Ther Adv Hematol
2021, Vol. 12: 1–19
DOI: 10.1177/20406207211013985

Treosulfan-based conditioning for inborn errors of immunity

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***Abstract*:** Inborn errors of immunity (IEI) 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 IEI 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. It is firmly incorporated into the conditioning guidelines of the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation. Unlike busulfan, pharmacokinetically guided dosing of treosulfan is not part of routine practice, but data are emerging which indicate that further improvements in outcome may be possible, particularly in infants who have a decreased clearance of treosulfan. It is likely that individualized dosing, not just of treosulfan, but of all agents used in conditioning regimens, will be developed and implemented in the future. 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 which are difficult to engraft or require high levels of donor chimerism, the dosing of individual cellular components within grafts, and effects of adjuvant cellular or immunotherapy post-transplant. This review documents the establishment of treosulfan worldwide, as a safe and effective agent for conditioning children with IEI prior to HSCT.

Keywords: inborn errors of immunity, haematopoietic stem cell transplantation, treosulfan

Received: 27 January 2021; revised manuscript accepted: 12 April 2021.

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Inborn errors of immunity

Inborn errors of immunity (IEI) are inherited disorders of development and/or function of the immune system. More than 450 single gene defects are identified.[1] Severe combined immunodeficiency (SCID), the most profound IEI characterized by impaired T- and B-lymphocyte function, presents in infancy with recurrent opportunistic infections and faltering growth: untreated, infants usually die within the first year of life.[2] The first successful bone-marrow transplant (BMT) in 1968 achieved immunological reconstitution in a patient with X-linked SCID,[3] followed by a second case published in 1969.[4] Other T-lymphocyte immunodeficiencies may present later. Another early BMT was performed in 1968 for a patient with Wiskott–Aldrich syndrome (WAS).[5] 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.[6–8] 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), experience recurrent infections, colitis and other

inflammatory manifestations, requiring frequent hospital admissions, surgery and leading to early death.[9] Quality of life has been shown superior in transplanted patients compared with those managed conservatively.[10]

Historically, haematopoietic stem-cell transplantation (HSCT) for SCID has led to a higher survival than for non-SCID IEI. In Europe, data from participating transplant centres are collected in the Stem Cell Transplantation for Immunodeficiencies in Europe registry, which show improved survival over time.[11–15] Importantly, patients with SCID diagnosed and transplanted before the development of infection have better outcomes, which has led to the introduction of newborn screening in many areas of the world.[16,17] Similarly in disease-specific series, transplant outcome is better for younger patients without pre-existing organ damage and infection.[18–21] Awareness of IEI has led to earlier diagnosis and treatment, enabling timely referral for HSCT. Incremental changes in methods of tissue typing resulting in better human leucocyte antigen (HLA)-matched donors, use of alternative donors such as cord blood (CB), and haplo-identical donors facilitated by improved methods of T-lymphocyte depletion, reduced toxicity of conditioning regimens, surveillance for viral infections by polymerase chain reaction, enabling pre-emptive treatment, better therapies for infections, and improved preventative strategies and treatments for complications such as graft-*versus*-host disease (GVHD) have improved transplant outcomes.

Historically, patients without a defined genetic defect had a poor outcome, maybe because they were only offered transplant as a last resort.[15] 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 developed as an alternative to HSCT for several IEI. Originally, gamma-retroviral vectors were used, but the development of insertional mutagenesis led to a number of patients developing leukaemia in those treated for X-linked SCID.[22] 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.[23] In 2016, the European Medicines Agency (EMA) approved the first stem-cell gene therapy product for ADA-SCID, (Strimvelis™), licensed by GSK.[24] Despite using a gamma-retroviral vector, only 1 case of T-cell leukaemia, possibly due to insertional mutagenesis, has occurred to date in ADA-SCID,[25] and worldwide, more than 150 cases have now been treated in various trials.[26] Successful gene therapy requires chemotherapy conditioning, and busulfan is administered at a lower dose than required prior to allogeneic HSCT.

While infants with SCID are 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 IEI such as CGD need to 'earn the right to transplantation by presenting with significant complications or infections'.[15] This is likely to have contributed to poorer outcome in these patients who were transplanted with organ damage and established infections, thus compounding the problem. As it became acceptable to offer transplant to a young patient, the transplant community was initially cautious to use any donor that was not an HLA-identical-matched sibling. It was demonstrated that a well-matched unrelated donor provides as good an outcome as a matched sibling for CGD[27] confirmed in the recent Inborn Errors Working Party Study of 712 patients transplanted for CGD from 101 different centres.[28] The new T-lymphocyte depletion technique of CD3+ TCRαβ/CD19+ depletion for mismatched donors, achieves improved survival in patients without an HLA-identical donor, not just for patients with CGD, but for a wide range of IEI.[19,29,30] As survival improves, quality of life and long-term immunoreconstitution are recognized as increasingly important.[10,31,32]

Conditioning in IEI

Preparative cyto-reductive chemotherapy is required to open osseo-thymic niches and create space for donor haematopoietic stem and precursor cells. Originally, conditioning therapy for children was most commonly based on the combination of two alkylating agents, usually: busulfan and cyclophosphamide, or of cyclophosphamide and total body irradiation (TBI). The goals were to predictably cause:

(1) host haematopoietic stem-cell toxicity to induce myeloablation;
(2) immunosuppression to enable engraftment and prevent rejection;
(3) 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 hepatic veno-occlusive disease (VOD), interstitial pneumonia, convulsions and mucositis. Busulfan is highly lipophilic with moderate plasma binding and achieves high levels in the liver where it undergoes enzymatic conversion. Results of busulfan use have improved with the introduction of an intravenous preparation and careful pharmacokinetic (PK)-guided dosing to achieve an area under the curve (AUC) within a targeted range.[33,34] However, PK variability remains substantial, particularly in young children.[35] Cyclophosphamide is associated with hepatotoxicity, as well as haemorrhagic cystitis and acute cardiotoxicity.[36]

The role of conditioning prior to HSCT in non-malignant diseases is seen as providing a platform for the development of donor chimerism and in the setting of malignancy, a graft-*versus*-malignancy effect, in addition to the antineoplastic activity of the conditioning itself. In patients with IEI there is no advantage in having GVHD because no graft-*versus*-malignancy effect is required and GVHD can have a negative effect on thymic reconstitution, leading to poor immune function.[37]

Myeloablative conditioning (MAC) results in irreversible cytopenia and the requirement for stem-cell rescue. Truly non-myeloablative regimens cause minimal cytopenia and theoretically, no need for 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 occasionally, cytopenia may be reversible without such support.[38]

The introduction of RIC regimens such as fludarabine and melphalan led to reduced treatment-related toxicity, but specific cardiac toxicities are associated with melphalan in infants,[39] reducing utility in infants with SCID. Minimal-intensity conditioning using an anti-CD45 antibody together with fludarabine and low-dose cyclophosphamide may also result in successful engraftment, but data are sparse and the availability of such antibodies is limited.[40] RIC regimens lead to a higher rate of mixed chimerism and efforts to improve chimerism such as using donor lymphocyte infusions risk causing GVHD, leading to immunosuppressive therapy and more infections.

Treosulfan has increased lymphotoxic and myelosuppressive properties compared with 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, while incurring the more limited non-haematological toxicity associated with RIC regimens. It can be defined as a reduced-toxicity myeloablative regimen.[41]

Conditioning regimens for children with IEI have 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 more durable thymopoiesis is obtained after the administration of chemotherapy.[31] Patients with deoxyribonucleic acid (DNA)-repair disorders are at risk of toxicity from alkylating agents, and a RIC regimen such as that with low-dose cyclophosphamide and fludarabine is generally recommended.[42]

Treosulfan

Treosulfan (*L*-treitol-1, 4-bis-methanesulphonate) is a water-soluble bifunctional alkylating agent,[43] that is a dihydroxy-busulfan derivative. The introduction of two hydroxyl components into the carbon chain confers different properties to busulfan. As a pro-drug, it undergoes a pH and temperature-dependent non-enzymatic conversion to a mono-epoxide [(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)]. At pH values below 6 and a temperature of 20°C, *in vitro*, almost no transformation of treosulfan occurs, but spontaneous conversion

into the derivatives occurs under physiological conditions. The monoepoxide intermediates and *L*-diepoxybutane alkylates DNA at guanine residues and produce DNA interstrand crosslinks, resulting in DNA fragmentation and apoptosis. It is water soluble and easily given intravenously, but hydrophilic properties confer less hepatic distribution than busulfan, reducing the severity and incidence of hepatic complications, particularly VOD. The absence of hepatic metabolism decreases the risk of interaction with concomitant medications such as glutathione level reducers (e.g. cyclophosphamide, paracetamol), hepatic enzyme inducers (e.g. itraconazole) and substrates such as methylprednisolone. Treosulfan is significantly less neurotoxic than busulfan, induces no seizures, and consequently, does not require prophylactic anticonvulsant therapy, which is mandatory for busulfan. The main toxicities are mucosal, and skin rashes, particularly in the perineal area, which may require pain relief. Most centres recommend frequent bathing and nappy changes in infants receiving treosulfan.

Treosulfan was designated an 'orphan medicine' (a medicine used in rare diseases) in 2004, given marketing authorization by the EMA in June 2019 for adult patients with malignant and non-malignant diseases, and in paediatric patients older than 1 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).

Use of treosulfan in conditioning

Treosulfan has been used to treat ovarian cancer since the 1990s,[44,45] and has a broad spectrum of anti-tumour activity, for example, in metastatic melanoma,[46] breast carcinoma[47] and acute lymphoblastic leukaemia (ALL).[48] When used as chemotherapy against malignancy, haematotoxicity limits its use above doses of 10 g/m^2. Two phase I studies with autologous blood stem-cell rescue demonstrated that it was possible to escalate the dose to almost five times conventional therapy before mucositis, stomatitis, diarrhoea, skin toxicity and acidosis became dose limiting.[49] Murine studies revealed the pronounced effect of treosulfan on haematopoietic stem cells[50] and showed that reliable donor-cell engraftment could be achieved using repeated dosing, which was as effective as busulfan or TBI,[51,52] associated with limited non-haematopoietic organ toxicity and was a promising alternative to traditionally used conditioning agents. In mice, treosulfan causes equivalent myeloablation to busulfan and cyclophosphamide, but additionally causes stronger splenic B- and T-lymphocyte depletion: this immunosuppressive effect is beneficial when used as a conditioning agent to suppress the host and facilitate successful engraftment.[53]

Thirty adults with haematological malignancies, considered high risk for conventional conditioning, in a phase I/II trial between 1999 and 2002 from three German centres, received treosulfan with fludarabine. The total treosulfan dose was 30 g/m^2 with 150 mg/m^2 fludarabine and antithymocyte globulin (ATG) was given to recipients of unrelated donors. Toxicity was low and no VOD occurred. Eight patients died: six (20%) of non-relapse causes and two (6.7%) of relapse, giving an estimated overall survival (OS) of 73% and event-free survival (EFS) of 49% after a median follow up of 22 months (range 7.4–33.4 months).[54] This landmark study initiated the use of treosulfan in conditioning for HSCT.

A low rate of organ toxicities and favourable 1-year non-relapse mortality was reported when 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 busulfan and cyclophosphamide. PK studies were incorporated, which showed predictable maximum concentration (*C*max) and AUC values with low inter-patient and inter-day variability.[55] Treosulfan and fludarabine were used for patients with myeloma before allogeneic HSCT, leading to an estimated OS of 58% at 2 years.[56] Six severe aplastic anaemia patients with a median age of 21 years (range 14–25 years) were transplanted using treosulfan, cyclophosphamide and ATG, who all engrafted with a good outcome.[57]

The first reported use exclusively in children was in three patients with Schwachman–Diamond syndrome (SDS), conditioned with fludarabine, treosulfan and melphalan to replace historically

used cyclophosphamide and busulfan or TBI. All engrafted, but one died post-cord-blood HSCT with idiopathic pneumonitis syndrome.[58] Therefore, it was not clear 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.

A phase I–II prospective trial reported 20 patients transplanted for thalassemia major in two Italian centres. Treosulfan is attractive for this disease because of the substantial risk of VOD in iron-overloaded patients. HSCT for thalassemia is associated with a substantial risk of graft failure. Median age at HSCT was 13 years (range 1–28 years). All received thiotepa, treosulfan and fludarabine, with ATG for the 17 unrelated donor recipients. This was well tolerated with no cases of VOD. The 2-year estimates of OS and transfusion-free survival were 95% confidence interval (CI) 85–100% and 85% CI 66–100%, respectively.[59] This experience has been confirmed in a larger series of 60 patients.[60]

Strocchio *et al.* reported 15 patients with sickle cell anaemia who underwent matched sibling donor (MSD) or matched unrelated donor (MUD) HSCT following treosulfan, thiotepa and fludarabine conditioning and compared patient outcomes with 15 patients from a historical cohort given a busulfan-based regimen. Engraftment was achieved in 28 out of 30 patients (93%), with one case of graft failure in both groups. The conditioning regimen was well tolerated in both groups, with no cases of grade III–IV regimen-related toxicity. The 7-year OS and disease-free survival (DFS) for the whole cohort were 100% and 93%, respectively, with a 93% DFS in both busulfan and treosulfan groups. No SCD-related adverse events occurred after engraftment in patients with complete or mixed donor chimerism. This retrospective analysis suggested that treosulfan-based conditioning is able to ensure engraftment with excellent outcomes in patients with sickle cell disease.[61]

A multicentre open-label, non-controlled prospective phase II study in children with haematological malignancies reported on 70 children with ALL, acute myeloid leukaemia, juvenile myelomonocytic leukaemia or myelodysplasia, 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 as follows:

- BSA $\leq 0.5\,m^2$ received a total dose of $30\,g/m^2$
- BSA > 0.5–$1.0\,m^2$ received a total dose of $36\,g/m^2$
- BSA $> 1.0\,m^2$ received a total dose of $42\,g/m^2$

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 compared favourably with other conditioning regimens. There were no primary graft failures; one secondary poor graft function was rescued with a stem-cell boost. There was one case of grade II hepatic sinusoidal obstruction syndrome that resolved. Treosulfan/fludarabine/thiotepa was recommended as a suitable myeloablative preparative treatment in children with malignant disorders.[62]

Peters *et al.* have recently reported a multinational, randomized, non-inferiority phase III study comparing preparative combination chemotherapy with TBI plus etoposide before HSCT in patients with ALL. OS was inferior in the chemotherapy recipients compared with the TBI based group, but there was no difference in outcomes in the chemotherapy arm between those who received treosulfan, thiotepa and fludarabine and those who received busulfan, thiotepa and fludarabine.[63]

Use of treosulfan in children with inborn errors of immunity

Table 1 summarizes the reports of treosulfan used for IEI including haemophagocytic lymphohistiocytosis (HLH) and osteopetrosis.

Greystoke et al

Thirty-two children received treosulfan prior to HSCT for a variety of non-malignant diseases in two UK centres. They received treosulfan rather than busulfan due to toxicity risks. Diagnoses included IEI (18), metabolic disorders (9), osteopetrosis (4) and beta-thalassaemia major (1). Skin toxicity was noted, usually nappy rash but with severe perineal ulceration in some cases.

Table 1. Use of treosulfan in patients with IEI.

Author(s), year, type of study	Number of patients, diagnoses, *n* (%)	Donor stem-cell source *n* (%)	Treo dose (mg), additional agents *n* (%)	GVHD aGVHD grade *n* (%)	Second procedures	Survival
Greystoke *et al.*, 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) [+Cyclo 120, 1 patient] or Cyclo 200, 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 *et al.*, 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) Cyclo 120, 20 (87) Cyclo 200, 3 (13) + Mel 140, 1 (4) Flu 180, 1 (4) Etop 30, 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 *et al.*, 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) Cyclo 200, 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 1–47 months)
Burroughs *et al.*, 2014, prospective	**31** PID 13 (42) HLH 6 (19) BMF 6 (19) RBC 6 (19)	MRD 4 (13) MUD 26 (84) MMUD 1 (3) BM 29 (94) PBSC 2 (6)	Tr 42, 31 (100) Flu 150, 31 (100) ATG 22 (71) None 9 (29)	I NA II 16 (52) III–IV 3 (10) cGVHD 2-year cumulative incidence 21%	2 HSCT	2 years 90% Day 100 TRM 0%
Beier *et al.*, 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 150-180, 49 (96) Flu 7.2, 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

(continued)

Table 1. (continued)

Author(s), year, type of study	Number of patients, diagnoses, *n* (%)	Donor stem-cell source *n* (%)	Treo dose (mg), additional agents *n* (%)	GVHD aGVHD grade *n* (%)	Second procedures	Survival
Lehmberg *et al.*, 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 *et al.*, 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) Hyper-eosinophilic 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%
Haskologlu *et al.*, 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)] Cyclo 200, 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

Alem, alemtuzumab; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; aGVHD, acute graft-*versus*-host disease; ATG, anti-thymocyte globulin; BM, bone marrow; BMF, bone marrow failure; CB, cord blood; CGD, chronic granulomatous disease; CID, combined immunodeficiency; CAMT, congenital amegakaryocytic thrombocytopenia; CD40L, CD40 ligand deficiency; cGVHD, chronic graft-*versus*-host disease; CHH, cartilage hair hypoplasia; Cy, cyclophosphamide mg/kg; DBA, Diamond–Blackfan anaemia; DLI, donor lymphocyte infusion; DOCK 8, dedicator of cytokinesis 8 deficiency; Etop30, etoposide 30 mg/kg; Flu, fludarabine mg/m^2; FU, follow up; GVHD, graft-*versus*-host disease; HLH, haemophagocytic lymphohistiocytosis; HSCT, haematopoietic stem-cell transplantation; IPEX, immunodeficiency polyendocrinopathy X-linked; ITK, interleukin-2 inducible T-cell kinase deficiency; JMML, juvenile myelomonocytic leukaemia; LAD, leukocyte adhesion deficiency; MDS, myelodysplasia; Mel, melphalan mg/m^2; MHC II, major histocompatibility type II; MMRD, mismatched related donor; MMUD, mismatched unrelated donor; MRD, matched related donor; MSMD, Mendelian susceptibility mycobacterial disease; MUD, matched unrelated donor; NA, not available; OP, osteopetrosis; PBSC, peripheral blood stem cell; PID, primary immunodeficiency; PNH, paroxysmal nocturnal haemoglobinuria; RBC, red blood cell disorder; SCC, sickle cell anaemia; SCID, severe combined immunodeficiency; SCN, severe congenital neutropenia; SDS, Schwachman–Diamond syndrome; SID, severe immune dysregulation; Thal, thalassemia; Tr, treosulfan; 30, 36 and 42, all total dose in g/m^2; TRM, transplant-related mortality; TT, thiotepa mg/kg; WAS, Wiskott–Aldrich syndrome; Y-RIT, yttrium coupled CD66 antibody radioimmunotherapy.

One patient with Wolman syndrome died at day +4 with VOD and there were four late deaths: one from continuing neurodegeneration from osteopetrosis, one HLH with chronic GVHD (cGVHD), rotavirus and HLH, one with mucopolysaccharidosis type 1 Hurler syndrome (MPS1H) who required two additional transplants died from GVHD and another child with

MPS1H lost donor T lymphocytes post-cord-blood transplant and died from adenovirus more than a year post-HSCT. One patient with primary graft failure was successfully re-transplanted, three had secondary graft failure, two of whom were successfully re-transplanted. 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 IEI did particularly well, with only one death in a patient with HLH and only two with low-level chimerism needing consideration of a second procedure.[64]

Cutting et al

Twenty-three children with a variety of malignant and non-malignant diseases had substituted treosulfan for conventional busulfan. Median age at transplant was 4.5 years (range 5 months–15 years). All received treosulfan with varying doses of cyclophosphamide according to disease: four received additional chemotherapeutic agents (see Table 1). There was one primary graft failure. The patient with osteopetrosis died of aGVHD and adenovirus infection. OS was 83%. Despite a heterogenous cohort of diseases with nine mismatched donors there was a high rate of engraftment and minimal toxicity. No patients had VOD and mucositis of at least grade II only occurred in three 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 (three relapses in seven patients) and planned to increase the dose of treosulfan from 36 g/m^2 to 42 g/m^2.[65]

Slatter et al

A retrospective study in 2011 reported 40 patients from Newcastle upon Tyne and 30 from Great Ormond Street Hospital transplanted between 2006 and 2009.[66] Patients were not randomized, and conditioning decisions were left to clinician discretion. Median age at transplant was 8.5 months (range 1.2–175 months), young compared with most transplant cohorts, reflecting the large number of infants who present with IEI and require curative therapy. A total of 66% were ⩽12 months at the time of HSCT. Fludarabine total dose 150 mg/m^2 was used in 40 and cyclophosphamide 200 mg/kg in 30. There were 13 deaths, giving an OS of 81%. There was no significant difference in survival between those who received fludarabine or received cyclophosphamide. Toxicity was not formally graded but skin toxicity was common, including perineal ulceration, and mucositis was mild. Deaths in four patients were possibly related to the conditioning drugs. Only two patients had severe VOD, both of whom had received treosulfan 42 g/m^2 with cyclophosphamide. One with CD40 ligand (CD40L) deficiency recovered after ventilation and dialysis, while the other with SCID died. There is a strong correlation between blood levels of cyclophosphamide metabolites and VOD.[36] Eighteen patients (26%) had aGVHD, but only seven (10%) greater than grade II. Four had limited skin cGVHD. There were three deaths associated with GVHD. Of 42 who were more than 1-year post-transplant, 24 (57%) had 100% donor chimerism. The rate of viral reactivation was 26%, similar to 33% reported by Rao *et al* in 33 children with primary immunodeficiency (PID) conditioned with melphalan, fludarabine and alemtuzumab.[67] This study demonstrated the safety of treosulfan in infants. Toxicity was lower when combined with fludarabine compared with cyclophosphamide and there was no difference in donor chimerism. Use of peripheral-blood stem cells (PBSCs) lead to better chimerism without the risk of severe GVHD when alemtuzumab was used.

Beier et al

Results of 109 patients, from 9 centres in Germany and Austria, transplanted between 2003 and 2009 for a variety of diseases using treosulfan-containing conditioning regimens, included 53 with non-malignant diseases (Table 1).[68] A total of 71% of the non-malignant group received thiotepa or melphalan with treosulfan and fludarabine. Toxicity was difficult to evaluate due to the retrospective nature of the study but VOD was seen in only three patients with myeloid malignancy who had also received either thiotepa or melphalan. A total of 13 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-immunoglobulin (Ig) M 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 and many relapsed. Good long-term survival with few late events was seen in non-malignant disease. In particular, of 31 patients with IEI including 2 with HLH, 27 survived (87%).[68]

Lehmberg et al

A total of 100% OS and DFS of 19 HLH patients who received treosulfan, fludarabine, alemtuzumab with additional thiotepa in 14, was reported from 9 German centres.[69] Median age at transplant was 3.9 years (range 0.3–22 years) with six infants <12 months and two young adults. Two required a second transplant; one for early graft rejection post CD34+-selected haplo-identical HSCT and one at day +125 for secondary graft failure. Five patients with low-level chimerism received donor lymphocyte infusion (DLI), with a stem-cell boost in one. Use of a mismatched donor was a risk factor on multivariate analysis, for requiring additional cellular therapy. Sustained engraftment in patients who had received thiotepa was superior on univariate analysis only. Sixteen patients received defibrotide prophylaxis due to the known risk of VOD in HLH. One developed VOD despite prophylaxis. A total of 37% patients were not in remission at time of HSCT, and 32% had had central nervous system (CNS) involvement. Alemtuzumab may suppress remaining HLH activity due to the widespread distribution of CD52 in T lymphocytes and antigen-presenting cells, and is likely to be important due to the hyperinflammatory nature of HLH.[70] Optimization of dose, 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.[69]

Burroughs et al

A prospective, multicentre, open-label trial to evaluate safety and engraftment efficacy of treosulfan with fludarabine as a conditioning regimen for patients with non-malignant diseases described 13 patients with PID and 6 with HLH (total 64%): all except 2 had bone marrow (BM). Because of a high incidence of grade III–IV aGVHD in the first nine patients, thymoglobulin (rabbit ATG) was added to the regimen 2 mg/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 0.4–30.5 years). Primary engraftment was achieved in all patients and only one had secondary graft failure. Grade 3 mucositis and grade 3 skin rash occurred in 10% of patients; no patients developed liver toxicity. Two patients aged 5.6 months and 23 months had a single focal seizure on day +8 and day +25, respectively. Two patients had grade 4 toxicity: one 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. Two of six 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. While the diseases treated in this study were quite diverse, of note, all patients with IEI or bone-marrow failure survived with disease resolution. Long-term stability of engraftment and late effects need to be studied.[71]

Dinur-Schejter et al

A report from 3 Israeli centres documented 44 patients who received treosulfan-based conditioning for non-malignant diseases. Median age at transplant was 18 months (range 1–181 months), with 37.8% ⩽1 year. Two patients had primary graft failure. A patient with WAS required a second transplant, and a patient with severe congenital neutropenia (SCN) rejected the graft and died; two patients died before engraftment. Pulmonary complications were common (52%) but mainly in those with pre-transplant respiratory problems. One patient developed VOD. A comparison in engraftment rates was made between those who received treosulfan and

fludarabine (66.7%), treosulfan and cyclophosphamide (16.7%) and treosulfan, fludarabine, thiotepa (94.7%). This did not show any difference in OS or DFS: stable mixed chimerism is sufficient to achieve cure in some non-malignant disorders, although for others, high-level donor chimerism is required and so this finding is important.[72]

Slatter et al

In 2015, use of treosulfan in children with non-malignant disorders was reported on behalf of the European society for Blood and Marrow Transplantation (EBMT) Inborn Errors and Paediatric Diseases Working Parties. 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 was analyzed to identify dose-related toxicity and determine incidence of engraftment, treatment-related mortality and OS. Results from 316 transplants from 11 different countries were included. A total of 144 of these were in patients with IEI; the remainder, in patients with metabolic, histiocytic or autoimmune disorders, haemoglobinopathies and BM failures. Treosulfan was shown to be safe and effective in infants: 30% of patients were <1 year of age at transplant and there was no significant difference in OS or EFS between these and older children. There was more respiratory acute toxicity in patients <1 year of age, likely due to the larger number of patients with SCID in this group with pre-existing respiratory impairment. Importantly, the addition of thiotepa, which may lead to improved engraftment and chimerism, did not increase acute toxicity, although incidence of late adverse late effects is unknown. Multivariate analysis showed no association of TRM with age at transplant, treosulfan dose, other agents used in combination with treosulfan, type of donor, stem-cell source or whether it was used for a second or subsequent transplant.[73]

Morillo-Gutierrez et al

Treosulfan-based conditioning was shown to be a safe treatment option in children with CGD even in patients with high-risk problems prior to HSCT, regardless of donor type.[74] A total of 70 patients from 16 centres worldwide transplanted for CGD between 2006 and 2015 with treosulfan-based conditioning were reported in 2016. The OS and EFS at a median follow up of 34 months were 91.4% and 81.4%, respectively. There were no serious non-haematological toxicities. In particular, there were no cases of VOD. For those in whom split chimerism was available, 80% had $\geqslant$95% donor myeloid chimerism.[73] Results were similar to those reported by Güngör *et al.*[75] 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.

Haskologlu et al

A single-centre retrospective series published in 2018 reported 15 children with PID conditioned with treosulfan and fludarabine or cyclophosphamide. Median age at transplant was 12 months (range 3–180 months). OS was 86.7% despite nine patients having documented hepatic problems and six with bronchiectasis pre-transplant. One patient with pre-existing bronchiectasis died of pulmonary failure 13 months post-transplant and a second patient with RAG1 deficiency died of sepsis and thrombotic microangiopathy 7 months post-HSCT. One had primary graft failure following a CD34+ selected haplo-identical 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 *Bacillus Calmette–Guérin*itis which was successful. Skin toxicity was common including severe perianal dermatitis and ulcers in three patients. Three patients with liver dysfunction developed VOD, which responded to defibrotide; one 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 high (53.3% aGVHD) but mainly aGVHD grade I–II, and cGVHD (20%). Only three patients received serotherapy, so a more comprehensive use of serotherapy even for MFD transplants, may have reduced this rate.[76]

Slatter et al

In 2018, a retrospective study on the outcome of 160 children who underwent HSCT for PID in two centres in the UK was reported.[77] All received homogenous conditioning chemotherapy prior to

transplant with treosulfan, fludarabine and in the majority of cases, alemtuzumab. Survival, need for second procedure, toxicity, GVHD, viral reactivation, chimerism and immune reconstitution were assessed. An excellent survival rate of 83% at median follow up of 4.3 years was achieved, with low toxicity and good levels of donor chimerism. Donor myeloid chimerism was significantly higher in recipients of PBSC compared with BM and CB, and 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 ⩽4 months 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 asymptomatic infants in early infancy before infection and organ damage. While HSCT is more successful if performed before infection and organ damage, there is debate as to the best approach in terms of conditioning these young infants.

In summary, since Greystoke and Cutting *et al.* published results in 2008 on the use of treosulfan in children with a variety of non-malignant and malignant disorders including IEI, successive publications have demonstrated safety and efficacy leading to widespread incorporation of treosulfan in conditioning for HSCT for IEI.

There is interest in the use of treosulfan prior to gene therapy instead of busulfan in IEI, but to date no clinical trials are in progress. The advantage of using busulfan is that TDM-based dosing is widely available and generally a much lower AUC is sufficient to achieve engraftment of genetically modified stem cells. Marktel *et al.*[78] used treosulfan and thiotepa and demonstrated safety and efficacy of intrabone haematopoietic stem-cell gene therapy in patients with beta thalassemia.

Pharmacokinetics of treosulfan

The PK profile of treosulfan is best fitted using a two-compartment model with first-order elimination. The main PK studies are summarized in Table 2. In 1998, the clinical PK of treosulfan after a single dose of 8 g/m^2 or 10 g/m^2 in 18 adults with advanced or resistant ovarian or small-cell lung cancer was reported, using a method based on separation by reverse-phase high-performance liquid chromatography 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 h and the AUC and plasma *C*max values were significantly higher in the 10 g/m^2 compared with the 8 g/m^2 recipients. Urinary excretion of the parent compound was nearly 30% of the total dose delivered over 48 h with about 25% being excreted in the first 6 h after administration.[79] Scheulen *et al.*[49] demonstrated a linear increase in AUC up to 56 g/m^2 in adult patients. Half-life, volume of distribution and renal elimination were independent of dose. Beelen *et al*, and Glowka *et al* also demonstrated that AUC increased linearly with treosulfan dose.[55,80] Glowka *et al.*[80] reported results of PK studies in seven patients aged 2–15 years, five of whom received 36 g/m^2, one 30 g/m^2 and one 42 g/m^2 and demonstrated a dose-dependent increase of AUC and *C*max, but with high variability (70%) in these parameters, suggesting that PK evaluation may be necessary in paediatric patients undergoing treosulfan-based conditioning. There was no correlation with outcome but it was a small study.

A bioanalytical method of measuring treosulfan levels, which quantifies treosulfan concentrations in serum, was used to develop a PK model to describe the concentration-time profile for treosulfan in 20 children with malignant and non-malignant diseases. All received 42 g/m^2 treosulfan. Limited interpatient variability (14%) was found in contrast to Glowka *et al.*'s results and there was no correlation with outcomes.[82]

PK results of treosulfan and the monoepoxide S,S-EBDM in 16 children aged 0.4–18 years with malignant and non-malignant haematological disorders showed 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.[83]

Van Der Stoep et al

PK studies were reported in 77 paediatric patients transplanted for haemoglobinopathies (40.3%), haematological malignancy (15.6%), PID (28.5%)

Table 2. PK studies of treosulfan.

Author(s), year, *n*	Diagnoses, *n* (%)	Median age (years)	Method	Regimen: drug, dose, *n* (%)	AUC, mg h/l (mean ± SD)	Comments
Beelen *et al.*, 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 *et al.*, 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 *et al.*, 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 *et al.*, 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 et al., 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 with 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 et al., 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 *et al.*, 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 *et al.*, 2020, 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

(continued)

Table 2. (Continued)

Author(s), year, *n*	Diagnoses, *n* (%)	Median age (years)	Method	Regimen: drug, dose, *n* (%)	AUC, mg h/l (mean ± SD)	Comments
Kalwak *et al.*, 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

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

and BM failure or metabolic disease (15.6%). All received treosulfan and fludarabine. Additional thiotepa was given to 67.5%. Median age at transplantation was 4.8 years (interquartile range 1.6–11.4 years). A total of 12 infants <1 year of age received 30 g/m^2, and 65 patients age ⩾1 year of age received 42 g/m^2 treosulfan. Donors were MSD (35%), ⩾9/10 allelic-matched unrelated donors (46.8%) and haplo-identical related donors (18.2%) following *in vitro* T-lymphocyte depletion with either CD34+ selection or TCR αβ+ and CD19+ depletion. Mean day 1 treosulfan exposure was 1744 ± 795 mg hour per litre (10 g/m^2) and 1561 ± 511 mg h/l (14 g/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 <1350 mg h/l. The risk of experiencing two or more toxicities was higher with AUC >1650 mg h/l compared with AUC <1350 mg h/l. No relationship between treosulfan exposure and chimerism, aGVHD, treatment-related mortality or OS was found.[85] This landmark study was the largest paediatric cohort reported and first to show that treosulfan exposure was associated with toxicity. Limitations included wide heterogeneity in the primary diseases treated, and non-uniform conditioning as 67.5% received additional thiotepa. Further studies are necessary to determine whether treosulfan exposure is related to long-term disease outcome and late treatment-related toxicity such as gonadal toxicity.

Danielak et al

Results of PK studies in 14 children looking at treosulfan and the mono-epoxytransformer S,S-EBDM found that the majority of treosulfan (approximately 68% of total treosulfan clearance) is transformed to S,S-EBDM 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 *et al.*'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, additional to the parent compound.[86]

Kalwak et al

In a recent study, there was limited variability in inter-individual 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.[62]

Chiesa et al

A prospective, open-label, phase II study of the PKs of treosulfan in infants and children investigated a relationship between PK and mortality or donor engraftment.[88] In each of the first 10 patients, 8 samples were taken post-dose of treosulfan, but an interim analysis showed that PK parameters could be estimated on 4 post-dose samples. A high treosulfan AUC was associated with mortality. Children with a cumulative treosulfan AUC >6000 mg h/l had a TRM of 39% compared with 3% below this level. The difference between the levels measured after the first and third doses of treosulfan was 14%, which is lower than the inter-individual difference of 30% and consistent with the study by van der Stoep *et al.*, with a more heterogenous population of diagnoses and conditioning treatment.[85] One patient had primary graft failure, but 12 had ⩽20% donor myeloid chimerism at last follow up. A low treosulfan AUC was associated with poor engraftment with 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 h/l of the three doses. Approximately 50% of the patients had a cumulative AUC within 80–125% of the target; therefore, despite adjustments to the dose based on age, 50% were outside this target. This suggests that TDM-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: 42 g/m^2, reduced to 36 g/m^2 if BSA<1 m^2, and 30 g/m^2 for those with a BSA <0.5 m^2. A comparison with the published dosing scheme showed a trend for reduced overexposure using the BSA dosing in the younger age groups. This 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.[88]

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. TDM-guided dosing should be explored particularly for infants and young children undergoing HSCT for non-malignant conditions.

Future research

Fertility

Alkylating agents impair gonadal function and fertility, and myeloablative busulfan regimens cause severe impairment, particularly in females.[89,90] Treosulfan may be less gonadotoxic than busulfan.[91] Serum concentrations of anti-Müllerian hormone (AMH) in females and inhibin B in males in survivors of HSCT in three different groups were measured: group A had received treosulfan-based conditioning, group B, fludarabine and melphalan, and group C, busulfan and cyclophosphamide. Serum AMH and inhibin B were significantly higher in group A compared with groups B and C, suggesting that treosulfan-based regimens confer a more favourable outlook for gonadal reserve.[92]

Need for additional agents

Despite widespread use of the addition of thiotepa to treosulfan and fludarabine, only one study has reported a higher incidence of complete engraftment compared with treosulfan and fludarabine alone or treosulfan and cyclophosphamide, and the numbers in each group were small.[71] Many reports indicate that the addition of thiotepa does not seem to increase short-term toxicity, but no formal studies have been done: thiotepa is an alkylating agent and would be expected to have an impact on fertility, so studies of late effects are also needed. 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.

DNA-repair defects

The approach to transplanting patients with DNA-repair disorders needs to be optimized, and it is unclear whether treosulfan may have a role in these patients. These patients are exquisitely sensitive to DNA-damaging chemotherapeutic agents. Regimens using RIC similar to that used for Fanconi anaemia have been successful.[42,93–95] A recent publication of 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.[96]

Conclusion

There are many factors that have contributed to improved outcomes of HSCT for IEI, but the introduction of less toxic conditioning regimens has been a fundamental change. The demonstration of superior T-lymphocyte chimerism and less toxicity when combined with fludarabine compared with cyclophosphamide led to a step change in confirming this combination. Early indications suggest that using PBSC with alemtuzumab serotherapy, with careful attention to the CD3+ content of grafts such as capping at 5×10^8/kg, may lead to higher myeloid chimerism than BM without an increase in grade III/IV aGVHD. An association with high AUC and increased mortality, and low AUC and poor engraftment, is leading to future studies to optimize the dosing of treosulfan.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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