Clinical and immunological outcome after paediatric stem cell transplantation in inborn errors of immunity

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Part I

General introduction

Chapter 1

General principles of haematopoietic cell transplantation for inborn errors of immunity

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Conditioning regimens for haematopoietic cell transplantation in primary immunodeficiency

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Introduction

Haematopoietic cell transplantation (HCT) describes the transplantation of haematopoietic stem cells (HSCs) with co-infusion of lymphocytes and other haematopoietic cells, which can be found in bone marrow, mobilised peripheral blood and umbilical cord blood. The concept of a stem cell was first introduced by Till and McCulloch following their pioneering studies in regeneration of the blood system *in vivo*. (1-3) They observed cellular colonies that formed in the spleens of recipient mice which were transplanted with a limiting number of syngeneic marrow cells. Analysis of these colonies demonstrated that a very small subpopulation of the donor marrow cells had two distinct properties: 1) the ability to self-replicate and 2) the ability to generate multiple types of blood cells. These findings introduced the two defining criteria of stem cells i.e. self-renewal and multi-potency. Self-renewal is the ability to give rise to a HSC itself without differentiation, while multi-potency refers to the ability to differentiate into all functional blood cells. HSCs have the ability to reconstitute normal bone marrow function. HCT can be used for malignant (leukaemia, lymphoma) and non-malignant disorders (inborn errors of immunity (IEI) or primary immunodeficiencies (PID), bone marrow failure, inborn errors of metabolism and haemoglobinopathies) that can be treated by replacing the haematopoietic system with a population of healthy donor cells. A healthy donor derived haematopoietic system is able to correct the diseased bone marrow and cellular immune system.

Bone marrow has been a source of nutrition for many centuries and xeno-transplants were described in an ancient Irish manuscript. (4) It was not until adverse haematological effects of ionizing radiation were known during World War II that the stimulus for bone marrow transplantation was established. (4) Massive radiation exposures provided an opportunity to advance/develop therapies for bone marrow failure syndromes and leukaemia. In 1949, studies found that shielding of the spleen of a mouse during otherwise lethal irradiation, permitted survival. (5) In the 1950s, extensive research in mice had shown that delivery of bone marrow could prevent death following lethal irradiation and could be used to cure murine leukaemia if bone marrow cells were infused following a dose of irradiation that was sufficient to eradicate leukaemic cells. (6-9). HCT was first pioneered in humans by Professor E. Donnall Thomas in 1957, though treatment with irradiation, chemotherapy and bone marrow infusion did not initially lead to either long-term development of donor chimerism or survival. Subsequently, a series of critical studies in mice, dogs and non-human primates that were subjected to high doses of radiation followed by transplantation of marrow grafts led to the understanding of the concepts of histocompatibility, conditioning, graft-versus-leukaemia effects and graft-versus-host disease. (9) Defining the genetic loci controlling acceptance and rejection, initially in mice, later in dogs and sub-human primates, was a

seminal event. The HLA system in humans was defined by Dausset, Benacerraf, Jan van Rood, Paul Teraski, Rose Payne, Felix Rapport and others and this led to the first WHO reference on HLA Nomenclature in 1968.

Advances in defining the HLA system allowed physicians to choose possible bone marrow donors. In 1968, De Koning and colleagues in Leiden, and Robert Good in Minneapolis reported the first successful allogeneic HCT from an HLA-identical sibling of a child with severe combined immunodeficiency (SCID). At the same time, Bach *et al*. reported the first successful in a child with Wiskott-Aldrich syndrome. (10) After the first successful HCT in SCID, HCT was subsequently used to treat severe aplastic anaemia (SAA) and as a rescue therapy for haematological malignancies. HCT has been used to treat haemoglobinopathies and inherited metabolic diseases since the 1980s. During the 1970s, donor selection, control of GvHD, and conditioning regimens became areas of interest in research. In the 1980s and 1990s, there was a rapid increase in the number of transplants performed, national and international bone marrow registries were established, cord blood was recognised as a source of stem cells and T-cell depletion methods were introduced. Over the years, transplant outcomes have improved dramatically and transplant survival using alternative donors, including unrelated donors and haploidentifical donors is now comparable to matched family donors (Figure 1).

This chapter will focus on the principles of HCT in IEI or PID, approach to HCT in IEI (including donor selection, stem cell source, graft engineering, and conditioning), early transplant complications and late effects.

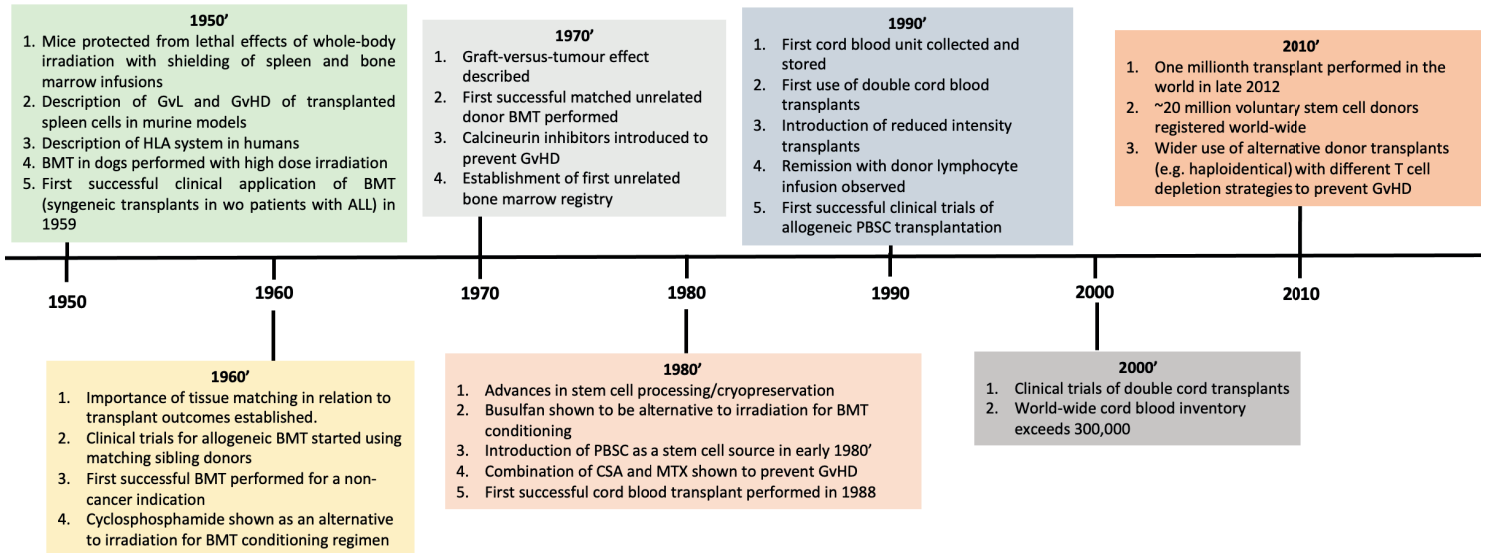

Figure 1: Developmental milestones in allogeneic haematopoietic cell transplantation (HCT)

Abbreviations: BMT: bone marrow transplantation; CSA: ciclosporin; GvHD: graft-versus-host disease; GvL: graft-versus-leukaemia; MTX: methotrexate; PBSC: peripheral blood stem cell

Principles of haematopoietic stem cell transplantation in inborn errors of immunity

Inborn errors of immunity (IEI) or primary immunodeficiencies (PID) comprise a large, heterogeneous group of disorders that result from mutations in genes involved in immune host defence and immunoregulation. Long considered as rare diseases, recent studies show that one in 2000-5000 children younger than 18 years is thought to have a IEI. There are now more than 400 single-gene IEI and the underlying phenotypes are as diverse as infection, allergy, autoimmunity, autoinflammation, lymphoproliferation and malignancy. Therefore, presenting features, severity and age of diagnosis varies immensely. Based on classification which is compiled by International Union of Immunological Societies (IUIS) (11), IEI comprises the following subgroups of conditions:

1. Severe combined immunodeficiency (SCID)
2. Combined immune deficiency (CID)
3. CID with associated features
4. Antibody deficiency
5. Immune dysregulation, including haemophagocytic disorders, lymphoproliferative disorders, autoimmune diseases, and early onset inflammatory bowel disease
6. Phagocytic cell disorders
7. Innate defects

Children born with severe IEI invariably died prematurely until three patients with IEI, two with SCID and one with Wiskott Aldrich syndrome (WAS), were transplanted in 1968. These three patients demonstrated sustained benefit and prolonged cure from a primary genetic defect following HCT. (10, 12, 13) Historically, the utility of HCT in non-SCID IEI was initially limited by high rate of graft failure and transplant-related morbidity and mortality. Transplant survival and graft outcomes in IEI have significantly improved, particularly since 2000. (10, 12) 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 anti-microbial therapy. In the modern era, graft engineering, additional cellular therapy and pharmacokinetic-guided conditioning regimens enable precise personalised transplant care including prescription of graft components, better cell-dosed grafts, and a patient-tailored conditioning regimen. (14-16) Today, haematopoietic cell transplantation (HCT) is a well-recognised and effective

therapy offering a potential cure for many IEI. Developmental millstones of HCT for IEI in Newcastle are summarized in figure 2.

Short-term transplant survival 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. The advances in the understanding the pathological bases of IEI and the general principles of HCT have been crucial for long-term disease outcomes. IEI stemming from defects in the HSCs can be corrected by HCT, but immunological diseases due to thymic stromal or other extra-haematopoietic defects are unlikely to be cured by HCT. The HSCs cannot undergo a functional maturation in the defective thymus and this results in poor immune reconstitution and poor long-term disease outcomes. Therefore, the decision for HCT in non-SCID IEI must take into consideration the risks of HCT and the risks of disease evolution, and must be individualized, not only on the basis of the specific IEI, but also on the characteristics of the individual patient. With regards to late effects of HCT, transplant recipients are at risk of premature onset of chronic health conditions such as malignancy post-transplant, coronary heart disease, and musculoskeletal abnormalities. This is caused by injury of normal tissues by chemotherapy, infection and graft-versus-host disease. Many of the late effects may not manifest for years or even decades after HCT, survivors need ongoing, life-long monitoring. Novel transplant strategies, including minimal toxic conditioning regimens, pharmacokinetic targeted drug doses, graft manipulation and better GvHD prevention, are required to prevent/reduce late effects.

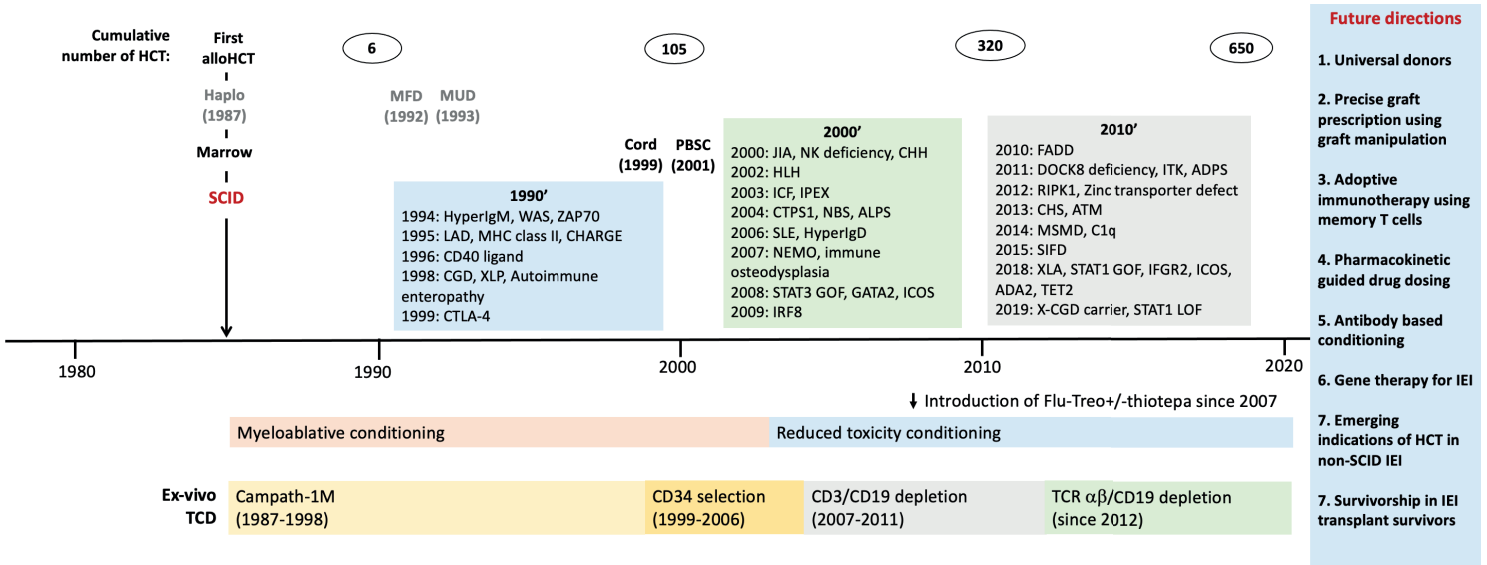

Figure 2: Developmental milestones of haematopoietic cell transplantation for inborn errors of immunity in Newcastle upon Tyne, United Kingdom

Approach to haematopoietic cell transplantation in inborn errors of immunity

HCT is a complex medical procedure which requires careful planning. Outcomes following HCT depend on the underlying disease, the timing of transplant, patient comorbidities, donor selection, and conditioning regimen. Transplant strategies to optimize the transplant outcomes of patients with IEI can be divided into three phrases: (1) pre-transplant phase, (2) transplant phase; and (3) post-transplant phase.

Pre-transplant phase

There are several considerations surrounding HCT for IEI including underlying diagnosis, patient health status, donor options, local transplant centre practice preferences and experience, and family understanding and willingness. A child with suspected IEI should be referred promptly to an expert team for evaluation and confirmation of the diagnosis. The transplant process should be initiated and performed as soon as possible. HCT should be performed as early as possible before the onset of organ damage from multiple infections and inflammation. Younger age at HCT has been consistently shown to be associated with improved survival in children with IEI. Early identification of SCID and non-SCID IEI, for example through a newborn screening programme, and prompt genetic diagnosis with next generation sequencing techniques, have also improved the transplant outcomes.

Patients might require treatment of infections, respiratory support and nutritional rehabilitation to optimize their organ function prior to HCT. A multidisciplinary team with participation of respiratory physicians, gastroenterologists, dietitians, psychologists, play therapists and other specialists is required in all the phases in order to achieve the best outcomes possible.

Transplant phase

This consists of donor selection, appropriate stem cell source and an optimal conditioning regimen.

Donor selection

The selection of a donor is a critical element that influences transplant outcomes. Matching donor and recipient for HLA class I (-A, -B, and -C) and class II (-DRB1 and -DQB1) is a key part of the success of an allogeneic HCT. Techniques for HLA typing and the HLA nomenclature have evolved in parallel with the increasing use of unrelated donors; the discovery of a growing number of new alleles, and improved methods of DNA-based HLA typing. Serological typing was used for antigen matching and detects HLA proteins using serological antibody-based assays. Serologic typing might be used for typing within families, but most centres use intermediate and high resolution nowadays. For unrelated donors, serologic typing alone is insufficient to ensure those donors and recipients share the same HLA genes. Serological typing has largely been replaced by the more specific molecular typing. Molecular typing is used for allele matching, defines HLA genes based on their DNA sequences. It is the preferred method and is required for optimal HLA matching in unrelated donor transplantation. It can differ by resolution. High resolution typing defines sets of alleles that encode the same protein sequence for the HLA molecule's antigen binding site, whilst low resolution typing provides information regarding the allele group, but not the specific HLA protein. (17, 18)

The direction of mismatches may impact patient outcome and can be further classified as being in the "graft-versus-host" direction or the" host-versus-graft" direction. A graft-versus-host direction mismatch means that the patient possesses one or more alleles not present in the donor. In case of such a mismatch, the donor T cells from the graft would be expected to mount an alloreactive response towards the host tissue. A host-versus-graft direction mismatch means that the donor possesses one or more alleles not present in the patient. In such a mismatch, the host T cells would be expected to mount an alloreactive response towards the graft cells. The location of mismatch within an HLA allele may also impact patient outcome. Specific peptide position involved in the HLA mismatch determine whether mismatch is permissive or non-permissive. Pidala *et al.* reported that amino acid substation at peptide-binding pockets of HLA class I molecules increases risk of severe acute GvHD and mortality. (19)

Class II HLA-DPBI has not been involved in donor selection but more attention has been given to HLA-DP haplotype recently. Retrospective analyses looking at the impact of HLA-DP haplotype on transplant outcomes suggested HLA-DP mismatch was not associated with lower survival, but was associated with an increased risk of acute GVHD and a low risk of leukaemic relapse. (20-22) Similar to class I and II HLA loci, An HLA-DP mismatch refers to either a single or a double HLA-DPB1 allele mismatch in the graft-versus-host or host-versus-graft direction, or both. HLA-DP mismatches can also be further divided into permissive or

non-permissive mismatches depending on the haplotypes involved and the direction of the mismatch.

There are several possible donor options: 1) an identical twin (syngeneic); 2) a sibling or matched relative/family donor; 3) unrelated donor; 4) mismatched family or unrelated donors. As with all allogeneic HCT, an HLA-matched family donor (MFD) is considered to be the optimal choice for HCT in IEI. (23) In some diseases such as X-linked chronic granulomatous disease, X-linked carrier family donors should be avoided as they have increased risk inflammatory and autoimmune symptoms and excessive fatigue due to raised serum IL-8. (24-26) As graft-versus-host disease confers no benefit to patients with IEI, a matched sibling or matched family donor usually remains the first choice. If no suitable family donor is available, a search of the national or international unrelated donor registries should be undertaken. Historically, transplants with a MFD have had the best overall survival, and success rates have been lower with alternative donor sources due to graft rejection and GvHD. However, transplant survival and graft outcome have improved dramatically over the past 10 years, due to reduced toxicity conditioning regimens, a detailed graft selection hierarchy, superior HLA matching technology, better cell-dosed grafts, greater availability of grafts, improved supportive care and more effective anti-microbial therapy. As an example, reduced toxicity conditioning in CGD and MHC class II deficiency observed comparable survival between MFD, MUD and haploidentical donors. (27-30) HLA-haploidentifical HCT is increasingly applied in patients lacking a matched donor. Improved ex vivo as well as in vivo T-cell depletion strategies have resulted in improved outcomes with mismatched donor HCT. In several recent studies outcomes with mismatched donors are comparable with matched unrelated donors which clearly has an impact on donor hierarchy.

Stem cell source

The multipotent HSCs required for HCT are usually obtained the bone marrow or peripheral blood or umbilical cord blood of a related or unrelated donor. The optimal source and composition of the HSC product continues to be an area of active clinical investigation. 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 IEI 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.(31) This is an important finding particularly for patients with diseases where a high level of chimerism is required to achieve complete cure. In Newcastle, PBSC is the preferred stem cell source except in matched sibling donors.

Haploidentical donor transplantation

MSD is available in less than 25% of cases and fewer than 70% of remaining patients will have a suitable fully 10/10 matched unrelated donor. This figure is even lower in patients belonging to ethnic groups poorly represented in donor registries. HLA-haploidentical HCT has emerged now as one of the most commonly employed alternative donor option, either using T-deplete or T-replete strategies. Over the past several decades, numerous approaches to HLA-haploidentical HCT have been developed. The three most commonly used approaches are: 1) T cell depletion 2) High dose post-transplantation cyclophosphamide (PTCy) 3) "GIAC" strategy which uses **G**CSF-stimulation of the donor; **I**ntensified immunosuppression post-transplantation; **A**nti-thymocyte globulin added to conditioning to help prevent GvHD and aid engraftment; and **C**ombination of peripheral blood stem cell and bone marrow allografts. The first two methods are more commonly used in primary immunodeficiency worldwide whilst the "GIAC" strategy is used in China and Italy.

Removal of T cells from the graft decreases the risk of GvHD. Early attempts to limit GvHD in haploidentical donor transplants utilizing ex vivo T-cell depletion were generally successful, but recipients were plagued by delayed donor T cell reconstitution and high rates of viral infections. (32) Newer methods of selective T cell depletion, including CD3/CD19 and TCRαβ/CD19 depletion, have observed an overall survival of approximately 80% in children with IEI. (16). The latter method does not eliminate gamma-delta-T cells and natural killer cells from the graft. While graft manipulation is laborious expensive, PTCy is relatively easy to implement and inexpensive and requires no graft manipulation. It was pioneered by the Johns Hopkins group and has been increasingly used in both malignant and non-malignant disorders, predominantly in adults abut increasingly in children as well. PTCY is selectively toxic to proliferating lymphocytes, depletes alloreactive T cells from the donor and the recipient, facilitates engraftment and prevents GvHD. Outcomes using this technique are promising in IEI. Neven *et a.l* reported a 2-year survival of 77.7% in children with IEI and Kurzay *et al.* reported a 2-year OS of 92% and EFS of 77% in children with inborn errors of immunity and metabolism. (33, 34) Hence, parental haploidentical donors with newer methods of T-lymphocyte depletion have emerged as promising alternative donors.(16, 35-37)

Conditioning regimens

The majority of children with IEI undergoing HCT require conditioning therapy for two purposes; 1) myeloablation – to create space for donor stem cells; 2) immunosuppression – to prevent rejection of donor stem cells. The exception is certain infants with SCID who have

limited or no ability to reject the donor graft. However, there has been increasing evidence that conditioning in infants with SCID is associated with better thymopoiesis, donor B-lymphocyte chimerism, cessation of immunoglobulin therapy and normal quality of life. (38) 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 (Figure 3). MAC, consisting of alkylating agents with, or without total body irradiation (TBI), is expected to myeloablate the recipient's haematopoiesis which does not allow for autologous haematological recovery. This aims to prevent rejection by the use of supra-lethal chemotherapy to remove host-versus-graft reactivity 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 is 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 haematopoiesis, and allows relatively rapid autologous haematopoietic recovery without a transplant, but adequately myelosuppresses the recipient to enable at least partial donor progenitor cell engraftment.

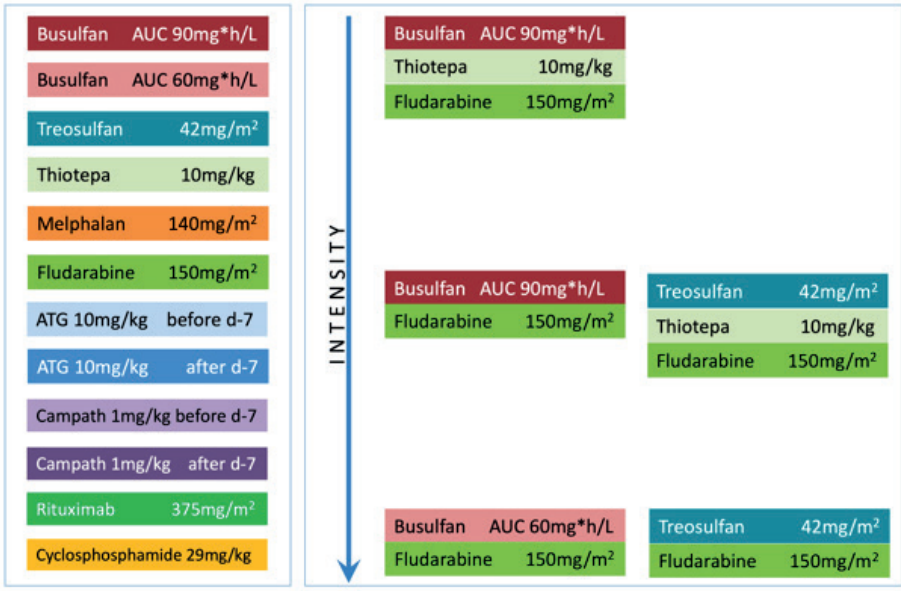

Figure 3: Intensity of conditioning regimen according to chemotherapy, pharmacokinetic guided dosing, timing of serotherapy and combination of chemotherapy, adapted from Lum *et al.* (39)

Myeloablative conditioning regimens in IEI

Historically, conditioning therapy prior to HCT in IEI was based on the combination of alkylators busulfan and cyclophosphamide. However, many children with IEI have significant co-morbidities 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. Whilst initial results may have been acceptable, appreciation of acute conditioning toxicities and recognition of long-term sequelae have increasingly resulted in only few centres now approaching transplantation of IEI patients with conventional myeloablative preparative regimens (Table 1).(36, 40-42)

Table 1: Outcome of HCT in IEI 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
Fischer 1994 (40)	1977-1991	149 non-SCID IEI 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 (36)	1981-1993	19 MHC class II deficiency (7 second HCT)	1.4 (0.5-9.5)	8 MFD marrow 1 MMFD marrow 10 HID marrow All 7 second HCT used HID	MFD Bu20mg/kg + Cy 200mg/kg or Cy 50mg/kg + ALG or Cy 50mg/kg + CCNU 300mg/m^2 + procarbazine 280mg/kg + ALG MMFD Bu 16mg/kg + Cy 200mg/kg or Bu 20mg/kg + Cy 200mg/kg + anti-LFA-1 antibody or Bu 20mg/kg + Cy 200mg/kg + anti-LFA-1 antibody + anti-CD2 antibody	47%

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen	OS
Antoine 2003 (41)	1968-1999	1082 HCT in 919 IEI patients 566 HCT in 475 SCID patients 512 HCT in 444 non-SCID PID patients	SCID: 5.5 mos Non-SCID: 34.6 mos	88% marrow 12% PBSC 0.7% CB T-cell depletion: 91% MD 41% UD marrow	205 SCID: unconditioned 361 SCID: Bu 8mg/kg + Cy 200mg/kg 512 non-SCID; Bu 16mg/kg + cy 200mg/kg	SCID: 77% MD vs 54% in MMD Non-SCID: 71% MFD vs 42% MUD vs 59% MMD
Renella 2006 (42)	1981-2004	15 MHC class II deficiency	1.5 (0.3-5.4)	13 MFD marrow 2 MUD marrow	Bu 16-20mg/kg + Cy 200mg/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; mos: months; 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

Reduced toxicity conditioning (RTC) regimens in IEI

The use of reduced toxicity conditioning regimens is now generally preferred for patients with IEI 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 co-morbidities. Additionally, many patients are infants at the time of transplant and may be more susceptible to toxicity. (43) Less toxic regimens may reduce early and late adverse effects, particularly infertility. (15) There are several reduced toxicity regimens that have been utilised by investigators in IEI (Table 2). (44-46)

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 HCT. In addition to myeloablative properties, it has marked immunosuppressive properties which contribute to the achievement of stable engraftment post-transplant. It causes relatively low organ toxicity compared to the combination of 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. (45, 47-53) Slatter *et al.* first published results of 70 children with IEI who received treosulfan in combination with either cyclophosphamide (n=30) or fludarabine (n=40) with an overall survival of 81% (median follow up 19 months) equivalent in those aged less or greater than one year at time of transplant.(47) Toxicity was low but worse after cyclophosphamide, and T cell chimerism was significantly better after fludarabine. (52) 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 lineage, sufficient to cure disease. (53) 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. (54, 55) A 100-day survival of 94% demonstrated the low toxicity of this regimen making it suitable for patients with IEI 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 GvHD. This highlights the importance of the whole transplant package including stem cell source and serotherapy when tailoring therapy. (31)

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.(51)

Haskologlu *et al.* reported 15 patients with IEI who had a high risk of developing transplant related toxicity due to previous lung and liver damages and were given treosulfan based conditioning.(56) 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 considered 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. (48)

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 paediatric patients. Cyclophosphamide is increasingly being substituted with fludarabine (Flu), a nucleoside analogue with immunosuppressive properties, to provide a less toxic but equally effective regimen.(57-59)

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. 803 had non-malignant disorders including 195 with IEI who received Bu-Cy and 86 who received Bu-Flu. 978 had malignant disorders. Children receiving Bu-Flu for non-malignant conditions experienced less toxicity than those receiving Bu-Cy, but survival was comparable. Children with malignancy had shorter post relapse survival with Bu-Flu than Bu-Cy although transplant-related mortality and relapse were similar. (60)

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). 56 children and young adults with CGD were reported, many of whom had high-risk features such as intractable infections and autoinflammation. 21 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 -99.99) and of EFS was 91% (79.78-16.17). 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. (29) Similar result was demonstrated by Felber *et al.* in 25 patients with haemophagocytic lymphohistiocytosis who received reduced intensity busulfan, fludarabine and alemtuzumab. All patients survived, none had grade III-IV acute GvHD, and 4% had limited chronic GvHD. (61)

Dvorak *et al.* has 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. (58)

Reduced intensity conditioning (RIC) in IEI

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 pre-existing co-morbidities, were striking with significantly improved early survival. (44, 62-65) However, donor chimerism was not always optimal, and there was a high incidence of late viral re-activation, and late onset acute GvHD. Furthermore, toxicities in infants < 1 year of age remained significant. (55) Melphalan in particular has been associated with cardiac toxicities.(66) Good results have been reported for patients with haemophagocytic lymphohistiocytosis. (67) 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. (68) It has been used in adults with IEI with good transplant survival. (63) Whilst 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 IEI

Fludarabine and low-dose TBI. Burroughs *et al.* from the Seattle group reported the transplant outcome of using fludarabine and low-dose TBI in 14 IEI patients with significant pre-existing organ dysfunction and infections. All received post-transplant 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%) (69). 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 IEI. (69, 70)

Antibody-based. Whilst 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.(71-73) 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 haematopoietic progenitors but is absent on non-haematopoietic tissues. Straathof and colleagues studied 16 patients with PID who were less than one year of age or had significant pre-existing co-morbidities and were felt not suitable for conventional reduced intensity conditioning. (54) 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 (YTH24·5 and 54·12) 0·4mg/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 (RIT) is an attractive concept for conditioning of patients with IEI 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 (74). Radioisotopes emitting α, β or γ-radiation of calculated intensity can be brought in direct proximity to the cells of interest. This accounts for malignant cells to be eradicated or benign haematopoietic 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 HCT without an increase in nonrelapse-mortality. Considerable clinical data 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.(74)

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 HCT (75, 76). CD45 is expressed on most AML and ALL blasts as well as on virtually all developing and mature cells of normal haematopoiesis. Radiolabeled anti-CD45 antibody doses up to 43Gy 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. (77) For children, limited published data exists for the use of RIT for pre-transplant 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 X-radiation with a maximum range of 11mm and a half-life of 2.7 days (78). With these qualities, no isolation of the peediatric 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 (74). In order to avoid graft rejection in unrelated or mismatched grafts, recipients received serotherapy with ATG in this setting. 15 of 16 children with non-malignant disease survived the procedure, 13/15 with complete donor chimerism. The Kaplan-Maier 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 haematopoietic stem cells at all stages of development. Interactions with the ligand of CD117, stem cell factor, are crucial for haematopoietic stem cell survival, and this signaling pathway plays a critical role in the homing, adhesion, maintenance, and survival of haematopoietic stem cells in the haematopoietic niche. Pre-clinical studies demonstrated that using an antibody against CD117 to impede CD117-stem cell factor signaling selectively depleted haematopoietic stem cells with no effect on differentiated progenitor or mature cell lineages, and enabled engraftment of donor cells.(79) 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. (80) These preliminary data are extremely exciting and potentially lead the way to a step change in approaches to conditioning in patients with IEI.

Table 2: Outcome of HCT in IEI 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 & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Fludarabine and Treosulfan														
Slatter 2018 (53)	2006 - 2013	**160** SCID: 39 WAS: 20 CGH: 17 HLH 18 Others: 66	1.36 (0.1-18.3)	29 MSD/ MFD 73 MUD 54MMUD 4 HID 49 marrow 70 PBSC 41 CB	Flu 150mg/m^2 + Treo 42g/m^2 (36g/ m^2 if < 1 yr; 30g/m^2 for SCID) + Alemtuzumab 0.3 to 1.0mg/kg GvHD prophylaxis: CSA/MMF	NA	0	IIII-IV: 9	15	2-yr OS: 88 5-yr OS: 78	2-yr ES: 88 5-yr ES: 78	3	4 second HCT	PBSC was associated with better donor chimerism
Morillo-Gutierrez 2016 (50)	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 αβ/ CD19 depleted PBSC 1 CB	46 Flu150mg/m^2 + Treo 42g/m^2 (36g/ m^2 if < 1 yr) Alemtuzumab (n=39) or ATG (n=18) 15 Flu + Treo + TT + alemtuzumab or ATG 9 other Treo-based conditioning regimen GvHD prophylaxis: CSA ± MMF or MTX	17 (IQR 15-35)	0	III-IV: 12	12.8	91.4	81.4	12	8 (2 unconditioned boost; 3 DLI; 5 conditioned 2nd HCT [2 had DLI])	Myeloid ≥ 95%: 80% surviving patients

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Slatter 2015 (47)	2005-2010	**316** 144 PID 39 IMD 70 H-globinopathy 32 histiocytic disorders 24 marrow failure 2 autoimmune disease 5 others	< 1 yr: 95 1-12 yr: 189 >12 yr: 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 150mg/m^2 + Treo 42g/m^2 98 Cy 200mg/kg + Treo 42g/m^2 104 Flu 150mg/m^2 + Treo 42g/m^2 + TT 8mg/kg 104 Flu 150mg/m^2 + Treo 42g/m^2 + Melphalan GvHD prophylaxis: 284 CSA alone 100 CSA + MMF 101 CSA + MTX	NA	0	III-IV: 10	NA	83	76	5.1	NA	57% full donor chimerism 43% stable mixed chimeris,
Burroughs 2014 (45)	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 150mg/m^2 + Treo 42g/m^2 Serotherapy: 22 ATG GvHD prophylaxis: Tacrolimus + MTX	21 (range, 12-46)	0	II-IV: 62 III-IV: 10	21	90	NA	3	2 second 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

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Dinur-Schejter 2015 (48)	2009-2013	44 12 SCID 5 severe congenital neutropenia 5 thalassaemia 2 WAS 2 CGD 10 PID 1 HLH 5 osteopetrosis 3 IMD 4 others	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 (Treo 36g/m^2 for < 1 year; 42g/m^2 for > 1 year) Serotherapy: 9 no serotherapy 26 ATG 8 Alemtuzumab 1 OKT3	Flu/Treo/TT: 18.4 Flu/Treo: 25.3 Cy/Treo: 19.5	3	III-IV: 27	18.9	71	55	14	4 second HCT (one had a further 3rd HCT)	Full: 31 (72%) Mixed: 6 (28%)
Lehmberg 2014 (51)	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	Flu150mg/m^2 (Days-7 to -3) + Treo 42g/m^2 (Days -6 to -4) (36g/m^2 if < 12kg) Alemtuzumab 0.3mg – 1.0mg/kg 14 had additional TT 10mg/kg (Day -3) (7mg/kg if <12kg) GvHD prophylaxis: 2 CSA alone 7 CSA + MMF 9 CSA + MTX 1 Tacrolimus + MMF	20 (range 11-62)	1	III-IV: 1 patient after DLI	no	100	NA	11	2 second HCT (1 1° graft failure after HID; 1 2° graft failure) 7 DLI	WB >95%: 10 WB 75-95%: 2 WB 20-74%: 4

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Beier 2013 (49)	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 had 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	III-IV: 6	6	87	NA	4	NA	Full: 46 (87%)
Slatter 2011 (52)	2006-2009	70 33 SCID 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	30 Flu150mg/m^2 + Treo 42g/m^2 30 Flu150mg/m^2 + Cy 200mg/kg	NA	2	II-IV: 10	6	81 Flu: 85% Cy: 77%	NA	3	1 had top-up and second conditioned HCT	

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Busulfan ± Fludarabine														
Dvorak 2019 (58)	2011-2017	10 4 typical SCID 6 leaky SCID	5 mos (range, 2-108 mos)	2 MUD 2MMUD 6 HID Marrow for MUD/ MMUD CD34 selected PBSC or HID	Bu with target AUC 30 mg*hr/LATG or Alemtuzumab For patients with any T cells: Additional Flu 160mg/m^2 For patients with NK cells: Additional TT 10mg/ kg 2 had plerixafor 9 hours prior to each dose of Bu	16 (range, 14-23)	0	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
Güngör 2015 (26)	2003-2015	56 CGD	12.7 (IQR 6.8-17.3)	21 MSD/ MFD 25 MUD 10MMUD 45 marrow 11 PBSC	Flu 150mg/m^2 Bu with target AUC 45-65 mg*hr/Lxh Serotherapy ATG for MFD Alemtuzumab for MUD	19 (IQR 16-22)	0	III-IV: 4	7	93	89	5	3 second HCT	Myeloid >90%: 52 (93%)
Jacobsohn, 2004 (59)	2000-2004	13 6 PID 4 H-glo-binopathy 3 IMD	5.2 (IQR, 0.6-11.1)	4 MSD 1MMFD 6 MUD 2 unrelated CB 11 PBSC	Flu 150mg/m^2 Bu with target AUC 3800 to 4200umol x min ATG GvHD prophylaxis CSA±MMF	18 (IQR, 14-25)	0	II-IV: 8	25	84	NA	15 (2 H-glo-binopathy)	none	NA

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Fludarabine and Melphalan														
Allen 2018 (62)	2013-2015	34 HLH 12 PID	2.3 (0.4-28)	7 MSD 1 MMRD 25 MUD 13 MMUD All had marrow	Flu 150mg/m^2 Melp 140mg/m^2 Alemtuzumab 1mg/kg GvHD prophylaxis CSA and steroid	13	0	II-IV: 17.4 III-IV: 10.9	26.7	18 month "OS: 66.9%	60.9% with second produces 39.1% without interven-tion	Primary: 4 Secondary: 4	2 second HCT	57% had full chimerism in all cell lines 42% had stable mixed chimerims
Fox 2018 (63)	2004-2014	29 PID	24 (17-50)	11 MFD 13 MUD 5 MMUD	Non-GCD Flu 150mg/m^2 Melph 140mg/m^2 Alemtuzumab 100mg CGD Flu 150mg/m^2 Meph 10mg/m^2 or Bu 9.6mg/kg Alemtuzumab or ATG GvHD prophylaxis CSA	13 (IQR, 11-17)	0	I-II: 45% III-IV: 3%	Limited: 34 Exten-sive: 1	85.2	NA	none	none	85% full chimerism 15% mixed chimerism

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Marsh 2010 (64)	2003-2009	40 HLH	?	7 MFD 33 MUD 36 marrow 2 PBSC 2 CB	26 RIC Flu 150mg/m^2 Melp 140mg/m^2 Alemtuzumab 1mg/kg 14 MAC Bu 14mg/kg Cy 200mg/kg 2 had additional etoposide 30mg/kg GvHD prophylaxis CSA or tacrolimus + steroid/MTX	MAC: 14.5 RIC: 10	NA	II-IV: 14	Limited: 12%	MAC: 43% MIC 89%	NA	?	5 had DIL	MAC: 18% mixed RIC: 65% mixed
Rao 2005 (44)	1998-2001	33 6 SCID 27 non-SCID	5.9 (0.19-18)	22 MUD 11 MMUD marrow	Fludarabine 150mg/m^2 Melphalan 1 40mg/m^2 Alemtuzumab 1mg/kg CSA	13 (range, 8-34)		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
Amrolia 2000 (65)	NA	8 3 SCID 1 XLP/HLH 2 CID 2 CD40 Ligand def.	6.5 (range, 0.75- 18)	2 MSD 6 MUD All had marrow	Flu 150mg/m^2 Melp 140mg/m^2 ALG 10mg/kg GvHD prophylaxis CSA and steroid	13 (range, 9-17)	0	I: 50 II-IV: 0	1 had limited cGvHD	88	NA	1 patient	none	4 had 100% donor chimerism 3 had mixed chimerism

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Fludarabine and low dose TBI														
Burroughs 2010 (70)	NA	2 IPEX	0.75, 16	2 MUD 1 marrow 1 PBSC	Flu 90mg/m^2 TBI 4Gy GvHD prophylaxis CSA and MMF	16, 17	0	2 had grade II	1 severe	Both alive	Both engrafted	none	none	Full immune function and normal FOXP3 protein expression
Burroughs 2007 (69)	1998-2006	14 PID	Range 0.5-30	8 MFD 8 MUD 8 marrow 5 PBSC 1 CB	Flu 90mg/m^2 (n=13) TBI 2Gy (n=14) GvHD prophylaxis CSA and MMF	15 (range 5-23)	0	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

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Antibody-based conditioning														
Schulz 2009 (78)	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 160mg/m^2 Melphalan 70-140mg/m^2 ATG for mismatched donor and unrelated donor	NA	0	II: 36 III-IV: 0	2 limited 3 extended	88	81	1 patient	1	9 had 100% chimerism 2 had mixed chimerism
Straathof 2009 (54)	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 1.6mg/kg (Day -5 to -2) Flu 150mg/m^2 Alemtuzumab 0.3 to 0.6mg/kg GvHD prophylaxis CSA and MMF	9.5 (range 1- 15)	0	II-IV: 38 III-IV: 19	31	81%	95%	3	1 second 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: haemoglobinopathy; HID: haploidentical donor; HLH: haemophagocytic lymphohistiocytosis; IMD: inherited metabolic disease; IQR: interquartile range; MMF: mycophenolate mofetil; MMRD: mismatched related donor; MMUD; mismatched unrelated donor; mos: months; 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; yr: year

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 IEI. Various non-myeloablative conditioning regimens have been used in T-deplete and T-replete haploidentical transplant (Table 3).(16, 81-83) The Great North Children's Hospital (GNCH) group in Newcastle has used fludarabine, treosulfan, ATG (Grafalon) and Rituximab for patients who received CD3 TCR αβ/CD19 depleted peripheral blood stem cells. Patients with non-SCID IEI received additional thiotepa. The overall survival was comparable with matched family and unrelated donor transplant using similar conditioning regimen. (52, 83). *Neven et al.* reported the outcome of Bu-Flu in 22 patients with IEI received haploidentical transplant using post-transplant 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, minimise 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). (84) 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 paediatric 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. Chiesa *et al*. showed that treosulfan AUC (area under the curve) was strongly associated with mortality (high AUC) and to a lesser extent poor engraftment (low AUC). (85)

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. (86)

Serotherapy levels. It is now well recognised 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 zero. (87) Willemsen *et al*. reported that children who received alemtuzumab (n=38) showed a trend to lower risk of acute GvHD but a slower T cell and NK cell reconstitution and higher viral infection, compared to children who received ATG (n=110). (88) Admiraal *et al.* demonstrated that exposure to alemtuzumab is highly variable upon empirical milligram/kilogram dosing due to the non-linear relationship between body weight and alemtuzumab pharmacokinetics. (89)

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 haematological

disorders. They found that ATG Fresenius was cleared rapidly and uniformly from the circulation whether they received 60mg/kg or 45mg/kg but there were significant differences in patients who received a high dose of ATG Genzyme (10mg/kg) who had significantly slower reconstitution for CD3, CD4, and CD8 T cells compared to patients who received a low dose of ATG genzyme (6-8mg/kg) or ATG Fresenius. (90)

Table 3: Outcome of haploidentical donor transplant in IEI 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 & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Fludarabine and Treosulfan														
Neven 2019 (82)	2014-2017	22 IEI 5 osteopetrosis 21 first HCT 6 second HCT	1.5 (0.2-17)	27 HID All marrow	20 MAC with Bu-pk + Flu 160mg/m^2 (4 received additional Cy 28mg/kg) Serotherapy: Rituximab plus alemtuzumab/ATG 7 had RIC (1 first HCT and 6 second HCT) GVHD prophylaxis CSA MMF PTCy 50mg/kg on Day 3 + 4	19 (14-36)	11	II-IV: 48 II: n=10 III: n=2	24.2	77.7	77.7	n=2	1	24 full chimerism 1 mixed chimerism

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
Shah 2018 (16)	2012-2016	25 IEI 3 for refractory GvHD	1.75 (0.28-10.3)	23 HID 2 MMUD TCR αβ/ CD 19 depleted PBSC	Flu 150mg/m^2 Treo 36-42mg/m^2 TT 10mg/kg 24 had serotherapy (ATG/ alemtuzumab) 6 had rituximab 3 SCID: unconditioned GvHD prophylaxis: CSF/MMF	25 (10-27)	0	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)
Rastogi 2017 (81)	2013-2016	8 IEI	4.9 (0.8-12)	7 HID 1 MUD Unmanipulated marrow/ PBSC	5 Flu 160mg/m^2 + Cy 29mg/kg + TBI 2Gy (3 had additional TT) + ATG/Alemtuzumab 2 Flu 160mg/m^2 + Treo 42mg/^{2} 1 Flu 160mg/m^2 + Bu 3.2mg/kg	Mean 17	NA	I-II: 3 patients II-IV: none	2 limited	75	75	none	none	All full donor chimerism

Author Year	Year of HCT	No of patients/ Diagnosis	Median age at HCT (range), years	Donor and stem cell source	Conditioning regimen & GvHD prophylaxis	Median day of N engraftment	VOD %	aGvHD %	cGvHD %	OS %	ES %	Graft failure %	Second procedure, n	Latest donor chimerism/ remarks
					GVHD prophylaxis Tacrolimus MMF PTCy 50mg/kg on Day 3 + 4									
Balashov 2015 (83)	2012-2014	37 IEI 5 SICD 32 non-SCID IEI	2.6 (0.2-17)	27 MUD 10 MMRD TCR αβ/ CD 19 depleted PBSC	Flu 150mg/m^2 Treo 36-42mg/m^2 8 had Melphalan 140mg/m^2 for high risk graft rejection 14 had rituximab 1 unconditioned Serotherapy 35 ATG 2 alemtuzumab	16 (range 11-28)	NA	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; mos: months; MSD: matched sibling donor; MUD: matched unrelated donor; N: neutrophil; NA: not available; OS: overall survival; PID: primary immunodeficiency disease ; RIC: reduced intensity conditioning; SCID: severe combined immunodeficiencies; Treo: Treosulfan; TT: thiotepa; WAS: Wiskott Aldrich syndrome; yr: year

Post-transplant phase

In this phase, the main tasks are supportive care, monitoring of donor graft and immune reconstitution, surveillance of transplant-related complications and rehabilitation of pre-transplant organ damage.

Donor chimerism

After allogeneic HCT, it is important to ascertain whether the newly developed haematopoietic system is of recipient or donor origin. The investigation of genotypic origin of post-HCT haematopoiesis is called chimerism analysis. "Chimera" refers to Greek mythology where Homer described a fire-spitting monster with the head of a lion, the body of a goat and the tail of a serpent terrorizing Lycia, a region in Asia Minor, and which was finally sacrificed by the ancient hero Bellorophon. (91) The term chimerism was first introduced into medicine by Anderson *et al*. who wrote that a chimera is an organism whose cells derive from two or more distinct zygote lineages. In 1956, it was first used in the field of transplantation by Ford. (7) Since chimerism analyses were first performed in HCT, many different methods have been developed, all following the same basic principle using differences in polymorphic genetic markers and their products. These methods include cytogenetics, red cell phenotyping, restriction fragment length polymorphism analysis (RELP) and fluorescence in situ hybridization of sex chromosomes. A major limitation of these methods is that they are time consuming and do not offer the possibility to study all patients. (92) The breakthrough for the clinical applicability came when polymerase chain reaction (PCR) was developed and also utilized for investigation of chimerism. These analyses are mainly performed by amplification of variable number of tandem repeats (VNTR) or by the characterization of short tandem repeat (STR) markers. (93) Analysis of STR is the predominant method for post-HCT monitoring of donor engraftment.

Historically, it was believed that complete donor haematopoiesis is essential to maintain engraftment after allogenic HCT in humans. In the last few decades, it became evident that donor and recipient haematopoiesis could co-exist after HCT in the recipient. This state of coexistence of haematopoietic cells is cell mixed chimerism. Mixed chimerism is often defined as having donor-derived cells that account for less than 95 percent of peripheral blood samples. It is common after non-myeloablative conditioning. Children with most IEI do not require complete donor chimerism for cure of the underlying disease but may develop symptoms of disease at ill-defined low levels of donor chimerism within relevant cell types (generally less than 5 to 20%).

Immune reconstitution

Following administration of chemotherapy with the goals of myeloablation and immunosuppression to prevent graft rejection, there is severe depletion of all haematopoietic cells of immune system. Recovery of the innate and adaptive immune systems occurs gradually during the post-HCT period. Innate immunity usually recovers over the first few months post-HCT whilst complete adaptive immunity reconstitution is often delayed, typically from months to even years, which can be further delayed by type of conditioning and serotherapy, *ex vivo* T cell depletion, GvHD, and treatment for transplant-related complications such as steroids and monoclonal antibodies.

Both non-haematopoietic and haematopoietic compartments of innate immunity are affected by the transplant process. The non-haematopoietic compartment, including physical barriers such as skin and mucosal surfaces, can be damaged by preparative chemotherapy and recovery can be delayed by gut infections and GvHD. Haematopoietic cells of innate immunity include neutrophils, macrophages, dendritic cells, innate lymphoid cells and natural killer cells. The first haematopoietic cells to engraft following HCT are monocytes, followed by neutrophils, platelets and natural killer (NK) cells. Neutrophil number may return to normal within 2 to 4 weeks after HCT, but the function lags behind and only reaches normal levels after 2 to 4 months (94, 95). NK cells are lymphocytes of the innate immune system, of which different subsets can broadly be characterized by expression of CD56 and CD16 in CD3-negative cells. NK cells recover both numerically and functionally within the first few weeks after HCT. (96, 97) Low NK cells in the first weeks after HCT are associated with lower overall survival and higher infection risk. (98) Monocytes may maintain a suboptimal in function for up to one year. (99, 100) Recipient's macrophages are not significantly ablated by the preparative regimen, they are gradually replaced by donor-derived macrophages after the first few months of HCT. (101)

The adaptive immune system is significantly impaired following HCT largely due to absence naïve T cells and reduced function of more differentiated effector T cells. There is a reversal of CD4:CD8 ratio. Recovery of T cell immunity occurs via the peripheral expansion of infused donor memory T cells and/or seeding of the thymus by infused donor haematopoietic progenitors. Development of new T cells from an allogeneic graft typically takes at least 3 months, but recovery of T cell function may begin earlier in patients who have received reduced intensity conditioning. T cell immune reconstitution generally takes 6 to 12 months. It might be delayed in patients who receive serotherapy, steroid and immunosuppressive therapy, or who develop graft versus host disease. Similar to T cells, B cell counts are completely depleted after transplant, but reach normal levels by nine months after HCT. (102)

The timing of immune reconstitution can be influenced by many factors in the transplant process and transplant-related complications, including stem cell source, degree of HLA match, conditioning regimen, graft manipulation and GvHD. A number of strategies have been proposed to fasten immune recovery, including cytokines such as keratinocyte growth factors (KGF), IL-7, Flt-3 ligand, growth hormone, and cellular therapies using memory T cells. (103-108)

Early transplant complications

Patients who undergo HCT are subject to numerous complications in the first year after HCT. Early complications include infections, toxicities from cytoreductive chemotherapy, graft failure, GvHD and post-transplant lymphoproliferative disease. (Table 5) These complications can affect many organ systems and they may influence the quality of life, duration of hospitalizations, longer-term complications, and HCT outcomes. In the context of HCT for IEI, factors that may contribute to early HCT complications include comorbid medical conditions (e.g. infection, nutritional status, organ function), history of cancer, conditioning regimen, immunosuppressive therapy, duration and degree of cytopenias and severity of GvHD.

Infection is one of the most common causes of mortality of after HCT. All patients who undergo allogeneic HCT are at risk of bacterial, fungal, and viral infections. Appropriate prophylactic antimicrobial therapy should be given during HCT. Viral screening and pre-emptive therapy are essential to reduce the severity of viral infection and organ damage. In IEI, all patients should be thoroughly evaluated for infections prior to transplant. Some IEI patients are at risk of particular infections. For examples, patients with chronic granulomatous disease (CGD) may have refractory fungal or bacterial infection and patients with interferon gamma receptor deficiencies may have atypical mycobacterial infections. Cryptosporidium is common in patients with CD40 ligand deficiency and DOCK8 deficiency. Pulmonary infection is frequent in IEI and some patients may have co-infection with several different pathogens. In Newcastle, all patients will undergo bronchoalveolar lavage (BAL) at the time of insertion of a central venous catheter before starting preparative chemotherapy. The BAL specimens are tested for viruses, bacterias, fungi and mycobacteria prior to transplant. In Slatter's report, of 69 patients who had a BAL, 39 (57%) were symptomatic and 30 (43%) were asymptomatic. Of 39 symptomatic patients, 20 (51%) had pathogens identified: 11 had *Pneumocystis jiroveci* (PCP) (3 had viral co-pathogens) and 9 had a virus and/or bacteria. In 30 asymptomatic patients, 6 (20%) had pathogens identified: 1 had PCP and 5 had bacteria. This study showed that routine BAL was safe in IEI patients about to undergo HCT and led to management changes. (109)

Chemotherapy related toxicities can be varied according to the intensity of conditioning regimens. An expected complication of cytoreductive chemotherapy is myelosuppression. Patients may experience symptoms from cytopenia such as anaemia, bleeding and infections related to neutropenia. Blood and platelet transfusions are required during the period of aplasia. Mucositis may result in pain and diarrhoea. Patients may require enteral supplementation via nasogastric tube or parenteral nutrition. Hepatic veno-occlusive disease is less common with reduced toxicity conditioning.

Graft failure

Graft failure is a serious complication of HCT. It is defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure). It may be due to rejection caused by recipient T-cells, NK cells or antibodies. The risk factors for graft failure include HLA-mismatched grafts, unrelated donor grafts, T-cell depleted transplants, sensitized patients from multiple transfusions and in patients treated with a reduced intensity conditioning regimen.

Graft-versus-host disease

Despite advances made in the various aspects of HCT, graft-versus-host disease (GvHD) remains a leading cause of morbidity and non-relapse mortality in patients after HCT. GvHD occurs when immune cells from the donor's graft recognize the recipient tissue as foreign, thereby initiating an immune reaction that causes disease in the recipient. GvHD has been classically divided into acute and chronic based upon the time of onset using a cutoff of 100 days. However, this conventional classification has been challenged by the recognition of signs of acute and chronic GvHD which may occur outside of these designated periods. This observation has led to the increased use of clinical findings, rather than a set time period, to differentiate between acute and chronic GvHD. (110)

The incidence of grade II-IV aGvHD in children ranges from 40%-85%, depending on patient factors, HLA-matching, conditioning regimen, in vivo and *ex vivo* T-cell depletion. (111)Acute GvHD (aGvHD) mainly affects skin, gut and liver. Acute GvHD is mediated by mature donor stem cell derived, donor thymus tolerized T-lymphocytes that recognize and attack disparate host antigens resulting in harmful inflammatory responses. The most important targets are the HLAs, encoded by major histocompatibility complex (MHC) located on the short arm of chromosome 6, which play a key role in tissue histocompatibility and T-lymphocyte recognition. The degree of MHC mismatch between donor and recipient is the most important determinant of aGvHD, most importantly at the HLA-A, -B, C and DRBI loci. However, even

in the setting of an HLA identical family HCT, alloreactivity can still occur due to mismatches between minor histocompatibility antigens.

Chronic GvHD (cGvHD) can occur after previous or ongoing aGvHD or in patients without a history of aGvHD. The skin, liver, gut, and lungs are the principal target organs involved in patients with cGvHD, but it can affect any other systems. The presenting symptoms of cGvHD are in some ways similar to those found in other well-established autoimmune syndromes, but there is no uniform presentation in cGvHD. This variation makes clinical therapeutic studies difficult

Transplant-associated thrombotic microangiopathy (TMA)

TMA is now recognised as a severe complication of HCT. It is a generalized endothelial dysfunction that leads to microangiopathic haemolytic anaemia, intravascular platelet activation, and formation of platelet-rich thrombi within the microcirculation, leading to organ failure. (112) It significantly affects patient's transplant outcomes and well-being. If not promptly diagnosed and treated, TMA can lead to significant morbidity such as permanent renal injury or mortality. The incidence of TMA is poorly defined, but likely ranges from 10 to 35%, this wide range of reported incidence is due to various definitions of syndrome in assorted studies (113) Multiple factors, such as chemotherapy, mismatched donors, viral infections, calcineurin inhibitors and GVHD have been implicated as TMA triggers. An analysis of risk factors for the development of TMA lends support to a "Three-Hit Hypothesis". First, patients with either an underlying predisposition to complement activation (e.g. racial or genetic) or a pre-existing endothelial injury (e.g. prior myeloablative HCT or prolonged calcineurin inhibitors use), are subject to further endothelial injury, mediated by a toxic conditioning regimen. Then, a third hit occurs which perpetuates continued endothelial injury which triggers activation of the complement system. This can be caused by medications, alloreactivity, infections and antibody mediated (114, 115)

While the hallmark of TMA is kidney involvement, it is important to recognize that other organs may also be compromised in TMA, especially the gastrointestinal tract (manifested as abdominal pain, nausea, bleeding, and ileus), serosal surface (manifested as pericardial and pleural effusion), the cardiopulmonary system (manifested as pulmonary arterial hypertension), and the neurological system (manifested as confusion, headaches, and seizures). The most reliable modality to diagnose TA-TMA is histological, but tissue biopsies in ill recipients with HCT carry significant risks. Clinical TMA diagnosis in recipients with HCT requires a high index of suspicion. Laboratory and clinical markers include: 1) LDH above normal for age; 2) schistocytes on blood smear; 3) de novo thrombocytopenia or increased

transfusion requirements; 4) de novo anaemia or increased transfusion requirements; 5) hypertension; 6) proteinuria ≥ 30mg/dL; 7) terminal complement activation, sC5b9. Presence of 5 of 7 markers are suggestive of TMA. (116, 117) Early therapy is predicted to result in improved survival and long-term outcomes. Therapy for TMA can broadly be classified into adjunctive treatment and specific therapy. Adjunctive treatment includes anti-hypertensives, withdrawal of ciclosporin, treatment of infection and control of GvHD. Various therapies have been considered for TMA, including eculizumab and defibrotide. Dvorak *et al*. has proposed potential approaches to TMA prophylaxis which include: 1) improving baseline endothelial health, e.g. vitamin D and eicosapenthaenoic acid (EPA); 2) limiting/repairing endothelial injury from conditioning e.g. individualized dosing, allopurinol, defibrotide, statins and N-acetly-L-cystine; 3) limiting the "Third-hit", e.g. calcineurin inhibitor free GvHD prophylaxis (such as ex vivo T cell depletion), improved GvHD prophylaxis regimens, improved antimicrobial prophylaxis and prophylactic rituximab. (114)

Table 5: Early transplant complications according to organ system

Organ system	Early complications	Contributing factors
Haematology	Cytopenias Bleeding	Chemotherapy
Infection	Bacteria, virus, fungus	Chemotherapy, serotherapy, T-cell depletion
Pulmonary	Pre-engraftment: infection, pulmonary oedema, engraftment syndrome Post-engraftment: infection, idiopathic pneumonia syndrome, diffuse alveolar haemorrhage, bronchiolitis obliterans	Drug toxicity and radiation
Gut	Mucositis, vomiting, diarrhoea	Chemotherapy, infection, graft-versus-host disease, cord colitis syndrome
Liver	Deranged liver enzyme Veno-occlusive disease	Chemotherapy Pre-existing liver diseae
Renal	Hypertension Acute kidney injury	Drugs e.g. ciclosporin, tacrolimus, steroid, amphotericin B
Neurology	Seizure, infection	chemotherapy
Multi-organ involvement	Transplant associated microangiopathy	Chemotherapy, infection, calcineurin inhibitors, GvHD

Late effects after haematopoietic cell transplantation for primary immunodeficiency

In the last several decades, HCT has become accepted as a standard-of-care treatment modality for an increasing number of diseased haematopoietic and immune systems, but HCT is associated with substantial toxicities. Advances in patient and donor selection, preparative regimen and supportive care have led to improved survival. As more children survive HCT, the price of survival has become increasingly apparent in the protean manifestations of late effects of HCT. Therefore, even patients who are cured of their underlying disease may not achieve full restoration of health after HCT. Late complications, those manifest beyond one year after HCT, can affect many organ systems, persist for the remainder of the affected child's life, impair quality of life and increase mortality. Some late complications (e.g. cGvHD) may have begun earlier, while others (e.g. malignancy post-HCT) are first diagnosed years after HCT. Some of the late complications (e.g. bronchiolitis obliterans, end-stage renal failure) contribute to late mortality, while others (e.g. avascular necrosis, dry mouth) may lead to substantial morbidity and impaired quality of life, but do not reduce transplant survival. Table 6 summarizes the late complications according to organ system.

Advances in HCT have led to an increasing number of transplant survivors. Based on a CIBMTR data and analysis, the number of HCT survivors is estimated to increase by five times by the year 2030 (502, 000 survivors) in the US. (118) Data on late complications in children with IEI are sparse, they differ from complications in children who have HCT for cancer, as children with IEI are not exposed to chemotherapy and/or radiation before HCT for management of primary cancer. Very little is known about late complications following reduced intensity conditioning regimen for IEI. (119)

Table 6 Late effects after HCT

Organ system	Late complications	Risk factors	Recommendations (119,120)
Quality of life	Functional impairment: Family, social Emotional, relationships Education, employment Sexual relationships	Any treatment Chronic transplant complications	Routine evaluation needed even in absence of overt symptoms
Neurocognitive	Cognitive impairment	Pre-transplant therapy with methotrexate (systemic or intrathecal) TBI Busulfan Younger age <3 at treatment Female	Routine evaluation needed even in absence of overt symptoms
Infection	Various infections	Type of transplant, cGvHD, asplenia, delayed immune reconstitution	Antibiotic prophylaxis in high risk patients e.g. asplenia, TBI
Eyes	Anterior segment: Cataract, keratoconjunctivitis sicca syndrome, may lead to corneal/conjunctival ulceration/scarring Posterior segment: Chorioretinitis	TBI Steroid therapy cGVHD	Regular assessment at follow-up Enquire regarding visual impairment, tear production, dry or painful eyes, photophobia Examine for signs of posterior subcapsular cataract or complications of lacrimal gland atrophy (i.e. corneal ulceration of scarring) Early referral to ophthalmologist
Auditory	Sensorineural hearing impairment Impaired speech development	Pre-transplant therapy with platinum agents and RT to fields including ears Aminoglycosides Younger age	Routine evaluation needed even in absence of overt symptoms

Organ system	Late complications	Risk factors	Recommendations (119,120)
Oral/Dental and craniofacial	Oral Reduced saliva ® xerostomia, difficult in mastication and swallowing Lichenoid lesions/leukoplakia Fibrosis of salivary gland Oral/salivary gland tumours Craniofacial/dental Impaired craniofacial skeletal growth Dental abnormalities, including root, enamel Dental caries, gingivitis Abnormal development of teeth (tooth agenesis, hypodontia, microdontia, enamel hypoplasia, malformed roots, taurodontia), delayed eruption, over-retention of primary teeth	Chemotherapy Radiotherapy Younger age at HCT (121)	Regular dental care
Cardiovascular	Myocardial toxicity ECG abnormalities Pericardia disease Coronary artery disease Valvular disease	Pre-transplant therapy (anthracyclines and exposure to radiation) High dose cyclophosphamide TBI Pre-/peri-HCT iron overload (thalassaemia, aplastic anaemia)	Regular assessment at long-term follow-up clinic Enquire regarding exercise tolerance, chest pain, palpitations, shortness of breath Measure blood pressure Echocardiography
Metabolic syndrome	Central obesity, insulin resistance, glucose intolerance, dyslipidaemia, hypertension	TBI cGVHD	Regular assessment at long-term follow-up clinic

Organ system	Late complications	Risk factors	Recommendations (119,120)
Pulmonary	Bronchiolitis obliterans, pulmonary hypertension	cGvHD, TBI, chemotherapy, infection, inflammatory pneumonitides	Regular assessment at long-term follow-up clinic Lung function test in selected patients
Hepatic	Iron overload, hepatitis	cGvHD, infection, iron overload, medications	Regular assessment at long-term follow-up clinic Venesection for patients with high serum ferritn
Renal	Hypertension, chronic kidney disease	Prior kidney disease, radiation injury, calcineurin inhibitors	Regular assessment at long-term follow-up clinic
Neurology	Leukoencephalopathy Vasculopathy – CVAs, vasculitis, "migraine-lie" episodes CNS infections Benign and malignant CNS tumours Neuropathy	Pre-transplant therapy with methotrexate (systemic or intrathecal) TBI cGVHD IST – thalidomide (neuropathy) Prolonged IST (CNS infection)	Routine evaluation needed even in absence of overt symptoms
Endocrine	Hypothalamic pituitary axis: growth hormone deficiency, attenuated pubertal growth spurt, early puberty, delayed puberty, multiple pituitary hormone deficiency	Radiotherapy Younger age at HCT Iron overload	Measure and chart height and weight at least six monthly until growth complete Measure sitting height at some time as height and weight if possible Pubertal staging at least six monthly Regular bone in recipients of TBI
	Thyroid dysfunction	Radiotherapy Busulfan-based conditioning	Annual TSH and T4 Neck examination for thyroid swelling
	Hypoadrenalism	Prolonged steroid use	Assessment for patients at risk
	Primary gonadal failure	Radiotherapy Alkylating agents	Pubertal staging at least six monthly Gonadal hormone when appropriate Semen analysis when appropriate

Organ system	Late complications	Risk factors	Recommendations (119,120)
Bone and joints	Osteoporosis, avascular necrosis		Regular assessment at long-term follow-up clinic DEXA scan in selected patients
Autoimmunity	Immune cytopenia, non-haematological autoimmune disease	Alemtuzumab, GvHD	Clinical evaluation Full blood count
Malignancy	Post-transplant lymphoproliferative disease AML/MDS Solid tumours	T cell depletion, alkylating agents, Topoisomerase II inhibitors, immunosuppressive treatment, young age, cGVHD	Routine evaluation needed even in absence of overt symptoms

Aims and outline of this thesis

This thesis examines the clinical and immunological outcome after paediatric stem cell transplantation in inherited immune disorders. First part (chapter 1) reviews the general principles of HCT in IEI.

The second part (chapter 2-4) of this thesis focuses on practice pattern changes over the past three decades in the field of transplant for non-SCID IEI. Chapter 2 focuses on transplant survival and its predictors in children with chronic granulomatous disease. In Chapter 3 we report the clinical features, transplant survival and long-term disease outcome in children with MHC class II deficiency. Chapter 4 we describe the role of HCT in monogenic autoimmune diseases.

The third part (chapter 5-6) of this thesis analyses the outcome after HCT in IEI using ex-vivo T cell depleted mismatched grafts. Over the past three decades, several methods of facilitating HLA-haploidentical donor transplant using ex-vivo T cell depletion have been developed. Chapter 5 examines the outcomes according to various T cell depletion methods. Chapter 6 compares the HCT outcomes between T deplete HLA-mismatched grafts and T-replete HLA-matched family/unrelated grafts.

Part 4 focuses on late effects of HCT. Chapter 7 reports the incidence, risk factors and outcome of autoimmune cytopenia (AIC) after HCT for IEI. Chapter 8 examines the incidence and risk factors of non-haematological autoimmunity post-HCT in IEI. Chapter 9 reviews current understanding and incidence of malignancy post-HCT in IEI.

Finally, chapters 10 and 11 contains the summary of the thesis and explores the potential future directions of research into the diversity in the use of HCT in IEI.

References

1. Till JE, Mc CE. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res. 1961;14:213-22.
2. Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature. 1963;197:452-4.
3. Till JE, Mc CE. Early repair processes in marrow cells irradiated and proliferating in vivo. Radiat Res. 1963;18:96-105.
4. McCann S, Gale RP. A brief history of bone marrow transplantation. Advances in Cell and Gene Therapy. 2018;1(1):e8.
5. Jacobson LO, Marks, E.K., Robson, M.J., Gaston, E.O., Zinckel, R.E. Effect of spleen protection on mortality following x□irradiation. Journal of Laboratory and Clinical Medicine. 1949;34:1538-42.
6. Lorenz E, Uphoff D, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J Natl Cancer Inst. 1951;12(1):197-201.
7. Ford CE, Hamerton JL, Barnes DW, Loutit JF. Cytological identification of radiation-chimaeras. Nature. 1956;177(4506):452-4.
8. Nowell PC, Cole LJ, Habermeyer JG, Roan PL. Growth and continued function of rat marrow cells in x-radiated mice. Cancer Res. 1956;16(3):258-61.
9. Thomas ED. A history of haemopoietic cell transplantation. BJH. 2005;105(2):330-9.
10. Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet. 1968;2(7583):1364-6.
11. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol. 2020;40(1):66-81.
12. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968;2(7583):1366-9.
13. De Koning J, Van Bekkum DW, Dicke KA, Dooren LJ, Radl J, Van Rood JJ. Transplantation of bone-marrow cells and fetal thymus in an infant with lymphopenic immunological deficiency. Lancet. 1969;1(7608):1223-7.
14. Slatter MA, Gennery AR. Hematopoietic cell transplantation in primary immunodeficiency - conventional and emerging indications. Expert Rev Clin Immunol. 2018;14(2):103-14.
15. Slatter MA, Gennery AR. Advances in hematopoietic stem cell transplantation for primary immunodeficiency. Expert Rev Clin Immunol. 2013;9(10):991-9.
16. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, et al. T-cell receptor alphabeta(+) and CD19(+) cell-depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J Allergy Clin Immunol. 2018;141(4):1417-26 e1.
17. Edgerly CH, Weimer ET. The Past, Present, and Future of HLA Typing in Transplantation. Methods Mol Biol. 2018;1802:1-10.
18. Erlich H. HLA DNA typing: past, present, and future. Tissue Antigens. 2012;80(1):1-11.
19. Pidala J, Wang T, Haagenson M, Spellman SR, Askar M, Battiwalla M, et al. Amino acid substitution at peptide-binding pockets of HLA class I molecules increases risk of severe acute GVHD and mortality. Blood. 2013;122(22):3651-8.
20. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110(13):4576-83.

21. Morishima Y, Kashiwase K, Matsuo K, Azuma F, Morishima S, Onizuka M, et al. Biological significance of HLA locus matching in unrelated donor bone marrow transplantation. Blood. 2015;125(7):1189-97.
22. Shaw BE, Gooley TA, Malkki M, Madrigal JA, Begovich AB, Horowitz MM, et al. The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation. Blood. 2007;110(13):4560-6.
23. Howard CA, Fernandez-Vina MA, Appelbaum FR, Confer DL, Devine SM, Horowitz MM, et al. Recommendations for donor human leukocyte antigen assessment and matching for allogeneic stem cell transplantation: consensus opinion of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant. 2015;21(1):4-7.
24. Battersby AC, Martin AJ, Tarn J, Ng FW, Cale CM, Goldblatt D, et al. Raised Serum IL-8 Levels Are Associated with Excessive Fatigue in Female Carriers of X-Linked Chronic Granulomatous Disease in the UK. J Clin Immunol. 2017;37(3):279-81.
25. Battersby AC, Braggins H, Pearce MS, Cale CM, Burns SO, Hackett S, et al. Inflammatory and autoimmune manifestations in X-linked carriers of chronic granulomatous disease in the United Kingdom. J Allergy Clin Immunol. 2017;140(2):628-30 e6.
26. Marciano BE, Zerbe CS, Falcone EL, Ding L, DeRavin SS, Daub J, et al. X-linked carriers of chronic granulomatous disease: Illness, lyonization, and stability. J Allergy Clin Immunol. 2018;141(1):365-71.
27. Lum SH, Flood T, Hambleton S, McNaughton P, Watson H, Abinun M, et al. Two decades of excellent transplant survival for chronic granulomatous disease: a supraregional immunology transplant center report. Blood. 2019;133(23):2546-9.
28. Lum SH, Anderson C, McNaughton P, Engelhardt KR, MacKenzie B, Watson H, et al. Improved transplant survival and long-term disease outcome in children with MHC class II deficiency. Blood. 2020;135(12):954-73.
29. Gungor T, Teira P, Slatter M, Stussi G, Stepensky P, Moshous D, et al. Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. Lancet. 2014;383(9915):436-48.
30. Chiesa R, Wang J, Blok HJ, Hazelaar S, Neven B, Moshous D, et al. Haematopoietic Cell Transplantation in Chronic Granulomatous Disease: a Study on 712 Children and Adults. Blood. 2020.
31. Unni MNM, Elfeky R, Rao K, Nademi Z, Chiesa R, Amrolia P, et al. Non-posttransplant lymphoproliferative disorder malignancy after hematopoietic stem cell transplantation in patients with primary immunodeficiency: UK experience. J Allergy Clin Immunol. 2018;141(6):2319-21 e1.
32. Or-Geva N, Reisner Y. The evolution of T-cell depletion in haploidentical stem-cell transplantation. Br J Haematol. 2016;172(5):667-84.
33. Neven B, Diana JS, Castelle M, Magnani A, Rosain J, Touzot F, et al. Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children. Biol Blood Marrow Transplant. 2019;25(7):1363-73.
34. Kurzay M, Hauck F, Schmid I, Wiebking V, Eichinger A, Jung E, et al. T-cell replete haploidentical bone marrow transplantation and post-transplant cyclophosphamide for patients with inborn errors. Haematologica. 2019;104(10):e478-e82.
35. Ouederni M, Vincent QB, Frange P, Touzot F, Scerra S, Bejaoui M, et al. Major histocompatibility complex class II expression deficiency caused by a RFXANK founder mutation: a survey of 35 patients. Blood. 2011;118(19):5108-18.
36. Klein C, Cavazzana-Calvo M, Le Deist F, Jabado N, Benkerrou M, Blanche S, et al. Bone marrow transplantation in major histocompatibility complex class II deficiency: a single-center study of 19 patients. Blood. 1995;85(2):580-7.

37. Small TN, Qasim W, Friedrich W, Chiesa R, Bleesing JJ, Scurlock A, et al. Alternative donor SCT for the treatment of MHC class II deficiency. Bone Marrow Transplant. 2013;48(2):226-32.
38. Abd Hamid IJ, Slatter MA, McKendrick F, Pearce MS, Gennery AR. Long-term outcome of hematopoietic stem cell transplantation for IL2RG/JAK3 SCID: a cohort report. Blood. 2017;129(15):2198-201.
39. Lum SH, Hoenig M, Gennery AR, Slatter MA. Conditioning Regimens for Hematopoietic Cell Transplantation in Primary Immunodeficiency. Curr Allergy Asthma Rep. 2019;19(11):52.
40. Fischer A, Landais P, Friedrich W, Gerritsen B, Fasth A, Porta F, et al. Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. Blood. 1994;83(4):1149-54.
41. Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet. 2003;361(9357):553-60.
42. Renella R, Picard C, Neven B, Ouachee-Chardin M, Casanova JL, Le Deist F, et al. Human leucocyte antigen-identical haematopoietic stem cell transplantation in major histocompatiblity complex class II immunodeficiency: reduced survival correlates with an increased incidence of acute graft-versus-host disease and pre-existing viral infections. Br J Haematol. 2006;134(5):510-6.
43. Malar R, Sjoo F, Rentsch K, Hassan M, Gungor T. Therapeutic drug monitoring is essential for intravenous busulfan therapy in pediatric hematopoietic stem cell recipients. Pediatr Transplant. 2011;15(6):580-8.
44. Rao K, Amrolia PJ, Jones A, Cale CM, Naik P, King D, et al. Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. Blood. 2005;105(2):879-85.
45. Burroughs LM, Nemecek ER, Torgerson TR, Storer BE, Talano JA, Domm J, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. Biol Blood Marrow Transplant. 2014;20(12):1996-2003.
46. Chiesa R, Veys P. Reduced-intensity conditioning for allogeneic stem cell transplant in primary immune deficiencies. Expert Rev Clin Immunol. 2012;8(3):255-66; quiz 67.
47. Slatter MA, Boztug H, Potschger U, Sykora KW, Lankester A, Yaniv I, et al. Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases. Bone Marrow Transplant. 2015;50(12):1536-41.
48. Dinur-Schejter Y, Krauss AC, Erlich O, Gorelik N, Yahel A, Porat I, et al. Bone marrow transplantation for non-malignant diseases using treosulfan-based conditioning. Pediatric blood & cancer. 2015;62(2):299-304.
49. Beier R, Schulz A, Honig M, Eyrich M, Schlegel PG, Holter W, et al. Long-term follow-up of children conditioned with Treosulfan: German and Austrian experience. Bone Marrow Transplant. 2013;48(4):491-501.
50. Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, et al. Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience. Blood. 2016;128(3):440-8.
51. Lehmberg K, Albert MH, Beier R, Beutel K, Gruhn B, Kroger N, et al. Treosulfan-based conditioning regimen for children and adolescents with hemophagocytic lymphohistiocytosis. Haematologica. 2014;99(1):180-4.
52. Slatter MA, Rao K, Amrolia P, Flood T, Abinun M, Hambleton S, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. Blood. 2011;117(16):4367-75.

53. Slatter MA, Rao K, Abd Hamid IJ, Nademi Z, Chiesa R, Elfeky R, et al. Treosulfan and Fludarabine Conditioning for Hematopoietic Stem Cell Transplantation in Children with Primary Immunodeficiency: UK Experience. Biol Blood Marrow Transplant. 2018;24(3):529-36.
54. Straathof KC, Rao K, Eyrich M, Hale G, Bird P, Berrie E, et al. Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study. Lancet. 2009;374(9693):912-20.
55. Ritchie DS, Morton J, Szer J, Roberts AW, Durrant S, Shuttleworth P, et al. Graft-versus-host disease, donor chimerism, and organ toxicity in stem cell transplantation after conditioning with fludarabine and melphalan. Biol Blood Marrow Transplant. 2003;9(7):435-42.
56. Haskologlu S, Kostel Bal S, Islamoglu C, Altun D, Kendirli T, Dogu EF, et al. Outcome of treosulfan-based reduced-toxicity conditioning regimens for HSCT in high-risk patients with primary immune deficiencies. Pediatr Transplant. 2018;22(7):e13266.
57. Ben-Barouch S, Cohen O, Vidal L, Avivi I, Ram R. Busulfan fludarabine vs busulfan cyclophosphamide as a preparative regimen before allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. Bone Marrow Transplant. 2016;51(2):232-40.
58. Dvorak CC, Long-Boyle J, Dara J, Melton A, Shimano KA, Huang JN, et al. Low Exposure Busulfan Conditioning to Achieve Sufficient Multilineage Chimerism in Patients with Severe Combined Immunodeficiency. Biol Blood Marrow Transplant. 2019.
59. Jacobsohn DA, Duerst R, Tse W, Kletzel M. Reduced intensity haemopoietic stem-cell transplantation for treatment of non-malignant diseases in children. Lancet. 2004;364(9429):156-62.
60. Harris AC, Boelens JJ, Ahn KW, Fei M, Abraham A, Artz A, et al. Comparison of pediatric allogeneic transplant outcomes using myeloablative busulfan with cyclophosphamide or fludarabine. Blood Adv. 2018;2(11):1198-206.
61. Felber M, Steward CG, Kentouche K, Fasth A, Wynn RF, Zeilhofer U, et al. Targeted busulfan-based reduced-intensity conditioning and HLA-matched HSCT cure hemophagocytic lymphohistiocytosis. Blood Adv. 2020;4(9):1998-2010.
62. Allen CE, Marsh R, Dawson P, Bollard CM, Shenoy S, Roehrs P, et al. Reduced-intensity conditioning for hematopoietic cell transplant for HLH and primary immune deficiencies. Blood. 2018;132(13):1438-51.
63. Fox TA, Chakraverty R, Burns S, Carpenter B, Thomson K, Lowe D, et al. Successful outcome following allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency. Blood. 2018;131(8):917-31.
64. Marsh RA, Vaughn G, Kim MO, Li D, Jodele S, Joshi S, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. Blood. 2010;116(26):5824-31.
65. Amrolia P, Gaspar HB, Hassan A, Webb D, Jones A, Sturt N, et al. Nonmyeloablative stem cell transplantation for congenital immunodeficiencies. Blood. 2000;96(4):1239-46.
66. Ritchie DS, Seymour JF, Roberts AW, Szer J, Grigg AP. Acute left ventricular failure following melphalan and fludarabine conditioning. Bone Marrow Transplant. 2001;28(1):101-3.
67. Marsh RA, Bleesing JJ, Chandrakasan S, Jordan MB, Davies SM, Filipovich AH. Reduced-intensity conditioning hematopoietic cell transplantation is an effective treatment for patients with SLAM-associated protein deficiency/X-linked lymphoproliferative disease type 1. Biol Blood Marrow Transplant. 2014;20(10):1641-5.
68. Ono S, Okano T, Hoshino A, Yanagimachi M, Hamamoto K, Nakazawa Y, et al. Hematopoietic Stem Cell Transplantation for XIAP Deficiency in Japan. J Clin Immunol. 2017;37(1):85-91.

69. Burroughs LM, Storb R, Leisenring WM, Pulsipher MA, Loken MR, Torgerson TR, et al. Intensive postgrafting immune suppression combined with nonmyeloablative conditioning for transplantation of HLA-identical hematopoietic cell grafts: results of a pilot study for treatment of primary immunodeficiency disorders. Bone Marrow Transplant. 2007;40(7):633-42.
70. Burroughs LM, Torgerson TR, Storb R, Carpenter PA, Rawlings DJ, Sanders J, et al. Stable hematopoietic cell engraftment after low-intensity nonmyeloablative conditioning in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. J Allergy Clin Immunol. 2010;126(5):1000-5.
71. Schuetz C, Neven B, Dvorak CC, Leroy S, Ege MJ, Pannicke U, et al. SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID. Blood. 2014;123(2):281-9.
72. Slack J, Albert MH, Balashov D, Belohradsky BH, Bertaina A, Bleesing J, et al. Outcome of hematopoietic cell transplantation for DNA double-strand break repair disorders. J Allergy Clin Immunol. 2018;141(1):322-8 e10.
73. Haddad E, Logan BR, Griffith LM, Buckley RH, Parrott RE, Prockop SE, et al. SCID genotype and 6-month posttransplant CD4 count predict survival and immune recovery. Blood. 2018;132(17):1737-49.
74. Ali AM, Dehdashti F, DiPersio JF, Cashen AF. Radioimmunotherapy-based conditioning for hematopoietic stem cell transplantation: Another step forward. Blood Rev. 2016;30(5):389-99.
75. Pagel JM, Appelbaum FR, Eary JF, Rajendran J, Fisher DR, Gooley T, et al. 131I-anti-CD45 antibody plus busulfan and cyclophosphamide before allogeneic hematopoietic cell transplantation for treatment of acute myeloid leukemia in first remission. Blood. 2006;107(5):2184-91.
76. Pagel JM, Gooley TA, Rajendran J, Fisher DR, Wilson WA, Sandmaier BM, et al. Allogeneic hematopoietic cell transplantation after conditioning with 131I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. Blood. 2009;114(27):5444-53.
77. Mawad R, Gooley TA, Rajendran JG, Fisher DR, Gopal AK, Shields AT, et al. Radiolabeled anti-CD45 antibody with reduced-intensity conditioning and allogeneic transplantation for younger patients with advanced acute myeloid leukemia or myelodysplastic syndrome. Biol Blood Marrow Transplant. 2014;20(9):1363-8.
78. Schulz AS, Glatting G, Hoenig M, Schuetz C, Gatz SA, Grewendorf S, et al. Radioimmunotherapy-based conditioning for hematopoietic cell transplantation in children with malignant and nonmalignant diseases. Blood. 2011;117(17):4642-50.
79. Derderian SC, Jeanty C, Walters MC, Vichinsky E, MacKenzie TC. In utero hematopoietic cell transplantation for hemoglobinopathies. Front Pharmacol. 2014;5:278.
80. Agarwal R DC, Proshaska S, et al. Toxicity-Free Hematopoietic Stem Cell Engraftment Achieved with Anti-CD117 Monoclonal Antibody Conditioning. Biol Blood Marrow Transplant 2019(25):S92.
81. Rastogi N, Katewa S, Thakkar D, Kohli S, Nivargi S, Yadav SP. Reduced-toxicity alternate-donor stem cell transplantation with posttransplant cyclophosphamide for primary immunodeficiency disorders. Pediatric blood & cancer. 2018;65(1).
82. Neven B, Diana JS, Castelle M, Magnani A, Rosain J, Touzot F, et al. Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children. Biol Blood Marrow Transplant. 2019.
83. Balashov D, Shcherbina A, Maschan M, Trakhtman P, Skvortsova Y, Shelikhova L, et al. Single-Center Experience of Unrelated and Haploidentical Stem Cell Transplantation with TCRalphabeta and CD19 Depletion in Children with Primary Immunodeficiency Syndromes. Biol Blood Marrow Transplant. 2015;21(11):1955-62.

84. Ivaturi V, Dvorak CC, Chan D, Liu T, Cowan MJ, Wahlstrom J, et al. Pharmacokinetics and Model-Based Dosing to Optimize Fludarabine Therapy in Pediatric Hematopoietic Cell Transplant Recipients. Biol Blood Marrow Transplant. 2017;23(10):1701-13.
85. Chiesa R, Standing JF, Winter R, Nademi Z, Chu J, Pinner D, et al. Proposed Therapeutic Range of Treosulfan in Reduced Toxicity Pediatric Allogeneic Hematopoietic Stem Cell Transplant Conditioning: Results From a Prospective Trial. Clin Pharmacol Ther. 2019.
86. Romanski M, Wachowiak J, Glowka FK. Treosulfan Pharmacokinetics and its Variability in Pediatric and Adult Patients Undergoing Conditioning Prior to Hematopoietic Stem Cell Transplantation: Current State of the Art, In-Depth Analysis, and Perspectives. Clin Pharmacokinet. 2018;57(10):1255-65.
87. Marsh RA, Lane A, Mehta PA, Neumeier L, Jodele S, Davies SM, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. Blood. 2016;127(4):503-12.
88. Willemsen L, Jol-van der Zijde CM, Admiraal R, Putter H, Jansen-Hoogendijk AM, Ostaijen-Ten Dam MM, et al. Impact of serotherapy on immune reconstitution and survival outcomes after stem cell transplantations in children: thymoglobulin versus alemtuzumab. Biol Blood Marrow Transplant. 2015;21(3):473-82.
89. Admiraal R, Jol-van der Zijde CM, Furtado Silva JM, Knibbe CAJ, Lankester AC, Boelens JJ, et al. Population Pharmacokinetics of Alemtuzumab (Campath) in Pediatric Hematopoietic Cell Transplantation: Towards Individualized Dosing to Improve Outcome. Clin Pharmacokinet. 2019;58(12):1609-20.
90. Oostenbrink LVE, Jol-van der Zijde CM, Kielsen K, Jansen-Hoogendijk AM, Ifversen M, Muller KG, et al. Differential Elimination of Anti-Thymocyte Globulin of Fresenius and Genzyme Impacts T-Cell Reconstitution After Hematopoietic Stem Cell Transplantation. Front Immunol. 2019;10:315.
91. P Bader DN, A Willasch, H Kreyenberg, T Klingebiel. How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplantation 2004;35:117-9.
92. Thiede C, Bornhauser M, Ehninger G. Strategies and clinical implications of chimerism diagnostics after allogeneic hematopoietic stem cell transplantation. Acta Haematol. 2004;112(1-2):16-23.
93. Clark JR, Scott SD, Jack AL, Lee H, Mason J, Carter GI, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. Br J Haematol. 2015;168(1):26-37.
94. Zimmerli W, Zarth A, Gratwohl A, Speck B. Neutrophil function and pyogenic infections in bone marrow transplant recipients. Blood. 1991;77(2):393-9.
95. Atkinson K, Biggs JC, Downs K, Juttner C, Bradstock K, Lowenthal RM, et al. GM-CSF after allogeneic bone marrow transplantation: accelerated recovery of neutrophils, monocytes and lymphocytes. Aust N Z J Med. 1991;21(5):686-92.
96. Hokland M, Jacobsen N, Ellegaard J, Hokland P. Natural killer function following allogeneic bone marrow transplantation. Very early reemergence but strong dependence of cytomegalovirus infection. Transplantation. 1988;45(6):1080-4.
97. Jacobs R, Stoll M, Stratmann G, Leo R, Link H, Schmidt RE. CD16- CD56+ natural killer cells after bone marrow transplantation. Blood. 1992;79(12):3239-44.
98. Bartelink IH, Belitser SV, Knibbe CA, Danhof M, de Pagter AJ, Egberts TC, et al. Immune reconstitution kinetics as an early predictor for mortality using various hematopoietic stem cell sources in children. Biol Blood Marrow Transplant. 2013;19(2):305-13.

99. Cayeux S, Meuer S, Pezzutto A, Korbling M, Haas R, Schulz R, et al. Allogeneic mixed lymphocyte reactions during a second round of ontogeny: normal accessory cells did not restore defective interleukin-2 (IL-2) synthesis in T cells but induced responsiveness to exogeneous IL-2. Blood. 1989;74(6):2278-84.
100. Sahdev I, O'Reilly R, Black P, Heller G, Hoffmann M. Interleukin-1 production following T-cell-depleted and unmodified marrow grafts. Pediatr Hematol Oncol. 1996;13(1):55-67.
101. Nakata K, Gotoh H, Watanabe J, Uetake T, Komuro I, Yuasa K, et al. Augmented proliferation of human alveolar macrophages after allogeneic bone marrow transplantation. Blood. 1999;93(2):667-73.
102. de Koning C, Plantinga M, Besseling P, Boelens JJ, Nierkens S. Immune Reconstitution after Allogeneic Hematopoietic Cell Transplantation in Children. Biol Blood Marrow Transplant. 2016;22(2):195-206.
103. Alpdogan O, Muriglan SJ, Eng JM, Willis LM, Greenberg AS, Kappel BJ, et al. IL-7 enhances peripheral T cell reconstitution after allogeneic hematopoietic stem cell transplantation. J Clin Invest. 2003;112(7):1095-107.
104. Lu H, Zhao Z, Kalina T, Gillespy T, 3rd, Liggitt D, Andrews RG, et al. Interleukin-7 improves reconstitution of antiviral CD4 T cells. Clin Immunol. 2005;114(1):30-41.
105. Napolitano LA, Schmidt D, Gotway MB, Ameli N, Filbert EL, Ng MM, et al. Growth hormone enhances thymic function in HIV-1-infected adults. J Clin Invest. 2008;118(3):1085-98.
106. Wils EJ, Braakman E, Verjans GM, Rombouts EJ, Broers AE, Niesters HG, et al. Flt3 ligand expands lymphoid progenitors prior to recovery of thymopoiesis and accelerates T cell reconstitution after bone marrow transplantation. J Immunol. 2007;178(6):3551-7.
107. Rossi S, Blazar BR, Farrell CL, Danilenko DM, Lacey DL, Weinberg KI, et al. Keratinocyte growth factor preserves normal thymopoiesis and thymic microenvironment during experimental graft-versus-host disease. Blood. 2002;100(2):682-91.
108. Triplett BM, Muller B, Kang G, Li Y, Cross SJ, Moen J, et al. Selective T-cell depletion targeting CD45RA reduces viremia and enhances early T-cell recovery compared with CD3-targeted T-cell depletion. Transpl Infect Dis. 2018;20(1).
109. Slatter MA, Rogerson EJ, Taylor CE, Galloway A, Clark JE, Flood TJ, et al. Value of bronchoalveolar lavage before haematopoietic stem cell transplantation for primary immunodeficiency or autoimmune diseases. Bone Marrow Transplant. 2007;40(6):529-33.
110. Choi SW, Levine JE, Ferrara JL. Pathogenesis and management of graft-versus-host disease. Immunol Allergy Clin North Am. 2010;30(1):75-101.
111. Gatza E, Reddy P, Choi SW. Prevention and Treatment of Acute Graft-versus-Host Disease in Children, Adolescents, and Young Adults. Biol Blood Marrow Transplant. 2020;26(5):e101-e12.
112. Jodele S, Davies SM, Lane A, Khoury J, Dandoy C, Goebel J, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood. 2014;124(4):645-53.
113. Jodele S, Laskin BL, Dandoy CE, Myers KC, El-Bietar J, Davies SM, et al. A new paradigm: Diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. Blood Rev. 2015;29(3):191-204.
114. Dvorak CC, Higham C, Shimano KA. Transplant-Associated Thrombotic Microangiopathy in Pediatric Hematopoietic Cell Transplant Recipients: A Practical Approach to Diagnosis and Management. Front Pediatr. 2019;7:133.
115. Yang S, McGookey M, Wang Y, Cataland SR, Wu HM. Effect of blood sampling, processing, and storage on the measurement of complement activation biomarkers. Am J Clin Pathol. 2015;143(4):558-65.

116. Jodele S. Complement in Pathophysiology and Treatment of Transplant-Associated Thrombotic Microangiopathies. Semin Hematol. 2018;55(3):159-66.

117. Jodele S, Dandoy CE, Lane A, Laskin BL, Teusink-Cross A, Myers KC, et al. Complement blockade for TA-TMA: lessons learned from a large pediatric cohort treated with eculizumab. Blood. 2020;135(13):1049-57.

118. Majhail NS, Tao L, Bredeson C, Davies S, Dehn J, Gajewski JL, et al. Prevalence of hematopoietic cell transplant survivors in the United States. Biol Blood Marrow Transplant. 2013;19(10):1498-501.

119. Heimall J, Buckley RH, Puck J, Fleisher TA, Gennery AR, Haddad E, et al. Recommendations for Screening and Management of Late Effects in Patients with Severe Combined Immunodeficiency after Allogenic Hematopoietic Cell Transplantation: A Consensus Statement from the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects after Pediatric HCT. Biol Blood Marrow Transplant. 2017;23(8):1229-40.

120. Skinner RWWLG. Therapy based long term follow up2005. 62 p.

121. Holtta P, Hovi L, Saarinen-Pihkala UM, Peltola J, Alaluusua S. Disturbed root development of permanent teeth after pediatric stem cell transplantation. Dental root development after SCT. Cancer. 2005;103(7):1484-93.