimmune hematological cytopenias [95]. Nademi et al. published the successful outcome of 5 patients following HSCT [96] and other centers have also recently published outcome data [97,98]. A recent multicenter survey published data on 97 patients with IPEX syndrome, of whom 58 received HSCT [99]. Overall survival was better in the HSCT group compared to those on chronic immunosuppression, particularly for those who had no or mild end-organ toxicity. Complete donor engraftment in all hematopoietic lineages may not be necessary, because the preferential engraftment of donor regulatory T lymphocytes seem to be sufficient to control the disease [100,101].

7.2. Cytotoxic T lymphocyte antigen 4 haploinsufficiency

Pathogenic mutations in cytotoxic T lymphocyte antigen 4 (*CTLA-4*) behave in an autosomal dominant manner with variable expressivity, resulting in a complex immune dysregulation syndrome with disrupted T- and B lymphocyte homeostasis [102–104]. Kuehn et al. identified 7 patients from 4 families with lymphoproliferation, organ infiltration, autoimmune cytopenias, and B cell abnormalities [102]. Schubert et al. identified 14 patients from 6 families, of whom 11 had enteropathy and 10 had hypogammaglobulinemia. Other manifestations included granulomatous lymphocytic interstitial lung disease, respiratory infections, organ infiltration, cytopenias, lymphadenopathy, skin diseases, autoimmune thyroiditis, arthritis and one case of solid cancer [103]. Eight patients with *CTLA4* haploinsufficiency who have undergone HSCT in 3 pediatric centers have been reported recently [105]. One patient died with severe acute gut GVHD and another died 2.5 years post-transplant from diabetic ketoacidosis highlighting that diabetes is irreversible and so early recognition and treatment of this disorder is important before irreversible end-organ damage. Five of 8 developed GvHD despite well-matched donors and use of serotherapy, highlighting the importance of strategies to prevent GvHD in patients who are highly inflamed going into HSCT. Seven of 8 patients had complete resolution of severe enteropathy and cytopenias following HSCT. Other therapeutic options proposed for *CTLA4*-haploinsufficient patients include soluble *CTLA4* fusion proteins (abatacept and belatacept), which bind to CD80 and CD86 and inhibit immune activation [106]. These were tried with probable benefit in the only patient to receive a molecular diagnosis prior to HSCT, but did not alter the indication for transplant, which was non-Hodgkin's lymphoma. *CTLA4*-haploinsufficiency shows a variable phenotype and further studies are needed to guide treatment selection including which patients could benefit from *CTLA4*-ligand-targeted immunomodulation rather than HSCT, optimal timing of HSCT and long-term outcome post-HSCT.

7.3. LPS-responsive beige-like anchor protein deficiency

LPS-responsive beige-like anchor protein (LRBA) deficiency is a severe PID, which manifests a variable clinical phenotype [107]. Many features overlap with common variable immunodeficiency, autoimmune lymphoproliferative syndrome, and IPEX syndrome and there is also an association with lymphoma [108–110].

The indication and optimal timing for HSCT in this condition are undetermined. LRBA is ubiquitously expressed, having roles in autophagy and intracellular vesicle trafficking, as well as facilitating cell-surface translocation of CTLA4 [107,109]. The relevance of LRBA in nonimmune cells is unknown.

Regulatory T lymphocyte function in LRBA-deficient patients exhibiting chronic enteropathy is augmentated by regular CTLA4-Ig infusions potentially inducing remission [106], although symptom resolution is variable and CTLA4-Ig is not universally available. Furthermore, such treatment is lifelong and additional immunosuppression including corticosteroids and sirolimus, may fail to halt progressive clinical deterioration. Several case reports have described HSCT in patients with LRBA deficiency, included in a recent report detailing transplant course and outcome in 12 patients [111]. Clinical features precipitating HSCT included refractory immune cytopenias, gastrointestinal complications, parenchymal lung disease, failure to thrive, as well as severe neurological and infectious complications. The overall survival was only 67%, which is relatively poor compared to outcome of HSCT for conventional PIDs. Deaths, which all occurred within 3 months of HSCT, were standard transplant-related mortality (pre-existing infections, graft failure, multiorgan failure, and thrombotic microangiopathy). Surviving patients had favorable disease remission (4 complete; 2 good partial [some mild or moderate potentially LRBA-related symptoms not requiring immunosuppressive therapy]; 2 partial [amelioration of disease but need of immunosuppression for potentially LRBA-related symptoms]). HSCT course and symptom recurrence or persistence was not dependent on the LRBA status of the donor or the conditioning regimen. Survivors in complete or good partial remission exhibited full (>95%) donor chimerism. Two patients with partial remission who required re-initiation of immunosuppressive treatment because of ongoing symptoms showed decreasing donor leukocyte chimerism of <90%. These early results demonstrate effectiveness of HSCT in curing symptoms, but suggest that complete donor chimerism is important to achieve this.

8. Gain-of-function immunodeficiencies

Primary immunodeficiencies are usually due to mutations preventing or reducing T lymphocyte activation or differentiation. Recently, disorders with gain-of-function mutations in pathways involving T lymphocyte activation have been implicated in PID.

8.1. Activated PI3K-δ syndrome

Activated PI3K-δ syndrome (APDS) is associated with dominantly inherited gain-of-function mutations in PIK3CD [112], but a similar clinical phenotype is also associated with a heterozygous loss-of-function splice site mutation in PIK3R1 [113]. Angulo et al. reported 17 patients from 7 unrelated families with the E1021K mutation [112], and Lucas et al. [113] described 14 patients from 7 families who bore heterozygous gain-of-function mutations in PIK3CD and termed this disease 'p110 δ – activating mutations causing senescent T lymphocytes cells, lymphadenopathy and immunodeficiency' (PASLI) [114]. Subsequently, a similar clinical phenotype was associated with a heterozygous splice site mutation in PIK3R1, reported by Deau et al. in 4 patients [115]. Lucas et al. subsequently described hyperactive PI3K signaling in 4 patients who experienced recurrent sino-pulmonary infections and lymphoproliferation [113]. Patients may benefit from rapamycin or other inhibitors of PI3K [112,114], but not all achieve durable disease remission. Chronic morbidity and death from severe infection and lymphoma are reported and so HSCT is a relevant option. Eleven patients who underwent HSCT in 7 centers have recently been reported [116]. Nine patients (81%) were alive with post-HSCT follow up of 8 months–16 years. One

patient died 75 days post-HSCT due to progressive renal failure, respiratory failure post-CMV and adenovirus chest infection and pulmonary hemorrhage. A second patient died 70 days posttransplant due to idiopathic pulmonary syndrome. The survival of 81% is similar to that seen after HSCT for other combined primary immunodeficiencies. However, because 2 patients developed low level mixed chimerism with disease recurrence documented in 1, further studies are required to determine the level of donor chimerism required to cure disease. The precise indications and optimal timing for HSCT need to be defined. In particular, the risks of transplant must be judged against the benefit of treatment with rapamycin or inhibitors of PI3K.

8.2. Signal transducer and activator of transcription 1 gain-of-function mutations

Gain-of-function mutations in signal transducer and activator of transcription 1 (STAT 1) are the most common genetic cause of inherited chronic mucocutaneous candidiasis, and lead to a wide variety of infectious and autoimmune features [117]. The enhanced autoimmunity of these patients is likely to result from stronger IFN-α/β signaling. Toubiana et al. reported 274 patients from 167 kindreds originating from 40 countries demonstrating an unexpectedly broad clinical phenotype [118]. Those with severe complications such as invasive infections, aneurysms and tumors had a significant risk of mortality. Leiding et al. reported 14 patients from 12 centers, all of whom had heterozygous missense mutations in either the coiled-coil or DNA-binding domain of STAT1 and underwent HSCT [119]. The indications for transplant were severe infections, combined immunodeficiency and/or an IPEX-like syndrome. Nine patients died, who were in a poor condition at transplant, were older in age and/or received myeloablative conditioning. Death was due to recognized causes of transplant-related mortality. Of 6 survivors, 2 had secondary graft loss highlighting the difficulty of obtaining good engraftment. Ruxolitinib, a Janus kinase (JAK) family tyrosine kinase inhibitor, targeting the JAK-STAT1 pathway has been used to successfully treat chronic mucocutaneous candidiasis and alopecia areata in a patient with GOF-STAT1 mutation [120], and was used to stabilize one of the patients prior to successful outcome from HSCT [119].

9. Expert commentary

The outcomes from HSCT for PID have dramatically improved over recent years, so that for many conditions survival is around 90%. Less toxic conditioning regimens have improved outcomes, particularly in patients with preexisting comorbidities. Improved treatments for transplant complications, in particular the use of specific antiviral cytotoxic T lymphocytes to counter viral infection, the use of defibrotide to treat veno-occlusive disease, and new modalities to treat aGvHD have made a significant impact on survival. However, the most significant impact on outcome has occurred with the introduction of new methods of graft manipulation. The most widely reported results are using the method of selective T lymphocyte depletion, whereby T lymphocytes bearing the γδ receptor are retained and those bearing the αβ receptor are removed. Since the introduction of this method, the range of PIDs treated using T lymphocyte depletion has significantly expanded.

With regard to emerging indications for transplantation, data are available from patients with newly described diseases, transplanted when the diagnosis was unknown. These disorders have variable phenotypes and clinical features, which may be due to the patient environment, infection and other modifier genes. They should be suspected in patients with a combination of any of the following features: lymphadenopathy, organomegaly, lymphoproliferation, cytopenias, skin rashes, enteropathy, endocrinopathies, joint inflammation and infections. If the defect is at least partially in the hematopoietic stem cell, the defect can be cured by HSCT in order to have better long-term outcomes, although other features of the disease will not be cured by HSCT. Low toxicity regimens are increasingly preferred but care needs to be taken to maximize GvHD prophylaxis but also to procure high levels of stable donor chimerism. Until now, often patients have been offered HSCT on the basis of clinico- and immuno-phenotype, and (often retrospective) genotype only provides reassurance that the correct therapeutic decision was made [121]. However, the advent of antenatal or early postnatal diagnosis and new generation sequencing, although extremely helpful in the field of diagnostic PID, does make decisions regarding HSCT more challenging. A patient, diagnosed in the first few weeks of life with, for instance, chronic granulomatous disease or CD40 ligand deficiency, and commenced on appropriate antimicrobial prophylaxis, is unlikely to present with a disease-defining illness. Survival, at least through childhood or early adulthood is likely to be similar with conventional therapy or HSCT [122,123]. Patients who are diagnosed on the basis of new-born screening may also present a conundrum – whilst mutations in SCID-associated genes usually indicate that HSCT or gene therapy is appropriate, it is well recognized, particularly in RAG-deficiency, that hypomorphic mutations may give rise to mild disease or be asymptomatic [124–132]. Ultimately the decision to transplant needs to be made according to the phenotype, and not the genotype, of the patient, following careful discussion with the patient and their family. Factors such as long-term requirement for medication, compliance and diminished quality of life may aid decision-making when a disease phenotype is not manifest because of prophylaxis. The ability to save gonadal tissue, and so provide therapeutic possibilities in future infertility or sub-fertility may also help with therapeutic decisions [133].

10. Five-year view

A number of challenges are on the horizon. The most pressing will be the management of young infants diagnosed with SCID by newborn screening, which is likely to be introduced in the near future in a number of countries. The dilemma is how to achieve durable immunity in the least toxic way. Whilst potential solutions lie in the use of antibody-based conditioning regimens, current evidence suggests that using compounds available today will not be enough to sustain long-term immunity.

In genetic disease, the occurrence of aGvHD is not welcome and maybe significantly detrimental as aGvHD and corticosteroid treatment cause profound depression in thymopoiesis [134]. For a number of years, the results of HSCT using a well matched unrelated donor have been similar to those using a sibling. With the advent of new T lymphocyte depletion techniques, survival using these modalities is approaching that of using matched sibling or matched unrelated donors. The use of posttransplant cyclophosphamide, particularly in resource-poor settings where there is limited access to unrelated donor registries, and *in vitro* methods of T lymphocyte depletion are often prohibitively expensive, is especially attractive. Given the reduction in risk of GvHD, and, in *in vitro* T lymphocyte depletion methods, the promise of adding back genetically modified T lymphocytes bearing the TCRαβ receptor to give additional antiviral effect, it is likely that the use of these products will continue to expand, and maybe replace the use of matched unrelated donors.

The advent of next-generation sequencing has created a revolution in diagnostic genetics for patients with PID. However, interpretation of results can be challenging, particularly if a novel sequence change is described. Thus, determining which changes are disease-causing and explain symptoms, and which are inconsequential will be an increasing challenge. In juxtaposition with this is the challenge to know which patients should be transplanted and at what point in the illness course. Transplantation of younger patients is better, before organ damage and severe infection and inflammation occur. However, for many of the new diseases described, it is not clear that all patients will require HSCT – thus, determining at an early stage, which patients would benefit from early HSCT will provide an increasing challenge. This issue is compounded by the introduction of specific immunomodulatory agents (such as ruxolitinib), which, if taken life-long, may provide long-term symptom reduction. It is necessary to balance the risks of HSCT against use of long-term targeted therapy. Importantly, these agents may be useful for optimizing the clinical condition of patients prior to HSCT, leading to better outcomes from HSCT. Further studies are needed on the long-term outcome of the non-transplanted and transplanted patients including quality of life, as well as long-term outcomes of transplanted patients and those on targeted therapies.

Key issues

- Introduction of newborn screening programmes will lead to early identification of patients before onset of infection or end-organ damage and will further improve HSCT outcomes.
- Less toxic conditioning regimens have improved outcomes – further refinements with targeted pharmokinetics of individual agents including serotherapy will reduce risk of rejection and aGvHD further.
- Specific lineage-related donor chimerism levels are important for different disease eg complete donor chimerism in Wiskott-Aldrich syndrome reduces the risk of future autoimmunity.
- Use of carrier donors may be associated with future disease risk for the patient eg X-linked chronic granulomatous disease.
- Transplantation of newly described disease with a predominance of auto-immune or inflammatory symptoms (eg STAT-1 or STAT-3 gain-of-function disease) may be associated with increased risk of graft rejection or aGvHD.
- Use of targeted therapies such as JAK inhibitors or CTLA-4 fusion proteins have a role in treatment – studies are required to determine whether they are most effective as sole treatment or as an adjunct and bridge to curative therapy.
- Next generation sequencing will reveal new variants in genes associated with immunodeficiency – calculating which are disease-causing and which are benign variants will be a major challenge.
- Next generation sequencing may uncover mild disease phenotypes – HSCT decisions should be based on clinical phenotype (± genotype) rather than genotype alone.

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2.1.6 Short discussion of strengths and limitations

This manuscript was not a systematic review, but focused on certain aspects of HSCT in PID, outcome in the more commonly transplanted disorders and some newly described PIDs. These are all areas of expertise in our unit which is one of the biggest transplant units for PID in the world. There was no detail about gene therapy or gene editing as an alternative to HSCT and, due to word count limitations, it was not possible to cover every PID which is amenable to treatment by HSCT.

2.2 PP2.

Lum SH *et al*. 2019 (Conditioning Regimens for Hematopoietic Cell Transplantation in Primary Immunodeficiency)

Title: Conditioning Regimens for Hematopoietic Cell Transplantation in Primary Immunodeficiency

Authors: Lum SH, Hoenig M, Gennery AR, **Slatter MA**

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2.2.1 Overview

In this article, which we were invited to write, the latest developments in conditioning regimens for Primary Immunodeficiencies were reviewed. It focused on data regarding transplant outcomes following newer reduced toxicity conditioning regimens.

2.2.2 What was known

- Conditioning chemotherapy for patients with PID before the year 2000 tended to follow conditioning regimens used for patients with malignancy. The most commonly used regimen combined 2 alkylating agents: busulfan and cyclophosphamide.
- In order to reduce toxicity the use of reduced intensity conditioning was explored predominantly using melphalan and fludarabine with serotherapy. This

led to a marked improvement in survival for patients unable to tolerate myeloablative regimens, but led to a high proportion of patients with mixed chimerism. Cardiac toxicity in infants under the age of 1 year was associated with melphalan, limiting its use in many patients with PID who need a HSCT in infancy.

- A number of reduced toxicity regimens are increasingly being employed. These are either treosulfan-based or busulfan-based, in combination with fludarabine.

2.2.3 What this study added

- A review of the literature focusing on reduced toxicity approaches.
- The importance of all agents used in a conditioning regimen including doses, timing before transplant and pharmacokinetic measurements or serum levels.
- Stem cell source can influence donor chimerism post HSCT.
- Minimal intensity conditioning regimens utilizing antibodies such as anti c-Kit receptor, to create space in the stem cell niche, are set to transform the future of cellular therapy for patients with PID.

2.2.4 Contribution of the candidate to this work

I was the senior author on this paper. I designed the structure of the manuscript in terms of content and wrote a significant contribution. SHL, MH and ARG also wrote the manuscript. I assisted in editing the manuscript following critical review by the co-authors and submitted the manuscript. A copy of the Newcastle University co-authorship form can be found in the Appendix.

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IMMUNE DEFICIENCY AND DYSREGULATION(C KUO, SECTION EDITOR)

Conditioning Regimens for Hematopoietic Cell Transplantation in Primary Immunodeficiency

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Abstract

Purpose of Review Hematopoietic cell transplantation (HCT) is an established curative treatment for children with primary immunodeficiencies. This article reviews the latest developments in conditioning regimens for primary immunodeficiency (PID). It focuses on data regarding transplant outcomes according to newer reduced toxicity conditioning regimens used in HCT for PID.

Recent Findings Conventional myeloablative conditioning regimens are associated with significant acute toxicities, transplant-related mortality, and late effects such as infertility. Reduced toxicity conditioning regimens have had significant positive impacts on HCT outcome, and there are now well-established strategies in children with PID. Treosulfan has emerged as a promising preparative agent. Use of a peripheral stem cell source has been shown to be associated with better donor chimerism in patients receiving reduced toxicity conditioning. Minimal conditioning regimens using monoclonal antibodies are in clinical trials with promising results thus far.

Summary Reduced toxicity conditioning has emerged as standard of care for PID and has resulted in improved transplant survival for patients with significant comorbidities.

Keywords Primary immunodeficiency · Hematopoietic cell transplantation · Reduced toxicity conditioning · HCT outcome · Transplant-related survival

Introduction

Primary immunodeficiency (PID) comprises a large, heterogeneous group of disorders that result from defects in immune system development and/or function. Long considered as rare diseases, recent studies show that one in 2000–5000 children younger than 18 years is thought to have a PID. There are now around 350 single-gene inborn errors of immunity and the underlying phenotypes are as diverse as infection, malignancy, allergy, autoimmunity, and autoinflammation. Therefore, presenting features, severity, and age of diagnosis vary immensely. Hematopoietic cell transplantation (HCT) is a well-recognized curative therapy for many of these PIDs. Since the first transplant took place in 1968, utility of HCT was initially limited by high rates of graft failure and transplant-related morbidity and mortality; however, transplant survival and graft outcomes have significantly improved, particularly since 2000 [1, 2]. Many factors have contributed to this improvement including earlier diagnosis, a detailed graft selection hierarchy, superior HLA matching technology, improved methods for graft manipulation, greater availability of grafts, improved supportive care, vigilant infection surveillance and pre-emptive treatment, and more effective antimicrobial therapy. In the modern era, graft engineering, additional cellular therapy, and pharmacokinetic-guided conditioning regimens enable precise personalized transplant care including prescription of graft components, better cell-dosed grafts, and a patient-tailored conditioning regimen [3, 4•, 5••].

This article is part of the Topical Collection on *Immune Deficiency and Dysregulation*

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Short-term transplant survival outcomes must be carefully distinguished from long-term disease outcomes and late effects of transplant. As survival from transplant has improved, more attention is now given to long-term disease outcomes and quality of life. Therefore, the goal of conditioning is to give the least toxic regimen with minimal short- and long-term side effects but still achieve cure of the underlying condition. This review will focus on newer conditioning regimens, how they have changed, and possible future directions. It is important to note that success does not simply depend on which conditioning chemotherapeutic agents are employed but on a combination of factors such as additional serotherapy, timing and dosage, and stem cell source. In almost all cases, preparative conditioning with a combination of chemotherapeutic agents, with or without monoclonal antibodies, is required for successful engraftment and stable robust long-term immune reconstitution.

Definition

The intensity of the conditioning regimen can vary substantially and has been classified as myeloablative conditioning (MAC), reduced toxicity conditioning (RTC), reduced intensity conditioning (RIC), and minimal intensity conditioning (MIC) in decreasing order (Fig. 1). MAC, consisting of alkylating agents with or without total body irradiation (TBI), is expected to myeloablate the recipient's hematopoiesis which does not allow for autologous hematological recovery. This aims to prevent rejection by the use of supralethal chemotherapy to remove host-versus-graft reaction and create marrow niche space for donor stem cells. Newer myeloablative chemotherapy agents are being explored to reduce toxicity and enable safer HCT. These reduced toxicity conditioning (RTC) regimens, including pharmacokinetic targeted busulfan-fludarabine (Bu-Flu) and treosulfan-fludarabine, have a comparable myeloablative effect with conventional MAC but reduced organ toxicities. Compared to MAC, RIC has been traditionally characterized by reversible myelosuppression in the absence of stem cell rescue, reduced regimen-related toxicity, and a higher incidence of mixed chimerism. MIC is strictly non-myeloablative, does not eradicate host hematopoiesis, and allows relatively rapid autologous hematopoietic recovery without a transplant, but adequately myelosuppresses the recipient to enable at least partial donor engraftment.

Myeloablative Conditioning Regimens in PID

Historically, conditioning therapy prior to HCT in PID was based on the combination of alkylators busulfan and cyclophosphamide. However, many children with PID have significant comorbidities at the time of HCT, and these conventional myeloablative preparative regimens are associated with significant toxicity and a relatively high incidence of transplant mortality, as well as long-term sequelae. While initial results may have been acceptable, appreciation of acute conditioning toxicities and recognition of long-term sequelae mean that few centers now approach transplantation of PID patients with conventional myeloablative preparative regimens (Table 1) [6–9].

RTC Regimens in PID

The use of reduced toxicity conditioning regimens are now generally preferred for patients with PID as there is no malignant disease to eradicate, stable mixed chimerism achieves cure for many diseases, and many patients enter HCT with chronic infections and end-organ comorbidities. Additionally, many patients are infants at the time of transplant and may be more susceptible to toxicity [10]. Less toxic regimens may reduce early and late adverse effects, particularly infertility [4•]. There are several reduced toxicity regimens that have been utilized by investigators in PID (Table 2) [14•, 49, 50].

Fludarabine and Treosulfan

Treosulfan (L-treitol-1,4-bis-methanesulfonate) is a prodrug and a water-soluble bifunctional alkylating agent which has been used for many years as treatment for various neoplasms, but more recently as part of conditioning for HSCT. In addition to myeloablative properties, it has marked immunosuppressive properties which contribute to the achievement of stable engraftment posttransplant. It causes relatively low organ toxicity compared to high-dose busulfan and cyclophosphamide leading to fewer complications such as veno-occlusive disease of the liver.

The first successful allogeneic transplant in a child using treosulfan was performed in 2000 and since then many reports have confirmed its efficacy and safety in both malignant and non-malignant disorders [11••, 12•, 13, 14•, 15–18]. Slatter et al. first 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 1 year at time of transplant [13]. Toxicity was low but worse after cyclophosphamide, and T cell chimerism was significantly better after fludarabine [18]. Slatter et al. more recently reported 160 patients who had received conditioning with treosulfan and fludarabine achieving a probability of 2-year survival of 87.1% with a high level of complete or stable mixed chimerism in the diseased cell

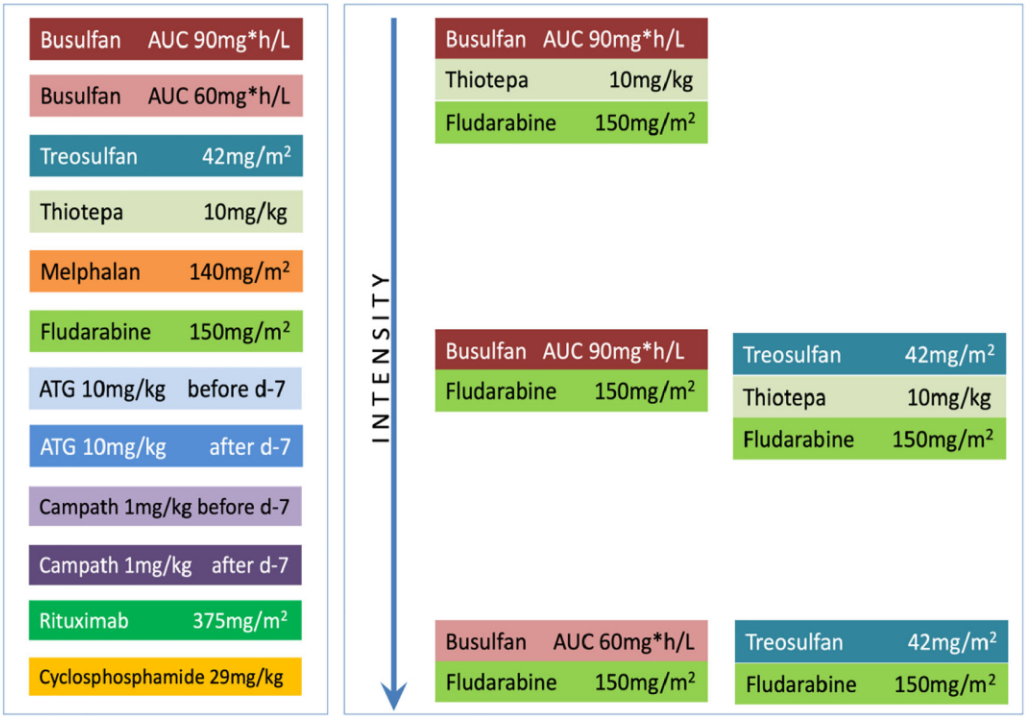

Fig. 1 Intensity of conditioning regimen according to chemotherapy, pharmacokinetic guided dosing, timing of serotherapy, and combination of chemotherapy

lineage, sufficient to cure disease [11••]. 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% demonstrated the low toxicity of this regimen making it suitable for patients with PID who often have infection and organ damage prior to HCT. In this series, a higher level of myeloid chimerism was found in recipients of PBSC compared to CB and BM, without an increased risk of grade III/IV acute or chronic graft-versus-host disease (GvHD). This highlights the importance of the whole transplant package including stem cell source and serotherapy when tailoring therapy [26].

Excellent results were reported by Lehmberg et al. in 19 patients with hemophagocytic lymphohistiocytosis (HLH) following HCT with treosulfan, fludarabine, alemtuzumab, with or without thiotepa, all of whom survived with a median follow-up of 16 months [16].

Haskologlu et al. reported 15 patients with PID who had a high risk of developing transplant-related toxicity due to previous lung and liver damages and were given treosulfan-based conditioning [27]. At 32 months follow-up, the overall survival was 86.7% with excellent chimerism and low conditioning associated morbidity despite the high-risk population.

Mixed chimerism is sufficient to achieve cure in some non-malignant disorders, but the specific diagnosis and level of chimerism needed to achieve cure must be taken into account when balancing the need for increased myeloablation against short- and long-term toxicities from the conditioning regimen. The addition of thiotepa is common in order to increase the intensity of the regimen, but there are few reports of any comparison in outcomes comparing treosulfan and fludarabine with or without additional thiotepa. Yael Dinur-Schejter et al. reported 44 patients with non-malignant diseases: 19 received treosulfan with fludarabine 66.7% of whom achieved complete engraftment compared to 94.7% of 20 patients who received additional thiotepa, but this did not translate into any significant difference in overall or event free survival [15].

Fludarabine and Busulfan

Traditionally, busulfan (Bu) was used in combination with cyclophosphamide (Cy) as the standard myeloablative conditioning regimen for HCT for both malignant and non-malignant disorders in both adult and pediatric patients. Cyclophosphamide is increasingly being substituted with fludarabine (Flu), a nucleoside analogue with immunosuppressive properties, to provide a less toxic but equally effective regimen [19, 21, 28].

Harris et al. compared 1400 children who received Bu-Cy to 381 who received Bu-Flu. Busulfan doses were comparable between the 2 groups and the majority had pharmacokinetic monitoring. Eight hundred and three had non-malignant disorders including 195 with PID who received Bu-Cy and 86 who received Bu-Flu. Nine hundred and seventy-eight had malignant disorders. Children receiving Bu-Flu for non-malignant

Table 1 Outcome of HCT in PID after myeloablative conditioning regimens

Author, Year	Year of HCT	No. of patients/diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen	OS
Fisher, 1994 [6]	1977–1991	149 non-SCID PID received 171 transplants	Range 0.1–16	65 MSD/MFD 6 MUD 78 MMUD	Bu+Cy 12 additional TBI	Before 1985, 51.7% After 1985, 81.5%
Klein, 1995 [7]	1981–1993	19 MHC class II deficiency (7 s HCT)	1.4 (0.5–9.5)	8 MFD marrow 1 MMFD marrow 10 HID marrow All 7 s HCT used HID	*MFD* Bu20mg/kg + Cy 200 mg/kg or Cy 50 mg/kg + ALG or Cy 50 mg/kg + CCNU 300 mg/m^2 + procarbazine 280 mg/kg + ALG *MMFD* Bu 16 mg/kg + Cy 200 mg/kg or Bu 20 mg/kg + Cy 200 mg/kg + anti-LFA-1 antibody or Bu 20 mg/kg + Cy 200 mg/kg + anti-LFA-1 antibody + anti-CD2 antibody	47%
Antoine, 2003 [8]	1968–1999	1082 HCT in 919 PID patients 566 HCT in 475 SCID patients 512 HCT in 444 non-SCID PID patients	SCID: 5.5 months Non-SCID: 34.6 months	88% marrow 12% PBSC 0.7% CB T cell depletion: 91% MD 41% UD marrow	205 SCID: unconditioned 361 SCID: Bu 8 mg/kg + Cy 200 mg/kg 512 non-SCID; Bu 16 mg/kg + cy 200 mg/kg	SCID: 77% MD vs 54% in MMD Non-SCID: 71% MFD vs 42% MUD vs 59% MMD
Renella, 2006 [9]	1981–2004	15 MHC class II deficiency	1.5 (0.3–5.4)	13 MFD marrow 2 MUD marrow	Bu 16-20 mg/kg + Cy 200 mg/kg + ATG in MUD	53%

ALG antilymphocyte globulin, *Bu* busulfan, *CB* cord blood, *CCNU* lomustine, *Cy* cyclophosphamide, *HID* haploidentical donor, *MD* matched donor, *MFD* matched family donor, *MMD* mismatched donor, *MSD* matched sibling donor, *MMUD* mismatched unrelated donor, *MUD* matched unrelated donor, *OS* overall survival, *PID* primary immunodeficiency, *SCID* severe combined immunodeficiency, *TBI* total body irradiation, *UD* unrelated donor, *WAS* Wiskott-Aldrich syndrome

Table 2 Outcome of HCT in PID according to reduced toxicity conditioning regimens

Author, year	Year of HCT	No of patients/diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen and GvHD prophylaxis	Median day of N engraftment	VOD, *n*
Fludarabine and treosulfan							
Slatter, 2018 [11]	2006–2013	160 39 SCID 20 WAS 17 CGD 18 HLH 66 Other PID:	1.36 (0.1–18.3)	29 MSD/MFD 73 MUD 54MMUD 4 HID 49 marrow 70 PBSC 41 CB	Flu 150 mg/m^2 + Treo 42 g/m^2 (36g/m^2 if < 1 year; 30 g/m^2 for SCID) + alemtuzumab 0.3 to 1.0 mg/kg GvHD prophylaxis: CSA/MMF	NA	0
Morillo-Gutierrez, 2016 [12]	2006–2015	70 CGD	8.9 (IQR 3.8–19.3)	13 MSD/MFD 44MUD 12 MMUD 1 HID 36 marrow 32 PBSC 1 TCR ∝β/CD19 depleted PBSC 1 CB	46 Flu150mg/m^2 + Treo 42 g/m^2 (36g/m^2 if < 1 year) Alemtuzumab ($n = 39$) or ATG ($n = 18$) or no serotherapy ($n = 13$) 15 Flu + Treo + TT + alemtuzumab or ATG 9 other Treo-based conditioning regimen GvHD prophylaxis: CSA ± MMF or MTX	17 (IQR 15–35)	0
Slatter, 2015 [13]	2005–2010	316 144 PID 39 IMD 70 H-globinopathy 32 histiocytic disorders 24 marrow failure 2 autoimmune disease 5 others	< 1 year, $n = 95$ 1–12 years, $n = 189$ > 12 years, $n = 32$	94 MSD/MFD 29 MMRD 39 MUD 16 MMUD 138 undefined UD 167 marrow 8 marrow + CB 3 marrow + PBSC 87 PBSC 1 PBSC + CB 50 CB	106 Flu 150 mg/m^2 + Treo 42 g/m^2 98 Cy 200 mg/kg + Treo 42 g/m^2 104 Flu 150 mg/m^2 + Treo 42 g/m^2 + TT 8 mg/kg 8 Flu 150 mg/m^2 + Treo 42 g/m^2 + melphalan GvHD prophylaxis: 284 CSA alone 100 CSA + MMF 101 CSA + MTX	NA	0
Burroughs, 2014 [14]	2009–2013	31 6 IPEX 5 CGD 2 other PID 6 HLH 6 BM failures 6 RBC disorders	10.7 (0.4–30.5)	4 MSD 27 MUD 29 marrow 2 PBSC	Flu 150 mg/m^2 + Treo 42 g/m^2 Serotherapy: 22 ATG GvHD prophylaxis: Tacrolimus + MTX	21 (range, 12–46)	0
Dinur-Schejter, 2015 [15]	2009–2013	45 HCT in 44 patients 12 SCID 5 severe congenital neutropenia	1.5 (0.1–15.1)	19 MSD/MFD 3 MMFD 14 MUD 9 unrelated CB	19 Flu + Treo 6 Cy + Treo 20 Flu + Treo + TT	Flu/Treo/TT: 18.4 Flu/Treo: 25.3 Cy/Treo: 19.5	1

Table 2 (continued)

		2 WAS 2 CGD 1 HLH 10 PID 5 thalassaemia 5 osteopetrosis 3 IMD 4 others			(Treo 36 g/m^2 for < 1 year; 42 g/m^2 for > 1 year) Serotherapy: 9 no serotherapy 26 ATG 8 Alemtuzumab 1 OKT3		
Lehmberg, 2014 [16]	2010–2012	19 HLH	3.9 (0.2–22)	1 MRD 6 MUD 9 MMUD HID 1 17 marrow 1 PBSC 1 CD34 selected PBSC for HID	16 Flu150mg/m^2 (3 Flu 160-180 mg/m^2) + Treo 42 g/m^2 (36g/m^2 if < 12 kg) Alemtuzumab 0.3 mg—1.0 mg/kg 14 additional TT 10 mg/kg (7 mg/kg if < 12 kg) GvHD prophylaxis: 2 CSA alone 7 CSA + MMF 9 CSA + MTX 1 Tacrolimus + MMF	20 (range 11–62)	1
Beier, 2013 [17]	2003–2009	53 non-malignant patients 10 SCID 4 CGD 2 HLH 2 WAS 11 other PID 3 osteopetrosis 9 H-globinopathy 9 BM failure 1 IMD 2 0thers	4.8 (0.1–20.1)	16 MSD/MFD 1 MMFD 1 MUD 25 MUD I HID 2 CB + HID 36 marrow 11 PBSC 1 CB 2 CB + PBSC 2 NA	15 Flu + Treo (1 additional radioimmunotherapy) 32 Flu + Treo + TT 5 Flu + Treo + melphalan Serotherapy 4 None 19 ATG 3 ATG + OKT3 1 ATG + alemtuzumab 16 alemtuzumab 1 alemtuzumab + rituximab 1 rituximab 5 OKT3	20	0
Slatter, 2011 [18]	2006–2009	70 26 SCID 7 Omenn syndrome 7 WAS 4 HLH 4 LAD 4 CGD 2 IPX 16 other PID	0.7 (0.1–14.6)	21 MSD/MFD 45 MUS 4 HID	40 Flu150mg/m^2 + Treo 42 g/m^2 30 Flu150mg/m^2 + Cy 200 mg/kg 53 alemtuzumab 0.3 to 1.0 mg/kg	NA	2 in Cy group
Busulfan ± fludarabine							
Dvorak, 2019 [19]	2011–2017	10 4 typical SCID 6 leaky SCID	5 mos (range, 2–108 mos)	2 MUD 2MMUD 6 HID	Bu with target AUC 30 mg*hr./L ATG or alemtuzumab	16 (range, 14–23)	0

Table 2 (continued)

				Marrow for MUD/MMUD CD34 selected PBSC or HID	For patients with any T cells: Additional Flu 160 mg/m^2 For patients with NK cells: Additional TT 10 mg/kg 2 had plerixafor 9 h prior to each dose of Bu		
Güngör, 2015 [20]	2003–2015	56 CGD	12.7 (IQR 6.8–17.3)	21 MSD/MFD 25 MUD 10MMUD 45 marrow 11 PBSC	Flu 150 mg/m^2 Bu with target AUC 45–65 mg*hr./Lxh Serotherapy ATG for MFD Alemtuzumab for MUD	19 (IQR 16–22)	0
Jacobsohn, 2004 [21]	2000–2004	13 6 PID 4 H-globionopathy 3 IMD	5.2 (IQR, 0.6–11.1)	4 MSD 1MMFD 6 MUD 2 unrelated CB 11 PBSC	Flu 150 mg/m^2 Bu with target AUC 3800 to 4200umol x min ATG GvHD prophylaxis CSA ± MMF	18 (IQR, 14–25)	0
Fludarabine and melphalan							
Allen, 2018 [22]	2013–2015	34 HLH 12 PID	2.3 (0.4–28)	7 MSD 1 MMRD 25 MUD 13 MMUD All had marrow	Flu 150 mg/m^2 Melp 140 mg/m^2 Alemtuzumab 1 mg/kg GvHD prophylaxis CSA and steroid	13	0
Fox, 2018 [23]	2004–2014	29 PID	24 [11, 12, 16, 18–48]	11 MFD 13 MUD 5 MMUD	Non-CGD Flu 150 mg/m^2 Melp 140 mg/m^2 Alemtuzumab 100 mg CGD Flu 150 mg/m^2 Meph 10 mg/m^2 or Bu 9.6 mg/kg Alemtuzumab or ATG GvHD prophylaxis CSA	13 (IQR, 11–17)	0
Marsh, 2010 [30]	2003–2009	40 HLH	1 (0.1–16)	7 MFD 33 MUD 36 marrow 2 PBSC 2 CB	26 RIC Flu 150 mg/m^2 (5mg/kg if < 10 kg) Melp 140 mg/m^2 (4.7 mg/kg if < 10 kg) Alemtuzumab 14 MAC Bu 14 mg/kg Cy 200 mg/kg 12 additional etoposide 30 mg/kg GvHD prophylaxis	MAC: 14.5 RIC: 10	NA

Table 2 (continued)

					CSA or tacrolimus + steroid/MTX		
Rao, 2005 [49]	1998–2001	33 6 SCID 27 non-SCID	5.9 (0.19–18)	22 MUD 11 MMUD All marrow	Flu 150 mg/m^2 Melp 140 mg/m^2 Alemtuzumab 1 mg/kg CSA	13 (range, 8–34)	0
Amrolia, 2000 [31]	NA	8 3 SCID 1 XLP/HLH 2 CID 2 CD40 ligand def	6.5 (range, 0.75–18)	2 MSD 6 MUD All marrow	Flu 150 mg/m^2 Melp 140 mg/m^2 ALG 10 mg/kg GvHD prophylaxis CSA and steroid	13 (range, 9–17)	0
Fludarabine and low-dose TBI							
Burroughs, 2010 [36]	NA	2 IPEX	0.75, 16	2 MUD 1 marrow 1 PBSC	Flu 90 mg/m^2 TBI 4Gy GvHD prophylaxis CSA and MMF	16, 17	0
Burroughs, 2007 [35]	1998–2006	14 PID	Range 0.5–30	8 MFD 8 MUD 8 marrow 5 PBSC 1 CB	Flu 90 mg/m^2 ($n = 13$) TBI 2Gy ($n = 14$) GvHD prophylaxis CSA and MMF	15 (range 5–23)	0
Antibody-based conditioning							
Schulz, 2011 [44]	2003–2007	14 non-malignant 4 SCID 2 CGD 2 Hyper IgM 2 other PID 4 H-globinopahty	7.5 (range, 1–20)	3 MFD 1 MMFD 8 MUD 2 HID 8 marrow 4 PBSC 2 TCD-PBSC	^{90}Y-labeled anti-CD66 antibody at Day −14 Fludarabine 160 mg/m^2 Melphalan 70-140 mg/m^2 ATG for mismatched donor and unrelated donor	NA	0
Straathof, 2009 [24]	1999–2002	16 8 SCID 1 MHC class II def. 1 IPEX 1 HLH 1 DKC + SCID 1 Ligase 4 def. 1 CD40 ligand def. 2 Other PIDs	0.7 (range, 0.4 to 11.4)	5 MSD 9 MUD 2 MMUDD 40 marrow 12 PBSC 1 PBSC + marrow 17 CB	Anti-CD45 1.6 mg/kg (day −5 to −2) Flu 150 mg/m^2 Alemtuzumab 0.3 to 0.6 mg/kg GvHD prophylaxis CSA and MMF	9.5 (range 1–15)	0

aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, *n*	Latest donor chimerism/remarks
Fludarabine and treosulfan						
I–IV: 46 III–IV: 9	15	2-year OS: 88 5-year OS: 78	2-year ES: 88 5-year ES: 78	3	4 s HCT for graft loss or poor immune reconstitution 5 unconditioned boost 3 DLI	PBSC was associated with better donor myeloid chimerism without an increased risk of GvHD

Table 2 (continued)

I–II: 39 III–IV: 12	13	91.4	81.4	12	8 (2 unconditioned boost; 3 DLI; 5 conditioned 2nd HCT [2 had DLI])	Myeloid ≥ 95%: 80% surviving patients
I–IV: 38 III–IV: 10	NA	83	76	5.1	NA	NA
II–IV: 62 III–IV: 10	21	90	NA	3	2 s HCT	*ATG patients:* 19 (86%) full or high level of mixed CD3 chimerism 3 (14%) low-level mixed donor CD3 chimerism *No ATG patients:* 6 full/high level of mixed CD3 chimerism 2 low-level mixed donor CD3 chimerism 1 graft failure
I–IV: 44.4 III–IV: 27	18.9	71	55	14	3 s HCT (one had a further 3rd HCT)	Full: 31 (72%) Mixed: 6 (28%)
I–II: 21 III–IV: 1 patient after DLI	No	100	NA	11 ($n = 2$)	2 s HCT (1 1° graft failure after HID; 1 2° graft failure) 6 DLI	WB > 95%: 10 WB 75–95%: 2 WB 20–74%: 4
I–IV: 32 III–IV: 4	6 ($n = 3$)	87	NA	4	NA	Full: 46 (87%)
I–IV: 26 III–IV: 10	6	81 Flu: 85% Cy: 77%	NA	3 ($n = 2$_	1 had both top-up and second conditioned HCT	57% full donor chimerism 43% stable mixed chimerism
Busulfan ± fludarabine						
II–IV: 2 patients	0	100	NA	10	1 additional HCT	Median myeloid at one-year post HCT 14% (range, 2–100%) 6 had full T- and B cell reconstitution 3 had no B cell recovery (2 had rituximab for autoimmunity post-HCT) 3 had B cell autoimmunity
III–IV: 4	7	93	89	5	3 s HCT	Myeloid > 90%: 52 (93%)
II–IV: 8	25	84	NA	15 (2 h-globinopathy)	none	72% full donor chimerism
Fludarabine and melphalan						
II–IV: 17.4 III–IV: 10.9	26.7	18-month OS: 66.9%	60.9% with second procedure 39.1% without intervention	Primary: 4 Secondary: 4	2 s HCT	57% had full chimerism in all cell lines 42% had stable mixed chimerism
I–II: 45 III–IV: 3	Limited: 34 Extensive: 1	1-yr: 85.2	1-year: 85.7	None	None	85% full chimerism
II-III MAC: 14 RIC: 8 ($p = 0.317$)	MAC: 0 RIC: 12% limited	MAC: 43% RIC 89% ($p = 0.0036$)	NA	None	3 CD34+ boost 14 DLI	MAC: 18% mixed RIC: 65% mixed Mixed chimerism in RIC was less in patients who received distal aleumtuzumab

Table 2 (continued)

						(29%) vs 79% in proximal alemtuzumab ($p = 0.02$)
II–IV: 9	Limited: 0 Extensive: 3	94%	NA	NA	NA	55% had full chimerism 32% had high level mixed chimerism 6.5% had low level mixed chimerism 6.5% very low mixed chimerism
I: 50 II–IV: 0	limited cGvHD, $n = 1$	88	NA	1 patient	None	4 had 100% donor chimerism 3 had mixed chimerism
Fludarabine and low-dose TBI						
2 had grade II	1 severe	Both alive	Both engrafted	None	None	Full immune function and normal FOXP3 protein expression
II: 71 III–IV: 7	Extensive: 47	62	62	1	1 unconditioned PBSC for slipping myeloid chimerism 1 conditioned HCT for persistent thrombocytopenia 1 DLI for low donor CD4 and CD8 chimerism 1 conditioned HCT for graft failure	5 mixed chimerism 8 full donor chimerism
Antibody-based conditioning						
II: 36 III–IV: 0	limited, $n = 2$ extended, $n = 3$	88	81	$n = 1$	1	9 had 100% chimerism 2 had mixed chimerism
II–IV: 38 III–IV: 19	31	81	95	3	1 s HCT	Median myeloid: 100% (range, 41–100%) Median lymphocyte: 100% (range, 54–100%)

1° primary, *2°* secondary, *aGvHD* acute graft-versus-host disease, *ALG* antilymphocyte globulin, *ATG* anti-thymocyte globulin, *AUC* area under curve, *BM* bone marrow, *BU* busulfan, *CB* cord blood, *CGD* chronic granulomatous disease, *cGvHD* chronic graft-versus-host disease, *CSA* ciclosporin, *def* deficiency, *DLI* donor lymphocyte infusion, *ES* engrafted survival, *Flu* fludarabine, *H-globinopathy* hemoglobinopathy, *HID* haploidentical donor, *HLH* hemophagocytic lymphohistiocytosis, *IMD* inherited metabolic disease, *IQR* interquartile range, *MMF* mycophenolate mofetil, *MMRD* mismatched related donor, *MMUD* mismatched unrelated donor, *MSD* matched sibling donor, *MUD* matched unrelated donor, *MTX* methotrexate, *N* neutrophil, *NA* not available, *OS* overall survival, *PID* primary immunodeficiency diseases, *SCID* severe combined immunodeficiencies, *Treo* treosulfan, *TT* thiotepa, *vs* versus, *WAS* Wiskott-Aldrich syndrome, *WB* whole blood

conditions experienced less toxicity than those receiving Bu-Cy, but survival was comparable. Children with malignancy had shorter postrelapse survival with Bu-Flu than Bu-Cy although transplant-related mortality and relapse were similar [29].

The pharmacokinetics of busulfan have been studied extensively and the use of a lower target area under the curve (45–65 mg/L × h) combined with fludarabine has been pioneered by Tayfun Güngör and colleagues in Zurich. Particularly impressive results have been seen using this regimen for patients with chronic granulomatous disease (CGD). Fifty-six children and young adults with CGD were reported, many of whom had high-risk features such as intractable infections and autoinflammation. Twenty-one HLA-matched related-donor and 35 HLA-matched unrelated-donor transplants were done. The 2-year probability of overall survival was 96% (95% CI 86·46–99·09), and of EFS was 91% (79·78–96·17). Graft-failure occurred in 5% (three of 56) of patients. The cumulative incidence of acute GvHD of grade III–IV was 4% (two of 56) and of chronic GvHD was 7% (four of 56). Stable (≥ 90%) myeloid donor chimerism was documented in 52 (93%) surviving patients [20••].

Dvorak et al. have recently reported the result of the use busulfan at a lower target area under the curve (30 mg/L × h) alone or in combination with fludarabine or thiotepa in 10 patients with severe combined immunodeficiency. All the patients survived, one patient required second HCT, and 3 had no B cell reconstitution [19].

RIC in PID

Fludarabine and Melphalan

Increasing recognition of the significant toxicities associated with conventional doses of busulfan and cyclophosphamide, particularly in very young infants and especially in those with pre-existing end organ damage, led to the adoption of immunosuppressive-based, rather than myelo-ablative-based regimens, with fludarabine and melphalan. The results, principally in those with significant preexisting comorbidities, were striking with significantly improved early survival [22, 23, 30, 31, 49]. However, donor chimerism was not always optimal, and there was a high incidence of late viral reactivation, and late onset acute GvHD. Furthermore, toxicities in infants < 1 year of age remained significant [25]. Melphalan in particular has been associated with cardiac toxicities [32]. Good results have been reported for patients with hemophagocytic lymphohistiocytosis [33]. Patients with X-linked inhibitor of apoptosis protein (XIAP) deficiency, which is difficult to transplant, also have good outcomes reported using fludarabine and melphalan-based regimens [34]. It has been used in adults with PID with good transplant survival [23]

While the approach remains attractive in terms of reduced toxicities, concerns regarding late graft failure and high mortality in the < 12-month-aged infants remain.

Minimal Intensity Conditioning for PID

Fludarabine and Low-Dose TBI

Burroughs et al. from the Seattle group have reported the transplant outcome of using fludarabine and low-dose TBI in 14 PID patients with significant preexisting organ dysfunction and infections. All received posttransplant GvHD prophylaxis with cyclosporin and mycophenolate mofetil but no serotherapy. Overall survival at 3 years was 62%, but there were high rates of acute (79%) and extensive chronic GvHD (47%) [35]. One had graft failure and an additional three patients required a second procedure for decreasing chimerism. Of 10 evaluable patients, 8 had correction of immune deficiency with stable chimerism. However, the high rate of GvHD has limited the broader use of this conditioning regimen in children with PID [35, 36].

Antibody-Based

While conditioning regimens have undoubtedly become less toxic, the ability to achieve donor chimerism without the use of chemotherapeutic agents, particularly in patients with non-malignant disease, is extremely attractive. Furthermore, some primary immunodeficiencies have significant toxicities associated with the administration of alkylating agents, due to the nature of the molecular defect, leading to serious long-term effects or early mortality [37–39]. A number of different strategies have been employed to minimize the exposure to chemotherapeutic agents by the use of antibodies to aid stem cell engraftment, with or without adjunct chemotherapy.

Anti-CD45 Antibodies

CD45 is selectively expressed on all leucocytes and hematopoietic progenitors but is absent on non-hematopoietic tissues. Straathoff and colleagues studied 16 patients with PID who were less than 1 year of age or had significant preexisting comorbidities and were felt not suitable for conventional reduced intensity conditioning [24]. The conditioning regimen was comprised of alemtuzumab 0.2 mg/kg daily for 3 days for unrelated donors, or 0.1 mg/kg daily for 3 days for matched sibling donors on day − 8 to day − 6, clinical grade rat anti-CD45 (YTH24·5and54·12) 0.4 mg/kg on day − 5 to day − 2, fludarabine (30 mg/m^2 daily for 5 days on day − 8 to day − 4) and cyclophosphamide (300 mg/m^2 daily for 4 days on day − 7 to day − 4). Twelve patients were alive and well at the end of the study, one failed to engraft and was successfully re-

transplanted, and 3 died—none of conditioning toxicity. Donor chimerism was variable but high level and sufficient to cure disease in the survivors.

Radioimmunotherapy

Radioimmunotherapy is an attractive concept for conditioning of patients with PIDs as it exploits of the physical cytotoxic effect of radiation and reduces the toxicity to other organ systems by its internal application and the conjugation of radioisotopes to specific antibodies [40]. Radioisotopes emitting α, β or γ-radiation of calculated intensity can be brought in direct proximity to the cells of interest. This enables malignant cells to be eradicated or benign hematopoietic cells to be depleted as part of conditioning before autologous or allogeneic HSCT. The method was developed to allow better and more specific control of malignant cells in the setting of HSCT without an increase in non-relapse mortality. Considerable clinical data was accumulated with conjugates of ^{90}Yttrium or ^{131}Iodine to anti-CD20 antibodies in the treatment of patients with refractory or recurrent B cell non-Hodgkin lymphoma (B-NHL). The drugs were used in combination with chemotherapy to prepare patients for autologous and allogeneic stem cell transplantation. This experience resulted in the approval of two drugs (Zevalin® and Bexxar®) by the FDA at the beginning of the century [40].

The use of RIT for the treatment of leukemias or for myeloablation in non-malignant disease until present is limited to clinical studies. A conjugate of ^{131}Iodine to anti-CD45-antibody was explored in the treatment of patients with AML and high-risk MDS, again a combination of RIT with conventional myeloablative or immunosuppressive drugs was used for conditioning before allogeneic HSCT [41, 42]. CD45 is expressed on most AML and ALL blasts as well as on virtually all developing and mature cells of normal hematopoiesis. Radiolabeled anti-CD45 antibody doses up to 43 Gy were administered to the bone marrow in combination with RIC and allogeneic transplantation with good tolerance and without additional toxicity in younger adult patients with AML and MDS [43]. For children, limited published data exists for the use of RIT for pretransplant conditioning. A conjugate of ^{90}Yttrium to an antibody targeting CD66 was used in combination with melphalan and fludarabine or TBI for the treatment of children with considerable comorbidities with malignant and non-malignant disease. ^{90}Yttrium emits pure β-radiation with a maximum range of 11 mm and a half-life of 2.7 days [44]. With these qualities, no isolation of the pediatric patients was necessary, but the dosimetry had to be performed with another isotope, emitting γ-radiation to be detected in a γ-camera. CD66 is abundantly present on mature myeloid cells but usually not expressed on malignant blasts. The therapeutic principle of RIT with this antibody in malignant disease therefore relies on the so-called cross-fire effect, which describes the indirect depletion of blasts by binding of the antibody to cells in close proximity [40]. In order to avoid graft rejection in unrelated or mismatched grafts, recipients received serotherapy with ATG in this setting. Fifteen of 16 children with non-malignant disease survived the procedure, 13/15 with complete donor chimerism. The Kaplan-Meier estimation for disease-free survival at 24 months was 94%. This clearly documented feasibility of and reliable myeloablation by RIT in children and young adults with non-malignant disease.

Anti-CD117 Antibodies

The molecule CD117 (c-Kit receptor) is expressed on hematopoietic stem cells at all stages of development. Interactions with the ligand of CD117, stem cell factor, are crucial for hematopoietic stem cell survival, and this signaling pathway plays a critical role in the homing, adhesion, maintenance, and survival of hematopoietic stem cells in the hematopoietic niche. Preclinical studies demonstrated that using an antibody against CD117 to impede CD117-stem cell factor signaling selectively depleted hematopoietic stem cells with no effect on differentiated progenitor or mature cell lineages, and enabled engraftment of donor cells [45]. A clinical trial is currently in progress using anti-CD117 antibody alone to treat patients with primary immunodeficiencies (AMG191 Conditioning/CD34 + CD90 Stem Cell Transplant Study for SCID Patients, ClinicalTrials.gov Identifier: NCT02963064). The early results of this dose finding study show that some donor stem cell chimerism, leading to donor T and B lymphocyte chimerism can be achieved [46]. These preliminary data are extremely exciting and potentially lead the way to a step change in approaches to conditioning in patients with PIDs.

Conditioning for Haploidentical Donor Transplant

As the outcomes of HCT using newer T cell depletion methods have improved, there is an increasing number of haploidentical transplants performed for both SCID and non-SCID PID. Various non-myeloablative conditioning regimens have been used in T-deplete and T-replete haploidentical transplant (Table 3) [5••, 47, 48, 51]. The Great North Children's Hospital (GNCH) group in Newcastle has used fludarabine, treosulfan, ATG (Grafalon), and ritixumab for patients who received CD3 TCR ab/CD19 depleted peripheral blood stem cells. Patients with non-SCID PID received additional thiotepa.

Table 3 Outcome of haploidentical donor transplant in PID using modern T lymphocyte depletion strategies and various conditioning regimens

Author, year	Year of HCT	No of patients/diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen and GvHD prophylaxis	Median day of N engraftment	VOD %
Fludarabine and treosulfan							
Neven, 2019 [48]	2014–2017	22 PID 5 osteopetrosis 21 first HCT 6 s HCT	1.5 (0.2–17)	27 HID All marrow	20 MAC with Bu-pk + Flu 160 mg/m^2 (4 received additional Cy 28 mg/kg) Serotherapy: rituximab plus alemtuzumab/ATG 7 had RIC (1 first HCT and 6 s HCT) GVHD prophylaxis CSA MMF PTCy 50 mg/kg on day 3 + 4	19 [11–13, 15–34]	11
Shah, 2018 [5]	2012–2016	25 PID 3 for refractory GvHD	1.75 (0.28–10.3)	23 HID 2 MMUD TCR ab/CD 19 depleted PBSC	Flu 150 mg/m^2 Treo 36-42 mg/m^2 TT 10 mg/kg 24 had serotherapy (ATG/alemtuzumab) 6 had rituximab 3 SCID: unconditioned GvHD prophylaxis: CSF/MMF	25 [10–19, 21, 24–28, 49, 50]	0
Rastogi, 2017 [47]	2013–2016	8 PID	4.9 (0.8–12)	7 HID 1 MUD Unmanipulated marrow/PBSC	5 Flu 160 mg/m^2 + Cy 29 mg/kg + TBI 2Gy (3 had additional TT) + ATG/alemtuzumab 2 Flu 160 mg/m^2 + Treo 42 mg/^{2} 1 Flu 160 mg/m^2 + Bu 3.2 mg/kg GVHD prophylaxis Tacrolimus MMF PTCy 50 mg/kg on Day 3 + 4	Mean 17	NA
Balashov, 2015 [51]	2012–2014	37 PID 5 SICD 32 non-SCID PID	2.6 (0.2–17)	27 MUD 10 MMRD TCR ab/CD 19 depleted PBSC	Flu 150 mg/m^2 Treo 36-42 mg/m^2 8 had Melphalan 140 mg/m^2 for high risk graft rejection	16 (range 11–28)	NA

Table 3 (continued)

				14 had rituximab 1 unconditioned Serotherapy 35 ATG 2 alemtuzumab		
aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, *n*	Latest donor chimerism/ remarks
Fludarabine and treosulfan						
II–IV: 48 II: *n* = 10 III: *n* = 2	24.2	77.7	77.7	*n* = 2	1	24 full chimerism 1 mixed chimerism
II–IV: 22	None	83.9	80.4	*n* = 1	1	76.1% full donor chimerism 5 had high T cell but mixed myeloid chimerism (2 unconditioned)
I–II: 3 patients II–IV: none	2 limited	75	75	None	None	All full donor chimerism
Max grade 2 in 7 patients Only one had grade IV (no conditioning)	1 patient (unconditioned)	96.7	67.7	27% HID: 36% MUD: 28%	10	NA

aGvHD acute graft-versus-host disease, *BU* busulfan, *cGvHD* chronic graft-versus-host disease, *CSA* ciclosporin, *ES* engrafted survival, *Flu* fludarabine, *HID* haploidentical donor, *MAC* myeloablative conditioning, *MMF* mycophenolate mofetil, *MMUD* mismatched unrelated donor, *MSD* matched sibling donor, *MUD* matched unrelated donor, *N* neutrophil, *NA* not available, *OS* overall survival, *PID* primary immunodeficiency diseases, *RIC* reduced intensity conditioning, *SCID* severe combined immunodeficiencies, *Treo* treosulfan, *TT* thiotepa, *WAS* Wiskott-Aldrich syndrome

The overall survival was comparable with family and unrelated donor transplant using a similar conditioning regimen [18, 51]. Neven et al. reported the outcome of Bu-Flu in 22 patients with PID received haploidentical transplant using posttransplant cyclophosphamide. The overall survival and donor chimerism were good, but 48% had acute GvHD and 24.2% had chronic GvHD.

Pharmacokinetic Studies

Although levels of busulfan have been measured for many years, to target the narrow myeloablative therapeutic window, minimize toxicity from supra-therapeutic levels and avoid sub-myelo-ablation and rejection, it is only recently that the importance of pharmacokinetic monitoring of other agents of the conditioning cocktail has been appreciated.

Fludarabine Pharmacokinetics

Ivaturi et al. prospectively studied the pharmacokinetics and pharmacodynamics of 133 children undergoing HCT for a variety of disorders with a variety of conditioning regimens but all included fludarabine. Young age and renal impairment were found to lead to an increased exposure. In the setting of malignancy, disease-free survival (DFS) was highest 1 year after HCT in subjects achieving a systemic fludarabine plasma (f-ara-a) cumulative area under the curve (cAUC) greater than 15 mg*hour/L compared to patients with a cAUC less than 15 mg*hour/L (82.6% versus 52.8%, $p = 0.04$) [52]. Further development of model-based dosing may minimize toxicity and maximize efficacy, resulting in superior outcomes for malignant and non-malignant patients.

Treosulfan Pharmacokinetics

Relatively high variability of treosulfan pharmacokinetics in pediatric patients may raise the need for implementing therapeutic drug monitoring and individual dose adjustment in this group. Vander Stoep et al. and Mohanan et al. recently published the first results of a relationship between the exposure of treosulfan and early toxicity, as well as clinical outcome, in children undergoing conditioning prior to HSCT. In the former study, patients with an AUC > 1650 mg h/L demonstrated a statistically higher incidence of mucosal and skin toxicity than those with an AUC 1350 mg h/L (odds ratio 4.4 and 4.5, respectively). The odds of developing hepato- and neurotoxicity were also higher in the former group, but the difference did not reach statistical significance. No association was found between treosulfan exposure and early clinical outcomes, i.e., engraftment, donor chimerism, acute graft-versus-host disease, treatment-related mortality, and overall survival. PK parameters were shown to be age-dependent, with higher AUC values in younger children (< 1 year old) and corresponding lower treosulfan clearance. A challenge in therapeutic monitoring of treosulfan within conditioning prior to HCT is a very brief course of treatment, consisting of three doses administered on 3 consecutive days. This allows personalization of only the second and third dose of the prodrug unless a test dose is applied prior to starting the actual regimen.

Since pharmacokinetic studies of treosulfan began, it has been assumed that plasma (serum) concentrations of the prodrug are a good representation of the alkylating activity of its epoxy transformers. However, for years, a correlation between treosulfan concentrations in plasma and levels of specific DNA adducts in tissues, for example the bone marrow, or clinical effects, have not been investigated. Therapeutic drug monitoring of not only prodrug but also its active epoxide might be needed. In addition blood pH, body temperature, and intravenous fluid delivery may influence glomerular filtration, tubular reabsorption, and nonenzymatic epoxy transformation of the prodrug [53].

Serotherapy Levels

It is now well recognized that type of serotherapy, dose and timing in relation to the transplant all have an impact on outcome of transplant in terms of occurrence of GVHD, immune reconstitution importantly in terms of viral reactivation, clearance of infection, and chimerism. Marsh RA et al. collected data from 105 patients to examine the influence of peritransplant alemtuzumab levels on acute GVHD, mixed chimerism, and lymphocyte recovery. Significantly higher levels of aGVHD but higher levels of donor chimerism, lymphocyte counts at D+30 and T cell counts at D+100 were associated with lower alemtuzumab levels at day 0 [54].

In a recent report, the clearance of the active components of the 2 widely used types of ATG (Fresenius/Grafalon and Genzyme) was studied in 38 children with malignant hematological disorders. They found that ATG Fresenius was cleared rapidly and uniformly from the circulation whether they received 60 mg/kg or 45 mg/kg, but there were significant differences in patients who received a high dose of ATG Genzyme (10 mg/kg) who had significantly slower reconstitution for CD3, CD4, and CD8 T cells compared to patients who received a low dose of ATG Genzyme (6–8 mg/kg) or ATG Fresenius [55].

Stem Cell Source in Non-MAC Conditioning

Historically bone marrow has been the preferred stem cell source for HCT in children due to concerns that peripheral blood stem cell products led to an increased risk of GVHD. In Slatter et al.'s report of 160 PID patients who received uniform conditioning with treosulfan and fludarabine, a higher level of myeloid chimerism was found in recipients of PBSC compared to CB and BM, without an increased risk of grade III/IV acute or chronic GvHD [26]. This is an important finding particularly for patients with diseases where a high level of chimerism is required to achieve complete cure.

Conclusions

The use of RTC and RIC has been a major paradigm shift in HCT for PID and may have contributed to improved survival through a reduction in early post-HSCT toxicities. Almost certainly, long-term toxicities will be reduced, although further data are required to confirm this. However, the use of antibody-based conditioning regimens is likely to transform the field in the future. The drive for this has been that PID can be completely cured by HCT, and as malignancy is rarely a feature of the disease, toxicity from the curative procedure should be minimized. More recently, newborn screening for severe combined immunodeficiencies has meant that these patients are now being identified by 2–3 weeks of age [56]. Rapid transplantation is preferred, as survival and neurological outcome results are best in patients with no preexisting infection [57, 58]. As gene therapy approaches become mainstream treatment, then a non-toxic conditioning approach followed by an autologous gene-corrected stem cell procedure should almost eliminate short- and long-term treatment-related morbidities for patients with SCID [59, 60]. These conditioning approaches will have to be modified for combined immunodeficiencies and gain-of-function diseases where high-level or complete donor chimerism is required to abolish disease manifestations [61–64]. However, combinations of antibody-based regimens and pharmacokinetically targeted reduced low-toxicity agents may help resolve these issues. The future for patients with PID looks extremely encouraging.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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2.2.6 Short discussion of strengths and limitations

This manuscript is a review which gives an overview of progress that has been made in conditioning regimens for HSCT in patients with PID over time. It offers new perspectives and future directions in the field. Although the literature was described, there were no systematic searching methods or evaluation of the papers included in the manuscript. Therefore there is a chance of bias and incomplete ascertainment of relevant papers.

2.3 PP3.

Slatter M *et al.* 2015 (Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases)

Title: Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases

Authors: **Slatter MA,** Boztug H, Potschger U, Sykora K-W, Lankester A, Yaniv I, Sedlacek P, Glogova E, Veys P, Gennery AR and Peters C on behalf of the EBMT Inborn Errors and Paediatric Diseases Working Parties

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2.3.1 Overview

I was honored to be asked to write this manuscript on behalf of the European society for Blood and Marrow Transplantation (EBMT) Inborn Errors and Paediatric Diseases Working Parties. It reports a retrospective analysis of children registered in the EBMT database, who received Treosulfan as part of conditioning for HSCT for non-malignant diseases between 2005 and 2010. The aim was to identify any possible dose-related toxicity and determine the incidence of engraftment, treatment-related mortality and OS. Results from 316 transplants from 11 different countries were included. One hundred and forty-four of these were in patients with PID, the remainder being in patients with metabolic, histiocytic or autoimmune disorders, haemoglobinopathies and bone marrow failures.

2.3.2 What was known

- Treosulfan has myeloablative and immunosuppressive effects and can be used as conditioning for HSCT.
- The use of Treosulfan for conditioning for HSCT in children with non-malignant disorders was increasing, particularly for inherited disorders including haemoglobinopathies.
- Treosulfan had been shown to cause less acute toxicity - particularly hepatic VOD, than traditionally used busulfan in combination with cyclophosphamide.
- Children with non-malignant disorders differ from those with malignant disease;
    - A high proportion of them are children with SCID who require transplant as young infants and often present with infection and organ damage.
    - GVHD which may be associated with a beneficial graft versus leukaemia effect in malignancy impairs immune reconstitution and is to be avoided if possible.
    - Although stable mixed chimerism may lead to cure, graft rejection can be a problem particularly in haemoglobinopathies.

2.3.3 What this study added

- This was and still is the largest study to date of children receiving Treosulfan as part of conditioning for HSCT.
- Treosulfan was shown to be safe and effective in infants. 30% of the treated children were under 1 year of age at transplant and there was no significant difference found in OS or EFS between these and older children.

- There was more respiratory acute toxicity reported in patients under 1 year of age. This is likely due to the larger number of patients with SCID in this group with pre-existing respiratory impairment.
- The addition of thiotepa did not increase acute toxicity. This is important because it may lead to improved engraftment and chimerism. However it is not known whether or not it may lead to an increase in adverse late effects.
- Multivariate analysis showed no association of transplant-related mortality with age at transplant, dose of treosulfan given, other agents used in combination with treosulfan, type of donor, stem cell source or whether it was used for a second or subsequent transplant.

2.3.4 Contribution of the candidate to this work

I was the lead writer and submitted the manuscript and contributed equally with HB, AL, PV, ARG, UP and CP. CP and UP designed the study. KWS and PS contributed to the study and manuscript. EG provided additional statistical support. A copy of the Newcastle University co-authorship form can be found in the Appendix.

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ORIGINAL ARTICLE

Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases

MA Slatter[1], H Boztug[2], U Pötschger[2], K-W Sykora[3], A Lankester[4], I Yaniv[5], P Sedlacek[6], E Glogova[2], P Veys[7], AR Gennery[1] and C Peters[2] on behalf of the EBMT Inborn Errors and Paediatric Diseases Working Parties

An increasing number of children with non-malignant diseases can be cured by allogeneic haematopoietic stem cell transplantation (HSCT). Treosulfan (L-treitol-1,4-bis-methanesulfonate) is being used more frequently for conditioning, owing to its' lower toxicity profile compared with conventional myeloablative regimens. A retrospective analysis was performed of children registered in the EBMT database, who received treosulfan before HSCT between January 2005 and 2010, to identify possible dose-related toxicity and determine the incidence of engraftment, treatment-related mortality and overall survival (OS). Results from 316 transplants from 11 different countries are presented. Ninety-five (30%) were under 1 year of age at the time of transplant. OS was 83% and event-free survival was 76%; 3-year OS and event-free survival of infants below 1 year were 79% and 73%, respectively. No association was found with age at transplant, dose of treosulfan given, other agents used in combination with treosulfan, donor type, stem cell source, or second or subsequent transplant. In this report of the largest number of children to date receiving treosulfan for non-malignant diseases, treosulfan is shown to be a safe and effective agent even for those under 1 year of age at the time of transplant. Further prospective studies are needed using precisely defined protocols with pharmacokinetic monitoring and detailed chimerism analysis. In addition, long-term studies will be vital to determine long-term effects, for example, on fertility in comparison with other regimens.

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INTRODUCTION

Treosulfan (L-treitol-1,4-bis-methanesulfonate) is the pro-drug of L-epoxybutane, a water-soluble bifunctional alkylating agent, which has been used as an antineoplastic agent for treating ovarian carcinoma in particular for many years. Owing to its' myeloablative and immunosuppressive properties, it has been shown to provide effective haematopoietic stem cell transplant (HSCT) conditioning with reduced risk of toxicities, in particular veno-occlusive disease (VOD), compared with traditional combinations of busulfan and cyclophosphamide.[1,2]

It was first used in an HSCT setting in a group of adult patients with haematologic malignancies considered ineligible for other myeloablative preparative regimens in combination with either cyclophosphamide[3] or fludarabine.[4] Good outcomes with respect to toxicity, achievement of complete donor chimerism, low GvHD rate and low treatment-related mortality and relapse rates were shown. Since then, treosulfan has increasingly been used for paediatric patients undergoing HSCT for both malignant and non-malignant diseases.[5–12]

An increasing number of patients with non-malignant disorders are eligible for HSCT and these patients present different challenges compared with those with malignant diseases: children with inherited disorders such as SCID often come to transplant as infants under 1 year of age with organ damage and co-morbidities. GvHD, which may be associated with a beneficial GvL effect in patients with high-risk haematological diseases, is of no added value in controlling the underlying genetic illness and may adversely affect subsequent immune reconstitution and have an unnecessarily negative impact on HSCT-related morbidity and quality of life in the short and long term. Concerns about relapse in high-risk malignant diseases do not apply[13] but mixed chimerism in high-risk haemoglobinopathies can be a problem.[14] Treosulfan-containing regimens achieving a high rate of stable donor engraftment in the required lineage, with a reduced regimen-related toxicity, and low rate of GvHD are therefore attractive.[5]

A retrospective study of children registered in the European Society for Blood and Marrow Transplantation (EBMT) database, who received treosulfan as part of their preparative regimen for HSCT between January 2005 and July 2010 was conducted. Patients with non-malignant disease are reported here on behalf of the Paediatric Disease and the Inborn Errors working parties of EBMT.

PATIENTS AND METHODS

There were 843 patients below the age of 18 years, who underwent HSCT between January 2005 and July 2010 registered in the EBMT database,

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who were eligible for this retrospective study. The survey was performed in the Autumn of 2011 and the analysis was done at the end of 2012. The study was conducted in accordance with the EBMT Guidelines for retrospective studies. Questionnaires were sent to the respective centres but no toxicity data were received for 211 patients and 6 patients were excluded for whom no treosulfan dose was given. Five hundred and thirty-three allogeneic and 93 autologous transplants from 13 countries were included in the analysis. Three hundred and sixteen transplants in patients with non-malignant diseases from 11 countries (UK, Germany, Italy, The Netherlands, Poland, Czech Republic, Israel, Russia, Austria, Spain and Australia) are presented here. There were no autologous transplants for non-malignant diseases. Patient characteristics according to age at transplant, gender and number of transplants are shown in Table 1.

Ninety-five (30%) children were under 1 year of age at transplant. The numbers undergoing first, second and third transplants were 290, 23 and 3, respectively.

Diagnoses of the patients were inherited disorders 188 (59%), including 144 with primary immunodeficiencies, 39 metabolic disorders and 5 not specified, haemoglobinopathies 70 (22%), histiocytic disorders 32 (10%), bone marrow failure 24 (8%) and autoimmune disease 2 (1%). Table 2 describes the age groups and the various disease categories.

Donors were matched sibling 69 (22%), matched other related 25 (8%), matched unrelated 39 (12%), mismatched related 29 (9%), mismatched unrelated 16 (5%) and unrelated not defined 138 (44%). Mismatch was defined as anything <9 out of 10 HLA identical. Stem cell sources were the bone marrow 167 (53%), bone marrow+cord progenitors 8 (2.5%), bone marrow+peripheral blood 3 (1%), cord progenitors 50 (15%), peripheral blood 87 (28%) and peripheral blood+cord progenitors 1 (0.5%).

The median total dose of treosulfan was 42 g/m^2 in three divided doses with a range from $<3\times11$ to $>3\times15$ g/m^2. Roughly equal numbers of patients received a median total dose of fludarabine 150 mg/m^2 (106), cyclophosphamide 200 mg/kg (98), or fludarabine+thiotepa median dose 8 mg/kg (104) in addition to treosulfan. Eight patients received fludarabine and melphalan in addition to treosulfan. Choice of additional agents was dependent on the centre preference, as there were no defined protocols for different diseases at the time beyond the inborn errors working party guidelines, which can be found on the EBMT website. Most children received GvHD prophylaxis containing CsA ($n=284$, 90%), combined with mycophenolate mofetil in ($n=110$, 35%) or methotrexate ($n=101$, 32%). Acute and chronic GvHD were graded according to the Seattle criteria.[15]

Early regimen-related toxicity until day +100 was defined and graded using the Short Name based on the Common Terminology Criteria for Adverse Events v3.0, available online at: http://ctep.cancer.gov/forms/. The median follow-up time calculated using the reverse Kaplan–Meier estimator, which also takes into account the patients who died, was 2.16 (range 0.02–6.37) years; the median follow-up time of all survivors was 2.08 years (range 0.09–6.37). There were eight survivors with follow-up of <0.5 years.

Table 1. Patient characteristics according to age at transplant, sex and number of transplants

	Total	*Number of HSCT*	
		First HSCT	*>First HSCT*
Total	316	290	26
Age (years)			
<0.5	40	39	1
	13%	13%	4%
0.5–1	55	54	1
	17%	19%	4%
1–12	189	169	20
	60%	58%	77%
>12	32	28	4
	10%	10%	15%
Gender			
Male	198	179	19
	63%	62%	73%
Female	118	111	7
	37%	38%	27%

Abbreviation: HSCT = haematopoietic stem cell transplantation.

Statistical methods

The univariate statistical analysis was performed in several prospectively identified subgroups defined by diagnosis, conditioning regimen, number of HSCT and patient age. Kaplan–Meier estimates and log-rank test[16,17] were used to evaluate overall survival (OS). For OS, deaths from any cause were considered an event. Cumulative incidences of events were calculated by the method of Fine and Gray[18] for censored data subject to competing risks, and compared using the Gray test.[19] The cumulative incidence of neutrophil engraftment was calculated taking into account the competing risks of lost graft, death without engraftment and subsequent HSCT, and the cumulative incidence of transplant-related mortality—taking into account the deaths after graft loss and subsequent HSCT without treosulfan-based conditioning. Mantel–Haenszel χ^2-test and χ^2-test were used to compare categorical non-time-to-event variables: toxicities and acute GvHD at day +100 and chronic GvHD at 1 year. Further, a multivariate analysis was performed to study the impact of possible confounding factors on the defined outcomes. Cox regression[11,12] was used to model the time to transplant-related mortality and OS. The statistical analysis was done with SAS System V9.2 (2008, SAS Institute, Cary, NC, USA). All *P*-values below 5% were considered significant.

RESULTS

Age, diagnosis, other conditioning drugs, GvHD prophylaxis and stem-cell source correlated significantly with treosulfan dose. Younger patients were more likely to receive a lower dose. More patients received the higher dose rather than the lower dose for all diagnoses. When thiotepa was added, it was almost always with the higher dose of treosulfan and fludarabine.

Table 2. Diagnosis according to age

Diagnosis/age	*Total*	*<0.5 Years*		*0.5–1 Year*		*1–12 Years*		*> 12 Years*	
	N	N	%	N	%	N	%	N	%
Total	316	40	13%	55	17%	189	60%	32	10%
Inherited disorders	188	35	19%	44	23%	99	53%	10	5%
Haemoglobinopathies	70	0	0%	2	3%	53	76%	15	21%
Histiocytic disorders	32	5	16%	8	25%	16	50%	3	9%
Bone marrow failure	24	0	0%	1	4%	19	79%	4	17%
Autoimmune disease	2	0	0%	0	0%	2	100%	0	0%

Abbreviation: *N* = number.

Table 3. Early regimen-related toxicity

	Number evaluated	*Grade 0*	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Stomatitis (%)	314	35	31	16	6	12
Diarrhoea (%)	316	31	22	20	13	14
Vomiting (%)	314	35	22	27	7	9
Respiratory (%)	313	72	4	12	2	10
Bilirubin (%)	315	66	16	10	7	1
AST (%)	315	27	29	22	19	3
VOD (%)	313	95	3	2	0	0
CNS (%)	314	87	3	6	1	3
PN (%)	310	92	4	1	1	2

Abbreviations: AST = aspartate transaminase; CNS = central nervous system; PN = peripheral neurological; VOD = veno-occlusive disease.

Early regimen-related toxicity

Early regimen-related toxicity is shown in detail in Table 3. Stomatitis, diarrhoea, vomiting, respiratory toxicity, elevated bilirubin, elevated aspartate transaminase, central nervous sytem (CNS) toxicity and peripheral neurological toxicity $\geqslant$ grade 3 occured in 18% (56/314), 27% (86/316), 16% (49/314), 12% (38/313), 8%(27/315), 22% (71/315), 4% (12/314) and 3% (8/310) of patients, respectively. Grade 4 toxicity of any kind was observed in $<20\%$ of patients.

Table 4 describes toxicity in correlation with treosulfan dose, age, diagnosis and number of HSCT.

Gastrointestinal. There was no association between grade 3 or 4 vomiting or diarrhoea with age, dose, diagnosis, first or subsequent transplant, donor type or additional conditioning agents.

Stomatitis was worse when an additional third conditioning agent, that is, thiotepa or melphalan was used (data not shown).

Respiratory. All grades of respiratory toxicity were higher in children below the age of 1 year. There was less respiratory toxicity in the children with haemoglobinopathies than in other diagnoses. For inherited disorders with the highest number of patients under 1 year of age (79), there was no significant difference between those less than or over 6 months of age but just for those less than 1 year compared with over a year of age.

Liver. There was no association between raised bilirubin and age, dose, diagnosis, first or subsequent transplant, donor type, stem cell source or additional conditioning agents.

Aspartate transaminase was more likely to be elevated in those with haemoglobinopathies and histiocytic disorders, and second transplants. There was no statistically significant association between VOD and age, dose, diagnosis, 1st or subsequent transplant, donor type, stem cell source or additional conditioning agents. Only 5% of patients developed VOD grade 1 or 2 and no patients had grade 3 or 4 VOD.

Neurological. Children under 6 months of age were more likely to have CNS toxicity limited to grade 1–2. There was no association between peripheral neurotoxicity and age, dose, diagnosis, first or subsequent transplant, donor type or additional conditioning agents.

GvHD

Thirty eight per cent (121/316) of patients had acute GvHD but only 10% had grade 3 or 4. There was no significant difference in the incidence of acute GvHD grade 3 or 4 between age groups, treosulfan dose, donor type, stem cell source and additional conditioning drugs used. There was a significantly higher incidence of chronic GvHD in histiocytic disorders than in other diseases as shown in the Supplementary Table.

Non-engraftment/graft loss

Incidence of graft failure was low ($n=16$, 5.1%) and no significant associated factors were found. One of four patients who had $<3\times11$ g/m^2 failed to engraft having received a cord blood for a metabolic disorder with fludarabine, although six of six, who received $<3\times9$ g/m^2, engrafted and only one of them had thiotepa in addition to fludarabine, one had treosulfan with cyclophospamide. The majority of mismatched family donor recipients had treosulfan with cyclophosphamide (12) or with fludarabine and thiotepa (11), one of whom had graft loss. One mismatched family donor recipient who had treosulfan with fludarabine and melphalan also had graft loss. Three recipients with fludarabine alone engrafted successfully. Further details are shown in the Supplementary Table.

Survival

There was no significant difference in OS regarding diagnosis, age at transplant or with dose of treosulfan given (Figures 1, 2, 3). The very small group of six patients with other combinations of conditioning drugs (these patients received additionally melphalan and fludarabine) for first transplant had significantly worse OS than the other patient subgroups (Figure 4). Figures for event-free survival are provided as Supplementary Material. The 3-year OS for 59 patients with thalassaemia was 91% with an event-free survival of 77%. The 3-year OS and event-free survival for 11 patients with sickle cell anaemia was 100%.

Data on the cause of death were missing for 5 of 51 deaths. Of the remaining 46, the following were the main causes: Infection (19), interstitial pneumonitis (7), multi-organ failure (6), haemorrhage (6), GvHD (5), graft rejection or poor graft function (3), pulmonary toxicity (3), cardiac toxicity (1) and CNS toxicity (1).

Using multivariate analysis, we could not identify any significant association of diagnosis, age, dose, number of HSCT donor or stem cell source with outcome (Table 5).

DISCUSSION

The use of treosulfan for conditioning for HSCT in children with non-malignant disorders is increasing, in particular in patients with inherited disorders and haemoglobinopathies.[5,6,9,14,20,21] In this retrospective EBMT study, we evaluated the toxicity profile and outcome of 316 patients with non-malignant diseases undergoing HSCT, following treosulfan-based conditioning. To our knowledge, this is the largest such study to date. The 3-year OS in this cohort was 0.83 (±0.02), which is consistent with other published series.[4,5,7,8,15–17] Treosulfan is shown to be a safe and effective

Table 4. Early regimen-related toxicity in correlation with treosulfan dose, age, diagnosis and number of HSCT

	Treosulfan dose(g/sqm)				*Age at HSCT (years)*					*Diagnosis*							*Number of HSCT*		
Toxicity ⩾ grade 3 (%)	*< 3*11* n = 15*	*3*11-3*13* n = 90*	*> 3*13* n = 211*	P	*< 0.5* n = 40*	*0.5–1* n = 55*	*1–12* n = 189*	*> 12* n = 32*	P	*Immune deficiencies* n = 144*	*Disorders of metabolism* n = 39*	*Thalas saemia* n = 59*	*Sickle cell anaemia* n = 11*	*Histiocytic disorders* n = 32*	*Bone mar. failure* n = 24*	P	*First* N = 290*	*> First* N = 26*	P
Stomatitis	4 (27)	17 (19)	35 (17)	0.60	3 (8)	11 (20)	33 (18)	9 (28)	0.16	22 (15)	9 (23)	12 (20)	2 (18)	7 (23)	4 (17)	0.85	52 (18)	4 (15)	0.73
Diarrhoea	4 (27)	22 (24)	60 (28)	0.78	8 (20)	14 (25)	58 (31)	6 (19)	0.33	43 (30)	6 (15)	17 (29)	2 (18)	10 (31)	7 (29)	0.54	79 (27)	7 (27)	0.97
Vomiting	3 (20)	9 (10)	37 (18)	0.23	4 (10)	7 (13)	31 (16)	7 (22)	0.52	26 (18)	6 (15)	8 (14)	1 (9)	4 (13)	4 (17)	0.92	45 (16)	4 (15)	0.97
Respiratory	2 (13)	14 (16)	22 (10)	0.48	9 (23)	10 (18)	17 (9)	2 (6)	0.04	23 (16)	3 (8)	1 (2)	0 (0)	7 (23)	4 (17)	0.02	36 (13)	2 (8)	0.47
Bilirubin	3 (20)	8 (9)	16 (8)	0.25	5 (13)	4 (7)	15 (8)	3 (9)	0.8	16 (11)	2 (5)	1 (2)	1 (9)	6 (19)	1 (4)	0.08	23 (8)	4 (15)	0.2
AST	4 (27)	17 (19)	50 (24)	0.63	7 (18)	8 (15)	47 (25)	9 (28)	0.3	27 (19)	9 (23)	20 (34)	3 (27)	10 (31)	2 (8)	0.09	59 (20)	12 (46)	0.003
CNS	1 (7)	3 (3)	8 (4)	0.82	2 (5)	2 (4)	7 (4)	1 (3)	0.97	6 (4)	1 (3)	2 (3)	1 (9)	1 (3)	1 (4)	0.96	11 (4)	1 (4)	1
PN	0	4 (5)	4 (2)	0.35	3 (8)	1 (2)	4 (2)	0	0.15	4 (3)	2 (5)	0 (0)	2 (18)	0 (0)	0 (0)	0.01	8 (3)	0	0.39

Abbreviations: AST = aspartate transaminase; CNS = central nervous system; PN = peripheral neurological.

Figure 1. No significant difference in 3-year OS with diagnosis.

Figure 2. No significant difference in 3-year OS with age at transplant.

Figure 3. There was no significant difference in OS with dose of treosulfan.

agent for such children, even those under 1 year of age at the time of transplant. Multivariate analysis showed no association of transplant-related mortality with age at transplant, dose of treosulfan given, other agents used in combination with treosulfan, type of donor, stem cell source or even second or subsequent transplant. It is of particular interest that the addition of thiotepa to treosulfan and fludarabine did not increase acute toxicity. This combination, which has gained popularity in matched donor transplantation in haemoglobinopathies, also facilitated the engraftment of mismatched donors in a variety of diseases. The very small group of six patients who received melphalan in addition to treosulfan and fludarabine for first transplant did significantly worse. It is not clear why this occurred as other patients have tolerated this combination quite well.[11]

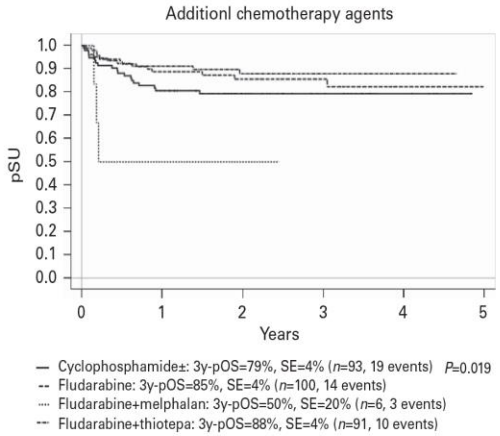

Figure 4. OS according to additional chemotherapy agents given for first transplants.

Toxicity was not associated with an increasing dose of treosulfan. There was more respiratory toxicity in the patients under 1 year of age, which may be due to the large number of primary immunodeficiencies in this group, who may have had respiratory infections. Pre-existing respiratory impairment in children with SCID is known to be associated with a worse outcome.[22]

CNS toxicity, although transient in all cases, was more common in the children under 6 months of age. These very young children are on many other drugs at the time of transplant, in particular calcineurin inhibitors. In addition, viral encephalitis as a consequence of SCID could have an additional role. Treosulfan and its' active monoepoxide have been found at low concentration in the cerebrospinal fluid of rats given treosulfan[23] and it is also possible that in very young babies the blood barrier may be less mature.

There was very little overall toxicity and very little graft loss, and therefore it is difficult to establish the optimal doses for different age groups of children. Our study has the limitations of being a registry-based retrospective study. There is a lack of data available on the use of serotherapy and levels of chimerism achieved. Further prospective studies are needed in non-malignant diseases using precisely defined protocols with pharmacokinetic monitoring and detailed chimerism analysis.[24] This study confirms a low incidence of CNS toxicity and VOD with treosulfan, both of which may give treosulfan an advantage over busulfan; however, there are recent promising results for some busulfan-containing regimens, in particular with carefully targeted low-dose busulfan in combination with fludarabine, which seems to be associated with very limited toxicity.[25] It is therefore essential that studies are performed looking at the long-term effects of these drugs, for example, on fertility, and comparing treosulfan-containing with busulfan-containing regimens.

Table 5. Multivariate analysis

	OS			*TRM*		
	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*
Diagnosis (vs inherited disorders)	0.13			0.87		
Bone marrow failure	0.35	0.6	0.2–2.0	0.87	0.9	0.2–3.3
Haemoglobinopathies	0.05	0.4	0.1–1.0	0.40	0.6	0.2–1.9
Histiocytic disorders	0.50	1.3	0.6–3.0	0.80	0.9	0.3–2.7
Number of HSCT (vs first HSCT)	0.94			0.59		
>First HSCT	0.94	1.0	0.4–2.9	0.59	0.7	0.2–2.9
Age (vs 1–12 years)	0.22			0.26		
0–1 Year	0.17	1.6	0.8–3.3	0.67	1.2	0.5–2.8
>12 Years	0.19	1.8	0.7–4.5	0.10	2.3	0.9–6.1
Treosulfan dose (vs> 3*11-3*13 g/sqm)	0.82			0.97		
3×11–3×13 g/sqm	0.53	0.8	0.4–1.6	0.82	0.9	0.4–2.0
<3×11 g/sqm	0.92	0.9	0.2–4.0	0.95	1.1	0.2–4.7
Conditioning (vs fludarabine+thiotepa)	0.003			0.13		
Cyclophosphamide±	0.67	1.2	0.5–2.7	0.25	1.8	0.7–4.6
Fludarabine	0.34	0.7	0.3–1.6	0.83	1.1	0.4–3.1
Other	0.00	7.3	2.1–26.0	0.03	6.0	1.2–30.1
Donor (vs other)	0.45			0.39		
MSD	0.45	0.7	0.3–1.8	0.39	0.6	0.2–1.9
Stem cell source (vs PB)	0.41			0.63		
BM	0.62	0.8		0.41	0.7	0.3–1.6
CB	0.18	0.5		0.45	0.7	0.3–1.9

Abbreviations: BM = bone marrow; CB = cord blood; CI = confidence interval; HR = hazard ratio; HSCT = haematopoietic stem cell transplantation; MSD = sibling donor; OS = overall survival; PB = peripheral blood; TRM = related mortality.

CONFLICT OF INTEREST

The EBMT received an unrestricted grant for the retrospective data collection and analysis from medac GmbH. CP received travel grants and study support from medac. MS received travel grants from medac. All other authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Arnaud Dalassier for data correction and management.

AUTHOR CONTRIBUTIONS

CP and UP designed the study. MS, HB, AL, PV, ARG, UP and CP contributed equally to this manuscript. KWS and PS contributed to the study and manuscript. EG provided additional statistical support.

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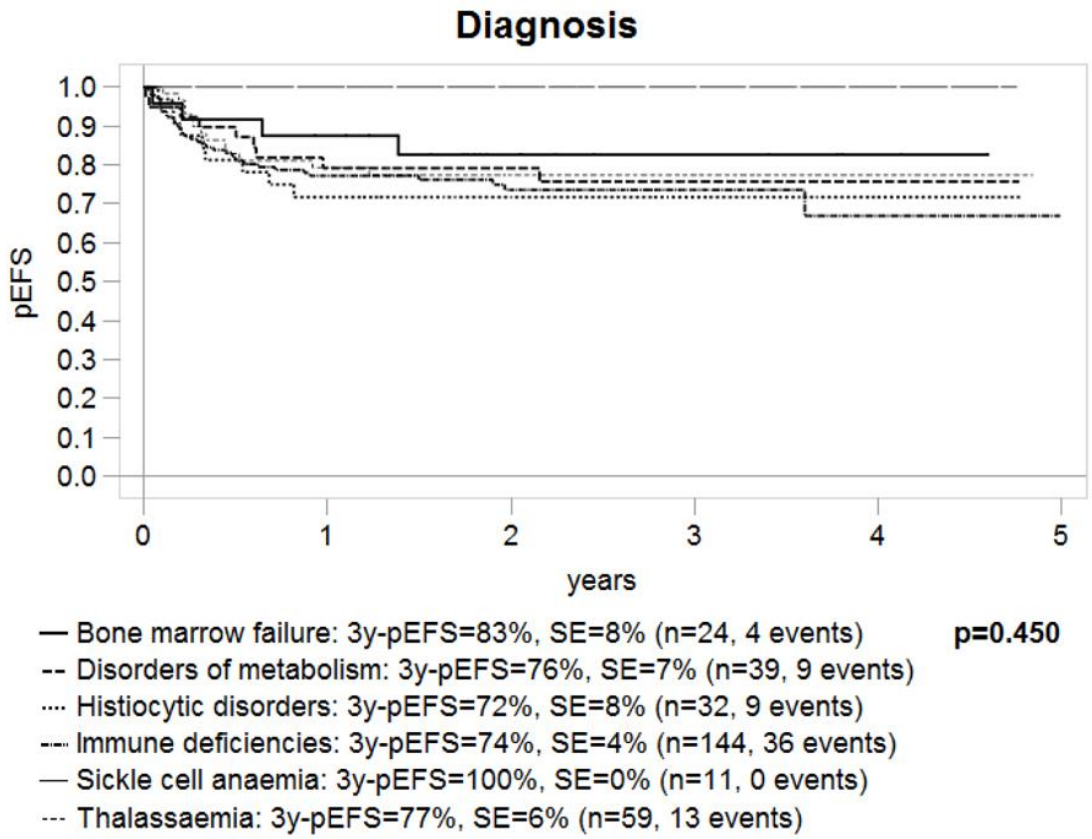
Supplementary Information accompanies this paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)

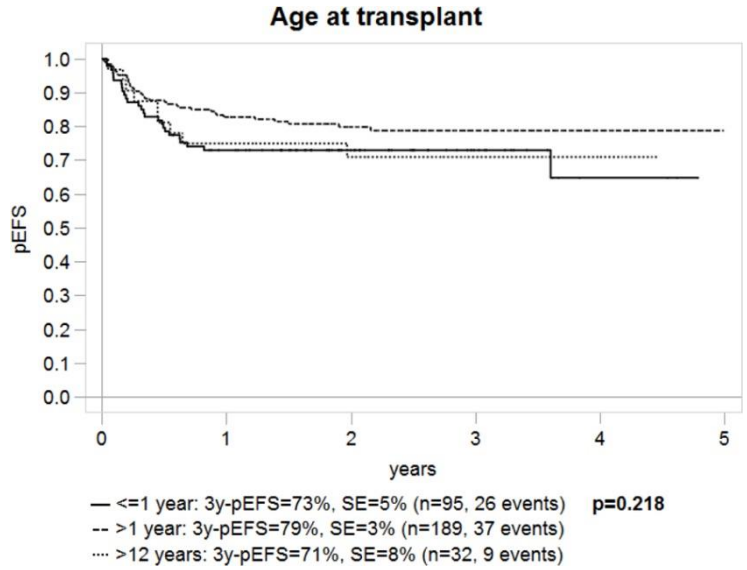
2.3.6 Supplementary Table. Graft failure

	Total n	No engraftment N	Graft loss n	p value Chi-Square test
All	316	6	10	0.115
Age <0.5 yr	40	0	0	
0.5-1yr	55	1	1	
1-12yr	189	3	7	
>12yr	32	2	2	
Dose				0.100
<=3*9g/sqm	6	0	0	
3*9-3*11g/sqm	9	2	0	
3*11-3*13g/sqm	90	1	1	
3*13-3*15g/sqm	207	3	9	
>3*15g/sqm	4	0	0	
Diagnoses				0.334
Inherited disorders	188	4	3	
Hemoglobinopathies	70	1	6	
Histiocytic disorders	32	0	1	
Bone marrow failure	24	1	0	
Auto-immune disease	2	0	0	
1st HSCT	290	5	8	0.119
>1st HSCT	26	1	2	
Donor				0.358
MSD	69	2	3	
Other	247	4	7	
Source				0.715
BM	167	3	5	
CP	50	2	0	
PB	87	1	5	
Other	12	0	0	
Additional agent				0.051
Cyclophosphamide±	98	2	3	
Fludarabine	106	3	0	
Fludarabine+Melphalan	8	1	1	
Fludarabine+Thiotepa	104	0	6	

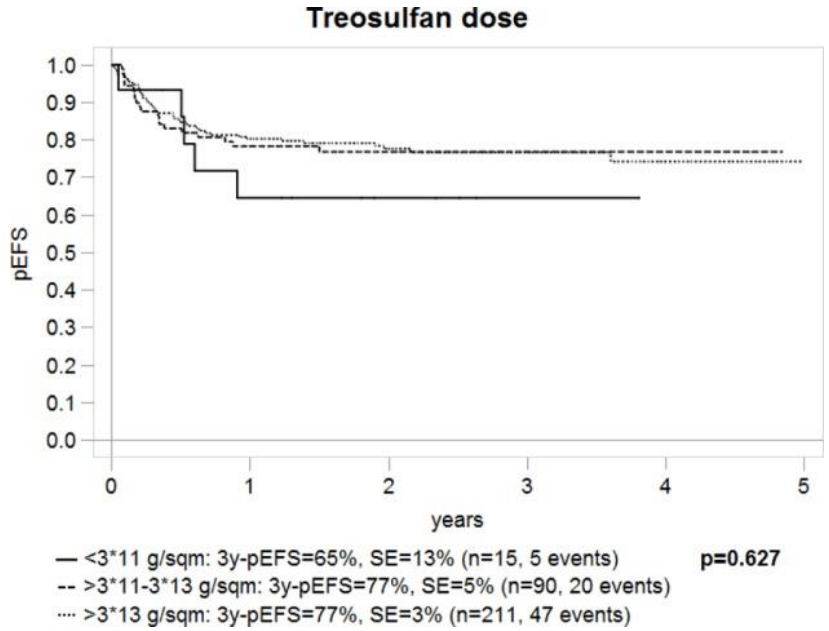
2.3.7 Supplementary Figure 1



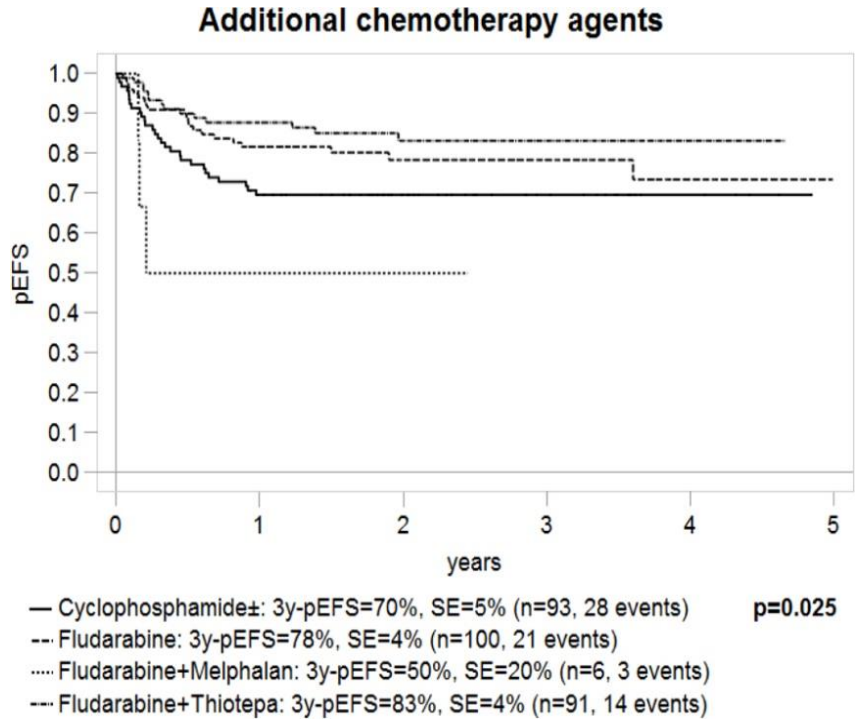
2.3.8 Supplementary Figure 2



2.3.9 *Supplementary Figure 3*



2.3.10 *Supplementary Figure 4*



2.3.11 Short discussion of strengths and limitations

- This study reported the largest number of children receiving treosulfan-based conditioning regimens for HSCT with non-malignant diseases. It was a collaboration between the Inborn errors and Paediatric diseases working parties of the EBMT and demonstrated the utility of the EBMT registry for studying a large group of patients from a large number of centres. It was highlighted in the ASBMT news (see Appendix B).
- This study was however limited by being a retrospective study. No data were available on the use of serotherapy, viral reactivation or on the levels of chimerism achieved. Choice of additional agents to treosulfan was according to centre preference so roughly a third received treosulfan with fludarabine, a third with cyclophosphamide and a third with fludarabine and thiotepa. GvHD prophylaxis also varied. There was little overall toxicity and only 5.1% graft failure and no correlation between dose of treosulfan given and outcome. Whilst the results were excellent showing safety and efficacy of treosulfan in a variety of non-malignant diseases, these variations make it difficult to make specific recommendations about precise components of protocols for transplant going forward.
- Further prospective studies are needed using precisely defined protocols with pharmacokinetic monitoring and detailed chimerism analysis together with long-term follow up.

2.4 PP4.

Morillo-Gutierrez B *et al.* 2016 (Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience)

Title: Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience

Authors: Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, Gibson B, Sedlacek P, Krol L, Strahm B, Zaidman I, Kalwak K, Talano JA, Woolfrey A, Fraser C, Meyts I, Muller I, Wachowiak J, Bernardo ME, Veys P, Sykora KW, Gennery AR and **Slatter M**

Journal: Blood Volume 128, Number 3, Pages 440-448

Impact factor: 17.543

Date of publication: 23 May 2016

2.4.1 Overview

CGD is an inherited disorder in which impaired microbial killing results in recurrent bacterial and fungal infections and impaired inflammatory cytokine regulation leads to granuloma formation and inflammation such as colitis. HSCT can cure CGD. Initial results using toxic myeloablative conditioning regimens had a high rate of mortality and morbidity and so reduced intensity or reduced toxicity regimens have been developed. In 2014 Güngör *et al* on behalf of the IEWP of EBMT, published impressive results using a reduced dose busulfan regimen in combination with fludarabine. In view of our good results using treosulfan in combination with fludarabine for a wide variety of PIDs including CGD we decided to gather the world-

wide experience of using treosulfan-based conditioning specifically for patients with CGD.

2.4.2 What was known

- There are many published studies on the value of HSCT as a curative treatment for patients with CGD.
- Patients with CGD often have severe co-morbidities such as bacterial and fungal infection together with inflammation such as colitis prior to transplant, which causes them to be at risk of toxicity, morbidity and mortality.
- Patients with CGD are at risk of rejecting a HSCT unless they are conditioned with a myeloablative regimen.
- Outcome from HSCT was shown to be excellent using a reduced busulfan regimen with fludarabine.
- A number of publications showed safety and efficacy of treosulfan with fludarabine for a variety of PIDs including a small number of patients with CGD.

2.4.3 What this study added

- This study reported the largest number to date of children with CGD who had undergone HSCT with treosulfan-based conditioning.
- The OS and EFS at a median follow up of 34 months were 91.4% and 81.4% respectively which were both excellent compared to previous literature.
- There were no serious toxicities with the exception of expected chemotherapy-related myelosuppression. In particular there were no cases of VOD.

- Results were similar to those reported by Güngör *et al.* who reported a study of 56 adults and children with CGD who received low dose busulfan combined with fludarabine prior to HSCT with an OS of 93% and EFS 89% at a median follow up of 21 months.
- Treosulfan-based conditioning is a safe treatment option in children with CGD even in patients with high risk problems prior to HSCT, regardless of donor type.

2.4.4 Contribution of the candidate to this work

I was the senior author on this paper. I designed the study, collected and analysed the data with BMG and ARG and wrote the manuscript. I assisted in editing the manuscript following critical review by the co-authors. A copy of the Newcastle University co-authorship form can be found in the Appendix.

Regular Article

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TRANSPLANTATION

Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience

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Key Points

- Treosulfan, a low-toxicity alkylating agent, can be used effectively as part of conditioning for HSCT in children with CGD.
- Long-term follow-up is required to ascertain effects, particularly on gonadal function and compare with other regimens.

Chronic granulomatous disease (CGD) can be cured by allogeneic hemopoietic stem cell transplantation (HSCT). Complications include graft failure, graft-versus-host disease (GVHD), infection, and transplant-related mortality; therefore, reduced-intensity conditioning regimens are being used to improve outcomes. In this retrospective study, the aim was to determine the outcome of treosulfan-based conditioning in HSCT for pediatric patients with CGD. The following data were collected: risk features pre-HSCT, additional conditioning agents, donor type and stem cell source, toxicity, engraftment, GVHD, chimerism, viral reactivation, post-HSCT complications, length of follow-up, and outcome. Seventy patients (median age, 107 months; interquartile range [IQR], 46-232 months) from 16 centers worldwide were transplanted between 2006 and 2015. Ninety-one percent had high-risk features. Fifty-seven HLA-matched donors, 12 HLA-mismatched donors, and 1 CD3$^+$TCR αβ/CD19 depleted parental haploidentical transplants were performed. No major toxicity was reported. Median times to neutrophil and platelet engraftment were 17 (IQR, 15-35) and 16 (IQR, 13-50) days. At a median follow-up of 34 months (IQR, 13-102 months), the overall survival was 91.4%, and event-free survival was 81.4%. The cumulative incidence of acute grade III-IV GVHD was 12%. Nine patients developed chronic GVHD. When split cell chimerism was available, 95% or more myeloid donor chimerism was documented in 80% of surviving patients. Secondary graft failure occurred in 12% of patients. Treosulfan-containing conditioning regimens can be used safely in HSCT for children with CGD and high-risk clinical features, achieving excellent survival with high myeloid chimerism. Further studies are needed to compare with other regimens and evaluate the long-term outcome, particularly on fertility. (*Blood*. 2016;128(3):440-448)

Introduction

Chronic granulomatous disease (CGD) is a primary immunodeficiency in which mutations in genes encoding 1 of the 5 subunits of the enzyme nicotinamide adenine dinucleotide phosphate oxidase lead to failure of microbicidal oxygen metabolite generation.[1] This causes impaired microbial killing, which leads to severe life-threatening bacterial and fungal infections. In addition, impairment in the regulation and termination of pro-inflammatory cytokine-mediated signals cause granuloma formation and inflammation.[1] Despite rigorous antibiotic and antifungal prophylaxis and treatment of inflammatory complications, ongoing medical problems are common in pediatric and adult patients, with a significant disease-related mortality.[2-5]

Hematopoietic stem cell transplantation (HSCT) can cure CGD with resolution of infections and inflammatory complications.[5-7] In addition, growth and quality of life are improved in transplanted patients compared with those treated conservatively.[4,8] Historically, high-risk patients with ongoing infectious or active inflammatory complications at HSCT had considerable transplant-related mortality up to 38%.[9,10] Efforts to reduce the toxicity of the conditioning regimen

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There is an Inside *Blood* Commentary on this article in this issue.

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were complicated by high rates of autologous reconstitution and graft-versus-host disease (GVHD).[10,11]

Recently, there has been increasing interest in the use of reduced-intensity conditioning regimens for patients with primary immunodeficiency,[12] and specifically for those with CGD.[13] These regimens cause minimal toxicity and achieve high rates of cure, even in patients with underlying infections and/or organ dysfunction.

Treosulfan, a bifunctional alkylating agent with myeloablative and immunosuppressive effects, has been increasingly used as 1 of the main conditioning agents for HSCT for children with malignant and non-malignant disorders in some European and US centers.[14-18] It has a low-toxicity profile, with the most commonly reported acute toxicities being skin, including nappy rash; diarrhea; mucositis; and hepatic toxicity; however, these are generally mild, and importantly, veno-occlusive disease (VOD) is very rare.[19] Long-term effects are not well-documented because of the relatively recent introduction of the drug for conditioning for HSCT.

The purpose of this retrospective analysis was to determine the outcome of treosulfan-based conditioning for patients with CGD. We report a multicenter pediatric series of 70 patients with CGD who underwent HSCT, using treosulfan as the main agent for conditioning.

Patients, materials, and methods

Data collection

Centers identified through the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation that had performed HSCT for CGD, using a treosulfan-based conditioning regimen, were asked to participate in the retrospective study.

Data were submitted for 70 patients from 16 centers in 9 countries worldwide (United Kingdom, Germany, Belgium, Poland, Czech Republic, Italy, Israel, United States, and Australia) after a questionnaire distributed by the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation.

The following data were collected: risk features before HSCT, additional conditioning agents to treosulfan, donor type, stem cell source and number, toxicity (presence of skin toxicity, neurotoxicity, or VOD), platelet and neutrophil engraftment, occurrence of acute GVHD (aGVHD) after Glucksberg criteria and/or chronic GVHD[20] (cGVHD), donor chimerism with lineage-specific chimerism CD3, CD19/CD20 and CD15/CD33 when available, viral reactivation (cytomegalovirus [CMV], Epstein-Barr virus, adenovirus, human herpesvirus 6), other HSCT complications, length of follow-up, and outcome.

Data submission and analysis were performed between February 2014 and October 2015. For the analysis of the parameters platelet and neutrophil engraftment, occurrence of GVHD, and donor chimerism, 1 patient was excluded, as he died on day +1. The 8 patients receiving a second procedure were included in the analysis of GVHD, as none of them developed either aGVHD or cGVHD after the first transplant.

All patients or their guardians gave written consent according to local center and European Society for Blood and Marrow Transplantation guidelines.

Patient characteristics

Sixty-six of the 70 patients were male. Two patients with long histories of recurrent infection were 19 years old (232 months) at the time of HSCT, the rest were younger than 18 years, with a median age of 9 years (107 months; interquartile range [IQR], 46-232 months). Fifty-six had X-linked CGD, and 11 were reported as having autosomal recessive (AR) disease: 4 cytochrome b-245, α polypeptide (CYBA); 4 neutrophil cytosolic factor 1; and 1 neutrophil cytosolic factor 2. For the other 2 patients, even after being extensively investigated, no mutation was found; however, based on the family history, females affected, and dihydrorhodamine pattern, X-linked inheritance was excluded. For 3 patients, this information was not available.

All except 6 patients had ongoing or previous radiologically and microbiologically proven infection or autoinflammation, defined as high-risk criteria pre-HSCT. Among the 64 (91%) patients who had these high-risk criteria, 34 patients had more than 1. The most frequently reported complication was infection in 52 patients, with previous microbiologically proven *Aspergillus* in 12, followed by colitis in 35, chronic lung disease in 15, and failure to thrive in 10 patients. Other significant symptoms reported in 10 patients were bladder inflammation, previous splenectomy, McLeod phenotype, pericardial effusion, thymus abscesses, allergic bronchopulmonary aspergillosis, recurrent hemophagocytic lymphohistiocytosis, and brain lesions with the syndrome of inappropriate antidiuretic hormone secretion. In addition, 5 patients had failed a previous transplant, 1 of whom has been previously reported.[21]

Transplantation

Fifty-six patients received a transplant from an unrelated donor (URD), 13 from a 10/10 HLA-matched related donor (MRD), (12 siblings [matched sibling donor] and 1 family [matched family donor]), and 1 received a $CD3^+$ TCR α $β^+/CD19^+$ depleted haploidentical parental transplant (Table 1). Among the URD recipients, 12 received grafts that were less than 10/10 HLA matched (11 URD [9/10] and 1 cord blood [4/6]), and 44 received 10/10 HLA-matched grafts.

Patients received bone marrow (n = 36), G-CSF mobilized peripheral blood stem cells (n = 33), or umbilical cord blood (n = 1) grafts. The median number of $CD34^+$ hematopoietic stem cells administered was 8.50×10^6/kg (IQR, 4.5-34.5 $\times 10^6$/kg).

The choice of conditioning regimen was institutionally dependent, with treosulfan as the primary myeloablative agent. There were 2 main groups: 46 (66%) patients received treosulfan, fludarabine ± serotherapy with either antithymocyte globulin (ATG) or alemtuzumab, and 24 patients received other regimens, with 15 patients receiving treosulfan, fludarabine, thiotepa ± ATG or alemtuzumab.

Standard total doses of treosulfan were 42 g/m^2 or 36 g/m^2, guided mainly by age and center preference. Fifty-nine patients older than 12 months received 42 g/m^2, and 7 received 36 g/m^2. All 4 patients younger than 12 months received 36 g/m^2. The administration was in 3 doses from day −6 to day −4. No pharmacodynamic parameters of treosulfan were evaluated.

Fifty-seven patients received either ATG (n = 18) or alemtuzumab (n = 39) compared with 13 who did not receive any serotherapy.

With regard to the dose and timing of additional conditioning agents and serotherapy, it was variable, depending on center preference.

For GVHD prophylaxis, 62 patients received cyclosporin A, alone or with either mycophenolate mofetil in 45 (1 with additional methylprednisolone) or methotrexate in 13 patients. Eight patients received tacrolimus and methotrexate.

Statistical analysis

Overall survival (OS) and event-free survival (EFS) were described by Kaplan-Meier estimates. The Log-rank test was applied for the comparison between transplants from HLA-matched related and unrelated donors. Significance of results was determined using χ-squared test with Yates correction, using 2×2 contingency tables (GraphPad Prism 6; GraphPad Software, Inc., La Jolla, CA).

Results

Survival

Sixty-four patients are alive, with a median follow-up of 34 (IQR, 13-102) months, giving an OS of 91.4% (Figure 1). The 2-year probability of survival was 90.48% (95% confidence interval, 79.86%-95.65%). There was no significant difference in OS between those who received an URD graft and those who received a MRD transplant (92.9% vs 85.7%; $P = .255$).

Of the 6 deaths resulting from transplant related mortality, only 2 occurred in the first 100 days post-HSCT, highlighting the low toxicity of the regimens (Table 2).

Table 1. Patient characteristics

Patient	CGD type	Sex	Age at HSCT, mo	High-risk clinical	Donor (HLA match)	Stem cell source	Conditioning used	CD34/kg body weight, $\times 10^6$	Toxicity	GHVD, grade	Complications	Latest chimerism, % donor	Outcome/length of follow-up, mo
1	CYBB	M	4,4	Yes	URD 10/10	PBSC	Treo Cy ATG	21.99	N	Skin II	CMV	100	Alive/102
2	CYBB	M	6	Yes	MSD	BM	Treo Flu TT	8.50	N	N	No major complication	74	Alive/39
3	CYBB	M	7	No	URD 10/10	BM	Treo Flu A	12.50	Resolved limited skin rash	N	Pneumonitis	T, 83; B, 100; CD15, 100	Alive/21
4	CYBB	M	7	Yes	MSD 10/10	BM	Treo Flu TT	15	N	Resolved limited cGVHD	EBV	T, 98; CD15, 96	Alive/7
5	CYBB	M	9,6	Yes	URD 9/10	PBSC	Treo Cy ATG	6.34	N	Skin II, generalized extensive cGVHD	CMV EBV	100	Died/11
6	CYBB	M	11	Yes	URD 10/10	PBSC	Treo Flu A	29.40	Resolved limited skin rash	N	No major complication	T, 71; B, 24; CD15, 66	Alive/32
7	CYBB	M	12	No	URD 10/10	BM	Treo Flu ATG	6.89	N	Skin II	No major complication	100	Alive/39
8	CYBB	M	14	Yes	URD 10/10	PBSC	Treo Cy A	5/3.3	N	N	Pneumonitis; adenovirus HHV6	100	Top up 2 mo; alive/79
9	CYBB	M	17	Yes	Cord 4/6	Cord	Treo Flu ATG TBI	1.11	N	Gut II	No major complication	100	Alive/25
10	CYBA	M	18	Yes	URD 10/10	BM	Treo Flu A	1.84	N	Skin, liver II	Adenovirus	31	Alive/13
11	CYBB	M	18	Yes	URD 10/10	PBSC	Treo Flu A	1.60	N	Skin II	No major complication	T, 40; CD15, 14	Alive/57
12	CYBB	M	18	Yes	URD 10/10	BM	Treo Flu ATG	20.45	N	Skin II	CMV EBV	T, 89; B, 86; CD15, 100	Alive/6
13	CYBB	M	12.8	Yes	URD 10/10	PBSC	Treo Flu Cy ATG	17.86	N	Skin/liver/gut IV	CMV	100	Died/2
14	CYBB	M	33	No	URD 10/10	BM	Treo Flu TT	3.53	N	N	No major complication	100	Alive/32
15	CYBB	M	35	Yes	URD 10/10	BM	Treo Flu ATG	8.83	N	N	No major complication	T, 95; B, 99; CD15, 100	Alive/5
16	NCF2	M	37	Yes	URD 10/10	PBSC	Treo Flu A	10.10	N	Skin I	CMV, Guillain Barre	100	Retransplanted 18 mo; alive/54
17	CYBA	M	44	Yes	α β TCR depleted father	PBSC	Treo Flu TT A	34.50	Resolved limited skin rash	Skin I	Adenovirus HHV6 CMV EBV	T, 84; B, 100; CD15, 100	Alive/11
18	CYBB	M	45	Yes	URD 10/10	PBSC	Treo Flu A	10.00	Resolved limited skin rash	Skin I	No major complication	100	Alive/46
19	CYBB	M	46	Yes	URD 10/10	BM	Treo Flu ATG	7.77	N	Skin II	Adenovirus CMV	100	Alive/34
20	CYBB	M	46	Yes	MSD	BM	Treo Cy	10.80	N	N	No major complication	54	Alive/47
21	NCF1	M	46	Yes	URD 10/10	BM	Treo Flu A	4.40	N	Skin II	Pneumonitis	T, 93; B, 100; CD15, 100	Alive/51
22	CYBB	M	47	Yes	URD 9/10	BM	Treo Flu A	2.34	Idiopathic epilepsy	N	No major complication	NA	DLI + retransplanted 3 m; alive/46
23	CYBB	M	49	Yes	URD 10/10	BM	Treo Flu A	10.67	N	N	No major complication	39	Alive/10
24	AR	M	55	Yes	URD 10/10	PBSC	Treo Flu A	9.95	N	N	No major complication	100	Alive/4
25	CYBB	M	55	Yes	MSD	BM	Treo Flu TT	15.00	N	N	EBV	100	Alive/76
26	CYBB	M	59	Yes	URD 10/10	PBSC	Treo Flu A	6.80	N	Gut II	Adenovirus CMV	100	Alive/21

A, alemtuzumab; AIHA, autoimmune hemolytic anemia; AR, autosomal recessive; ATG, anti-thymocyte globulin; AVN, avascular necrosis; BM, bone marrow; CGD, chronic granulomatous disease; CMV, cytomegalovirus; Cy, cyclophosphamide; CYBA, cytochrome b-245, α polypeptide; CYBB, cytochrome b-245, β polypeptide; DLI, donor lymphocyte infusion; EBV, Epstein-Barr virus; F, female; Flu, fludarabine; GVHD, graft-versus-host disease (C, chronic); HHV6, human herpesvirus 6; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; M, male; MFD, matched family donor; MSD, matched sibling donor; N, none; NA, not applicable; NCF1, neutrophil cytosolic factor 1; ND, not determined; PBSC, peripheral blood stem cells; RI, radioimmunotherapy; TBI, total body irradiation; TCR, T cell receptor; TMA, thrombotic microangiopathy; Treo, treosulfan; TT, thiotepa; URD, unrelated donor.

Table 1. (continued)

Patient	CGD type	Sex	Age at HSCT, mo	High-risk clinical	Donor (HLA match)	Stem cell source	Conditioning used	CD34/kg body weight, $\times 10^6$	Toxicity	GHVD, grade	Complications	Latest chimerism, % donor	Outcome/length of follow-up, mo
27	CYBB	M	60	Yes	URD 10/10	PBSC	Treo Flu A	20.04	N	Skin II	No major complication	100	Alive/39
28	CYBB	M	63	Yes	URD 9/10	BM	Treo Flu A	6.10	N	N	AIHA CMV	NA	Retransplanted 46 mo; alive/50
29	CYBB	M	64	Yes	URD 10/10	BM	Treo Flu TT ATG	3.75	N	N	EBV	100	Alive/5
30	NCF1	F	74	Yes	MFD	BM	RI Treo Flu	10.40	N	cGVHD, resolved	CMV colitis and pneumonitis; agranulocytosis	100	Alive/75
31	CYBB	M	75	No	MSD	BM	Treo Flu TT	12.00	N	N	No major complication	100	Alive/69
32	CYBB	M	76	Yes	MFD	BM	Treo Flu	3.09	N	N	No major complication	T, 47; B, 31; CD15, 40	Alive/34
33	CYBB	M	79	Yes	URD 10/10	BM	Treo Flu	7.42	N	Gut III	No major complication	100	Alive/56
34	CYBB	M	86	Yes	URD 10/10	PBSC	Treo Flu	9.40	N	Skin II	Disseminated *Aspergillus*	100	Alive/54
35	CYBB	M	88	Yes	URD 10/10	PBSC	Treo Flu A	13.00	N	Skin II	No major complication	T, 98; B, 100; CD15, 100	Alive/35
36	CYBB	M	103	Yes	URD 10/10	BM	Treo Flu TT ATG	1.70	N	Skin I	Recurrent bilateral otitis media	100	Alive/71
37	CYBB	M	106	Yes	URD 10/10	PBSC	Treo Flu A	14.00	Resolved limited skin rash	Skin I	CMV	100	Alive/39
38	CYBA	M	107	No	URD 9/10	BM	Treo Flu TT	6.94	N	IV	Adenovirus HHV6 CMV EBV	100	Alive/18
39	CYBB	M	110	Yes	MSD	BM	Treo Flu	2.60	NA	NA -event-	Capillary leak; macrohematuria	NA	Died day +1
40	CYBB	M	113	Yes	URD 9/10	BM	Treo Flu A	0.60	N	N	Immune pancytopenia	89	Alive/54
41	CYBB	M	114	No	URD 10/10	PBSC	Treo Flu A	10.50	N	N	Pneumonitis	T, 89; B, 85; CD15, 87	Alive/49
42	CYBB	M	118	Yes	URD 10/10	PBSC	Treo Flu A	4.50	N	N	No major complication	100	Alive/29
43	CYBB	M	119	Yes	MSD	BM	Treo Flu	7.50	N	Skin/liver/gut IV; extensive cGVHD	Adenovirus	100	Died/10
44	CYBB	M	128	Yes	URD 10/10	PBSC	Treo Flu A	16.00	N	Skin/liver/gut III	Adenovirus	100	Top up 23 m; died/23
45	CYBB	M	129	Yes	URD 10/10	PBSC	Treo Flu A	28.78	N	cGVHD, resolved	No major complication	100	Alive/60
46	ND	M	132	Yes	URD 10/10	PBSC	Treo Flu A	5.29	N	N	CMV	100	Alive/46
47	CYBB	M	132	Yes	URD 10/10	PBSC	Treo Flu TT A	10.00	Resolved severe perineal rash	N	No major complication	NA	DLI + retransplanted 5 mo; alive/23
48	CYBB	M	133	Yes	URD 9/10	BM	Treo Flu A	3.90	Proximal myopathy	N	CMV	100	Alive/46
49	CYBB	M	139	Yes	URD 10/10	PBSC	Treo Flu A	15.72	N	N	No major complication	100	Alive/4
50	CYBB	M	141	Yes	URD 10/10	PBSC	Treo Flu A	7.65	N	N	AIHA	NA	Retransplanted 7 mo; alive/34
51	CYBB	M	144	Yes	URD 10/10	PBSC	Treo Flu A	5.10	N	Skin II, resolved limited cGVHD	CMV HHV6	100	Alive/46
52	CYBB	M	145	Yes	URD 9/10	BM	Treo Flu TT ATG	3.40	N	N	no major complication	NA	DLI 7 mo; alive/30

A, alemtuzumab; AIHA, autoimmune hemolytic anemia; AR, autosomal recessive; ATG, anti-thymocyte globulin; AVN, avascular necrosis; BM, bone marrow; CGD, chronic granulomatous disease; CMV, cytomegalovirus; Cy, cyclophosphamide; CYBA, cytochrome b-245, α polypeptide; CYBB, cytochrome b-245, β polypeptide; DLI, donor lymphocyte infusion; EBV, Epstein-Barr virus; F, female; Flu, fludarabine; GVHD, graft-versus-host disease (C, chronic); HHV6, human herpesvirus 6; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; M, male; MFD, matched family donor; MSD, matched sibling donor; N, none; NA, not applicable; NCF1, neutrophil cytosolic factor 1; ND, not determined; PBSC, peripheral blood stem cells; RI, radioimmunotherapy; TBI, total body irradiation; TCR, T cell receptor; TMA, thrombotic microangiopathy; Treo, treosulfan; TT, thiotepa; URD, unrelated donor.

Table 1. (continued)

Patient	CGD type	Sex	Age at HSCT, mo	High-risk clinical	Donor (HLA match)	Stem cell source	Conditioning used	CD34/kg body weight, $\times 10^6$	Toxicity	GHVD, grade	Complications	Latest chimerism, % donor	Outcome/length of follow-up, mo
53	NCF1	F	147	Yes	URD 10/10	PBSC	Treo Flu A	10.49	N	Skin I	Bilateral maxillary sinusitis	100	Alive/11
54	CYBB	M	147	Yes	MSD	BM	Treo Cy	4.40	N	N	No major complication	35	Alive/38
55	CYBB	M	163	Yes	URD 10/10	PBSC	Treo Flu A	16.27	N	N	EBV, red cell aplasia	CD3, 61; CD15, 100	Alive/14
56	NCF1	F	165	Yes	URD 10/10	BM	RI Treo Flu	4.90	N	Skin I	EBV, CMV enteritis	100	Alive/75
57	ND	M	173	Yes	URD 10/10	BM	Treo Flu ATG	8.28	N	Skin II	No major complication	T, 95; CD15, 100	Alive/33
58	CYBB	M	176	Yes	URD 9/10	PBSC	Treo Flu A	8.80	N	cGVHD, resolved	EBV	100	Alive/68
59	CYBB	M	177	Yes	MSD	BM	Treo Flu TT	11.00	N	N	No major complication	100	Alive/10
60	CYBB	M	180	Yes	URD 9/10	PBSC	Treo Flu A	17.78	N	N	CMV, EBV	100	Alive/64
61	CYBB	M	190	Yes	URD 10/10	PBSC	Treo Flu TT C	13.07	Resolved abdominal rash	N	No major complication	100	Alive/45
62	AR	M	191	Yes	MSD	BM	Treo Flu ATG	2.97	N	Skin II, controlled cGVHD on treatment	Multifocal AVN	100	Alive/33
63	CYBB	M	198	Yes	URD 10/10	PBSC	Treo Flu A	16.65	Resolved generalized rash	Skin I	CMV	100	Alive/15
64	ND	M	198	Yes	URD 9/10	PBSC	Treo Flu A	5.90	N	N	TMA CMV	100	Alive/34
65	CYBB	M	200	Yes	MSD	BM	Treo Flu A	5.30	N	N	CMV	T, 72; B, 82; CD15, 100	Alive/14
66	CYBB	M	202	Yes	URD 10/10	PBSC	Treo Flu A	13.39	N	Skin II	CMV	100	Alive/12
67	CYBB	M	206	Yes	URD 10/10	BM	Treo Flu TT ATG	2.10	N	N	CMV EBV PTLD	100	Alive/10
68	CYBB	M	208	Yes	URD 9/10	PBSC	Treo Flu A	18.45	Resolved generalized rash	Skin, gut III	Adenovirus, disseminated aspergillosis	NA	Died/4
69	CYBA	F	232	Yes	URD 10/10	BM	Treo Flu TT ATG	5.00	N	Skin, gut III	EBV -Rituximab- Suspected pulmonary aspergillosis	100	Alive/28
70	CYBB	M	232	Yes	URD 10/10	BM	Treo Flu ATG	3.37	N	Skin II; ongoing cGVHD on treatment	No major complication	100	Alive/41

A, alemtuzumab; AIHA, autoimmune hemolytic anemia; AR, autosomal recessive; ATG, anti-thymocyte globulin; AVN, avascular necrosis; BM, bone marrow; CGD, chronic granulomatous disease; CMV, cytomegalovirus; Cy, cyclophosphamide; CYBA, cytochrome b-245, α polypeptide; CYBB, cytochrome b-245, β polypeptide; DLI, donor lymphocyte infusion; EBV, Epstein-Barr virus; F, female; Flu, fludarabine; GVHD, graft-versus-host disease (C, chronic); HHV6, human herpesvirus 6; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; M, male; MFD, matched family donor; MSD, matched sibling donor; N, none; NA, not applicable; NCF1, neutrophil cytosolic factor 1; ND, not determined; PBSC, peripheral blood stem cells; RI, radioimmunotherapy; TBI, total body irradiation; TCR, T cell receptor; TMA, thrombotic microangiopathy; Treo, treosulfan; TT, thiotepa; URD, unrelated donor.

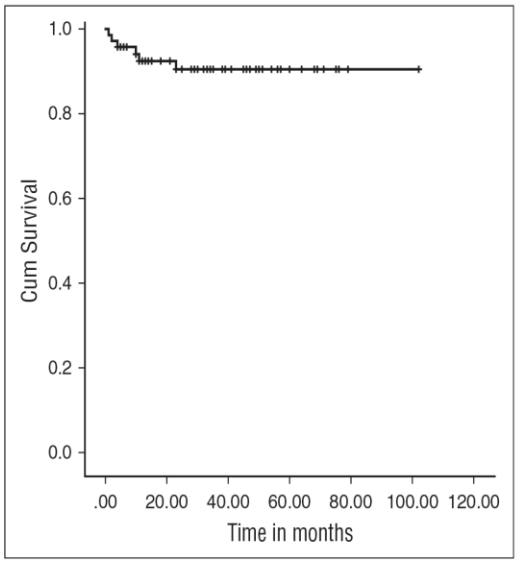

Figure 1. Kaplan-Meier survival curve. OS was 91.4% at a median follow-up of 34 months (IQR, 13-102 months).

One patient with severe multisystem inflammatory disease and previous *Aspergillus* infection developed multiorgan failure during conditioning and died on day +1. The remaining 5 deaths were associated with severe GVHD, severe infection, or both (Table 2).

Toxicity

There were no serious toxicities with the exception of the expected chemotherapy-related myelosuppression. Nine patients had limited skin toxicity, including perianal ulceration, pigment changes, and occasional peeling. Two had central nervous system toxicity other than tremor related to cyclosporin A (1 idiopathic epilepsy, 1 proximal myopathy).

No VOD occurred, even in patients who had undergone previous HSCT.

Engraftment

Platelet engraftment (first day of platelet $>20 \times 10^9$/L for 3 consecutive days) occurred at a median of 16 days (IQR, 13-50 days), and neutrophil engraftment (first day of neutrophils $>0.5 \times 10^9$/L for 3 consecutive days) at 17 days (IQR, 15-35 days) post-HSCT. One patient died on day +1, and 1 patient who died at 10 months post-HSCT did not achieve platelet engraftment, despite 100% donor chimerism.

Viral reactivations

Thirty-four patients (48.5%) had viral reactivation, with 11 of them having more than 1 virus isolated in the blood. CMV was present in 22, Epstein-Barr virus in 14, adenovirus in 8, and human herpesvirus 6 in 4 patients. Disseminated adenovirus infection contributed to the death in 2 patients, and influenza pneumonitis in 1. Among these 34 patients, 29 had received previous serotherapy, either with alemtuzumab (n = 19) or ATG (n = 10).

Graft-versus-host disease

Twenty-seven patients (39%) developed aGVHD grade I-II, and the cumulative incidence of GVHD grade III-IV was 12% (8 patients). The incidence of grade III-IV GVHD was 7.6% for the MRD recipients (n = 1) and 12.5% for the URD recipients (n = 7). This difference was not statistically significant ($P = .082$).

Nine patients (13%) developed cGVHD: in 4 this was limited to the skin; in 3 it was extensive in the skin, joints, and muscle; and in 2 it was extensive in the skin, gut, and liver. In 5 patients, cGVHD has resolved and patients are off immunosuppression, 1 is receiving a weaning dose of immunosuppressive treatment with no symptoms, 1 has ongoing symptoms in spite of treatment, and 2 with extensive disease of skin, gut, and liver died. There was an unexpectedly higher cumulative incidence of cGVHD in the MRD group, at 30.7% (n = 4), compared with the URD group, at 16% (n = 9; $P = .035$). Three of these MRD recipients did not receive any serotherapy.

Among the URD recipients, there was no statistically significant difference in incidence of death, second procedures, severe aGVHD, or cGVHD between those recipients of mismatched unrelated donor and those who received matched unrelated donor grafts: there were 2 deaths in each group ($P = .20$) and 3 procedures in the mismatched unrelated donor compared with 5 in the matched unrelated donor recipients ($P = .35$). Two patients in the mismatched unrelated donor group developed aGVHD III-IV compared with 5 in the matched unrelated donor group ($P = .64$), and 2 in each group had cGVHD ($P = .2$).

There was a statistically significant difference in incidence of grade I-II aGVHD comparing serotherapy use versus none (ATG [n = 11] vs alemtuzumab [n = 16] vs nil [n = 0]; $P = .002$); this difference was not significant, depending on the serotherapy agent (ATG vs nil, $P < .0001$; alemtuzumab vs nil, $P = .006$; ATG vs alemtuzumab, $P = .158$).

The patients who received serotherapy and developed grade III-IV aGVHD or cGVHD were n = 5 and n = 6, respectively, compared with those who did not receive any; n = 3 for each type of GVHD, but the differences were not statistically significant ($P = .19$ for grade III-IV aGVHD and $P = .305$ for cGVHD).

Complications

Other significant complications reported were autoimmune hemolytic anemia (2 patients), immune pancytopenia (1 patient), thrombotic microangiopathy (1 patient), multifocal avascular necrosis (1 patient), Guillain-Barré syndrome (1 patient), and disseminated *Aspergillus* infection (1 patient).

Events and chimerism

Eight patients required second procedures, resulting in an EFS of 81.4% at the median follow-up of 34 months (IQR, 13-102) (Figure 2). The 2-year probability of EFS was 81.03%, (95% confidence interval, 68.79%-88.85%). All these patients had received a total dose of treosulfan of 42 g/m^2 with the exception of 1 who received 36 g/m^2.

There was no significant difference between URD and MRD recipients (80.4% vs 85.7%; $P = .490$). Moreover, we found there was no difference in incidence of secondary graft failure with progressive decreasing chimerism in those patients with (4/22) or without (9/48) CMV reactivation ($P = .9$).

Second procedures included 2 boosts from the original donor without further conditioning; 1 of the patients died after developing severe extensive GVHD and disseminated adenovirus infection. Three received donor lymphocyte infusions, and 5 underwent a second HSCT (of whom 2 had received donor lymphocyte infusions) (Table 3).

In 64 patients for whom data were available, last reported donor myeloid chimerism was higher than 95% in 51 patients (80%), and T lymphoid chimerism was higher than 95% in 48 patients (75%) (Figure 3). The remaining 6 patients were excluded for the following reasons: the patient who died on day +1; 1 of the patients who received an additional procedure for decreasing chimerism, whose chimerism

Table 2. Deaths

Age, mo	Time posttransplant, mo	High-risk features	Donor	Chimerism (%)	Cause of death
110	D+1	Y	MSD	NA	Severe MOF Stroke
114	11	Y	URD 9/10	100	Pneumonia cGVHD
119	10	Y	MSD	100	Adenovirus a/cGVHD G-IV
128	23	Y	MUD	100	Influenza pneumonitis aGVHD G-III
152	2	Y	MUD	100	Acute cardiac/pulmonary failure aGVHD G-IV
208	4	Y	URD 9/10	100	Disseminated *Aspergillus* Disseminated Adenovirus

GVHD, graft-versus-host-disease (a: acute; c: chronic); MOF, multiorgan failure; MSD, matched sibling donor; MUD, matched unrelated donor; URD, unrelated donor.

pretransplant was not available; and 4 who had whole-blood chimerism below 100% but split chimerism was not reported. For these 4 patients, the whole-blood chimerism was 89%, 74%, 54%, and 39% respectively.

The patients did not have a neutrophil oxidative function performed routinely; myeloid chimerism higher than 50% can be considered enough for recovery of neutrophil function in the CGD.

Conditioning agents and serotherapy

All the centers used treosulfan as the main condition agent, but otherwise the regimens were quite heterogeneous; 15 patients had additional thiotepa, but there was no significant difference in terms of graft failure (n = 2; $P = .794$), higher percentage of myeloid chimerism (n = 12; $P = .3146$), or deaths (n = 0; $P = .3289$) compared with those who did not receive thiotepa (n = 6, n = 34, and n = 6, respectively).

Fifty-seven patients received either ATG or alemtuzumab compared with 13 who did not receive any serotherapy.

No significant differences were found in deaths (n = 4 in the serotherapy group compared with n = 2 in nonserotherapy; $P = .470$) or graft failures (n = 8 vs n = 0, respectively; $P = .140$).

Genetic type

As stated earlier, there were 56 patients with X-linked CGD and 11 patients with autosomal recessive CGD. For 3 patients, the information was not available, so they have been excluded from this analysis. Of the X-linked patients with CGD, 51 had high-risk features pretransplant, as did 10 of the patients with AR disease. All deceased patients had X-linked CGD, as did 7 of the 8 patients who needed a second procedure. There is a 100% survival and 91% EFS in the AR group compared with 89.3% and 87.5%, respectively, in the X-linked group; these differences were not significant when analyzed (survival $P = .255$; EFS $P = .75$).

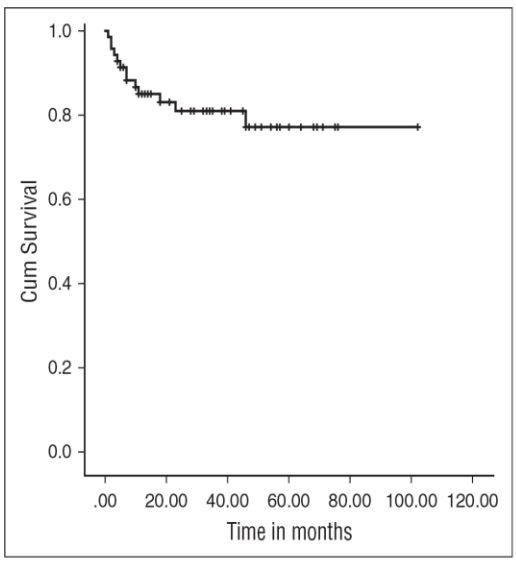

Figure 2. Kaplan-Meier EFS curve. EFS was 81.4% at a median follow-up of 34 months (IQR, 13-102 months).

Discussion

Without HSCT, patients with CGD face life-threatening infections and other complications that lead to shortened life span and diminished life quality.[2-5] Although HSCT is the only curative therapy, patients often have significant comorbidities, particularly molds and other infections that may contribute to transplant-related mortality. During the last 10 years, there have been multiple reports suggesting that treosulfan-based regimens were associated with reduced incidence of transplant-related mortality when used for conditioning patients with hematologic malignancies or other life-threatening disorders, including CGD.[14,16-18] However, we report the largest series to date of children with CGD who underwent allogeneic HSCT using treosulfan-based conditioning.

With a median follow-up of 34 months, the OS was excellent, at 91.4%, with an EFS of 81.4% in a high-risk group of patients in this study (nearly all of the patients had significant pre-HSCT risk factors including infection or infections and/or inflammation). In addition, there were no differences in OS or EFS between matched and mismatched donor grafts or the addition of thiotepa to the conditioning regimen.

Because of the retrospective nature of the study, no toxicity scale was used, but in the reported cases, the toxicity was minimal; in particular, no VOD occurred even in patients receiving a second transplant. Two patients had neurotoxicity, and 9 had mild skin toxicity, which resolved.

Some comparisons can be made with the results shown by Güngör et al,[13] who published the largest prospective study of a reduced-intensity conditioning regimen for HSCT for this disease in a cohort of pediatric and adult patients (median age, 12.7 years; IQR, 6.8-17.3 years). Using a sub-myeloablative total dose of busulfan, and at a median follow-up of 21 months, the OS was 93% and EFS 89%, with very promising results in terms of low toxicity, sustained chimerism, and therefore, recovery of neutrophil function.

Eight patients in our cohort experienced secondary graft failure, 1 of whom died. The remaining patients had stable donor chimerism in myeloid and lymphoid lineages at last follow-up, compatible with cure of the underlying disease. We did not find a relationship between graft failure and CMV reactivation, as was recently reported.[22] In the study published by Güngör et al,[13] 3 patients experienced graft failure

Table 3. Second procedures

Age, mo	Time of event posttransplant, mo	High-risk features	Conditioning	CD34/kg body weight (×106)	Stem cell source	Donor	Type of event	Reason	Outcome
14	2	Y	Treo Cy A	5.00	PBSC	MUD	Top up	Decreasing donor chimerism	Alive, 100% chimerism
37	18	Y	Treo Flu A	10.10	PBSC	MUD	Second HCT: Bu/Flu	Decreasing donor chimerism	Alive, 100% chimerism
47	3	Y	Treo Flu A	2.34	BM	URD 9/10	DLI ×3 + second HCT: 9/10 Cord, Bu/Flu	Decreasing donor chimerism (0%)	Alive, 100% chimerism
63	46	Y	Treo Flu A	6.10	BM	URD 9/10	Second HCT: Bu/Flu	Decreasing donor chimerism	Alive. Stable donor chimerism
128	23	Y	Treo Flu A	16.00	PBSC	MUD	Top up	Pancytopenia with hypocellular marrow	Deceased
132	5	Y	Treo Flu TT A	10.00	PBSC	MUD	DLI ×3 + second HCT: Same donor Bu/Flu	Decreasing donor chimerism (20%)	Alive, 100% chimerism
141	7	Y	Treo Flu A	7.65	PBSC	MUD	Second HCT: Same donor Bu/Flu	Decreasing donor chimerism	Alive, stable donor chimerism
145	7	Y	Treo Flu TT ATG	3.40	BM	URD 9/10	DLI ×5	Decreasing donor chimerism	Alive, decreasing donor chimerism (CD15, 20%-40%; CD3, 70%-80%)

A, alemtuzumab; BM, bone marrow; Bu, busulfan; Cy, cyclophosphamide; DLI, donor lymphocyte infusion; Flu, fludarabine; MUD, matched unrelated donor; PBSC, peripheral blood stem cells; Treo, treosulfan; TT, thiotepa; URD, unrelated donor transplant.

and received a second HSCT; 2 of them successfully and 1 who died at day 10 after HSCT. In this series, 5 patients had previous graft failure after conditioning with fludarabine and melphalan (n = 1) and busulfan (n = 4).

There were 6 deaths in our cohort, 2 in the first 100 days, and all but 1 were associated either with severe GVHD or severe viral infection, or both. We cannot draw any conclusions regarding differences in age, other conditioning agents used, or donor type.

Although the overall incidence of aGVHD was high, at 51%, the incidence of severe acute grade III-IV GVHD was low, at 8%, compared with 16% in a report of children with malignancy, using treosulfan-based conditioning.[17] cGVHD developed in 9 patients (13%), 6 of whom are now free of symptoms. GVHD was not associated with use of serotherapy or the donor source. Further work needs to be done to determine optimal timing and dosing of serotherapy to minimize the risks for GVHD and viral reactivation.[23]

In Güngör's[13] study, grade III-IV aGVHD was only 4%, and cGVHD was present in 4 of 56 patients (7%), 3 of which were of pediatric age. Viral reactivation was reported in almost half of the patients, but resolved with appropriate therapy, and contributed to death in 2 patients. This highlights the importance of monitoring and preemptive therapy for viral reactivation in patients receiving serotherapy.

These results show that HSCT using a treosulfan-based conditioning regimen is a safe treatment option in pediatric patients with CGD, even in those with high-risk clinical features pre-HSCT or those with no HLA-identical family donor, as has been previously recommended. We observed high curative rates and minimal toxicity with excellent survival, comparable to low-intensity conditioning regimens.[24] A third of the patients in this study had additional agents to treosulfan, fludarabine, and serotherapy, and so further studies are needed to establish a consistent approach. The other main study using reduced-intensity conditioning published by Güngör et al[13] is prospective and includes a number of adult patients (11 out of 56 were aged 19 years or older) and a smaller proportion of X-linked patients with CGD than our cohort (60% vs 83%). Our results suggest that patients with X-linked CGD have a somewhat higher mortality and less successful transplants than those with AR-CGD, although this did not reach statistical significance. This could contribute to the small differences in survival, transplant success, and GVHD between the 2 studies. Prospective studies will be required comparing treosulfan with low-dose busulfan-based regimens, but will need to have lengthy follow-up to determine any differences in chimerism and long-term toxicity. Further studies are also needed to evaluate the pharmacokinetics of treosulfan to determine any correlation with area under the curve and donor chimerism,[19] and finally, studies are required to evaluate long-term toxicity, particularly looking at gonadal function.[25]

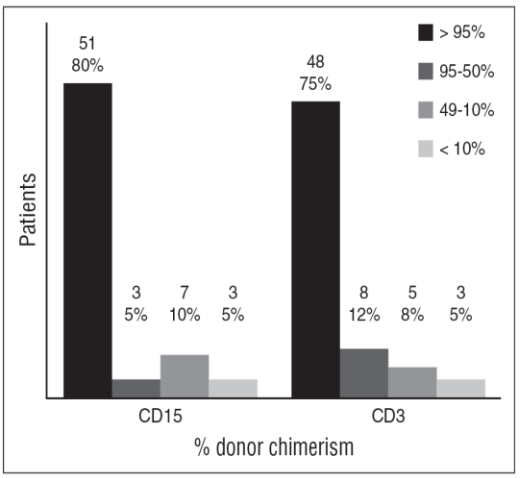

Figure 3. Split cell chimerism for CD15$^+$ and CD3$^+$ cells at last follow-up. Results given in absolute number and percentage over the 64 patients with available split chimerism. Those who had second procedures were included with their last result before the event. Those who died had the last result available before the death.

Acknowledgments

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Authorship

Contribution: B.M.-G., M.S., and A.R.G. designed the study, analyzed data, and wrote the paper; R.B., K.R., L.B., A.S., A.-M.E., B.G., P.S., L.K., B.S., I.Z., K.K., J.-A.T., A.W., C.F., I. Meyts, I. Müller, J.W., M.E.B., P.V., and K.-W.S. submitted data from their patients and reviewed and corrected the paper.

Conflict-of-interest disclosure: The patients from the United States were enrolled in a clinical trial that was supported in part by research funding from Medac, GmbH (Hamburg, Germany). In addition, Medac, GmbH provided Treosulfan for the study in the United States. J.W., K.-W.S., B.M.-G., A.R.G., and M.S. received travel grants from Medac.

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2.4.6 Short discussion of strengths and limitations

This manuscript was chosen for an expert commentary (Appendix C). Seventy patients with CGD from 16 centres worldwide were included in this study which represents a large number for such a rare disease. This study secured the incorporation of treosulfan-based conditioning into the IEWP of EBMT guidelines for conditioning for PID. However the retrospective nature of the study did limit some aspects of the results as centres vary in their practice. Additional agents used with treosulfan such as fludarabine, cyclophosphamide and thiotepa varied. GVHD prophylaxis also varied. Formal toxicity grading was not carried out and lineage specific chimerism was not available in all patients.

Further studies are needed to establish a consistent approach and long-term follow up is required to record late effects such as fertility. Formal prospective randomized trials comparing treosulfan- to busulfan-based conditioning are in progress for malignant and non-malignant conditions.

2.5 PP5.

Slatter M *et al.* 2018 (Treosulfan and Fludarabine conditioning for Hematopoietic stem cell transplantation in children with Primary Immunodeficiency: UK experience)

Title: Treosulfan and Fludarabine conditioning for Hematopoietic stem cell transplantation in children with Primary Immunodeficiency: UK experience

Authors: **Slatter MA,** Rao K, Abd Hamid IJ, Nademi Z, Chiesa R, Elfeky R, Pearce MS, Amrolia P, Worth A, Flood T, Abinun M, Hambleton S, Qasim W, Gaspar HB, Cant AJ, Gennery AR, Veys P

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2.5.1 Overview

This manuscript reported a retrospective study on the outcome of 160 children who underwent HSCT for PID in the Paediatric stem cell transplant unit in either Newcastle upon Tyne or Great Ormond Street Hospital in London. These units are the 2 supraregionally designated units for transplanting such children in the UK. All patients received homogenous conditioning chemotherapy prior to transplant with treosulfan, fludarabine and in the majority of cases Alemtuzumab. The following

outcomes were assessed: survival, need for second procedure, toxicity, GVHD, viral reactivation, chimerism and immune reconstitution. In addition a number of specific patient cohorts were studied.

2.5.2 What was known

- The use of reduced toxicity conditioning in patients with PID is preferred to avoid short and long-term complications from more intense historically used regimens.
- Stable mixed donor chimerism post-transplant can achieve cure in patients with PID.
- A number of studies had shown that Treosulfan is a safe and efficacious agent for conditioning children with non-malignant diseases.
- We had previously published results of 70 children with PID who received Treosulfan in combination either with cyclophosphamide or fludarabine. This study demonstrated that toxicity was worse and T cell chimerism lower when combined with cyclophosphamide. Therefore we had stopped using cyclophosphamide in combination with Treosulfan.

2.5.3 What this study added

- An excellent survival rate was achieved using the combination of treosulfan and fludarabine with low toxicity and good levels of donor chimerism.
- Donor myeloid chimerism was higher in recipients of PBSC compared to BM and CB. This is important in many diseases, but particularly in neutrophil disorders such as CGD and WAS who need good levels of myeloid chimerism to cure the underlying thrombocytopenia and tendency to autoimmunity.

Despite using PBSC which has been associated with an increased risk of cGVHD, in association with Alemtuzumab there was no increased risk of severe aGVHD or cGVHD.

- This regimen was shown to be safe for very young infants. Eleven patients with SCID diagnosed at birth due to previous family history were transplanted at the age of 4 months or younger and all survived with good immune reconstitution in 10. This is important because newborn screening for SCID is being introduced in many countries which will allow detection of babies with SCID at a younger age before infection and organ damage. HSCT is more successful if performed before infection and organ damage, but debate is ongoing within the transplant community as to the best approach in terms of conditioning these young infants.
- Patients with HLH had poor survival compared to other published studies. Of 16 patients only 7 survived (OS 44%). In particular the combination of using a CB for transplant without serotherapy led to a poor outcome and therefore we recommend serotherapy for all patients with HLH prior to transplant due to the hyperinflammatory nature of this disease.

2.5.4 Contribution of the candidate to this work

I designed the study with PV, ARG and KR. I collected all the data for the Newcastle patients and collated the data from Great Ormond Street Hospital. I analysed the data with IJAH and MSP. I wrote the whole manuscript and edited it after critical review by the co-authors. A copy of the Newcastle University co-authorship form can be found in the Appendix.

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Treosulfan and Fludarabine Conditioning for Hematopoietic Stem Cell Transplantation in Children with Primary Immunodeficiency: UK Experience

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A B S T R A C T

We previously published results for 70 children who received conditioning with treosulfan and cyclophosphamide (n = 30) or fludarabine (n = 40) before undergoing hematopoietic stem cell transplantation (HSCT) for primary immunodeficiency (PID). Toxicity was lower and T cell chimerism was better in the patients receiving fludarabine, but cohort numbers were relatively small and follow-up was short. Here we report outcomes of 160 children who received homogeneous conditioning with treosulfan, fludarabine, and, in most cases, alemtuzumab (n = 124). The median age at transplantation was 1.36 years (range, .09 to 18.25 years). Donors included 73 matched unrelated, 54 1 to 3 antigen-mismatched unrelated, 12 matched sibling, 17 other matched family, and 4 haploidentical donors. Stem cell source was peripheral blood stem cells (PBSCs) in 70, bone marrow in 49, and cord blood in 41. Median duration of follow-up was 4.3 years (range, .8 to 9.4 years). Overall survival was 83%. No patients had veno-occlusive disease. Seventy-four patients (46%) had acute GVHD, but only 14 (9%) greater than grade II. Four patients underwent successful retransplantation for graft loss or poor immune reconstitution. Another patient experienced graft rejection and died. There was no association between T cell chimerism >95% and stem cell source, but a significant association was seen between myeloid chimerism >95% and use of PBSCs without an increased risk of significant GVHD compared with other sources. All 11 patients with severe combined immunodeficiency diagnosed at birth were alive at up to 8.7 years of follow-up. Long-term studies are needed to determine late gonadotoxic effects, and pharmacokinetic studies are needed to identify whether specific targeting is advantageous. The combination of treosulfan, fludarabine, and alemtuzumab is associated with excellent results in HSCT for PID.

INTRODUCTION

The use of treosulfan as part of conditioning for hematopoietic stem cell transplantation (HSCT) in pediatric practice is increasing for both malignant [1-4] and nonmalignant disorders [5-15]. Treosulfan (L-treitol-1,4-bis-methanesulfonate) is the prodrug of L-epoxybutane, a water-soluble bifunctional alkylating agent with myeloablative and immunosuppressive properties [16] but with less systemic toxicity compared with standard doses of busulfan [17].

The use of reduced-toxicity conditioning is preferred in patients with primary immune deficiency (PID), in whom 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 comorbidities. In addition, many HSCT recipients are infants, who may be more susceptible to toxicity [18]. Less-toxic regimens may reduce early and late adverse effects, particularly effects on fertility [19,20]. Several reduced-toxicity regimens have been investigated in patients with PID [21-23]; initial results suggest

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that specific conditioning regimens may be preferable in certain PID diseases with severe comorbidities [24], with specific donor types and stem cell sources, and appear to have enhanced toxicity in children age <1 year [25].

We previously published results for 70 children with PID who received treosulfan in combination with either cyclophosphamide (n = 30) or fludarabine (n = 40), with an overall survival (OS) of 81% (median follow-up, 19 months), equivalent in those age <1 year and those age >1 year at time of transplantation. 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 was fairly short. Here we report 160 consecutive patients with prolonged follow-up who received homogeneous conditioning with treosulfan and fludarabine without additional agents such as thiotepa for a wide variety of PID diagnoses, using different types of donors and stem cell sources.

METHODS

Patients

We performed a retrospective study of 160 consecutive patients with PID who underwent HSCT at the 2 UK supraregional referral centers 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 on patient demographics, diagnosis, donor match and stem cell source, conditioning regimen, transplantation-related complications, graft-versus-host disease (GVHD), chimerism, immune reconstitution, outcome, and duration of follow-up. Patients were not randomized to receive a specific conditioning regimen and the conditioning regimen was chosen by the treating medical team. Informed consent was obtained from all parents according to the local center and European Society for Blood and Marrow Transplantation and Declaration of Helsinki guidelines.

HLA typing was performed by molecular typing for HLA class I and II loci. The unrelated donors were all 7 to 10/10 HLA-matched. The stem cell source was bone marrow (BM) in 49 patients, peripheral blood stem cells (PBSCs) in 70, and cord blood (CB) in 41. Peripheral blood was used for the 4 haploidentical transplants, using the CliniMACS system (Miltenyi Biotech, Surrey, UK) for CD3/CD19 depletion.

Treosulfan was given at a dose of 42 g/m^2 (n = 102), 36 g/m^2 (n = 54), or 30 g/m^2 (n = 4) in divided doses on 3 consecutive days. The lower dose of 36 g/m^2 was given to infants age <1 year and 30 g/m^2 to patients with severe combined immunodeficiency (SCID) who were diagnosed at birth and had undergone transplantation very early. All patients received fludarabine 150 mg/m^2 in 5 divided doses on consecutive days. Alemtuzumab at a total dose of .3 to 1.0 mg/kg was given to all patients except for 6 recipients of a matched sibling donor (MSD) graft, 1 recipient of haploidentical CD3/CD19-depleted PBSCs, and 30 recipients of CB grafts, including 3 who received ATG and 27 who received no serotherapy. This reflects the different approaches to the use of cord blood in the 2 centers [26,27]. In the majority of patients, GVHD prophylaxis consisted of cyclosporine with mycophenolate mofetil, which was weaned starting on day +28 in the absence of GVHD. Patients underwent weekly polymerase chain reaction (PCR) analysis 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 benchtop magnetic cell sorter (Miltenyi Biotec). Separated cells were assayed using variable number of tandem repeats (VNTR) or XY fluorescence in situ hybridization analysis for donor-recipient sex-mismatched transplants.

Statistics

Statistical analysis was performed using Stata version 15 (StataCorp, College Station, TX). Descriptive analyses were performed using frequency, mean, and median and range. Data were analyzed using Pearson chi-square and Kruskall-Wallis tests. Survival outcomes were evaluated with Kaplan-Meier estimates and the log-rank test. Censoring of patients was defined at time of death or last follow-up or of a second procedure for event-free survival. Multivariable logistic regression analysis was performed to evaluate factors influencing aGVHD and chimerism at last follow-up.

RESULTS

Our cohort comprised 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 1. The median age at transplantation was 1.36 years (range, .09 to 18.25 years). Seventy-six patients underwent HSCT at age ≤12 months. There was no significant difference in survival between these 76 children and children who underwent HSCT at age >12 months ($P = .30$).

Patients underwent HSCT from a 10/10 HLA-matched unrelated donor (MUD; n = 73), a 1 to 3 mismatched unrelated donor (MMUD; n = 54), MSD (n = 12), another matched family donor (MFD; n = 17), or haploidentical mismatched family donor (MMFD; n = 4) using treosulfan in combination with fludarabine 150 mg/m^2.

Survival

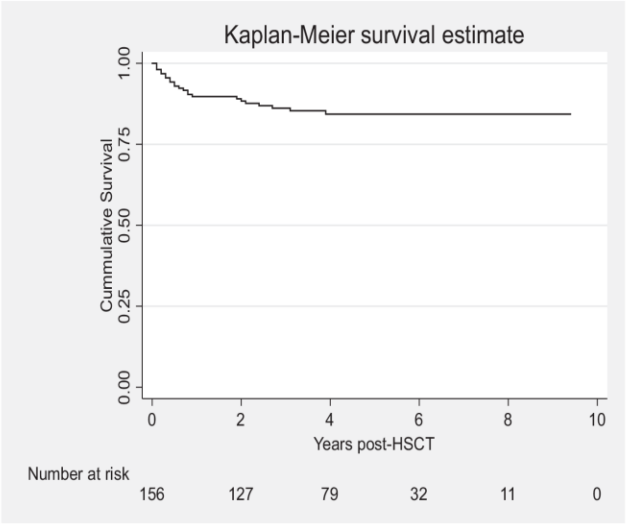
The median duration of follow-up was 4.3 years (range, .8 to 9.4 years). OS is shown in Figure 1. Twenty-seven children died, giving an OS of 83%. Only 10 died in the first 100 days (100-day survival, 94%). The probability of 2-year OS was 88.3% (95% confidence interval [CI], 82.1% to 92.5%).

Most deaths were associated with infection and/or GVHD and are detailed in Table 2. 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 tumor necrosis factor α inhibitor (infliximab) before transplantation.

Table 1
Patient Diagnoses

Diagnosis	Number
SCID	39
WAS	20
CGD	17
HLH	18
Major histocompatibility class II deficiency	7
Omenn syndrome	5
Cartilage hair hypoplasia	4
IPEX	3
CD40 ligand deficiency	3
Dedicator of cytokinesis 8 deficiency	3
Colitis	3
Leukocyte adhesion deficiency	3
Natural killer T cell deficiency	2
Zeta-chain-associated protein kinase 70 deficiency	2
Phosphatidylinositide 3-kinase deficiency	2
Severe immune dysregulation	9
Combined immunodeficiency	8
X-linked inhibitor of apoptosis deficiency	1
X lymphoproliferative-like syndrome	1
Autoimmune lymphoproliferative syndrome	1
Cytotoxic T lymphocyte antigen 4 deficiency	1
Interferon regulatory factor 8 deficiency	1
Fas-associated death domain protein deficiency	1
IL-2–inducible T cell kinase deficiency	1
Nuclear factor-κB essential modulator deficiency	1
Undefined neutrophil disorder	1
Hyper-IgE	1
CTP synthase1	1
Juvenile idiopathic arthritis	1

IPEX indicates immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.



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%)

Figure 1. OS.

Event-free survival is shown in Figure 2. An event was defined as death or an additional procedure. Four patients underwent successful retransplantation 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 undergoing retransplantation. An additional 5 patients received a boost without conditioning from the original donor, and another 3 patients received donor lymphocyte infusion. Details are shown in Table 4.

Table 2
Deaths

Diagnosis	Donor	Time post-HSCT	Cause (s)
HLH	MUD BM	Day -2	HLH, toxicity
CGD	MSD BM	Day +1	Severe inflammation, toxicity
HLH	MMUD CB	Day +7	Infection (parainfluenza 3)
Autoimmune enteropathy	MMUD CB	Day +23	Pulmonary hemorrhage
SCID, intestinal atresias	MMUD CB	1 month	Infection (*Pseudomonas*)
HLH	MUD CB	Day +34	Pulmonary hemorrhage
HLH	MMUD CB	1.4 months	Infection (parainfluenza 3)
HLH	MMUD CB	2 months	Infection
CID	MMUD CB	2 months	Multiorgan failure
Omenn syndrome	MUD CB	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 PTLD, EBV
HLH	MFD BM	8 months	GVHD
Autoimmune enteropathy	MSD BM	10 months	Infection (adenovirus), respiratory failure
HLH	MMUD CB	10 months	GVHD, infection (RSV)
IPEX	MMUD PBSC	11 months	Respiratory failure
Omenn syndrome	MUD BM	11 months	GVHD, cerebral infarcts
CGD	MUD PBSC	23 months	Infection (influenza) GVHD
X-linked inhibitor of apoptosis deficiency	MUD PBSC	24 months	Infection (JC virus, leukoencephalopathy)
CγC SCID thymectomy due to cardiac surgery	MFD BM	24 months	Respiratory failure after donor lymphocyte infusion
Omenn syndrome, RAG 1	MSD BM	25 mo	Pneumonitis, chronic lung disease
RAG SCID	MSD BM	33 mo	Infection while being treated for Ph+ pre-B cell ALL (absent donor myeloid and B cell chimerism)
HLH	MSD BM	36 mo	MDS/AML
SCID	MMUD CB	48 mo	Infection

CγC indicates common gamma chain; RAG, recombinatin activating gene; PTLD, post-transplantation lymphopoliferative disease; RSV, respiratory syncytial virus; JC, John Cunningham; Ph, Philadelphia chromosome; ALL, acute lymphocytic leukemia; MDS, myelodysplasic syndrome; AML, acute myelogenous leukemia.

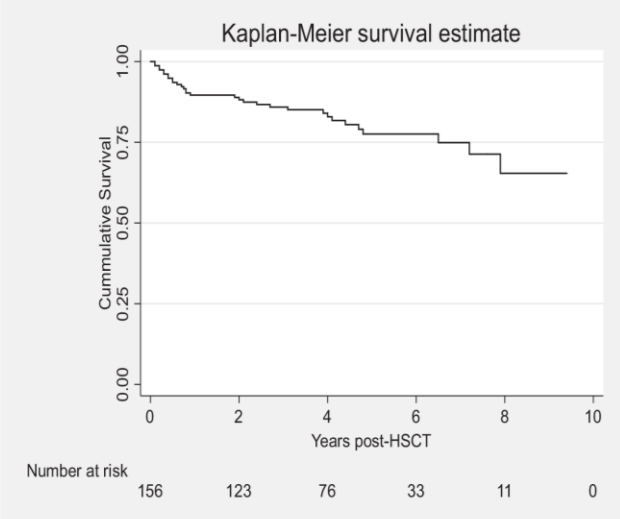


Figure 2. Event-free survival. An event was death or additional procedure.

Donor and Stem Cell Source

Survival according to type of donor and stem cell source is shown in Table 3. There was no significant difference in survival according to type of donor ($P = .5$) or stem cell source ($P = .23$).

There has been an increase in the use of PBSCs compared with BM (44% and 30.5%, respectively) compared with our previously published series (17% and 57%, respectively) [9]. The rate of use of CB has remained the same at 26%.

There were significant differences in median CD34$^+$ stem cell dose according to stem cell source ($P < .0001$). The median dose in CB was $.4 \times 10^6$/kg (range, .05 to 6.3×10^6/kg), that in BM was 5.8×10^6/kg (range, 1.1 to 19.5×10^6/kg) and that in PBSC was 13.7×10^6/kg (range, 2.0 to 63.8×10^6/kg).

Toxicity

Formal grading using the National Cancer Institute's toxicity criteria was not carried out, because it was not standard practice at the time in our centers. 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 occurred.

GVHD

Seventy-four patients (46%) 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 the incidence of aGVHD or cGVHD and stem cell source ($P = .37$). Twenty-seven of the 41 patients who received CB stem cells did not receive serotherapy and experienced a particularly high rate of both aGVHD (22 [82%], but only 2 [7%] with grade III/IV) and cGVHD (9 of 27; 33%). There was a significantly higher incidence of cGVHD in recipients of MMUD grafts compared with recipients of MUD grafts ($P = .04$), but no significant difference in aGVHD, either grade I/II or III/IV, between MMUD and MUD graft recipients.

Viral Reactivation

Fifty-six patients had evidence of CMV, EBV, and/or adenovirus replication (35%) detected by PCR in blood post-transplantation. CMV was detected in 30 patients (27 of whom

Table 3
Survival According to Donor Type and Stem Cell Source

Stem Cell Source/Donor	PBSC, n (%)	BM, n (%)	CB, n (%)	Total, n (%)	Survival, n (%)
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)	

There was no significant difference in survival according to type of donor ($P = .50$) or stem cell source ($P = .23$). Four mismatched family donor recipients were excluded due to the small number.

Table 4
Second Procedures

Diagnosis	First HSCT	Indication	Time to/Type of Second Procedure	Outcome
Undefined neutrophil disorder	MSD, BM	25% myeloid chimerism. Abnormal neutrophils	10 mo; MUD, PBSC, busulfan/ fludarabine/ alemtuzumab	Alive and well
CGD	MUD, PBSC	Dropped to 0% myeloid chimerism	DLI for slipping chimerism—no effect; then 19 mo; MUD, PBSC, busulfan/ fludarabine/ alemtuzumab	Alive and well
ADA	MUD, CB	Poor immune reconstitution	12 mo; MUD, PBSC, fludarabine/ melphalan/ alemtuzumab	Alive and well
CHH	MMUD, CB	Poor immune reconstitution	16 mo; MMUD, PBSC, fludarabine/ melphalan/ alemtuzumab	Alive and well
HLH	MUD, PBSC	Secondary graft failure	4 mo; unconditioned unmanipulated boost	Died of infection (*Aspergillus*) at 5 mo after first HSCT
FADD	MFD, PBSC	Low-level mixed chimerism	10 mo; unconditioned unmanipulated boost	Stable low-level mixed chimerism; alive
CHH	MMUD, BM	Aplasia despite 100% donor chimerism	7 mo; unconditioned unmanipulated boost	100% donor; alive and well
CGD	MUD, PBSC	GVHD, hypocellular	22 mo; unconditioned unmanipulated boost	Died of infection (influenza), GVHD at 23 mo after first HSCT
XIAP	MUD, PBSC	Hypocellular	17 mo; DLI then unconditioned unmanipulated boost	Died of infection (JC leukoencephalopathy) at 2 yr after first HSCT
SCID, thymectomy	MFD, BM	Poor immune reconstitution	1 yr post-DLI	Died of respiratory failure at 2 yr post-HSCT
Autoimmune enteropathy	MSD, BM	Poor immune reconstitution; adenovirus	5 mo post-DLI	Died of infection (adenovirus), respiratory failure at 10 mo post-HSCT
SCID	MUD, BM	Poor immune reconstitution despite 100% donor chimerism	33 mo post-DLI	Liver aGVHD grade III post- DLI, resolved. Alive and well but with ongoing poor immune reconstitution

FADD indicates Fas-associated death domain protein deficiency; DLI, donor lymphocyte infusion.

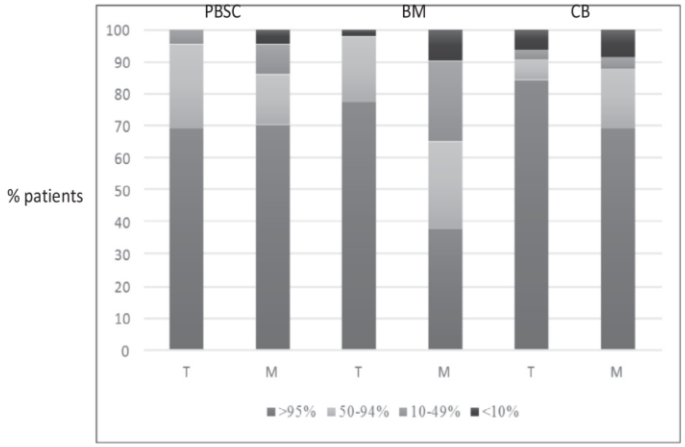
received treatment with foscarnet, ganciclovir, or cidofovir); EBV, in 21 (6 treated with rituximab, 1 with ofatumumab, and 1 with EBV cytotoxic T lymphocytes); and adenovirus, in 24 (19 of whom received treatment with cidofovir). In 4 cases, these viral infections contributed to the death of the child.

Chimerism

There was no association between latest T chimerism >95% and stem cell source ($P = .20$). However, there was a significant overall association with myeloid chimerism ($P = .005$). The odds of having myeloid chimerism > 95% were highest in the PBSC recipients, followed by the CB recipients and then the BM recipients (Figure 3).

There was no significant difference between MUD and MFD graft recipients in donor T cell (odds ratio [OR], .9; 95% CI, .26 to 3.21; $P = .90$) or myeloid cell chimerism (OR, 1.52; 95% CI, .52 to 4.46; $P = .43$).

There was no significant difference between those who received 36 g/m^2 and 42 g/m^2 treosulfan in terms of achiev-



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

Figure 3. T and myeloid cell chimerism according to stem cell source. All patients who survived longer than 1 year post-HSCT were included. Four patients without split cell lineage chimerism data were excluded.

ing T cell or myeloid cell chimerism > 95% ($P = .34$ and .22, respectively).

Immune Reconstitution

Data on lymphocyte reconstitution are presented 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, 6, 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 (1 mg/kg) was also associated with delayed B cell reconstitution, which ceased to be significant by 6 months post-HSCT.

Seven survivors remained on immunoglobulin replacement because of ongoing immunosuppression in 5 patients, recipient myeloid chimerism with absent B cells in 1 patient with Omenn syndrome patient, and poor immune reconstitution despite 100% donor chimerism in 1 patient with SCID.

Newborn SCIDs

Eleven patients with SCID diagnosed at birth due to positive family history underwent transplantation using treosulfan 36 g/m^2 (n = 8) or 30 g/m^2 (n = 3) at less than 5 months of age. All are alive with 15 to 104 months of follow-up (median, 55 months). All patients are off immunoglobulin prophylaxis except 1 patient who was given rituximab for autoimmune hemolytic anemia 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 also underwent HSCT at age ≤4 months. Diagnoses were SCID in 6, Omenn syndrome in 2, ZAP 70 in 2, HLH in 1, LAD in 1, and severe immune dysregulation in 1. Eight are alive and well, with a median follow-up of 76 months (range, 40 to 107 months). The 5 deaths are detailed in Table 2.

Wiskott-Aldrich Syndrome

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

CGD

Seventeen patients underwent HSCT for CGD: 1 with an MSD, 12 with a MUD, and 4 with a MMUD, with 13 PBSC and 4 BM grafts. Six patients had fungal disease before transplantation, 9 had colitis, and 4 were undergoing a second transplantation. Two patients died, 1 on day + 1 post-transplantation from multiorgan failure and the other at 23 months post-transplantation from grade III aGVHD. Fifteen patients are alive and well, with a median follow-up of 53 months (range, 24 to 66 months). 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 underwent successful retransplantation.

HLH

Sixteen patients underwent HSCT for HLH, with only 7 survivors (OS 44%). Six patients received CB with no serotherapy, 5 of whom died. An additional MSD BM recipient who did not receive serotherapy also died. The numbers are small, but 6 of 9 patients who received serotherapy are alive (69%). One patient died on day 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 4. Survival at 2 years post-HSCT was 94.6% (95% CI,

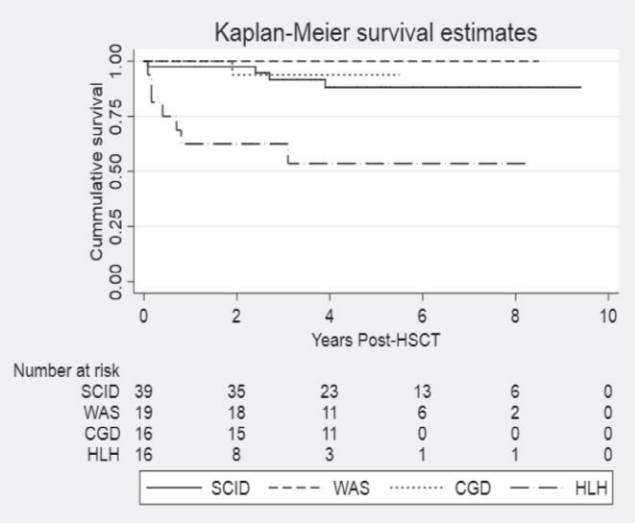


Figure 4. OS by diagnosis. Survival at 2 years post-HSCT: SCID, 94.6% (95% CI, 80.2% to 98.6%); WAS, 100%; CGD, 93.7% (95% CI, 63.2% to 99.1%); HLH, 62.5% (95% CI, 34.8% to 81.0%). $P = .0001$, log-rank test.

80.2% to 98.6%) for SCID, 100% for WAS, 93.7% (95% CI, 63.2% to 99.1%) for CGD, and 62.5% (95% CI, 34.8% to 81.0%) for HLH ($P = .0001$, log-rank test).

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 previously published series, there was a high survival rate in children who underwent HSCT at age <1 year, 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 before undergoing HSCT. In particular, in this series we have demonstrated a higher level of myeloid chimerism in recipients of PBSCs grafts compared with CB and BM recipients, without an increased risk of grade III/IV aGVHD or cGVHD. There was no significant difference in survival according to type of donor or stem cell source, although it would be interesting to evaluate this in a larger number of patients.

With the advent of newborn screening for SCID, and knowing that the outcome of HSCT is better for those who undergo transplantation 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 provided [32,33]. This report provides evidence supporting the safety of using treosulfan in very young infants. Eleven patients with SCID diagnosed at birth owing to previous family history and HSCT at age ≤4 months are alive, 10 with good immune reconstitution.

The outcomes in our patients with HLH were poor in contrast to those reported by Lehmberg et al. [8] in 19 patients with HLH following HSCT with treosulfan, fludarabine, and alemtuzumab, with or without thiotepa, who achieved 100% survival. Of note, in that study, all patients, including MSD graft recipients, were given alemtuzumab, which is likely important owing to the hyperinflammatory nature of the disease. In particular, in our series, the combination of CB without serotherapy was associated with poor outcomes, and we strongly recommend the inclusion of serotherapy 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 comorbidities leading to transplantation-related mortality, but also by failure to attain complete remission at the time of HSCT.

Although 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 in which 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 with no 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 centers may have been labeled as an engraftment rash. Further work is needed to determine the optimal timing and dosing of serotherapy to minimize the risks of GVHD and viral reactivation [35]. Although there was no significant difference in the incidence of aGVHD between MUD and MMUD donors, there was a significantly greater risk of cGVHD with MMUD. Newer techniques of T cell depletion such as $CD3^+TCR\text{-}\alpha/\beta$ together with $CD19^+$ depletion are enabling a wider spectrum of patients with PID to receive successful haploidentical grafts and will lead to a decreased use of MMUD grafts [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 regimen with treosulfan and fludarabine. Data on the long-term effects of treosulfan on fertility are lacking and are needed for comparison with other agents [19]. In addition, further pharmacokinetic studies on treosulfan are needed to identify whether specific pharmacokinetic targeting is advantageous, as for busulfan [41-43]. Many centers are using additional thiotepa in combination with treosulfan and fludarabine, but in a recent multicenter study of patients with CGD, this did not produce superior results in terms of OS, 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 pre-HSCT conditioning in patients with a diverse range of PID diseases, regardless of age, and with all types of donor and stem cell sources, providing a uniformly applicable conditioning strategy in PID. One caveat to this may be children with DNA repair disorders, in whom there are little data [44,45].

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2017.11.009.

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

2.5.7 Supplementary 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.

2.5.8 Supplementary 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%)**	

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

2.5.9 Supplementary Table IV. Second procedures

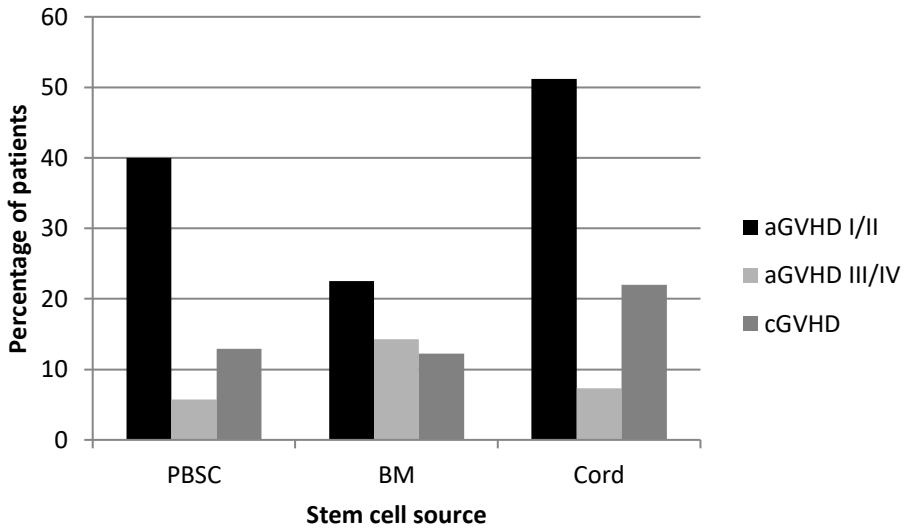
Diagnosis	1st HSCT	Indication	Time to/type 2nd procedure	Outcome
Undefined neutrophil disorder	MSD BM	25% myeloid chimerism. Abnormal neutrophils	10m MUD PBSC Bu/flu/alem	Alive and well
CGD	MUD PBSC	Dropped to 0% myeloid chimerism	DLI for slipping chimerism - no effect, then 19m MUD PBSC Bu/flu/alem	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 boost 4m	Died infection (Aspergillus) 5m post 1st 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.

2.5.10 Supplementary Figure S1.

Graft versus host disease according to stem cell source



Abbreviations: PBSC Peripheral blood stem cells, BM Bone marrow, Cord Cord blood. There was no significant association between acute or chronic GVHD and stem cell source (p=0.37).

2.5.11 Short discussion of strengths and limitations

This study includes a large cohort of children with a variety of PIDs. Almost all children with PID who undergo HSCT in the UK are cared for either in Newcastle or at Great Ormond Street Hospital and the 2 units work very closely to ensure uniformity and best practice. Therefore one of the strengths of this report is that it is not just a single centre report but provides the UK experience.

In the treatment of these very rare diseases there is tremendous variation in practice across the world. The IEWP of EBMT produces guidelines for transplanting different conditions with different types of donor, but prospective studies using a completely uniform approach are lacking. Therefore although this study is a retrospective study without randomisation, the fact that all patients received homogenous conditioning with treosulfan and fludarabine without e.g. additional thiotepa in some patients but not others, is a great strength.

Follow up is relatively short and it will be important to conduct long-term follow up studies to determine late effects such as fertility, durability of donor chimerism and immune function.

2.6 PP6.

Chiesa R *et al.* 2019 (Proposed Therapeutic Range of Treosulfan in Reduced Toxicity Pediatric Allogeneic Hematopoietic Stem Cell Transplant Conditioning: Results From a Prospective Trial)

Title: Proposed Therapeutic Range of Treosulfan in Reduced Toxicity Pediatric Allogeneic Hematopoietic Stem Cell Transplant Conditioning: Results From a Prospective Trial

Authors: Chiesa R, Standing JF, Winter R, Nademi Z, Chu J, Pinner Danielle, Kloprogge F, McLellen S, Amrolia PJ, Rao K, Lucchini G, Silva J, Ciocarlie O, Lazareva A, Gennery AR, Doncheva B, Cant AJ, Hambleton S, Flood T, Rogerson E, Devine K, Prunty H, Heales S, Veys P and **Slatter M**

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2.6.1 Overview

PK samples were taken from 87 children who received treosulfan with fludarabine for conditioning prior to allo-HSCT at either the Great North Children's Hospital, Newcastle upon Tyne or Great Ormond Street Hospital, London between January 2013 and December 2016. Seventy-nine of these patients had PID, 5 an inflammatory bowel disorder, 2 Juvenile myelomonocytic leukaemia and 1 Osteopetrosis.

Patients received a total treosulfan dose of 42g/m^2 if over 12 months of age at the time of transplant, 36g/m^2 if 3-12 months and 30g/m^2 if 3 months or younger in 3 divided doses. Thirty patients were enrolled in the pilot phase in which PK sampling was undertaken following the 1st dose and 57 patients were enrolled in the main trial in which samples were taken after the 1st and 3rd doses to see if there was any intra-patient variability. In addition in the main study detailed short-term toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Advance Events criteria. The median follow up was 16 months.

The aim was to evaluate the PKs of treosulfan in infants and children and to see if there was any relationship between PK and mortality or donor engraftment. This was a prospective, open-label, phase II study (ClinicalTrials.gov, NCT02048800; EudraCT number: 2013-003257-20).

2.6.2 What was known

- Busulfan (Bu; Busulfex® for injection) shows large PK variability between children. Therapeutic drug monitoring (TDM)-based dosing is therefore often performed in children undergoing HSCT. Target AUC varies between centres

and between specific diseases. Higher exposure is associated with an increased risk of toxicity: e.g. mucositis, GVHD, VOD and TRM. Low busulfan AUC has been associated with a higher probability of graft rejection or disease relapse (Long-Boyle et al., 2015). Bartelink et al. reported results of 674 children in 2016, of whom 274 (41%) had malignant and 400 (59%), non-malignant disease. An optimal target busulfan AUC of 78-101mg × h/l predicted higher EFS in children/young adults, compared to lower and higher exposure groups so they suggested that improved clinical outcomes are likely to be achieved by using a new validated pharmacokinetic model for all indications (Bartelink et al., 2016).

- Treosulfan has become more widely used due to its low toxicity profile in particular the low risk of VOD of the liver.
- Data on treosulfan PK and toxicity in children were limited to mainly observational or retrospective studies. High interpatient variability of treosulfan exposure was shown to be associated with early toxicity in skin and mucosa in 77 children undergoing HSCT published in 2017.
- It was not known whether there is a relationship between treosulfan PK and mortality or donor engraftment.

2.6.3 What this study added

- The PK of treosulfan in children was characterised.
- In each of the first 10 patients, 8 samples were taken post dose of treosulfan but an interim analysis showed that PK parameters could still be estimated on 4 post dose samples.

- A high treosulfan AUC was found to be associated with mortality. Children with a cumulative treosulfan AUC > 6000 mg hour/L had a transplant related mortality of 39% compared with 3% below this level.
- The difference between the levels measured after the 1st and 3rd doses of treosulfan was 14% which is much lower than the interindividual difference of 30% and consistent with the study by van der Stoep et al. with a more heterogenous population of diagnoses and conditioning treatment.
- Only 1 patient had primary graft failure, but 12 patients had ≤ 20% donor myeloid chimerism at last follow up. A low treosulfan AUC was found to be associated with poor engraftment as defined by low myeloid chimerism.
- Grade II and grade III-IV aGVHD occurred in 28% and 3% respectively with no strong relationship to treosulfan AUC. Only 2% developed cGVHD.
- A model was created defining the probability of success as being alive at last follow-up with myeloid engraftment of > 20% donor. Maximum success of 82% occurred with a treosulfan cumulative AUC of 4829 mg hour/L of the 3 doses.
- Approximately 50% of the patients had a cumulative AUC within 80 to 125% of the target, therefore despite adjustments to the dose based on age 50% were outside this target. This indicates that therapeutic drug monitoring-guided dose individualization should be considered in infants and children undergoing allo-HSCT for non-malignant conditions.
- During the course of the trial the company manufacturing treosulfan (Medac PHARMA) suggested a BSA scheme: 42g/m^2, reduced to 36g/m^2 if BSA <1m^2, and 30g/m^2 for those with a BSA <0.5m^2. A comparison with our dosing scheme

showed a trend for reduced over-exposure using their BSA dosing in the younger age groups.

- A prospective study on TDM-guided personalisation is required.

2.6.4 Contribution of the candidate to this work

I wrote the manuscript together with RC, JFS and RW. I designed the research with RC, JFS, HP and PV. I was the PI in Newcastle for the study which was performed together with RC, RW, ZN, JC, DP, SM, PJA, KR, GL, JS, OC, AL, ARG, BD, AJC, SH, TF, ER, KD and PV. RC, JFS and FK analysed the data. HP, RW and SH contributed analytical tools. A copy of the Newcastle University co-authorship form can be found in the Appendix.

ARTICLE

Proposed Therapeutic Range of Treosulfan in Reduced Toxicity Pediatric Allogeneic Hematopoietic Stem Cell Transplant Conditioning: Results From a Prospective Trial

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Treosulfan is given off-label in pediatric allogeneic hematopoietic stem cell transplant. This study investigated treosulfan's pharmacokinetics (PKs), efficacy, and safety in a prospective trial. Pediatric patients (n = 87) receiving treosulfan-fludarabine conditioning were followed for at least 1 year posttransplant. PKs were described with a two-compartment model. During follow-up, 11 of 87 patients died and 12 of 87 patients had low engraftment (≤ 20% myeloid chimerism). For each increase in treosulfan area under the curve from zero to infinity ($AUC_{(0-\infty)}$) of 1,000 mg hour/L the hazard ratio (95% confidence interval) for mortality increase was 1.46 (1.23–1.74), and the hazard ratio for low engraftment was 0.61 (0.36–1.04). A cumulative $AUC_{(0-\infty)}$ of 4,800 mg hour/L maximized the probability of success (> 20% engraftment and no mortality) at 82%. Probability of success with $AUC_{(0-\infty)}$ between 80% and 125% of this target were 78% and 79%. Measuring PK at the first dose and individualizing the third dose may be required in nonmalignant disease.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Unlike busulfan, it is thought that treosulfan does not require dose individualization by therapeutic drug monitoring (TDM) in pediatric allogeneic hematopoietic stem cell transplant (allo-HSCT). A recent study, finding increased mortality with increased treosulfan area under the curve from zero to infinity ($AUC_{(0-\infty)}$). Another including a heterogeneous group of diagnoses and conditioning regimens found no trend.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Pharmacokinetic (PK) and long-term allo-HSCT outcome were studied in children receiving treosulfan-fludarabine conditioning. The questions were: What are the PKs of treosulfan in infants and children? What is the relationship between treosulfan PK ($AUC_{(0-\infty)}$) and mortality and donor engraftment?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Treosulfan $AUC_{(0-\infty)}$ was strongly associated with mortality (high $AUC_{(0-\infty)}$), and to a lesser extent poor engraftment (low $AUC_{(0-\infty)}$). A target treosulfan $AUC_{(0-\infty)}$ of 4,800 mg hour/L was defined. Interoccasion variability on clearance was low.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ TDM-guided treosulfan dose individualization should be considered in infants and children undergoing allo-HSCT for nonmalignant conditions.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is used in children for relapsed malignancies and nonmalignant conditions, such as primary immune deficiency.[1] To deplete host immune cells and facilitate donor engraftment, children usually receive conditioning consisting of combination cytotoxic chemotherapy. Conditioning regimen intensity varies depending on the

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disease being treated. Malignant conditions tend to be treated with high intensity myeloablation, whereas nonmalignant conditions may be treated with lower intensity (lower dosing and/or fewer agents). Nevertheless, even with reduced toxicity conditioning, transplant-related morbidity and mortality remain significant.[1–9]

Busulfan is commonly used in allo-HSCT conditioning and studies have demonstrated therapeutic drug monitoring (TDM) and dose adjustment are associated with reduced transplant-related mortality.[10–12] The target area under the curve (AUC) and therapeutic range of busulfan was recently revised in a pharmacokinetic/pharmacodynamic (PK/PD) meta-analysis and methods for personalizing exposure by measuring busulfan PK after the first dose and adjusting later dose(s) is now well established.[10]

Treosulfan is a busulfan analogue but, although busulfan causes direct DNA alkylation, treosulfan is a prodrug with alkylating activity mediated by its main epoxybutane derivatives.[13] Since the first report of treosulfan-based conditioning in pediatric allo-HSCT in 2002, it has been increasingly used off-label in children, largely due to a perceived wider therapeutic index and a lower propensity to cause veno-occlusive disease/sinusoidal obstruction syndrome than busulfan.[14] Data on treosulfan PK and toxicity in childhood are limited to mainly observational or retrospective studies,[15–19] meaning the therapeutic range in this population is poorly defined.

This study aimed to characterize the PK/PD profile of treosulfan in children undergoing allo-HSCT in an investigator-initiated, multicenter phase II clinical trial. The primary end point was to measure treosulfan PK and the secondary end point was to assess its association with short-term toxicity, graft failure, and mortality.

METHODS

Ethics and patient recruitment

This was a prospective, open-label, phase II study (ClinicalTrials.gov, NCT02048800; EudraCT number 2013-003257-20) conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. Patients aged 28 days to 18 years old were eligible if they were scheduled to receive treosulfan-fludarabine conditioning prior to allo-HSCT. Patients and/or their legal guardians were asked to provide written informed consent and assent where appropriate at two centers in the United Kingdom: the Bone Marrow Transplant Department in Great Ormond Street Hospital, London, and the Bone Marrow Transplant Department in Great North Children's Hospital, Newcastle upon Tyne.

The study was split into an initial pilot phase and the main trial. In the pilot phase, PK sampling was undertaken following the first dose, whereas in the main study PK samples were taken after the first and third dose and detailed study of short-term toxicity was performed. All patients were followed up for at least 1 year for survival and engraftment. Around 50 patients are required to capture important covariate effects in PK studies[20] so we aimed to recruit at least 50 to the main study.

Study conditioning regimen

The chemotherapy protocol consisted of treosulfan and fludarabine for all patients. Treosulfan was administered by 2-hour i.v. infusion on days -7, -6, and -5 prior to allo-HSCT at a total dose of 42 g/m^2 (14 g/m^2/dose) in children aged > 12 months, 36 g/m^2 (12 g/m^2/dose) in children aged 3–12 months, and 30 g/m^2 (10 g/m^2/dose) in children ≤ 3 months. Fludarabine was given from day -7 to day -3 prior to allo-HSCT, at a total dose of 150 mg/m^2. *In vivo* T-cell depletion with alemtuzumab or antithymocyte globulin (ATG) was administered according to donor type and stem cell source. Further details of transplant procedures are given in the **Supplementary Materials**.

Toxicity monitoring

In addition to mortality, in the main study, acute transplant-related toxicity was assessed up to 1 month post-allo-HSCT, graded according to the National Cancer Institute Common Terminology Criteria for Advance Events criteria.[21]

The diagnosis of acute graft-vs.-host disease (GVHD) was made clinically and confirmed pathologically with skin, mucosal, or liver biopsy whenever possible. Grading of acute GVHD was performed according to the Seattle criteria.[22] Chronic GVHD was assessed and scored according to the National Institute of Health (NIH) criteria.[23]

Blood sample collection and treosulfan determination

Patients had indwelling multilumen central venous catheters *in situ*. Treosulfan was administered over 2 hours down one lumen and the line flushed. Following the end of the flush, blood was taken from a different lumen of the central venous catheter. A minimum of 3 mL of dead space blood was drawn and discarded prior to sampling. Initially, samples were drawn at the following times after completion of the flush postinfusion: 5, 15, and 30 minutes, and 1, 2, 3, 4, and 8 hours after the end of the infusion. To limit invasiveness and after confirming PK parameters (in particular $AUC_{(0-\infty)}$) could still be estimated, an interim analysis showed sampling could be reduced to 4 postdose samples at: end of infusion, and 1, 2, and 4 hours after the end of the infusion.

Treosulfan concentrations in plasma were determined using a validated reverse-phase high-performance liquid chromatography method with refractometric detection in the Chemical Pathology Laboratory at Great Ormond Street Hospital.[15] Further details are given in the **Supplementary Materials**.

PK model building

Parameters for both one-compartment and two-compartment models assuming linear or nonlinear (Michaelis-Menten) elimination were estimated using nonlinear mixed effects modeling with NONMEM version 7.4, using the first order conditional estimation algorithm.[24] Interindividual variability was tested for all parameters assuming a log-normal distribution, and interoccasion variability was tested for clearance and central volume. The residual error included additive and proportional terms.

Allometric size scaling of clearance and volume terms were added *a priori*, and addition of a sigmoidal postmenstrual age maturation function tested.[25] Biomarkers relating to hepatic function (bilirubin and ALT), renal function (serum creatinine), and blood pH were tested on clearance. These covariates entered the model in the following form:

$$p_i = \theta_p \left(\frac{c_i}{\bar{c}} \right)^{\theta_c}$$

where p_i is the individual parameter of interest, c_i is the individual value of the covariate and $\bar{c}$ is the typical value of the covariate in the population. In the fixed allometric weight scaling, c_i was the individual body weight, $\bar{c}$ was set to 70 kg, and θ_c was 0.75 for clearance and intercompartmental clearance, and 1 for central and peripheral volume. For bilirubin, ALT and pH, $\bar{c}$ was set to the median observed value, whereas for serum creatinine (because it is known to change with age) $\bar{c}$ was set to the median expected for age, as reported by Ceriotti *et al.*[26] In adolescents aged 15–18, a sex-specific linear extrapolation was used to link the end of the Ceriotti *et al.*[26] function and the adult expected values, as previously reported by Johansson *et al.*[27] The sigmoidal age function scaling clearance used postmenstrual age (assumed a gestational age of 40 weeks when this was

unavailable) and contained two estimated parameters, so the model took the following form:

$$p_i = \theta_p \frac{1}{1+\left(\theta_a/a_i\right)^{\theta_\gamma}}$$

where a_i is an individual's postmenstrual age in weeks, θ_a is the age at which clearance is 50% mature, and θ_γ is a shape parameter.

The following categorical covariates were also tested: use of T-cell depletion in the conditioning regime, whether patients were in the pilot or the main study, and study site. These categorical covariates entered the model as follows:

$$p_i = \theta_p(1+\theta_c I)$$

with I the indicator taking values of 1 when the covariate is present, and zero otherwise, and θ_c now being the fractional parameter change in the presence of the covariate, and allowed to take values of ≥−1.

For nested models, significance of the additional parameters was evaluated with the likelihood ratio test, the difference in -2 log-likelihood (objective function value (OFV) in NONMEM) of the models being asymptotically χ_1^2 distributed. Covariates were added if the likelihood ratio test indicated a significant improvement in fit at the level of $P < 0.01$. Further model evaluation consisted of plotting predictions vs. observations, and standardized residuals vs. time and predictions, a visual predictive check (1,000 samples) and a nonparametric bootstrap (1,000 samples). A cumulative $AUC_{(0\text{-}\infty)}$ calculated from the sum of all three doses administered divided by the individual clearance estimate was generated for each patient.

Statistical analysis of PDs

Two Cox proportional hazard survival analyses were performed in R, one to assess time to graft failure (chimerism in myeloid engraftment ≤ 20%) and one to assess time to mortality. Covariates considered were: cumulative treosulfan $AUC_{(0\text{-}\infty)}$ for the three doses, age, use of T-cell depletion (alemtuzumab or ATG) in conditioning, donor source and matching, diagnosis, and CD34 dose. Univariable analysis was performed, and if two or more were significant ($P < 0.05$) these were taken forward to a multivariable analysis.

Upon finding low $AUC_{(0\text{-}\infty)}$ to be associated with engraftment ≤ 20%, and high $AUC_{(0\text{-}\infty)}$ to be associated with mortality, a therapeutic target was derived by fitting a quadratic model of cumulative $AUC_{(0\text{-}\infty)}$ vs. probability of success (defined as being alive at last follow-up, with a myeloid engraftment > 20%). The linear predictor was defined as follows:

$$\rho = \beta_0 + \beta_1 \log \mathrm{AUC} + \beta_2 \log \mathrm{AUC}^2$$

where logAUC was the natural logarithm of cumulative $AUC_{(0\text{-}\infty)}$. This model was fitted to the binomial probability of success (defined as engraftment > 20% and being alive) with a generalized linear model in R. Logit, probit, and complimentary log–log canonical link functions were tested, the model with the lowest Akaike Information Criteria being chosen. Target concentration was defined as the logAUC, which maximized the probability of success, which by differentiating the expression above, yields:

$$\mathrm{AUC}_{\max} = \frac{\beta_1}{2\beta_2}$$

where AUC_{max} is the natural logarithm of cumulative $AUC_{(0\text{-}\infty)}$, which maximizes probability of success. A nonparametric bootstrap with 10,000 samples was used to derive a 95% confidence interval (CI) on AUC_{max}.

In the main study, an analysis investigating the relationship between cumulative $AUC_{(0\text{-}\infty)}$ and National Cancer Institute (NCI) common toxicity criteria grade (0–5) for all major toxicity types was undertaken. The relationship with $AUC_{(0\text{-}\infty)}$ and NCI grade was analyzed using the Kruskal–Wallis test by rank.

Dosing simulations

During the course of our study, the company manufacturing treosulfan (Medac) suggested the following body surface area (BSA)-based dosing scheme: 42 g/m^2, reduced to 36 g/m^2 for those with a BSA < 1 m^2, and 30 g/m^2 for those with a BSA < 0.5 m^2. Ten thousand hypothetical patient characteristics (age 1 month–16 years) were generated using a published weight for age model[28] and each was randomly assigned a serum creatinine value based on their age and sex by sampling from the model by Ceriotti *et al.*[26] Using the PK model, the cumulative $AUC_{(0\text{-}\infty)}$ was simulated for our dosing scheme, the Medac scheme and doses derived from our final model accounting for age, weight, and serum creatinine, or only age and weight. These were compared with the target concentration derived from the quadratic model.

RESULTS

Patients, donors, and transplant characteristics

A total of 87 children (30 in the pilot phase and 57 in the main study) receiving treosulfan as the sole alkylating agent in conditioning for allo-HSCT between January 2013 and December 2016 were enrolled and followed up for at least 1 year posttransplant. The median follow-up was 16 months (range 1247 months for surviving patients), baseline characteristics are detailed in **Table 1**. A total of 633 PK samples were obtained following the first and third doses, and no sample was below the assay lower limit of quantification. A total of 10 patients underwent the full PK sampling schedule (8 postdose samples), the remaining patients contributing 4 PK samples per occasion following the prespecified interim PK analysis.

PK modeling

Treosulfan was given once daily for 3 days with a cumulative dose of 42 g/m^2 (14 g/m^2/dose) in children aged > 12 months, 36 g/m^2 (12 g/m^2/dose) in children aged 3–12 months, and 30 g/m^2 (10 g/m^2/dose) in children ≤ 3 months. The corresponding cumulative median (range) treosulfan $AUC_{(0\text{-}\infty)}$ for the 3 doses was: 4,521 (4,352–4,740), 5,204 (2,321–9,023), and 4,590 (2,880–14,647) mg hour/L for the 4, 23, and 60 patients receiving these doses.

A two-compartment model provided a superior fit to the one-compartment ($P < 0.01$). The MichaelisMenten elimination did not result in successful minimization (Kaplan–Meier value became very large) or lower OFV, indicating linear clearance in the dose range studied. The addition of a sigmoidal maturation function decreased the OFV by 29 points. Serum creatinine was the only other covariate that significantly ($P < 0.01$) improved model fit. A scatter plot of correlations in the continuous covariates is given in **Figure S1**. **Table 2** gives PK model parameters, a visual predictive check is given in **Figure 1**, and further goodness-of-fit and covariate plots are shown in the **Supplementary Figure S2–S4**. Parameter estimates are provided in **Table 2**.

Toxicity, survival, and engraftment

At last follow-up, 76 of 87 children were alive. The causes of death from transplant-related complications were: adenovirus infection ($n = 3$), Epstein–Barr virus-related lymphoproliferative disease ($n = 2$), sepsis ($n = 2$), transplant-associated micro-angiopathy/veno-occlusive disease ($n = 1$), multiorgan failure ($n = 2$), and progressive encephalopathy ($n = 1$).

Table 1 Patient characteristics

Characteristics	All patients	Died	Poor engraftment (< 20%)	Pilot study	Main study
Number of patients	87	11	12	30	57
Median age, months, at transplant (range)	19 (2–200)	18 (4–121)	24 (7–182)	39 (2–200)	16 (2–195)
Median weight, kg	10 (4.3–55.5)	10 (4.74–40)	11 (5.12–44.3)	13 (4.3–55.5)	10 (4.4–54.3)
Diagnosis					
Primary immune deficiency	79/87 (91%)	9/11 (82%)	12/12 (100%)	24/30 (80%)	55/57 (96%)
Inflammatory bowel disorder	5/87 (6%)	2/11 (18%)	0/12 (0%)	4/30 (13%)	1/57 (2%)
Juvenile myelomonocytic leukemia	2/87 (2%)	0/11 (0%)	0/12 (0%)	2/30 (7%)	0/57 (0%)
Inborn error of metabolism	1/87 (1%)	0/11 (0%)	0/12 (0%)	0/30 (0%)	1/57 (2%)
Donor type					
MSD	12/85 (14%)	1/9 (11%)	2/11 (18%)	8/29 (28%)	4/56 (7%)
MFD	4/85 (5%)	1/9 (11%)	1/11 (9%)	2/29 (7%)	2/56 (4%)
MUD	52/85 (61%)	2/9 (22%)	8/11 (73%)	11/29 (38%)	41/56 (73%)
MMUD	15/85 (18%)	4/9 (44%)	0/11 (0%)	8/29 (28%)	7/56 (12%)
MMFD	2/85 (2%)	1/9 (11%)	1/11 (9%)	0/29 (0%)	2/56 (4%)
Stem cell source					
Peripheral blood	53/85 (62%)	4/9 (44%)	7/11 (64%)	12/29 (41%)	41/56 (73%)
Bone marrow	22/85 (26%)	4/9 (44%)	4/11 (36%)	14/29 (48%)	8/56 (14%)
Umbilical cord blood	10/85 (12%)	1/9 (11%)	1/11 (9%)	3/29 (10%)	7/56 (12%)
Median CD34 + cell dose × 10^6/kg (range)	11.7 (0.04–87)	7.5 (0.37–87)	8.9 (0.5–21.7)	7.5 (0.21–87)	13.5 (0.04–50.86)
Conditioning regimen					
Treosulfan + fludarabine	87/87 (100%)	11/11 (100%)	12/12 (100%)	30/30 (100%)	57/57 (100%)
Treosulfan dose					
30 g/m^2	4/87 (5%)	0/11 (0%)	0/12 (0%)	1/30 (3%)	3/57 (5%)
36 g/m^2	23/87 (26%)	4/11 (36%)	3/12 (25%)	7/30 (23%)	16/57 (28%)
42 g/m^2	60/87 (69%)	7/11 (64%)	9/12 (75%)	22/30 (73%)	38/57 (67%)
In vivo T-cell depletion					
Alemtuzumab	76/87 (87%)	10/11 (91%)	11/12 (92%)	25/30 (83%)	51/57 (89%)
Antithymocyte globulin	1/87 (1%)	0/11 (0%)	0/12 (0%)	0/30 (0%)	1/57 (2%)
GVHD prophylaxis					
Ciclosporin + mycophenolate	84/85 (99%)	9/9 (100%)	12/11 (109%)	29/29 (100%)	55/56 (98%)
Ciclosporin	1/85 (1%)	0/9 (0%)	0/11 (0%)	0/29 (0%)	1/56 (2%)

GVHD, graft-vs.-host disease; MFD, matched family donor; MMFD, mismatched family donor; MMUD, mismatched unrelated donor; MSD, matched sibling donor; MUD, matched unrelated donor.

Figure 2 summarizes organ toxicity within 30 days posttransplant in the main study ($n = 57$), graded according to the NCI criteria. Overall treosulfan was well tolerated, although gastrointestinal toxicity was common. Grade II and grade III–IV acute GVHD occurred in 24 patients (28%) and 3 patients (3%), respectively, with no strong relationship to treosulfan $AUC_{(0\text{-}\infty)}$ (**Figure S5**). Two patients (2%) developed chronic GVHD.

Median neutrophil recovery time was 16 days (range 8–33 days). Median platelet recovery time was 12 days (range 5–101 days). Only 1 of 85 patients who received the allo-HSCT presented primary engraftment failure. At last follow-up, myeloid (CD15+ cells) donor engraftment was ≥ 95% in 52 children, 21–94% in 21 children, and ≤ 20% in 12 patients. Three patients had very poor donor engraftment (≤ 5%). T-cell (CD3+ cells) donor engraftment was ≥ 95% in 57 children, 21–94% in 27 children, and ≤ 20% in 1 patient.

PD modeling and dosing simulations

Survival and ≤ 20% engraftment were modeled in a stepwise manner using Cox proportional hazards. First, univariable analysis was performed and significant covariates ($P < 0.05$) taken forward to a multivariable analysis (**Table 3**). For mortality, two covariates were significant ($AUC_{(0\text{-}\infty)}$) and being in receipt of a mismatched donor, whereas for engraftment there was a trend for low $AUC_{(0\text{-}\infty)}$ to be associated with poor engraftment (hazard ratio (95% CI) = 0.61 (0.361.04); $P = 0.072$).

Upon finding low treosulfan $AUC_{(0\text{-}\infty)}$ associated with poor engraftment and high treosulfan $AUC_{(0\text{-}\infty)}$ with mortality, we

Table 2 Pharmacokinetic model parameter estimates: All parameters being centered on a 70 kg individual using allometric scaling with exponents of 1 for volume terms and 0.75 for clearance terms

Parameter	Estimate (%RSE)	IIV %CV (%RSE)	IOV %CV (%RSE)	Bootstrap median (95% CI)	Bootstrap IIV %CV (95% CI)	Bootstrap IOV %CV (95% CI)
Pharmacokinetic model parameters						
CL (L/hour)	17.31 (5.6)	30% (25.1)	14% (49.8)	17.33 (15.38, 20.66)	30% (22, 37%)	13% (7, 18%)
V1 (L)	35.55 (4.7)	38% (27.1)	–	35.95 (30.54, 41.55)	38% (27, 47%)	–
Covariance of CL + V	–	0.95 (25.1)	–	–	0.943 (0.941, 0.947)	–
Q (L/hour)	9.36 (12.7)	–	–	8.99 (3.17, 13.13)	–	–
V2 (L)	9.89 (8.4)	43% (38.4)	–	9.51 (5.74, 11.9)	42% (20, 64%)	–
θ_a (postmenstrual age in weeks at 50% mature)	38.01 (4.6)	–	–	38.87 (28.17, 45.38)	–	–
θ_γ (shape parameter on age)	2.12 (3.2)	–	–	2.24 (0.79, 4.41)	–	–
θ_c (creatinine power)	−0.3 (30.7)	–	–	−0.31 (−0.49, −0.12)	–	–
Proportional error %	13.51 (0.2)	–	–	13.09 (10.07, 15.48)	–	–
Additive error (mg/L)	0.92 (61.6)	–	–	0.02 (0.01, 49.67)	–	–

Parameter	Estimate (%RSE)	95% CI	*P* value
Quadratic model parameters			
β_0	−138 (56%)	−310, −2.81	0.076
β_1	32.7 (55%)	1.27, 72.7	0.07
β_2	−1.9 (54%)	−4.25, −0.105	0.066

Quadratic model parameter estimates (see Methods for description of parameters) with generalized linear model and complimentary log–log link function. θ_a, postmenstrual age in weeks to reach 50% of the mature value; θ_c, allometric exponent of serum creatinine scaling for CL; θ_γ, shape parameter in the maturation function; %CV, percentage of coefficient of variation; CI, confidence interval; CL, clearance; IIV, interindividual variability; IOV, interoccasion variability; Q, intercompartment clearance; V, volume; V1, central volume; V2, peripheral volume.

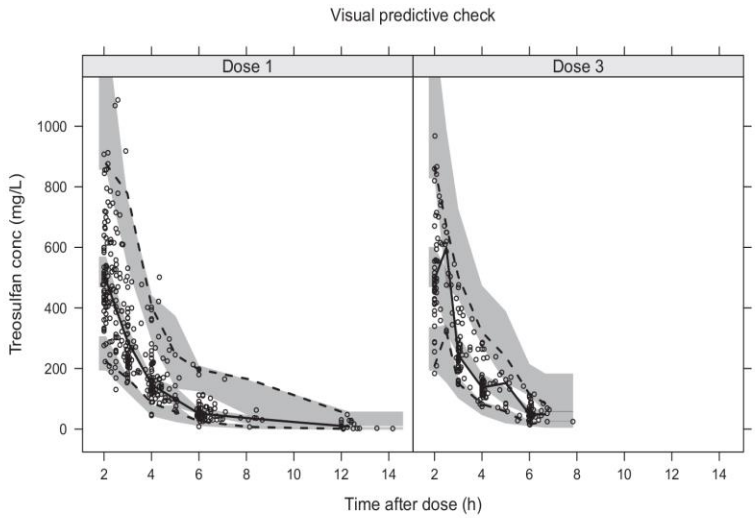

Figure 1 Visual predictive check of the final treosulfan pharmacokinetic model stratified for first and third doses. Shaded areas are the 95% confidence intervals of the 2.5th, 50th, and 97.5th percentiles of the model simulated data; lines are the corresponding percentiles of the raw data.

modeled the probability of success (alive at last follow-up, with a myeloid engraftment > 20%) with a quadratic generalized linear model in R (see Methods section). The lowest Akaike Information Criteria was found with the complimentary log-log canonical link. The model fit is shown in **Figure 3a** and parameter estimates are presented in **Table 2**. The probability of success was maximized at 82% for a treosulfan $AUC_{(0\text{-}\infty)}$ of 4,829 mg hour/L (cumulative of the 3 doses). A nonparametric bootstrap revealed

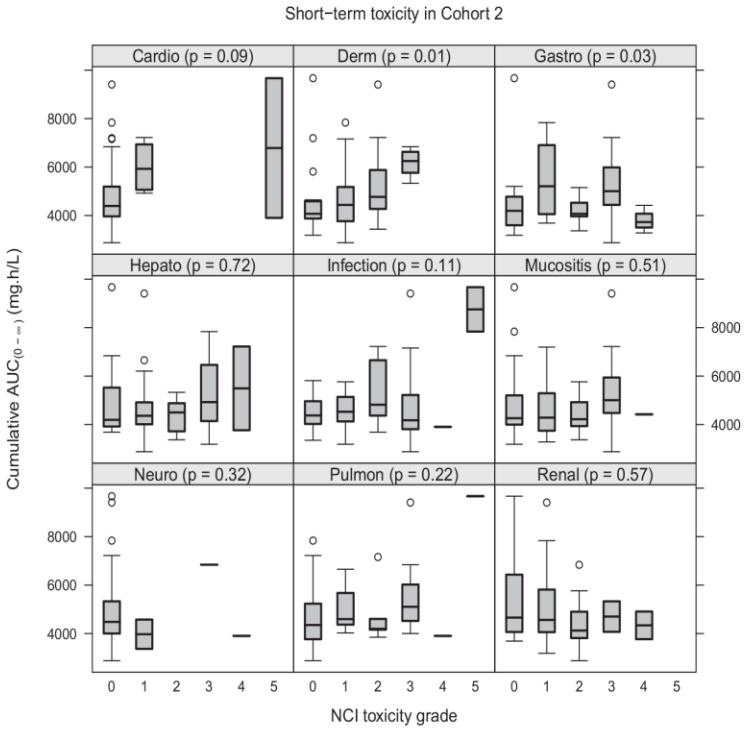

Figure 2 Short-term toxicity National Cancer Institute (NCI) grade in the main study vs. cumulative area under the curve from zero to infinity ($AUC_{(0-\infty)}$). Significance according to the Kruskal–Wallis test by rank shown in brackets.

Table 3 Univariable Cox proportional hazards model for mortality and engraftment

Covariate	Mortality hazard ratio	Mortality *P* value	Engraftment hazard ratio	Engraftment *P* value
Cumulative treosulfan $AUC_{(0-\infty)}$ g hour/L	1.46 (1.23, 1.74)	0.000021	0.61 (0.36, 1.04)	0.072
Age, months	1 (0.98, 1.01)	0.50	1 (0.99, 1.01)	0.750
CD34 + dose ($\times 10^6$/kg)	1.03 (0.99, 1.07)	0.16	0.95 (0.89, 1.02)	0.160
Stem cell source – BM	2.43 (0.61, 9.74)	0.21	2.13 (0.61, 7.43)	0.240
Stem cell source – UCB	1.33 (0.15, 11.97)	0.80	0.64 (0.08, 5.35)	0.680
Received ATG/alemtuzumab	1.72 (0.22, 13.5)	0.61	2.39 (0.3, 19)	0.410
Donor – MFD/MSD	3.27 (0.46, 23.25)	0.24	1.61 (0.42, 6.18)	0.490
Donor – MMFD/MMUD	8.98 (1.74, 46.42)	0.0088	0.45 (0.06, 3.6)	0.450
Diagnosis – not PID	2.61 (0.56, 12.12)	0.22	0 (0, Inf)	1.000
Multivariable analysis	**Multivariable mortality hazard ratio**	**Multivariable mortality *P* value**		
Cumulative treosulfan $AUC_{(0-\infty)}$ g hour/L	1.32 (1.07, 1.64)	0.0093		
Donor – MMFD/MMUD	3.35 (0.74, 15.23)	0.1200		

For the binary variables the result relates to a patient not receiving ATG or alemtuzumab with a matched unrelated donor, with peripheral blood stem cell source, and a diagnosis of primary immune deficiency.
ATG, antithymocyte globulin; $AUC_{(0-\infty)}$, area under the curve from zero to infinity; BM, bone marrow; MFD, matched family donor; MMFD, mismatched family donor; MMUD, mismatched unrelated donor; MSD, matched sibling donor; PID, primary immune deficiency; UCB, umbilical cord blood.

this estimate to be unbiased but imprecise with bootstrap median (95% CI) of 4,876 (1,623–10,839) mg hour/L. The target was, therefore, rounded to two significant figures to 4,800, which also gives an 82% probability of success, whereas the interval between 3,863 and 6,037 mg hour/L represents the treosulfan $AUC_{(0-\infty)}$ interval suggested for narrow therapeutic index drugs,[29] which gives corresponding probabilities of success of 78% and 79%, respectively.

In our study, only 57% of children achieved this $AUC_{(0-\infty)}$ range, whereas 16% had a treosulfan $AUC_{(0-\infty)}$ below the lower cutoff (3,840 mg hour/L) and 26% patients had a treosulfan $AUC_{(0-\infty)}$ above the upper cutoff (6,000 mg hour/L).

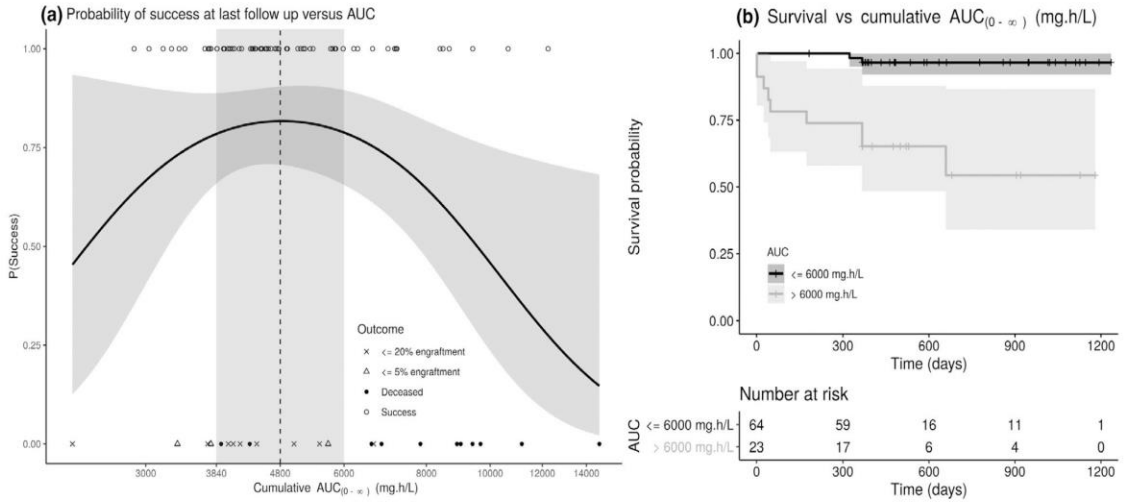

Figure 3 Left side: Pharmacodynamic model fit of the quadratic expression describing the change in probability of success (vertical axis) with increasing cumulative area under the curve from zero to infinity ($AUC_{(0-\infty)}$) (horizontal axis). Black line and associated shaded area is the model fit and 95% confidence interval, open circles are $AUC_{(0-\infty)}$ for patients with successful outcomes; crosses are for patients with ≤ 20% engraftment, triangles are for patients with < 5% engraftment, and black points are patients who died. Vertical dashed line gives $AUC_{(0-\infty)}$ at which probability of success is maximized, vertical shaded area gives $AUC_{(0-\infty)}$ region covering 80% probability of success. Right side: Kaplan–Meier curve for 12-month overall survival in patients above and below the upper success probability $AUC_{(0-\infty)}$ cutoff.

Transplant-related mortality in patients with treosulfan $AUC_{(0-\infty)}$ > 6,000 mg hour/L was 39% compared with 3% in those with a treosulfan $AUC_{(0-\infty)}$ < 6,000 mg hour/L. The corresponding survival was significantly lower ($P < 0.0001$) in patients with $AUC_{(0-\infty)}$ values above and below this cutoff (**Figure 3b**).

Simulated treosulfan $AUC_{(0-\infty)}$ for our dosing scheme (30, 36, and 42 g/m^2 for patients < 3 months, 3–12 months, and > 12 months, respectively) and the Medac dosing scheme (30, 36, and 42 g/m^2 for BSA ≤ 0.5, 0.5–1 and > 1 m^2, respectively) are shown in **Figure 4**. In addition, calculating the dose from our

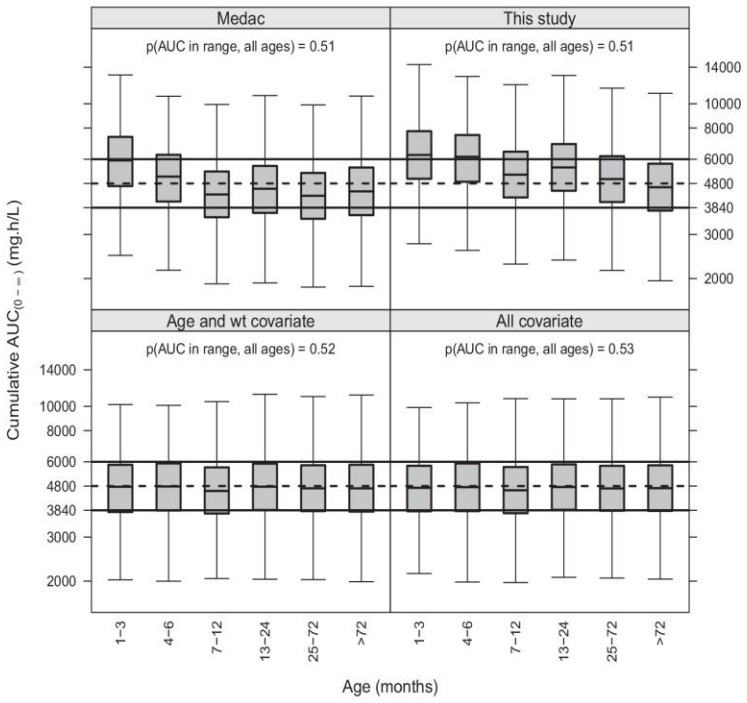

Figure 4 Simulated comparison of dosing used in our study against dosing proposed by Medac on cumulative area under the curve from zero to infinity ($AUC_{(0-\infty)}$) with age. The lower two plots give target attainment if doses were based on the covariates in the pharmacokinetic model (either age and weight, or age, weight, and creatinine). Dashed horizontal lines give the upper and lower cumulative $AUC_{(0-\infty)}$ targets with overall probability of target attainment printed on each plot.

model with and without the creatinine covariate is presented. This was achieved by taking the typical clearance for a patient based on their covariates and defining the dose to target a cumulative $AUC_{(0\text{-}\infty)}$ of 4,800 mg hour/L as follows:

$$\text{Dose} = 4800 \times \text{CL}_{\text{PRED}}$$

where

$$\text{CL}_{\text{PRED}} = 17.31 \left(\frac{\text{wt}_i}{70}\right)^{0.75} \frac{1}{1+\left(38.01/a_i\right)^{2.12}} \left(\frac{\text{sec}\, r_i}{\text{mscr}}\right)^{-0.3}$$

with wt_i being the individual's weight in kg, a_i the postmenstrual age in weeks, secr_i the individual's serum creatinine in μmol/L, and mscr is the median creatinine for age predicted from the Ceriotti model.[26]

DISCUSSION

In a prospective clinical trial of treosulfan PK in pediatric allo-HSCT, treosulfan $AUC_{(0\text{-}\infty)}$ was associated with poor donor engraftment and mortality. Because all but two patients in our study had nonmalignant disease, our findings should be inferred only to apply to this group. This has facilitated the proposal of a therapeutic target of cumulative $AUC_{(0\text{-}\infty)}$ 4,800 mg hour/L. Being within 80–125% of this target cannot be met in ~ 50% of patients through dosing by covariates alone (**Figure 4**), hence, the major finding is that a TDM-guided treosulfan dose adjustment should be explored.

Children with a cumulative treosulfan $AUC_{(0\text{-}\infty)}$ > 6,000 mg hour/L had transplant-related mortality of 39%, whereas patients with $AUC_{(0\text{-}\infty)}$ below this had transplant-related mortality of 3%. The only other significant relationship with mortality in the univariable analysis was receiving a mismatched donor, but upon multivariable analysis the strength of this association was reduced (**Table 3**). Our choice of using all transplant-related mortality could be questioned given that three patients died beyond 100 days of viral-associated complications. It could be argued that because treosulfan is mainly myeloablative, high $AUC_{(0\text{-}\infty)}$ may not be related to these cases, and fludarabine, lymphocyte depletion, or the patient's underlying immune deficiency are more important. However, treosulfan does have broader immunosuppressive effects than simple myeloabaltion[30] and its role in the establishment and longer term effects from viral complications cannot be completely ruled out. Truncating the survival analysis at 100 days and 6 months shows the effect is less strong early on but by 6 months is similar to the overall effect (100-day hazard ratio (95% CI): 1.18 (0.93, 1.51); 6-month hazard ratio: 1.3 (1.06, 1.6)).

Although an observational study of 77 children by van der Stoep *et al.*[19] did not find a correlation between treosulfan $AUC_{(0\text{-}\infty)}$ and mortality, this may be due to the heterogeneity of conditioning as 67.5% patients also received thiotepa. In contrast, children enrolled in our prospective clinical trial received homogeneous conditioning of treosulfan and fludarabine only. Recently, an observational study in children undergoing treosulfan conditioning for allo-HSCT in thalassemia major found a trend of 82% survival in patients with an $AUC_{(0\text{-}\infty)}$ of < 5,484 mg hour/L compared with only 68% in patients above this threshold.[31] Taken together with our result possibly indicates the need to individualize doses in patients with nonmalignant disease. To draw firm conclusions on causation a prospective study is required, because patients who enter the conditioning period with lower treosulfan clearance may have comorbidities predisposing them to mortality, which would not be prevented by lowering treosulfan $AUC_{(0\text{-}\infty)}$.

The association between conditioning drug exposure and clinical outcome in pediatric allo-HSCT has been explored in a number of studies. Busulfan studies have shown an association among exposure and toxicity and engraftment[32,33] with TDM and personalization utilized for a number of years. The US Food and Drug Administration (FDA) proposes a target of 900–1,350 μM minutes[34] whereas the European Medicines Agency (EMA) proposes 900–1,500 μM minutes.[35] Surprisingly, these targets are based on small observational studies.[11,12] A larger but retrospective study on 674 patients with malignant and nonmalignant conditions recently derived a higher target of 1,225–1,575 μM minutes.[10] Likewise, Admiraal *et al.*[36] recently demonstrated that an optimal exposure to ATG is associated with higher event-free survival and lower risk of acute GVHD in adults undergoing allo-HSCT.

Early reports on treosulfan in pediatric allo-HSCT by Glowka *et al.*[15] showed children receiving treosulfan demonstrated large variability in $AUC_{(0\text{-}\infty)}$, suggesting that TDM may be needed. More recently, van der Stoep *et al.*[19] described treosulfan PK in 77 children undergoing allo-HSCT, showing interindividual and interoccasion clearance variability of 33–56% and 13.9%, respectively. Our results (30% and 14%, respectively) are similar.

Our target was found to be rather imprecise (95% CI 1,623–10,839 mg hour/L) upon nonparametric bootstrap but the median (4,876 mg hour/L) was close to our estimate, suggesting it is unbiased. The imprecision is likely due to the small number of events but for now our data remain one of the largest to date. It has been proposed that an acceptable range is being within 80% and 125% of a target value, and if the log-normal distribution is assumed this translates to 90% of patients achieving that range if unexplained variability is 13.6% coefficient of variance.[29] Comparing our interindividual and interoccasion variability values on clearance shows dosing by covariates alone will not achieve this target (**Figure 4**), because the first dose AUC could be measured and the interoccasion variability is 14%, it is likely the cumulative $AUC_{(0\text{-}\infty)}$ for the three doses could readily be targeted. Our future work will include a detailed optimal design and simulation-estimation study to evaluate the potential of treosulfan TDM.

The clearance estimate in our model was scaled by both weight and age. Weight scaling used a fixed allometric model, which approximately follows BSA and for older children and has recently been shown to apply for most drug classes.[37] Hence, BSA-based dosing should give similar $AUC_{(0\text{-}\infty)}$ for children older than around 2 years. It is also well known that in the first year of life BSA-based dosing leads to higher $AUC_{(0\text{-}\infty)}$ due to immaturity in clearance.[37] There are a number of ways to model declining clearance with younger age and recently it was shown that most give equivalent results,[25] and, hence, we used the standard method proposed by Holford *et al.*[38] The major benefit of using this standard method is that it is then very straightforward to compare clearance values between different studies. Our

estimate of clearance was 17.31 L/hour/70 kg, which is similar to that in a recent treosulfan observational study (17.9 L/hour/70 kg),[19] and the surface-area scaled value in the recent thalassemia study (20.07 L/hour/1.73 m^2),[31] all of which are somewhat higher than the value recently estimated by Danielak *et al.*[18] (14.7 L/hour/70 kg). The likely reason is the latter study only included 15 patients with a wide age range, the youngest of whom was < 6 months old, yet no age-related maturation term was used. Recently, a model-based reanalysis of the data by van der Stoep *et al.* in 2017[19] found a similar maturation half-time to ours (38 weeks) with a lower shape parameter (1.2).[39] It is likely our shape parameter is more reliable because our patients were, on average, 19 months old whereas in that study the median age was 52 months,[39] and, furthermore, the busulfan maturation half time and the shape parameter were 40 weeks and 2.2 in a large meta-analysis.[40]

A possible reason for decreased clearance in younger patients is immaturity in glomerular filtration rate because around 40% is excreted renally. During the covariate analysis we found serum creatinine to be inversely correlated with clearance. This was modeled by multiplying clearance by the ratio of serum creatinine to the age-expected serum creatinine raised to an estimated power.[27] Although this relationship was statistically significant and so retained in the model, the covariate power estimate of −0.3 means even in a child with a twofold higher than age-expected creatinine, this would only decrease clearance by around 19%.

Target attainment through dosing by covariates was similar from each of the tested dosing regimens (text probabilities in **Figure 4**). Because the simulated population had a uniform distribution of ages, it seems that surface area or allometric dosing gives very similar target attainment in patients aged > 2 years. The differences come in the younger age groups where dosing by age and weight seems optimal, the addition of creatinine adding little. The Medac scheme showed a trend for reduced overexposure compared with dosing in our study. However, because all covariate-based dosing gives target attainment of around 50%, TDM will still be required.

In conclusion, the PK of treosulfan in children have been characterized and an association with high $AUC_{(0-\infty)}$ and mortality and low $AUC_{(0-\infty)}$ and poor engraftment was found. A prospective study on TDM-guided personalization is warranted.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Supplementary Methods, Figures S1-S7, Treosulfan NONMEM PK model code.

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CONFLICT OF INTEREST

M.S. has received travel grants to attend meetings by Medac and honoraria for speaking engagements. A.G. has received travel grants to attend meetings by Medac. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

R.C., J.F.S., R.W., and M.S. wrote the manuscript. R.C., J.F.S., H.P., P.V., and M.S. designed the research. R.C., R.W., Z.N., J.C., D.P., S.M., P.J.A., K.R., G.L., J.S., O.C., A.L., A.R.G., B.D., A.J.C., S.H., T.F., E.R., K.D., P.V., and M.S. performed the research. R.C., J.F.S., and F.K. analyzed the data. H.P., R.W., and S.H. contributed new analytical tools.

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SUPPLEMENTARY MATERIAL: Proposed therapeutic range of treosulfan in reduced toxicity pediatric allogeneic hematopoietic stem cell transplant conditioning: results from a prospective trial

Contents

1 Supplementary Methods

1.1 Transplant procedures

HLA typing was performed by molecular typing for HLA class I and II loci; mismatch was defined as anything < 9 out of 10 HLA identical. Graft *versus* host disease (GvHD) prophylaxis consisted of ciclosporin (CsA, from day – 3 with doses adjusted to a trough whole blood level of 100-250 ng/mL with TDM) and mycophenolate mofetil (MMF, from day 0 to day +28, then weaned over 3 weeks in the absence of GvHD).

Antimicrobial prophylaxis consisted of either ciprofloxacin (from day -10, until neutrophil count - 1 x 10^9/L) or co-trimoxazole, aciclovir (from day -10, until 1 year post-transplant) and itraconazole or liposomal amphotericin B (from day -11, until neutrophil count - 1 x 10^9/L and no steroid treatment). Prophylaxis of *Pneumocystis jiroveci* pneumonia was with co-trimoxazole daily from day -10 to day -1 and then, after myeloid recovery, until a CD4+ T-cell count > 0.3 x 10^9/L and absence of chronic GvHD/immunosuppressive treatment or throughout the transplant period.

Myeloid recovery was defined as the first of 3 consecutive days with an absolute neutrophil count exceeding $0 \cdot 5$ x 10^9/L, while platelet recovery was defined as an unsupported (by transfusion) platelet count exceeding 20 x 10^9/L. Engraftment was monitored by short tandem repeat variability on peripheral blood in the T-cell (CD3+ cells) and myeloid (CD15+ cells) compartment.

1.2 Sample preparation and analysis of treosulfan concentrations

Blood samples were adjusted to a final pH of 5.5 by the addition of 50 µL of 1 M citric acid per 1 mL of blood immediately after collection to avoid artificial ex vivo degradation of treosulfan and then centrifuged to obtain plasma. The resulting plasma samples were frozen at -20°C until analysis.

Treosulfan concentrations in plasma were determined using a validated reverse-phase HPLC method with refractometric detection in the Chemical Pathology laboratory at Great Ormond Street Hospital. This method was validated in-house with a limit of quantitation of 10 µg/mL, within batch imprecision of 4.5% and inter-day imprecision of 7.5%. Plasma samples were removed from the freezer and allowed to come to room temperature over an hour. The 10 g/L sodium barbital ISTD was removed from the freezer and allowed to defrost over this time. From the sample 200 µL was taken by fixed volume pipette into Millipore centrifugal filter tubes (10,000 kDa cut off). This was followed by 25L ISTD and 25L distilled water. The tubes were briefly vortexed and then placed into a centrifuge and spun at 13,000 rpm for 15 minutes. The filtered solution was then transferred into UHPLC vials. The UHPLC system was set up at the beginning of the day to allow time for the baseline to stabilise.

2 Correlations in continuous covariates

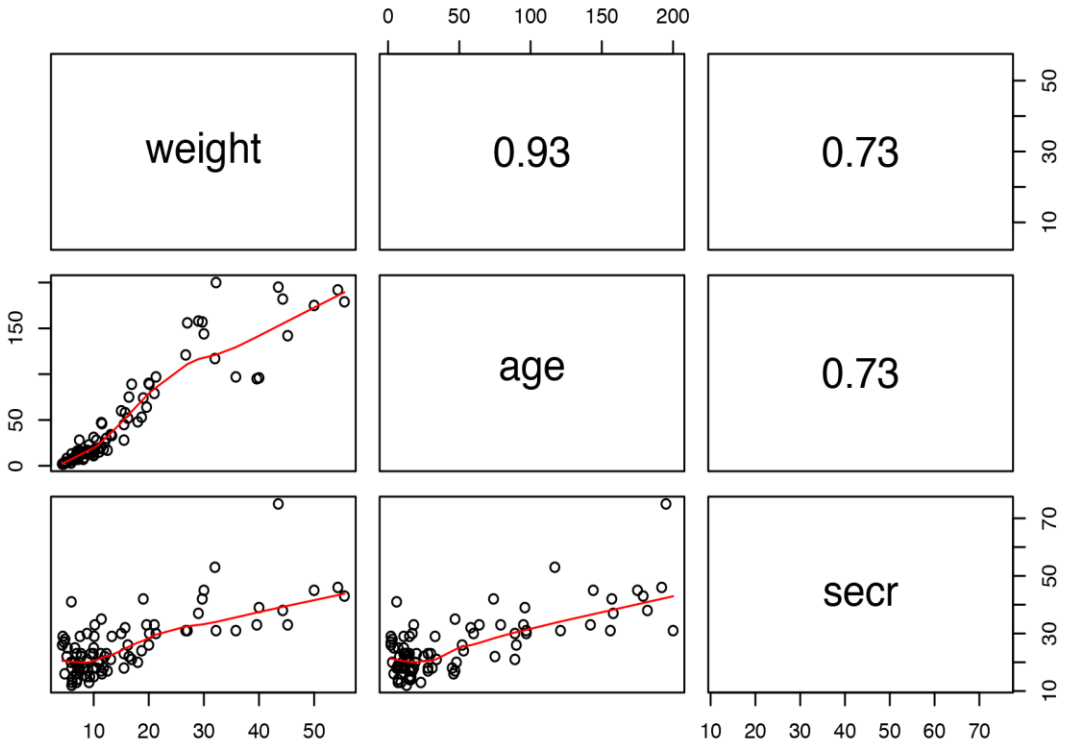


Figure S1: Correlations in covariates, plots in lower triangle, correlation coefficients in upper triangle

3 Further pharmacokinetic results

3.1 Treosulfan model basic goodness-of-fit

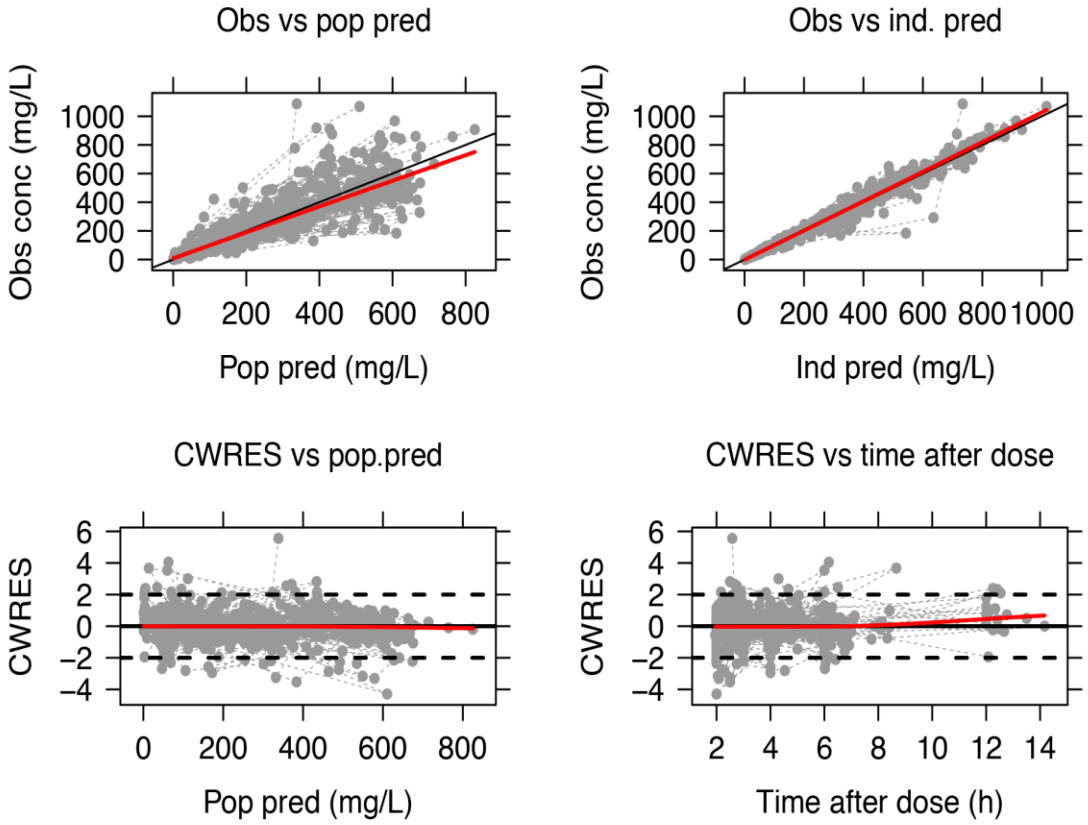


Figure S2: Population and individual predictions plotted against observed concentrations, and conditonal weighted residuals versus population prediction and time after dose

3.2 AUC and clearance relationship with age

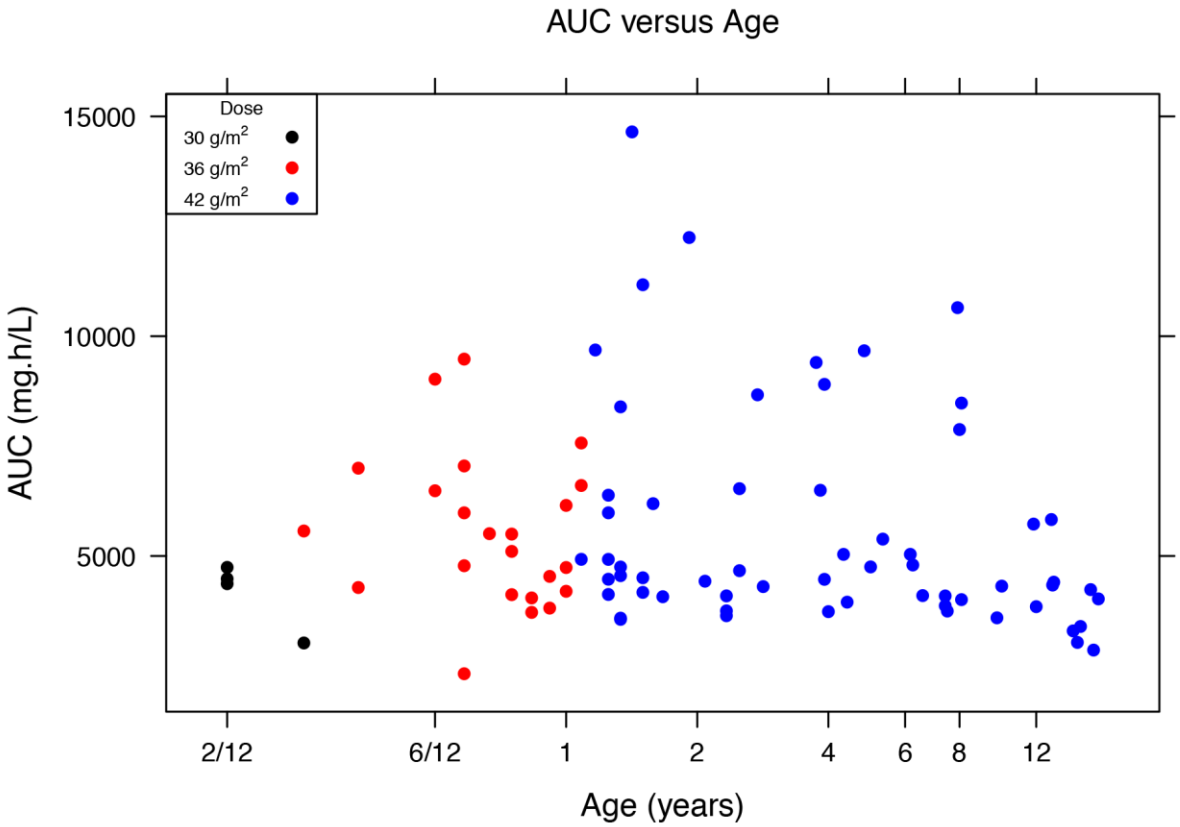


Figure S3: Relationship between AUC and age

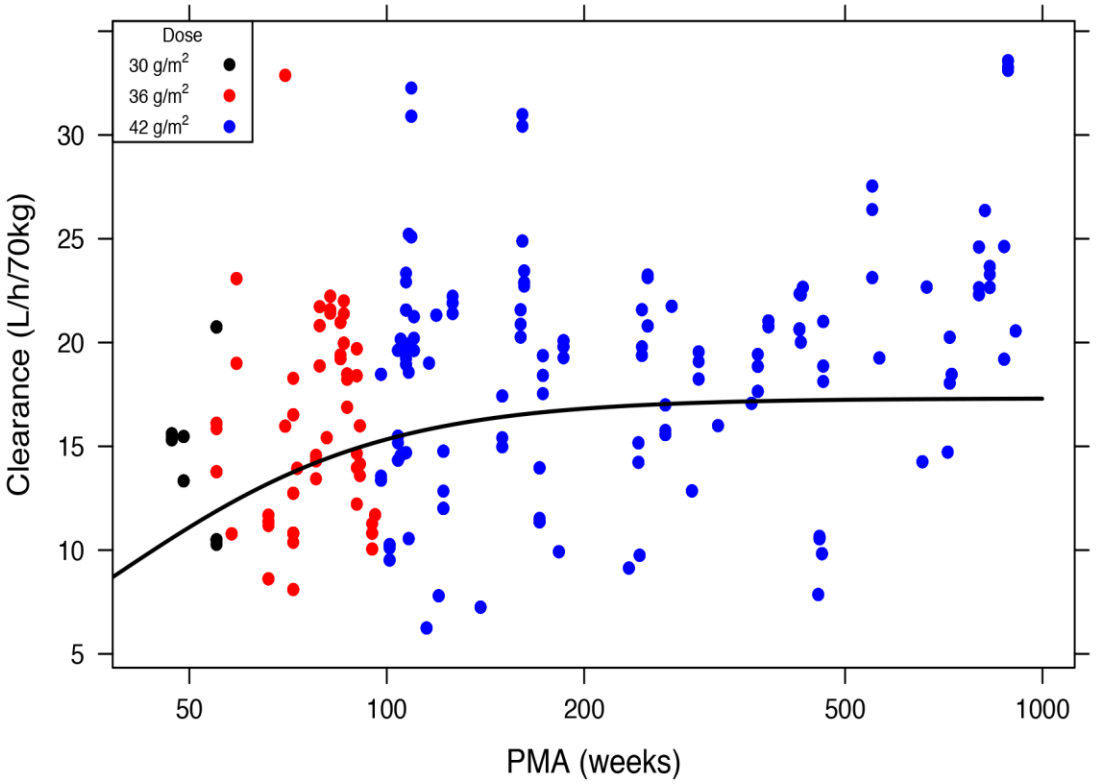


Figure S4: Relationship between size standardised clearance and age, line denotes model maturation function

4 Further Pharmacodynamic results

4.1 Graft versus Host Disease and AUC

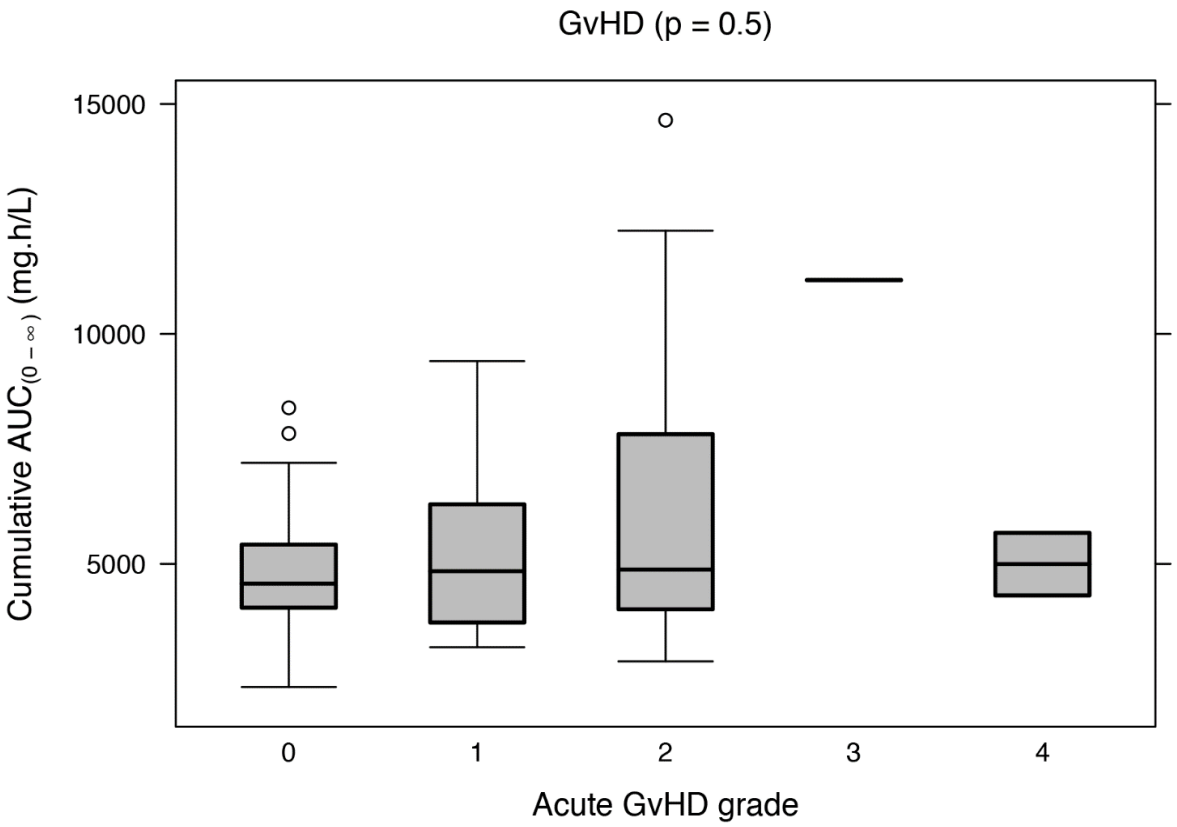


Figure S5: Relationship between acute GvHD and AUC with Kruskal Wallis test

4.2 Neutrophil recovery and AUC

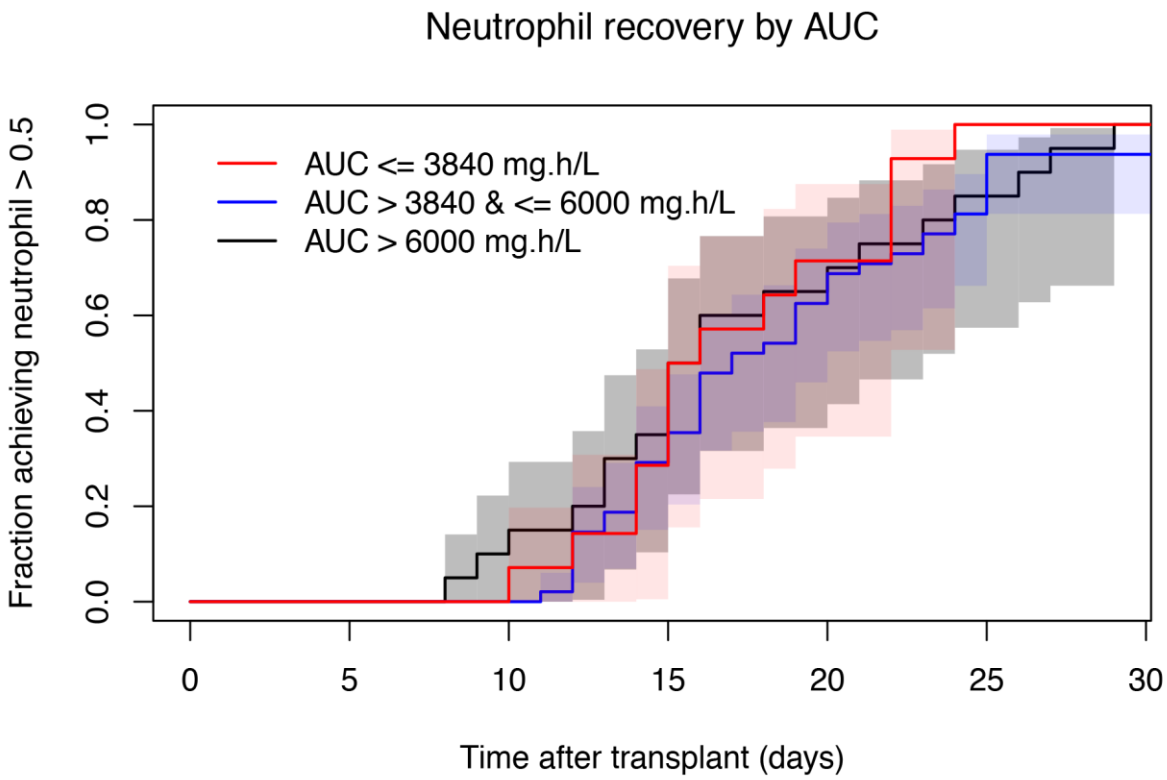


Figure S6: Time to neutrophil engraftment for patients below, in or above AUC target range

4.3 Platelet recovery and AUC

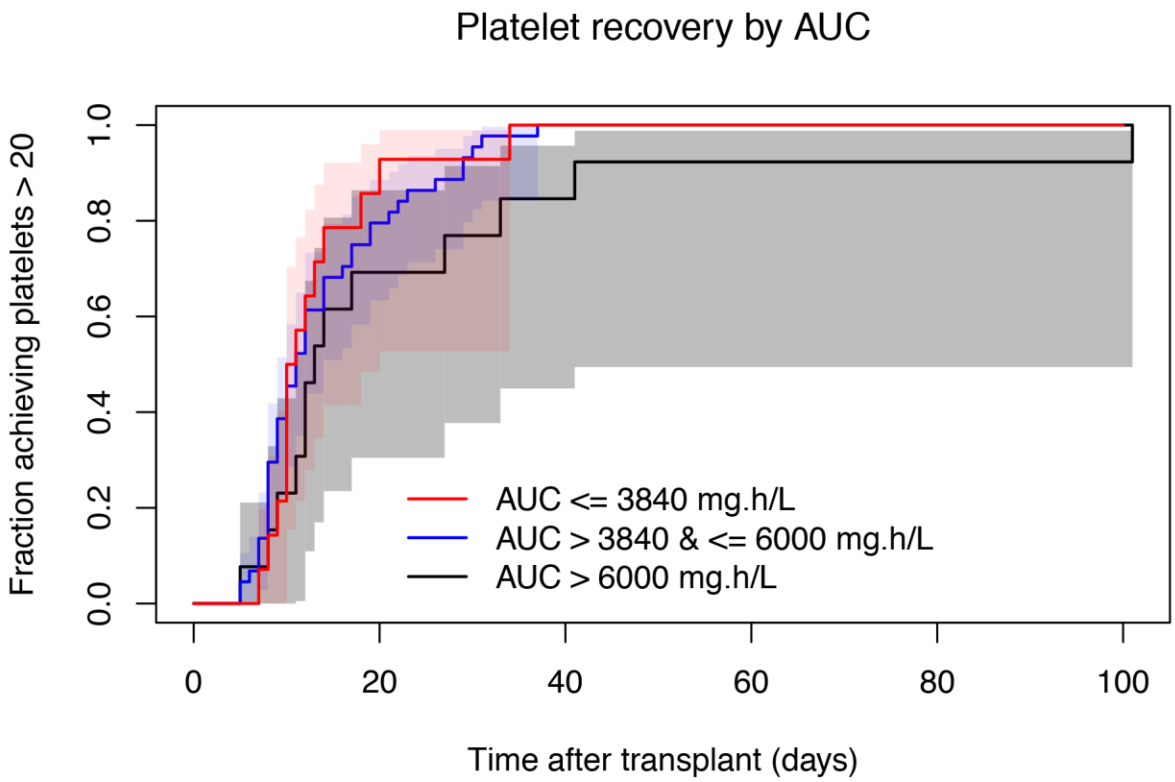


Figure S7: Time to platelet engraftment for patients below, in or above AUC target range

5 NONMEM pharmacokinetic model code

```
$PROBLEM    Treosulfan pk
$INPUT      ID TIME TAD AMT RATE DV EVID OCC BLQ WT AGEMO PMAW BSA
            DOSE AUCE HLE BILI ALT SECR SERO PH NEW COND SEX COHORT
            PIMM
$DATA       ./data/Treo_nonmem_180621.csv IGNORE=@
$SUBROUTINE ADVAN3 TRANS1
$PK
;--- TV param
 TVCL = THETA(1)
 TVV1 = THETA(2)
 TVQ2 = THETA(3)
 TVV2 = THETA(4)
 PM50 = THETA(5)
 HILL = THETA(6)
;--- MU param
 MU_1 = DLOG(TVCL)
 MU_2 = DLOG(TVV1)
 MU_3 = DLOG(TVQ2)
 MU_4 = DLOG(TVV2)
;--- Covariate functions
;--- Allometric and age scaling
 WTCL = (WT/70)**0.75
 WTV  = (WT/70)
 AGEF = 1/(1+(PM50/PMAW)**HILL)
;--- Mean expected creatinine for age
;--- (F. Ceriotti et al, Clinical Chemistry 54:3 559-566 (2008))
AGEY  = AGEMO/12
MSCR  = -2.37330-12.91367*DLOG(AGEY)+23.93581*AGEY**0.5
IF(AGEY>15)THEN
   IF(SEX==0)THEN
         MSCR = 9.5471*AGEY-87.847
   ELSE
         MSCR = 4.7137*AGEY-15.347
   ENDIF
ENDIF
;--- Creatinine covariate
 SCOV = (SECR / MSCR)**THETA(7)
;--- Between occasion variability
 BOV = 0
 IF(OCC==1) BOV = DEXP(ETA(5))
 IF(OCC==2) BOV = DEXP(ETA(6))
;--- Individual paramaters
 CL = WTCL * AGEF * BOV * SCOV * DEXP(MU_1 + ETA(1))
 V1 = WTV * DEXP(MU_2 + ETA(2))
 Q2 = WTCL * DEXP(MU_3 + ETA(3))
 V2 = WTV * DEXP(MU_4 + ETA(4))
;--- Rate constants
 K10 = CL/V1
 K   = K10
 K12 = Q2/V1
 K21 = Q2/V2
```

```
 BETA = 1/2*((K12+K21+K10)-SQRT((K12+K21+K10)**2-(4*K21*K10)))
 V    = V1
;
$ERROR
  IPRED = A(1) / V1
  Y     = IPRED * (1 + EPS(1)) + EPS(2)
  PROP  = SQRT(SIGMA(1,1))*IPRED
  ADD   = SQRT(SIGMA(2,2))
  SD    = SQRT(PROP*PROP + ADD*ADD) ; Standard deviation
  IRES  = DV - IPRED
  IWRES = IRES/SD
;--- Final estimates
$THETA  (0,17.3139) ; 1. CL
$THETA  (0,35.5482) ; 2. V1
$THETA  (0,9.36276) ; 3. Q2
$THETA  (0,9.89182) ; 4. V2
$THETA  (20,38.0144,80)  ; 5. PM50
$THETA  (0.5,2.11914,10) ; 6. HILL
$THETA  -0.30042         ; 7. SCOV
$OMEGA  BLOCK(2)
 0.09057
 0.108607 0.144922
$OMEGA  0  FIX
$OMEGA  0.18708
$OMEGA  BLOCK(1) 0.0206426
$OMEGA  BLOCK(1) SAME
$SIGMA  0.0182599
$SIGMA  0.920867
;--- Estimation method
$ESTIMATION METHOD=1 INTER MAXEVAL=0 PRINT=1
;$COVARIANCE
;--- Tables
$TABLE      ID AMT TIME TAD IPRED CWRES TIME IWRES NOPRINT ONEHEADER
            FILE=sdtab24
$TABLE      ID CL V1 Q2 V2 ETAS(1:5) BETA NOPRINT ONEHEADER
            FILE=patab24
$TABLE      ID TIME EVID OCC BLQ WT AGEMO PMAW BSA BILI ALT SECR SERO
            PH NEW COND RATE DOSE AUCE HLE COHORT PIMM SECR MSCR SECRN
            NOPRINT ONEHEADER FILE=cotab24
```

2.6.7 Short discussion of strengths and limitations

- This is the largest prospective, open-label, phase II study to date, of children receiving treosulfan for conditioning for allo-HSCT with PK monitoring. It was conducted in the 2 largest Paediatric transplant centres in the UK who use treosulfan. The conditioning regimen was uniform treosulfan and fludarabine without additional alkylating agents such as thiotepa. All but 2 of the 87 patients had a non-malignant disorder, 91% had a PID. Other studies have a more heterogenous population and less standardization of the chemotherapy employed. In Van der Stoep's study 67.5% received additional thiotepa and of 77 patients 12 had malignancy, 31 haemoglobinopathies, 22 PID and 12 bone marrow failure or other conditions.
- The median age of children in our study was 19 months compared to 52 months in Van der Stoep's study. It is important to study infants who are known to have lower clearance of treosulfan.
- The simulated comparison of dosing used in our study against dosing proposed by Medac based on BSA in our patients, revealed a trend for more overexposure in the younger age group in our patients. This has led to a change in practice at both centres who have adopted the Medac recommended dosing which may lead to a reduction in mortality.
- There are a number of limitations: the 30 patients in the pilot phase did not have prospective data on toxicity collected. Longer follow up is needed to see if there is any correlation with late effects such as gonadal function and treosulfan exposure. Some studies suggest that the PK of the active epoxides of treosulfan are necessary to understand clinical observations. For a personalized approach to conditioning we need to know more about all the drugs involved: an

increasing number of studies are looking at fludarabine PK and levels of serotherapy agents. A Pan PK study to perform PK on all agents used is warranted.

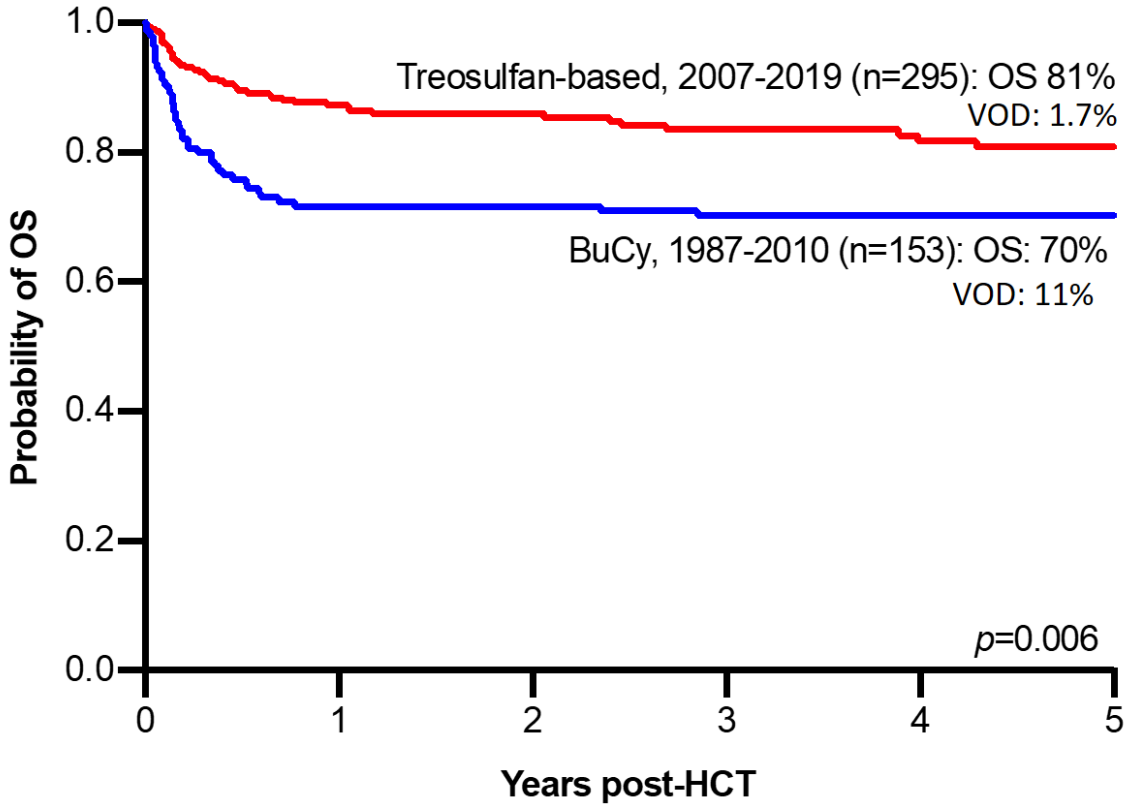
CHAPTER 3: DISCUSSION

3.1 Implications

Treosulfan has been established as a safe and effective agent for conditioning prior to HSCT for children with PID and related disorders (MF Silva et al., 2018). Greystoke et al reported 32 children who had received treosulfan including 13 with PID and 5 with HLH in 2008 (Greystoke et al., 2008). The same year Cutting et al reported 23 children including 2 with HLH (Cutting et al., 2008). We reported our first 70 children with PID who received treosulfan between 2006 and 2009 either in Newcastle or Great Ormond Street Hospitals (Supplementary paper 1). The OS was 81% with a median follow up of 19 months in a cohort of children with a particularly young age (median 8.5 months) at HSCT. This demonstrated the safety of using treosulfan in infants, toxicity was lower when treosulfan was combined with fludarabine compared to cyclophosphamide and there was no difference in chimerism. There was a trend towards better chimerism when patients received PBSC without an increased risk of severe GVHD (Slatter et al., 2011).

Figure 3 shows the improvement in overall survival and decrease in incidence of VOD with treosulfan-based compared to busulfan and cyclophosphamide conditioning in our unit.

Figure 3: Overall survival and incidence of VOD in treosulfan-based compared to busulfan-cyclophosphamide conditioning



Use of treosulfan across Europe has been growing since 2005. In PP3 we reported 316 transplant recipients with non-malignant diseases from 11 different countries. One hundred and forty-four of these were in patients with PID, the remainder being in patients with metabolic, histiocytic or autoimmune disorders, haemoglobinopathies and bone marrow failures. Ninety-five (30%) were under 1 year of age at transplant. OS was 83% and EFS was 76%. This confirmed that treosulfan is a safe and effective agent in a wide range of non-malignant diseases, even for those under 1 year of age.

3.1.1 Treosulfan in conditioning for HSCT in CGD

Newcastle has for many years promoted HSCT as a cure for patients with CGD. Supplementary paper 2 reports 55 children who have undergone HSCT in our centre up to the end of 2017 with a 5 year OS of 89%. For 28 children transplanted at, or under 5 years of age OS was 100% versus 81% for the 27 children above 5 years of age confirming the importance of early transplant before recurrent infections such as Aspergillus, inflammatory complications and organ damage (Lum et al., 2019a). Early reports of transplant for CGD reported high TRM for high risk patients (Seger et al., 2002, Horwitz et al., 2001), and reduced intensity regimens led to high rates of mixed chimerism and GVHD (Horwitz et al., 2001, Segal et al., 2011). There has therefore been a specific interest in reduced toxicity conditioning for patients with CGD and a very successful regimen was developed by Tayfun Güngör and colleagues in Zurich in collaboration with the Karolinska Institute in Sweden using TDM of busulfan to target exposure to a cumulative AUC of 45–65 mg/L × hour, equivalent to 55–75% of standard full myeloablative cumulative AUC of 80–100 mg/L × hour in combination with fludarabine and serotherapy (Gungor et al., 2014). At the same time as this regimen was being increasingly used, other centres were using treosulfan and so we collected the data of 70 patients from 16 centres in 9 countries worldwide reported in PP4. Results were similar with OS and EFS at a median follow up of 34 months of 91.4% and 81.4% respectively, compared to Güngör's OS of 93% and EFS 89% at a median follow up of 21 months. Further studies are needed to evaluate long-term outcome and late effects particularly on fertility comparing these regimens.

3.1.2 Treosulfan and fertility

Alkylating agents impair gonadal function and fertility and myeloablative busulphan regimens cause severe impairment particularly in females (Borgmann-Staudt et al., 2012, Bresters et al., 2014). Treosulfan may have less gonadotoxicity than busulphan (Faraci et al., 2019). A recent

paper compared serum concentrations of Anti-Müllerian hormone (AMH) in females and Inhibin B in males in survivors of HSCT in 3 different groups: group A had received treosulfan-based conditioning, group B, fludarabine and melphalan, and group C, busulphan and cyclophosphamide. Serum AMH and Inhibin B were significantly higher in group A compared to groups B and C suggesting that treosulfan-based regimens confer a more favourable outlook for gonadal reserve than fludarabine/melphalan and busulphan/cyclophosphamide (Leiper et al., 2020). In the 2014 study Güngör reported that 2 of 13 male patients transplanted over the age of 16 years had fathered children. There are no studies to date comparing fertility after the use of treosulfan/fludarabine with low dose busulfan/fludarabine.

3.1.3 Treosulfan in conditioning for HSCT in PID in the UK

The results of our early experience of using treosulfan published in 2011 (Supplementary paper 1) led to our practise of always combining treosulfan with fludarabine and using PBSC in preference to BM or CB when a 10 out of 10 HLA-matched donor is available. This practise was reported in PP5 in 2018. One hundred and sixty children with PID received treosulfan and fludarabine (without additional agents such as thiotepa) either in Newcastle or Great Ormond Street Hospitals. OS was 83% with a longer median follow up of 4.3 years and median age at transplant of 1.36 years. A significant association was seen between myeloid chimerism > 95% and use of PBSC without an increased risk of significant GVHD compared with other sources.

3.1.4 The importance of donor chimerism

Although mixed chimerism can lead to cure in many patients with PID, a degree of donor myeloid chimerism is associated with superior thymopoiesis which ensures better long-term immune reconstitution in SCID (Abd Hamid et al., 2017, Cavazzana-Calvo et al., 2007). Durable T-lymphocyte reconstitution is important for survival (Haddad et al., 1998, Haddad et

al., 2018) and prevention of late complications such as cGVHD and autoimmunity (Neven et al., 2009). In addition the presence of donor myeloid chimerism correlates with donor B-lymphocyte chimerism and it has been shown that freedom from intravenous immunoglobulin prophylaxis is associated with superior quality of life (Abd Hamid et al., 2018). A higher level of donor myeloid chimerism is required for long-term cure in disorders such as CGD and WAS. Female carriers of X-linked CGD are at risk of autoimmune problems, increased fatigue, anxiety and depression and neutrophil function of less than 10% is associated with risk of infection (Battersby et al., 2017, Battersby et al., 2019, Marciano et al., 2018). Incomplete donor chimerism in patients with WAS post HSCT has been associated with autoimmunity (Ozsahin et al., 2008, Moratto et al., 2011). For newer emerging disorders such as Activated PI3K Delta syndrome, Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and Lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency, Signal transducer and activator of transcription 1 (STAT1) and STAT3 gain of function diseases, it is currently unknown what level of donor chimerism is required to ensure long-term cure, but it is likely to be a high level (Nademi et al., 2017, Slatter et al., 2016, Seidel et al., 2018, Leiding et al., 2018, Milner et al., 2015). Therefore finding that the use of PBSC leads to a higher level of chimerism without any increase in grade III/IV acute GVHD and no chronic GVHD is important. The reason PBSC leads to better chimerism is likely to be due to the higher number of CD34+ stem cells compared to BM and CB. Traditionally the use of BM is preferred by Paediatric transplanters for treating malignant disease. A European retrospective study included 2584 patients transplanted from 2003 to 2012 for ALL. Use of PBSC was associated with a higher incidence of chronic GVHD and a higher TRM compared to other stem cell sources, although the OS was similar. No details of in vivo T-lymphocyte depletion were given (Simonin et al., 2017). However, in a prospective study of 411 paediatric patients with high risk ALL, no difference was found in outcomes between patients receiving BM or PBSC,

whichever end-point was considered, that is, OS, leukaemia-free-survival, non-relapse-mortality and cGVHD (Peters et al., 2015). We pay careful attention to the numbers of CD3+ cells in the product and limit this to 5 x 10^8/kg of the recipient. All patients, even MFD recipients receive serotherapy. These details are likely to be as important as the choice of conditioning agents and are part of the personalised medicine approach to each patient.

3.1.5 Treosulfan PK study

The PK study PP6 for the first time showed a relationship between high AUC and mortality and low AUC and chimerism. Children with a cumulative treosulfan AUC >6000mg hour/l had a TRM of 39%, whereas patients with AUC below this had a TRM of only 3%. A low treosulfan AUC was found to be associated with poor engraftment as defined by ≤ 20% donor myeloid chimerism. Forty-three percent of patients were outside the target of being within 80-125% of an AUC of 4800mg hour/l. Outcome might further be improved by TDM-based dosing particularly in infants who have a lower clearance due to immaturity in glomerular filtration rate, and lower central volume of distribution. The Medac BSA-based dosing showed a trend for reduced overexposure compared with weight-based dosing used in our study. As a result of this we have changed our practise and adopted the BSA-based dosing.

3.2 Future research

A prospective clinical trial with TDM of treosulfan to target the established AUC is warranted. Outcomes, particularly mortality and levels of chimerism are important factors which may be influenced. One of the challenges is that as treosulfan is given once daily for only 3 days, results of PK following the 1st dose need to be rapidly available to enable any change in the 2nd or 3rd doses to achieve the desired cumulative AUC.

Despite widespread use of the addition of thiotepa to treosulfan and fludarabine only Dinur-Schejter has reported a higher incidence of complete engraftment compared to treosulfan and fludarabine alone or treosulfan and cyclophosphamide and the numbers in each group were small. Many reports indicate that the addition of thiotepa does not seem to increase short-term toxicity, but no formal studies have been done and as thiotepa is an alkylating agent it would be expected to have an impact on fertility, so studies of late effects are also needed. A retrospective study is underway between Newcastle and Great Ormond Street Hospital to compare treosulfan/fludarabine with and without additional thiotepa in patients with PID. A prospective randomised trial comparing the 2 regimens would be ideal. It may be that additional thiotepa would be of benefit in some diseases, which are more difficult to engraft or require full donor chimerism in all cell lineages, but not be required for other disorders.

Further studies are required to investigate the late effects of treosulfan-based regimens particularly on fertility.

It is becoming increasingly recognized that the success of transplant depends on multiple factors in the transplant package. Simply performing TDM-guided personalization of one of the components of a conditioning regimen is only one aspect of the package. Interest is growing in the influence of fludarabine PK on outcome. Ivaturi et al reported results of 133 children with a variety of diseases that underwent HSCT with a variety of conditioning regimens which all included fludarabine. Young age and renal impairment were found to be associated with increased exposure. No association with TRM was found, but in the malignant setting disease free survival was highest 1 year post HSCT in those who had a systemic fludarabine plasma cumulative AUC greater than 15 mg x hour/l compared to patients with a level below this and this was statistically significant (Ivaturi et al., 2017). Mohanan et al studied 53 patients with

severe aplastic anaemia and Fanconi anaemia. Fludarabine given intravenously as fludarabine monophosphate is converted to fludarabine by the enzyme ecto-5'-nucleotidase or NT5E. Patients with a polymorphism in the NT5E gene had a lower clearance of fludarabine. They found a positive association with aGVHD in patients with a high plasma cumulative AUC (Mohanan et al., 2017). Chung et al found no association with clinical outcomes and fludarabine plasma AUC in 43 children undergoing HSCT with mainly busulfan and fludarabine based conditioning (Chung et al., 2019).

The effect of serotherapy on host lymphocytes and the incoming donor lymphocytes is extremely important and an increasing number of studies are focusing on levels of serotherapy and the impact they have on GVHD, engraftment, chimerism, immune reconstitution and infection. Marsh et al. reported data from 105 patients who had received alemtuzumab as part of conditioning. They found that lower levels of alemtuzumab at day zero were associated with significantly higher levels of aGVHD, but also higher levels of donor chimerism, lymphocyte counts at day + 30 and T lymphocytes at day + 100 (Marsh et al., 2016). Admiraal et al. reported results of 251 recipients of ATG. They defined successful immune reconstitution as a CD4+ lymphocyte count of more than 50 cells per microliter on 2 occasions by day + 100 post HSCT. The chance of successful immune reconstitution decreased with increasing AUC of ATG. Successful immune reconstitution was associated with increased OS caused by decreased non-relapse and relapse-related mortality. A high ATG AUC was associated with lower incidences of aGVHD, cGVHD and graft failure (Admiraal et al., 2015). Oostenbrink et al. reported results of 38 children with malignant haematological disorders who received different brands of ATG. Fresenius was cleared rapidly and uniformly whether they received 60mg/kg or 45mg/kg, but those who received 10mg/kg Genzyme had significantly slower reconstitution in CD3, CD4

and CD8 T lymphocytes compared to those who received a lower dose of 6-8mg/kg or Fresenius (Oostenbrink et al., 2019).

Traditionally post HSCT GVHD prophylaxis was given with cyclosporine A (CSA) and methotrexate. Some centres use tacrolimus as an alternative calcineurin inhibitor, but when intravenous administration is required, this is given as a 24 hour infusion which can be difficult. Calcineurin inhibitors inhibit GVHD by preventing the activation of the nuclear factor of activated T-cell family of transcription factors, which reduces the transcription of interleukin-2-dependent anti-inflammatory T regs. From the early development of reduced intensity regimens methotrexate has largely been replaced by mycophenolate mofetil (MMF) in combination with CSA (Zeiser et al., 2017). MMF is usually tapered and stopped a month post HSCT in the absence of GVHD. Lawitschka et al. recently reported approaches for paediatric aGVHD prophylaxis and treatment from 75 EBMT centres in 26 countries. CSA was continued for longer post HSCT, and there was more heterogeneity in additional agents, in non-malignant compared to malignant diseases. The majority of the centres aimed for a post-transplant target level of CSA of <200 ng/ml with an equal distribution between 100–150 and 160–200 ng/ml (Lawitschka et al., 2020) highlighting the need for standardised approaches towards aGVHD prophylaxis aswell as management. Further studies are needed to investigate differences in outcome such as GVHD or rejection, according to CSA levels, specifically for children undergoing HSCT for PID.

Therefore a Pan PK study to include TDM of treosulfan, fludarabine, thiotepa, together with serotherapy and calcineurin inhibitor levels, would enhance our understanding of the impact of different agents on outcomes.

Individualised dosing of all drugs used in the conditioning regimen is one aspect of the transplant package. We have shown that use of PBSC with a high number of CD34+ cells leads to higher myeloid chimerism compared to BM and CB without increasing the risk of severe acute or chronic GVHD. In the UK the transplant community has agreed not to give G-CSF to children as allogeneic donors due to the small risk of splenic rupture and possible, but not proven, increased risk of leukaemia developing in the future (Tigue et al., 2007), thus limiting them to donating BM. Therefore in some cases if a well matched unrelated donor is available who can donate PBSC, this may be preferable to a MSD. When choosing a donor for patients with inherited disease care needs to be taken when considering family donors who may be carriers which is to be avoided for example, in X-linked CGD, or who have not been tested for certain genetic disorders which may manifest later or show different phenotypes even within families, such as STAT 1 gain of function.

Current and future research planned will build on the cellular content of the harvest product and additional cellular therapy which may enhance immune reconstitution in particular to counteract infections in patients with PID, but which could also enhance the anti-tumour effect in the malignant setting.

An exciting development currently used for patients who lack an HLA matched donor uses a mismatched donor, most commonly a parent, who undergoes a mobilized PBSC harvest which is then manipulated in the laboratory using a CliniMACS machine which removes T-cell receptor (TCR) αβ bearing cells which are the effectors of GVHD together with CD19+ cells to reduce the risk of EBV driven post-transplant lymphoproliferative disease. The remaining product contains CD34+ progenitors, TCRγδ T cells, natural killer and dendritic cells, which enhance engraftment and early immune reconstitution. This has revolutionized the practice of

haploidentical HSCT for patients with PID (Lum et al., 2020b). Historically results were poor for non-SCID PID (Antoine et al., 2003, Gennery et al., 2010), but now a wide range of conditions have been successfully transplanted using this technique (Shah et al., 2018, (Supplementary paper 3), Balashov et al., 2015, Bertaina et al., 2014). Viral infection remains a significant problem and additional strategies are needed to accelerate immune recovery. Depletion of naïve CD45RA+ T lymphocytes significantly reduces alloreactivity whilst preserving memory CD45RO+ T lymphocytes with antiviral properties. A small study published results in 5 patients with PID and chronic viral infection – 4 patients engrafted and cleared virus within 2 months of HSCT (Touzot et al., 2015). A study using low dose memory T cell infusion after TCR αβ/CD19+ depleted grafts in 53 paediatric patients with malignant and non-malignant diseases showed safety and expansion of CMV specific T lymphocytes in 64% of patients within 100 days (Maschan et al., 2018). Another study using CD45RA+ depleted grafts from mismatched family donors in 26 children with leukaemia showed significantly higher T lymphocyte counts at Day + 30 post HSCT and a significant reduction in incidence, and duration of viraemia compared to CD3+/CD19+ depleted grafts (Triplett et al., 2018). We are currently designing a study to establish whether CD45RO+ memory T lymphocyte infusion following mismatched TCR αβ/CD19+ depleted HSCT is safe, and if it enhances immune recovery with reduced incidence, and severity of viral infection. The study cohort will be compared to historical cases without CD45RO+ additional cell infusion, and to a prospective cohort of MUD and MFD recipients.

An alternative is to infuse genetically modified αβ T lymphocyte bearing cells with an added caspase suicide gene. If aGVHD occurs, the cells can be removed by administration of rimiducid which is an inert compound that activates the suicide gene (Di Stasi et al., 2011). Two clinical trials have been performed, sponsored by Bellicum: BP-004 a phase I/II clinical

trial in children with malignant and non-malignant disorders following partially-matched, related, TCR αβ cell-depleted HSCT, and C-004, an observational trial evaluating outcomes in similar patients who received MUD donors, in order to compare with the mismatched donors who received T-depleted grafts with additional gene modified cells in the BP-004 trial (EudraCTnumber: 2014-000584-41 and 2017-002828-25 respectively). Results are awaited.

These approaches may enhance engraftment and immune reconstitution following matched donor transplants, or it may be that in time using a suitable parental haploidentical donor with one of these approaches which have a minimal risk of causing GVHD, may supersede the use of unrelated donors which are costly and take time to procure.

A simple and less costly approach to minimize the risk of GVHD post mismatched donor transplant is to give 2 doses of cyclophosphamide 3 to 4 days post infusion of replete mismatched cells. Rapidly proliferating cells are preferentially targeted by cyclophosphamide which depletes alloreactive donor T lymphocytes, leaving viral specific T lymphocytes and lymphocyte precursor cells. Use of this technique is increasing (Kreetapirom et al., 2017, Shah et al., 2017, Ouederni et al., 2016, Neven et al., 2019, Kurzay et al., 2019, Uppuluri et al., 2019). A current IEWP study is comparing the outcome of mismatched donor HSCT for PID using either post-transplant cyclophosphamide or TCR αβ/CD19+ depleted HSCT.

The use of T lymphocytes specifically directed at viral epitopes is another way of combating viral infections post HSCT (Ip et al., 2018, Naik et al., 2016), but donor banks of virus-specific or multi-virus specific T lymphocytes are not widespread. TRACE (Transfer of Adenovirus, CMV and EBV specific T cells) is a multi-national clinical phase III trial to prove efficacy and

safety of adoptive T cell transfer in immunocompromised individuals funded by the European commission.

A medac sponsored trial MC-FludT.16/NM is ongoing which is enrolling paediatric patients with non-malignant disorders in a randomised trial comparing treosulfan to busulfan. Newcastle decided not to participate in this trial as we did not want to randomise children to receive busulfan. However results of this trial will be important and may enable licensing of treosulfan for use in non-malignant disorders.

Further work needs to be done to optimize our approach to transplanting patients with DNA repair disorders and it is unclear whether treosulfan may have a role in these patients. These patients are exquisitively sensitive to DNA damaging chemotherapeutic agents. Regimens using reduced intensity conditioning similar to that used for Fanconi anaemia have been successful (Slack et al., 2018, Wolska-Kusnierz et al., 2015, Deripapa et al., 2017, Albert et al., 2010). In a recent publication a treosulfan-based conditioning regimen followed by TCRαβ/CD19-depleted HSCT in 10 patients with Nijmegen breakage syndrome demonstrated a low level of early transplant-associated toxicity and enhanced graft function with stable donor chimerism (Laberko et al., 2020).

There is interest in the use of treosulfan prior to gene therapy instead of busulfan but to date no clinical trials are in progress. The advantage of busulfan is that TDM-based dosing is widely available and generally a much lower AUC is sufficient.

Further efforts to reduce the toxicity of conditioning regimens include the use of antibodies and radioimmunotherapy. Straathof et al combined 2 anti-CD45 antibodies together with

alemtuzumab, fludarabine and low dose cyclophosphamide and achieved successful engraftment in 15 of 16 high risk patients with PID. CD45 is an attractive candidate because it is selectively expressed on all leucocytes and haemopoietic progenitors, but is absent on non-haemopoietic tissues (Straathof et al., 2009). CD117 (c-Kit receptor) is expressed on haematopoietic stem cells and studies have shown that an antibody against CD117 depleted haematopoietic stem cells and enabled engraftment of donor cells (Agarwal R., 2019). A clinical trial in patients with SCID is ongoing (Clinicaltrials.gov, 2020). Radiolabeled anti-CD45 or anti-CD66 have also been used more commonly in patients with malignant diseases to target haematopoietic cells and reduce toxicity to other organs (Ali et al., 2016, Pagel et al., 2006, Pagel et al., 2009, Schulz et al., 2011).

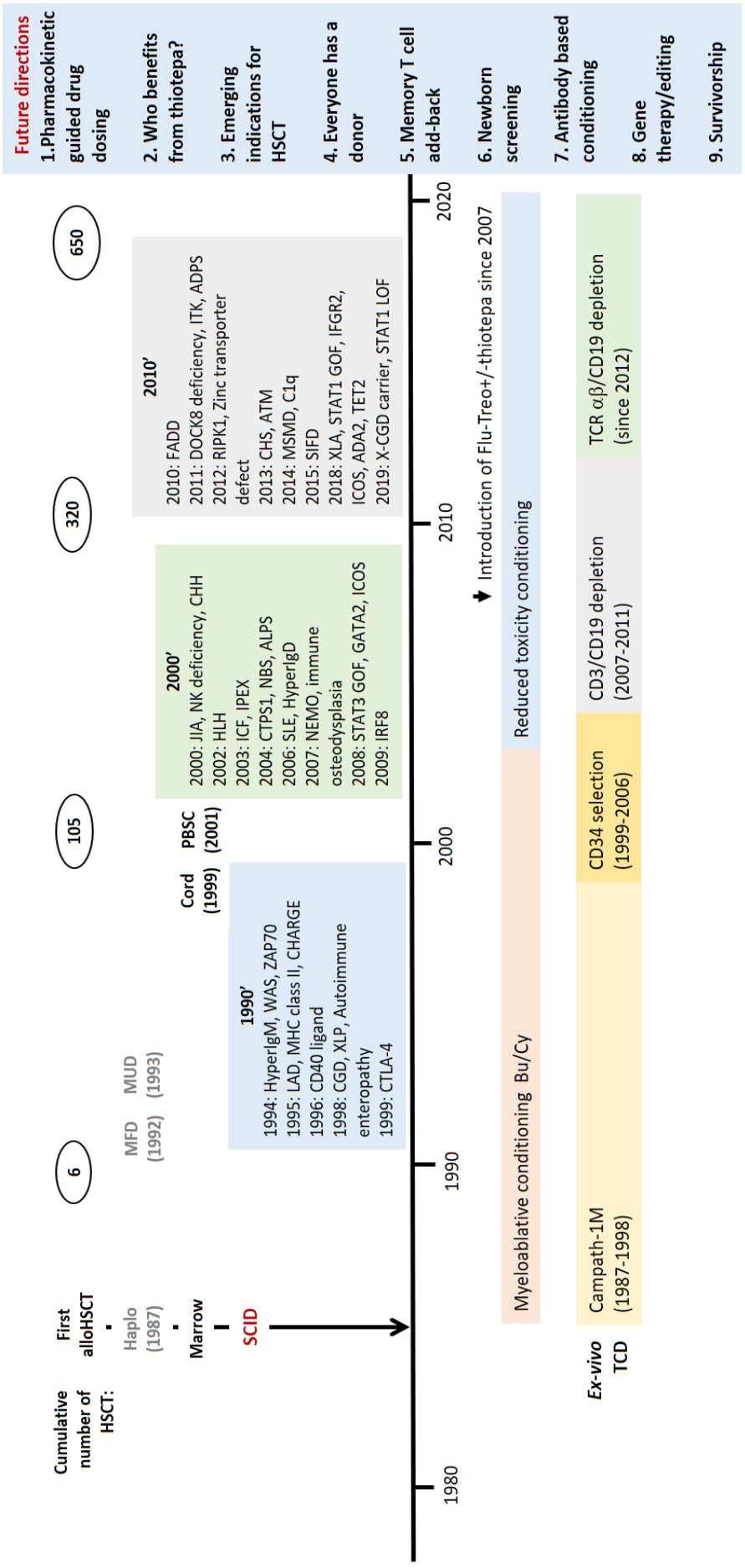
3.3 Conclusions

Increased awareness of PIDs and the advent of next-generation-sequencing have revolutionized the diagnosis of affected patients. The field of HSCT for PID has advanced tremendously over the last 20 years. Newcastle's clinical research, and specifically the presented publications, have made a major contribution to these improved outcomes. In Newcastle the OS was in the region of 50% in the 1980s and 1990s when 5 – 15 patients were transplanted each year. Now, approximately 40 children are transplanted each year with an OS of between 85 and 90%. There are many factors that have influenced these improvements, but the introduction of less toxic conditioning regimens has been a fundamental change. We have used treosulfan in preference to busulfan since 2007 and have rigorously documented the outcome of the patients treated in Newcastle and Great Ormond Street Hospitals. The demonstration of superior T cell chimerism and less toxicity when combined with fludarabine compared to cyclophosphamide led to a step

change in confirming this combination (Appendix D1). Early indications that using PBSC with Alemtuzumab serotherapy and capping the CD3+ content of grafts at 5 x 10^8/kg leads to higher myeloid chimerism than BM without an increase in grade III/IV aGVHD, were reported in PP5. Our prospective phase II trial is the first to demonstrate an association with high AUC and increased mortality, and low AUC and poor engraftment, paving the way for future studies to optimize the dosing of treosulfan. At the same time, use of treosulfan has increased for a wide range of non-malignant and malignant disorders. PP3 reported its use in non-malignant disorders from EBMT centres demonstrating safety and efficacy. PP4 reported excellent OS and EFS for patients with CGD treated with treosulfan-based conditioning in a worldwide study.

These works have firmly established the place of treosulfan in conditioning regimens used for children with PID across the world. They have also paved the way for future studies by demonstrating the need to explore personalized dosing of all conditioning agents prior to HSCT, together with precise measurement of the cellular components of graft material. These vital aspects of transplant provide a platform for further improvements to be made post-transplant, e.g. additional cellular therapy to improve immune reconstitution.

Figure 4: Evolution of HSCT in children with PID in Newcastle



Adapted from Figure by Su Han Lum

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REFLECTION

Following what would now be called Core Paediatric Training, my family and I spent 5 months in France at the beginning of 1994 to brush up our French before moving to the Democratic Republic of Congo (then Zaire), to work in a mission hospital. In 1996 war broke out and, after the birth of my daughter Maddie here in the UK, we were unable to return until August 1997 for a final year of work there. We moved to Newcastle in 1998 for my husband Justin to take up an SpR post in Adult Infectious Diseases and Tropical Medicine. Paediatric training had changed, I had no national training number and competition for SpR posts was high. I was not in a rush to be an SHO again with a young family and a busy husband. A passing conversation between Justin and Terry Flood, who asked if his wife would like any sessions on the Children's Bone Marrow Transplant Unit, led to me taking a 6 week part-time locum Staff Grade Post at the end of 1999, which I assumed would help me get an SpR post and become a "proper" General Paediatrician in time. And yet here I am running a world-renowned Transplant Unit for children with PID!

The route that I took to becoming a Consultant through the CESR pathway was a huge struggle. Today more than ever, the medical system does not lend itself kindly to people who do not tick the boxes. In my view there is no substitute for training on the job, which needs to be balanced with the current emphasis on reducing trainees working hours, protected time for education and training, supervision etc.

I was fortunate to find myself in a close-knit supportive team with a methodical, systematic approach to caring for children undergoing HSCT on a daily basis which I enjoyed. At the same time, I was thrown into a world of opportunities to critically appraise what we do, put data together, present and publish in order to improve the outcome for these children. I was so nervous about presenting at conferences that I took singing lessons, figuring that if I could sing, I should definitely be able to talk.

For many years as a Staff Grade/Associate Specialist working less than full-time, I felt second-class to Consultants. I was not a "trainee" and did not feel like an expert in what is a very academic department. I have never thought of taking 3 years out of clinical medicine to do a PhD, which despite the challenges, seemed like a luxury. There is a danger that departments

can become 2 tiered with the real “academics” and everybody else. Nevertheless, I have accrued more publications than many Professors I know and find myself giving advice to physicians all over the world about HSCT for children with PID, on an almost daily basis.

So why write a thesis on the use of treosulfan? . . . . . Because it’s a story worth telling.
I have been instrumental in introducing the use of treosulfan around the world through a consistent approach, leading to good data and publications in high impact journals. It is only recently that we have been involved in prospective trials and whilst retrospective series are frequently criticized, we have included all patients no matter what risks etc. they came to transplant with.

I am now thoroughly enjoying developing our department, sharing our experience with trainees, clinical observers and fellows from overseas, through presentations, publications and email requests from all over the world. There has been a huge improvement in the outcome of our patients in 20 years and there are so many exciting developments on the horizon.

Still my favourite days are those on the ward, looking after patients and being part of a highly motivated multi-disciplinary team.

APPENDICES

Appendix A *Candidates' contributions to papers and co-authorship forms*

SUBMISSION BY STAFF CANDIDATES FOR THE DEGREE OF PHD BY PUBLISHED WORK

CO-AUTHORSHIP FORM

This form must accompany any submission of a joint authored publication for the degree of Doctor of Philosophy on the basis of published work.

A candidate should submit a separate form for each jointly authored work which is submitted for the degree.

TITLE OF PUBLICATION (article, book, chapter, monograph)

Hematopoietic Cell Transplantation for Primary Immunodeficiency – Conventional and Emerging Indications

DATE OF PUBLICATION **2018**

NAME AND VOLUME OF JOURNAL (where appropriate)

Expert Review in Clinical Immunology 2018 Feb;14(2):103-114

PUBLISHER (for book, chapter or monograph)

EDITORS (chapter only)

ISBN (where appropriate)

If the work has not been published but has been accepted for publication please attach a statement from the Editor or Publisher which confirms the intention to publish the work.

NAMES OF JOINT AUTHORS	INSTITUTION
1. **Andrew Gennery**	**Newcastle University**

CONTRIBUTION OF THE CANDIDATE TO THIS WORK (%)

Design of investigation	**50%**
Conduct of research	**50%**

Analysis of outcome	**50%**
Preparation for publication	**50%**
TOTAL	**50%**

(To be an average of, or at least consistent with, the above figures)

This statement should be endorsed by all of the co-authors.

I confirm that the above is a true estimate of the candidate's contribution to this work.

Signature 1: 

(Professor Andrew Gennery)

SUBMISSION BY STAFF CANDIDATES FOR THE DEGREE OF PHD BY PUBLISHED WORK

CO-AUTHORSHIP FORM

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A candidate should submit a separate form for each jointly authored work which is submitted for the degree.

TITLE OF PUBLICATION (article, book, chapter, monograph)

Conditioning Regimens for Hematopoietic Cell Transplantation in Primary Immunodeficiency

DATE OF PUBLICATION **18 November 2019**

NAME AND VOLUME OF JOURNAL (where appropriate)

Current Allergy and Asthma Reports (2019) 19:52
https://doi.org/10.1007/s11882-019-0883-1
Immune Deficiency and Dysregulation

PUBLISHER (for book, chapter or monograph)

EDITORS (chapter only)

ISBN (where appropriate)

If the work has not been published but has been accepted for publication please attach a statement from the Editor or Publisher which confirms the intention to publish the work.

NAMES OF JOINT AUTHORS	INSTITUTION
1. Su Han Lum	**Great North Children's Hospital, Newcastle**
2. Manfred Hoenig	**University Medical Centre, Ulm**
3. Andrew Gennery	**Newcastle University**

CONTRIBUTION OF THE CANDIDATE TO THIS WORK (%)

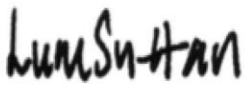
Design of investigation	**50%**
Conduct of research	**50%**
Analysis of outcome	**50%**
Preparation for publication	**50%**
TOTAL	**50%**

(To be an average of, or at least consistent with, the above figures)

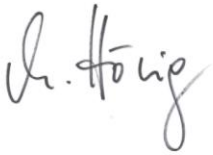
This statement should be endorsed by all of the co-authors.

I confirm that the above is a true estimate of the candidate's contribution to this work.

Signature 1: **Su Han Lum**



Signature 2: **Manfred Hoenig**



Signature 3: **Andrew Gennery**

SUBMISSION BY STAFF CANDIDATES FOR THE DEGREE OF PHD BY PUBLISHED WORK

CO-AUTHORSHIP FORM

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A candidate should submit a separate form for each jointly authored work which is submitted for the degree.

TITLE OF PUBLICATION (article, book, chapter, monograph)

Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases

DATE OF PUBLICATION **10 August 2015**

NAME AND VOLUME OF JOURNAL (where appropriate)

Bone Marrow Transplantation 2015;50:1536-1541

PUBLISHER (for book, chapter or monograph)

EDITORS (chapter only)

ISBN (where appropriate)

If the work has not been published but has been accepted for publication please attach a statement from the Editor or Publisher which confirms the intention to publish the work.

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9. A Gennery	**Newcastle University**
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CONTRIBUTION OF THE CANDIDATE TO THIS WORK (%)

Design of investigation	**50%**
Conduct of research	**50%**
Analysis of outcome	**20%**
Preparation for publication	**90%**
TOTAL	**55%**

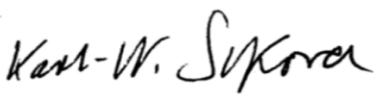
(To be an average of, or at least consistent with, the above figures)

This statement should be endorsed by all of the co-authors.

I confirm that the above is a true estimate of the candidate's contribution to this work.

Signature 1: **H Boztug**

Signature 2: **U Potschger**



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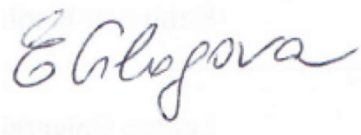
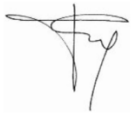


Signature 8: **P Veys**

Signature 9: **A Gennery**

Signature 10: **C Peters**



SUBMISSION BY STAFF CANDIDATES FOR THE DEGREE OF PHD BY PUBLISHED WORK

CO-AUTHORSHIP FORM

This form must accompany any submission of a joint authored publication for the degree of Doctor of Philosophy on the basis of published work.

A candidate should submit a separate form for each jointly authored work which is submitted for the degree.

TITLE OF PUBLICATION (article, book, chapter, monograph)

Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicentre experience

DATE OF PUBLICATION **23 May 2016**

NAME AND VOLUME OF JOURNAL (where appropriate)

Blood 21 July 2016;128(3):440-448

PUBLISHER (for book, chapter or monograph)

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21. Karl-Walter Sykora	**Hannover Medical School**
22. Andrew Gennery	**Newcastle University**

CONTRIBUTION OF THE CANDIDATE TO THIS WORK (%)

Design of investigation	**75%**
Conduct of research	**70%**
Analysis of outcome	**50%**
Preparation for publication	**40%**
TOTAL	**60%**

(To be an average of, or at least consistent with, the above figures)

This statement should be endorsed by all of the co-authors.

I confirm that the above is a true estimate of the candidate's contribution to this work.

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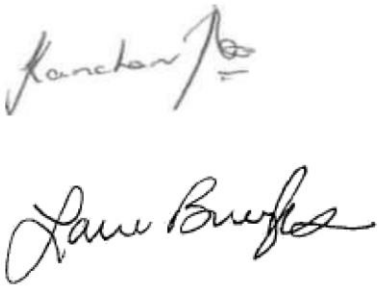

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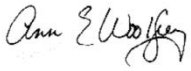

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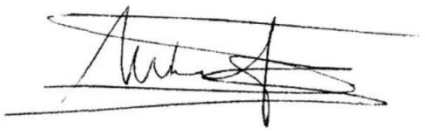

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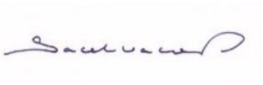

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TITLE OF PUBLICATION (article, book, chapter, monograph)

Treosulfan and Fludarabine for Hematopoietic Stem Cell Transplantation in Children with Primary Immunodeficiency: UK experience

DATE OF PUBLICATION **8 November 2017**

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If the work has not been published but has been accepted for publication please attach a statement from the Editor or Publisher which confirms the intention to publish the work.

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Conduct of research	**60%**
Analysis of outcome	**50%**
Preparation for publication	**90%**
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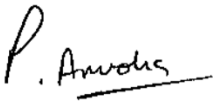

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Conduct of research	**50%**
Analysis of outcome	**30%**
Preparation for publication	**40%**
TOTAL	**40%**

(To be an average of, or at least consistent with, the above figures)

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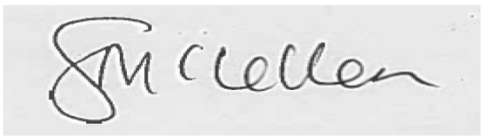

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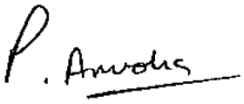

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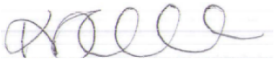
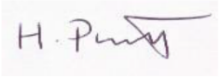
Signature 21: **Kirsty Devine** 

Signature 22: **Helen Prunty** 

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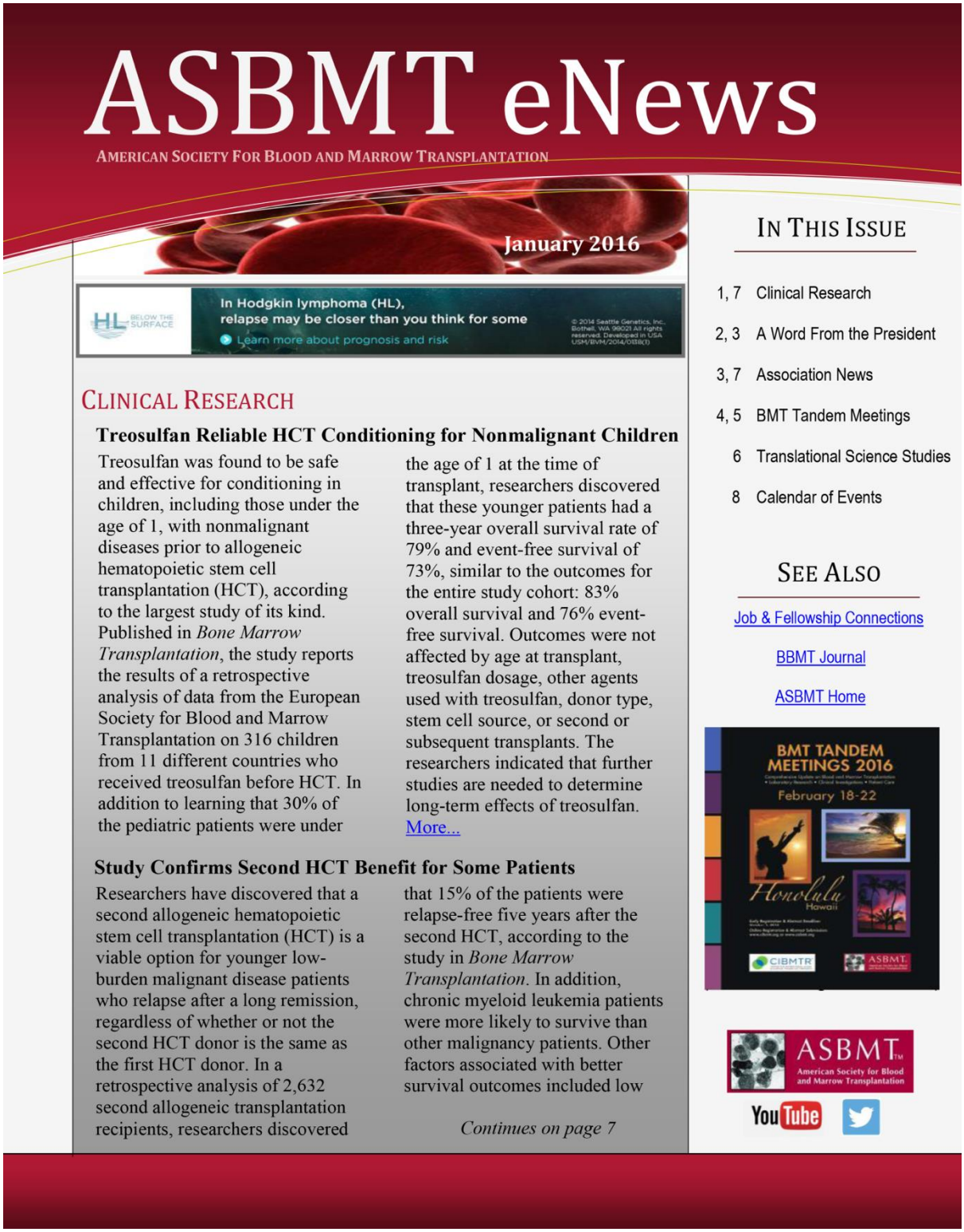
Appendix B ***ASBMT eNEWS January 2016. Slatter et al. 2015. 'Treosulfan Reliable HSCT Conditioning for Nonmalignant Children'***



ASBMT eNews

AMERICAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION

January 2016

CLINICAL RESEARCH

Treosulfan Reliable HCT Conditioning for Nonmalignant Children

Treosulfan was found to be safe and effective for conditioning in children, including those under the age of 1, with nonmalignant diseases prior to allogeneic hematopoietic stem cell transplantation (HCT), according to the largest study of its kind. Published in *Bone Marrow Transplantation*, the study reports the results of a retrospective analysis of data from the European Society for Blood and Marrow Transplantation on 316 children from 11 different countries who received treosulfan before HCT. In addition to learning that 30% of the pediatric patients were under the age of 1 at the time of transplant, researchers discovered that these younger patients had a three-year overall survival rate of 79% and event-free survival of 73%, similar to the outcomes for the entire study cohort: 83% overall survival and 76% event-free survival. Outcomes were not affected by age at transplant, treosulfan dosage, other agents used with treosulfan, donor type, stem cell source, or second or subsequent transplants. The researchers indicated that further studies are needed to determine long-term effects of treosulfan. More...

Study Confirms Second HCT Benefit for Some Patients

Researchers have discovered that a second allogeneic hematopoietic stem cell transplantation (HCT) is a viable option for younger low-burden malignant disease patients who relapse after a long remission, regardless of whether or not the second HCT donor is the same as the first HCT donor. In a retrospective analysis of 2,632 second allogeneic transplantation recipients, researchers discovered that 15% of the patients were relapse-free five years after the second HCT, according to the study in *Bone Marrow Transplantation*. In addition, chronic myeloid leukemia patients were more likely to survive than other malignancy patients. Other factors associated with better survival outcomes included low

Continues on page 7

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DOI 10.1182/blood-2016-05-717488

TRANSPLANTATION

Comment on Morillo-Gutierrez et al, page 440

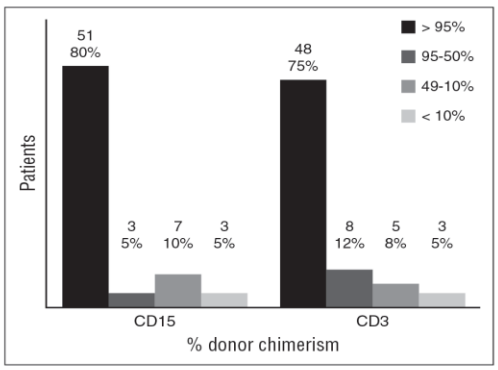
Progress toward less toxic conditioning

Rebecca H. Buckley DUKE UNIVERSITY MEDICAL CENTER

In this issue of *Blood*, Morillo-Gutierrez et al report findings from the largest multicenter retrospective study on the use of treosulfan as the primary conditioning agent for hematopoietic stem cell transplantation (HSCT) in children with chronic granulomatous disease (CGD).[1]

Their main discovery in the 70 patients studied is that treosulfan, a low toxicity alkylating agent, can be used effectively as part of conditioning for HSCT in high-risk children with CGD. This represents a major breakthrough for the treatment of this condition, which is complicated by a lifetime of serious bacterial and fungal infections that also put these patients at high risk for transplantation. In the few reported cases of toxicity in these authors' study, it was minimal, and primarily involved the skin and resolved spontaneously. In particular, no veno-occlusive disease occurred, even in patients receiving a second transplant.

It has been recognized for some time that patients with genetic defects in the immune system do not require the same conditioning that patients with malignancies do, prior to HSCT. Many, if not most of these immunodeficient patients are already seriously and chronically infected by the time transplantation is considered, so their chances of surviving standard conditioning regimens are low. A variety of reduced-intensity conditioning regimens have, therefore, been tried for primary immunodeficiency patients undergoing HSCT, with less overall toxicity but with varying success in preventing autologous recovery or mixed chimerism.[2] However, it is often not appreciated that the type of conditioning needed (if any) depends upon the genetic defect being treated. If the defect is primarily in T-lineage cells, the conditioning required is much less, as shown by the many patients with severe combined immunodeficiency (SCID) who had successful T-cell reconstitution with no[3] or low-dose conditioning because their T-cell defects prevented graft rejection. Because SCID patients' myeloid cells are usually normal in number and function, there is no need to achieve myeloid chimerism. However, in CGD, the defect is in the myeloid lineage, so the assumption has been that full myeloablative conditioning prior to HSCT is needed to cure this condition. Importantly, regarding the latter requirement, a major finding in the Morillo-Gutierrez et al study was that the level of chimerism achieved in both myeloid and T-cell lineages in these treosulfan-conditioned HSCT-treated CGD patients was excellent (see figure).



Split cell chimerism for $CD15^+$ and $CD3^+$ cells at last follow up. Results given in absolute number and percentage over the 64 patients with available split chimerism. Those who had second procedures were included with their last result before the event. Those who died had the last result available before their death. See Figure 3 in the article by Morillo-Gutierrez et al that begins on page 440.

The authors rightfully caution, however, that long-term follow up is required to compare with other regimens and to ascertain late effects of the agent, particularly on gonadal function. The largest prospective study of reduced-intensity conditioning for CGD, published by Güngör et al[4] in 2014, included 56 adults and children from 16 centers, and used sub-myeloablative doses of busulfan, an alkylating agent known to have greater toxicity than treosulfan. The results of the latter study compare favorably with the results of the Morillo-Gutierrez et al study. The 21-month median overall survival (OS) and event-free survival (EFS) rates in that study were 93% and 89%, respectively, similar to the OS and EFS rates of 91.4% and 81.4% found by Morillo-Gutierrez et al, at a median follow-up of 34 months. The cumulative incidence of acute grade 3-4 graft-versus-host disease (GVHD) was 4% in the Güngör at al[4] study compared with 12% in the Morillo-Gutierrez et al study. Nine patients (13%) developed chronic GVHD in the Morillo-Gutierrez et al study (although 6 of them later became asymptomatic) and 4 patients (7%) in the Güngör et al[4] study. Graft failure occurred in 5% of patients in the Güngör et al[4] study and in 12% of patients in the Morillo-Gutierrez

et al study. Stable (≥90%) donor myeloid chimerism was documented in 93% of the surviving patients in the Güngör et al[4] study.

The problems with analyzing data from the above and all other transplant outcome studies, retrospective or prospective, are the concomitant use of other conditioning agents and the use of various types of donors. In the Morillo-Gutierrez et al study, it could be shown that that there were no differences between matched and mismatched donor grafts in either OS or EFS, and the addition of thiotepa had no effect. The increasing use of serotherapy, however, is another variable that confounds the outcomes of HSCT studies. Serotherapy agents, such as antithymocyte globulin (ATG) and alemtuzumab, are used primarily to enhance engraftment and suppress GVHD. However, the long-term effects of use of these agents need to be carefully studied, particularly for alemtuzumab. In a recent study of different doses of alemtuzumab, patients receiving the higher doses had lower lymphocyte counts at days 30 and 100 than did those receiving lower doses.[5] It is well-recognized that the use of this agent is associated with a high incidence of viral reactivation posttransplantation.[6] In addition, Dvorak et al and others have observed long-term delays in T-cell recovery and in the production of recent thymic emigrants in patients receiving this agent.[7] In the Morillo-Gutierrez et al study, 57 patients received either ATG or alemtuzumab compared with 13 who did not receive any serotherapy and there was no significant effect of the use of these agents on the outcome measures, but no separate analysis was presented on the effects of use of alemtuzumab.

Finally, one of the well-recognized late toxicities of standard conditioning regimens is gonadal dysfunction. In a recent comparison study of busulfan-containing regimens vs reduced-intensity regimens containing other agents, there was a much higher incidence of gonadal dysfunction in those receiving busulfan-containing regimens.[4] It will take several years before it is known whether treosulfan causes this problem.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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TRANSPLANTATION

Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience

Mary A. Slatter,[1] Kanchan Rao,[2] Persis Amrolia,[2] Terry Flood,[1] Mario Abinun,[1] Sophie Hambleton,[1] Zohreh Nademi,[1] Nick Goulden,[2] Graham Davies,[2] Waseem Qasim,[2] Hubert B. Gaspar,[2] Andrew Cant,[1] Andrew R. Gennery,[1] and Paul Veys[2]

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Children with primary immunodeficiency diseases, particularly those less than 1 year of age, experience significant toxicity after hematopoietic stem cell transplantation, with busulfan- or melphalan-based conditioning. Treosulfan causes less veno-occlusive disease than busulfan and does not require pharmacokinetic monitoring. We report its use in 70 children. Children received 42 g/m² or 36 g/m² with cyclophosphamide 200 mg/kg (n = 30) or fludarabine 150 mg/m² (n = 40), with alemtuzumab in most. Median age at transplantation was 8.5 months (range, 1.2-175 months); 46 (66%) patients were 12 months of age or younger. Donors were as follows: matched sibling donor, 8; matched family donor, 13; haploidentical, 4; and unrelated, 45. Median follow-up was 19 months (range, 1-47 months). Overall survival was 81%, equivalent in those age less or greater than 1 year. Skin toxicity was common. Veno-occlusive disease occurred twice with cyclophosphamide. Eighteen patients (26%) had graft-versus-host disease, and only 7 (10%) greater than grade 2. Two patients rejected; 24 of 42 more than 1 year after transplantation had 100% donor chimerism. The remainder had stable mixed chimerism. T-cell chimerism was significantly better with fludarabine. Long-term follow-up is required, but in combination with fludarabine, treosulfan is a good choice of conditioning for hematopoietic stem cell transplantation in primary immunodeficiency disease. (*Blood.* 2011;117(16):4367-4375)

Introduction

Hematopoietic stem cell transplantation (HSCT) remains the only curative option for many children with primary immunodeficiency disorders (PIDs) or severe immune dysregulatory disorders. The aim of HSCT is to produce stable donor engraftment after partial or full ablation of the recipient's marrow and immune system using a combination of chemotherapy, antibody therapy, and a graft-versus-marrow effect.[1] Apart from a graft-versus-marrow effect, there is no advantage in producing significant graft-versus-host disease (GVHD) in these patients as no graft-versus-tumor effect is required, GVHD can adversely affect thymic function, and stable mixed chimerism can lead to cure.[2] In recent years, survival rates for allogeneic HSCT have improved because of a number of factors, including better human leukocyte antigen (HLA) matching and greater availability of closely matched unrelated donors, including the use of cord blood donations, improved monitoring for viral and fungal infections with preemptive treatment, and better supportive care.[3] The introduction of reduced intensity conditioning regimens, such as fludarabine and melphalan, has reduced treatment-related toxicity in some PID patients,[4] but toxicity remains a problem for children less than 1 year of age[5] and there have been specific cardiac toxicities associated with melphalan.[6] Minimal intensity conditioning, for instance, with fludarabine and cyclophosphamide can reduce toxicity even further but may be associated with poor donor myeloid chimerism or an increased incidence of GVHD.[7] Consequently, there is a need to explore new conditioning regimens for PID, which enable adequate myeloablation with limited toxicity, particularly in patients less than 1 year of age.

Treosulfan (L-treitol-1,4-bis-methanesulfonate) is the prodrug of L-epoxybutane, a water-soluble bifunctional alkylating agent with myeloablative and immunosuppressive properties,[8] and has been shown to provide effective HSCT conditioning with reduced risk of toxicities, particularly veno-occlusive disease (VOD), compared with busulfan.[9-13] In addition, unlike busulfan, it may not be necessary to measure drug levels, as stable linear pharmacokinetics up to the clinically effective dose of 42 g/m² have been shown.[10] Beelen et al demonstrated a low rate of organ toxicities and favorable one-year nonrelapse mortality rate combining treosulfan with cyclophosphamide in a group of 18 adult patients with hematologic malignancies considered ineligible for other myeloablative preparative regimens.[10] Casper et al combined treosulfan with fludarabine in a group of 30 adult patients with hematologic malignancies considered as unacceptable risks for conventional conditioning and achieved a good outcome with respect to toxicity, achievement of complete donor chimerism, low GVHD rate, and low treatment-related mortality and relapse rate.[12] There is less published evidence on the use of treosulfan in children,[13] in particular those with PID. Early studies using treosulfan for HSCT in children with PID (n = 18) look promising with 17 of 18 surviving to a median follow-up of 429 days (range, 156-722 days),[9] including successful engraftment in some forms of PID prone to graft rejection,

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Table 1. Characteristics of patients who received fludarabine

Diagnosis	Age at transplantation, mo	Total dose treosulfan, g/m² serotherapy	Donor	Stem cell source	Toxicity	GVHD, grade	Complications	Latest chimerism, % donor	IVIg/CD4	Outcome/length of follow-up, mo
Patients who received fludarabine										
1. HLH	8	42, alemtuzumab	MFD	BM	Rash	Liver III	Adenovirus cGVHD	100	NA	Died 16 mo after transplantation
2. HLH	8	42, alemtuzumab	URD	BM*	No	No	Multiorgan failure	NA	NA	Died day −2 before transplantation, HLH
3. ALPS	8	42, alemtuzumab	Haplo CD3/CD19 depletion	PBSCs	No	No	CMV	Rejected	NA	Died 6 mo after transplantation
4. HLH/Griscelli syndrome	8	42	MSD	BM	No	No	MDS/AML	100 slipped, monosomy 7 recipient cells	NA	Died 3 ys after transplantation
5. SCID Intestinal atresias	8	36, ATG	URD 9/10	Cord	No	No	Pseudomonal sepsis	100	NA	Died 1 month after transplantation
6. Omenn syndrome	4	36, alemtuzumab	URD	BM	Seizures	Liver + gut IV	GVHD, cerebral infarcts	100	NA	Died 11 mo after transplantation
7. SCID	5	42, ATG	URD 7/10	Cord	No	No	No	100	Off/N	Alive 47 mo
8. WAS	15	42, alemtuzumab	URD	BM	No	No	Low platelets, splenectomy	T 100, B 40, CD15 0	Off/N	Alive 43 mo
9. HLH	7	42, alemtuzumab	URD 8/10	BM	No	Gut III	No	100	Off/N	Alive 41 mo
10. SCID	8	42, alemtuzumab	URD 9/10	BM	No	No	No	100	Off/N	Alive 41 mo
11. WAS	47	42, alemtuzumab	URD	BM	No	No	CMV retinitis	100	Off/N	Alive 39 mo
12. SCID	7	42, alemtuzumab	URD 7/10	BM	No	No	No	T 100, B 0, CD15 0	On/N	Alive 38 mo
13. LAD	9	42, alemtuzumab	URD 9/10	BM	No	No	CMV retinitis + viremia	100	Off/N	Alive 37 mo
14. LAD	7	42, ATG	URD 7/10	Cord	No	No	No	T + B 14, CD15 9	Off/N	Alive 36 mo
15. WAS	32	42, alemtuzumab	URD	BM	No	No	No	100%	Off/N	Alive 34 mo
16. LAD	3	42, alemtuzumab	MFD	BM	Perineal ulceration	No	No	T 96, B 43, CD15 62	Off/N	Alive 30 mo
17. WAS	19	42, alemtuzumab	URD	PBSCs	No	No	No	100	Off/N	Alive 28 mo
18. SCID	10	42, alemtuzumab	URD	BM	No	No	Nephrotic syndrome	100	On/low	Alive 28 mo
19. WAS	21	42, alemtuzumab	URD 9/10	PBSCs	No	No	Nephrotic syndrome	T 100, B97, CD15 44	Off/N	Alive 28 mo
20. T-cell activation defect	3	42, alemtuzumab	URD	PBSCs	Perineal ulceration	No	No	100	Off/N	Alive 27 mo
21. SCID	8	42, alemtuzumab	URD	Cord	No	No	No	T 100, B 100, CD15 93	Off/N	Alive 26 mo
22. SCID	1.5	36, alemtuzumab	URD 8/10	Cord	Perineal ulceration	No	No	T 100, B 94, CD15 91	Off/N	Alive 26 mo
23. SCID	2.5	36, alemtuzumab	MFD	BM	Perineal ulceration	No	Pneumonitis	100	Off/N	Alive 26 mo
24. SCID	1.6	36, alemtuzumab	MFD	BM	Perineal ulceration, seizures	No	No	100	Off/N	Alive 25 mo
25. SCID	1.6	36, alemtuzumab	MFD	BM	Perineal ulceration	Skin I	No	T 100, B 61, CD15 84	Off/N	Alive 25 mo
26. SCID	12	42, alemtuzumab	URD	Cord	No	No	No	T 100, B 97, M 98	Off/N	Alive 23 mo
27. Omenn syndrome β-thalassemia	6	36, alemtuzumab	URD 9/10	PBSCs	Rash	No	AIHA	100	On/N	Alive 21 mo
28. Intractable colitis of infancy	10	42, alemtuzumab	MFD	PBSCs	No	No	No	100	Off/N	Alive 20 mo
29. SCID	9	36	URD 9/10	Cord	No	Skin II	No	100	On/N	Alive 19 mo
						cGVHD				
30. SCID/CHH	8	36, alemtuzumab	URD 9/10	BM	No	No	No	T 100, B NA, CD15 0	Off/N	Alive 16 mo
31. SCID	10	36, alemtuzumab	MFD	BM	No	Skin II, cGVHD	No	T 100, B 0, CD15 0	On/N	Alive 13 mo
32. CGD	127	42, alemtuzumab	URD	PBSCs	No	Skin II	Pneumatosis, renal dysfunction	100	Off/low	Alive 11 mo
33. SID	31	42, alemtuzumab	URD	BM	Rash	No	Adenovirus	T 95, B 100, CD15 100	On/N	Alive 8 mo
34. WAS	43	42, alemtuzumab	URD	BM	No	No	No	100	Off/N	Alive 8 mo
35. CGD	175	42, alemtuzumab	URD 9/10	PBSCs	No	No	No	100	Off/N	Alive 7 mo
36. WAS	17	42, alemtuzumab	URD	BM	No	No	EBV	100	On/N	Alive 6 mo
37. IPEX	6	36, alemtuzumab	URD	Cord	No	No	Tracheostomy, gut resection, nephrotic syndrome	T 94, B 82, CD15 58	On	Alive 6 mo

HLH indicates hemophagocytic lymphohistiocytosis; MFD, matched family donor; BM, bone marrow; NA, not applicable; cGVHD, chronic graft-versus-host disease; URD, unrelated donor; 10/10, HLA match; ALPS, autoimmune lymphoproliferative syndrome; PBSCs, peripheral blood stem cells; MSD, matched sibling donor; MDS, myelodysplasia; AML, acute myeloid leukemia; SCID, severe combined immunodeficiency; ATG, antithymocyte globulin; EBV, Epstein-Barr virus; WAS, Wiskott-Aldrich syndrome; LAD, leukocyte adhesion deficiency; AIHA, autoimmune hemolytic anemia; CHH, cartilage hair hypoplasia; CGD, chronic granulomatous disease; SID, severe immune dysregulation; IPEX, immune deficiency polyendocrinopathy X-linked; HOCM, hypertrophic obstructive cardiomyopathy; HHV6, human herpesvirus 6; XLP, X-linked lymphoproliferative syndrome; MHC II, major histocompatibility class II deficiency; CID, combined immune deficiency; CVA, cerebrovascular attack; VOD, veno-occlusive disease; SCN, severe congenital neutropenia; Bu/Cy, busulfan/cyclophosphamide; ICF, immunodeficiency centromeric instability facial dysmorphism syndrome; CD40L, CD40 ligand deficiency; and CVVH, continuous veno-venous hemofiltration.

*Patient died before transplantation.

†Patient 50 had haploidentical maternal CD34⁺ selected PBSCs plus BM from MSD who had had a haploidentical transplantation from the mother 15 years earlier.

Table 1. (Continued)

Diagnosis	Age at transplantation, mo	Total dose treosulfan, g/m² serotherapy	Donor	Stem cell source	Toxicity	GVHD, grade	Complications	Latest chimerism, % donor	IVIg/CD4	Outcome/length of follow-up, mo
38. SCID	1. 4	36, alemtuzumab	URD 9/10	Cord	Perineal ulceration	No	No	100	On	Alive 5 mo
39. SCID	3	36, alemtuzumab	MFD	BM	No	No	Pneumonitis, pulmonary hemorrhage, HOCM	100	On	Alive 5 mo
40. XLP-like	42	42, alemtuzumab	URD	BM	Rash	No	No	100	On	Alive 1 month
Patients who received cyclophosphamide										
41. MHC II	17	42	MSD	BM	No	No	HHV6, lungs	100	On/low	Died 5 mo after transplantation
42. CID	96	42	MSD	BM	No	No	CVA	NA	NA	Died day 7
43. CID	81	42, alemtuzumab	URD	BM	No	No	Pneumonitis, renal failure, HHV6	100	NA	Died day 34
44. SCID	6	42, alemtuzumab	MFD	BM	VOD	No	Sudden liver failure VOD	NA	NA	Died day 13
45. SCID	1. 2	36	MSD	Cord	Seizures	No	Severe pneumonitis, pulmonary hypertension	100	NA	Died day 64
46. CHH	42	36, alemtuzumab	URD 9/10	BM	Skin rash	No	Pulmonary hemorrhage, CMV, adenovirus	100	NA	Died day 32
47. SID	134	36, alemtuzumab	URD	BM	No	Gut + skin IV	Adenovirus, GVHD, candida	100	NA	Died day 139
48. SCN	13	42	MSD	BM	No	No	Ongoing neutropenia,	Mixed	(Off/N)	Retransplantation, Bu/Cy alive
49. MHC II	11	36	MSD	BM	No	No	↓ chimerism, top-up day 54, graft loss	Mixed	(On/N)	Retransplantation different donor, Bu/Cy alive
50. SCID	3	36	Haplo + MSD†	PBSCs + BM	No	No	CMV retinitis, EBV	100	Off/N	Alive 25 mo
51. Omenn syndrome	2	36, alemtuzumab	MFD	BM	No	No	CMV	T 97, B 99, CD15 1	Off/N	Alive 21 mo
52. SCID	11	42	Haplo CD3/CD19 depleted	PBSCs	No	No	No	100	Off/N	Alive 21 mo
53. SCID	5	42, alemtuzumab	URD	BM	Perineal ulceration	Skin I	No	T 99, B100, CD15 99	Off/N	Alive 20 mo
54. SCID	2	36, alemtuzumab	URD	Cord	Perineal ulceration	No	No	100	Off/N	Alive 19 mo
55. CGD	14	42, alemtuzumab	URD	PBSCs	No	Liver, skin ,gut IV after top-up	Pneumonitis, adenovirus, HHV6, chimerism slipped, top-up	100	On/N	Alive 18 mo
56. CID	75	42	MSD	BM	No	Skin, gut, liver III	CMV, pneumonitis	T 96, B 9, CD15 3	On/N	Alive 18 mo
57. SCID	48	42	MFD	BM	Perineal ulceration	No	CMV	Top-up day 126, T 91, B 76, CD15 11	Off/N	Alive 17 mo
58. CID	104	42, alemtuzumab	URD	BM	No	Skin I	Adenovirus, EBV	100	Off/N	Alive 15 mo
59. Omenn syndrome	4	42, alemtuzumab	URD 9/10	Cord	Seizures	No	No	100	Off/N	Alive 15 mo
60. LAD	4	42, alemtuzumab	URD	Cord	Perineal ulceration	No	No	T 96, B 99, CD15 100	Off/N	Alive 15 mo
61. Omenn syndrome	9	36, OKT3	Haplo CD34⁺	PBSCs	No	Skin + gut III	No	100	On/N	Alive 15 mo
62. Omenn syndrome	7	36	URD 9/10	Cord	No	Skin I	No	T 100, B 100, CD15 71	On/N	Alive 14 mo
63. IPEX	9	42, alemtuzumab	URD 8/10	Cord	Perineal ulceration	gut, skin III	Nephrotic syndrome	T 35, B 45, CD15 38	On/N	Alive 13 mo
64. SCID	9	36	Haplo CD3/CD19 depleted	PBSCs	No	No	Adenovirus	T100, B 94, CD15 87	Off/N	Alive 13 mo
65. SCID	6	36, alemtuzumab	MFD	BM	No	No	No	100	Off/N	Alive 13 mo
66. ICF	17	36, alemtuzumab	URD 9/10	BM	Skin rash	Skin I	No	100	Off/N	Alive 11 mo
67. SCID	12	36	URD 9/10	Cord	Skin rash	cGVHDskin	Engraftment syndrome	100	On/N	Alive 10 mo
68. Omenn syndrome	3	36, alemtuzumab	MFD	BM	Perineal ulceration	No	Pneumonitis, adenovirus	T 99, B 71, CD15 4	Off/N	Alive 9 mo
69. SCID	15	42	URD 9/10	Cord	Skin rash	Skin II	No	100	Off/N	Alive 9 mo
70. CD40L	13	42, alemtuzumab	URD	BM	VOD	No	VOD, ventilated, CVVH, EBV	100	On/N	Alive 9 mo

HLH indicates hemophagocytic lymphohistiocytosis; MFD, matched family donor; BM, bone marrow; NA, not applicable; cGVHD, chronic graft-versus-host disease; URD, unrelated donor; 10/10, HLA match; ALPS, autoimmune lymphoproliferative syndrome; PBSCs, peripheral blood stem cells; MSD, matched sibling donor; MDS, myelodysplasia; AML, acute myeloid leukemia; SCID, severe combined immunodeficiency; ATG, antithymocyte globulin; EBV, Epstein-Barr virus; WAS, Wiskott-Aldrich syndrome; LAD, leukocyte adhesion deficiency; AIHA, autoimmune hemolytic anemia; CHH, cartilage hair hypoplasia; CGD, chronic granulomatous disease; SID, severe immune dysregulation; IPEX, immune deficiency polyendocrinopathy X-linked; HOCM, hypertrophic obstructive cardiomyopathy; HHV6, human herpesvirus 6; XLP, X-linked lymphoproliferative syndrome; MHC II, major histocompatibility class II deficiency; CID, combined immune deficiency; CVA, cerebrovascular attack; VOD, veno-occlusive disease; SCN, severe congenital neutropenia; Bu/Cy, busulfan/cyclophosphamide; ICF, immunodeficiency centromeric instability facial dysmorphism syndrome; CD40L, CD40 ligand deficiency; and CVVH, continuous veno-venous hemofiltration.

*Patient died before transplantation.

†Patient 50 had haploidentical maternal CD34⁺ selected PBSCs plus BM from MSD who had had a haploidentical transplantation from the mother 15 years earlier.

Table 2. Transplantation-related complications

	Fludarabine (n = 40)	Cyclophosphamide (n = 30)
Deaths	6	7
Survival	85%	77%
Seizures	2	2
VOD	0	2
Rejection	1	1
GVHD > grade 2	6/3	9/3
Viral reactivation	5	13

Four children had seizures. All were age 4 months or less. Two were already on cyclosporine. Patients 3 and 20 (Table 1) rejected their grafts.

such as chronic granulomatous disease.[9,14] Here we describe a large cohort of children who have received treosulfan-based conditioning regimens for PID and examine outcomes with particular reference to age younger or older than 1 year.

Methods

Patients

A retrospective study of 70 consecutive patients with PID or severe immune dysregulatory disorder who underwent HSCT at United Kingdom supra-regional referral centers for PID, Newcastle on Tyne General Hospital (n = 40) and Great Ormond Street Hospital (n = 30), between February 2006 and December 2009 was performed. Information was collected regarding patient demographics, diagnosis, donor match and stem cell source, conditioning regimen, transplantation-related complications, GVHD, chimerism, immune reconstitution, outcome, and length of follow-up. Patients were not randomized, and the choice of conditioning was based on clinical decision. Informed consent was taken from all parents according to the local center and European Blood and Marrow Transplantation guidelines and the Declaration of Helsinki.

HLA typing was performed by molecular typing for HLA class I and II loci. The unrelated donors were all 7 to 10 of 10 HLA matched. Bone marrow (BM, n = 40), peripheral blood stem cells (PBSCs, n = 9), and cord blood (CB, n = 17) were used as a stem cell source. Peripheral blood was used for the 4 haploidentical transplants, using the Clinimacs (Miltenyi Biotec) systems for CD3/CD19 depletion in 3 and $CD34^+$ stem cell selection in one.

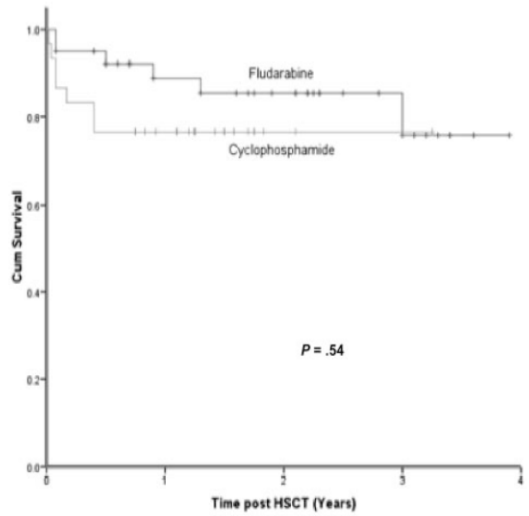

Figure 1. Kaplan-Meier survival curve. There was no significant difference in survival between those that received fludarabine compared with cyclophosphamide.

Treosulfan was given at a dose of 42 g/m^2 (n = 43) or 36 g/m^2 (n = 27) in 3 divided doses on 3 consecutive days. Sixteen of 30 patients (53%) who received cyclophosphamide were given the higher dose of treosulfan as were 24 of 40 (68%) who received fludarabine. The lower dose was given in young babies generally less than 1 year of age. Alemtuzumab 0.3 to 1.0 mg/kg total dose was given to all the patients except those who received a matched sibling donor (MSD) graft (n = 8), 1 who had a second transplant from an matched family donor (MFD) after a previous unconditioned transplant, recipients of haploidentical CD3/CD19-depleted PBSCs (n = 3), or haploidentical $CD34^+$ selected PBSCs who received OKT3 (n = 1) and 7 recipients of CB, 3 of whom received antithymocyte globulin. GVHD prophylaxis in the majority of patients (53) consisted of cyclosporine with mycophenolate mofetil, which was weaned from day 28 in the absence of any GVHD. Ten received cyclosporine alone, 3 cyclosporine and methotrexate, 2 cord transplant recipients cyclosporine and methylprednisolone, 1 haploidentical recipient mycophenolate mofetil and OKT3, and 1 died before transplantation.

Patients had weekly polymerase chain reaction testing of blood for adenovirus, Epstein-Barr virus (EBV), and cytomegalovirus (CMV) in both centers and human herpesvirus 6 (HHV6) in Newcastle. Acute GVHD was assessed using the Seattle criteria. Chronic GVHD was defined as GVHD occurring 100 days or more after HSCT and was graded as extensive or limited.

Chimerism

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

Statistics

Groups were compared using Fisher exact test with a 2-tailed *P* value, except where numbers were small, when the χ^2 test with Yates correction was used (GraphPad Prism, Version 5, GraphPad Software). *P* values equal to or less than .05 were considered statistically significant.

Results

Diagnoses were as follows: severe combined immunodeficiency (SCID, n = 26), Wiskott-Aldrich syndrome (WAS, n = 7), Omenn syndrome (n = 7), hemophagocytic lymphohistiocytosis (HLH, n = 4), combined immunodeficiency (CID, n = 4), leukocyte adhesion deficiency (LAD, n = 4), chronic granulomatous disease (CGD, n = 3), severe immune dysregulation (n = 3), cartilage hair hypoplasia (CHH, n = 2), immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX syndrome, n = 2), major histocompatibility class II deficiency (MHC II, n = 2), and 1 of each: T-cell activation defect, X lymphoproliferative-like syndrome (XLP-like), CD40 ligand deficiency, autoimmune lymphoproliferative syndrome (ALPS), severe congenital neutropenia (SCN), and immunodeficiency, centromeric instability, facial dysmorphism (ICF) syndrome.

The median age at transplantation was 8.5 months (range, 1.2-175 months). Forty-six of 70 (66%) were 12 months or younger at the time of transplantation. Patients received HSCT from an unrelated donor (n = 45), MSD (n = 8), MFD (n = 13), or haploidentical donor (n = 4) using treosulfan in combination with fludarabine 150 mg/m^2 (n = 40) or cyclophosphamide 200 mg/kg (n = 30; Table 1). Transplantation-related complications are summarized in Table 2.

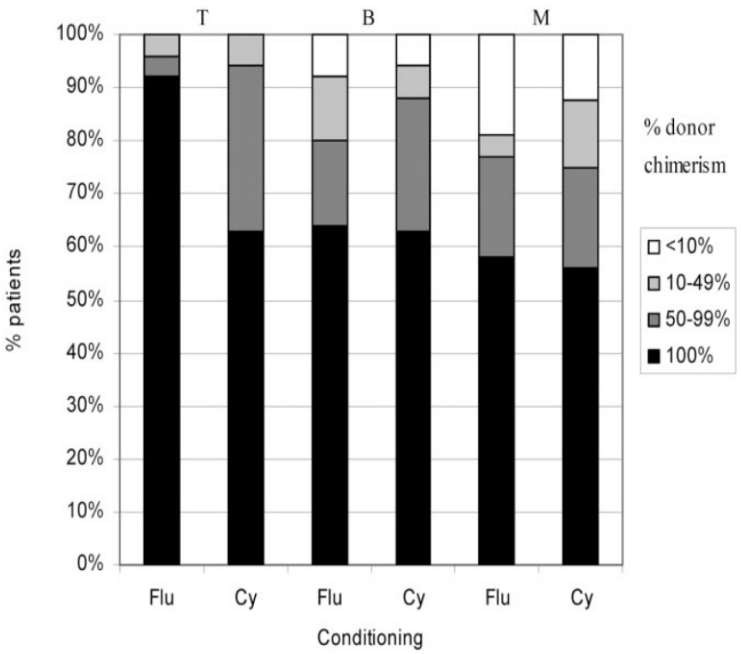

Figure 2. Split cell chimerism in patients more than 1 year after HSCT. Of 42 patients, 24 (57%) had 100% donor chimerism in all cell lines: 15 of 26 (58%) in the fludarabine group and 9 of 16 (56%) in the cyclophosphamide group. The rest had stable mixed chimerism. There was no very low level chimerism (< 10%) in the T-cell lineage and very little in the B and myeloid cell lineages. There was significantly better T-cell chimerism in the group receiving fludarabine ($P = .038$). T indicates T-cell lymphocytes; B, B-cell lymphocytes; M, myeloid cells; Flu, fludarabine; and Cy, cyclophosphamide.

Survival

Thirteen children died, giving an overall survival (OS) of 81%. There was no significant difference in survival between those who received fludarabine compared with those who received cyclophosphamide (Figure 1). In the fludarabine group, there were 6 deaths (OS = 85%). None of these appeared to be directly related to toxicity of the conditioning regimen: patient 1 (Table 1) died 16 months after transplantation of chronic GVHD and infection; patient 2 died of refractory HLH on day −1; patient 3 with ALPS rejected a haploidentical transplant, reactivated CMV, and died before retransplant; patient 4 after transplantation for Griscelli syndrome developed myelodysplasia/acute myeloid leukemia with monosomy 7 in recipient cells, declined a second HSCT, and died 3 years after transplantation; patient 5 with T-B-SCID and intestinal atresia died of Pseudomona sepsis at 2 months; and patient 6 with Omenn syndrome died 11 months after transplantation with GVHD and cerebral infarcts. In the cyclophosphamide group, there were 7 deaths (OS = 77%). The first 4 of these were possibly related to the conditioning drugs: patient 42 (Table 1) died on day 7 with a cerebral hemorrhage; patient 43 died of multiorgan failure in association with HHV6 disease; patient 44 died of VOD of the liver; patient 45 had severe pneumonitis and pulmonary hypertension; patient 41 died 5 months after transplantation with HHV6; patient 46 had chronic lung damage before transplantation because of multiple infections and died of pulmonary hemorrhage; and patient 47 had severe gut GVHD after withdrawal of cyclosporine because of mixed chimerism and died.

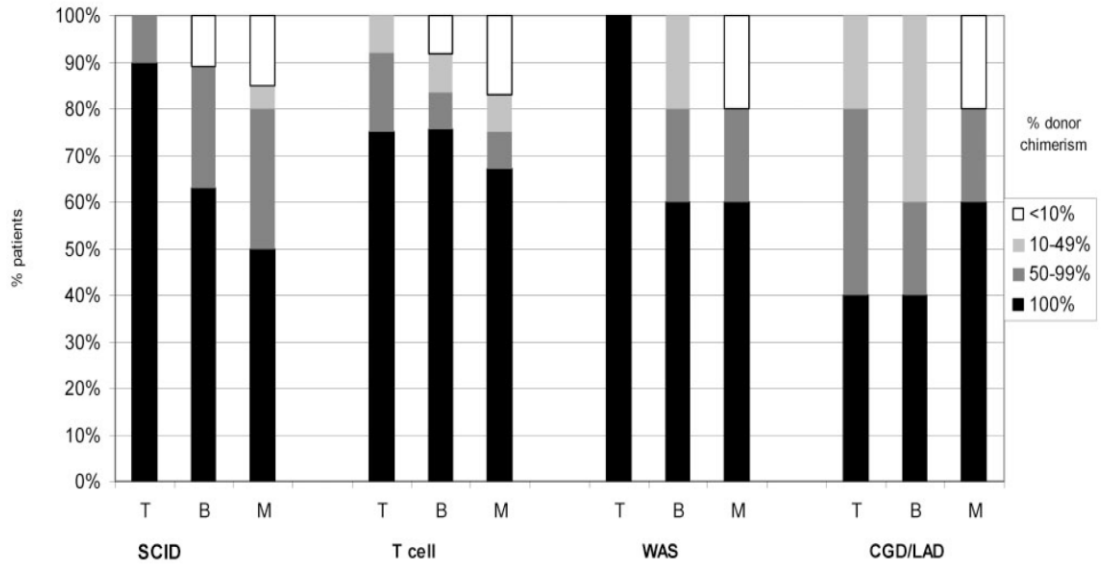

Figure 3. Split cell chimerism in 42 patients more than 1 year after HSCT by disease. Numbers are small, but there is a tendency to greater donor T-cell chimerism in T cell-deficient diseases, SCID, other T-cell deficiency, and WAS compared with CGD/LAD. Donor myeloid chimerism is similar between the 4 groups and B-cell chimerism approximately mirrors myeloid chimerism.

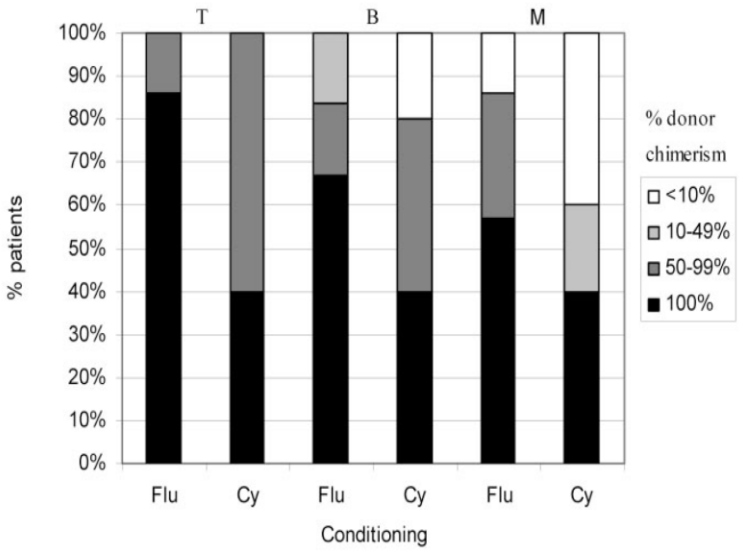

Figure 4. Split cell chimerism in MSD/MFD recipients more than 1 year after HSCT. Numbers are small (7 in fludarabine group and 5 in cyclophosphamide group), but there is a suggestion that chimerism is better after fludarabine in all cell lineages.

In those 12 months of age or younger at the time of transplantation, there were 8 of 46 deaths (OS = 83%), which was not statistically different compared with 5 of 24 deaths (OS = 79%) in those older than 12 months. Twelve of 44 patients (27%) transplanted at age less than 1 year and 5 of 26 (19%) age more than 1 year required admission to an intensive care unit.

Toxicity

Skin toxicity was common, including perianal ulceration, pigment changes, and occasional peeling. Mucositis was mild. Four children had seizures after cessation of treosulfan: 2 were already on cyclosporine at the time of seizures, and all were less than 4 months of age. Two patients had severe VOD: both received the higher dose of treosulfan in combination with cyclophosphamide, and both had enterovirus in their feces. Both patients were treated with defibrotide: 1 recovered after ventilation and dialysis, but the other died and VOD was confirmed at postmortem.

GVHD

Eighteen (26%) patients had GVHD, but only 7 (10%) had greater than grade 2 GVHD. There were 3 deaths from GVHD. Four patients had limited chronic skin GVHD.

Viral reactivation

Eighteen patients had evidence of 1 or more viruses (26%). CMV was detected in 7, EBV in 4, adenovirus in 8, and HHV6 in 3 children after transplantation. In 5 cases, the viral infection contributed to the death of the child.

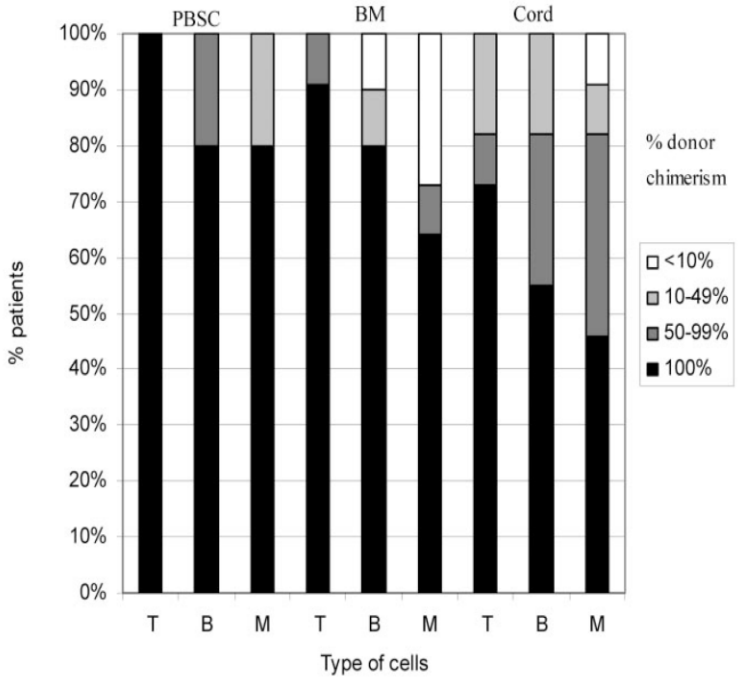

Figure 5. Split cell chimerism in unrelated donor recipients more than 1 year after HSCT according to stem cell source. Numbers are small (PBSCs, n = 5; BM, n = 11; CB, n = 11), but there is a suggestion that chimerism is better with PBSCs.

Chimerism

Median follow-up is 19 months (range, 1-47 months). Two patients had graft rejection: Patient 20 with MHC II deficiency rejected marrow from an MSD despite a top-up procedure but was successfully retransplanted using busulfan and cyclophosphamide; patient 3 had ALPS and rejected after a haploidentical transplant with CMV reactivation and died before retransplantation. Patient 8 with SCN had mixed chimerism after MSD marrow, with ongoing neutropenia and was successfully retransplanted using busulfan and cyclophosphamide. Two further patients had top-up procedures because of mixed chimerism; patient 14 with CGD had a neutrophil oxidative burst of 27% continuing to fall and an unconditioned PBSC top-up and developed grade 4 GVHD, but recovered well with 100% donor chimerism; and patient 16 with RAG SCID had stable mixed chimerism after top-up, with good immune reconstitution.

Forty-two were more than 1 year after transplantation, including patient 1 who died 16 months after transplantation. Twenty-four (57%) had 100% donor chimerism in all cell lines: 15 of 26 (58%) in the fludarabine group and 9 of 16 (56%) in the cyclophosphamide group. The rest had stable mixed chimerism. There was no very low level chimerism (< 10%) in the T-cell lineage and very little in the B and myeloid cell lineages (Figure 2).

There was significantly better T-cell chimerism in the group receiving fludarabine (24 of 26 had 100% donor) compared with cyclophosphamide (10 of 16; $P = .038$).

Lineage specific chimerism by disease type for 42 patients with at least 1 year of follow-up is shown in Figure 3. Although it is difficult to draw firm conclusions from small numbers, as might be expected, there was a tendency to greater donor T-cell chimerism in T-cell deficient diseases, SCID, other T-cell deficiency, and WAS compared with CGD and LAD. Donor myeloid chimerism was similar between the 4 groups, and B-cell chimerism roughly mirrored myeloid chimerism.

Donor and stem cell source

Twenty-one patients had MSD (n = 8) or MFD (n = 13) transplants (BM, n = 19; PBSCs, n = 1; CB, n = 1). There were 6 deaths (OS = 71%). Patients 20 and 8 required a second procedure and were successfully retransplanted using busulfan and cyclophosphamide as described in "Chimerism." Two are less than 12 months after transplantation. Six of 12 (50%) have 100% donor chimerism in all cell lineages. There was no significant difference in chimerism between this group and those that had unrelated donor transplants. There are only 7 in the fludarabine group (including 1 death) and 5 in the cyclophosphamide group who are more than 12 months after transplantation (Figure 4). There was no significant difference in chimerism between these 2 groups.

Fifteen of 17 patients who had CB survived (OS = 88%). There was no significant difference in survival between this group, and 42 of 53 who survived after BM or peripheral blood. There are 6 in the fludarabine group and 5 in the cyclophosphamide group who are more than 12 months after transplantation. Four are less than 12 months after transplantation.

Forty-five patients had unrelated donor transplants. Survival was as follows: PBSCs, 8 of 8; BM, 17 of 21; and CB, 15 of 16. Of these, 27 are more than 12 months after transplantation (PBSCs, n = 5; BM, n = 11; and CB, n = 11). Chimerism is shown in Figure 5. Numbers are small, but there is a suggestion that chimerism is better with PBSCs without a significant increase in GVHD (1 > grade 2 after top-up in the PBSCs and 3 > grade 2 in the BM group).

Immune reconstitution

Of 41 who are more than 1 year after transplantation, 9 currently remain on IVIg. They have all had GVHD, except 1 common γ-chain-deficient SCID with recipient B cells. CD4 counts are all normal at last follow-up, except patient 18 who has poor immune reconstitution despite having 100% donor chimerism.

Discussion

HSCT with treosulfan-based conditioning regimens achieved excellent OS of more than 80% in this group of children with PID or severe immune dysregulatory disorder with a high level of complete or stable mixed chimerism in the diseased lineage sufficient to cure disease; this includes specific types of PID (eg, CGD), in whom secure engraftment has been compromised with other reduced intensity conditioning regimens as reported previously.[2,14] In particular, there was a high survival rate in children transplanted less than 1 year of age in whom toxicity continues to be a problem with conventional and other reduced intensity conditioning regimens.

Toxicity was generally low. Perineal ulceration was common, presumably because of the urinary excretion of active treosulfan metabolites, but resolved in all cases with frequent napkin changes, use of barrier creams, and pain relief. Four babies had seizures; and although it cannot be proved that treosulfan was the cause, the use of clonazepam prophylaxis for those younger than 1 year might be considered. The combination of treosulfan with fludarabine was particularly well tolerated with no occurrence of VOD, which has been confirmed in other studies.[9,15] There were no toxic deaths related to the combination of treosulfan and fludarabine. Two patients who developed VOD received treosulfan in combination with cyclophosphamide, and a strong correlation between blood levels of cyclophosphamide metabolites and VOD has previously been shown as a result of depletion of glutathione from the liver.[16] Combinations of cyclophosphamide with reduced-dose busulfan may also lead to severe hepatic toxicity and VOD.[17,18] There was no cardiac toxicity, which can occur with melphalan, and no pulmonary fibrosis, which can be a complication of the use of busulfan.[19-21] Twelve of 44 patients (27%) transplanted at age younger than 1 year and 5 of 26 (19%) at age more than 1 year required admission to an intensive care unit. As a comparison, 146 patients undergoing fludarabine with melphalan conditioning largely for PID have recently been analyzed (Rao et al, manuscript in preparation): although the survival (76%) of those transplanted aged less than 1 year was similar to that of the rest of the group, the incidence of serious events needing intensive care support was higher in this group: 17 of 30 patients (57%) needed intensive care management. Bacterial and viral infections were the most common reasons for transfer to intensive care (n = 11) followed by conditioning-related toxicity (n = 4) and T-cell lung sequestration (n = 2). The incidence of PICU admission in the rest of the group was 26 of 118 (22% $P < .0001$). There were significantly fewer less than 1 year olds admitted to intensive care in this study (12 of 44) compared with those who received melphalan (17 of 30; $P = .0155$).

Rates of GVHD were generally low using this protocol. Standard conditioning agents lead to tissue damage, which causes

cytokine release, which is involved in the pathogenesis of GVHD and VOD. It has been suggested that the immunosuppressive properties of treosulfan coupled with less tissue destruction decrease the likelihood of a cytokine storm.[8,22,23] This may explain why, unlike other reports using PBSCs with modified conditioning,[24] the use of PBSCs in this study was not associated with an increased incidence of GVHD.

There were significantly more patients with 100% donor T-cell chimerism after the combination treosulfan with fludarabine rather than cyclophosphamide. T-cell chimerism is important for the majority of patients with PID apart from those with phagocytic disorders in whom secure donor myeloid engraftment was also achieved. Outcome in terms of survival with donor chimerism remained good regardless of stem cell source used. The number of patients receiving PBSCs was fairly small, and firm conclusions are difficult, but there was a suggestion that these patients were more likely to achieve full donor chimerism. Conversely, patients receiving cord blood stem cells in conjunction with serotherapy had a tendency to more mixed donor chimerism. Although donor myeloid chimerism was sufficiently high to cure most patients with CGD and WAS with all donor types/stem cell sources, where 100% donor chimerism might be preferred in all cell lineages (eg, WAS),[25] then the use of PBSCs or full myeloablation with busulfan might be required.

By chance, there were more HLH and WAS patients in the treosulfan plus fludarabine group than the treosulfan plus cyclophosphamide group. Rather than reducing toxicity, the increase in HLH patients in the former group might have been expected to increase transplantation-related toxicity because of the propensity of HLH patients to autoinflammatory responses. It is doubtful that the inclusion of these 2 conditions would explain an increase in donor T-cell chimerism in the treosulfan plus fludarabine group.

Viral infection before transplantation and viral reactivation after transplantation is common in patients with primary immunodeficiency; the rate of viral reactivation in this study (26%) was similar to that of 33% found in a group of 33 children with PID who received fludarabine, melphalan, and alemtuzumab in a previous study.[4]

There are only 2 published reports concerning treosulfan pharmacokinetics in children,[26,27] and further studies are warranted to determine whether pharmacokinetic studies could further increase efficacy and reduce toxicity. However, even without pharmacokinetic data, the combination of treosulfan and fludarabine appears to be a suitable choice of conditioning for HSCT in PID, regardless of age and stem cell source. Prospective studies are required comparing this combination with more conventional busulfan and cyclophosphamide, or perhaps with busulfan and fludarabine[28-30] where early reports also indicate reduced transplantation-related toxicities. In this latter respect, long-term follow-up is required with the treosulfan cohort to monitor long-term toxicity, and in particular infertility, as the gonadal toxicity of busulfan is already well documented.

Authorship

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

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TO THE EDITOR:

Two decades of excellent transplant survival for chronic granulomatous disease: a supraregional immunology transplant center report

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Chronic granulomatous disease (CGD), an inherited immunodeficiency resulting from defects in the reduced NAD phosphate oxidase complex rendering phagocytes deficient in producing the superoxide anion necessary for normal killing of bacterial and fungal microorganisms, leads to severe and recurrent infections, inflammatory granulomatous complications, and autoimmune diseases.[1] It is associated with substantial morbidity and premature death. Hematopoietic cell transplantation (HCT) is the only long-term curative therapy; however, historically, utility was limited by high rates of graft failure and transplant-related morbidity and mortality. Transplant survival and graft outcome have improved dramatically over the past 10 years as a result of reduced-toxicity conditioning (RTC) regimens, detailed graft-selection hierarchy, superior HLA-matching technology, better cell-dosed grafts, greater availability of grafts, improved supportive care, and more effective antimicrobial therapy. We studied transplant survival post-HCT for CGD in a supraregional immunology transplant center in the United Kingdom.

We report outcomes of patients with CGD who received their first HCT between 1998 and 2017 at the Great North Children's Hospital, 1 of 2 supraregional transplant centers in the United Kingdom for children with primary immunodeficiency. Outcomes of interest were overall survival (OS), event-free survival (EFS), hematopoietic recovery, toxicities, graft-versus-host disease (GvHD), long-term survival, graft function, posttransplant immune reconstitution, and autoimmune disease posttransplant. OS was defined as survival from first HCT to last follow-up or death. An event was defined as death or second procedure for decreasing chimerism. Cox proportional-hazard modeling was used to analyze predictors of OS and EFS. Variables included for predictor analysis were year of transplant (1998-2007 vs 2008-2017), age at transplant, donor (family vs unrelated donor), conditioning (myeloablative conditioning [MAC] vs RTC), stem cell source (marrow vs peripheral blood), total nucleated cell dose (TNC), and CD34 cell dose. Multiple linear-regression modeling was used to analyze the impact of conditioning, stem cell source, and cell doses on long-term graft function after first successful HCT. CD3, CD4, CD8, CD19, CD27/CD45RA, and CD16/56 enumeration was measured routinely. All data analyses were performed using STATA version 14.2.

Fifty-five children with CGD were included; none were excluded. A detailed description of transplant characteristics is summarized in Table 1. Median age at transplant was 5.3 years (range, 0.6-18.0). Forty-five (82%) had X-linked CGD, and 10 (18%) had autosomal-recessive CGD. Twelve (22%) had growth failure (defined as weight less than the fifth centile for age and sex) at the time of transplant. Of 34 who underwent colonoscopy, 31 (91%) had biopsy-proven colitis. Four (7%) had biopsy-proven granulomatous lung inflammation. One had undefined acute necrotizing encephalitis. Prior to 2007, various conditioning regimens were used, with 21 (38%) patients receiving pharmacokinetically guided IV busulfan and IV cyclophosphamide, with or without serotherapy. From 2007 on, the conditioning regimen was switched to fludarabine–treosulfan–alemtuzumab, with post-HCT GvHD prophylaxis comprising cyclosporine (CSA) and mycophenolate mofetil (MMF) for family and unrelated donors (n = 24; 44%). Fludarabine–treosulfan–thiotepa–anti-thymocyte globulin–rituximab was used for CD3 TCRαβ CD19–depleted haploidentical grafts (n = 4; 7%). Of these 4 patients, 1 received CSA/MMF, and the remaining 3 did not receive any post-HSCT GvHD prophylaxis. The median day to neutrophil and platelet engraftment was 16 days (range, 9-37) and 18 days (range, 10-139), respectively. Ten (20%) patients had grade II-IV acute GvHD, and 5 (9%) patients had grade III-IV acute GvHD. None had chronic GvHD. Only 1 patient who received busulfan-based conditioning had veno-occlusive disease. Eleven (20%) had cytomegalovirus viremia, 5 (9%) had adenoviremia, and 5 (9%) had Epstein-Barr virus viremia.

The 5-year OS was 89% (95% confidence interval [CI], 67-95%), increasing to 100% for children transplanted at ≤5 years of age (n = 28) vs 81% (95% CI, 60-92%) for children transplanted at >5 years of age (n = 27; $P = .04$) (Figure 1A-B). The OS was comparable between matched family (88%; 95% CI, 61-97%) and unrelated donor transplants (89%; 95% CI, 71-95%) (Figure 1C). The 4 haploidentical transplants were successful. Year of transplant ($P = .13$), conditioning ($P = .58$), stem cell source ($P = .57$), TNC ($P = .69$), and CD34 cell dose ($P = .52$) were not associated with OS. All 5 deaths were due to transplant-related complications. The median age at transplant for deceased patients was 10.0 years (range, 8.4-18). The 5-year EFS for the cohort was 77% (95% CI, 62-87%). No variables were associated with EFS. All 7 patients with decreasing chimerism received successful second procedures (3 had unconditioned stem cell boost infusion and 4 had fully conditioned second transplant).

The median age of long-term survivors was 14 years (range, 2-36), with a median duration of follow-up of 6.5 years (range, 0.32-19.5). There were no late deaths. None had clinical evidence of colitis. Of the 11 survivors who were >21 years of age, 6 (55%)

Table 1. Patient and transplantation characteristics and outcome after first HCT in children with CGD (N = 55)

	Data
Patient characteristics	
Year of transplant	
1998-2007	17 (31)
2008-2017	38 (69)
Males	51 (93)
Inheritance	
X-linked CGD	45 (82)
Autosomal recessive CGD	10 (18)
Median age at transplant (range), y	5.3 (0.6-18.0)
Donor characteristics	
Type of donor	
Matched family donor	20 (36)
Unrelated donor	31 (56)
Haploidentical donor	4 (8)
Stem cell source	
Marrow	29 (53)
Peripheral blood	24 (43)
Cord blood	2 (4)
Stem cell dose, median (range)	
Marrow	
TNC, × 10^8/kg	5.0 (1.6-24.4)
CD34, × 10^6/kg	4.3 (0.75-53.0)
PB	
TNC, × 10^8/kg	10.3 (1.2-22.8)
CD34, × 10^6/kg	8.0 (3.0-29.2)
Transplant characteristics	
Conditioning regimen	
MAC	
Busulfan-cyclophosphamide	21 (38)
Fludarabine-treosulfan-thiotepa	4 (7)
RTC	
Treosulfan-fludarabine	24 (45)
Busulfan-fludarabine	4 (7)
Treosulfan-cyclophosphamide	1 (2)
Fludarabine-melphalan	1 (2)
Serotherapy	
None	9 (16)
ATG	4 (8)
Alemtuzumab	42 (76)
GVHD prophylaxis	
None	3 (5)
CSA alone	2 (4)
CSA + MTX	16 (29)
CSA + MMF	33 (60)
CSA + steroid (for cord blood)	1 (2)
Hematopoietic recovery	
Days to neutrophil recovery, median (range)	16 (9-37)
Days to platelet recovery, median (range)	18 (10-139)

had unassisted successful pregnancy or fathered children (4 received busulfan-cyclophosphamide, 1 received busulfan-fludarabine, and 1 received fludarabine-melphalan). Median donor myeloid chimerism after first successful HCT (n = 43) was

Table 1. (continued)

	Data
Transplant-related complications	
Acute GvHD	
Grade II-IV	10 (20)
Grade III-IV	5 (9)
Chronic GvHD[2]	0
Veno-occlusive disease, n (%)	1 (2)
Patient receiving second procedures for decreasing chimerism	7 (13)
Patients receiving unconditioned stem cell boost infusion, n	3
Median myeloid chimerism prior to unconditioned stem cell boost infusion (range), %	17 (15-23)
Patients receiving fully conditioned second transplant, n	4
Median myeloid chimerism prior to fully conditioned second transplant (range), %	11 (0-13)
Cause of death (n = 5), n	
Multiorgan failure	2
Grade IV acute GvHD	1
Pulmonary hemorrhage	1
Posttransplant lymphoproliferative disease	1

Unless otherwise noted, data are n (%).

ATG, anti-thymocyte globulin (Grafalon) for haploidentical transplant; MTX, methotrexate.

92% (range, 30-100%). MAC was the only variable associated with higher donor myeloid chimerism (P = .036). MAC (P = .002) and TNC (P = .03) were associated with higher donor T-lymphocyte chimerism (supplemental Figure 1; supplemental Table 1 available on the *Blood* Web site). For patients who received a second procedure with unconditioned stem cell infusion (n = 3), the median myeloid chimerism was 52% (range, 30-100%), and the median T-lymphocyte chimerism was 26% (range, 16-100%). For patients who received a conditioned second transplant (n = 4), the median myeloid chimerism was higher (89%; range, 30-100%), and the median T-lymphocyte chimerism was 65% (range, 26-100%). Longitudinal immune-reconstitution results were available for 42 patients after first successful HCT (supplemental Figure 2).

The 1- and 5-year cumulative incidence of autoimmune diseases was 9% and 12%, respectively, significantly less than that reported in a recent study.[2] Three (5%) had immune cytopenia in the first year post-HCT, whereas 3 (5%) had autoimmune endocrinopathy (2 with thyroid dysfunction; 1 with type 1 diabetes mellitus). Two patients with immune hemolysis achieved remission with IV immunoglobulin and steroid, whereas 1 patient with immune hemolysis and thrombocytopenia needed additional rituximab. None had evidence of immune cytopenia at last follow-up. Autoimmune endocrinopathy occurred 1 year post-HCT (median, 3.9 years; range, 1.4-7.1). One patient with hypothyroidism received thyroxine replacement, 1 patient with Grave's disease was treated with radioiodine, and 1 patient with type 1 diabetes mellitus received insulin.

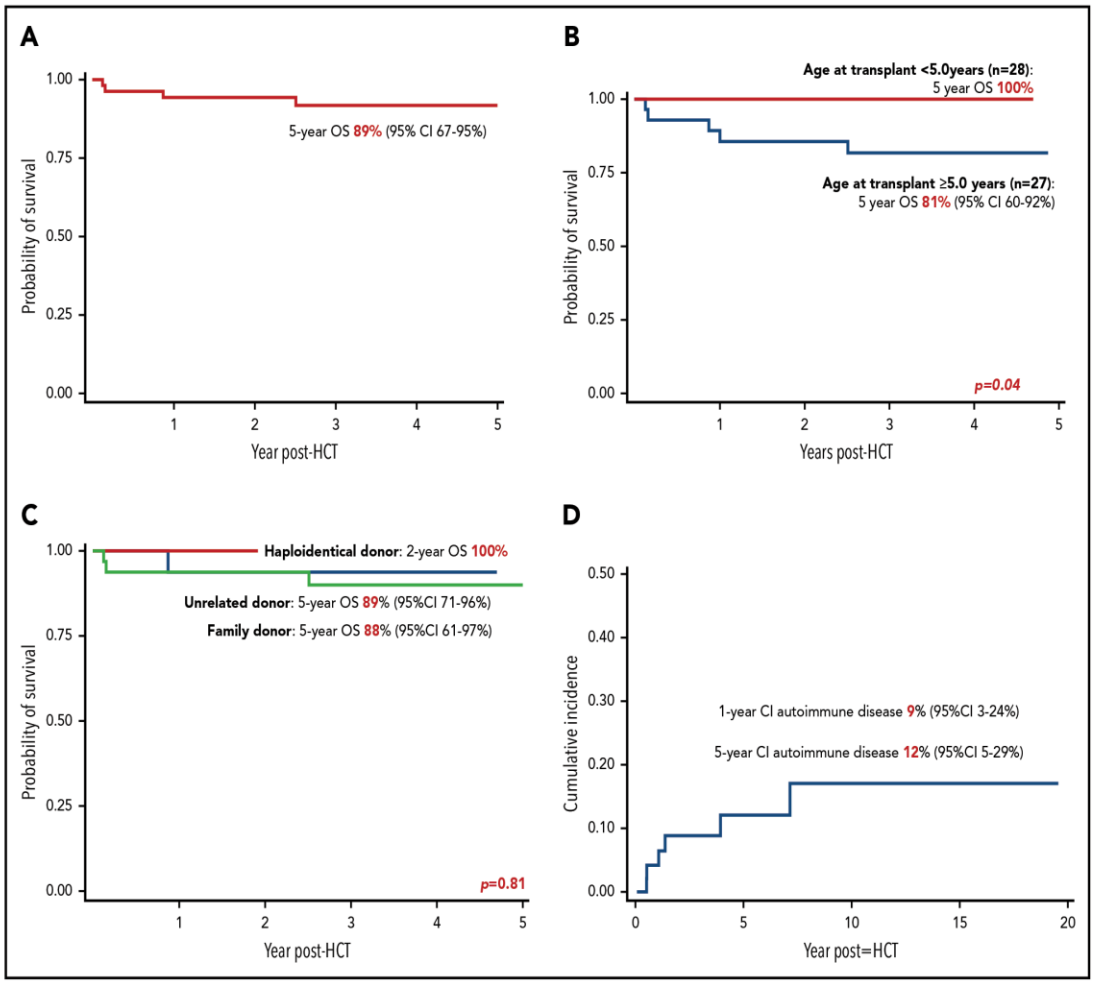

Figure 1. Outcomes after HCT for CGD. (A) OS of entire cohort; (B) OS according to age at transplant; (C) OS according to donor type; (D) cumulative incidence of autoimmune disease after HCT for CGD.

The ability of donor-derived neutrophils to replace the recipient's defective neutrophils makes HCT a superior therapy compared with conventional standard of care using antimicrobial therapy. Previous studies demonstrated that nontransplanted children have more serious infections, more episodes of surgery, poorer growth, and reduced quality of life compared with transplanted children.[3-5] Estimated survival of nontransplanted patients was 88% at age 10 years and 55% at age 30 years.[6] In this study, our findings emphasize that HCT performed in an experienced immunology transplant center is safe and provides a long-term cure for children with CGD. Alternative donors (unrelated and parental haploidentical donors) are associated with an excellent survival that is comparable to family donors. X-linked carrier family donors should be avoided because they have inflammatory and autoimmune symptoms and excessive fatigue.[7-9] Because young age at HCT is associated with a favorable outcome, HCT should be performed as early as possible before the onset of disease-related organ damage. In our center, children with newly diagnosed CGD and neonates that are diagnosed at birth based on family history are recommended for HCT with a matched donor or a haploidentical donor if no suitable matched donor is available. Family screening plays an important role in advancing the transplant care for children with CGD.

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Authorship

Contribution: S.H.L. collected the data, performed the statistical analysis, interpreted the data, and prepared the manuscript; P.M., H.W., and N.C. collected the data; M.S. and A.R.G. contributed equally to the conceptualization of the research, statistical analysis, interpretation of the data, manuscript writing, and critical review at every stage of the research; and S.O., T.F., S.H., A.C., and M.A. critically reviewed the manuscript.

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Footnote

The online version of this article contains a data supplement.

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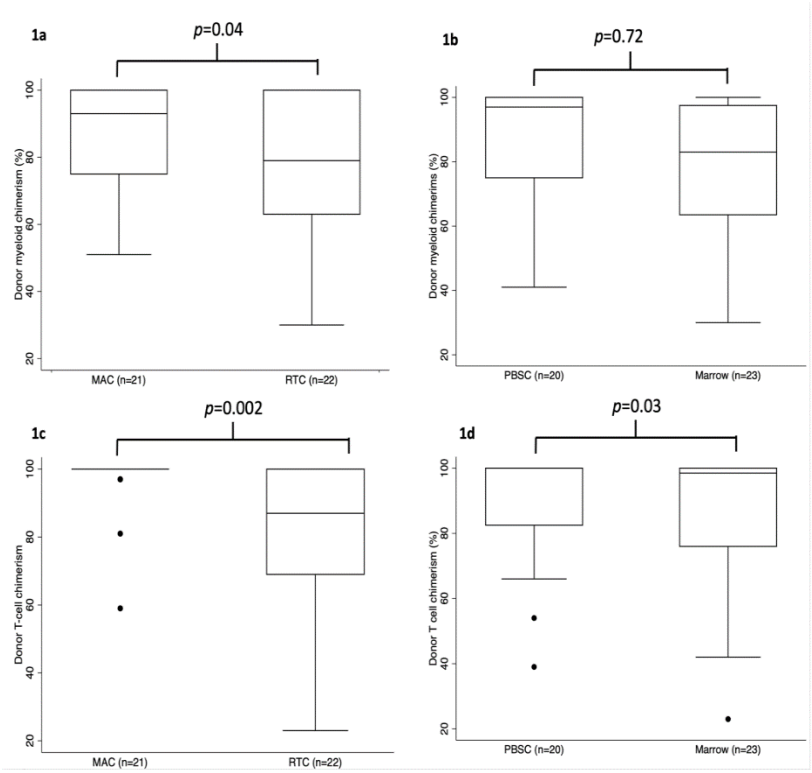
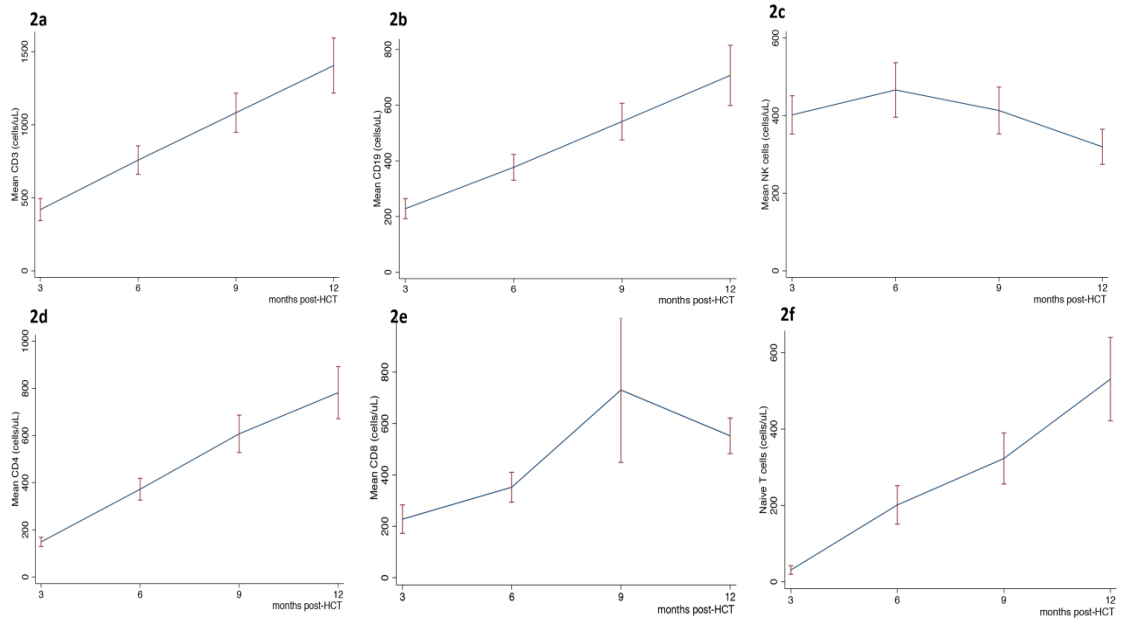


Supplemental figure 1: Impact on conditioning and stem cell source on donor myeloid chimerism (1a and 1b) and donor T cell chimerism (1c and 1d)



Supplemental figure 2: Mean and standard error (SE) of CD3+ cells (2a), CD19+ cells (2b), NK cells (2c), CD4 cells (2d), CD8 cells (2e) and naïve T cells (2f) measured at different time point post-transplant

Supplemental table 1: Impact of conditioning, stem cell source and cell doses on donor chimerism after first successful HCT for patients with CGD (n=43)

	Coefficient	95% confidence interval	p-value
Donor myeloid chimerism			
MAC vs RTC	-13.6	-25.4 to -0.92	0.04
PBSC vs marrow	-2.3	-15.5 to 10.8	0.72
TNC	1.12	-0.12 to 2.37	0.08
CD34	-0.01	-0.99 to 0.95	0.97
Donor T cell chimerism			
MAC vs RTC	-21.5	-34.4 to -8.74	0.002
PBSC vs marrow	-0.48	-20.1 to 8.40	0.41
TNC	1.37	0.13 to 2.61	0.03
CD34	-0.67	-1.64 to 0.20	0.17

T-cell receptor αβ$^+$ and CD19$^+$ cell–depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is used as a therapeutic approach for primary immunodeficiencies (PIDs). The best outcomes have been achieved with HLA-matched donors, but when a matched donor is not available, a haploidentical or mismatched unrelated donor (mMUD) can be useful. Various strategies are used to mitigate the risk of graft-versus-host disease (GvHD) and rejection associated with such transplants.

Objective: We sought to evaluate the outcomes of haploidentical or mMUD HSCT after depleting GvHD-causing T-cell receptor (TCR) αβ CD3$^+$ cells from the graft.

Methods: CD3$^+$TCRαβ$^+$/CD19$^+$ depleted grafts were given in conditioned (except 3) children with PIDs. Treosulfan (busulfan in 1 patient), fludarabine, thiotepa, and anti-thymocyte globulin or alemtuzumab conditioning were used in 77% of cases, and all but 4 received GvHD prophylaxis.

Results: Twenty-five patients with 12 types of PIDs received 26 HSCTs. Three underwent transplantation for refractory GvHD that developed after the first cord transplantation. At a median follow-up of 20.8 months (range, 5 month-3.3 years), 21 of 25 patients survived and were cured of underlying immunodeficiency. Overall and event-free survival at 3 years were 83.9% and 80.4%, respectively. Cumulative incidence of grade II to IV acute GvHD was 22% ± 8.7%. No case of visceral or chronic GvHD was seen. Cumulative incidences of graft failure, cytomegalovirus, and/or adenoviral infections and transplant-related mortality at 1 year were 4.2% ± 4.1%, 58.8% ± 9.8%, and 16.1% ± 7.4%, respectively. Patients undergoing transplantation with systemic viral infections had poor survival in comparison with those with absent or resolved infections (33.3% vs 100%).

Conclusion: CD3$^+$TCRαβ$^+$ and CD19$^+$ cell–depleted haploidentical or mMUD HSCT is a practical and viable alternative for children with a range of PIDs. (J Allergy Clin Immunol 2018;141:1417-26.)

Key words: *Primary immunodeficiency, haploidentical, mismatched unrelated, hematopoietic stem cell transplantation, CD3$^+$ T-cell receptor αβ$^+$ cell depletion*

Hematopoietic stem cell transplantation (HSCT) is curative for many primary immunodeficiencies (PIDs); however an HLA-matched donor might not be available, and in this case a mismatch related (haploidentical) donor or mismatched unrelated donor (mMUD) can be useful. Because of significant HLA donor-recipient disparities, these options bring higher rates of graft failure and graft-versus-host disease (GvHD), which contribute to poor survival.[1] Various approaches have been tried to mitigate these risks, such as T-cell depletion,[2] T- and B-cell depletion,[3] megadose CD34$^+$ cell selection,[4] and, recently, posttransplantation cyclophosphamide[5] and CD3$^+$ T-cell receptor (TCR) αβ[6,7] and naive T-cell[8] depletion. Commonly used methods, such as CD34$^+$ selection, decrease the risk of GvHD, but the risk of delayed viral clearance and immune recovery remains.[9] Graft manipulation by depleting GvHD-inducing α/β receptor T cells offers a reduction in GvHD while retaining populations of γ/δ receptor T cells[10] in

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Abbreviations used
aGvHD: Acute graft-versus-host disease
ATG: Anti-thymocyte globulin
CMV: Cytomegalovirus
CTL: Cytotoxic T lymphocyte
EFS: Event-free survival
GvHD: Graft-versus-host disease
HHV-6: Human herpesvirus 6
HSCT: Hematopoietic stem cell transplantation
mMUD: Mismatched unrelated donor
NK: Natural killer
PID: Primary immunodeficiency
SCID: Severe combined immunodeficiency
TCR: T-cell receptor
TMA: Thrombotic microangiopathy
TPN: Total parenteral nutrition

the graft, which might contribute to early immune reconstitution and viral or tumor clearance[11,12] after HSCT. Recent studies have shown beneficial effects of this approach in both patients with malignant[12-14] and those with nonmalignant[15-17] disorders. Using this technique to transplant from an immediately available haploidentical donor seems appealing, but more data are required not only on survival but also on morbidities, long-term immune reconstitution, and cost effectiveness in comparison with other modalities of transplantation. We report the outcome of this method in 25 patients with PIDs undergoing mismatched HSCT when a suitably matched donor was not available.

METHODS

A retrospective review of consecutive patient records was performed. Pretransplantation data were collected regarding patients' demographics, infections, donor-recipient matching, and conditioning regimens. Posttransplantation data were collected on engraftment, transfusion requirement, immune reconstitution, GvHD, and infection. Chimerism analysis was performed by using PCR-based amplification of short tandem repeat sequences. Various cell types in blood were assessed by using flow cytometry with mAbs against lineage-specific surface molecules (T cells, $CD3^+$; natural killer [NK] cells, $CD16^+/CD56^+$; B cells, $CD19^+$; and naive T cells, $CD45RA^+/CD27^+$). In addition, morbidities (pain, organ toxicities, and nutritional support), length of hospitalization, and long-term complications were recorded. All parents provided consent according to the European Group for Blood and Marrow Transplantation and local center regulations. Information regarding patients 1, 7, and 20 has been published previously.[18-20]

Patients

Patients' demographics and transplantation characteristics are shown in Table I. Twenty-five patients underwent 26 transplantations at the 2 HSCT centers for PIDs in the United Kingdom (Great North Children's Hospital, Newcastle [n = 18], and Great Ormond Street Hospital, London [n = 8]) between October 2012 and September 2016. The median age at transplantation was 1.75 years (range, 0.28-10.3 years). Infants (≤1 year) comprised 34.6% of the cohort. Two patients received a graft from mMUDs: one with an A mismatch and another with DR and DQ mismatches. The median interval between referral and transplantation was 4 months (range, 0.5-16 months; n = 21). Three patients underwent a second conditioned transplantation for refractory GvHD after matched (10/10) cord blood HSCT. Regarding their first HSCT, 2 of 3 were conditioned. All 3 had skin GvHD, whereas patient 8 also had GvHD affecting the joints and bone marrow, resulting in aplasia, and patient 9 had grade III liver and gut GvHD (biopsy proved). One patient received $CD3^+TCR\alpha\beta^+/CD19^+$ cell–depleted HSCT twice because of primary graft failure after the first transplantation. All patients underwent blood, bronchoalveolar lavage (Newcastle only), nasopharyngeal aspirate, stool, and urine screening for infections within 10 days before transplantation.

Transplantation characteristics

The source of stem cells was granulocyte colony-stimulating factor–mobilized peripheral blood in all patients. All grafts were $TCR\alpha\beta^+/CD19^+$ depleted by using an immunomagnetic method, according to the manufacturer's recommendations (Miltenyi Biotec, Bergisch Gladbach, Germany). Most patients (80.7%) received reduced-toxicity myeloablative conditioning consisting of treosulfan (36-42 g/m^2 total dose; days −7 to −5 [busulfan in one]), fludarabine (160 mg/m^2 total dose; days −7 to −4), and thiotepa (10 mg/kg; day −4). Three patients with severe combined immunodeficiency (SCID) did not receive any conditioning (1 each because of disseminated BCG, prematurity, and disseminated cytomegalovirus [CMV]). All conditioned patients except 1 (with drug-resistant CMV) received serotherapy (anti-thymocyte globulin [ATG]; Fresenius [rabbit], n = 18; and alemtuzumab, n = 4). ATG (15 mg/kg total) was given over days −5 to −3, and alemtuzumab (1 mg/kg total) was given over days −8 to −4. Six patients received 200 mg/m^2 rituximab (in addition to ATG) on day −1 to reduce the risk of EBV-related posttransplantation lymphoproliferative disease. Posttransplantation GvHD prophylaxis was given in 22 of 26 transplantations (Table I). Cyclosporine (trough target 50-100 μmol/L) and mycophenolate mofetil were used for 12 and 6 weeks after HSCT, respectively, unless required longer for GvHD. Steroid (1 mg/kg/d) was added for GvHD prophylaxis in patients with refractory GvHD and Omenn syndrome for around 2 weeks after HSCT and then tapered off over the next 4 to 6 weeks. GvHD was graded according to the modified Seattle Glucksberg criteria.[21] All patients received ursodeoxycholic acid, and 5 patients received defibrotide as veno-occlusive disease prophylaxis (1 patient with transaminitis receiving busulfan conditioning, 1 with liver involvement because of hemophagocytic lymphohistiocytosis, 1 with grade III GvHD affecting the liver, and 2 with abnormal liver function test results). All patients received immunoglobulin replacement after HSCT until sustained IgM production was demonstrated.

Viral infections were treated pre-emptively. CMV, EBV, and adenovirus (and human herpesvirus 6 [HHV-6] only in Newcastle) were monitored weekly in the blood by using quantitative PCR. Respiratory (nasopharyngeal aspirate) and gut (feces) viral screens were performed weekly.

Statistical analysis

Data were analyzed as of February 20, 2017, with STATA version 14.1 software (StataCorp, College Station, Tex). Patients were censored at the time of death or last follow-up. The probabilities of overall survival and event-free survival (EFS) were estimated by using the Kaplan-Meier product limit method. For calculation of EFS, death, disease persistence, and graft failure were considered as events. Primary graft failure was defined as the neutrophil count never reaching 0.5×10^9/L or greater. The probabilities of GVHD, graft failure, viral infections, and transplant-related mortality were calculated as cumulative incidence curves ± SEs to adjust the analysis for competing risks. Multivariate Cox regression models examining risk factors for GvHD were built with the use of stepwise forward selection, with a P value of .05 or less considered to indicate statistical significance. Variables considered were use of different serotherapy in conditioning, viral reactivation status, and doses of various cells in the graft.

RESULTS

Data on different cell populations in processed grafts are presented in Table II.[15,16] The median proportion of remaining TCRαβ T cells in the product after depletion was 0.28% (range, 0.08% to 9.7%). No patients experienced adverse reactions to the infusion of stem cell product.

Neutrophil count recovery occurred in all but 2 conditioned patients at a median of 15 days (range, 10-27 days). One patient

experienced primary graft failure, and another died on day +13. The cause of graft failure could not be determined because virology test results were negative and anti-HLA antibodies were present at a low level but were unreactive with the patient's cells. This patient underwent successful retransplantation with $CD3^+TCR\alpha\beta^+/CD19^+$ depleted stem cells from an mMUD donor. None of the patients required granulocyte colony-stimulating factor to achieve engraftment or had secondary graft failure. Patient 8 (Table I) was given buffy coat granulocyte infusions in the peritransplantation period because of pre-existing fungal infection. The median time to platelet engraftment was 9.5 days (range, 0-24 days), which was defined as a platelet count of greater than 20×10^9/L for 7 days (>50×10^9/L for patients with Wiskott-Aldrich syndrome).

Acute graft-versus-host disease (aGvHD) developed in 11 of 23 patients (Table II; 3 cases are excluded: 1 with graft failure and 2 with early death); however, 10 of 11 had only grade I/II skin GvHD and responded to first-line treatment. Two of 10 patients had a recurrence of skin GvHD after cyclosporine discontinuation, and both responded to a short course of steroids. On multivariate analysis, GvHD was not associated with the dose of $CD34^+$, T, $TCR\alpha\beta^+$, $CD19^+$, and NK cells in the graft or the different serotherapy in conditioning or systemic adenovirus or CMV infections.

As of February 2017, 21 of 25 patients are alive and disease free, with a median follow-up of 20.8 months. The probability of overall survival and EFS at 3 years for the whole cohort was 83.9% ± 7.4% (95% CI, 69.3% to 98.4%) and 80.4% ± 7.9% (95% CI, 64.9% to 95.8%), respectively. As of the last follow-up, 76.1% of survivors had full donor chimerism, and 5 patients had high T-cell but mixed myeloid chimerism (2 unconditioned, Table I).

Transplant-related mortality and morbidities

Transplant-related mortality at 100 days was 11.7% (95% CI, 4.0% to 33.9%), and that at 1 year was 16.1% (95% CI, 6.5% to 39.7%), with 4 deaths. Patient 5 with drug-resistant CMV did not receive serotherapy during conditioning and had grade III skin GvHD requiring prolonged immune suppression, but this patient died from aspergillosis. Two infants (nos. 10 and 21) with disseminated CMV before transplantation died of pulmonary complications soon after transplantation. Patient 12 died 2 months after transplantation because of pulmonary hemorrhage and pulmonary hypertension in the presence of ongoing adenoviremia. Patients going into transplantation with active CMV or adenovirus infection had significantly worse survival in comparison with those with no active viral infection (33.3% vs 100%, $P < .0001$; Fig 1).

Early conditioning-related toxicities, such as treosulfan-related skin rash, mucositis, and engraftment syndrome, were mild in most cases. Patient 9 with pre-existing skin GvHD had a severe treosulfan-related rash, which required dressings under sedation. Patients 7, 14, 15, and 21 had severe mucositis. Among conditioned patients, 56.5% required systemic analgesic infusion for at least 72 hours. Patients 7 and 12 had capillary leak syndrome around engraftment and needed oxygen support and diuretics. The most significant noninfectious complications were immune reconstitution inflammatory syndrome (n = 2) and thrombotic microangiopathy (TMA; n = 2). Patient 5 with disseminated BCG before transplantation and patient 20 with disseminated enterovirus infection after transplantation had immune reconstitution inflammatory syndrome.[20] TMA developed in patients 7 and 26, who responded to supportive measures with defibrotide[19] in 1 and eculizumab[22] in another. Three patients had features of posterior reversible encephalopathy syndrome, which responded to discontinuation of cyclosporine. None of the patients had veno-occlusive disease. The median number of blood and platelet transfusions after transplantation was 4 (range, 0-29) and 2 (range, 0-46), respectively. Patients with Wiskott-Aldrich syndrome became platelet transfusion independent within 1 week after transplantation. The median day 0 discharge interval in survivors was 86 days (range, 34-327 days).

Viral infections or reactivations after transplantation

Twelve patients had viral infections requiring antiviral therapy within 10 days before transplantation, and 50% were able to clear their virus by day 0. Six children went into transplantation with systemic viremia (CMV, 4; adenovirus, 2), 4 of whom died. Of these, the blood viral PCR titer decreased to less than 2×10^3 copies/mL by day 0 in 3 patients with antiviral drugs (1 died and 2 are surviving), and blood viral PCR remained at a higher level of greater than 10×10^3/mL in the remaining patients; all 3 died. Eighteen patients had 26 systemic viral infections or reactivations after transplantation (CMV, 10; adenovirus, 9; HHV-6, 5; EBV, 1; and enterovirus, 1). Among these, 6 infection episodes (33.3%; HHV-6, 3; adenovirus, 2; and EBV, 1) in 3 patients did not result in any complications or require treatment because the titers decreased with immune recovery. The cumulative incidence of any significant viral infection after transplantation (increasing blood titers or disseminated or symptomatic infection) was 59.9% ± 11.1% (Table II). The median time to clear the virus from blood was 75.5 days (range, 18-210 days). Patients 3, 4, and 14 received CMV-specific cytotoxic T lymphocytes (CTLs) on days +34, +31, and +7, respectively, at a dose of 1×10^4 CD3 cells/kg because their viral titers remained greater than 100,000 copies/mL despite antiviral treatment. Patient 19 received adeno-specific CTLs on day +47 as a part of the ASPIRE (adenovirus-specific T cells in pediatric patients after allo-HSCT) trial. Except patient 14, none of these patients had evidence of CMV or adenoviral disease at the time of CTL infusion; however, patients 3 and 4 had CMV retinitis around 2 to 3 weeks after CTL infusion and were managed with intraocular foscarnet along with systemic antivirals. Five patients (3 with CMV and 2 with adenovirus) did not clear viruses after transplantation despite treatment, and 4 of 5 died. To date, no patient has had EBV-related posttransplantation lymphoproliferative disease.

Twelve patients went into transplantation with enteric viral infections (norovirus, 7; adenovirus, 5; and vaccine-associated rotavirus, 3). Five additional patients were found to have viral infections after transplantation (norovirus, 4; enterovirus, 1; and sapovirus, 1). Most of these patients had feeding intolerance and poor weight gain. Of 11 patients with norovirus infection, 9 could clear it at a median of 243 days (range, 122-590 days) after transplantation. All patients had symptomatic improvement before viral clearance from the gut. Patients were managed with enteral feeding (oral or nasogastric tube) or total parenteral nutrition (TPN), as required. Of the total patient population, 80.7% required TPN at some point during the posttransplantation period. Of these, 7 (33.3%) patients required TPN for more than

TABLE I. Demographics and transplantation characteristics of patients

No.	Age	Sex	Diagnosis	Immediate pretransplantation morbidities	Donor	Conditioning
1	1 y	M	WAS (c.778-1G>A mutation in intron 8)	Disseminated CMV	Father	Bu (80 mg/L/h)/Flu/TT rATG‡
2	5 mo	M	WAS (c.1480delp.[leu470*] hemizygote exon 11 of WAS gene)	Developmental delay and hypotonia (? cause), pulmonary hemorrhage	Mother	Treo/Flu/TT rATG/rituximab
3	1.17 y	M	WAS (c777+1 G>A splice site mutation in exon 7)	ALL in morphological remission/high risk-MRD after induction; prolonged cytopenias after chemotherapy	Mother	Treo/Flu/TT rATG
4	1.7 y	M	WAS (c777+1 G>A splice site mutation in exon 7)	Autoimmune neutropenia	Mother	Treo/Flu/TT rATG
5	6 mo	M	CγC SCID (*IL2 RG* gene c.294dup p.[Val992serf*s11] hemizygote)	Disseminated BCG	Father	None
6	3 mo	F	SCID (T−B+NK+)	None	Father	None
7.	1.7 y	F	Artemis-deficient SCID (homozygous c.103C>G exon 1 DCLRE1C) Refractory GvHD (11 mo after cord SCT)	Refractory skin GvHD (11 mo after cord SCT), TPN dependent (diarrhea)	Mother	Treo/Flu/TT alemtuzumab
8	5.3 y	M	CγC SCID (c.854G>A in exon 6 of *IL2RGC*) + GvHD (9 mo after cord SCT)	Refractory skin GvHD with joint involvement and marrow aplasia ?Fungal pneumonia	Father	Treo/Flu alemtuzumab
9	1.2 y	F	SCID-RAG2 (heterozygous variant C.457>C [6 mo after cord SCT)	Refractory grade III skin, gut, and liver GvHD	Father	Treo/Flu/TT rATG/rituximab
10	5 mo	F	SCID-RAG2 (c1247G>T in exon 2)	Disseminated CMV (pneumonitis/encephalitis): T cell–mediated pneumonitis? On ventilator	Father	Alemtuzumab
11	10 mo	M	Artemis-deficient SCID	Pneumonia (cause?)	Mother	Treo/Flu/TT rATG/rituximab
12	8 mo	M	Omenn syndrome (RAG1 SCID)	Adenoviremia, glaucoma on day−2	Mother	Treo/Flu/TT rATG
13	4 mo	F	Omenn syndrome (*IL7R* compound heterozygous mutation)	Skin rash, diarrhea	Mother	Treo/Flu/TT rATG
14	1 y	M	CID	Disseminated resistant CMV, renal tubulopathy	Mother	Treo/Flu/TT
15	7.4 y	M	DOCK8 deficiency (homozygous deletion of exon 8)	Disseminated adenovirus and CMV, warts	Mother	Treo/Flu/TT Alemtuzumab
16	3.7 y	M	CGD (p22 deficiency)	Disseminated BCG, granulomatous pneumonitis (nodules)	Father	Treo/Flu/TT Alemtuzumab
17	2.4 y	M	CGD (X-linked)	Enteropathy on PEG feed	Father	Treo/Flu/TT rATG
18	1.9 y	M	CD40 ligand deficiency (C.540del.[Arg181Glyfs*10] exon5)	None	Father	Treo/Flu/TT rATG
19	2.6 y	M	MHC-II deficiency (RFXANK homozygous del-c.362A>Tp.D121V)	None	Mother	Treo/Flu/TT rATG
20	1.8 y	F	MHC-II deficiency (RFXP2c.362 mutation)	None	Father	Treo/Flu/TT rATG/ rituximab
21	4 mo	F	HLH (MUNC13-4; c817c>tpR273x)	Disseminated CMV (CMV pneumonitis)	Father	Treo/Flu/TT rATG
22	2.7 y	F	HLH	Transaminitis	Father	Treo/Flu/TT rATG/ rituximab
23	10.3 y	F	STAT3 GOF (exon 22c.2144C>T)	Neutropenia, autoimmune enteropathy Disseminated adenovirus	mMUD	Treo/Flu/TT rATG/rituximab
24	2.7 y	M	IPEX syndrome	Failed mMUD cord transplantation	Mother	Treo/Flu/TT rATG
25	9.3 y	M	SCN with MDS (ELANEC.688DELG)	MDS + enteropathy	Mother	Treo/Flu/TT
26	9.4 y		Same patient	Failed first transplantation	mMUD	rATG TBI (4Gy)/Flu/TT/Cyclo (60) rATG‡

ALI, Acute lung injury; *ALL*, acute lymphoblastic leukemia; *Bu*, busulfan; *CγC*, common γ chain; *CGD*, chronic granulomatous disease; *CID*, combined immune deficiency; *CsA*, cyclosporine; *DOCK8*, dedicator of cytokinesis 8; *F*, female; *Flu*, fludarabine; *GOF*, gain of function; *HHV-6*, human herpes virus type 6; *HLH*, hemophagocytic lymphohistiocytosis; *HR*, high risk; *IPEX*, immunodysregulation, polyendocrinopathy, enteropathy, X-linked; *IS*, immunosuppression; *M*, male; *MMF*, mycophenolate mofetil; *MDR*, multi-drug-resistant; *MDS*, myelodysplastic syndrome; *MRD*, minimal residual disease; *NA*, not applicable; *PEG*, percutaneous endoscopic gastrostomy; *PHTN*, pulmonary hypertension; *RAG*, recombination-activating gene; *rATG*, rabbit ATG; *SCID*, severe combined immune deficiency; *SCN*, severe congenital neutropenia; *SCT*, stem cell transplant; *TBI*, total body irradiation; *TMA*, thrombotic microangiopathy; *Treo*, treosulfan; *TT*, thiotepa; *WAS*, Wiskott-Aldrich syndrome.

*Platelet engraftment: platelet count greater than 20×10^9/L for 7 consecutive days without transfusion support; (for patients with WAS, the cutoff was greater than 50×10^9/L for 3 days without transfusion support).

†Neutrophil engraftment: absolute neutrophil count greater than 0.5×10^9/L for 3 consecutive days.

‡ATG dose was 15 mg/kg, except for patient 26, in whom a lower dose was used (6 mg/kg).

§No specific antiviral treatment given.

TABLE I. *(Continued)*

GvHD prophylaxis	Engraftment day after day 0 (neutrophil†/platelet*)	GvHD (grade)	Systemic viral infection after day 0	Latest chimerism	Last follow-up day outcome
CsA + MMF	15/17	Never	CMV	100%	1060/Alive and well
CsA	11/9	Never	None	100%	368/Alive and well
CsA	15/18	Never	CMV retinitis CMV CTLs	100%	1131/Alive and well
None	21/10	Grade II skin	CMV CTLs	100%	935/Alive and well
CsA + MMF	0/0	Never	None	T: 97% B: 10% Myeloid: 6%	965/Alive and well
CsA + MMF	0/0	Never	None	T: 94% B: 0% Myeloid: 0%	798/Alive and well
Tac/CsA + MMF + steroids	12/12	Skin (II)	Adenovirus	100%	1217/ Alive and well Mild renal insufficiency (TMA injury)
CsA + MMF + steroids	13/24	Skin (I)	HHV-6 Adenovirus	100%	634/Alive and well Joint Problems resolved
CsA + MMF + steroids	15/08	Skin (I)	None	100%	155/Alive and well
CsA	Early death	NA		NA	9/Died of ALI (T-cell infiltration in biopsy specimen)
CsA	15/10	Never		T: 100% B: 92% Myeloid: 55%	130/Alive and well
CsA + steroids	10/13	Never		NA	51/Died because of ALI/PHTN
CsA steroids	27/12	Never	Adenovirus	T: 100% B: 34% Myeloid: 25%	530/Alive and well
CsA + MMF	11/0	Skin (III)	CMV§ (MDR) Maternal CTLs on day +7	100% until death	287/Died (prolonged IS and aspergillosis)
CsA + MMF	12/5	Never	CMV gut disease	100%	1150/Alive and well, mild bronchiectasis for a year after HSCT (now resolved) warts started to decrease after day +11
CsA + MMF	14/10	Skin (I)	CMV Adenovirus§ HHV-6	100%	750/Alive and well
CsA	15/0	Skin (II)	Adenovirus HHV-6§	100%	388/Alive and well
CsA	12/8	Skin (I)	CMV Adenovirus HHV-6	T: 96% B: 96% Myeloid: 83%	698/Alive and well
CsA	16/8	Skin (I)	Adenovirus (maternal CTLs on day 47)	100%	413/Alive and well
CsA	15/9	Never	Enterovirus§ Meningoencephalitis-hydrocephalus with immune reconstitution	100%	334/Alive and and doing well, poor weight gain (norovirus- and enterovirus-related enteropathy?)
None		NA	CMV pneumonitis	NA	13/Died becausse of severe PHTN
CsA + MMF	18/10	Never	None	100%	483/Alive and well
CsA + steroids	20/7	Never	HHV-6§	100%	475/Alive and well
None	18/0	Grade I skin		100%	365/Alive and well
None	Primary graft failure	Grade II skin	CMV	NA	29/Primary graft failure
CsA + MMF	10/0		Pneumonitis	100%	850/Alive and well

TABLE II. Comparison of current study with previously published evidence using the same method of T-cell depletion in children with PIDs

	Bertaina et al[16]	Balashov et al[15]	Current study
No. of patients	23 (PID = 11)	37	25
Median age (y)	3.3 (0.4-12)	2.6 (0.2-17)	1.75 (0.2-10.3)
Donors (no.)	Haplo (23)	MUD (27) Haplo (10)	mMUD (2) Haplo (24)
Serotherapy	rATG, 12 mg/kg, + rituximab, 200 mg/m^2 (all)	rATG, 10 mg/kg (20) rATG + rituximab, 100 mg/m^2 (14) Alemtuzumab (2)	rATG, 15 mg/kg (11) rATG + rituximab, 200 mg/m^2 (6) Alemtuzumab (5) None (3)
Median cell dose (range)			
CD34 cells × 10^6/kg BW	15.8 (10.2-40)	11.7 (5.9-21.3)	17.8 × 10^6 (4.7-50.9)
TCRαβ cells ×10^4/kg BW	4 (1-9.5)	1.6 (0.08-36.8)	3.3 (0.075-9.5)
B cells × 10^5/kg BW	0.4 (0.05-1.5)	NA	4 (0.038-11)
NK cells × 10^7/kg	3.82 (1.57-17.6)	NA	5.15 (0.13-15)
GvHD prophylaxis	None	Tac + MTX (34) Tac + MMF (2) CsA + MTX (1)	CsA or Tac + MMF (11) CsA (11) None (4)
Neutrophil engraftment (d)	13 (10-20)	16 (11-28)	15 (10-27)*
Cumulative incidence (%), primary/ secondary graft failure	8.7/8.7	5.4/21.6	4.2/0
Cumulative incidence (%), aGvHD	Grade I/II: 13.1 Grade III/IV: 0	Grade II: 21.5 Grade III/IV: 2.8	Grade I/II: 44.1 (95% CI, 27.7-70.2) Grade III-IV: 4.3 (95% CI, 0.6-29.6)
cGvHD	None	1 patient	None
Cumulative incidence (%), systemic viral infection/reactivations	38% for CMV and adeno	46% for CMV	35.0% ± 9.4% for CMV 33.2% ± 9.7% for adenovirus 26.3% ± 10.4% for HHV-6
TRM at 1 year (%)	9.3	3.3	16.4
Median follow-up (mo [range])	18 (5-40)	13 (4-29)	20.8 (5-40.0)
EFS/OS (%)	74/91.1	67.7/96.5	80.4/83.9

BW, Body weight; *cGvHD*, chronic graft-versus-host disease; *Haplo*, haploidentical; *MMF*, mycophenolate mofetil; *MTX*, methotrexate; *NA*, not applicable; *OS*, overall survival; *Tac*, tacrolimus; *TRM*, transplant-related mortality.
*Unconditioned data are excluded.

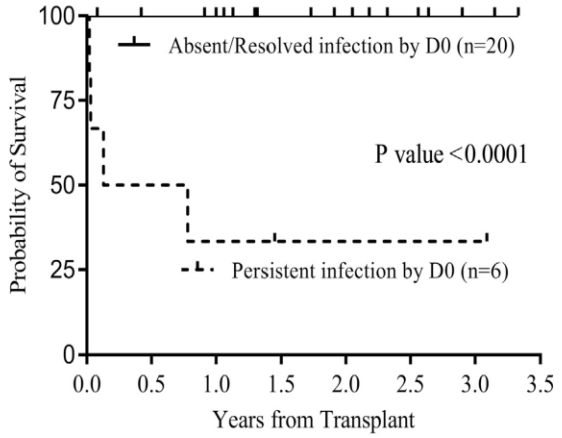

FIG 1. Survival according to CMV or adenovirus infection status on day 0.

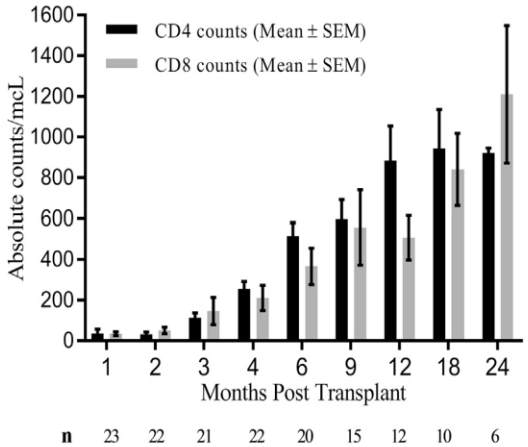

FIG 2. CD4 and CD8 cell reconstitution after transplantation.

80 days after transplantation, and all had gut viral infections (norovirus, 4; rotavirus, 2; and norovirus plus enterovirus, 1).

Immune reconstitution

Cotransfused NK cells were detectable in the first week after transplantation, and NK cell counts remained greater than 200 cells/μL in most patients after day 30. Median time to CD4 recovery (Fig 2) (absolute count ≥200 on 2 consecutive tests) was 129 days (range, 79-515) in 20 patients (3 were early deaths, and data for 1 patient are not available). Naive T cells appeared at 4 months after transplantation in most patients (Fig 3). Median

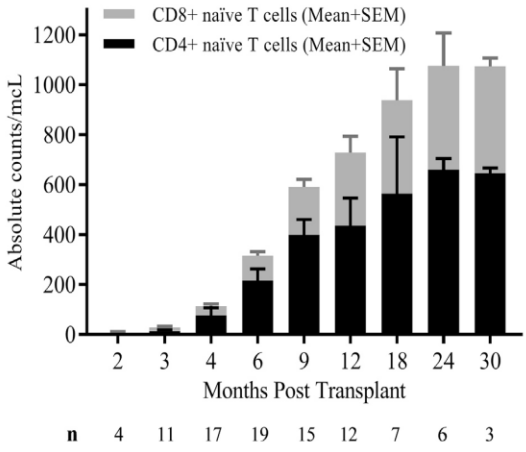

FIG 3. Naive T-cell recovery.

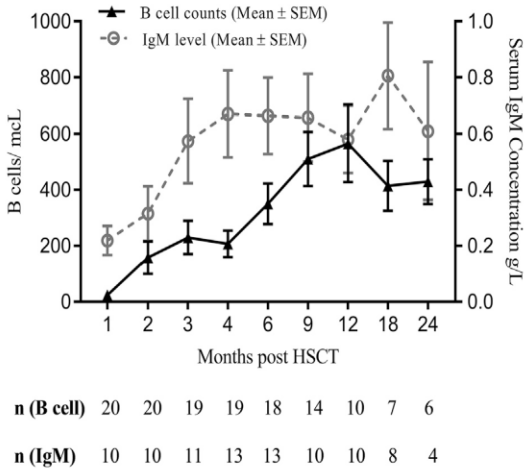

FIG 4. B-cell (B cell–positive SCIDs excluded) and IgM recovery after transplantation.

time to B-cell recovery (count ≥200 cells/μL on 2 consecutive tests) in all but 2 conditioned patients (n = 17) was 85 days (range, 41-367 days). The B-cell count of patient 13 remained zero until his death 9 months after HSCT despite good T-cell recovery and 100% donor chimerism. Class-switched memory B-cell ($CD27^+IgD^-$) testing at 6 months after transplantation showed a median of 1% (range, 0% to 9%) subpopulation of these cells in 14 patients, and the figures at 1 year and 1.5 years were 2% (range, 1% to 4%) and 3% (range, 1% to 5%), respectively. Considering robust absolute B-cell numbers at these time points (Fig 4), this proportion of class-switched memory B cells heralds good immune recovery. The median time to achieve normal IgM level (>0.5 g/L on 2 separate occasions) in 15 survivors was 97 days (range, 62-187 days) (Fig 4). Only 5 surviving patients have IgM levels of less than 0.5 g/L; 2 of them are less than 6 months after transplantation. Flow cytometric analyses performed on peripheral blood samples 4 weeks after HSCT showed that γδ T cells represented the majority of T cells (mean, 81.5%; range, 32% to 100%). Subsequently, the αβ T-cell proportions increased gradually (Fig 5). A normal αβ/γδ ratio was reached around 1 year after transplantation.

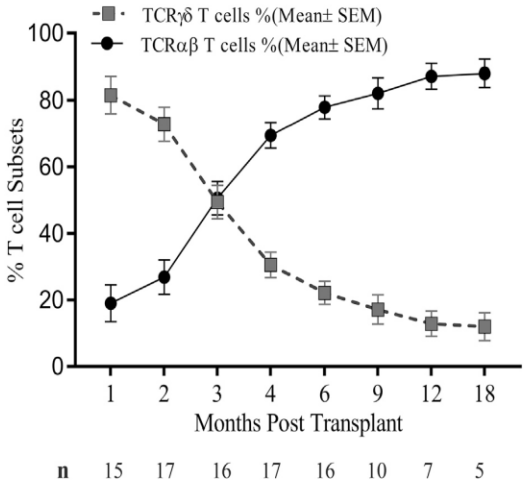

FIG 5. αβ and γδ T-cell reconstitution after HSCT.

Long-term follow-up and complications

All surviving patients are doing well. Four patients had autoimmune cytopenias 4 to 17 months after transplantation and responded to steroids, immunoglobulins, or both. One patient has mild chronic renal insufficiency and is receiving antihypertensive therapy. One patient had prolonged (8 months) renal TMA, which has now resolved. Of available data on 17 patients surviving more than 1 year after transplantation, 14 stopped requiring immunoglobulin replacement (indirect evidence of class-switched memory B-cell function) and commenced primary immunizations.

DISCUSSION

We report a 3-year EFS of 80.4% after $CD3^+TCR\alpha\beta^+/CD19^+$ cell–depleted HSCT in the most diverse group of patients with PIDs reported to date, some of whom historically had poor outcomes with haploidentical donors.[1] Two studies[15,16] have been published using the same approach for PIDs (Table II), and the major difference in comparison with our study is the rate of graft failures. Balashov et al[15] showed a high frequency of graft failure, which might be related to the use of only 1 alkylating agent for conditioning in 9 of 10 patients with graft failure. In the study by Bertaina et al,[16] 17.4% of patients had graft failure despite receiving 2 alkylating agents, but all were in patients with diseases known to be associated with an increased risk of rejection (thalassemia, osteopetrosis, and severe aplastic anemia). Use of an additional alkylating agent might have helped clear host stem cell niches, as evident by only one case of graft failure in our study. The addition of a second alkylating agent did not seem to result in additional short-term toxicities in both previous studies and the current study in which about half of conditioned patients did not even require systemic analgesia.

Despite using GvHD prophylaxis in our cohort, 47.8% of patients had acute graft-versus-host disease (aGvHD; mild in 10/11). It is important to note that 5 of 11 patients had skin GvHD around the time of engraftment (range, day +13 to day +17). We did not perform skin biopsies and have a lower threshold of reporting them as skin GvHD when many centers would report them as engraftment syndrome. In the Italian study only 3 of 23 patients had mild GvHD despite no use of GvHD prophylaxis. Rituximab has been shown to reduce the incidence of aGvHD,[23]

and rituximab was used in all of their patients; however, both B-cell dose in the graft and use of rituximab were not found to be associated with GvHD on multivariate analysis in our study. However, the number of patients receiving rituximab is small (n = 6) to derive any definite conclusion. Interestingly, the median dose of B cells in the grafts in the Italian study was a log lower compared with that in our study, although the reason for this is not clear. Although we were able to restrict the TCRαβ cell dose to less than 1×10^5/kg, it is possible that remaining TCRαβ or TCRγδ cells in the graft might have played a role in the occurrence of aGvHD. Although αβ T-cell subsets are known effectors of GvHD, the role of γδ T cells in GvHD is unclear, with conflicting results from animal and human studies and some showing a beneficial role in reducing GvHD[24,25] while others suggest pro-GvHD effects.[26-28] A recent study[29] showed a lower frequency of naive γδ T cells in the peripheral blood of donors corresponding to patients who later had grade II to IV aGVHD.

Interestingly, we saw only skin GvHD in the current study, as in the Italian cohort.[16] A similar trend was noticed in a study in patients with leukemia by Maschan et al[13]: 40% cumulative incidence of isolated skin GvHD in the TCRαβ-depleted group versus 6% in the matched unrelated transplantation group, although this was not statistically significant. TCRγδ cells are found in high concentrations in skin epithelia and gut mucosa under physiologic conditions, and it is possible that TCRγδ T cells might have homed into the skin or gut after transplantation, but this does not explain the absence of gut GvHD in our cohort. On the contrary, it is likely that a small amount of TCRαβ cells in the graft were able to induce mild skin GvHD but did not result in any systemic GvHD. This finding needs further investigation because we have not done subset analysis of infiltrating T cells in GvHD lesions. Moreover, 3 patients underwent transplantation for refractory GvHD, and considering their underlying inflammatory milieu, they had a very high risk of significant GvHD; still, none of them had visceral or severe skin GvHD. This suggests that TCRγδ cells are less likely to cause GvHD and that TCRαβ depletion is a good method to prevent GvHD. With the possible effect of GvHD on the thymus and other detrimental effects on quality of life, any GvHD is undesirable in a patient with nonmalignant disease. Thymic damage caused by GVHD[30] could result in mature medullary epithelial cell depletion and a defect in negative selection,[31] which might lead to a higher risk of chronic GvHD[30] and possibly autoimmune illnesses. Therefore we favored using low-dose cyclosporine. Although we have not seen chronic GvHD in our cohort, 4 patients had autoimmune cytopenias after transplantation, 3 of whom had aGvHD.

It is clear from the evidence that T-cell depletion by means of negative selection (T-cell depletion of the graft) is a better method to reduce the risk of GvHD compared with positive CD34 selection but is associated with an increased risk of graft failure. It is difficult to compare the current study with studies using different *ex vivo* T-cell depletion strategies for HSCT in children with PIDs because our cohort comprises patients with diverse non-SCID PIDs (which have a higher risk of graft failure vs SCID population) compared with the majority of patients with SCID in previous studies (see Table E1 in this article's Online Repository at www.jacionline.org).[32-34] However, based on our data and previous reports using TCRαβ depletion, it is clear that the graft failure rate is low with this technique and that rapid engraftment is achieved in most patients. We suspect that apart from the conditioning, residual TCRγδ cells and other immune cells contribute to robust engraftment and a very low rate of graft failure. It has been shown that infusion of activated γδ T cells can promote engraftment[35,36] without causing GvHD, even in the MHC-mismatched situation.[37]

Patients going into transplantation with active viral infections had the worst outcomes in our cohort, as shown previously in patients with SCID undergoing HSCT in which infants (>3.5 months of age) with infection had a poor survival in comparison with those with resolved infections or those without prior infection (50% vs 82% vs 90%).[33] One of the deaths in our cohort was at 9 months after transplantation because of aspergillosis resulting from prolonged immune suppression for GvHD. This patient had persistent low-grade CMV viremia at the time of death, which is well known to contribute to both immune suppression[38,39] and heightened the risk of GvHD[40] requiring immunosuppression, both of which are risk factors for fungal infections. Viral infections in HSCT are known to have unfavorable consequences and increased cost with longer hospital inpatient days.[41] We do not think the use of low-dose cyclosporine in our cohort contributed to the occurrence of viral infections, which is comparable with the data from Bertaina et al[16] (no use of cyclosporine) and the published frequency of viral infections (51.6%) in pediatric patients with nonmalignant illnesses after matched sibling donor or MUD transplantation using reduced-intensity conditioning with regular-dose GvHD prophylaxis.[42] Extensive weekly virology screening, including HHV-6 and enterovirus, might have picked up a higher number of asymptomatic infections in our cohort. An active role of γδ T cells in the immune response against CMV[43,44] has been suggested. Although there might be some beneficial anti-CMV effect from infused γδ T cells (as shown by us previously[18] in patient 1), not all patients were able to clear CMV. Of 6 patients with disseminated CMV or adenovirus before transplantation, only 2 survived HSCT despite the use of antivirals. A recent study[45] showed that although the TCRαβ depletion strategy is associated with high CMV reactivation rates, it does not affect the outcomes of such transplantations.

Similar to previous studies,[34] we have shown robust immune reconstitution 4 months after HSCT. Additionally, cyclosporine was stopped at a median of 4 months (range, 2.5-9 months) after HSCT, and a majority in longer follow-up are no longer receiving immunoglobulins (see Table E1). Assuming most children will have a haploidentical donor available, use of this technique will result in a shorter diagnosis-to-transplantation interval, which in turn can lead to fewer pretransplantation infections, which are known to contribute to mortality and morbidity. Availability of donor cells for CTL therapy at a later date will be an additional advantage.[46] The cost of the consumables per $CD3^+TCR\alpha\beta^+/CD19^+$ depletion procedure (including apheresis cost) is around £10,000 which is cheaper than procuring an MUD (£15,000-£20,000) or umbilical cord blood (£17,000-£35,000) donor.

We conclude that use of $CD3^+TCR\alpha\beta^+$ and $CD19^+$ depletion for *ex vivo* processing of mismatched donor grafts ensures a high engraftment rate; good immune reconstitution; low incidence of significant aGvHD, especially visceral GvHD; and acceptable posttransplantation morbidity in children with a range of PIDs, even when used for second HSCT. $CD3^+TCR\alpha\beta^+/CD19^+$ depleted HSCT from an HLA-haploidentical parent is a viable

option for children with PIDs and can also be considered a therapeutic modality for patients with refractory GvHD. Prevention and successful treatment of viral infections are the primary determinants of a healthy transplantation outcome. Although our study confirms the short-term tolerability of reduced-toxicity myeloablative conditioning regimens for patients with PIDs, longer follow-up is needed to evaluate long-term effects on, for example, fertility.

Clinical implications: This article supports the use of $CD3^+TCR\alpha\beta^+$ and $CD19^+$ cell–depleted haploidentical or mMUD HSCT across a range of PIDs and should be considered in children with PIDs lacking an HLA-matched donor or when urgent HSCT is indicated.

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TABLE E1. Comparison of the current study with previously published evidence using different methods of TCD in children with PIDs

	Pai et al[33]	Buckley et al[32]	Current study
No. of mismatched transplants	138 (SCID)	77 (SCID)	26 (PID)
TCD method	Soybean agglutination and E-rosette TCD (51%) CD34 selection (36%)	Soybean agglutination and E-rosette TCD of marrow	TCRαβ $CD3^+$ cell and $CD19^+$ cell depletion of PBSCs
Conditioning	MA (18%), RIC (7%) Immune suppression (12%) None (63%)	None except 2 patients who had previous transplantations	MA (88.5%) None (11.5%)
GvHD prophylaxis	4%	None except 2 who received conditioning	84.6%
OS	66% with conditioning 79% without conditioning	83.9%	83.9%
CI (%), grade III-IV, aGvHD	10	10.4 (involving skin and gut)	4.3 (no visceral GvHD)
CI (%), chronic GvHD	16	NA	0
CI (%), graft failure	24	19.5	4.2
B-cell chimerism	39% (13/36)	NA	81.5% (18/22)
CD4 T cells, >500 cells/μL at >2-y follow-up	30%	NA	83.3% (5/6)
Off immunoglobulin replacement at 2-5 y	37%	49.5%	82.3%

CI, Cumulative incidence; *MA*, myeloablative; *NA*, not applicable; *OS*, overall survival; *PBSC*, peripheral blood stem cell; *PID*, primary immune deficiency; *RIC*, reduced-intensity conditioning; *TCD*, T-cell depletion.