Clinical, Immunological and Psycho-Social Outcome of SCID Patients Who underwent Hematopoietic Stem Cell Transplantation in Newcastle, UK, 1987 – 2012 and Long-term Outcome of the UK SCID Cohort.

Volume 1 of 1

Intan Juliana Abd Hamid

120426454

Thesis submitted for Doctor of Philosophy

Faculty of Medical Sciences

Institute of Cellular Medicine

May 2017

Abstract

Background

Severe Combined Immunodeficiencies (SCID) are primary immunodeficiencies with defective development and/or/function of T-lymphocyte, B-lymphocyte and Natural Killer cells. Hematopoietic stem cell transplantation (HSCT) corrects immunodeficiency but long-term impact of pre-HSCT chemotherapy, and immunoreconstitution are poorly documented.

We explored: clinical outcome, immunoreconstitution, and quality of life (QoL) in SCID survivors >2 years post-HSCT (Newcastle cohort), newborn SCID (Newcastle cohort) and >20 years post-HSCT (Newcastle and London cohort).

Methods

A retrospective longitudinal study of long-term outcome of post-HSCT SCID patients by genotype (Newcastle), newborn diagnosis of SCID and >20 years long-term outcome of UK SCID HSCT patients.

Clinico-immunological data from London and Newcastle were retrospectively collated. Patients and families attending the Newcastle HSCT follow-up clinic were invited to complete PedsQL questionnaires.

Descriptive analyses were performed for clinical outcome. Longitudinal analyses assessed immunoreconstitution changes post-HSCT. Health-related questionnaire results were compared to UK norms.

Results

102 patients were identified from Newcastle with 49 patients were diagnosed during neonatal period and 74 patients for the UK study of >20 years post-HSCT long-term outcome.

Many patients have on-going medical issues at latest follow-up [IL2RG/JAK3 (68%), IL7Rα (73%), Artemis (85%), RAG 1/2 (55%) and ADA SCID (87%)]. Some issues were genotype-specific; papillomata in IL2RG/JAK3/IL7Rα SCID, neurocognitive issues and hearing loss in ADA SCID. Artemis SCID patients experienced more sequalae than RAG 1/2 SCID.

Conditioned recipients with IL2RG/JAK3 SCID, ADA, Artemis and RAG SCID had more CD4+ naïve lymphocytes compared to unconditioned recipients. B-

lymphocyte chimerism mirrored myeloid chimerism and those with more than 50% donor chimerism were more likely to be immunoglobulin independent. All parents except those of IL7Rα SCID reported lower QoL; further subset group analysis showed parents and IL2RG/JAK3 SCID immunoglobulin-independent survivors plus Artemis/RAG1/2 survivors without on-going medical issues reported normal QoL. Both parents and ADA SCID survivors reported lower QoL.

Conclusions

Conditioned recipients have superior long-term thymopoiesis, chimerism and immunoglobulin-independence. Quality of life was normal in those who were immunoglobulin-independent or normal health.

318 words

Dedication

To my family, the love of my life, without whom I will not be who I am now

Mariani Abd Hamid
Mustafa Kamal Mohd Samuri
Muhammad Aiman Mustafa Kamal
Amiruddin Mustafa Kamal
Muhammad Amin Mustafa Kamal
Muhammad Arif Mustafa Kamal

Acknowledgements

My PhD study was funded by Universiti Sains Malaysia and Ministry of Higher Education, Malaysia.

I would like to thank my supervisors, Dr Andy Gennery, Dr Mary Slatter and Prof Mark Pearce; for their unwavering encouragement, guidance and patience. A big thank you to Dr Siobhan Burns in facilitating my access for data collection in London.

I would also extend my gratitude to Prof Lokman Mohd Noh, Prof Hans van Rostenberghe, Dr Noorizan Abd Majid and Prof Abdul Aziz Tajuddin, who believe in me and support me when the time gets rough.

Lastly, thank you to all the patients and families with SCID and the medical staff and nursing staff in Ward 3, BMT Unit of the Great North Children's Hospital.

Table of Contents

List of Abbreviations

ADA	Adenosine deaminase
APC	Antigen presenting cell
AR	Autosomal recessive
BCG	Bacillus Calmette-Guérin
BMT	Bone marrow transplantation
BMDW	Bone Marrow Donor Worldwide
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein Barr virus
EDTA	Ethylene-diamineteraacetic acid
ESID	European Society for Immunodeficiencies
FEV1	Forced expiratory volume at 1 minute
FISH	Fluorescent in situ hybridization
FVC	Full vital capacity
GCSF	Growth colony stimulating factor
GNCH	Great North Children's Hospital
GOSH	Great Ormond Street Hospital
GVHD	Graft versus host disease
HLA	Human leukocyte antigen
HRQoL	Health related quality of life
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
IgG	Immunoglobulin G
IL-7	Interleukin-7
IUIS	International Union of Immunological Societies
JAK3	Janus kinase 3
LT MAC	Low toxicity myeloablative conditioning
MAC	Myeloablative conditioning
MAC8	Myeloablative conditioning (busulfan 8mg/kg)
MIC	Minimal intensity conditioning
MUD	Matched unrelated donor

MSD	Matched sibling donor
MRD	Matched related donor
MMUD	Mismatched unrelated donor
NIH	National Institute of Health
NK	Natural Killer cells
NMA	Non-myeloablative conditioning
OR	Odd ratio
PBSC	Peripheral blood stem cell
PEG-ADA	Polyethylene glycol-modified adenosine deaminase
PID	Primary immunodeficiency diseases
QoL	Quality of life
RAG1	Recombination activating gene 1
RAG2	Recombination activating gene 2
RFH	Royal Free Hospital
RIC	Reduced intensity conditioning
TCR α/β	T-cell receptor α/β
TRECs	T-lymphocyte receptors excision circles
TRM	Transplant related mortality
SCF	Stem cell factor
SD	Standard deviation
SE	Standard error
SCETIDE	The stem cell transplantation for immnuodeficiencies
SCID	Severe combined immunodeficiencies
UK	United Kingdom
USA	United States of America
UCBT	Umbilical cord blood transplantation
VOD	Veno-occlusive disease
WHO	World Health Organization
XL	X-linked

List of Figures

List of Tables

Chapter 1 Introduction

This chapter will focus on an overview of severe combined immunodeficiencies (SCID) and hematopoietic stem cell transplantation (HSCT).

1.1 Severe Combined Immunodeficiencies (SCID)

1.1.1 Epidemiology

Severe Combined Immunodeficiencies (SCID) are a group of devastating inherited illnesses affecting children worldwide. The incidence of SCID is estimated to be 1 in 30,000 live births [1]. They are a group of T-lymphocyte developmental dysfunction or defects, which subsequently causes severe lymphopenia and may be accompanied by absent or non-functioning B-lymphocytes and natural killer cells (NK cells). It is considered to be pediatric emergency, as the outcome of survival is directly dependent on the speed of diagnosis and treatment instituted. Most patients do not survive until their first birthday if left untreated [2].

1.1.2 Clinical features of SCID

The hallmark feature of SCID is the defect in T-lymphocyte development leading to recurrent, eventually fatal infections. Patients may come to clinical attention along a wide spectrum of presentation. Those with a positive family history (either by having an earlier deceased family member or diagnosed siblings) may be diagnosed at the time of birth and consequently have a better outcome compared to an index case [3]. Population based newborn screening programs in many states of the USA also allow early detection of infants [4]. This poses a new challenge of re-defining the disease itself, as usually, these infants are healthy at birth.

However, in most countries, patients may present with end organ damage as a consequence of recurrent infections and some of the patients died even before receiving transplantation [5-8]. Classically, patients with SCID usually present during infancy with persistent and recurrent infections (viral respiratory ± chronic

diarrhoea ± cutaneous fungal infection) [9]. They may have opportunistic infections (e.g. *Pneumocystis jiroveci, Candida albicans*, varicella, adenovirus, respiratory syncytial virus, cytomegalovirus, Epstein-Barr virus) or disseminated BCG infection. Failure to thrive is also a common feature, secondary to viral enteritis and malabsorption.

In recent years, there has been an increasing interest in guidelines for diagnosing SCID. One of the warning signs for SCID is lymphopenia and/or absent thymus shadow in babies [10]. As a general rule, patients suspected of having SCID should have several laboratory investigations depending on their history and on clinical findings [11]. Screening investigations include; full blood count, quantification of the T lymphocyte, B lymphocyte and NK cells via flow-cytometry and serum immunoglobulins. Severe lymphopenia (adjusted according to age-related normal range), reduced or absent major lymphocyte subpopulations may direct the need for further advanced investigations such as lymphocyte proliferation test against mitogens and antigens and specific antibody response. Serum immunoglobulins may be reduced or normal due to trans-placental transfer of maternal IgG during the intra-uterine period. In patients with SCID, there is a profoundly decreased or absent response of lymphocyte proliferation towards mitogen. Specific antibody responses are usually absent. Confirmatory tests are by detection of the genetic defect either by targeted DNA analysis or whole genome exome sequencing [8].

1.1.3 Historical perspective of SCID

The first description of a Primary Immunodeficiency goes back 60 years. In 1952, Colonel Ogden Bruton published the first case report of a boy with agammaglubulinemia [12]. He presented to Walter Reed Army Hospital in Washington D.C. with a history of 18 episodes of pneumonia. Out of curiosity, Colonel Bruton requested for quantification of gammaglobulin using the then newly acquired Tiselius electrophoresis apparatus. He postulated the child would have high antibody and gammaglobulin levels because of the exposure to multiple episodes of infections. However, the result came back with no gammaglobulin detected at all. Initially, it was thought that the machine was faulty as flat tracing

was noted at the gammaglobulin fraction site. Significantly, Colonel Bruton inferred the concept of 'no gammaglobulin, no antibody' [13].

He also pioneered the intervention of immunoglobulin administration for agammaglobulinemia. After the discovery of the flat tracing gammaglobulin, he proceeded with subcutaneous administration of gammaglobulin prophylaxis (in the forms of Cohn fraction II) at about 100mg/kg per month. He demonstrated that this measure protected the boy from further infections and there were successful attempts of quantification of small peaks of gammaglobulin levels in him [14].

The first descriptions of patients with alymphocytosis were published earlier than Bruton's case, in 1950 by Glanzmann and Riniker [15]. In their seminal paper, they had reported cases of two related babies, who had severe *Candida albicans* infection coupled with alymphocytosis and were progressively terminal. However, they concluded severe candidiasis as the cause of the profound lymphocytopenia, instead of an absence of lymphocytes leading to severe opportunistic infections. No causal relationship was linked to the defective immunity system.

The term 'Swiss type agammaglobulinemia' was introduced in the 1960s [16]. Hitzig and Willi (1961) [16] reported infants who presented with alymphocytosis, severe fungal and bacterial infections, leading to death in early life. They identified five characteristics of the main differentiating features that set 'Swiss type agammaglobulinemia' apart from the agammaglobulinemia described by Bruton. The features are; onset of symptoms during infancy, severe fungal infections, profound lymphopenia, extreme atrophy or absence of all lymphatic tissue and 'dystrophic and atopic' thymus (autopsy finding). Positive family history was also noted, as two patients from these series were cousins of the first series described by Glanzmann and Riniker. They linked the concepts of agammaglobulinemia with lymphopenia to a defective humoral-cellular immune system; leading to deadly consequences. However, later on the term 'Swiss type agammaglobulinemia' was abandoned as it is only applicable to the X-linked types of SCID and does not explain the other types of SCID. It was replaced with the term 'Severe Combined Immunodeficiency Disease' in 1970 during the first World Health Organization meeting on primary immunodeficiency [17].

By the late 1980s, new developments in molecular genetic fields had enabled further delineation of other types of SCID [18]. Analysis of the genes causing SCID was carried out by many researchers. The first gene identified was the gene coding for the enzyme ADA in 1983. The gene mutation of IL2RG for X-linked SCID was found in 1992 [18].

One of the most important events of the 1960s was the first successful attempt of Hematopoietic stem cell transplant for a patient with SCID. Gatti et al., (1968) reported on immune restoration after a lymphopenic SCID infant received bone marrow stem cells from his HLA-matched sibling [19]. There were many successful attempts of bone marrow transplantation from 1968-1980s, but these were exclusive to HLA-identical sibling donors [20]. A varying severity of graft versus host disease (GVHD) was noted with non-matched HLA donors, and were mostly fatal.

1.1.4 Pathogenesis of various SCID genotypes

Lymphopoiesis is the mechanism of lymphocyte development from uncommitted progenitor cells and occurs both in bone marrow and thymus. Lymphoid lineage stem cells will further differentiate to B-lymphocyte (bone marrow derived cells), T-lymphocyte (thymus derived cells) and NK cells. Interleukin-7 (IL-7) and stem cell factor (SCF) are two of the main cytokines involved and are necessary for both B- and T-lymphocyte differentiation. This explains why any gene defect affecting IL-7 will cause arrest of T-lymphocyte development and give rise to T- B+ NK+ SCID phenotype (Figure 1.1).

T-lymphopoiesis continues in the thymus. T-lymphocyte pre-cursors in the thymus are called thymocytes, and reside at the thymus cortex. With the influence of Interleukin-2, Interleukin-7, SCF and thymic factors; thymocytes further differentiate to naïve T-lymphocytes and are released to the peripheral circulation. This involves many steps of differentiation; i.e. from progeny of replicating cells to double negative thymocytes (CD4-CD8-), then to double positive thymocytes (CD4+CD8+), then to single positive thymocytes (CD4+ or CD8+). Throughout this whole process, the thymocytes also progressively

migrate from the outer cortex of the thymus to the inner cortex and finally the medulla of thymus.

Figure 1.1 T-lymphocyte, B-lymphocyte and NK cell Ontogeny in SCID [21]

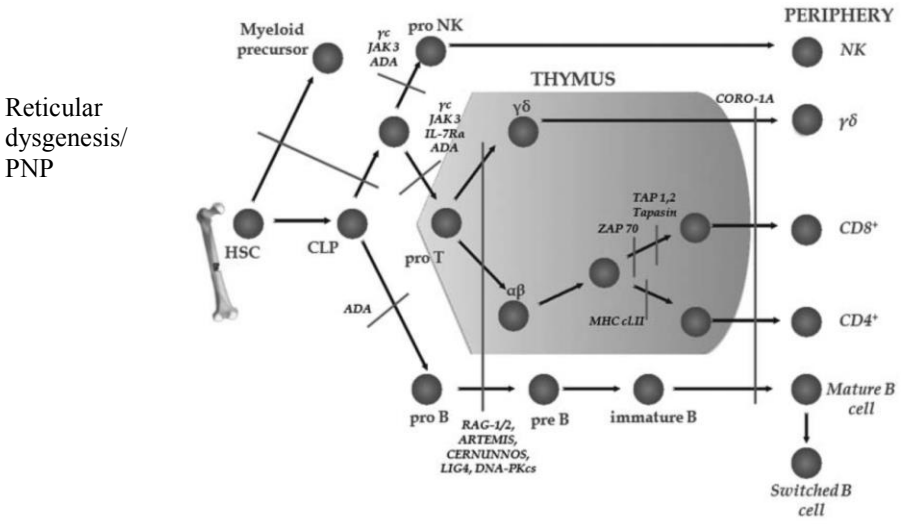


Figure 1.1 HSC = hematopoietic stem cells, CLP = common lymphoid progenitor

Recent developments in the field of molecular and genetic diagnostics have led to identification and further characterisation of various SCID immuno-phenotypes. Despite the diversity of molecular defects in SCID, all have similar outcomes, which are defective T-lymphocyte repertoires. Twenty gene defects have been identified that cause SCID [22]. The primary importance of getting the molecular diagnosis is that it confirms the diagnosis accurately, enabling appropriate genetic counselling and projection for future pregnancies, further enabling prediction of the outcome based on the genotype-phenotype association and finally allowing specifically tailored potential therapy according to the genotype characteristics (such as myeloablative conditioning which are best avoided for those with Artemis SCID undergoing stem cell transplantation) [23].

Common γ chain SCID is caused by *IL2RG* gene mutations (chromosome position Xq13.1) [22, 24]. The mutation leads to a defect in the γ chain of receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, causes absence of IL-7 and IL-21 signalling which is critical in T-lymphocyte and B-lymphocyte development (Figure 1.2) [25, 26]. It is inherited in an X-linked fashion and only found in

males. Usually patients have markedly decreased circulating T-lymphocyte, normal or increased circulating B-lymphocyte and markedly decreased Natural killer cells (T- B+ NK- immunophenotype) [27].

Figure 1.2 Pathway for cytokine receptors of common gamma chain and JAK3 tyrosine kinase in T-lymphocyte differentiation [25].

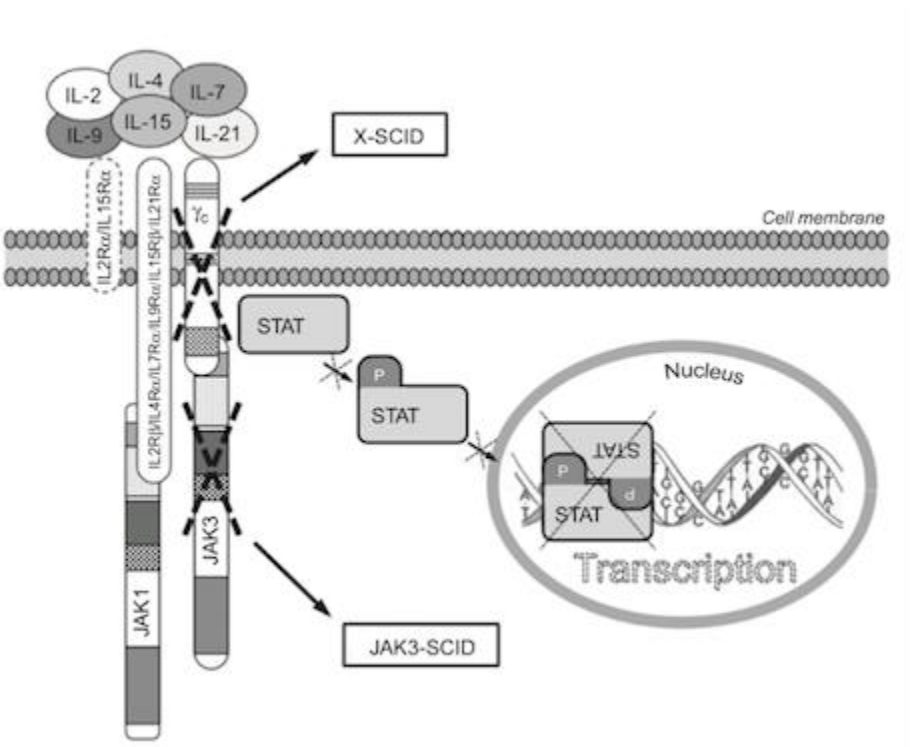


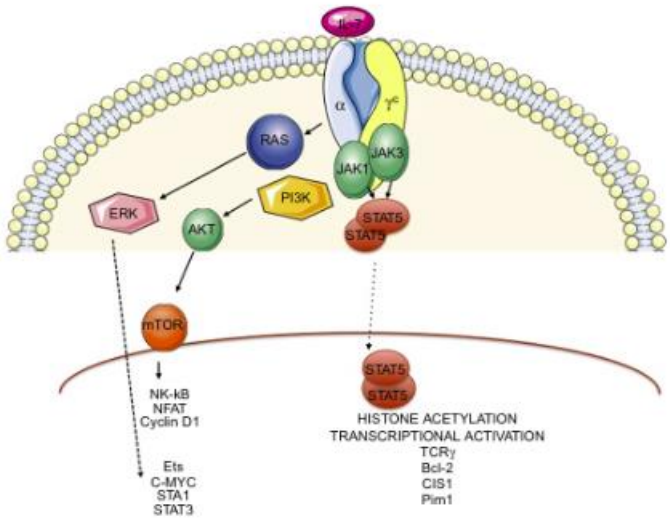
Figure 1.2. Defect in the IL2RG gene and JAK3 gene leads to defective cytokine signalling. X-SCID = Common gamma chain SCID or IL2RG SCID.

Patients diagnosed with JAK3 SCID have a mutation in the *JAK3* gene (chromosome position 19p13.11), which leads to a defect in the Janus activating kinase 3 protein [22, 24]. This defect affects some of the downstream signalling of the cytokine receptors involved in the common gamma chain pathway, such as IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (Figure 1.2). This explains why the clinical features of JAK3 SCID are similar to IL2RG SCID [28]. However, as the mode of inheritance is autosomal recessive, it can occur in both females and males. The immunophenotype features are markedly decreased circulating T-lymphocytes, normal or increased circulating B-lymphocyte and markedly

decreased Natural killer cells (T- B+ NK- phenotype). B-lymphocytes are present but are non-functional in both IL2RG SCID and JAK3 SCID.

In IL7Rα deficient SCID, patients have a mutation of the *IL7Rα* gene (chromosome position 5p13.2) and defect in the IL-7 receptor α chain protein [22, 24]. IL-7 receptor α is involved in at least three signalling pathways namely the JAK-STAT pathway, PI3K activation and the Ras-MAPK-ERK pathway (Figure 1.3) [25]. These pathways are important in thymocyte differentiation from double negative to double positive T-lymphocyte differentiation via V(D)J recombinase and TCR gamma locus regulation and control of T-lymphocyte quantity in the peripheral circulation. Thus, defects in IL-7 receptor α will lead to defects in the IL-7 receptor α mediated signalling pathway and subsequently compromising T-lymphocyte differentiation [29]. It is an autosomal recessive disease and patients have a T- B+ NK+ phenotype (markedly decreased circulating T-lymphocytes, normal or increased circulating B-lymphocytes and normal Natural killer cells).

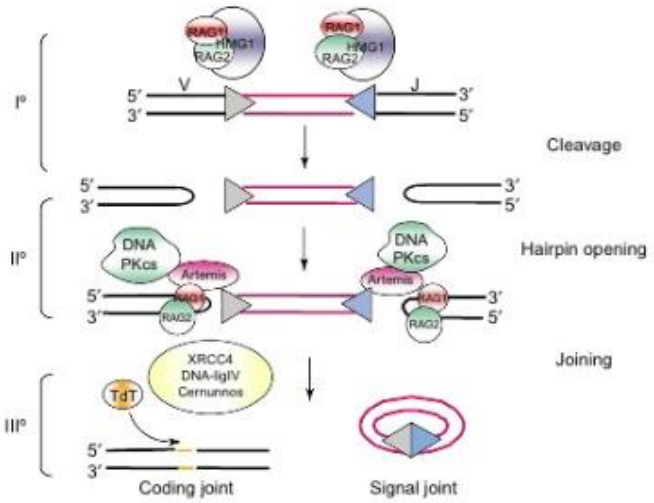
Figure 1.3 Schematic presentation of the IL7Rα mediated signalling pathway [25].



Mutations in the *RAG1* gene (chromosome position 11p12) causes RAG1 SCID and mutations in the *RAG2* gene (chromosome position 11p12) causes RAG2 SCID [22, 24]. Both RAG1 and RAG2 defects result in defective V(D)J recombination defects and are inherited in an autosomal recessive fashion. V(D)J recombination is the important step in the production of antigen receptor diversity

and early T-lymphocyte and B-lymphocyte development [25]. RAG1 and RAG2 are involved in the early steps of the V(D)J recombination pathway, specifically in cleavage of DNA to initiate V(D)J recombination (Figure 1.4). Both SCID genotypes patients have a T- B- NK+ immunophenotype (markedly decreased circulating T-lymphocytes, markedly decreased circulating B-lymphocyte and presence of Natural killer cells).

Figure 1.4 Schematic pathway of 3 steps in V(D)J recombination with involvement of RAG1, RAG2 and Artemis gene [25].

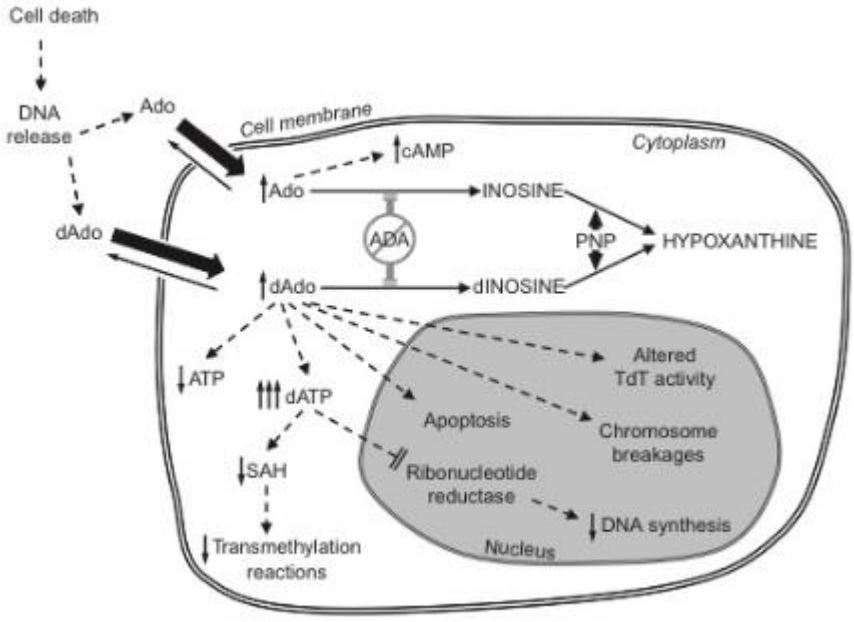


Mutations in *DCLRE1C* (chromosome position 10p13) cause Artemis SCID [22, 24]. Patients with the Artemis gene defect have defective V(D)J recombination [25]. Artemis is essential in the later part of the V(D)J recombination pathway which is at the opening of the hairpin coding ends of the DNA (Figure 1.4). It has autosomal recessive inheritance and patients have a T- B- NK+ immunophenotype (markedly decreased circulating T-lymphocytes, markedly decreased circulating B-lymphocyte and normal Natural killer cells). Patients with Artemis SCID have radiation sensitivity, because Artemis operates in all nucleated cells, which differentiates this defect from RAG1 and RAG2 SCID patients.

The *ADA* gene is located at chromosome position 20q13.12. Mutations of the *ADA* gene cause autosomal recessive ADA SCID [22, 24]. The main

differentiating feature of ADA SCID compared to other genotypes is that it is predominantly a systemic metabolic disorder. Mutations in the *ADA* gene lead to a defect in the expression and function of the purine salvage pathway enzyme adenosine deaminase. Usually patients present with absent ADA activity [elevated lymphotoxic metabolites (dATP, S-adenosylhomocysteine)] (Figure 1.5). The manifestation severity of the disease depends on types of mutations [30]. Patients have a T-B-NK- immunophenotype (absent circulating T-lymphocytes from birth (null mutation) or progressively decreased, absent from birth or progressively decreasing circulating B-lymphocyte and decreased Natural killer cells). Patients with ADA SCID do manifest other systemic features, such as costochondral junction flaring, neurological features, hearing impairment, lung, liver and behavioural issues.

Figure 1.5 Schematic diagram of the ADA SCID metabolic pathway [25].



1.2 Hematopoietic Stem Cell Transplantation (HSCT)

1.2.1 Definition

The accreditation subcommittee of the European Society for Blood and Marrow Transplantation (EBMT) (2006) has defined hematopoietic stem cell transplantation as any procedure where hematopoietic stem cells of any donor type and any source are given to a recipient with the intention of repopulating and replacing the hematopoietic system in total or in part [31].

1.2.2 History of HSCT for SCID

The concept of transferring other cells or tissue to achieve correction of defects or replace a missing entity has been proposed since early in the 19th century. The first reported paper on a skin graft was published in 1905 [18]. Important concepts noted were; engraftment depends on the type of donor for the skin graft, where autologous skin grafts were tolerated well, allogeneic skin grafts (unrelated human) were usually rejected and skin xenografts (different species) were always rejected and a connection between the presence of the donor's lymphocytes and rejection in the recipient was identified [13, 18, 32].

One of the most important events of the 1960s was the first successful hematopoietic stem cell transplant for a patient with SCID. Gatti et al., (1968) reported on immune restoration after a lymphopenic SCID infant received bone marrow stem cells from his HLA-matched sibling [19]. Another successful marrow transplantation was in a patient with Wiskott Aldrich Syndrome. There were many successful attempts of bone marrow transplantations from 1968-1980s, but these were exclusively with HLA-identical sibling donors. A varying severity of GVHD was noted with non-matched HLA donors, which were mostly fatal.

The development of the T-lymphocyte depletion methods has become the fundamental basis for HSCT with haploidentical donors. The first T-lymphocyte depleted technique described by Reisner et al., (1976) was the soybean lectin, sheep erythrocyte agglutination method [33]. In this method, soybean lectin was used to agglutinate mature donor's marrow cell in the *ex vivo* environment. The clumped cells were sedimented and removed. Mature T-lymphocytes in the

unagglutinated marrow cells were further removed by sheep erythrocyte rosetting and density-gradient centrifugation. The end product contained immature marrow cells. Infusion of these marrow cells with the absence of post-thymic T-lymphocytes to lethally irradiated animals have been shown to reconstitute the defect without causing GVHD in mouse model [33]. HSCT with this source of stem cells allows effective immune-reconstitution with a significantly reduced risk of GVHD [34].

The second technique of T-lymphocyte depletion is monoclonal antibody and complement lysis. Donor marrow cells are incubated with monoclonal antibodies (Campath 1M) to human T-lymphocytes and complement. However, engraftment failure remains a major issue as reported by Gennery et al (2001) [35]. Possible explanations for engraftment failure seen were immunological rejection of the donor's graft or due to reduced CD34+ cell counts due to the T-lymphocyte depletion manipulation of ensuring CD3+ lymphocyte less than 5×10^5/kg.

The third T-lymphocyte depletion method is CD34+ cell selection by magnetic columns. The cell is selected by use of an antibody attaching to the cell, with a magnetic bead on the other end of the antibody which sticks to the magnetic column. It was introduced in the 2000s. The advantages are a highly purified end product and removal of the need for post-transplant GVHD prophylaxis. However, the disadvantages are, delayed immune-reconstitution achievement that may be compromised with fatal viral infections. Other methods which have been described are, CD3/CD19 depletion and TCR α/β depletion which are evolutions of the CD34+ selection method.

Another important discovery from the T-lymphocyte depleted method is the ability to outsource the stem cell source from the peripheral blood and from cord blood. The initiative of cord blood banks and donor marrow cells bank has made the option of unrelated donors readily available. EBMT established the Bone Marrow Donor Worldwide (BMDW) in 1988. The current BMDW registries have 23,828,686 marrow and cord blood donors in their system [36].

1.2.3 Hematopoietic stem cells and stem cell niche

The stem cell is defined as an undifferentiated cell capable to divide for indefinite periods, to self-renew and to generate functional progeny of highly specialized cells [37]. Stem cells are further divided into several categories according to their developmental potential and physical location. Totipotent stem cells (zygote) are the highest in the hierarchical developmental process, where they have unrestricted differentiation potential into all types of cell. Pluripotent stem cells (embryonic stem cells) have ability to differentiate into a variety of specialized cell types except a fetus. Multipotent stem cells give rise to specific tissues such as hematopoietic stem cells (HSC), muscle, eye, neural and skin.

Hematopoiesis is a process of production and differentiation of blood cells in the bone marrow. Hematopoietic stem cells (HSC) further differentiate and produce committed oligopotent progeny of the lymphoid and myeloid lineages, which will further differentiate into lineage restricted unipotent precursors of mature blood cells [38].

During the intra-uterine period, hematopoietic stem cells (HSC) may be identified in the mesoderm of the yolk sac as early as the first week of the embryonic stage. By 8 weeks of gestation, they migrate to the fetal liver which becomes the major site for hematopoiesis. HSC start to migrate to the marrow spaces of long bones by the third trimester until birth. This remains the main site for hematopoiesis until puberty, where the axial skeleton takes over.

The stem cell niche is defined as a microenvironment that provides hematopoietic stem cells and their descendants with regulatory signals that are essential for their quiescence, self-renewal, proliferation and differentiation, in order to produce appropriate numbers of mature cells throughout life [38]. HSC remain quiescent in the bone marrow [37], but will proliferate and differentiate upon response to stress triggered by infections, GCSF or myeloablative treatment.

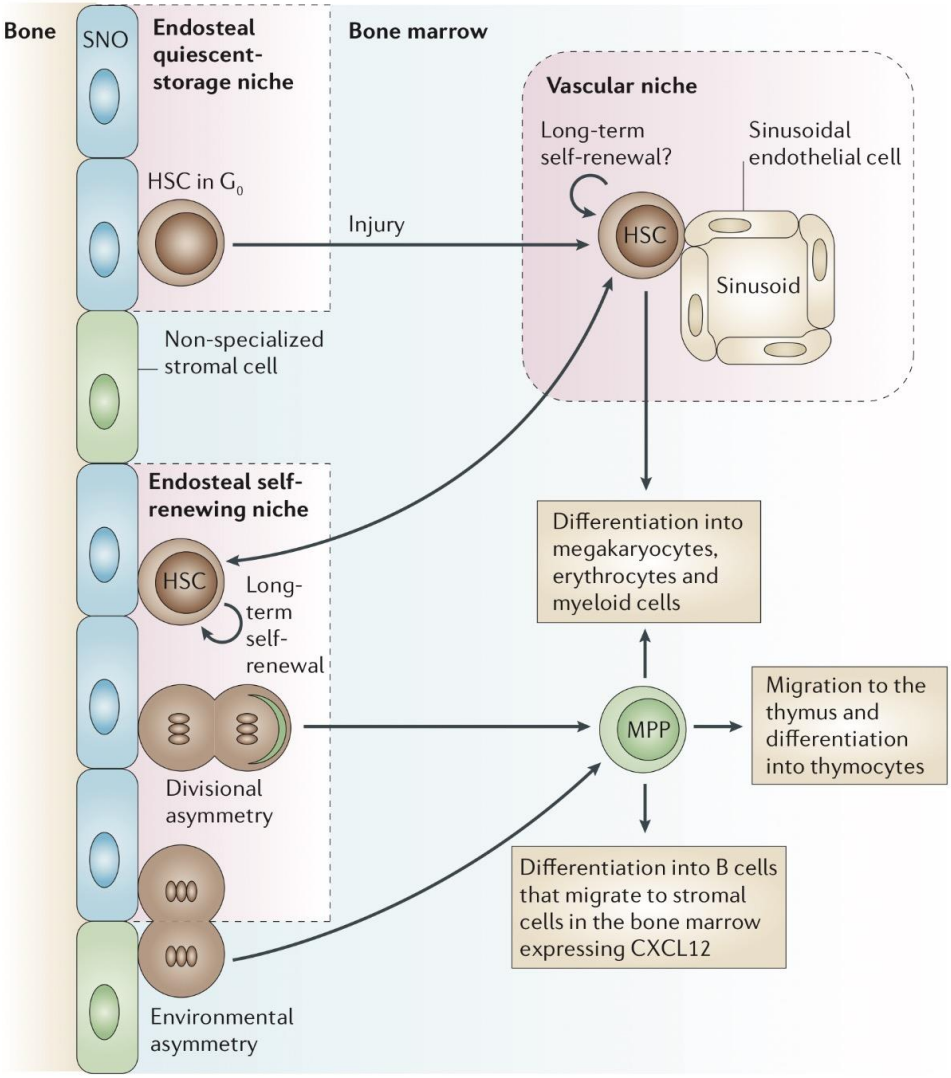
The stem cell niche has been divided into 2 niches according to their functions, which are the endosteal niche and vascular niche [39]. The endosteal niche is made up of trabecular endosteum. It consists of osteoblastic cells outlining the inner surface of the bone and provides a place for quiescent HSC. Actively dividing HSC stay in the vascular niche. The vascular niche also provides

channels of trafficking movements for HSC in and out of bone marrow (Figure 1.6).

The concept of emptying the stem cell niche via a conditioning regimen has been proposed to achieve maximal donor stem cell engraftment post-transplantation [40]. In T-B+NK- SCID phenotypes, the stem cell niche is full with recipient's HSC and mature B-lymphocytes; whereas the thymus is empty of T-lymphocyte precursors. When the patient receives an unconditioned HSCT, engraftment of the thymus with donor T-lymphocyte progenitors readily occurs but B-lymphocyte engraftment is problematic as the recipient marrow is occupied by recipient HSC and mature B-lymphocytes. Patients will develop donor T-lymphocyte progenitor engraftment in the thymus leading to thymopoiesis and long-term production of T-lymphocytes with a diverse receptor repertoire. Conditioning prior to HSCT will empty the stem cell niche permitting engraftment of donor HSC and B-lymphocyte progenitors.

In cases of T-B-NK+ SCID phenotypes because of the stage of developmental arrest, both stem cell niche environment and thymus are occupied with recipient HSC, B-lymphocyte and T-lymphocyte precursors. Thus unconditioned HSCT will only allow engraftment of mature peripheral donor T-lymphocytes which produces a finite and unreplenished source of limited T-lymphocyte receptor repertoire. Engraftment of donor T-lymphocyte and B-lymphocyte progenitor cells will be difficult as both bone marrow and thymus are occupied by recipient HSC and lymphocyte progenitors. This emphasizes the need for conditioning in HSCT for T-B-NK+ SCID phenotype patients.

Figure 1.6 Model of bone marrow niche [37]

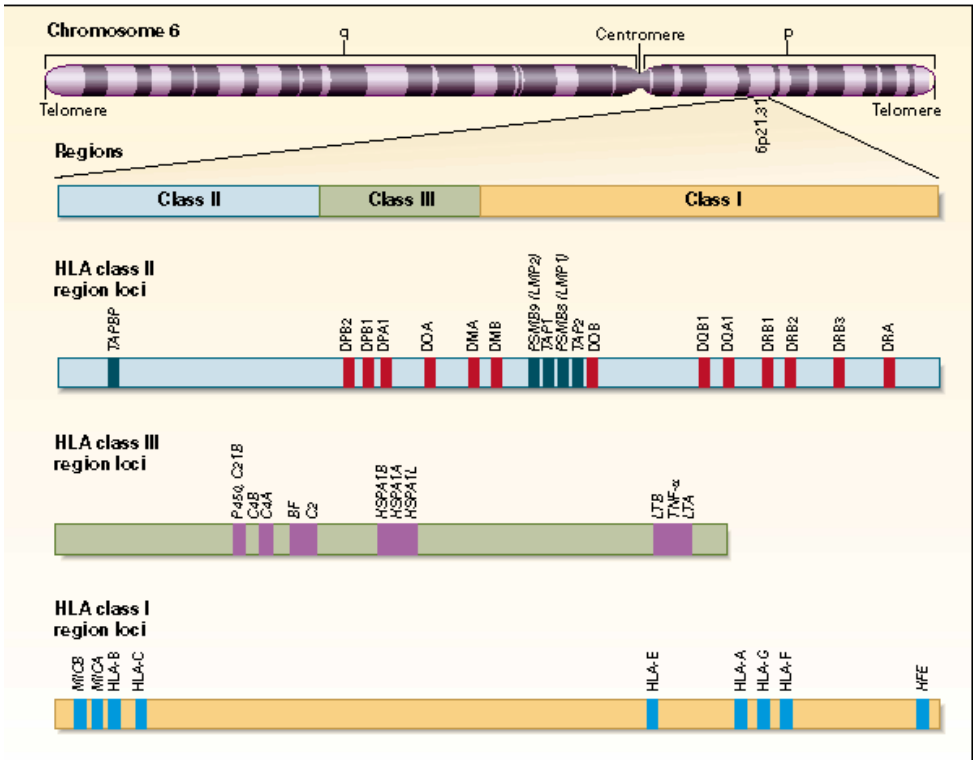


1.2.4 Donor categories

One of the important components in HSCT is the donor. After the decision for HSCT has been made, a donor search begins immediately. The human leukocyte antigen (HLA) system formed the basis for immunogenicity and tolerance of donor stem cells in recipients and is the most important donor selection marker [41]. The HLA system is responsible for antigen recognition and tolerance mechanisms. Mismatching between donor and recipient HLA is detrimental and leads to GVHD in the recipient.

The HLA system is highly polymorphic and diverse. It is located at chromosome 6 with over 200 genes identified [42]. The HLA complex is further divided into 3 regions depending on their locus; HLA Class I (HLA-A, -B and –C genes), HLA Class II (HLA-DR, -DQ and –DP) and HLA Class III (which are not related to Class I and Class II in structure and function) (Figure 1.7). HLA class I and Class II are specific for the immune response, in which they are involved in presenting pathogen derived peptides to T-lymphocytes.

Figure 1.7 Location and organisation of the HLA Complex on Chromosome 6. Adapted from Klein J. & Sato A. [42]



The gold standard for donor choice selection is the most exact matching between donor and recipients. This can be easily achieved with a matched sibling donor as HLA gene inheritance follows Mendelian principles and gene products are co-dominantly expressed [41]. Superior survival outcomes were demonstrated with MSD as commonly the transplantation can be performed without any prior conditioning therapy. However, the availability of MSD donor is limited, ranging from 18-20% [2, 43, 44].

In situations where a MSD is not available, another option would be a matched unrelated donor, matched family related donor or haploidentical donor. Similar principles apply here where the best HLA matching is considered as the best option. Improved techniques for HLA type identification (DNA high resolution sequence typing) and the availability of donor registries relatively contributes to better survival post-transplantation with matched unrelated donors across different time periods [45]. A worse survival outcome was noted in those with single HLA mismatches at HLA-A, HLA-B, HLA-C or HLA-DRB1 [45, 46]. The gold standard for matched unrelated donor is a 10/10 donor with HLA-matching at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 and HLA-DPB1 [47]. Other added decision factors are gender (male is preferred), age (younger age of donor is preferred), ABO matched, CMV status (to be matched according to recipient's CMV status) and urgency of timing for HSCT in particular diagnoses especially with SCID.

A haploidentical related donor offers an attractive solution in situations where MSD and MUD are not available or the urgency for HSCT is imminent. The pros for haploidentical donor are that they are readily available, highly motivated and available if the patient needs an added HSCT in the future. However, the graft needs to be T-lymphocyte depleted to reduce the risk of GVHD, which may confer a higher risk of mortality from serious infection due to delayed immune reconstitution (up till 120 days) [2, 48, 49].

1.2.5 Conditioning regimen

The major aims of conditioning prior to transplantation are to achieve maximal engraftment by creating marrow space for donor cells and prevention of rejection. Quests for better conditioning regimens have introduced the use of chemotherapy drugs such as busulfan, etoposide, cytosine arabinoside and melphalan. Busulfan is a bifunctional DNA alkylating agent with myeloablative capacity. Cyclophosphamide combined with busulfan became the widely used conditioning regimen for allogenic HSCT [50]. However, a strong association between busulfan and cyclophosphamide regimen and veno-occlusive disease (VOD) has been identified, introducing cautions regarding its use [51]. Further adding to the

problem is that busulfan was only available as an oral preparation in the 1980s, hence causing the unpredictable bio-availability in the systemic circulation. Introduction of an intravenous busulfan preparation and methods for busulfan plasma level monitoring in the late 2000s has enabled better dose adjustment and monitoring, and subsequently reduced the accompanying side effects [52]. In view of strong association between busulfan and cyclophosphamide conditioning regimen with veno-occlusive disease, the Inborn Error Working Party has recommended to stop using it [51].

Reduced intensity conditioning (RIC) has been introduced as an attempt to improve engraftment and at the same time minimise the treatment-related toxicity side effects. A few characteristics have been used to describe RIC regimen, one of which is that it has reversible myelosuppression in the absence of stem cell support (usually within 28 days). Secondly, there is noticeably reduced regimen-related toxicity and finally, there is a higher incidence of mixed donor haematopoiesis [53]. Treosulfan and melphalan are parts of the RIC regimen. Another subtype of the RIC conditioning regimen is Minimal-Intensity Conditioning (MIC). It is exclusively comprised of immunosuppressive agents such as fludarabine plus cyclophosphamide, anti CD45 antibody or anti C-Kit and does not have any myeloablative effects.

According to the EBMT/ESID's consensus [51], recommended conditioning regimen guidelines for SCID patients receiving stem cells from matched unrelated donors or phenotypically identical family donors are either Protocol A, B or D plus serotherapy treatment (Figure 1.8 and Figure 1.9). For SCID patients receiving umbilical cord as a stem cell source Protocol A, B, or D with serotherapy treatment are recommended either by using Alemtuzumab or r-ATG. Serotherapy may be omitted in cases where a well-matched donor is available and/or there is concern of viral infection. For haploidentical HSCT, the recommendation is for Protocol A with a T depleted graft (CD34+ selection). Those receiving grafts from HLA matched MSD donors especially in Common γ chain SCID or ADA SCID may receive no conditioning + no T-lymphocyte depletion method + no GVHD prophylaxis.

Other important exceptions are in SCID with radio-sensitivity disorders, where full conventional conditioning should be strongly avoided. This is because they are very sensitive and intolerant to the myeloablative conditioning regimens. Outcome results from the multi-centre transplantation of 69 patients with Artemis-deficient SCID demonstrated poorer long-term outcome such as growth retardation, late-onset endocrine deficiencies and dental abnormalities in those patients treated with myeloablative alkylating agents [54].

Figure 1.8 Myeloablative conditioning protocol as in the EBMT/ESID Guideline for HSCT in primary immunodeficiency disorder, including SCID [51].

Myeloablative Conditioning

PROTOCOL	CHEMOTHERAPY	SEROTHERAPY	GVHD PROPHYLAXIS
A	Busulfan (iv) (wt or AUC dosing)[1] Fludarabine 160 mg/m2	†Campath 1H (TD 0.6-1mg/kg) OR ††ATG (TD 10mg/kg)	CyA or CyA + MMF or MTX (as 2nd agent)

- [1]AUC dosing for iv Bu - = 90+/- 5 mg*h/L. (see appendix for specific protocols for different donor sources and dosing)
- †*Campath 1H – Alemtuzumab*
- ††*ATG – Genzyme rabbit ATG*
- Busulfan/Cyclophosphamide conditioning is no longer recommended by the IEWP because of the increased risk of VOD

Protocol A is aimed at PID (inc HLH) patients with standard risk and where a greater degree of myeloablation is required to promote increased donor engraftment than protocol B (for haplo-identical T cell depleted grafts Thiotepa needs to be added in NON-SCID patients to achieve engraftment)

Protocol B is aimed at PID (inc HLH) patients with organ toxicity / reduced performance scale and CGD patients (see CGD specific guidelines)

Figure 1.9 Reduced intensity conditioning protocol according to EBMT/ESID guideline for HSCT in primary immunodeficiency disorder, including SCID [51].

Reduced Intensity Conditioning

PROTOCOL	CHEMOTHERAPY	SEROTHERAPY	GVHD PROPHYLAXIS
B	Busulfan (iv) (AUC dosing)[2] Fludarabine 180 mg/m2	†Campath 1H (TD 0.6-1mg/kg) OR ††ATG (TD 7.5-10mg/kg)	CyA or CyA + MMF or MTX (as 2nd agent)
C	Fludarabine 150 mg/m2 Melphalan 140 mg/m2	Campath 1H (TD 0.6-1mg/kg)	CyA or CyA/MMF
D	Treosulphan 42 g/m2 Fludarabine 150 mg/m2	None or Campath 1H(0.6-1mg/kg)	CyA or CyA/MMF

- [2]AUC dosing for iv Bu - = 60+/- 5 mg*h/L. (see appendix for specific protocols for different donor sources and dosing)
- Avoid Melphalan 140mg/m^2 < 1 year of age unless HLH
- Treosulphan 36g/m^2 < 1 year of age (see appendix for specific protocols)
- If using ATG with protocols C or D – be aware of increased incidence of EBV-PTLD
- For these protocols if using matched UD or MFD – PBSCs are stem cell source of choice
- If using BM consider decrease in Campath 1H dose to 0.6mg/kg esp if condition requires full donor chimaerism as in WAS or MHC class II deficiency

1.2.6 Graft Source

There are three options available for graft source, which are bone marrow, peripheral blood stem cell (PBSC) derived from mobilization with GCSF (Growth colony stimulating factor) and umbilical cord blood. Donor factor is the main decisive factor for the selection of graft source. According to UK Human Tissue Act (2004), children aged less than 18 years old need to be assessed by an independent assessor before they can donate their bone marrow stem cells [55]. In situations where donors prefer to avoid, or have a contraindication for general anaesthesia, the option of PBSC is available for them. However, the option of PBSC is not available for paediatric donors due to risks associated with GCSF usage (such as splenic rupture).

The UK UCB (Umbilical Cord Blood) working group has proposed an algorithm for donor and stem cell source selection (Table 1.1) [56]. A matched family donor or MSD or umbilical cord stem cell from MSD remains the 1st choice for donor selection. Matched unrelated donor (10/10, high resolution HLA-matching) and/or umbilical cord blood from matched unrelated donor (6/6, low resolution for HLA-A, HLA-B and intermediate/high resolution for HLA-DRB1) are the second choice. Third choice are MUD with 9/10 HLA-matching and unrelated umbilical cord blood with 5/6 HLA-matching and the total nucleated cell more than 3 x 10^7/kg. The last option would be either haploidentical donor or unrelated umbilical cord blood with 5/6 HLA-matching and the total nucleated cell more than 3 x 10^7/kg or unrelated umbilical cord blood with 4/6 HLA-matching.

Table 1.1 Donor selection algorithm. Adapted from B.E. Shaw et al [56]

Choice	Family donor	Volunteer unrelated donor	Unrelated cord
Immunodeficiency/metabolic diseases			
1st	Matched family donor Matched cord (sibling)	-	-
2nd	-	10/10	6/6
3rd	-	9/10	5/6 (>3 x 10^7 TNC/kg)
4th	≤ 4/6	-	5/6 (>3 x 10^7 TNC/kg) 4/6

1.2.7 Summary

The availability of hematopoietic stem cell transplantation (HSCT) offers hope of a cure for SCID patients. Major advancements have led to safer HSCT with an improved immune re-constitution profile for the survivors. What is now needed is further study to objectively evaluate specific long-term outcome post-transplantation; focusing on impact of genotype-phenotype diagnosis and conditioning regimens on the long-term outcome and quality of life. Furthermore, introduction of newborn screening will improve the rate of detection, but at the same time create a major dilemma for choices of conditioning as SCID infants are usually healthy and infection free at presentation and are also very young. Concerns have been raised about the safety of using a toxic myeloablative regimen in these very young SCID infants. As such, long-term outcome of these patients is an extremely important area to be explore.

1.3 Outline of thesis

Following are the outline of the thesis. Chapter 2 will review the literature on the long-term outcome of HSCT for SCID. Objectives of the study are listed in Chapter 3. Chapter 4 will outline the methodology in detail. Results will be presented in Chapters 5-10. A decision has been made to present the results in accordance with each specific SCID genotype, one per chapter (Chapter 5-8). Chapter 9 will present the result of long-term outcome of newborn SCID of the Newcastle cohort and chapter 10 will present the outcome of more than 20 years for the Newcastle and London cohort. Chapter 11 will discuss the results and Chapter 12 will conclude the research. All appendixes were listed in Chapter 13.

Chapter 2 Literature Review

This chapter discusses the literature review of the long-term outcome of HSCT for SCID focusing on overall long-term outcome depending on disease specific outcome, immune reconstitution and conditioning, donor and graft sources and quality of life post-transplantation.

2.1 Long-term outcome of HSCT for SCID

2.1.1 Earlier publications of HSCT for SCID

The Stem Cell Transplantation for Immunodeficiencies (SCETIDE) registry is an electronic database for outcome of HSCT for primary immunodeficiency (PID) patients. SCETIDE have produced many publications on the outcome of allogenic HSCT in PID. Four important publications concerning post-transplantation outcome in SCID will be discussed in detail. These are available from the SCETIDE database and worldwide.

The first publication of SCETIDE data was by Fischer et al., (1986) [20]. This was a retrospective analysis of 162 patients with PID who underwent HSCT in 14 centres in Europe over 16 years (from 1969 until 1985). Eighty-seven patients had SCID, with 41 of 87 (47%) receiving HLA-matched HSCT and the remainder receiving T-lymphocyte depleted HLA-mismatched HSCT. Conditioning regimens were only instituted for five patients receiving T-lymphocyte depleted HLA-mismatched HSCT. Disease-free survival was better in the HLA-matched HSCT group, at 68% compared to the T-lymphocyte depleted HLA-mismatched HSCT group, which was 57%. Overall outcome was better in the HLA-matched HSCT recipients in terms of complete engraftment; with no severe acute GVHD episodes and absence of late graft failure and late complications. For T-lymphocyte depleted HLA-mismatched HSCT recipients, the main problem was failure of engraftment. Even from this first seminal publication, B-lymphocyte

chimerism was observed to be poorer in the non-conditioned recipients and ADA SCID tended to have poorer outcomes [20].

Buckley et al., (1999) reported their experience of SCID HSCT undertaken at Duke University Medical Centre, USA from 1982 until 1998 [57]. A total of 89 SCID infants were transplanted over the 16 year period. The majority were male patients (84%), X-linked SCID (48%) and received T-lymphocyte depleted marrow from haploidentical donors. Only 13% received transplants from an HLA-identical related donor. All were unconditioned except for three patients. Seventy-two patients (81%) were still alive at the time of follow-up with a median follow-up time of 5.6 years. The findings of this study are consistent with those of Fischer et al., (1986), where B-lymphocyte engraftment and function were worse than the T-lymphocyte, especially in unconditioned recipients. Up to 62% of patients still require regular intravenous immunoglobulin replacement therapy; as compared to almost 95% of the patients who achieved T lymphocyte engraftment post-transplantation. Another important finding was that T-lymphocyte function after T-lymphocyte depleted marrow HSCT was noted to be delayed up to 3-4 months post-transplant.

As time has moved on to the next millennium, patients' survival has improved tremendously. Multiple factors have been identified in contributing to better survival outcome such as an improvement in medical/intensive care, better quantification of CD34+ hematopoietic stem cells, better donor selection (development of high-resolution HLA tissue typing), application of reduced-intensity conditioning and better treatment of GVHD and infections [58].

Gennery et al., (2010) reported the outcomes of the SCETIDE database of HSCT for SCID and other PID from 1968 until 2005 [2]. A total of 699 SCID patients from 37 centres in Europe were studied (including the 87 patients from the first report). Better prognostic factors identified were transplantation after 1995, younger age at transplantation, B+ SCID phenotype, genoidentical and phenoidentical donors, absence of respiratory impairment and viral infection before HSCT. However, the main limitation of this study was the absence of data on immune reconstitution and B-lymphocyte function (immunoglobulin levels and antibody response to specific protein and polysaccharide antigens). Thus, even

though survival outcome had generally improved, the long-term immune reconstitution remains unknown.

Pai et al., (2014) described the outcome of 240 SCID infants who underwent HSCT in 25 centres in United States of America, between 2000 until 2009 [44]. The majority of the patients in this cohort were male (72%) and identifiable SCID mutations were found in 69% of the patients. Common γ chain remained the commonest SCID mutation identified. MSD was available for only 32 out of 240 patients (13%). Younger age at transplantation (less than 3.5 months old) and infection status pre-transplant were the significant positive prognostic factors identified, irrespective of donor type. Conditioned recipients were found to have an increased likelihood for higher CD3+ lymphocyte count, freedom from immunoglobulin replacement therapy and IgA recovery.

2.1.2 Disease specific outcomes

It is important to describe the long-term outcome of HSCT according to specific SCID genotype as the prognosis may differ and necessitate different strategies for HSCT depending on genotype [59]. Furthermore, this provides evidence based information useful for family counselling.

Earlier publications prior to 2010 focused on the comparison of outcome between B+ SCID and B- SCID, where findings consistently showed that B- SCID had a poorer outcome compared to B+ SCID [2, 57, 60]. SCID genotypes classified as B+ SCID were *IL2RG*, *JAK3*, *IL7Rα*, *CD45* SCID genetic mutations and those classified as B-SCID were *ADA*, *RAG1*, *RAG2*, *DCLRE1C* (Artemis) SCID. A few explanations have been proposed to clarify why B-SCID have a worse outcome. Firstly, the presence of double negative thymocytes in the thymus predisposed them to competition with the donors' stem cells. Secondly, presence of recipient NK cells causing rejection has been attributed to the engraftment failure [61]. Thirdly, presence of NHEJ complex deficiencies associated with B-SCID has been shown to have higher transplantation related complications due to a defective DNA repair mechanism especially after exposure to myeloablative alkylating agents [62].

Common γ chain deficient SCID (IL2RG SCID) has the most favourable T-lymphocyte engraftment outcome, even without conditioning or GVHD prophylaxis. This could be owing to the thymic niche availability concept. The defect is early in the lymphoid differentiation; hence the thymus is almost completely empty of double negative thymocytes. In relation to the highly conducive stromal environment, engraftment may occur readily [62]. B-lymphocyte engraftment remains an issue as those without conditioning tend to achieve lower B-lymphocyte chimerism compared to conditioned recipients [20, 63, 64]. Nevertheless, a series of publications demonstrated the occurrence of viral cutaneous warts in the IL2RG and IL7Rα SCID survivors' post-transplantation [43, 65-67]. No definitive causes or explanations have been offered as to why warts were found in IL2RG SCID and not in other SCID genotypes. A few theories have been postulated but not confirmed such as; association between the low number of NK cells in survivors, the role of Langerhan cells, and defective host keratinocytes [65, 67, 68]. It is important to look for warts in this cohort of patients as this might pose a future risk for malignancy as reported by previous studies [65, 67].

In terms of specific molecular defects, some of the SCID genotypes have their own associated features. Due to ubiquitous ADA gene expression, a profile of non-immunological manifestations have been described and some may not be corrected with HSCT [8, 69, 70]. The non-immunological manifestations include costochondral and skeletal dysplasia, neurologic deficits, bilateral sensorineural deafness, hepatic dysfunction and cognitive/behavioural deficits. Dermatofibrosarcoma protuberans have been reported in ADA SCID patients' post-transplantation [71]. A large series of 106 ADA SCID patients outcome post-HSCT from Europe, North America and Middle East has been published by Hassan et al (2012) [70]. Overall survival was influenced mainly by donor types (better in MSD and MRD, worse in haploidentical donor). Yet, the long-term immune reconstitutions were good with freedom from immunoglobulin replacement therapy seen; irrespective of donor types and conditioning regimen. However, caution is needed for the interpretation as data on immunoglobulin replacement were only available for 46 out of 106 patients (43%). The other limitation identified was the absence of a clinical outcome post-HSCT description.

Both Artemis SCID and RAG1/2 SCID are B- SCID with poorer outcomes compared to B+ SCID, in terms of overall survival and immune reconstitution. A multicentre study comparing 145 patients with ARTEMIS SCID and RAG1/2 deficiencies SCID found no significant differences in survival, early toxicity, or occurrence of tumours following HSCT; but ARTEMIS SCID developed more late complications post-transplantation associated with exposure to alkylating agents during conditioning [54]. A possible explanation is that Artemis SCID have a DNA repair defect affecting all cells, not just hematopoietic cells, which are sensitive to alkylating myeloablative conditioning. The study also showed that unconditioned recipients of RAG1/2 and Artemis SCID achieved lesser CD4+ T-lymphocyte count and were more likely to receive on going immunoglobulin replacement therapy, suggesting poorer myeloid engraftment post-HSCT. However, those who received conditioning had poorer overall survival and higher long-term side effects but better immune reconstitution. This study highlighted the need for safer conditioning to minimise long-term side effects, but at the same time ensuring maximal engraftment and better immune reconstitution.

2.1.3 Immune reconstitution and conditioning

The primary aim of hematopoietic stem cell transplantation for SCID is to cure the immune defect and achieve long lasting immune reconstitution of normal and functional T-, B- and NK-cells. Therefore, another important aspect of long-term outcome is the T-lymphocyte and B-lymphocyte profiles. Correlation between poor long-term T-lymphocyte function and poor early grafting has been suggested. CD4+ lymphocyte counts at 1-2 years post-HSCT were found to be a significant predictive factor for long-term CD4+ naïve lymphocyte numbers. [43, 72, 73]. Thymopoiesis, thymic output and T-lymphocyte diversity have been shown to continue until the second decade post-HSCT [49, 74].

Nevertheless, T-lymphocyte reconstitution is considered better compared to the B-lymphocyte outcome, even after allowing for all the differences of molecular type, transplant techniques and conditioning regimens involved [2, 57, 72].

In a single centre series, reporting outcomes of B-lymphocyte function on 125 patients with SCID, where all received non-conditioned HSCT and no GVHD

prophylaxis post-transplantation; only 29% achieved B-lymphocyte donor chimerism with the highest percentage seen in those with Common γ chain SCID (36%) and ADA SCID (33%) [63]. The authors concluded that B-lymphocyte outcome depends on the molecular phenotype, where in cases of IL-7Receptor α-Deficient, ADA SCID and CD3-Deficient SCIDs does not requires B-lymphocyte chimerism for normal B-lymphocyte function development. Whereas donor B-lymphocyte chimerism is essential in cases of Common γ chain SCID, JAK3 SCID and those with V(D)J recombination defects, possibly due to the defective cytokine receptors on the host B-lymphocytes [26].

Suggestions have been proposed for the role of conditioning in achieving higher myeloid and B-lymphocyte engraftment [75, 76]. From the literature analysis by Haddad et al., (2014), normal B-lymphocyte function was associated with busulfan and cycophosphamide conditioning [76]. The main limitation of this study was missing/incomplete data due to the retrospective review of multiple published evidence, and not being subjected to meta-analysis.

Debates continue about the best strategies for the conditioning options in HSCT and answers for these questions are to review the available evidence from published papers. However, as mentioned before, most of the published evidence is from observational studies, which have reduced strength, and the cohort populations are diverse, making direct comparisons impossible. The best would be for prospective observational studies or clinical trials that compare the efficacy and safety of many types and regimes of conditioning.

2.1.4 Donors and Graft Sources

There may be some overlap between the effect of donor and graft sources on HSCT outcome. Hence, both factors will be discussed together.

Donors remain the most important determinant factors of survival outcome but not in immune reconstitution outcome post-HSCT. Data from multiple cohort analysis studies have showed MSD as the best donor for most of the SCID genotype with survival outcome in the modern era approaching 90% [2, 44, 72]. The survival outcome of MUD was consistently better than haploidentical donors

across era of transplantation, however focusing only on the haploidentical HSCT data, there is an increasing better survival outcome trend [2, 20, 77]. This has been attributed to better HLA matching (high resolution DNA typing) and better T-lymphocyte depletion methods.

A relationship between timing of HSCT, patients' infection status before transplantation and survival outcome has been identified by Pai et al., (2014) [44]. Survival of alternative donor recipients was noted to be comparable to MSD recipients if HSCT were performed early and there was no infection prior to HSCT.

As previously mentioned, donor types are important for determination of survival outcome but not long-term immune reconstitution. Dovrak et al (2014) performed a direct comparison between unconditioned MUD and MSD HSCT [78]. There was no difference in T-lymphocyte engraftment, but unconditioned MUD was noted to have higher Grade II-IV acute GVHD, lesser myeloid and B-lymphocyte immune reconstitution. Even attempts of transfusing mega dose CD34+ cell grafts in unconditioned haploidentical recipients did not improve B-lymphocyte function in SCID patients post-HSCT [79]. These emphasize the importance of conditioning in ensuring better long-term immune reconstitution outcomes.

Bone marrow is the classical option for graft source in HSCT. The availability of donor registries have expanded the donor option to MUD and umbilical cord blood HSCT. Fernandes et al., (2012) compared the outcomes of patients with SCID or Omenn Syndrome undergoing HSCT, receiving mismatched related-donor transplantation versus unrelated-donor umbilical cord blood transplantation [80]. Data were retrospectively collected from the Eurocord & SCETIDE and EBMT. There were no significant differences in T-lymphocyte engraftment, CD4+ and CD3+ lymphocyte recoveries between both groups. However, a significantly higher frequency of complete donor chimerism, faster total lymphocyte count recovery, increased Grades II-IV acute GVHD, and chronic GVHD were noted in the unrelated cord blood recipients. As a conclusion, there were no differences in the engraftment performance in either unrelated cord blood transplantation or haploidentical related HSCT. However, higher occurrence of more severe acute GVHD and chronic GVHD were seen in unrelated cord blood HSCT.

Encouraging data have been published in identifying new efforts to improve HSCT techniques. Improved manipulation of the stem cell graft by TCRαβ and CD19 depletion has shown to speed up the T-lymphocyte immune reconstitution of the haploidentical recipients due to the TCR γδ in the graft that confers viral protection to the recipient [81-83]. This has successfully met the major shortcoming of delayed immune reconstitution found in haploidentical HSCT, and offers improved viral clearance during immediate post-transplantation.

Another improvement is the development of adoptive transfer of *ex vivo* selected donor derived T-lymphocytes in combination with the suicide gene, which offers the option of controlling the viral infection during the immediate post-HSCT period before thymus-derived T-lymphocyte immune recovery occurs [84]. The availability of suicide genes enables recognition and apoptosis of the donor T-lymphocyte if acute GVHD occurs; thus avoiding risk of GVHD and concurrently offering viral protection. Currently, it is still in clinical trial phase I-II and preliminary results have been promising [85].

2.1.5 Quality of Life post-HSCT

One important aspect of long-term outcome for SCID patients is their quality of life. As the immune defect have been corrected, the expectation is that SCID survivors would lead an optimal normal life. There are limited available publications on the quality of life (QoL) - most address HSCT survivors of haematological and malignancy diagnosis [86-88].

ADA SCID survivors have been found to have significant behavioural issues and cognitive disturbance, which diminish quality of life [69, 89]. Skucek et al (2011) have described social functioning difficulties in HSCT survivors for congenital immunodeficiency but mainly reported by parents and teachers and not the patients themselves [90].

A serial assessment of neurocognitive function in SCID patients comparing before and after HSCT showed that there was a delayed in developmental skills acquisitions compared to normal infant and toddlers [91].

A study of the UK's Chronic Granulomatous Disorder cohort showed better QoL in HSCT survivors compared to non-HSCT patients [92]. Even though chronic granulomatous disease is a PID, direct comparison is impossible as no SCID patients will survive more than 18-24 months if not transplanted.

Titman et al (2014) have demonstrated lower QoL in those receiving immunoglobulin replacement therapy for Primary Antibody Deficiency diseases in UK [93]. This is interesting as some SCID HSCT survivors do receive ongoing immunoglobulin replacement therapy, even though the clinical diagnosis is not similar for direct comparisons.

The French cohort of adults with PID diagnosed during childhood showed strong association between those with high disease burden and lower quality of life [94]. Thus it is important for this cohort of patients to be follow up lifelong so that early recognition and interventions can be offered.

Chapter 3 Objectives of the Study

3.1 Rationale for study

To date so far, numerous publications and reports on long-term outcome post-HSCT for SCID patients have been published [2, 20, 43, 44, 57, 60, 95]. Although the studies are multi-centre and involved larger cohorts of patients, the results are limited, because most are confined to descriptions of outcome of SCID as a single cohort, rather than separate analyses based on genotype. This is limiting, considering that more SCID patients are expected to survive the HSCT procedure itself. It is important for patient benefit, not only to understand what percentage will survive, but how that relates to genetic diagnosis. Furthermore the quality of life of survivors long after HSCT is important in relation to specific SCID genotypes. This information is important in guiding clinicians towards safer HSCT techniques with better long-term outcome.

3.2 Hypotheses

The main hypotheses for the study were:

- Survival outcome of conditioned SCID patients are lower than unconditioned recipients.
- Long-term medical issues in SCID survivors are influenced by their specific SCID genotypes.
- Conditioned recipients experience more long-term medical issues than unconditioned recipients.
- Thymopoiesis is better in conditioned recipients than unconditioned SCID patients.
- There is a correlation between myeloid chimerism and B-lymphocyte donor chimerism post-transplantation.
- Quality of life of SCID patients post-HSCT is equal to the UK's normal population.
- Newborn SCID have better survival and thymopoeisis compared to those who were diagnosed beyond neonatal period.

3.3 General Objective

- To examine the long-term outcome of patients with SCID who have undergone hematopoietic stem cell transplant (HSCT) in Newcastle over a 25 year period from 1987 - 2012.

- To examine the very long-term outcome of patients with SCID who have undergone hematopoietic stem cell transplant (HSCT), (more than 20 years post-transplantation) in Newcastle and London.

3.4 Specific Objectives

- To study the effect of SCID genotype and conditioning regimens prior to HSCT on the long-term immunological reconstitution and physical health (including growth, respiratory function, gastrointestinal, cardiac, dental and endocrine parameters)
- To evaluate the psychological development and quality of life in the Newcastle cohort.
- To identify associated factors influencing the outcome for those who had been transplanted.

Chapter 4 Methodology

The present study's methodology will be covered in this chapter. The study is a cohort study of the long-term outcome of SCID patients who underwent HSCT in Newcastle from 1987 until 2014, according to specific SCID genotypes (IL2RG/JAK3, IL7Rα SCID, Artemis/RAG 1/2 and ADA SCID). The second part of the study is about the more than 20 years outcome for all SCID patients, who underwent HSCT in Newcastle and London, UK.

4.1 Inclusion Criteria

With regards to the long-term outcome of the Newcastle SCID cohort; all patients with a SCID diagnosis of Common γ Chain deficiency, JAK3 deficiency, ADA SCID, IL7Rα deficiency, ARTEMIS SCID, RAG1 deficiency and RAG2 deficiency, according to the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015, were included in the study [22].

The International Union of Immunological Societies (IUIS) is an established expert committee on Primary Immunodeficiency Disorder. Introduction of the IUIS PID Classification has allowed for the standardisation of terminology, thus enabling everybody in clinical and research fields to communicate clearly and understand the same thing. This classification is being revised biennially and the latest publication was in 2015 [22]. It suggests classification according to the clinical and immunological phenotype of the disease. Table 4.1 further describes the genetic defects and clinical features associated with different SCID genotypes.

Patients who were at least 2 years post-transplantation were identified from the database available in the BMT unit, Ward 3, of the Great North Children's Hospital, Newcastle upon Tyne. A total of 120 SCID patients (77 males and 43 females) who had undergone a total of 146 transplants performed between 1987 and December 2012, were identified.

For the analysis of very long-term outcome UK SCID experience (Chapter 8); all SCID patients (irrespective of their SCID genotypes); who were more than 20

years post-transplantation at the Great North Children's Hospital , Great Ormond Street Hospital, London and the Royal Free Hospital, London were included in this study.

A group of patients who demonstrated immunophenotypic features similar to SCID but did not have proven underlying genetic defects were classified as "Undefined SCID". Additionally, for simplicity, all patients with a known gene defect of SCID but where there are less than 5 cases, were classified into "Others" [Newcastle cohort: CHARGE syndrome (4 patients), Reticular dysgenesis (2 patients), Zeta chain deficient SCID (1 patient), Cernunnos deficiency SCID (1 patient), CD3 Epsilon deficient SCID (1 patient)].

Table 4.1 The genetic defects and clinical features of various SCID genotypes (Adapted from Picard et al., 2015 [22])

Disease	Genetic defect/Presumed pathogenesis Gene OMIM	Inheritance	Circulating T-lymphocytes	Circulating B-lymphocytes	Serum Ig	Associated Features	Phenotype OMIM Number
T-B+ Severe Combined Immunodeficiency (SCID)							
γc deficiency	Mutation of IL2RG Defect in γ chain of receptors for IL-2, -4, -7, -9, -15, -21 308380	XL	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	300400
JAK3 deficiency	Mutation of JAK3 Defect in Janus activating kinase 3 600173	AR	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	600802
IL7Rα deficiency	Mutation of IL7RA Defect in IL-7 receptor α chain 146661	AR	Markedly decreased	Normal or increased	Decreased	Normal NK cells	608971
CD45 deficiency	Mutation of PTPRC Defect in CD45 151460	AR	Markedly decreased	Normal	Decreased	Normal γ/δ T-lymphocytes	608971

CD3δ deficiency	Mutation of CD3D Defect in CD3δ, chain of T-lymphocyte antigen receptor complex 186790	AR	Markedly decreased	Normal	Decreased	Normal NK cells No γ/δ T-lymphocytes	615617
CD3ε deficiency	Mutation of CD3E Defect in CD3ε chain of T-lymphocyte antigen receptor complex 186830	AR	Markedly decreased	Normal	Decreased	Normal NK cells No γ/δ T-lymphocytes	615615
CD3ζ deficiency	Mutation of CD3Z Defect in CD3ζ chain of T-lymphocyte antigen receptor complex 186780	AR	Markedly decreased	Normal	Decreased	Normal NK cells No γ/δ T-lymphocytes	610163
Coronin-1-A deficiency	Mutation of CORO1A Defective thymic egress of T-lymphocytes and defective T-lymphocyte locomotion 605000	AR	Markedly decreased	Normal	Decreased	Detectable thymus EBV-associated B-lymphocyte lymphoproliferation	615401

T-B- SCID							
DNA recombination defects							
RAG1 deficiency	Mutation of RAG1 Defective VDJ recombination; defect of recombinase activating gene (RAG)1 179615	AR	Markedly decreased	Markedly decreased	Decreased		601457
RAG2 deficiency	Mutation of RAG2 Defective VDJ recombination; defect of recombinase activating gene (RAG)2 179616	AR	Markedly decreased	Markedly decreased	Decreased		601457
DCLRE1C (Artemis) deficiency	Mutation of ARTEMIS Defective VDJ recombination; defects in Artemis DNA recombinase-repair protein 605988	AR	Markedly decreased	Markedly decreased	Decreased	Radiation sensitivity	602450
DNA PKcs deficiency	Mutation of PRKDC Defective VDJ recombination; defect in DNA PKcs	AR	Markedly decreased	Markedly decreased	Variable	Radiation sensitivity, microcephaly and	615966

	Recombinase repair protein 600899					developmental defects Autoimmunity and granuloma	
Cernunnos/XLF deficiency	Mutation of Cernunnos Defective VDJ recombination; defect in Cernunnos 611290	AR	Markedly decreased	Markedly decreased	Decreased	Radiation sensitivity, microcephaly and developmental defects	611291
DNA ligase IV deficiency	Mutation of LIG4 Defective VDJ recombination; defect in DNA ligase IV 601837	AR	Markedly decreased	Markedly decreased	Decreased	Radiation sensitivity, microcephaly and developmental defects	606593
Reticular dysgenesis, AK2 deficiency	Mutation of AK2 Defective maturation of lymphoid and myeloid cells (stem cell defect) Defect in mitochondrial adenylate kinase 2 103020	AR	Markedly decreased	Decreased or normal	Decreased	Granulocytopenia and deafness	267500

Adenosine deaminase (ADA) deficiency	Mutation of ADA Absent ADA activity, elevated lymphotoxic metabolites (dATP, S-adenosyl homocysteine) 608958	AR	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Decreased NK cells. Often with costochondral junction flaring, neurological features, hearing impairment, lung and liver manifestations; partial ADA deficiency may lead to delayed or milder presentation	102700

XL=X-linked, AR=Autosomal recessive

4.2 Exclusion Criteria

Omenn's Syndrome or Leaky SCID patients were excluded from the study because they are classified as having a combined immunodeficiency according to the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency (2015) [22].

Patients who had undergone another mode of therapy such as gene therapy, or thymic transplantation were also excluded from this study as they did not undergo hematopoietic stem cell transplantation as part of their treatment.

4.3 Cohort Characteristics

The Great Ormond Street Hospital, London and the Great North Children's Hospital, Newcastle upon Tyne perform HSCT specifically for primary immuno-deficiency diseases in the United Kingdom.

The paediatric Bone Marrow Transplantation (BMT) Unit at the Great North Children's Hospital (GNCH) was established in 1987. It is a supra-regional centre providing immunodiagnostic services and hematopoietic stem cell transplantation for primary immunodeficiency patients. Services are provided to patients from Northern England, the Midlands, Wales, Scotland, Northern Ireland and Eire. This centre performs approximately 40-60 hematopoietic stem cell transplantations per year, mainly for children with primary immunodeficiencies. All SCID patients who are more than 2 years post-transplantation attended the yearly follow up at the BMT Clinic, GNCH.

The Bone Marrow Transplant Unit in Great Ormond Street Hospital was established in the late 1970s. It is the largest paediatric BMT centre in the UK and performed 100 HSCT in 2013 [96]. The majority of transplanted SCID patients in Great Ormond Street Hospital aged more than 18 years old were referred to the Royal Free Hospital, London for transitional follow up services and further lifelong follow up. The frequency of clinic visits is yearly and may be more frequent depending on circumstances.

The transplant procedures, conditioning regimen and donor choices were chosen following EBMT Guidelines available at the time of transplant [51]. The gold standard for donor types is matched sibling donor (MSD). However, the MSD option was only available in 20% of cases most of the time. Other options of donor types are matched related donor (MRD), matched unrelated donor (MUD), mismatched unrelated donor (MMUD) and haploidentical donor. The decision for conditioning regimen options mainly depended on the donor type available and SCID genotypes.

There were 5 types of conditioning regimen available which were; myeloablative (MAC), low toxicity myeloablative (low toxicity MAC), (reduced intensity conditioning (RIC), non-myeloablative conditioning (NMA) and no conditioning received. Myeloablative conditioning consisted of busulfan (8 or 16mg/kg) and cyclophosphamide 200mg/kg. Low toxicity myeloablative conditioning consisted of either treosulfan and fludarabine (150mg/m^2) or treosulfan and cyclophosphamide (200mg/kg). Reduced intensity conditioning (RIC) consisted of fludarabine (150mg/m^2) and melphalan (140mg/m^2). Non-myeloablative conditioning consisted of non-myeloablative chemotherapy regimens such as Fanconi Anemia protocol. The addition of serotherapy (Alemtuzumab or rATG) were performed according to the ESID/EBMT Guideline protocol at the time [51].

4.4 Study Flow

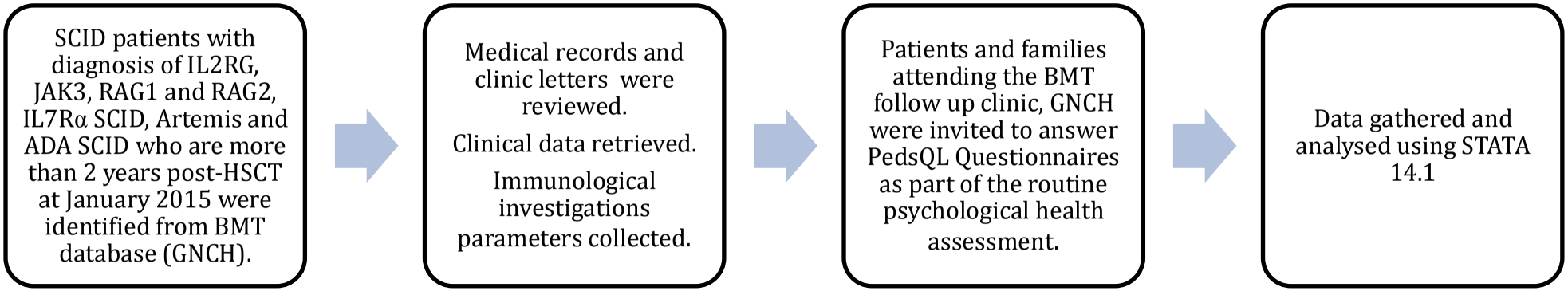
This study was divided into 2 phases. The first phase was to study and describe the long-term outcome of post-transplantation SCID patients in a Newcastle cohort, according to the specific SCID genotypes and newborn SCID. Newborn SCID was defined as those who were diagnosed or referred within neonatal period.

The second phase was to study and describe the more than 20 years long-term outcome of all SCID patients who had undergone HSCT before 1994 in Newcastle and Great Ormond Street Hospital, London and are currently being follow up at the Great North Children's Hospital, Newcastle upon Tyne or the Royal Free Hospital, London.

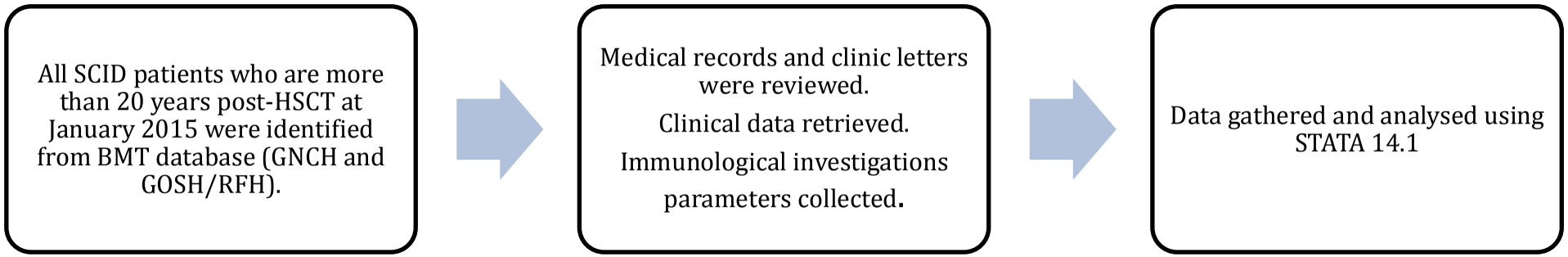
Patients' clinical records and clinic letters in all centres were retrospectively reviewed and collected into a specified proforma data. Clinical data and immunological investigation results were retrieved. With regards to the Newcastle cohort, patients and families attending the BMT follow up clinic in the GNCH were invited to complete the PedsQL questionnaires. This is performed concurrently with a psychological health assessment which was part of the routine check-up at the BMT clinic, GNCH. Figure 4.1 represents the graphic explanation of the study's flow.

Figure 4.1 Flow chart of the study (Phase I and Phase II).

Phase I (Newcastle cohort)



Phase II (Newcastle and London cohort)



4.5 Clinical Outcome

4.5.1 Survival Outcome

Overall survival was measured from the time of first transplant until the date of the last known follow up, or date of death. Transplant Related Mortality (TRM) was defined as all causes of death due to transplant, irrespective of time after transplant. The cause of death was retrieved from the patient's medical record available in the database.

4.5.2 Immediate post-HSCT Outcome

The time frame of immediate post-transplant outcome was defined as from 1 month post-transplant up to 2 years post-transplant. Variables measured as an immediate post-transplant outcome were; engraftment (neutrophil and CD3+ lymphocyte), the requirement for added interventions (e.g. boost or second transplant), acute GVHD and chronic GVHD. Donor lymphocyte engraftment was defined as presence of donor lymphocyte in the recipients system at day 30 post-transplantation. In context of this study, all variables for immediate transplant outcome were studied in first HSCT only. Neutrophil engraftment was defined as the time interval taken from the day of transplant (day 0) until the neutrophil count was more than 0.5 x 10^9/L for more than 3 consecutive days. The time taken for CD3+ lymphocyte recovery of more than 200 cells/µl was measured.

Patients who needed additional interventions after the first HSCT were quantified. Added intervention was defined as patients who needed further procedures such as a boost, second HSCT or donor lymphocyte infusions after their first HSCT. A boost is defined as a subsequent transfusion of allogenic or autologous hematopoietic stem cells, where the infusion is not preceded by a conditioning regimen [97]. Second HSCT is defined as HSCT after undergoing a previous transplantation. It requires re-qualification of a patient for transplantation and commonly involves a conditioning regimen directly followed by stem cell infusion [97]. Donor lymphocyte infusion is defined as an infusion of lymphocytes or T-lymphocytes from any source typically given after HSCT [97].

There is no preparative regimen or conditioning given to recipients prior to donor lymphocyte infusion.

Acute GVHD is a condition where the donor's immunologically competent T-lymphocytes mounted an inflammatory and cytotoxic immune response towards the target antigens on the recipient T-lymphocytes. Three phases have been identified in the pathogenesis of Acute GVHD. The first phase begins with the exposure of conditioning regimen causing tissue damage. This leads to the second phase which is the afferent phase, where both activation of host's antigen presenting ells (APC) and donor's T-lymphocyte occurs. Finally is the efferent phase, where marked release of inflammatory cytokines (TNF-α, IL-1, IL-6, IL-10) leads to tissue necrosis [98].

Acute GVHD occurs from the starting of HSCT until 100 days post-transplantation. In certain conditions, it may occur early during the neonatal period prior to transplantation due to materno-fetal lymphoid engraftment [99]. However, debates have been raised as to whether 100 days post-transplantation remains significant in differentiating between acute and chronic GVHD [98]. This is because patients have presented with acute GVHD-like illness beyond 100 days post-transplantation after the implementation of the RIC regimen.

Four organs involved in acute GVHD are skin, gastrointestinal system, liver and lung. Interestingly, kidneys are usually spared and the reasons are still unknown. Acute GVHD was identified and classified according to the Glucksberg Staging of Acute GVHD and Modified Glucksberg Grading Scale [98] (Table 4.2 and Table 4.3). Chronic GVHD was defined according to the NIH Classification of graft versus host disease (Table 4.4) [100].

Table 4.2 The Glucksberg Staging of Acute GVHD [98].

Stage	Skin based on maculopapular rash	Liver based on serum bilirubin (μmol/L)	Gastrointestinal tract based on quantity of diarrhea
+	<25% of body surface	34 - 50	>500 - <1000 ml
++	25 – 50% of body surface	51 – 102	>1000 - <1500 ml
+++	Generalised erythroderma	103 – 255	>1500 ml
++++	Generalised erythroderma with bullae and desquamation	>255	Severe abdominal pain with or without ileus

Table 4.3 Modified Glucksberg Grading staging of Acute GVHD [100].

Grade	Organ and stage of involvement
Grade I	Skin + to ++
Grade II	Skin + to +++
	Gastrointestinal tract and/or liver +
	Mild decrease in clinical performance
Grade III	Skin ++ to +++
	Gastrointestinal tract and/or liver ++ to +++
	Marked decrease in clinical performance
Grade IV	Skin ++ to ++++
	Gastrointestinal tract and/or liver ++ to ++++
	Extreme decrease in clinical performance

Table 4.4 NIH Classification of Acute and Chronic Graft versus Host Disease [100]

Category	Time of Symptoms after HSCT or DLI	Features of Acute GVHD	Features of Chronic GVHD
Acute GVHD			
Classic	<100 days	Present	Absent
Persistent, recurrent or late onset	>100 days	Present	Absent
Chronic GVHD			
Classic	No time limit	Absent	Present
Overlap syndrome	No time limit	Present	Present

4.5.3 Long-term clinical outcome

A complete physical examination and health assessment was performed by the attending consultants during each clinic visit. The patients' general well-being and any issues or concerns were addressed and explored. Medical issues were defined as any medical/health related problems that needed assessment and intervention. Patients receiving medications such as immunoglobulin replacement therapy, hormonal treatment, steroids and antibiotic prophylaxis were included in the on-going medication group.

Puberty status was assessed by the attending consultant during clinic follow up in all patients over 12 years old. Whenever possible, puberty status was recorded according to Tanner Staging [101]. However, if the information was not available from clinic letters, the puberty status was recorded as present or absent after consulting the consultation in charge of that patient. Documentation in the clinic letters was recorded in the proforma. Menarche status was assessed as present or

absent for all female patients aged more than 13 years old. Primary amenorrhea was considered in all female patients who had not achieved menses at the age of 16 years old [101]. Height (cm) and weight (kg) were measured during each clinic visit and were plotted in weight for age and height for age growth charts. Short stature was defined as height at or less than 2 standard deviation of the normal paediatric population at the specified age and gender [102].

The pulmonary function was assessed at the Great North Children's Hospital during the BMT follow up clinic, although for a few, the pulmonary function assessment was arranged locally in view of logistic issues. The results were sent from the local health facilities and were kept in the patient's medical record at the GNCH. The measurement of forced expiratory volume (FEV_1), full vital capacity (FVC) and FEV_1/FVC was recorded whenever available. The interpretation of the results was based on comparison between the observed values from patients to predicted normal range reference available in the individual centre. The pulmonary function result was further classified into normal, restrictive pattern, obstructive pattern or mixed pattern. According to the American Thoracic Society [103], for patients aged 5 to 18 years old; a normal pattern is defined as FEV_1/FVC > 85% of predicted value for height. Restrictive pattern is defined as FEV_1/FVC >85% of predicted for height and FVC < 80% of predicted value. An obstructive pattern is indicated by FEV_1/FVC < 85% of predicted value and FVC > 80% of predicted value. A mixed pattern is seen in those with FEV_1/FVC < 85% of predicted value and FVC < 80% of predicted value for height.

The accuracy of the all outcome data were verified with the consultants in charge taking care of the patients in this study (Dr Andrew Gennery and Dr Mary Slatter at the Great North Children's Hospital, Newcastle upon Tyne and Dr Siobhan Burns at the Royal Free Hospital, London).

4.6 Immunologic investigations and chimerisms

Longitudinal measurement of several immunological markers was performed; namely CD3+, CD19+, CD16/56+, CD4+, CD3+CD4+CD45RA+ (CD4+ Naïve lymphocyte), IgG, IgA and IgM. Serial baseline measurements were taken (pre-HSCT), 6 months after transplant, 1 year after transplant, 2 years after transplant

and at 5-yearly interval thereafter. For 5, 10, 15, 20 and 25 years post-transplant, measurements were taken at the nearest clinic follow up to the time for the above mentioned period.

The lymphocyte subset analysis was measured according to the Immunology Diagnostic Laboratory's protocol using 4-colour flow cytometry. In summary, lymphocyte surface marker studies were performed on fresh whole blood collected in EDTA by using appropriate markers (CD45 peridinin-chlorophyll-protein complex [PerCP], CD3 fluorescein isothiocyanate [FITC], CD4 allophycocyanin [APC], CD8 phyco- erythrin [PE], CD19 APC, CD16/CD56 PE, CD3 PerCP/CD4 APC/CD45RA- FITC/CD27 PE, CD19 PerCP/CD27 FITC/IgM APC/IgD PE; Becton Dickinson, UK Ltd, Oxford, United Kingdom) and analysed on a Becton Dickinson FACSCalibur flow cytometer. The T- and B-lymphocyte numbers were defined as normal or low by using age-specific reference ranges. The markers CD3+CD4+CD45RA+ were used as surrogates for thymic output.

There are 2 possible pathways of proliferation of donor T-lymphocytes in the recipient [49]. Firstly, is the peripheral expansion of the mature donor T-lymphocytes which may not last lifelong and has limited T-lymphocyte repertoire diversity. Secondly, is the thymopoiesis process; whereby the donor's progenitor cells seed the recipient's marrow and undergo intra-thymus differentiation, which has been shown to be sustained with high diversity of T-lymphocyte repertoire. The presence of CD3+CD4+CD45RA+ indicates the T-lymphocyte most likely but not exclusively originated from the graft as it can increase through an antigen independent pathway [104]. T-lymphocyte receptors excision circles (TRECSs) have been showed to be a more accurate marker of T-lymphocytes that have undergone recent intra-thymus differentiation [105, 106]. This is because TRECSs are a by-product produced during T-lymphocyte re-arrangements in the thymus. However, only CD3+CD4+CD45RA+ measurements were available for SCID survivors in follow up in GNCH from 1987 until 2015. Furthermore, earlier finding by Cavazzana-Calvo et al (2007) proved a strong correlation between the absolute counts of naïve CD31+CD45RA+CD4+ T-lymphocyte and TRECSs [104]. Thus, the markers CD3+CD4+CD45RA+ were used as surrogates for thymic output due to the retrospective nature of this study.

Chimerism analysis is a method of measuring the hematopoietic stem cell origin responsible for post-transplantation hematopoiesis [107]. This has been done routinely during early post-transplant period for the engraftment assessment and early detection of graft failure. In cases of long-term follow up, chimerism analysis is useful in delineating the success of transplant according to conditioning regimen and donor type.

Multiple assay techniques for chimerism analysis with varying sensitivity have been introduced since the 1960s. The principle basis of all these assay techniques is in differentiation of the genetic polymorphism between recipient and donor. The chimerism analysis in GNCH was based on XY-fluorescent in situ hybridization (FISH) or polymerase chain reaction amplification of short tandem repeats techniques, with sensitivity of 0.1 - 0.001% and 1 – 5%, respectively [107].

Cross-sectional measurement of donor cell chimerism analysis was analysed at the latest follow up available. This was chosen in view of standardisation of the techniques for ease of interpretation and to reduce the risk of missing data. The chimerism analysis result was presented as a percentage of donor cells according to specific lineage whenever available (T-lymphocyte, B-lymphocyte and myeloid cell). Full donor chimerism is defined as a state where all cells are of donor origin following allogenic HSCT, or in other words, 100% of donor cells [108]. Mixed donor chimerism was defined as a percentage of donor chimerism ranging from 20 – 95% and those with less than 20% of donor cell chimerism are categorised as recipient in origin.

4.7 Evaluation of Health Related Quality of Life

The World Health Organization (WHO) has defined quality of life as an individual's perception of their position in life in the context of culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns [109].

Health related quality of life (HRQoL) is another spectrum of multidimensional quality of life measurements in children apart from social indicators and subjective well-being [110]. PedsQL 4.0 Generic Core Scale Questionnaires was chosen as a tool to measure health related quality of life in this cohort [111]. It

was chosen in view of being validated, reliable and having the ability to differentiate between healthy children and children with chronic health conditions [111]. Furthermore, the availability of published UK normal population findings can be used as a comparison [112]. Approval to use the PedsQL questionnaire was obtained on 5th February 2014. The signed agreement and the PedsQL questionnaires are attached in Appendix A.

Patients and parents/caretakers were invited to answer the PedsQL questionnaires during their long-term clinic follow up at the GNCH. It was performed routinely as part of the psychological health assessment in the unit. Postal questionnaires were sent to those who failed to attend follow up sessions, with a return addressed and stamped envelope provided.

PedsQL 4.0 Generic Core Scale Questionnaires is a self-reported measure with 23 items (Appendix B). It also has a corresponding parent proxy-reported measure with similar formats. The children-report questionnaires were available for children between 5 – 18 years old. Similar questionnaires were also available for young adults aged from 18 years old onwards. Parent proxy-reports were available for children aged 2-4 years old and above. Due to age constraints, only parents answered for children aged 2 – 4 years old.

The PedsQL questionnaires comprised of assessment in 4 major domains, which were; Physical Functioning domains, Emotional Functioning domains, Social Functioning domains and School Functioning domains. The Total Summary Score (23 items) was recorded from the imputation of all 4 domains scores and divided by the number of items answered. The Psychosocial Health Summary Score was derived from the addition of 3 domains (Emotional Functioning domains, Social Functioning domains and School Functioning domains) and divided by number of items answered. Composite scores for each domain, total summary score and psychosocial health summary score were calculated as means as proposed by the original questionnaires developer [111]. The higher the value, the better health related quality of life is indicated. However, this questionnaires does not address the particular issue of employment in the young adults.

4.8 Statistical Analysis

The decision was made to analyse the long-term outcome for the SCID cohort in Newcastle according to SCID genotype in view of the complexity of the issues and difficulties in determination of causal association/relationship between donor type, conditioning regimen, graft source and the timing of HSCT. Division according to SCID genotypes distinctively characterizes each subgroup of patients in this SCID cohort.

The long-term outcome for IL2RG and JAK3 SCID patients post-transplantation were analysed together because patients with IL2RG SCID or JAK3-deficient SCID present with a similar immunophenotype, and the gene products are in the same signalling pathway. The long-term outcome post-transplantation for Artemis and RAG 1/2 SCID in Newcastle were also analysed together. Both SCID genotypes were presented and compared directly with each other; in view of both being T- B- SCID immunophenotype and involving defects in the V(D)J recombination pathway. However, the main differentiating feature is that Artemis SCID exhibits radiation sensitivity, which is not found in RAG 1/2 SCID. The ADA SCID and IL7Rα SCID cohort was analysed individually, due to the distinctive clinical manifestations and immunophenotype.

With regards to the outcome of newborn SCID, the results were analysed as a single cohort and comparisons were performed between newborn SCID and those who were diagnosed later. Whenever possible, detailed descriptions and comparison according to specific SCID genotypes were performed.

However, for the very long-term outcome post-transplantation of the Newcastle and London cohort, all patients were analysed as one single cohort irrespective of their SCID genotypes due to the small sample size.

All statistical analyses were undertaken using STATA version 14.1. Graphic figures were prepared using Graphpad Prism 7. Statistical significance of $p < 0.05$ was applied for all statistical analysis tests.

The normally distributed quantitative data are presented as mean with standard deviation. Parametric statistical analysis such as the T-test was performed to look for mean differences between groups of patients. Comparison of mean

differences with published data were performed using the one sample T-test. The non-normally distributed data were presented as median with range, and the differences between groups were analysed using a median test. The Fisher exact test was performed for measuring associations between group participants for $n<5$ and Pearson chi square for $n>5$. Spearman's correlation (rho) was performed to analyse correlation between variables (when the data are not normally distributed).

Survival outcome analysis was performed using Kaplan Meier Survival. Group comparisons of survival outcome were made between specific SCID genotypes and other SCID genotypes, and between those who were conditioned and those not receiving any conditioning regimen with a log rank test. An event was defined as death. Time until event was defined as the time interval from the date of first transplantation until the date of the last follow up or date of death. Outcome survival was calculated at 2 years and 10 years post-transplant. Patients who are still alive but lost to follow up by 31st January 2015 were censored from the analysis.

Multiple linear regression analysis (for continuous dependant outcome) and multiple logistic regression analysis (for binary dependant outcome) were used to assess associations and adjust for potential confounding factors. Variables were included in the analysis in multiple forward steps; considering those where the p value equal or less than 0.05. Several parameters such as likelihood ratio test, F-test, OR, coefficients and 95% confidence interval were presented in the result section.

Multi-level mixed modelling analyses was used to study the effect of conditioning on the longitudinal immune parameters reconstitution post-transplant. This is a hierarchal marginal model involving 2-level modelling analysis. The first level consists of individual SCID patients which were nested according to conditioning regimen group received (second level). The cluster was defined as the conditioning regimen group received (conditioned and unconditioned).

Multi-level mixed modelling analysis was chosen due to several factors. This analysis enabled study of the changes across time in each individual (level 1) and also between individuals (level 2) [113-115]. Importantly, it allows inference

about the sub-population averages and answers the research questions on the effect of conditioning on the longitudinal immune reconstitution post-transplantation [115]. It also caters for unbalanced data which are very common in this nature of study.

The following are the steps performed for multi-level mixed models to study the effect of conditioning on immunological parameters re-constitution across time.

1. Variables and data entered into STATA in wide format and re-shaped to long format.
2. Marginal models with immune re-constitution parameters (example: CD3+ lymphocyte count) as a continuous variable.
3. Plotting of original data and fitted lines for each sample to visually check the model
4. Generation of margins contrast and plot with original data mean values to visually check the model
5. Group comparisons of contrast mean at each point of time and overall time.

Due to the retrospective nature of the study, missing data were commonly found. Two types of missing data were identified in this study. Most of the data are missing completely at random especially with the immunological parameters post-transplantation. However, there is some possibility of missing data that depends on unobserved predictors. For example, missing data of clinical status and immunological parameters in those who defaulted at follow up.

Multiple steps were taken to minimize the impact of missing data [116]. Firstly, a trawl of information from multiple resources for each subject (clinic follow up letters, electronic databases of investigations results and individual patient hardcopy medical notes) was undertaken. Secondly, by using available-case analysis, analysis was done with the available data, ignoring the missing data. Thirdly, by choosing a statistical model such as multi-level mixed effect models, which was able to adjust for unbalanced longitudinal data. Of note, there were no missing data in the PedsQL questionnaires of quality of life. All PedsQL questionnaires respondents gave complete answers to all questions.

Chapter 5 Result - Long-term outcome of IL2RG/JAK3 SCID post-HSCT

This chapter will present the result of the analysis of long-term outcome for IL2RG and JAK3 SCID patients post-transplantation in Newcastle cohort. The common γ chain of the interleukin receptors -2, -4, -7, -9, -15 and -21 is critical for T lymphocyte development (through signalling via the γ chain receptors). Defective signalling through these molecular pathways leads to early arrest of T lymphocyte and NK cell development. Signalling through IL-4 is important for terminal B-lymphocyte differentiation and isotype switching. JAK3 is downstream of the common γ chain signalling pathways and so patients with common γ chain or JAK3-deficient SCID present with a similar immune-phenotype. Therefore, these two SCID variants were analysed as a single cohort.

5.1 Cohort Characteristics

Forty-three patients were identified from the database with a total of 49 transplants performed, constituting 35% of the Newcastle SCID cohort. Thirty-one of the 43 patients were alive in January 2015, with 100% attendance rate at the long-term follow up clinic at the GNCH.

The majority of patients received stem cells from a haploidentical donor (23 patients, 53%), followed by MRD (8 patients, 18%), MSD (5 patients, 12%), MUD (5 patients, 12%) and MMUD (2 patients, 5%). Table 5.1 shows details of donor type against the conditioning regimen used. No MSD recipients received conditioning.

Table 5.1 Conditioning regimen and donor type for each patient.

Conditioning regimen	MSD	MRD	MUD	Haploidentical	MMUD
None	5	1	2	6	0
Reduced intensity conditioning (RIC)	0	0	1	0	1
Low toxicity MAC	0	5	2	1	1
Myeloablative (MAC)	0	1	0	16	0
Non-myeloablative (NMA)	0	1	0	0	0
Total	5	8	5	23	2

Table 5.1 showed the distribution of conditioning regimen and donor type for IL2RG/JAK3 SCID patients in the Newcastle cohort. MSD = Matched sibling donor, MRD = matched related donor, MUD = matched unrelated donor and MMUD = mismatched unrelated donor.

The majority of patients (36, 83%) received bone marrow as the graft source. Five patients received umbilical cord blood stem cells and two received peripheral blood stem cells. Twenty patients received an un-manipulated graft. Twenty-three patients received a T-lymphocyte depleted graft (14 had CD34+ positively selected cells, 9 patients received monoclonal antibody and complement-depleted and 1 patient received CD3+/CD19+ depleted cells). Further details of median dose for CD34+ cells, CD3+ lymphocytes and CD19+ cells are listed in Table 5.2.

Table 5.2 The median value of recipients' weight, graft volume and stem cell doses.

Parameters	Median (Range)
Weight of Recipient (kg)	6.3 (3.5 – 8.7)
Volume Graft (ml)	63.5 (42.0 - 130)
Mononuclear cells ($x10^8$/kg)	2.7 (0.09 – 10.00)
CD34 cells ($x10^6$/kg)	7.8 (0.35 – 14.70)
CD3 cells ($x10^8$/kg)	0.02 (0.0001 - 1)
CD19 cells ($x10^7$/kg)	0.88 (0.11 – 3.8)

All data were shown as median value due to not normally distributed.

5.2 Immediate Outcome at less than 2 years post-HSCT

Thirty-eight patients were engrafted at one month after their first transplant. There was no evidence of T-lymphocyte engraftment after the first HSCT in 4 patients [NMA/MRD recipient (1 patient); unconditioned/haploidentical recipient (1 patient) and MAC/haploidentical recipients (2 patients)]. Five patients needed a second procedure in the form of a second HSCT. Further details of the reasons, conditioning and donor type for the first and second HSCT for these patients are listed in Table 5.3.

Table 5.3 Reasons, donor type and conditioning regimen of IL2RG/JAK3 SCID patients with added intervention.

Subject ID	Reason for added intervention	Conditioning/ Donor type for 1st HSCT	Conditioning/Donor type for added intervention	Status
42	Low T-lymphocyte numbers	RIC/ Haploidentical (father)	Unconditioned/ Haploidentical (father)	Died
48	Myeloid graft failure	MAC/ Haploidentical (father)	Unconditioned/ Haploidentical (father)	Alive
58	Falling T-lymphocyte chimerism	MAC/ Haploidentical (father)	Unconditioned/ Haploidentical (father)	Died
70	Graft failure	Unconditioned / haploidentical (mother)	Unconditioned/ haploidentical (mother)	Alive
76	Primary graft rejection and autologus reconstitution	NMA/ haploidentical (mother) Campath 1H=1mg/kg, Cy=200mg/kg, Anti LFA 2.8mg/kg, Anti CD2 2.8mg/kg	NMA/ haploidentical (mother) Campath 1G 1mg/kg, Cyclophosphamide 200mg/kg	Died

The median time taken for neutrophil recovery of more than 0.5 x 10^9/L was 18.5 days (range, 11-30). The unconditioned recipients did not experience a neutrophil count of less than 0.5 x 10^9/L, as expected. The fastest neutrophil recovery was seen in the MRD recipients with low toxicity MAC conditioning. The longest neutrophil recovery was seen in the MMUD recipients with low toxicity MAC (Figure 5.1). A two-way ANOVA was performed on a sample of 27 patients to examine the potential effect of donor and conditioning regimens (excluding unconditioned recipients as the neutrophil count was never below 0.5 x 10^9/L) on days taken for neutrophil recovery of more than 0.5 x 10^9/L post-transplant. There were no differences in neutrophil recovery between donor type ($p = 0.52$) and between conditioning regimens ($p = 0.63$). There was no significant interaction between donor type and conditioning regimens on neutrophil recovery ($p = 0.70$). There were no significant differences in neutrophil recovery between donor type ($p = 0.52$) and between conditioning regimens ($p = 0.63$).

Figure 5.1 Mean duration for neutrophil recovery according to donor type and conditioning regimen.

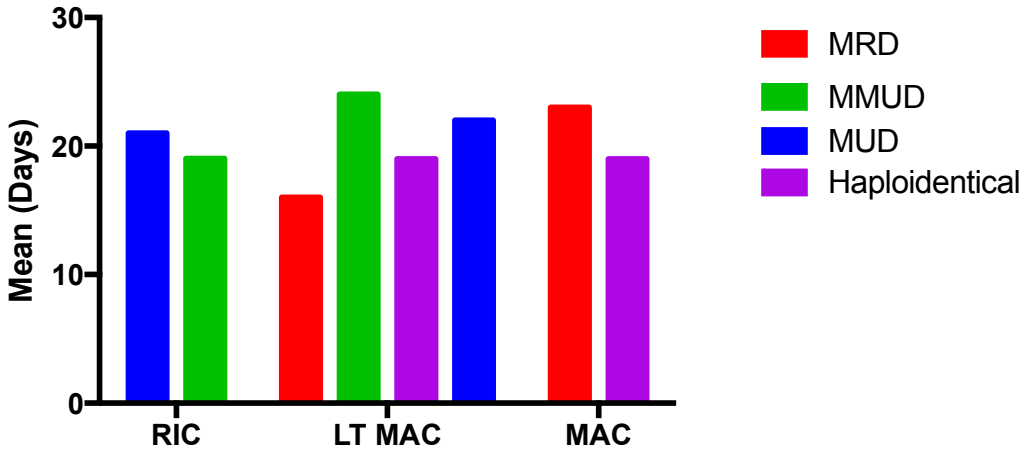


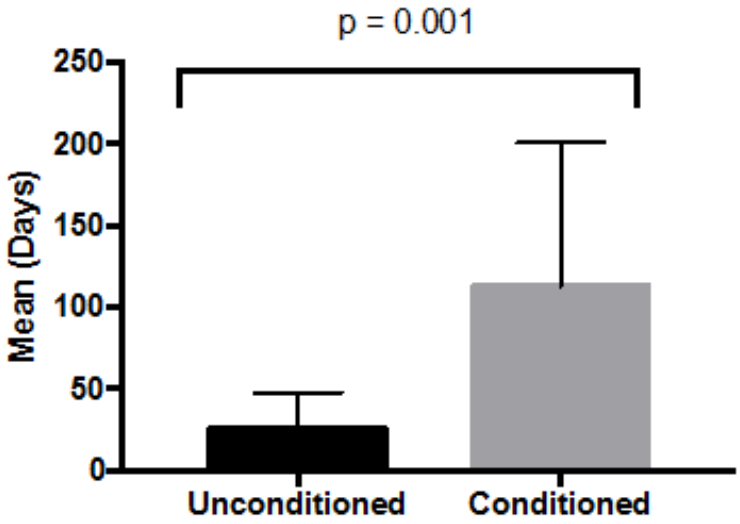
Figure 5.1 Matched related donor (MRD) receiving low toxicity MAC conditioning had the fastest neutrophil recovery and the slowest rate of neutrophil recovery was seen in mis-matched unrelated donor (MMUD) receiving low toxicity MAC regimen. Unconditioned recipients were not included as they never had neutrophil count less than 0.5 x 10^9/L.

The shortest time taken for CD3+ lymphocyte recovery of more than 200 cells/µl was 18 days and this was seen in unconditioned recipients (range, 11 – 83). The mean time taken for the CD3+ lymphocyte counts of more than 200 cells/µl in conditioned recipients was significantly longer, compared to unconditioned recipients; [113 days (SD, 88) versus 26 days (SD, 22), respectively, p = 0.001] (

Figure 5.2).

Figure 5.2 Comparison of mean days taken for CD3+ lymphocyte recovery of more than 200cells/µl between unconditioned and conditioned recipients.

Bars indicate standard deviation for each group.



Among the conditioned recipients, MRD with MAC conditioning had the fastest recovery and MURD with RIC conditioning had the slowest CD3+ lymphocyte recovery (Figure 5.3). A two-way ANOVA was performed on a sample of 23 patients to examine the effect of donor and conditioning regimens (excluding unconditioned recipients as the CD3+ lymphocyte count was never below 200 cells/µl) on days taken for CD3+ lymphocyte recovery of more than 200 cells/µl post-transplant. There were no significant differences in CD3+ lymphocyte recovery between donor type (p = 0.97) and between conditioning regimens (p = 0.84).

Figure 5.3 The mean duration of days taken for CD3+ lymphocyte recovery according to donor type and conditioning regimen.

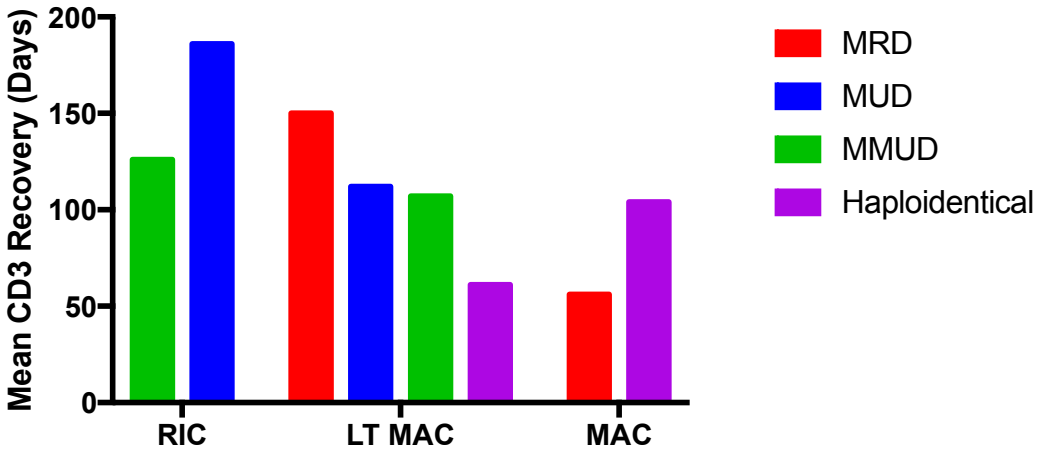


Figure 5.3 Matched related donor (MRD) receiving myeloablative (MAC) conditioning had the fastest CD3+ lymphocyte recovery. The slowest rate of CD3+ lymphcoyte recovery was seen in matched unrelated donor (MUD) receiving RIC regimen.

Twenty-four patients (56%) did not develop acute GVHD) after the first transplant. Nine patients developed Grade I acute GVHD. Eight patients developed Grade II acute GVHD. There were two patients with Grade III-IV acute GVHD (1 patient was an unconditioned haploidentical recipient and 1 patient received a MRD graft with non-myeloablative conditioning) (Figure 5.4). Two patients experienced chronic GVHD at 2 years post-HSCT [Unconditioned MURD (1 patient), haploidentical with MAC (1 patient)]. Further analysis showed that the time for CD3+ lymphocyte recovery was significantly shorter in those without acute GVHD compared to those with acute GVHD, mean 56.1 days (SD 55.6) vs 111 days (SD 98.2), ($p = 0.05$).

Figure 5.4 Numbers of patients experiencing Acute GVHD (Grade I, II, III, IV) after first HSCT according to donor type and conditioning regimen.

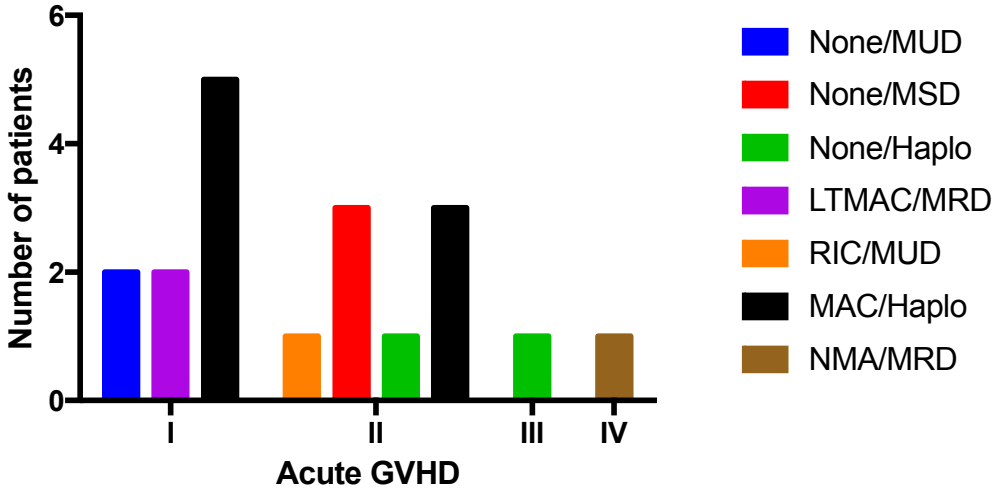


Figure 5.4 Seventeen out of 41 patient developed acute GVHD Grade I – II and only 2 patients developer severe acute GVHD Grade III-IV. Majority of acute GVHD Grade I – II patients received haploidentical donor and MAC conditioning regimen.

5.3 Survival Outcome

Thirty-one patients survived to January 2015 and twelve patients (28%) had died by this point. The median age at the last follow up was 10 years (range, 2-25 years). The majority of deaths occurred within 1 year after the transplant (10 patients, 83%). The causes of death were: infection (4 patients), hemorrhage following liver biopsy (2 patients), pneumonitis (3 patients), GVHD (1 patient) and Sudden Infant Death Syndrome (1 patient).

The survival outcome at 2 years post-HSCT was 74.4% and at 10 years was 71.9% for all IL2RG/JAK3 SCID patients in Newcastle. Transplant related mortality (TRM) was defined as all causes of death related to the transplant procedure, irrespective of time of the event. The TRM for this cohort was 23.3%. There was no significant difference in survival outcome between IL2RG/JAK3 SCID as compared to other SCID genotypes (71.8% versus 74.6%, respectively, p = 0.71) (Figure 5.5). There was no significant difference in survival outcome

comparing the unconditioned versus conditioned IL2RG/JAK3 SCID recipients; (69.8% versus 72.4%, respectively, p = 0.91) (Figure 5.6).

Figure 5.5 Comparison of survival outcome between IL2RG/JAK3 SCID and other SCID genotypes.

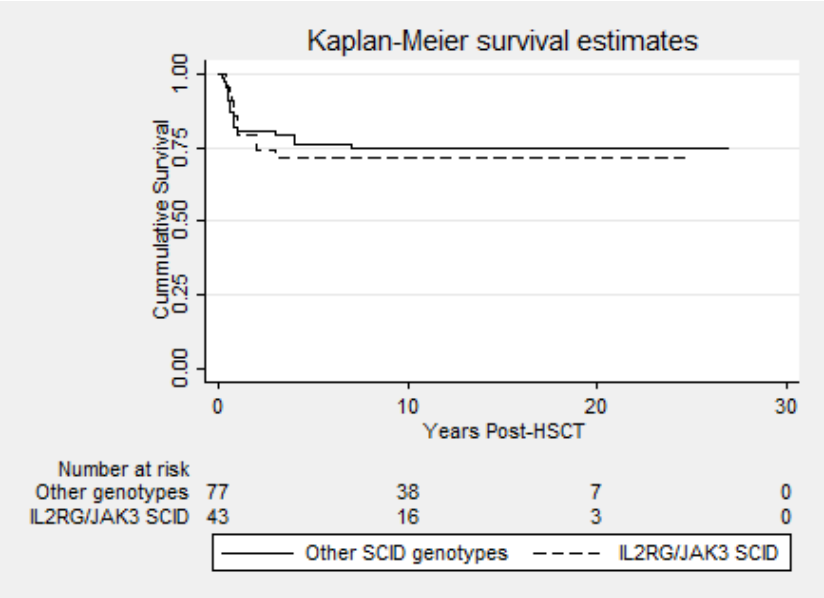
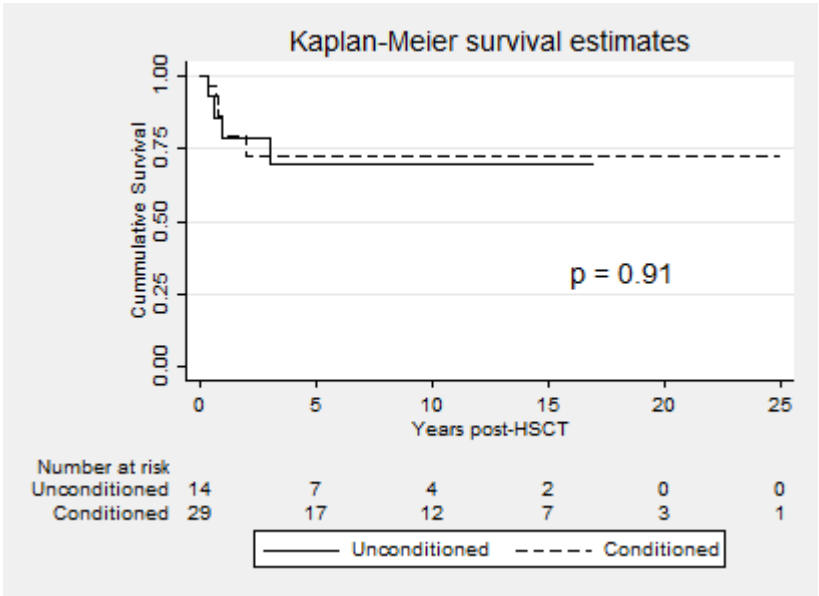


Figure 5.6 Comparison of survival outcome among the unconditioned and conditioned recipients of IL2RG/JAK3 SCID.



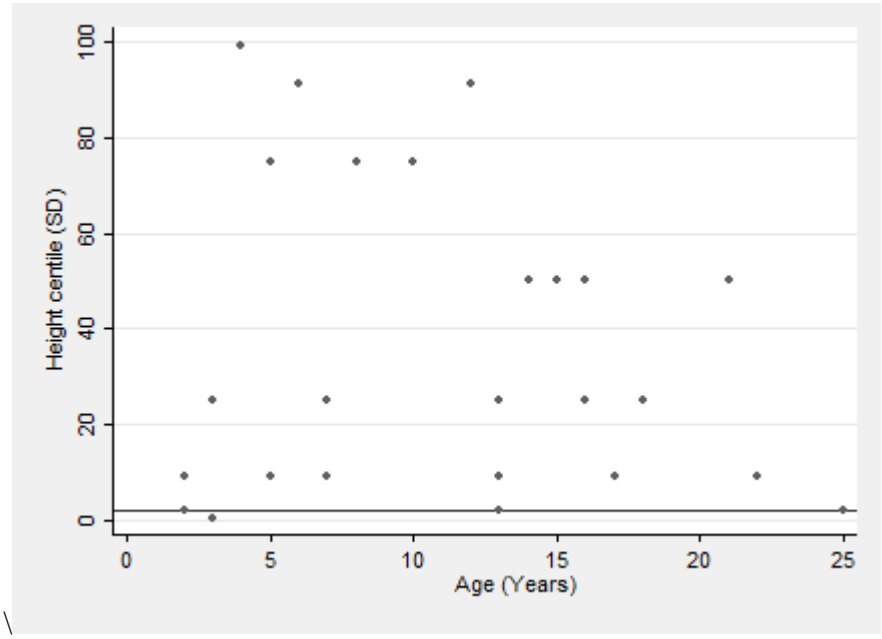
5.4 Long-term clinical outcome

5.4.1 Clinical outcome

Twenty-one patients (68%) had on-going medical issues at the last follow up in January 2015. Two patients had bronchiectasis. Lung function results were available for 6 patients including those with bronchiectasis, and all were normal.

Four patients had short stature (Height centile for age ≤ 2SD). Two of these 4 patients received low toxicity MAC and another 2 patients received MAC (busulfan 8mg/kg). However, there was no association between short stature and conditioning regimen, Fisher exact test, p = 0.49. Of the 4 patients with short stature, only one was aged more than 15 years (Figure 5.7).

Figure 5.7 A scatter plot of IL2RG/JAK3 SCID patients' height centile (SD) and age at last follow up.



*The red line indicates the reference range of the height centile = 2SD according to the normal population [102].

Thirteen patients (87%) aged above 13 years had achieved puberty according to assessment by the attending consultant paediatrician during post-transplantation clinic follow up. Three patients had pre-existing limb lymphoedema. One patient

developed autoimmune hemolytic anemia and another patient had delayed developmental milestones (Table 5.4). All 31 surviving patients were normal in the following clinical systems: endocrine, hearing, cardiovascular, renal and gastro-intestinal systems.

Seven patients had warts and there was no significant difference in the number of patients with warts between unconditioned and conditioned recipients (p = 0.70). There was also no significant difference in NK cells value at the latest follow up between those with warts and those without, p = 0.53 (Figure 5.8).

Figure 5.8 NK cells at latest follow up according to those with warts and those without.

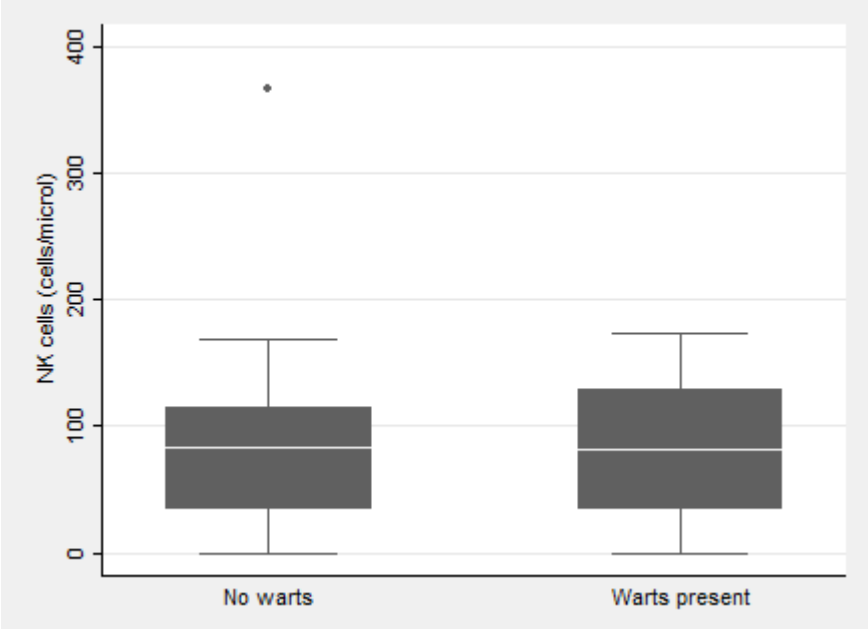


Table 5.4 Summary of long-term clinical outcome of IL2RG/JAK3 SCID post-transplant.

Clinical Outcome	% (n/N)
10 years survival	71.9 (31/43)
On-going Medical Issues	68% (21/31)
On-going IVIG Replacement Therapy	45% (14/31))
Bronchiectasis	7% (2/29)
Short Stature	14% (4/28)
Lymphoedema of the limbs	10% (3/29)
Warts	24% (7/29)
Auto-immune Haemolytic Anaemia (AIHA)	3% (1/29)
Delayed developmental milestone	3% (1/29)
Normal Lung Function	100% (6/6)
Normal Endocrine	100% (31/31)
Achieved Puberty	87% (13/15)
Normal Hearing	100% (31/31)
Normal Cardiovascular	100% (31/31)
Normal Renal System	100% (31/31)
Normal Gastro-intestinal System	100% (31/31)

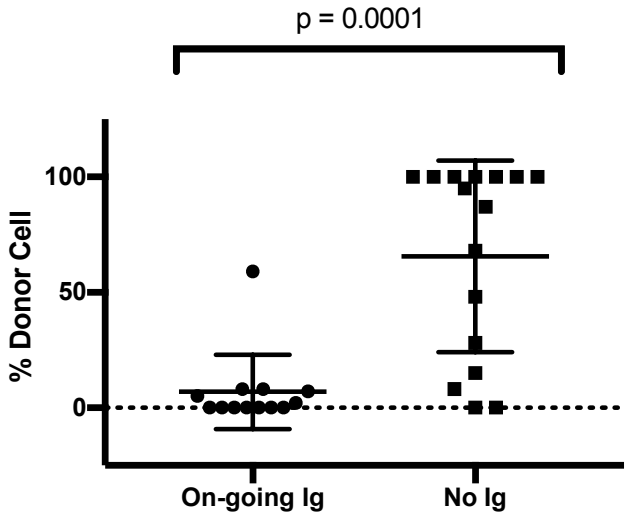
5.5 B-lymphocyte function at latest follow up

5.5.1 Immunoglobulin replacement therapy at last follow up

Seventeen of the surviving patients (55%) were free from immunoglobulin replacement therapy at the last follow up. All survivors of low toxicity MAC were free from immunoglobulin replacement therapy and had donor B-lymphocyte chimerism of more than 50%, irrespective of donor type. Notably, 3 patients with poor B-lymphocyte donor chimerism (less than 10% donor) were not receiving immunoglobulin replacement (2 of these 3 patients had acute GVHD Grade II-III). However, one patient was not included as the latest B-lymphocyte donor chimerism was not available.

Fourteen patients (45%) were receiving on-going immunoglobulin replacement therapy at the last follow up in 2015. Most had poor B-lymphocyte chimerism at the last follow up, except for one patient who received a haploidentical donor with MAC conditioning and had mixed B-lymphocyte donor chimerism (50 – 99% donor). There is a significant association between immunoglobulin replacement therapy status at last follow up and B-lymphocyte donor chimerism, p = 0.0001 (Figure 5.9).

Figure 5.9 B-lymphocyte donor chimerism at last follow up according to the immunoglobulin replacement therapy status.



5.5.2 B- lymphocyte and myeloid Chimerism at last follow up

Donor cell chimerism analysis was performed as part of the routine post-HSCT follow up for IL2RG/JAK3 SCID patients. Results were available for 29 out of 31 patients. Good T-lymphocyte donor chimerism was observed for all patients, irrespective of conditioning or donor type.

The donor B-lymphocyte and myeloid donor chimerism distribution varied depending on the donor group and conditioning regimen received. However, donor B-lymphocyte and myeloid donor chimerism tended to mirror each other in distribution. Figure 5.10, clearly demonstrates that patients with low B-lymphocyte donor chimerism tended to have low myeloid chimerism. Low toxicity MAC recipients tended to have better B-lymphocyte and myeloid donor chimerism irrespective of donor type. Unconditioned MSD transplants and haploidentical transplants with MAC conditioning demonstrated poor B-lymphocyte and myeloid donor chimerism at the last follow up (less than 20% donor chimerism).

Figure 5.10 B-lymphocyte and myeloid donor chimerism at last follow up according to corresponding donor groups and conditioning regimens received for each IL2RG/JAK3 SCID patient at last follow up.

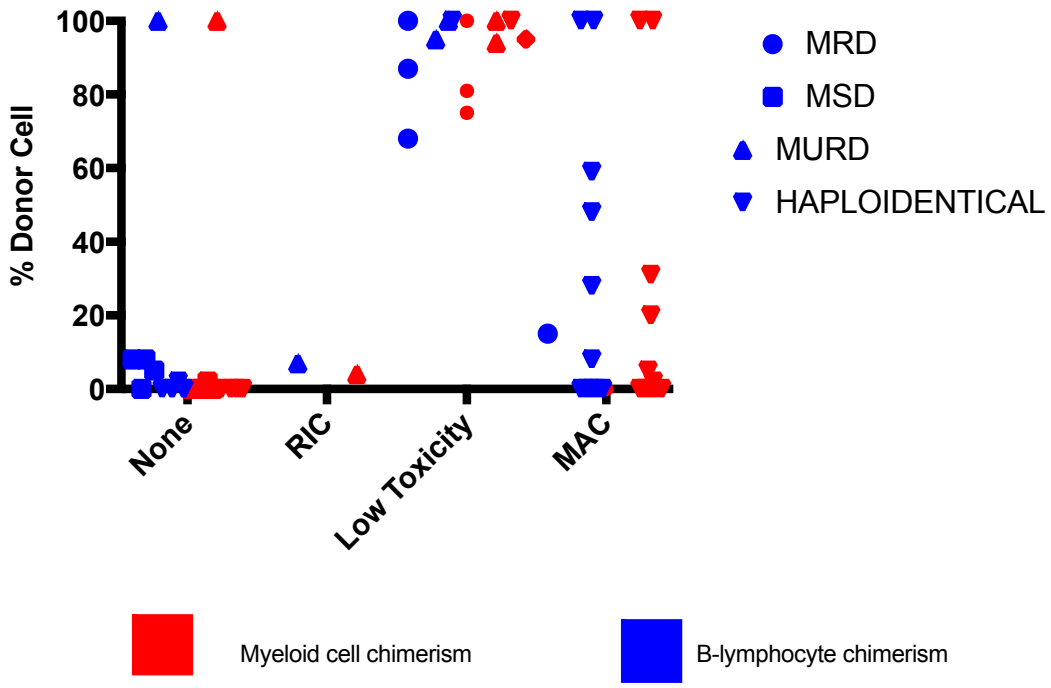
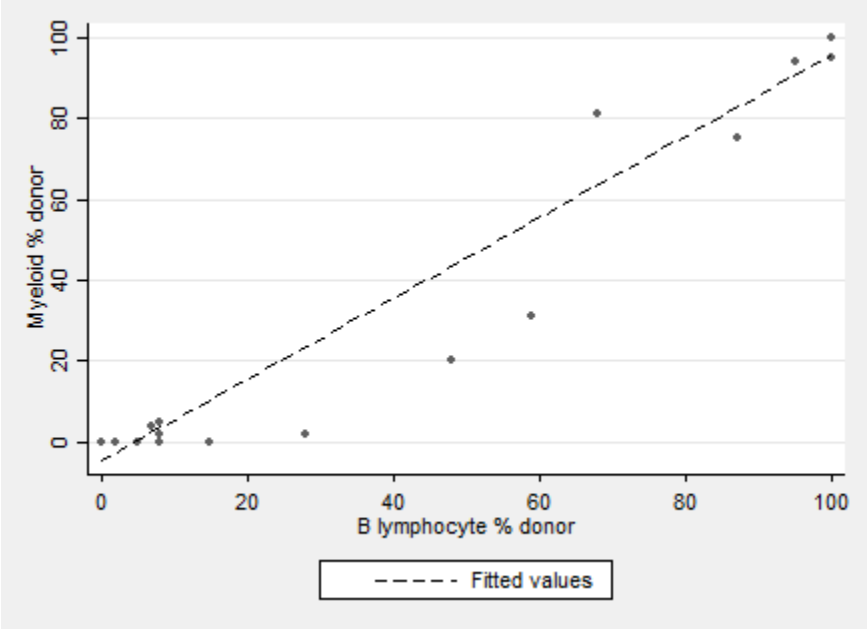


Figure 5.10. Low toxicity MAC recipients demonstrated higher B-lymphocyte and myeloid donor Chimerism, irrespective of donor types. Unconditioned MSD mostly had poor donor B-lymphocyte and myeloid donor chimerism (less than 20%). Haploidentical MAC recipients showed a wide range of donor B-lymphocyte and myeloid chimerism.

There was a strong positive correlation between donor B-lymphocyte chimerism and myeloid cell donor chimerism which was statistically significant, rho 0.98, p < 0.001 (Figure 5.11).

Figure 5.11 Scatter plot of donor B-lymphocyte chimerism and myeloid donor chimerism at last follow up.



The multivariable linear regression analysis showed that low toxicity MAC recipients had a significantly higher percentage of myeloid donor chimerism compared to unconditioned and other types of conditioning recipients (RIC & MAC8), after controlling for donor type, graft source and stem cell dose. The variables included in the final model explained 97.6% of the variation in myeloid donor chimerism (Table 5.5).

Table 5.5 Results of the multiple linear regression analysis of different variables on the percentage of myeloid donor chimerism at last follow up

Myeloid donor %	Coefficient	95% CI	p value
CD34 dose	-0.4	-1.8 – 1.0	0.56
CD3 dose	3.1	-23.0 – 29.3	0.79
CD19 dose	-1.0	-7.7 – 5.7	0.75
Donor types			
MSD	1.0	Reference	
MRD	-2.2	-31.8 – 27.4	0.87
MUD	78.7	-63.2 – 220.6	0.25
MMUD	75.8	-70.1 – 221.7	0.28
Haploidentical	-1.8	-17.6 -13.8	0.79
Conditioning regimen			
Unconditioned	1.0	Reference	
RIC	-8.5	-50.9 – 33.8	0.66
Low Toxicity MAC	85.5	52.7 – 118.4	< 0.001
MAC8	6.1	-10.5 – 22.8	0.43
Graft source			
BM	1.0	Reference	
PBSC	15.0	-26.5 – 56.6	0.44
UCBT	-71.2	-225.3 – 82.8	0.33

CI indicates confidence interval
p value < 0.05 was considered significant

5.6 Long-term immune reconstitution post-HSCT

5.6.1 Longitudinal analysis of CD3+ lymphocyte reconstitution post-HSCT

T-lymphocyte, B-lymphocyte and NK cell enumeration and CD4+ Naïve lymphocyte measurement was performed for all IL2RG/JAK3 SCID during their post-HSCT follow up visits to the Great North Children's Hospital in Newcastle. Results were available for 29 out of 31 patients. A serial measurement for every patient was recorded at intervals of 6 months, 1 year, 2 years and subsequent 5 yearly intervals post-transplant.

Multi-level mixed modelling analysis was used to study the effect of conditioning on the longitudinal immune reconstitution. The degree of differences in immune parameters' mean values between conditioned and unconditioned groups at each time point are presented as contrast (Table 5.6). Negative values imply the inverse relationship, where unconditioned groups have a higher immune parameter value compared to the conditioned group. SE indicates standard error and it serves as an indicator for precision of mean values as population parameters [117].

There was no significant difference in the overall trend of circulating CD3+ lymphocyte numbers between conditioned versus unconditioned IL2RG/JAK3 SCID patients, ($p = 0.38$) and at each time point post-transplant (Figure 5.12 and Table 5.6).

Figure 5.12 Longitudinal analysis of CD3+ lymphocyte output for IL2RG/JAK3 SCID patients post-HSCT according to conditioned and unconditioned recipients.

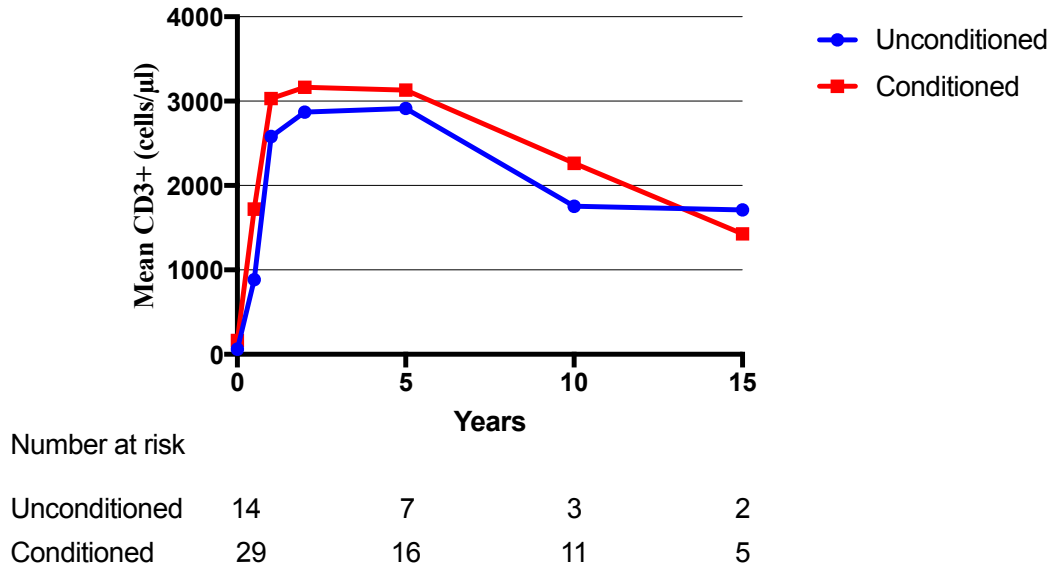


Figure 5.12. CD3+ lymphocyte trend during post-HSCT period. There was no significant difference in CD3+ lymphocyte trend between conditioned and unconditioned recipients of IL2RG/JAK3 SCID patients.

Table 5.6 Multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-transplant for IL2RG/JAK3 SCID patients.

Time (Years)	Contrast	SE	p value
0	503.2	365.2	0.16
0.5	355.2	300.9	0.23
1	207.1	309.2	0.50
2	59.1	385.4	0.87
5	-88.9	499.4	0.85
10	-236.9	630.9	0.70
15	-385.0	771.2	0.61
Overall trend			0.38

SE indicates standard error.
P value < 0.05 is considered significant.

Sustained CD3+ lymphocyte levels are seen even after 20 years post-HSCT, irrespective of donor and conditioning regimen received by IL2RG/JAK3 SCID patients (Figure 5.13 and Figure 5.14).

Figure 5.13 Longitudinal analysis of CD3+ lymphocyte output of IL2RG/JAK3 post-HSCT according to the donor type.

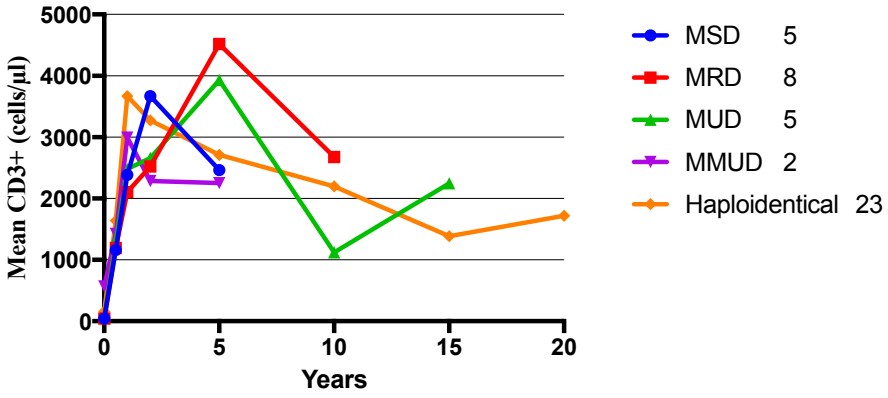


Figure 5.13. Mean value of CD3+ lymphocyte count during post-HSCT period according to the different donor types. The CD3+ lymphocyte tends to peak during the second year post-HSCT and is trending downward from 5 years post-HSCT.

Figure 5.14 Longitudinal analysis of CD3+ lymphocyte output of IL2RG/JAK3 SCID patients post-HSCT according to the conditioning regimen.

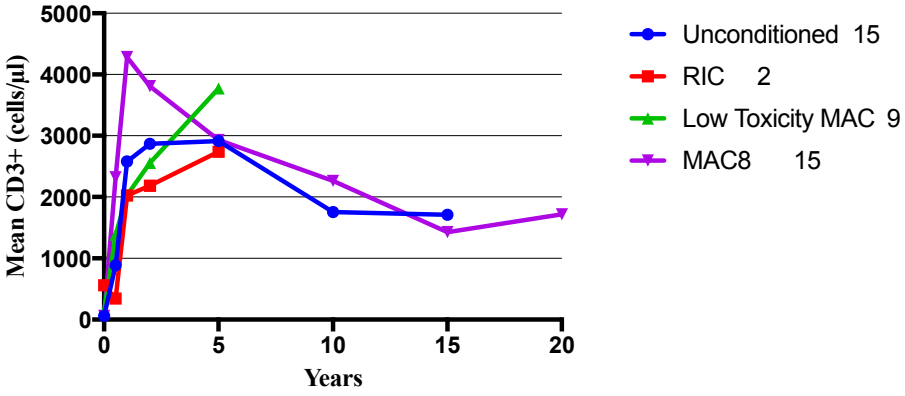


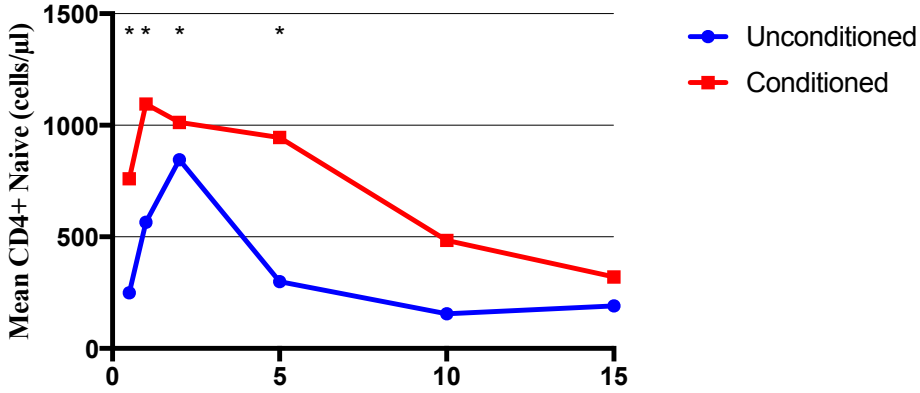
Figure 5.14. Mean value of CD3+ lymphocyte count during post-HSCT period according to the different conditioning regimens. The CD3+ lymphocyte tends to peak during the second year post-HSCT and is trending downward from 5 years post-HSCT.

5.6.2 Longitudinal analysis of CD4+ Naïve lymphocyte reconstitution post-HSCT

CD4+ naïve lymphocyte (CD3+CD4+45RA+) count was used as an indicator of thymopoiesis post-transplantation. The multi-level mixed effect analysis demonstrated that conditioned recipients had an overall trend to higher CD4+ naïve lymphocyte output compared to unconditioned recipients, ($p = 0.06$) (Figure 5.15 and Table 5.7).

Conditioned recipients had a better CD4+ naïve lymphocyte output at early time points after transplant compared to unconditioned recipients [0.5 years post-transplant ($p = 0.03$), 1 year post-transplant ($p = 0.02$), 2 years post-transplant ($p = 0.01$) and 5 years post-transplant ($p = 0.04$); respectively] (Table 5.7). However, this significant difference is lost in between groups from 10 to 15 years post-HSCT.

Figure 5.15 Longitudinal analysis of CD4+ Naive lymphocyte output for IL2RG/JAK3 SCID patients according to unconditioned versus conditioned recipients.



Number at risk				
Unconditioned	8	6	3	2
Conditioned	12	13	10	6

* indicates $p < 0.05$

Table 5.7 Multi-level mixed effect model analysis of conditioning on CD4+ Naive lymphocyte output with time post-transplant for IL2RG/JAK3 SCID patients.

Time (Years)	Contrast	SE	p value
0.5	611.1	286.1	0.03
1	513.1	224.8	0.02
2	415.1	178.0	0.01
5	317.0	159.0	0.04
10	219.0	177.1	0.21
15	121.0	223.4	0.58
Overall trend			0.06

SE indicates standard error.
P value < 0.05 is considered significant.

Sustained thymic output was seen at 15 years in MURD and haploidentical donor recipients. However, the best thymic output was seen in the MRD recipients, but the data were only available up to 10 years post-transplant (Figure 5.16). All except unconditioned recipients showed a mean of CD4+ Naïve lymphocyte of more than 500 cells; and low toxicity MAC recipients had the highest mean value 5 years post-transplantation (Figure 5.17).

Figure 5.16 Longitudinal analysis of CD4+ Naive lymphocyte for IL2RG/JAK3 SCID according to donor type.

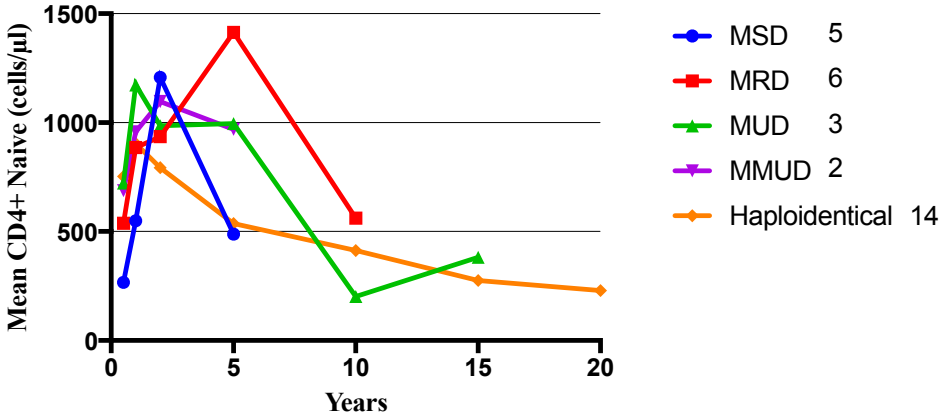
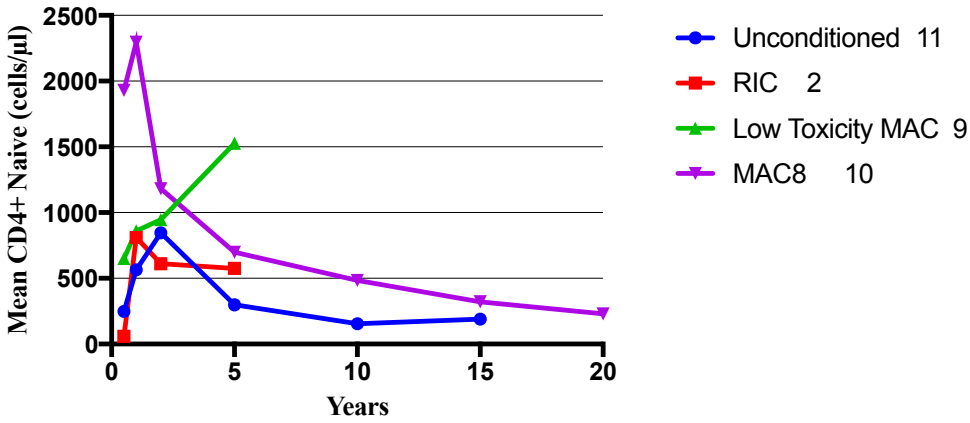


Figure 5.17 Longitudinal analysis of CD4+ naive lymphocyte of IL2RG/JAK3 SCID patients post-HSCT according to conditioning regimen.



5.6.3 Longitudinal analysis of CD19+ lymphocyte reconstitution post-HSCT

There was a reduction in the mean CD19+ lymphocyte output in both unconditioned and conditioned recipients at 0.5 years post-transplantation. There was no significant difference between conditioned and unconditioned recipients in the overall trend of CD19+ lymphocyte after transplantation, ($p = 0.47$). There was no significant difference in CD19+ output at any time point post-transplantation (Figure 5.18 and Table 5.8).

Figure 5.18 Longitudinal analysis of CD19+ lymphocyte output for IL2RG/JAK3 SCID patients post-transplant according to conditioned and unconditioned recipients.

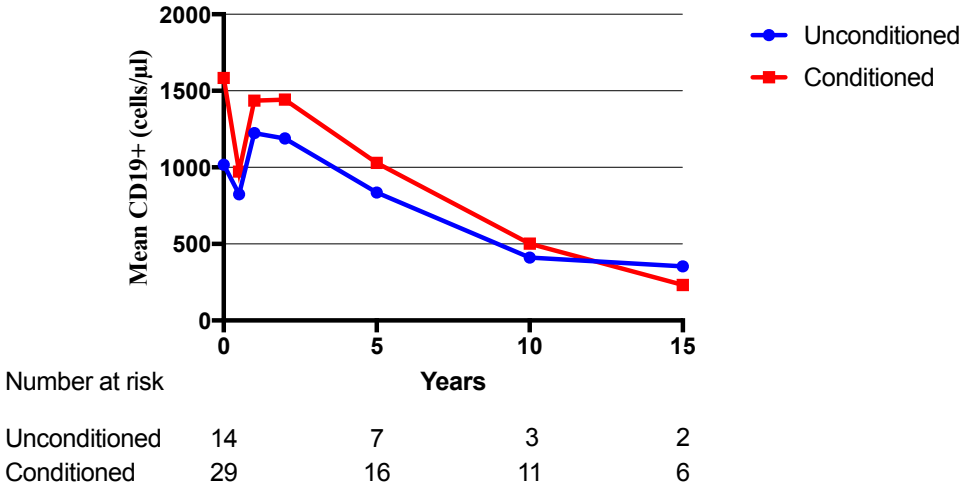


Table 5.8 Multi-level mixed effect model analysis of conditioning on CD19+ lymphocyte output with time post-transplant for IL2RG/JAK3 SCID patients.

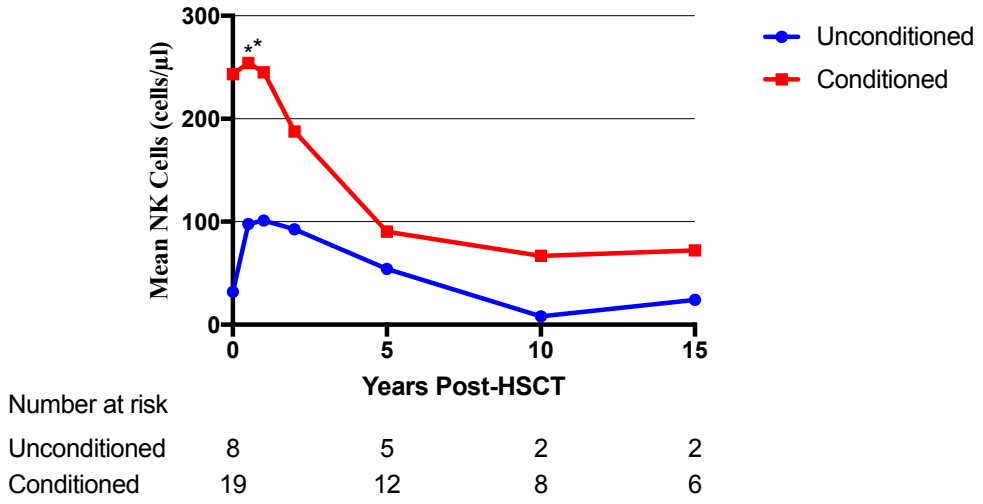
Time (Years)	Contrast	SE	p value
0	422.7	356.9	0.23
0.5	378.4	312.7	0.22
1	334.0	298.3	0.26
2	289.6	317.8	0.36
5	245.3	365.8	0.50
10	200.7	432.9	0.64
15	156.6	511.7	0.75
Overall trend			0.47

SE indicates standard error. p value < 0.05 is considered significant.

5.6.4 Longitudinal analysis of NK cell reconstitution post-HSCT

The conditioned recipients had a non-significantly higher overall NK cell number compared to unconditioned recipients, (p = 0.15). However, conditioned recipients did have a significantly higher NK cell number compared to unconditioned recipients; at baseline (p = 0.05) and 0.5 years post-transplant (p = 0.05) (Figure 5.19 and Table 5.9).

Figure 5.19 Longitudinal analysis of NK cell output for IL2RG/JAK3 SCID patients post-transplant according to conditioned and unconditioned recipients.



* indicates p < 0.05

Table 5.9 Multi-level mixed effect model analysis of conditioning on NK cell output with time post-transplant for IL2RG/JAK3 SCID patients.

Time (Years)	Contrast	SE	p value
0	126.5	66.1	0.05
0.5	112.1	59.3	0.05
1	97.7	57.2	0.08
2	83.3	60.0	0.16
5	68.9	67.3	0.30
10	54.4	77.8	0.48
15	40.0	90.4	0.65
Overall trend			0.15

SE indicates standard error. p value < 0.05 is considered significant.

5.7 Quality of Life IL2RG/JAK3 SCID post-HSCT

A total of 20 out of 31 patients (65%) agreed to the request to take part in this survey. All comparisons were made with published UK normal values (Table 5.10) [112]. Generally, parents of IL2RG/JAK3 SCID patients reported significantly lower QoL in three domains (total, psychosocial and school domains). However, there were no significant differences between the child reports of IL2RG/JAK3 SCID and UK published norms.

Further subgroup analysis of parent and child reports revealed that IL2RG/JAK3 SCID patients who were free from immunoglobulin replacement therapy had no significant difference in QoL compared to published UK norms (mean scores: total 75.0, $p = 0.06$; psychosocial 73.8, $p = 0.08$; school 68.0, $p = 0.09$). Both children, and parents of children with on-going immunoglobulin replacement therapy reported significantly lower QoL in a few domains (total, psychosocial and school domains). Similar findings were noted in parent reports of IL2RG/JAK3 SCID with on-going medical issues at the last follow up. However, no significant difference was noted in the child report between UK published norms and the IL2RG/JAK3 SCID patients with on-going medical issues (Table 5.10).

Table 5.10 Mean PedsQL Scores for IL2RG/JAK3 SCID patient post-HSCT (Parent and Child Report)

	UK Norm [112]	IL2RG/JAK3 SCID, Mean (p value)	On-going IG, Mean (p value)	No IG, Mean (p value)	On-going Medical issues (p value)	No Medical Issues (p value)
Parent Report		N = 19	N = 8	N = 11	N = 12	N = 7
Total	84.6	**70.9 (0.009)**	**63.9 (0.02)**	75.0 (0.06)	**70.0 (0.02)**	**72.3 (0.04)**
Psychosocial	82.2	**66.5 (0.007)**	**56.5 (0.01)**	73.8 (0.08)	**64.7 (0.01)**	69.5 (0.06)
Physical	89.1	82.4 (0.19)	77.7 (0.12)	85.8 (0.28)	79.9 (0.10)	86.6 (0.37)
Emotional	78.3	72.9 (0.34)	60.0 (0.06)	82.3 (0.81)	69.6 (0.15)	78.6 (0.52)
Social	86.8	77.4 (0.13)	70.0 (0.09)	82.7 (0.24)	77.5 (0.15)	77.1 (0.12)
School	81.5	**63.5 (0.02)**	**55.3 (0.01)**	68.0 (0.09)	**58.8 (0.01)**	70.2 (0.19)
Child Report		N = 15	N = 5	N = 10	N = 8	N = 7
Total	83.9	77.8 (0.23)	71.7 (0.16)	80.8 (0.28)	77.6 (0.23)	77.9 (0.17)
Psychosocial	81.8	74.1 (0.17)	67.3 (0.13)	77.5 (0.23)	75.2 (0.23)	72.9 (0.12)
Physical	88.5	84.6 (0.45)	80.0 (0.23)	86.9 (0.33)	82.0 (0.24)	87.5 (0.42)
Emotional	78.5	79.3 (0.89)	67.0 (0.24)	85.5 (0.88)	75.0 (0.37)	84.3 (0.79)
Social	87.7	75.7 (0.09)	77.0 (0.17)	75.0 (0.09)	81.3 (0.21)	69.3 (0.08)
School	78.9	67.3 (0.08)	**58.0 (0.05)**	72.0 (0.19)	69.4 (0.15)	65.0 (0.09)

Bold indicates p value < 0.05 and is considered significant.
IG indicates immunoglobulin replacement therapy.
All comparisons were made to UK published normal value using one sample T-test.

5.8 Summary of IL2RG and JAK3 SCID long-term outcome post-transplantation

Survival outcome of IL2RG/JAK3 SCID patients was 83%, but a significant number of patients experienced on-going medical issues requiring treatment and monitoring. Major clinical issues seen in IL2RG/JAK3 SCID patients post-transplant were on-going immunoglobulin replacement therapy (45%) and viral cutaneous warts (24%). With regards to long-term immune reconstitution, sustained CD3+ lymphocyte, CD4+ Naïve lymphocyte, CD19+ lymphocyte and NK cell levels were demonstrated even after 20 years post-HSCT. Conditioned recipients had better long-term thymopoiesis compared to unconditioned recipients. Good T lymphocyte donor chimerism was seen and not influenced by conditioning or donor types. B lymphocyte and myeloid donor chimerism were highly correlated. Low toxicity MAC conditioning appeared to give better B-lymphocyte and myeloid cell chimerism irrespective of donor type, graft source and stem cell doses. IL2RG/JAK3 SCID survivors who are free from immunoglobulin replacement therapy at the last follow up have a normal quality of life.

Important findings:

- Conditioned recipients have better thymopoiesis compared to unconditioned recipients in long-term post-HSCT.
- Low toxicity MAC conditioning appeared to give better B-lymphocyte and myeloid cell chimerism; irrespective of donor type, graft source and stem cell doses.
- Freedom from immunoglobulin replacement therapy was associated with normal quality of life.

Chapter 6 Results - Long-term Outcome for IL7Rα SCID Post-HSCT

This chapter presents the results on the long-term outcome for IL7Rα SCID patients post-transplantation. Due to the distinctive immune-phenotype, the IL7Rα cohort was analysed on its own.

6.1 Cohort Characteristics

A total of 18 patients were diagnosed with IL7Rα SCID, which was 15% of the Newcastle SCID cohort. Fifteen patients out of the 18 (83%) were still alive at the last follow up in 2015. Median age at the last follow up was 14 years (range 4 – 27).

Two patients did not receive conditioning chemotherapy pre-transplant [1 MSD and 1 MRD]. Donor type and conditioning regimen for all patients are shown in Table 6.1. In relation to busulfan doses, 5 patients received busulfan 16mg/kg and 6 patients received busulfan 8mg/kg. Only one haploidentical recipient received a NMA regimen (consisting of cyclophosphamide 200mg/kg, anti-LFA1 0.8mg/kg). No patients received a RIC regimen. Thirteen patients did not receive any serotherapy pre-transplant. Two patients received campath 1H and one patient received rATG. The majority of patients (12, 66.7%) received bone marrow as the graft source. Two patients (11.1%) received PBSC and 4 (22.2%) received umbilical cord blood. Table 6.2 summarises the parameters and cell dose for grafts received by patients in this cohort.

Table 6.1 Conditioning regimen, donor type and serotherapy for IL7Rα SCID patients.

Conditioning	MSD	MRD	MUD	MMUD	Haploidentical
Unconditioned	1	1	0	0	0
Low Toxicity	0	0	1	1	2
MAC					
MAC	0	0	1	1	9
NMA	0	0	0	0	1
Serotherapy					
No serotherapy	1	1	1	1	9
Campath 1H	0	0	1	1	0
rATG	0	0	0	0	1

Table 6.2 Characteristics of graft received during first HSCT for IL7Rα SCID patients.

Parameters	Value Median (range)
Patients' weight	5 kg (range, 3.9 – 9.1)
Graft volume	70 ml (range, 30 – 125).
CD34+ cell	2.8 x 10^6/kg (range, 0.05 – 24.1).
CD3+ cell	0.002 x 10^8/kg (range, 0.0002 – 1.1).
CD19+ cell	1 x 10^7/kg (range, 0.0009 – 8.9).

6.2 Immediate Outcome (less than 2 years post-HSCT)

A total of 18 patients underwent HSCT and 3 needed added interventions (second HSCT) (Table 6.3). Seventeen out of 18 patients achieved engraftment at 1 month post-transplantation.

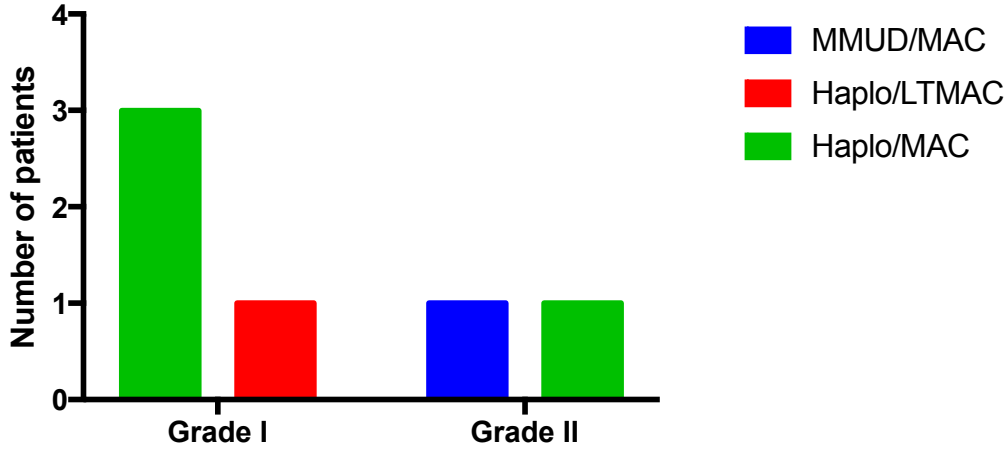
Table 6.3 Reasons, conditioning regimen and donor type for IL7Rα SCID with added intervention post-HSCT.

Subject ID	Reason for added intervention	Conditioning/Donor type for 1st HSCT	Conditioning/ Donor type for added intervention	Status
9	No engraftment after 120 days	NMA/ Haploidentical (father) Cyclophosphamide 200mg/kg, Anti-LFA1	MAC/ Haploidentical (father)	Died
91	Loss of engraftment	MAC/ Haploidentical (father)	Unconditioned/ Haploidentical (father)	Alive
96	Poor immune reconstitution with bronchiectasis	MAC/ Haploidentical (father)	RIC/ Haploidentical (father)	Alive

The median days taken for neutrophil recovery to more than 0.5 x 10^9/L was 21, range 13 – 60 days. The median days taken for CD3+ lymphocyte recovery was significantly shorter in the unconditioned recipients compared to conditioned recipients, 18 days (range, 11 – 83) vs 102 days (range, 19 – 407); p = 0.001.

Six out of the 18 patients developed Grade 1-II Acute GVHD. No patients developed Grade III-IV Acute GVHD. Four patients developed Grade I Acute GVHD [haploidentical & MAC recipients (3 patients), haploidentical & Low toxicity MAC recipient (1 patient)]. Two patients developed Grade II Acute GVHD [haploidentical & MAC recipient (1 patient) and MMUD & MAC recipient (1 patient)] (Figure 6.1).

Figure 6.1 Numbers of patients with Acute GVHD (Grade I and II) after first HSCT according to donor type and conditioning regimens.



6.3 Survival Outcome

Ten year survival for IL7Rα SCID was 83.3% (95% CI: 56.7 – 94.3%). There was no significant difference in survival outcome when compared to other SCID genotypes, 71.6% (95% CI: 61.5 – 79.5%), p = 0.3 (Figure 6.2). In the subgroup analysis, 10 year survival for unconditioned recipients was 100% compared to 81.2% (95% CI: 52.4 – 93.5%); p = 0.52 of conditioned recipients (Figure 6.3). Three deaths occurred (TRM 16.7%), all during the 1st year post-transplantation. Causes of death were infections (2 patients, 1 was a busulfan 16mg/kg recipient and the other a NMA recipient) and veno-occlusive disease (1 patient, a busulfan 16mg/kg recipient).

Figure 6.2 Comparison of survival outcome between IL7Rα SCID and other SCID genotypes.

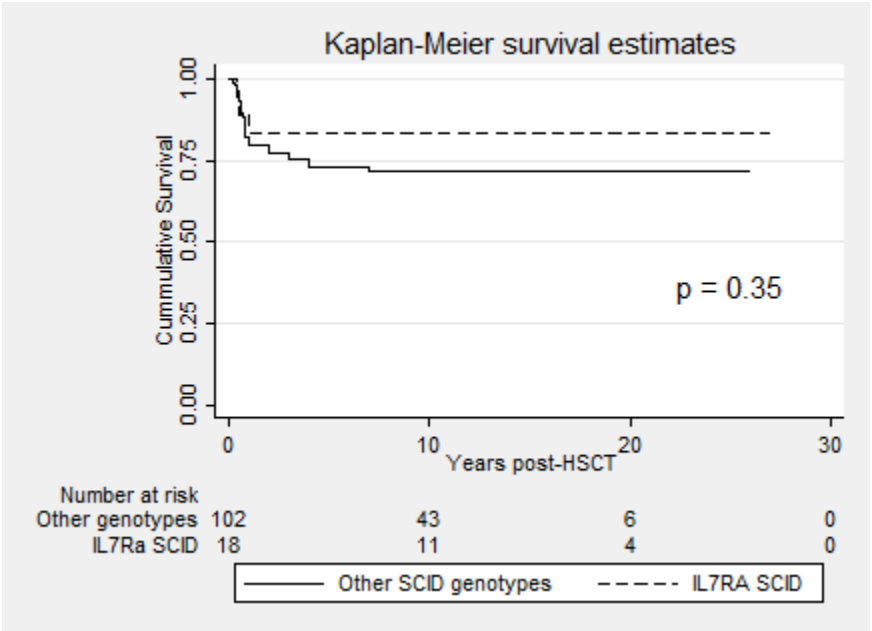
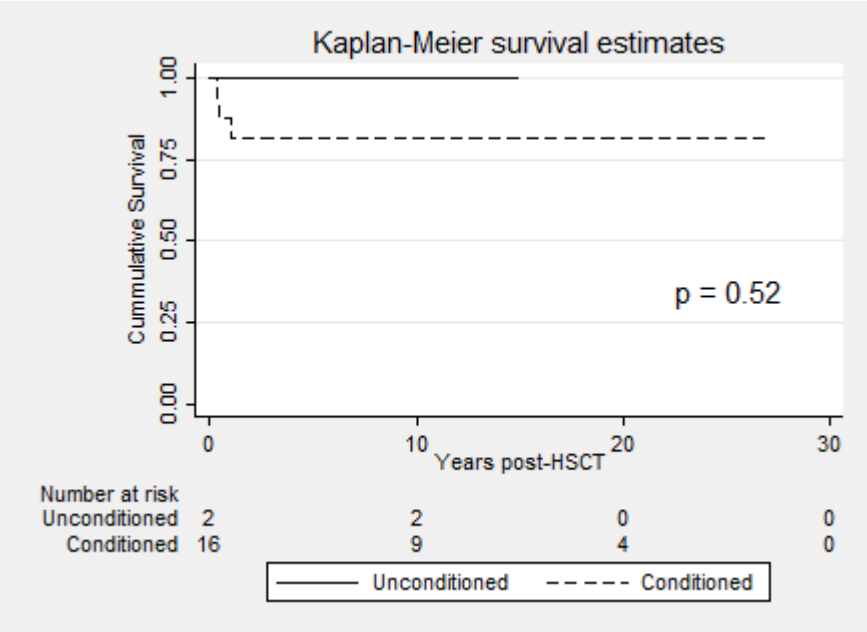


Figure 6.3 Comparison of survival outcome of unconditioned and conditioned recipients of IL7Rα SCID.



6.4 Long-term Clinical Outcome

6.4.1 Clinical Outcome

Fifteen out of eighteen patients were alive at the last follow up. A summary of on-going medical issues is given in Table 6.4. Eleven patients (73%) had on-going medical issues. Fourteen out of 15 patients (93%) were able to stop immunoglobulin replacement therapy. Two patients developed bronchiectasis and another patient had chronic pulmonary disease with a major restrictive defect demonstrated via lung function tests. Six other patients had normal lung function tests, including 3 patients with bronchiectasis.

Five patients developed cutaneous warts and further analysis showed no significant difference of mean NK values at latest follow up, comparing between those with warts versus none ($p = 0.40$).

Four patients experienced short stature (height $\leq$ 2 SD). However, all four patients with short stature were aged less than 15 years old. There was no significant relationship between those with short stature and conditioning regimen received; Fisher exact test, $p = 0.54$.

Table 6.4 Long-term clinical outcome of IL7Rα SCID post-transplant.

Clinical Outcome	% (n/N)
10 years survival	83.3% (15/18)
On-going Medical Issues	73% (11/15)
On-going IVIG Replacement Therapy	7% (1/15)
Bronchiectasis	13% (2/15)
Short Stature	27% (4/15)
Chronic Pulmonary Disease	14% (1/15)
Warts	33% (5/15)
Normal Lung Function	85% (6/7) 1 patient had major restrictive defect
Normal Endocrine	100% (15/15)
Normal Hearing	100% (15/15)
Normal Cardiovascular	100% (15/15)
Normal Renal System	100% (15/15)
Normal Gastro-intestinal System	100% (15/15)

6.4.2 Immunoglobulin replacement therapy at last follow up

IL7Rα SCID survivors post-HSCT have the highest proportion of patients free from immunoglobulin replacement therapy; in comparison to other SCID genotypes (IL7Rα 93%, IL2RG/JAK3 SCID (55%), RAG 1 and RAG 2 (77%), Artemis SCID (57%), ADA SCID (81%). All low toxicity MAC recipients were able to discontinue the immunoglobulin replacement therapy irrespective of donor type. Despite mixed B-lymphocyte donor chimerism, all MAC8 (busulfan 8mg/kg) recipients had discontinued immunoglobulin replacement therapy after 2 years post-HSCT. Only one IL7Rα patient required ongoing immunoglobulin

replacement therapy (1 out of 15 patients). She received a MUD transplant with MAC and despite good B-lymphocyte function, immunoglobulin replacement therapy was restarted after a diagnosis of chronic pulmonary disease had been made.

6.5 Chimerism at last follow up

Spearman's correlation analysis was used to assess the degree of correlation between B-lymphocyte donor and myeloid donor chimerism. There was a highly positive and significant correlation between both parameters, rho = 0.9452, $p < 0.0001$ (Figure 6.5).

Figure 6.4 Scatter plot of donor B-lymphocyte chimerism and myeloid donor chimerism at last follow up.

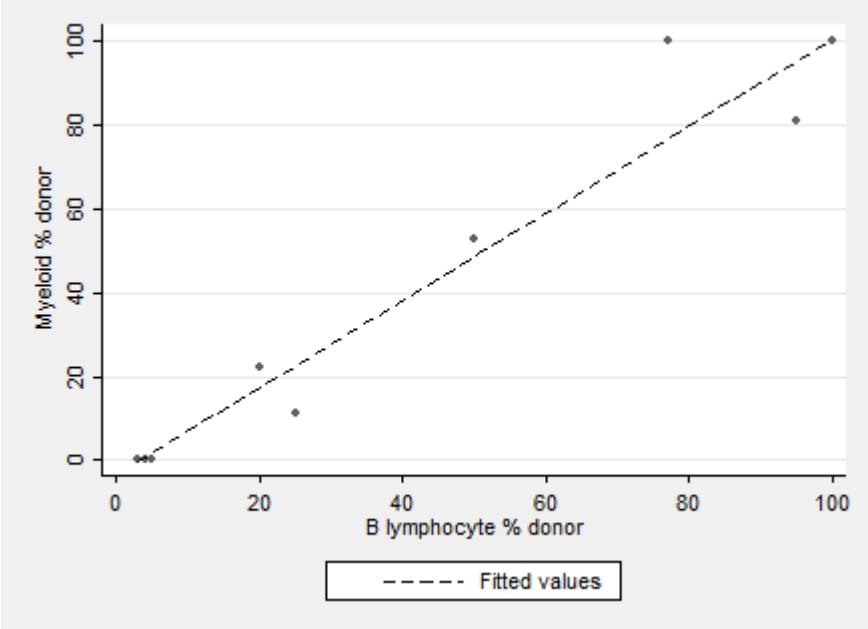


Figure 6.5 Myeloid and B-lymphocyte donor chimerism at last follow up according to donor type and conditioning regimen.

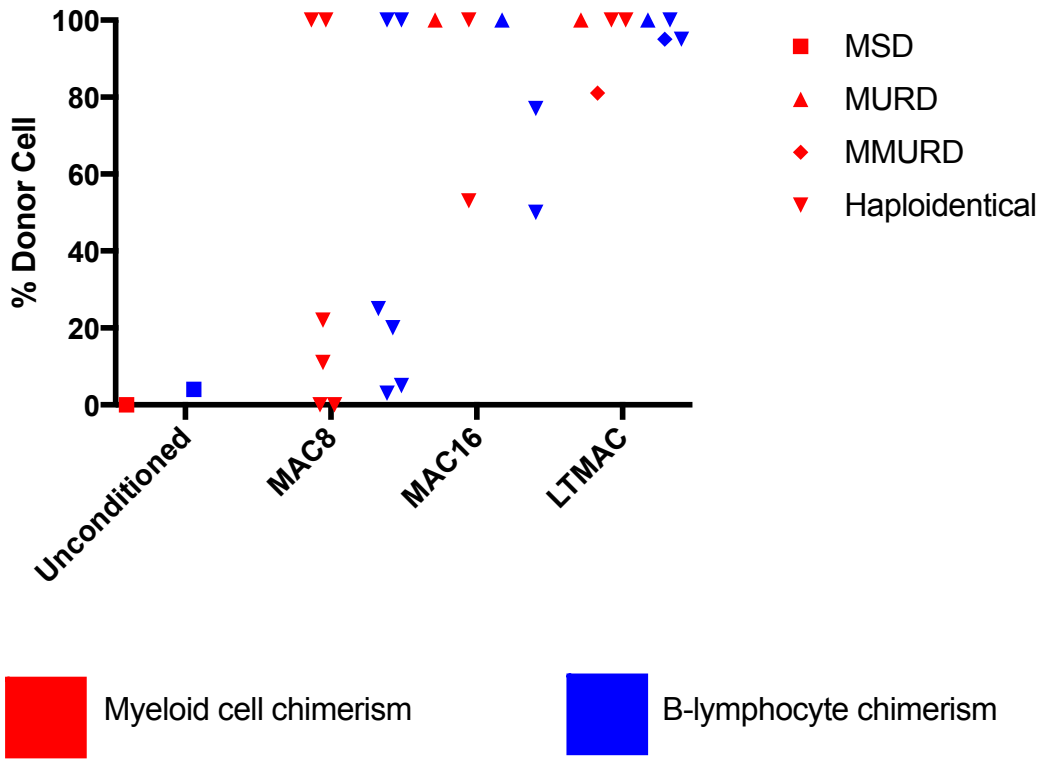


Figure 6.6 illustrates the distribution of myeloid donor chimerism and B-lymphocyte donor chimerism at the last follow up according to the donor type and conditioning regimen received. The myeloid chimerism and B-lymphocyte chimerism tend to mirror each other as observed in the IL2RG/JAK3 SCID cohort

All low toxicity MAC recipients had myeloid and B-lymphocyte donor chimerism of more than 80%. Haploidentical MAC16 (busulfan 16mg/kg) recipients demonstrated better donor B-lymphocyte and myeloid donor chimerism compared to haploidentical MAC8 (busulfan 8 mg/kg) recipients. Unconditioned and haploidentical MAC8 recipients had B-lymphocyte and myeloid donor cell chimerism of less than 20%.

The variables included in the final model explained 68.3% variation of myeloid donor chimerism at the last follow up and there was no significant predictive association between the myeloid donor chimerism percentage and independent factors such as stem cell doses, donor types, conditioning regimens and graft sources (Table 6.5).

Table 6.5 Results of multivariable linear regression analysis of the myeloid donor chimerism percentage at last follow up.

Myeloid donor %	Coefficient	95% CI	p value
CD34 cell dose	-1.9	-43.8 – 39.9	0.86
Mononucleated cell dose	11.2	-60.9 – 83.4	0.57
Donor type			
MSD	1.0	Reference	
MUD	136.1	-440.0 – 712.2	0.41
MMUD	124.9	-590.0 – 840.0	0.53
Haploidentical	95.5	-510.8 – 701.9	0.56
Conditioning regimen			
Unconditioned	1.0	Reference	
Low Toxicity MAC	-14.8	-366.4 – 336.8	0.87
MAC8	-14.2	-365.2 – 336.7	0.87
Graft Source			
BM	1.0	Reference	
PBSC	33.0	-653.5 – 719.5	0.85

$R^2 = 68.3\%$
CI indicates confidence interval
p value < 0.05 was considered significant

6.6 Long-term immune reconstitution post-HSCT

6.6.1 Longitudinal analysis of CD3+ lymphocyte reconstitution post-HSCT

There was sustained CD3+ lymphocyte output seen post-HSCT for both groups. There was no significant difference in the overall trend of circulating CD3+ lymphocyte numbers between conditioned versus unconditioned recipients, p = 0.92 at each time point post-transplant (Figure 6.6 and Table 6.6).

Figure 6.6 Longitudinal analysis of CD3+ lymphocyte output for IL7Rα SCID patients post-HSCT according to conditioned and unconditioned recipients.

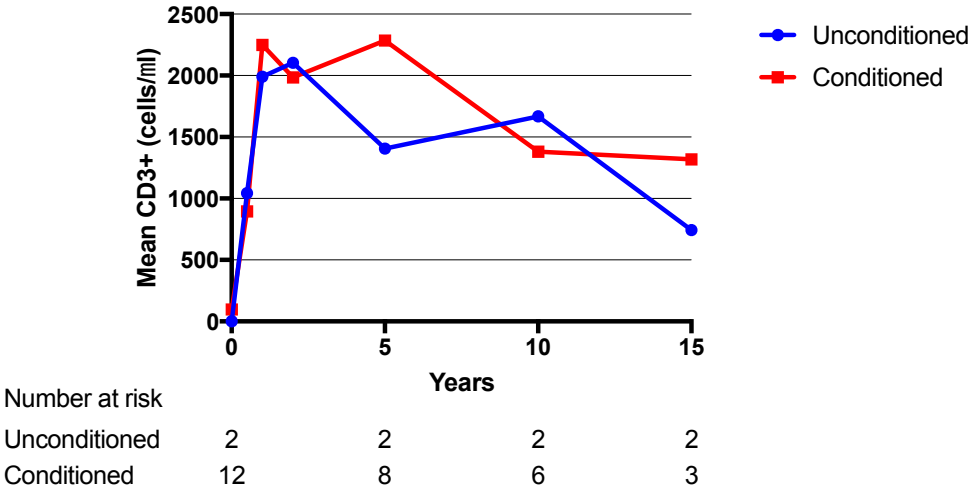


Table 6.6 Multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-HSCT for IL7Rα SCID patients.

Time (Years)	Contrast	SE	p value
0	60.4	60.5.6	0.92
0.5	141.4	544.1	0.79
1	222.5	612.9	0.71
2	303.5	778.3	0.69
5	384.5	993.1	0.69
10	465.5	1231.7	0.70
15	546.57	1482.6	0.71
Overall trend			0.92

SE indicates standard error.
p value < 0.05 is considered significant.

6.6.2 Longitudinal analysis of CD4+ Naïve lymphocyte reconstitution post-HSCT

The data for CD4+ Naïve lymphocyte was only available up until 10 years post-HSCT for comparison between conditioned and unconditioned recipients. The overall trend showed that the mean for CD4+ naïve lymphocyte output was non-significantly higher in conditioned recipients compared to unconditioned recipients, p = 0.45 (Figure 6.7 and Table 6.7). Comparison at each time point post-HSCT revealed a non-significantly higher mean CD4+ Naïve lymphocyte output in conditioned compared to unconditioned recipients. The unconditioned recipients' mean CD4+ Naïve lymphocyte number was below 500 cells/µl from 1 year post-HSCT till 10 years post-HSCT.

Figure 6.7 Longitudinal analysis of CD4+ Naive lymphocyte output for IL7Rα SCID patients according to unconditioned versus conditioned recipients.

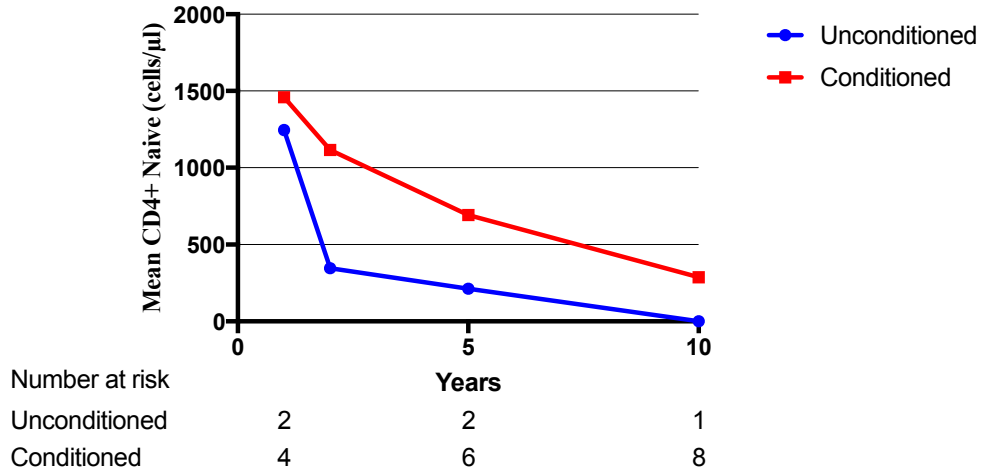


Table 6.7 Multi-level mixed effect model analysis of conditioning on CD4+ Naive lymphocyte output with time post-HSCT for IL7Rα SCID patients.

Time (Years)	Contrast	SE	p value
0.5	-530.5	570.8	0.35
1	-338.1	437.9	0.44
2	46.5	327.9	0.65
5	238.8	270.1	0.86
10	431.2	296.9	0.42
Overall trend			0.45

SE indicates standard error. p value < 0.05 is considered significant.

6.6.3 Longitudinal analysis of CD19+ lymphocyte reconstitution post-HSCT

There was a sustained CD19+ lymphocyte output with time post-HSCT. The overall trend showed that conditioned recipients had a non-significantly higher mean CD19+ lymphocyte output with time compared to unconditioned recipients, p = 0.75 (Figure 6.8 and Table 6.8).

Figure 6.8 Longitudinal analysis of CD19+ lymphocyte output for IL7Rα SCID patients according to unconditioned versus conditioned recipients.

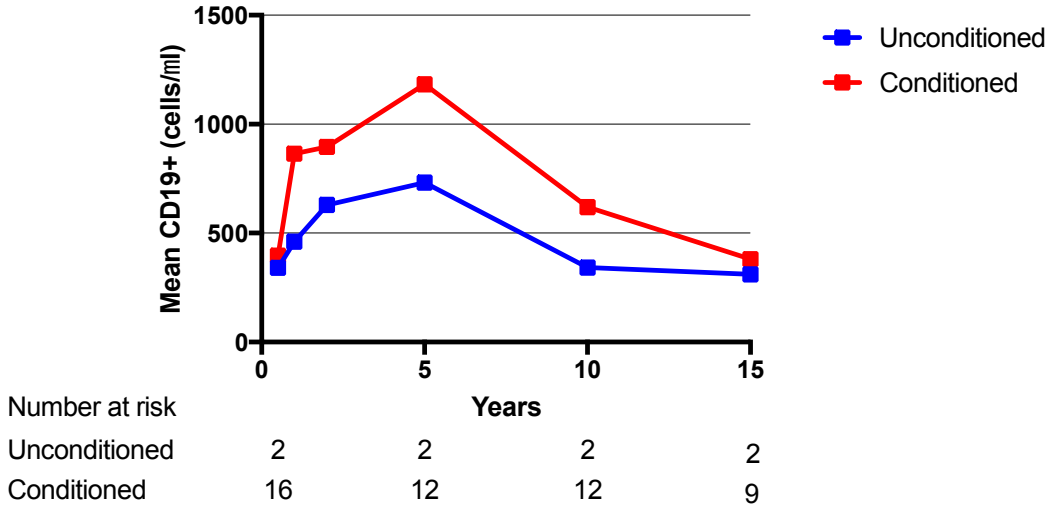


Table 6.8 Multi-level mixed effect model analysis of conditioning on CD19+ lymphocyte output for IL7Rα SCID patients according to unconditioned versus conditioned recipients.

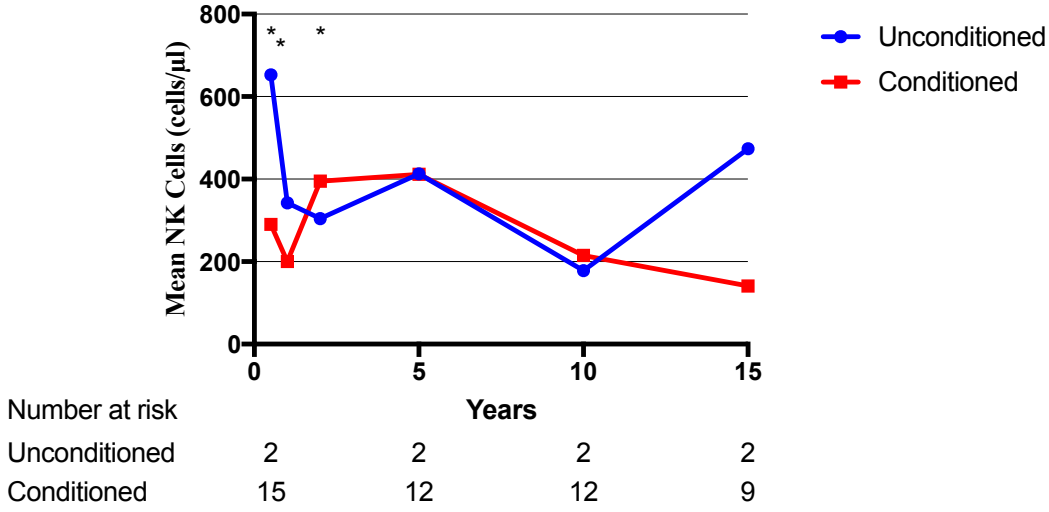
Time (Years)	Contrast	SE	p value
0	215.3	368.0	0.55
0.5	214.6	319.7	0.50
1	213.9	289.7	0.46
2	213.1	283.9	0.45
5	212.4	303.7	0.48
10	211.7	344.7	0.53
15	210.9	400.4	0.59
Overall trend			0.75

SE indicates standard error.
p value < 0.05 is considered significant

6.6.4 Longitudinal analysis of NK cell reconstitution post-HSCT

The mean NK cell output was significantly higher in the unconditioned recipients in the early time points after the transplant; 0.5 years (p = 0.0001), 1 year post-HSCT (p = 0.0001), 2 years post-HSCT (p = 0.0002) and 5 years post-HSCT (p = 0.007) (Table 6.9). The unconditioned recipients demonstrated a significantly higher overall number of NK cells with time post-HSCT, p = 0.0004 (Figure 6.9 and Table 6.9).

Figure 6.9 Longitudinal analysis of NK cell output for IL7Rα SCID patients according to unconditioned versus conditioned recipient



* indicates p < 0.05

Table 6.9 Multi-level mixed effect model analysis of conditioning on NK cell output with time post-HSCT for IL7Rα SCID patients.

Time (Years)	Contrast	SE	p value
0.5	-2462.0	646.2	0.0001
1	-1971.1	500.3	0.0001
2	-1480.3	396.0	0.0002
5	-989.4	370.2	0.007
10	-498.6	437.2	0.25
15	-7.7	564.8	0.98
Overall trend			0.0004

SE indicates standard error.
p value < 0.05 is considered significant.

6.7 Quality of Life post-HSCT

A total of 11 out of 15 patients and families (73%) responded to the PedsQL questionnaires. The median age of responders was 15 years (range, 6 – 27 years). All results were compared to published UK normal values using one sample T-test [112]. Both parents and patients reported no significant difference in the mean scores of all domains, in comparison to UK normal values (Table 6.10).

Table 6.10 Mean PedsQL Scores for IL7Rα SCID patient post-HSCT (Parent and Children's Report)

	UK Norms[112] Mean	IL7Rα SCID Mean	p value
Parent Report		N = 6	
Total	84.6	68.3	0.07
Psychosocial	82.2	65.0	0.07
Physical	89.1	74.5	0.11
Emotional	78.3	62.5	0.21
Social	86.8	78.3	0.52
School	81.5	67.0	0.19
Child Report		N = 9	
Total	83.9	76.7	0.33
Psychosocial	81.8	74.3	0.21
Physical	88.5	81.3	0.22
Emotional	78.5	66.7	0.29
Social	87.7	85.6	0.73
School	78.9	70.6	0.24

Bold indicates p value < 0.05 and is considered significant.
All comparisons were made to UK published normal value using one sample T-test.

6.8 Summary of IL7Rα SCID long-term outcome post-transplantation

IL7Rα SCID patients had good long-term survival, especially the unconditioned recipients with a survival rate of 100% at 10 years post-HSCT. IL7Rα SCID survivors demonstrated the highest percentage of patients free from immunoglobulin replacement therapy compared to other SCID genotypes. Normal quality of life was seen in IL7Rα SCID patients' post-HSCT. Warts did occur in this cohort. The long-term immune-reconstitution of CD4+ Naïve and CD19+ lymphocytes was non-significantly higher in conditioned recipients compared to unconditioned recipients, except for longitudinal NK cell output.

Important findings:

- Incidence of viral cutaneous wart was present in this cohort.
- IL7Rα SCID survivors had the highest percentage of freedom from immunoglobulin replacement therapy.
- Both parents and children reported normal quality of life.

Chapter 7 Long-term Outcome for Artemis and RAG 1/2 SCID Post-HSCT

This chapter presents the results of the long-term outcome post-transplantation for patients with Artemis and RAG 1/2 SCID in Newcastle. Both SCID genotypes were presented and compared directly with each other as both have a T- B- NK+ SCID immune-phenotype. However, the main differentiating feature is that Artemis SCID patients exhibited radiation sensitivity, which was not found in RAG 1/2 SCID patients [54]. This may explain why Artemis SCID patients exhibit more long-term effects compared to RAG 1/2 SCID.

7.1 Cohort Characteristics

A total of 21 patients with a diagnosis of Artemis SCID (8 patients) and RAG 1/2 SCID (13 patients) had undergone 28 HSCT in the Newcastle SCID cohort. The median age at the last follow up in 2015 was 10 years, range 2 – 18.

The unconditioned recipients with Artemis and RAG 1/2 SCID received the graft from various donor types [MSD (2 patients), MRD (1 patient), haploidentical (2 patients)]; in comparison to other SCID genotypes in which all unconditioned recipients received MSD donor type. Almost all MAC recipients received haploidentical donors. Seven patients received low toxicity MAC conditioning. The majority of patients (57%) did not receive any serotherapy before their transplant. Further details on conditioning regimen, donor type and serotherapy are listed in Table 7.1. Of note, all unconditioned recipients, except for 2 MSD needed added HSCT due to poor engraftment. Bone marrow was the main graft source in the cohort (16 patients). Three patients received umbilical cord blood and two patients had PBSC. Further comparison of characteristics of the transplant and graft dose between Artemis and RAG1/2 SCID are summarized in Table 7.2.

Table 7.1 Conditioning regimen, donor type and serotherapy for Artemis and RAG 1/2 SCID patients.

Conditioning	MSD	MRD	MUD	MMUD	Haploidentical
Unconditioned	2	1	0	0	2
RIC	0	2	0	0	0
Low Toxicity MAC	2	2	1	1	1
MAC	0	0	0	1	6
Serotherapy					
No serotherapy	4	1	0	1	6
Campath 1H	0	4	1	1	0
Campath 1G	0	0	0	0	2
rATG	0	0	0	0	1

Table 7.2 Comparison of HSCT parameters and graft dose between Artemis and RAG1/2 SCID during first HSCT.

Parameters	Artemis	RAG1/2
	Value	Value
	Median (range)	Median (range)
Patients' weight	5.4 kg (2 – 6.9)	5 kg (3.8 – 12)
Age at first HSCT	14 weeks (4 – 32)	25 weeks (4 – 84)
Graft volume	55ml (20 – 390)	100ml (30 – 234)
Mononucleated cell	3.5 x 10^8/kg	5.8 x 10^8/kg
	(0.14 – 33)	(0.22 – 17.2)
CD34+ cell	4 x 10^6/kg	5.7 x 10^6/kg
	(0.16 – 15.2)	(0.17 – 20.0)
CD3+ cell	0.01 x 10^8/kg	0.93 x 10^8/kg
	(0.0001 – 3.1)	(0.00002 – 7.6)
CD19+ cell	0.6 x 10^7/kg	3 x 10^7/kg
	(0.28 – 1.4).	(0.0006 – 60).

Data presented as median due to not normally distributed.

7.2 Immediate Outcome (within two years post-HSCT)

The median number of days taken for neutrophil recovery of more than 0.5 x 10^9/L was 20 days, (range 10 – 35) for Artemis and RAG 1/2 SCID conditioned recipients. All unconditioned recipients of Artemis and RAG 1/2 SCID never had neutrophils below 0.5 x 10^9/L. A median test was performed to compare days taken for neutrophil recovery to more than 0.5 x 10^9/L between conditioned Artemis and RAG 1/2 SCID recipients as the results were not normally distributed. There was no significant difference in median days for neutrophil recovery between conditioned RAG 1/2 SCID recipients and conditioned Artemis SCID recipients; 16 days (range, 10 – 24) vs. 23 days (range, 15 – 35), p = 0.21.

The median number of days taken for CD3+ lymphocyte recovery to more than 200 cells/µl was 47 days (range, 11 – 168) for all Artemis and RAG 1/2 SCID post-HSCT. However, there was a significant difference in median days for CD3+ lymphocyte recovery in unconditioned and conditioned recipients of Artemis and RAG 1/2 SCID recipients; 22 days (range, 11 – 39) vs. 61 days (range, 25 - 168), p = 0.03.

Eight out of 21 patients from this cohort developed acute GVHD after their first transplantation. Most had acute GVHD Grade I –II (6 patients, 75%) and only two patients had acute GVHD Grade III (an Artemis SCID patient who received MUD/low toxicity MAC HSCT and an unconditioned/haploidentical RAG SCID patient). More acute GVHD was seen in RAG 1/2 SCID patients (5 out of 13, 55%) than Artemis (3 out of 8, 43%). All Artemis patients with acute GVHD were conditioned recipients [low toxicity MAC/MUD (1), MAC/MMUD (1), MAC/Haploidentical (1)]. Most of RAG 1/2 SCID patients with acute GVHD were conditioned recipients [RIC/MRD (1), low toxicity/MRD (1), low toxicity/MMUD (1), MAC/haploidentical (1)] and one unconditioned/haploidentical recipient. Multiple logistic regression analysis did not reveal any significant association between the incidence of acute GVHD and conditioning regimen, donor type and SCID genotype (Artemis and RAG 1/2) (Table 7.3).

Table 7.3 Result of multiple logistic regression analysis of different independent factors on the incidence of acute GVHD post-HSCT.

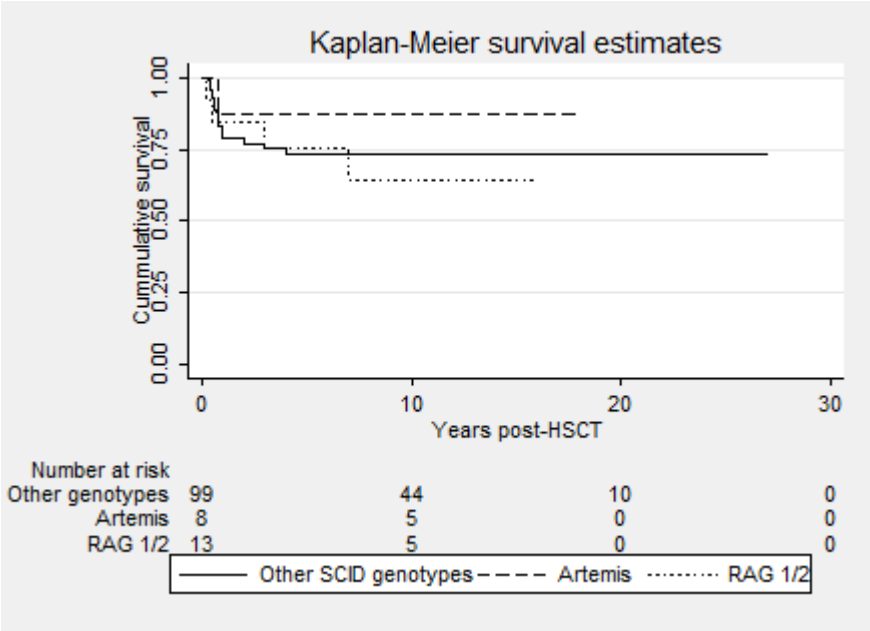
Acute GVHD	Odd Ratio	95% Confidence Interval	p-value
Artemis and RAG 1/2	0.71	0.09 – 5.27	0.74
Conditioning regimen	0.81	0.32 – 2.05	0.66
Donor Group	0.80	0.43 – 1.50	0.50

7.3 Survival Outcome

Five deaths occurred by the time of the last follow-up in January 2015. Causes of early death (less than 2 years post-HSCT) were infection (1 patient), veno-occlusive disease (1 patient) and severe pneumonitis (1 patient). There were 2 deaths after two years post-HSCT both in RAG patients. The causes of late death were Epstein-Barr virus infection of the central nervous system (1 patient), occurred 7 years post-HSCT and another patient developed pre-B Acute Lymphoblastic Leukemia (ALL) in recipient T-lymphocytes; and died from sepsis during chemotherapy 3 years post-HSCT.

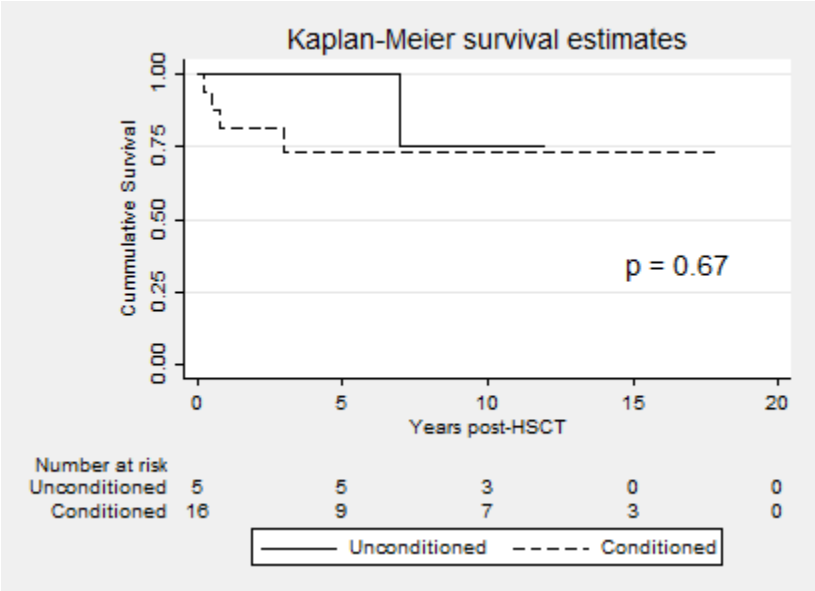
The overall 10 year survival for Artemis and RAG 1/2 SCID patients was 73.3% (95% CI: 46.6 – 88.1%) with a TRM of 21.5% (excluding the ALL death). However, when the SCID genotype was analysed individually, the 10 year survival outcome of Artemis, 87.5% (95% CI: 38.7 – 98.1%) was non-significantly higher compared to RAG 1/2 SCID, 64.4% (95% CI: 29.7 – 85.2%) and other SCID genotypes, 73.4% (95% CI: 63.5 – 81.1%); p = 0.64 (Figure 7.1).

Figure 7.1 Comparison of survival outcome between Artemis & RAG 1/2 SCID and other SCID genotypes.



Further subgroup comparisons of survival outcome between unconditioned and conditioned recipients of both genotypes were undertaken (Figure 7.2). The 10 year survival outcome of unconditioned Artemis & RAG 1/2 SCID was 75.0% (95% CI: 12.7 – 96.1%) and for conditioned recipients of Artemis & RAG 1/2 SCID was 73.1 % (95% CI: 42.6 – 89.1%) and the difference was not statistically significant; (log rank test analysis, p = 0.67).

Figure 7.2 Comparison of survival outcome among the unconditioned and conditioned recipients of Artemis & RAG 1/2 SCID.



7.4 Long-term Clinical Outcome

7.4.1 Clinical Outcome

Six out of 7 Artemis patients (85%) had on-going medical issues at the last follow up in January 2015, compared to 5 out of 9 RAG 1/2 SCID patients (55%). With regards to preparative regimen, all conditioned Artemis SCID recipients experienced on-going medical issues compared to 4 out of 7 conditioned RAG SCID patients. The number of unconditioned Artemis and RAG SCID are too small for comparisons; 1 out of 2 unconditioned recipients have on going medical issues for both genotypes. All the long-term clinical outcomes for both Artemis and RAG 1/2 SCID post-HSCT are detailed in Table 7.4.

Major clinical issues in Artemis patients post-HSCT were dental issues (3 patients, 43%) [manifested as damaged dental enamel (1), widely spaced teeth (1), edentulous (1) and all three patients received busulfan], short stature (3 patients, 43%), post- transplant autoimmune hemolytic anemia (3 patients, 43%), and dermatology issues (3 patients, 43%) [Vitiligo (1), psoriasis (1), eczema (1)]. Other medical issues were hearing loss (1 patient), autoimmune hypothyroidism (1 patient) and chronic renal failure (1 patient who received MMUD, busulfan 8mg/kg).

Only 55% of RAG 1/2 SCID survivors had on-going medical issues at the last follow-up in January 2015. These were dermatology issues (3 patients, 33%) [Vitiligo (2), Rash (1)], immunoglobulin replacement therapy (2 patients, 22%), short stature (1 patient, 11%), post- transplant autoimmune hemolytic anemia (1 patient, 11%) and autoimmune hypothyroidism (1 patient, 11%). No RAG 1/2 SCID survivors had dental issues or hearing loss. Lung function testing was not available for this group of patients.

In relation to short stature, four patients had a height centile at or below 2 SD [conditioned RAG 1/2 (1 patient), unconditioned Artemis (1 patient) and conditioned Artemis (2 patients)].

Table 7.4 Long-term clinical outcome of Artemis and RAG 1/2 SCID post-transplant.

Clinical Outcome	Artemis % (n/N)	RAG 1/2 % (n/N)
10 years survival	87.5% (1/8)	64.4% (4/13)
On-going medical issues	85% (6/7)	55% (5/9)
On-going Immunoglobulin replacement therapy	43% (3/7)	23% (2/9)
Hearing loss	14% (1/7)	0% (0/9)
Dental issues	43% (3/7)	0% (0/9)
Short stature	43% (3/7)	11% (1/9)
Chronic renal failure	14% (1/7)	0% (0/9)
Autoimmune hemolytic anemia	43% (3/7)	11% (1/9)
Autoimmune hypothyroidism	14% (1/7)	11% (1/9)
Dermatology issues	43% (3/7)	33% (3/9)
Lung function test	Not available	Not available
No Cardiovascular Issues	100% (7/7)	100% (9/9)
No Neurocognitive Issues	100% (7/7)	100% (9/9)
No Respiratory Issues	100% (7/7)	100% (9/9)

7.4.2 Immunoglobulin replacement therapy at last follow up

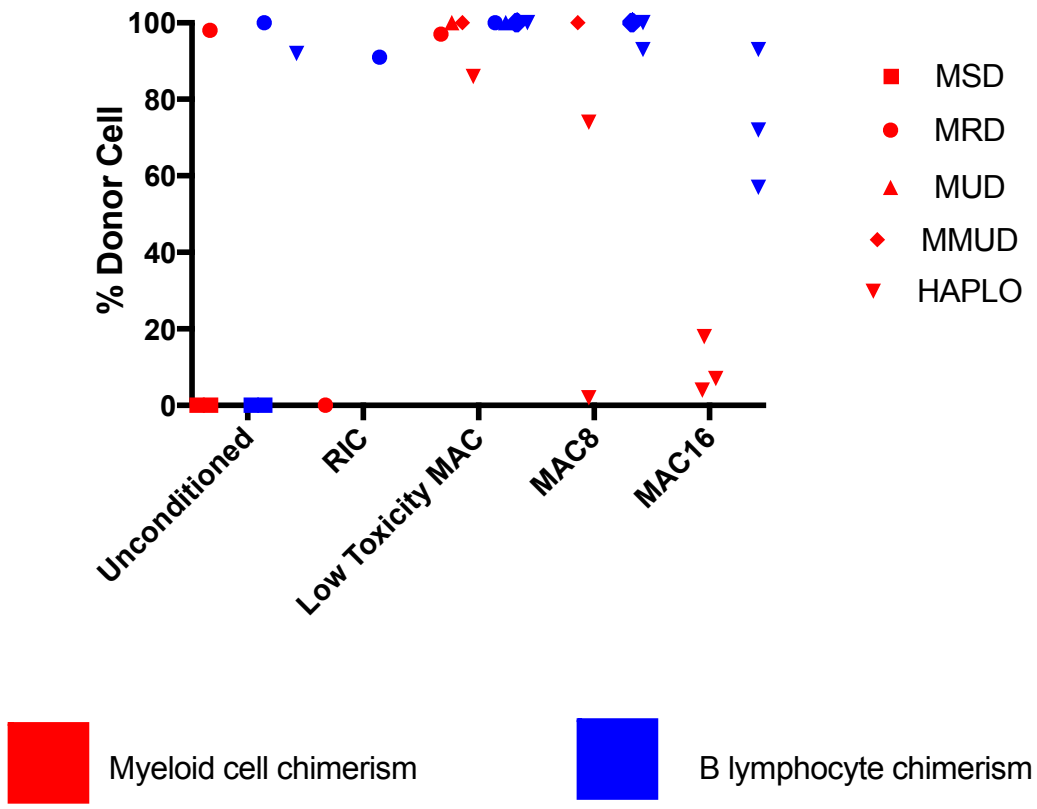
RAG 1/2 SCID survivors had a higher proportion of patients free from immunoglobulin replacement therapy compared to Artemis, 77% and 57%, respectively, $p = 0.36$. Considering both Artemis and RAG 1/2 SCID, almost all patients with full donor and mixed donor B-lymphocyte chimerism were able to discontinue immunoglobulin replacement, except for 2 patients with 100% donor B-lymphocyte chimerism still needed immunoglobulin replacement As expected, two unconditioned MSD recipients with poor donor B-lymphocyte chimerism (< 10%) still receive on-going immunoglobulin therapy. However, 2 conditioned patients with 100% donor B-lymphocyte Chimerism still needed on-going immunoglobulin replacement.

More conditioned Artemis and RAG 1/2 SCID recipients were able to be free from immunoglobulin replacement therapy compared to unconditioned recipients, (conditioned: 10 out of 12 patients, unconditioned: 1 out of 4 patients, Fisher exact test, p = 0.06).

7.5 Chimerism at last follow-up

The myeloid and B-lymphocyte donor chimerism values were available for 15 out of 16 Artemis and RAG 1/2 SCID patients post-HSCT. B-lymphocyte and myeloid donor chimerism mirrored each other in all donor types and conditioning regimens; except for haploidentical/MAC recipients (Figure 7.3). Low toxicity MAC recipients demonstrated better B/myeloid donor chimerism across all donor types. Poor B/myeloid donor chimerism percentage (<10%) was seen in unconditioned MSD recipients.

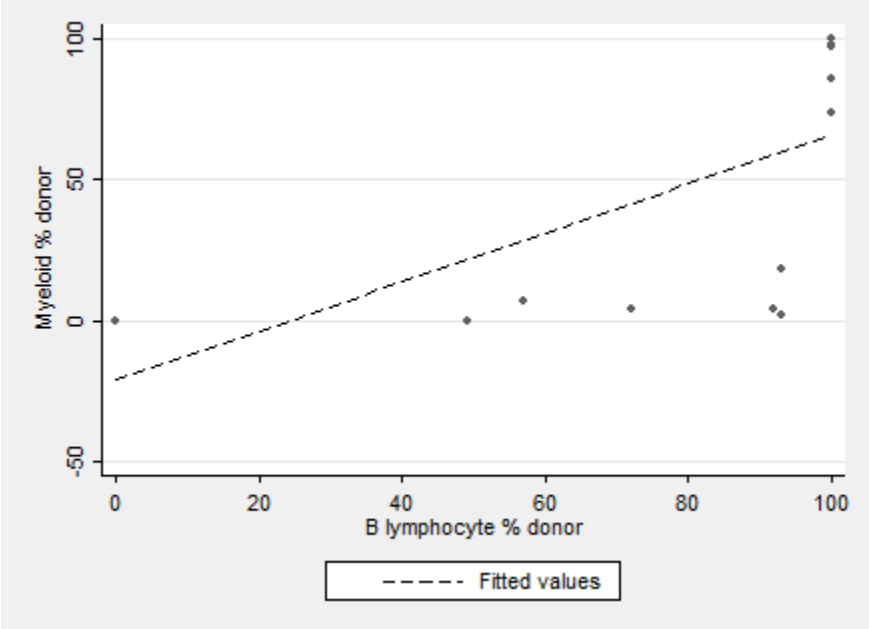
Figure 7.3 A scatter plot of myeloid and B-lymphocyte donor chimerism (at last follow up) according to donor type and conditioning regimen.



Low toxicity MAC recipients demonstrated better B/myeloid donor chimerism across all donor types. Poor B/myeloid donor chimerism percentage (<10%) was seen in unconditioned MSD recipients.

Spearman correlation analysis showed a high degree of positive correlation between B-lymphocyte and myeloid donor chimerism, rho 0.9070, $p < 0.0001$ (Figure 7.4).

Figure 7.4 Scatter plot of B-lymphocyte donor chimerism and myeloid donor chimerism at last follow up for Artemis and RAG 1/2 SCID patients.



There was no significant predictive association between myeloid donor chimerism (at the last follow up) and independent factors such as; graft doses, donor type, conditioning regimen and graft source (Table 7.5). The variables included in the final model explained 84.5% variation of myeloid donor chimerism.

Table 7.5 Results of multivariable linear regression analysis of the myeloid donor chimerism percentage (at last follow up).

Myeloid donor %	Coefficient	95% CI	p-value
CD34 cell dose	1.1	-13.7 – 15.9	0.82
Mononucleated cell dose	-2.2	-30.5 – 26.0	0.81
SCID Genotypes			
Artemis	1.0	Reference	
RAG 1/2	11.8	-149.1 – 172.7	0.83
Donor Types			
MSD	1.0	Reference	
MRD	75.7	-84.7 – 236.1	0.23
MUD	120.1	-177.4 – 417.7	0.28
MMUD	108.2	-178.1 – 394.6	0.31
Haploidentical	11.8	-174.7 – 198.5	0.85
Conditioning Regimen			
Unconditioned	1.0	Reference	
Low Toxicity MAC	-17.4	-253.2 – 218.3	0.82
RIC	-87.0	-313.6 – 139.6	0.30
MAC	-7.1	-243.0 – 228.6	0.92
Graft Source			
BM	1.0	Reference	
PBSC	52.3	-169.9 – 274.5	0.95

$R^2 = 84.5\%$
CI indicates confidence interval
p value < 0.05 was considered significant

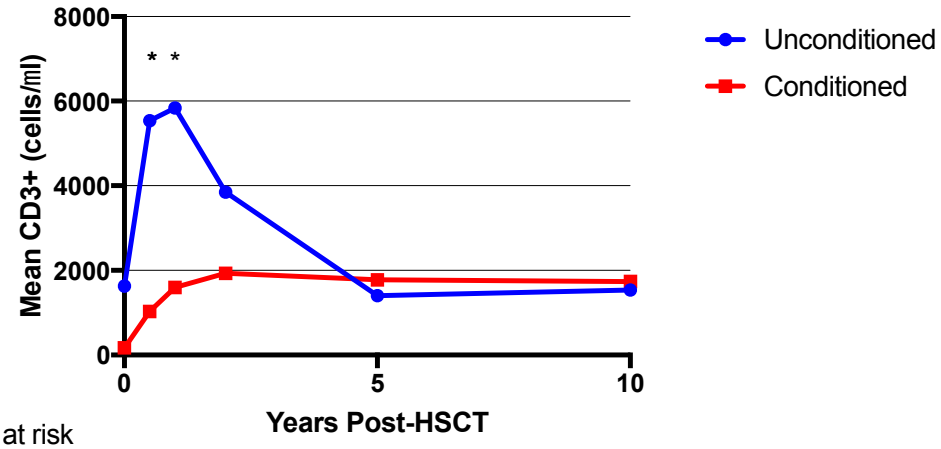
7.6 Long-term immune reconstitution post-HSCT

7.6.1 Longitudinal analysis of CD3+ lymphocyte reconstitution post-HSCT

Longitudinal analysis of all immune reconstitution parameters was analysed collectively for both Artemis and RAG 1/2 SCID patients due to the small sample size. A comparison of trend changes with time was performed between conditioned and unconditioned recipients.

In the initial first years post-transplant, unconditioned recipients had a higher CD3+ lymphocyte count compared to conditioned recipients, and it was significant at 3 time points (baseline, p = 0.001, 6 months post-transplant, p = 0.003 and 1 year post-transplant, p = 0.02) (Figure 7.5 and Table 7.6). However, after 5 years post-transplant, there was no significant difference in the CD3+ lymphocyte count between both groups (5 years post-transplant, p = 0.76 and 10 years post-transplant, p = 0.71). Multi-level mixed effect model analysis demonstrated a significant difference in overall trend changes between both groups, (p = 0.004).

Figure 7.5 Longitudinal analysis of CD3+ lymphocyte output for Artemis and RAG 1/2 SCID patients post-HSCT according to conditioned and unconditioned recipients.



Number at risk			
Unconditioned	5	3	3
Conditioned	16	7	5

* indicates p < 0.05

Table 7.6 Multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-HSCT for ARTEMIS and RAG 1/2 SCID patients.

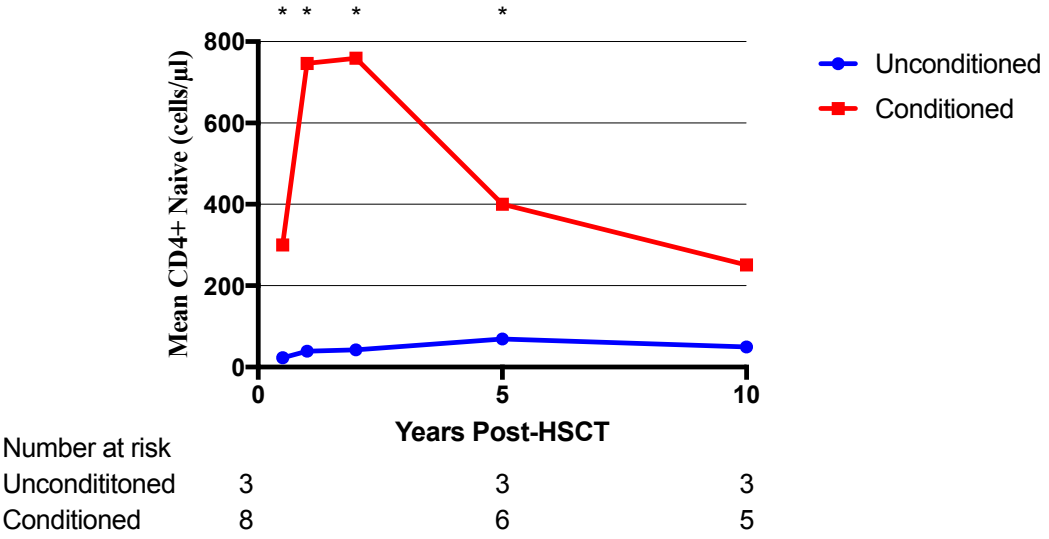
Time (Years)	Contrast	SE	p value
0	-3501.9	1077.8	0.001
0.5	-2706.6	922.7	0.003
1	-1911.3	866.0	0.02
2	-111.6	925.8	0.22
5	-320.7	1083.1	0.76
10	474.6	1303.1	0.71
Overall trend			0.004

SE indicates standard error.
p value < 0.05 is considered significant.

7.6.2 Longitudinal analysis of CD4+ Naïve lymphocyte reconstitution post-HSCT

Conditioned recipients had significantly higher CD4+ naïve lymphocyte numbers than unconditioned recipients across all time points post-transplant except at 10 years post-transplant (Figure 7.6 and Table 7.7). The conditioned recipients had a significantly higher overall trend of CD4+ naïve lymphocyte compared to unconditioned recipients, p = 0.04. The CD4+ naïve lymphocyte trend remained low throughout time post-transplant for unconditioned recipients at a level of less than 200 cells/μl.

Figure 7.6 Longitudinal analysis of CD4+ Naive lymphocyte output for Artemis & RAG 1/2 SCID patients according to unconditioned versus conditioned recipients.



* indicates $p < 0.05$

Table 7.7 Multi-level mixed effect model analysis of conditioning on CD4+ Naive lymphocyte output with time post-HSCT for Artemis and RAG 1/2 SCID patients.

Time	Contrast	SE	p-value
0.5	524.7	226.7	0.02
1	482.4	192.1	0.01
2	440.1	180.3	0.01
5	397.8	195.5	0.04
10	355.5	232.4	0.12
Overall trend			0.04

SE indicates standard error.
p value < 0.05 is considered significant.

7.6.3 Longitudinal analysis of CD19+ lymphocyte reconstitution post-HSCT

The conditioned recipients had non-significantly higher CD19+ lymphocyte counts with time post-transplant compared to unconditioned recipients, p = 0.16 (Figure 7.7 and Table 7.8).

Figure 7.7 Longitudinal analysis of CD19+ lymphocyte output for Artemis and RAG 1/2 SCID patients according to unconditioned versus conditioned recipients.

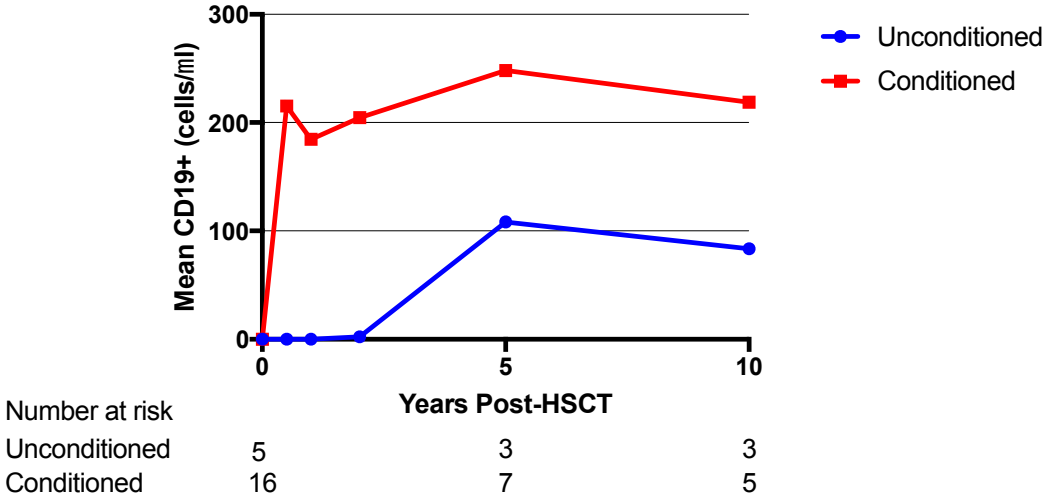


Table 7.8 Multi-level mixed effect model analysis of conditioning on CD19+ lymphocyte output for Artemis and RAG 1/2 SCID patients according to unconditioned versus conditioned recipients.

Time	Contrast	SE	p-value
0	76.6	64.3	0.23
0.5	110.5	60.9	0.06
1	144.4	78.0	0.06
2	178.2	106.0	0.09
5	212.1	138.5	0.12
10	246.0	173.0	0.15
15	210.9	400.4	0.59
Overall trend			0.16

SE indicates standard error.
p value < 0.05 is considered significant.

7.6.4 Longitudinal analysis of NK cell reconstitution post-HSCT

The NK cell level of conditioned recipients remained static with time post-transplant. During the first year after transplant, unconditioned recipients had significantly higher NK cells compared to conditioned recipients. The NK cells further decreased in value resulting in no significant difference between both groups from the second year post-transplant onwards. Regardless, there was a significant difference observed in the overall trend of NK cells between groups, p = 0.003 (Figure 7.8 and Table 7.9).

Figure 7.8 Longitudinal analysis of NK cell output for Artemis and RAG 1/2 SCID patients according to unconditioned versus conditioned recipients.

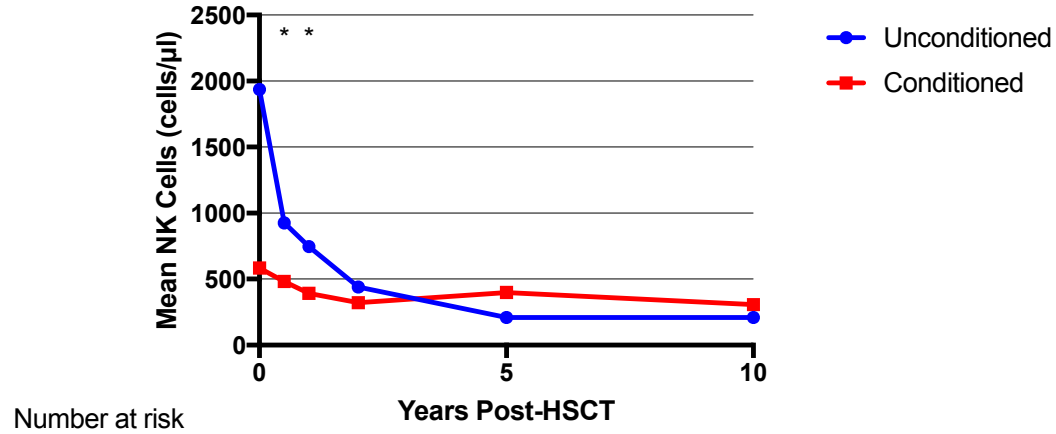


Table 7.9 Multi-level mixed effect model analysis of conditioning on NK cell output with time post-HSCT for Artemis and RAG 1/2 SCID patients.

Time	Contrast	SE	p-value
0	-1046.4	312.7	0.0008
0.5	-762.2	234.6	0.001
1	-478.0	195.4	0.01
2	-193.8	217.3	0.37
5	90.2	286.5	0.75
10	374.4	378.0	0.32
Overall trend			0.003

SE indicates standard error. p value < 0.05 is considered significant.

7.7 Quality of Life post-HSCT

Eleven out of sixteen (68%) Artemis and RAG 1/2 SCID patients and families answered the PedsQL questionnaires. The median duration of post-transplant follow-up for responders was 10 years, (range 2 – 18). A comparison of all mean scores was performed against mean scores of published normal values in the UK [112].

Parents of Artemis and RAG 1/2 SCID patients reported no significant difference in mean scores in all 5 domains in comparison to published normal values in the UK. The mean scores for parent reports were as follows: Total domain (72.4, $p = 0.15$), psychosocial domain (70.0, $p = 0.18$), physical domain (76.7, $p = 0.17$), emotional domain (70.7, $p = 0.40$), social domain (81.4, $p = 0.46$), school domain (67.5, $p = 0.08$) (Table 7.10). There was no significant difference in the PedsQL mean scores of domains for Artemis and RAG 1/2 SCID patients, except for the school domain (62.5, $p = 0.03$).

Further subgroup comparison was performed between those with on-going medical issues and those without on-going medical issues, against published normal values in the UK. Both parent and child reports of Artemis and RAG 1/2 SCID patients without on-going medical issues reported no significant difference in all domains compared to the UK's normal values. Artemis and RAG 1/2 SCID patients with on-going medical issues reported significantly lower mean scores in 2 domains; physical (72.3, $p = 0.04$) and school (60.8, $p = 0.03$). Parents of those with on-going medical issues reported no significant difference in mean scores.

Table 7.10 Mean PedsQL Scores for Artemis and RAG 1/2 SCID patients post-HSCT (Parent and Child Report)

	UK Norms[112] Mean	Artemis & RAG 1/2 SCID Mean (p-value)	On-going medical issues Mean (p-value)	No on-going medical issues Mean (p-value)
Parent Report		**N = 7**	**N = 5**	**N = 2**
Total	84.6	72.4 (0.15)	70.0 (0.20)	78.2 (0.74)
Psychosocial	82.2	70.0 (0.18)	68.6 (0.26)	73.3 (0.68)
Physical	89.1	76.7 (0.17)	72.5 (0.17)	87.5 (0.91)
Emotional	78.3	70.7 (0.40)	69.0 (0.37)	75.0 (0.91)
Social	86.8	81.4 (0.46)	82.0 (0.66)	80.0 (0.94)
School	81.5	67.5 (0.08)	68.7 (0.40)	65.0 (0.22)
Child Report		**N = 9**	**N = 6**	**N = 3**
Total	83.9	73.0 (0.13)	71.3 (0.13)	76.3 (0.68)
Psychosocial	81.8	73.5 (0.20)	71.9 (0.21)	76.6 (0.73)
Physical	88.5	73.6 (0.07)	**72.3 (0.04)**	76.0 (0.61)
Emotional	78.5	78.6 (0.98)	77.5 (0.90)	80.8 (0.87)
Social	87.7	79.4 (0.31)	77.5 (0.31)	83.3 (0.81)
School	78.9	**62.5 (0.03)**	**60.8 (0.03)**	66.1 (0.18)

Bold indicates p value < 0.05 and considered significant
All comparisons were made to UK published noral value using one sample T-test.

7.8 Summary of Artemis and RAG 1/2 SCID long-term outcome post-transplantation

Following is the summary of long-term outcome for Artemis and RAG 1/2 SCID patients post-transplant. CD3+ lymphocyte post-transplant recoveries were significantly shorter in unconditioned recipients compared to conditioned Artemis and RAG 1/2 SCID patients. Acute GVHD was seen more in RAG 1/2 SCID (5, 55%) than Artemis (3, 43%) and most were mild acute GVHD Grade I – II (75%).

No significant difference in 10 year survival between Artemis, RAG 1/2 SCID and other SCID genotypes was identified.

Artemis survivors experienced more on-going medical issues than RAG 1/2 SCID, 85% and 55%, respectively. The main medical issues seen in Artemis were dental issues (3 patients, 43%), short stature (3 patients, 43%), post- transplant autoimmune hemolytic anemia (3 patients, 43%), and dermatology issues (3 patients, 43%). Chronic renal failure was diagnosed in 1 patient who received MMUD and busulfan 8mg/kg. The main medical issues seen in RAG 1/2 SCID were dermatology issues (3 patients, 33%), immunoglobulin replacement therapy (2 patients, 22%), short stature (1 patient, 11%), post- transplant autoimmune hemolytic anemia (1 patient, 11%) and autoimmune hypothyroidism (1 patient, 11%). More conditioned Artemis and RAG 1/2 SCID recipients were able to be free from immunoglobulin replacement therapy compared to unconditioned recipients, (conditioned: 10 out of 12 patients, unconditioned: 1 out of 4 patients, Fisher exact test, $p = 0.06$).

Considering long-term immune reconstitution for both Artemis and RAG 1/2 SCID; B-lymphocyte and myeloid donor chimerism mirrored each other in all donor types and conditioning regimens; except for haploidentical/MAC recipients. Low toxicity MAC recipients demonstrated better B/myeloid donor chimerism across all donor types. Poor B/myeloid donor chimerism percentage (<10%) was seen in Unconditioned MSD recipients. There was sustained output for CD3+ lymphocytes, CD19+ lymphocytes and NK cells irrespective of conditioning regimen. CD4+ Naïve reconstitution was significantly better in conditioned Artemis and RAG 1/2 SCID recipients. CD4+ Naïve lymphocyte output of

unconditioned recipients remained < 500 cells/l throughout all time points post-transplantation.

Both parent and child reports of patients with Artemis and RAG 1/2 SCID without on-going medical issues reported a normal quality of life.

Important findings:

- Artemis survivors experienced more on-going medical issues than RAG 1/2 SCID, 85% and 55%, respectively.

- Conditioned Artemis and RAG 1/2 SCID recipients had better B-lymphocyte chimerism, CD4+ naïve/CD19+ lymphocyte reconstitution and were able to be free from immunoglobulin replacement therapy compared to unconditioned recipients, but conditioning causes more on-going medical issues in Artemis SCID patients at latest follow up during post-HSCT period.

- Both parent and child reports of patients with Artemis and RAG 1/2 SCID without on-going medical issues reported a normal quality of life.

Chapter 8 Long-term Outcome for ADA SCID Post-HSCT

This chapter presents the results of the long-term outcome post-transplantation for ADA SCID patients in Newcastle. The ADA SCID cohort was analysed individually, due to the distinctive clinical manifestations.

8.1 Cohort Characteristics

A total of 19 ADA SCID patients had undergone 20 HSCT in Newcastle between 1987 and 2012. ADA SCID accounted for 16% of the overall Newcastle SCID cohort. The median age at the last follow-up in January 2015 was 11.5 years (range 3 – 25). Sixteen out of 19 patients (84%) were alive at the last follow-up. The median age at first transplant was 12 weeks, range 1 – 47; and 52% of the cohort received their first transplant at, or less than 3 months of age. Only four out of 19 ADA SCID patients received PEG-ADA prior to their first HSCT.

Eleven out of nineteen patients (58%) did not receive any conditioning prior to their transplant [MSD (5 patients), MUD (4 patients) and MMUD (2 patients)]. For those who were conditioned, 3 patients received low toxicity MAC and 5 patients received MAC (Table 8.1). Only one third of the cohort had serotherapy, rATG (1 patient) and Campath 1H (4 patients).

Most patients received bone marrow (11 patients, 58%) as their graft source, followed by umbilical cord blood (7 patients, 37%) and peripheral blood stem cells (1 patient, 5%). The median weight of the recipients was 3.8 kg, (range 3 – 7.2). The median volume of the graft was 77.5ml, (range 35 – 180). The following are the median values for all the specific parameters concerning the graft cell doses: mononucleated cell dose 4.3 x 10^8/kg (range, 0.04 – 25.6), CD34+ cell dose 4.8 x 10^6/kg (range, 0.48 – 42.8), CD3+ lymphocyte dose 0.67 x 10^8/kg (range, 0.0001 – 3.1) and CD19+ lymphocyte dose 2.0 x 10^7/kg (range, 1.6 – 3).

Table 8.1 Conditioning regimen, donor type and serotherapy for ADA SCID patients.

Conditioning	MSD	MRD	MUD	MMUD	Haploidentical
Unconditioned	5	0	4	2	0
Low Toxicity MAC	0	2	1	0	0
MAC	1	0	0	2	2
Serotherapy					
No serotherapy	5	0	4	4	1
Campath 1H	0	2	1	0	1
rATG	1	0	0	0	0

8.2 Immediate Outcome (within two years post-HSCT)

All ADA SCID patients achieved T-lymphocyte engraftment at day 30 post-transplant (1st HSCT). However, one patient (a haploidentical recipient with Busulfan 16mg/kg) needed a second transplant due to secondary graft failure (as evidenced by slipping in chimerism from full donor to full recipient by 14 months post-transplant).

The median number of days taken for a neutrophil recovery of more than 0.5 x 10^9/L, among the conditioned ADA recipients was 18 days (range 11 – 31). No unconditioned recipients experienced a neutrophil count of less than 0.5 x 10^9/L

CD3+ lymphocyte recovery was significantly shorter in the unconditioned recipients compared to conditioned, (median 17 days, range 7 – 25 vs. median 78 days, range 25 – 167; $p = 0.03$).

Nine out of nineteen patients (47%) developed acute GVHD during the immediate post-transplant period. Seven patients developed acute GVHD Grade II (2

patients = unconditioned/MSD recipients, 1 patient = unconditioned/MUD recipient, 1 patient = unconditioned/MMUD recipient, 1 patient = low toxicity MAC/MUD recipient and 2 patients = haploidentical/MMUD recipients). Only one unconditioned MSD recipient developed acute GVHD Grade I and one unconditioned MUD with acute GVHD Grade III. From the multiple logistic regression analysis, no significant association between acute GVHD and donor type or conditioning regimen was identified (Table 8.2).

Table 8.2 Result of multiple logistic regression analysis of different independent factors on the incidence of acute GVHD post-HSCT.

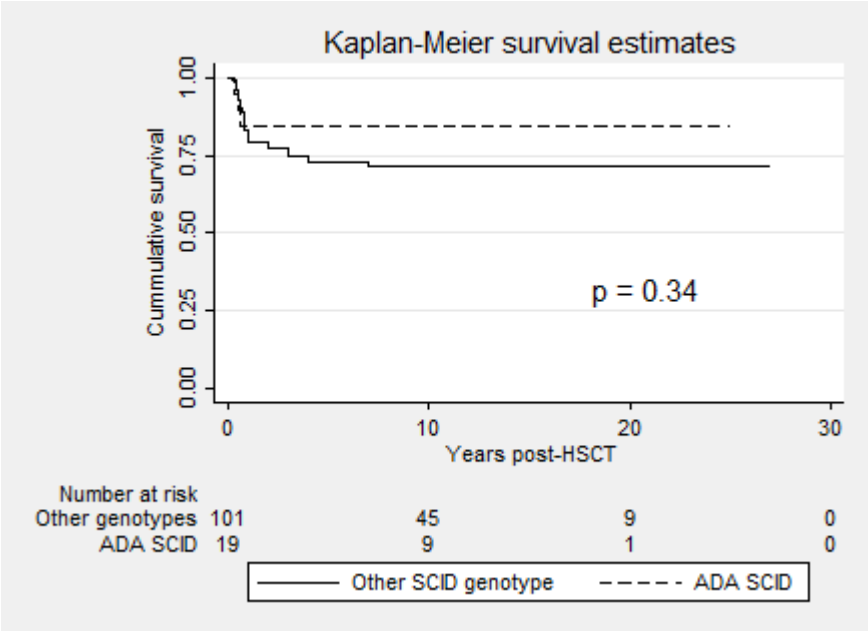
Acute GVHD	OR	95% CI	p-value
Conditioning regimen			
Unconditioned	1.0	Reference	
Conditioned	0.49	0.03 – 7.18	0.60
Donor Group			
MSD	1.0	Reference	
MUD	0.67	0.06 – 7.62	0.75
MMUD	0.40	0.02 – 7.32	0.54

CI indicates confidence interval
p value < 0.05 was considered significant

8.3 Survival Outcome

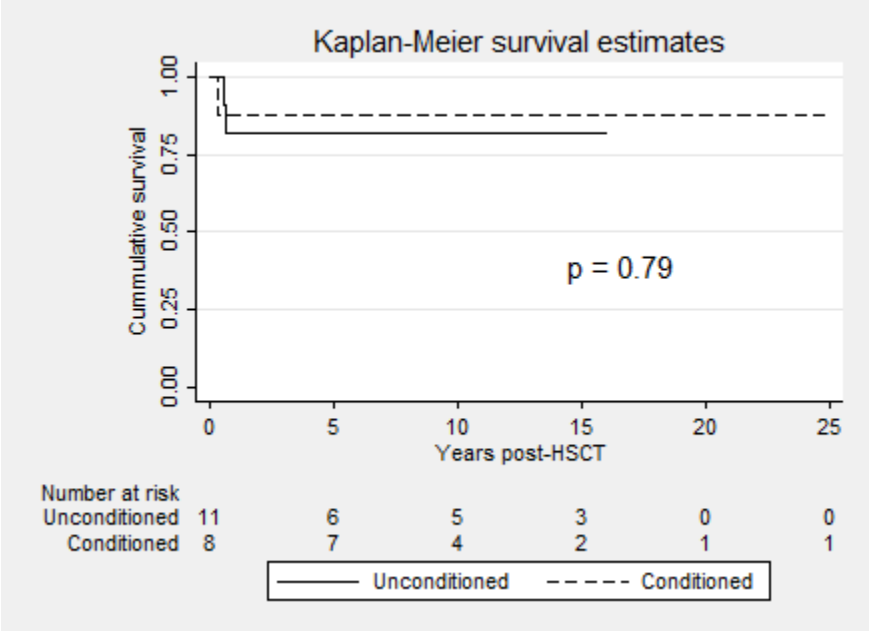
Ten year survival for the ADA SCID cohort was 84.2% (95% CI: 58.6 – 94.6%) with a TRM of 10.9%. There was no significant difference in survival compared to other SCID genotypes, 71.5% (95% CI: 61.4 – 79.4%), p = 0.34 (Figure 8.1). Three deaths were recorded and all were during the first year post-transplant. The causes of death were multi-organ failure (2 patients) and idiopathic neuro-degenerative disease associated with central hypoventilation (1 patient).

Figure 8.1 Comparison of survival outcome between ADA SCID and other SCID genotypes.



Further sub-group comparison of survival outcome was performed between conditioned and unconditioned recipients. There was no significant difference in 10 year survival outcome between both groups; conditioned recipients 87.5% (95% CI: 38.7 – 98.1%) vs. unconditioned recipients 81.8% (95% CI: 44.7 – 95.1%), p = 0.79 (Figure 8.2).

Figure 8.2 Comparison of survival outcome among the unconditioned and conditioned recipients in ADA SCID patients.



8.4 Long-term Clinical Outcome

8.4.1 Clinical Outcome

Fourteen out of sixteen (87%) surviving ADA SCID patients had on-going medical issues at the last follow-up in January 2015. Table 8.3 summarises these on-going medical issues.

Table 8.3 Long-term clinical outcome of ADA SCID post-transplant.

Clinical Outcome	ADA SCID % (n/N)
10 years survival	84.2% (16/19)
On-going medical issues	87% (14/16)
On-going Immunoglobulin replacement therapy	19% (3/16)
Neurocognitive issues	44% (7/16)
Short stature	47% (7/15)
Hearing loss	40% (6/15)
Hyper-pigmented rash	19% (3/16)
Endocrine issues	18% (3/16)
Dental issues	6% (1/15)
Lung function test	100% (1/1)
No Cardiovascular Issues	100% (16/16)
No Gastrointestinal Issues	100% (16/16)
No Respiratory Issues	100% (16/16)

Neurocognitive issues were the most common medical on-going problem in the ADA SCID cohort, seen in 7 out of 16 patients (44%). Further characterization of the neurocognitive issues showed learning difficulties (3 patients), attention deficit hyperactive disorder (2 patients), cerebral palsy (1 patient) and low mood (1 patient). There was a significant association between neurocognitive issues and ADA SCID genotype; Pearson chi square test, $p < 0.001$.

Short stature (Height $\leq$ 2SD) was found in 47% of the surviving ADA SCID cohort. However, there was no signifcant association between short stature and SCID genotypes; Pearson chi square test, $p = 0.08$. Of seven patients with short stature, five were conditioned recipients. There was no significant association between short stature and conditioning pre-transplant; Fisher exact test, $p = 0.10$.

A high proportion of ADA SCID patients developed hearing loss and wear hearing aids as a consequence, (6 out of 15 patients (40%)). There was a significant association between hearing loss and SCID genotype; Pearson chi square test, $p = 0.001$.

Three patients had hyperpigmented skin rash noted during their routine clinical follow up. They were referred for dermatological assessment to rule out the possibility of dermatofibrosarcoma protuberans, which has been reported as exclusively found in ADA SCID patients post-transplant [118].

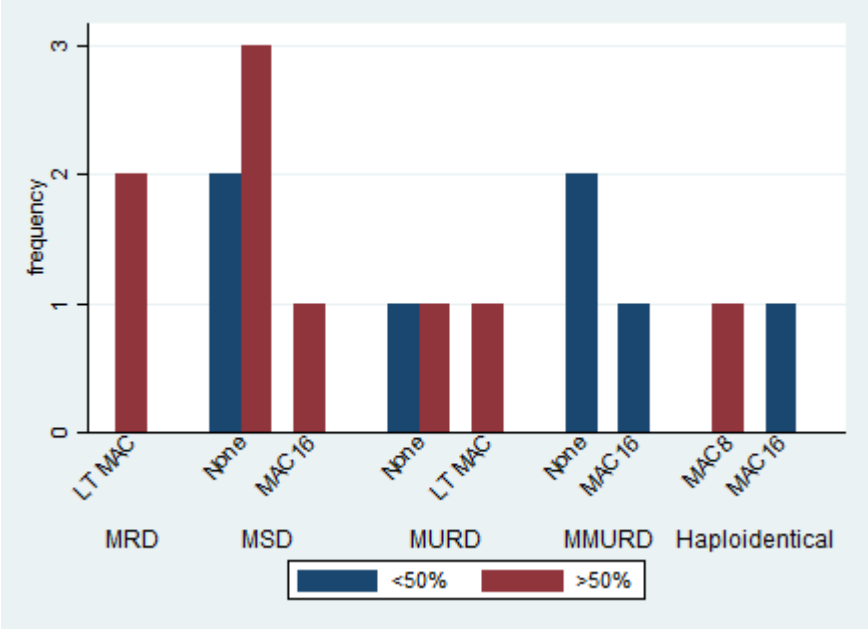
Two patients had endocrine issues which were central precocious puberty and hypogonadism. One patient had dental issues and another patient had autoimmune hypothyroidism. Lung function testing was available for one patient and it was normal. All patients aged more than 13 years old had achieved puberty except for 2 female patients aged 13 and 14 years old. No pregnancy was reported in this cohort.

8.4.2 Immunoglobulin replacement therapy at last follow up

Eighty-one percent of patients were free from immunoglobulin replacement therapy at the last review in January 2015. The main differentiating feature observed in this cohort is that most of the patients with mixed or poor B lymphocyte donor chimerism were able to discontinue immunoglobulin replacement (Figure 8.3). Three patients with less than 10% B lymphocyte donor

chimerism still needed on-going immunoglobulin replacement [unconditioned MSD (1) and MMUD (2) recipients]..

Figure 8.3 Number of ADA SCID patients free from immunoglobulin replacement therapy at last follow up according to the donor type, conditioning regimen and B-lymphocyte donor chimerism.



8.5 Chimerism at last follow-up

The result of myeloid and B lymphocyte donor chimerism was available for 14 out of 16 patients. Myeloid and B lymphocyte donor chimerism were found to be positively correlated which was statistically significant. (Spearman rho = 0.8493, p = 0.0001) (Figure 8.4). All low toxicity MAC recipients had a B/myeloid donor chimerism percentage of more than 80%, except for one haploidentical donor (Figure 8.5). A similar observation to the other SCID types was seen, in which unconditioned recipients had poor B/myeloid donor chimerism at the last follow up.

Figure 8.4 Scatter plot of B-lymphocyte donor chimerism and myeloid donor chimerism at last follow up.

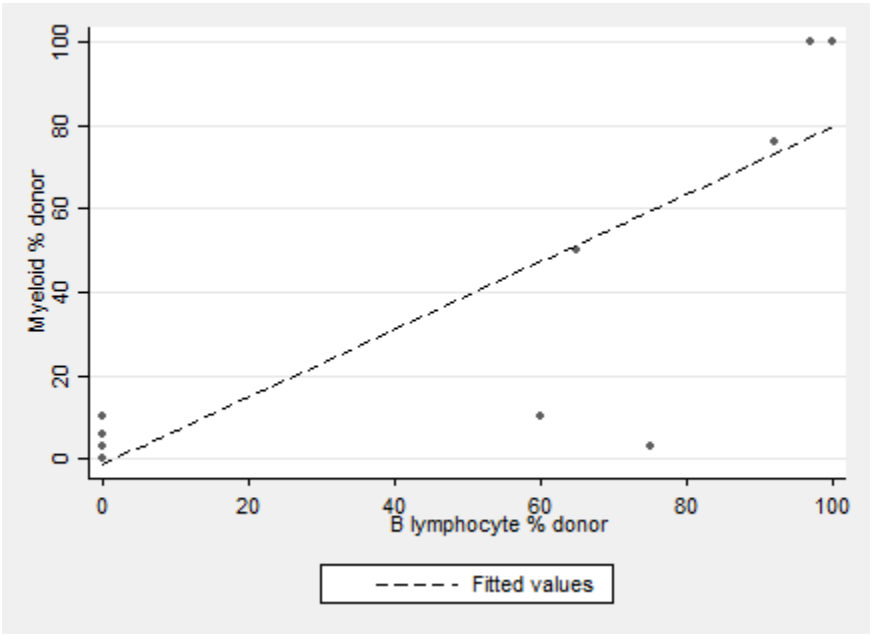
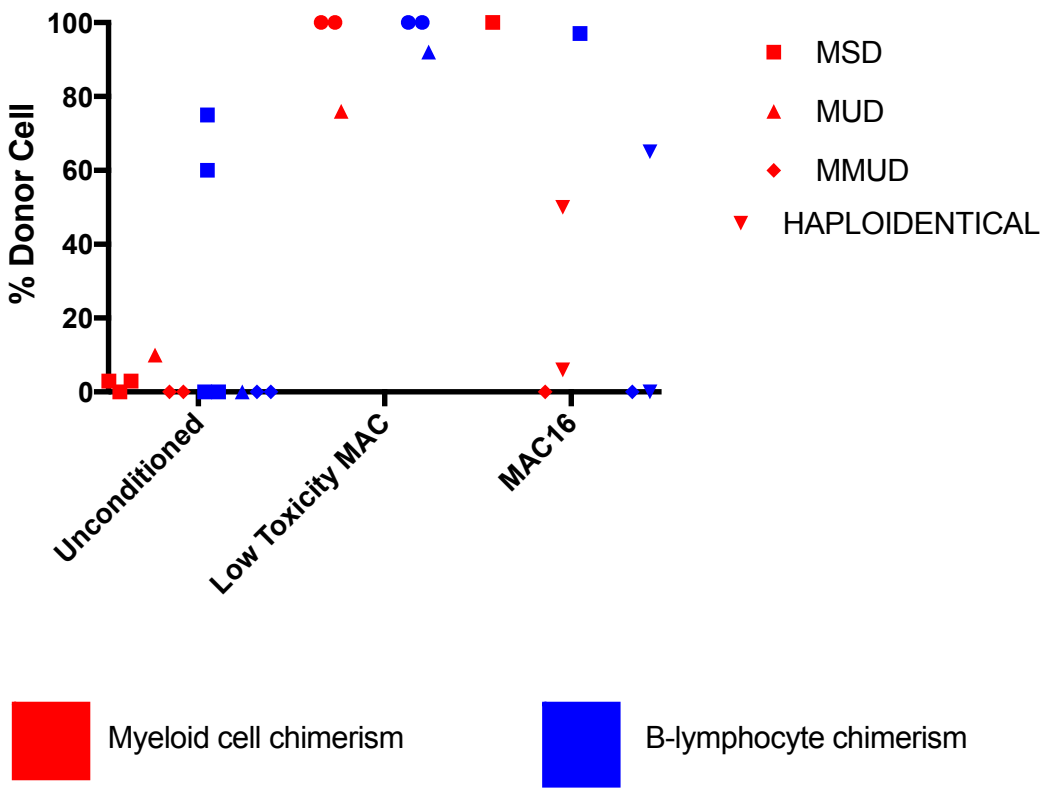


Figure 8.5 Myeloid and B lymphocyte donor chimerism (at last follow up) according to donor type and conditioning regimen.



All low toxicity MAC recipients had a B/myeloid donor chimerism percentage of more than 80%, except for one haploidentical donor. Most of the unconditioned MSD recipients had poor B and myeloid donor chimerism.

These variables in final models explains 100% of myeloid donor chimerism at the last follow up. Both low toxicity MAC and MAC conditioning were significantly predictive of higher myeloid donor chimerism compared to those who did not receive any conditioning, after controlling for stem cell dose, graft source and donor type (Table 8.4).

Table 8.4 Results of multivariable linear regression analysis of the myeloid donor chimerism percentage (at last follow up).

Myeloid donor %	Coefficient	95% CI	p-value
CD34 cell dose	-1.1	-3.2 – 0.9	0.09
Mononucleated cell dose	-0.09	-2.0 – 1.8	0.65
Donor type			
MSD	1.0	Reference	
MRD	-70.0	-76.2 – -63.7	0.004
MUD	-93.8	-99.4 – -88.3	0.003
MMUD	-104.2	-111.9 – -96.6	0.004
Haploidentical	-56.3	-76.1 – -36.4	0.01
Conditioning regimen			
Unconditioned	1.0	Reference	
Low Toxicity MAC	164.3	160.1 – 168.6	0.001
MAC	99.0	96.0 – 102.0	0.002
Graft source			
BM	1.0	Reference	
UCBT	93.8	81.6 – 105.9	0.006

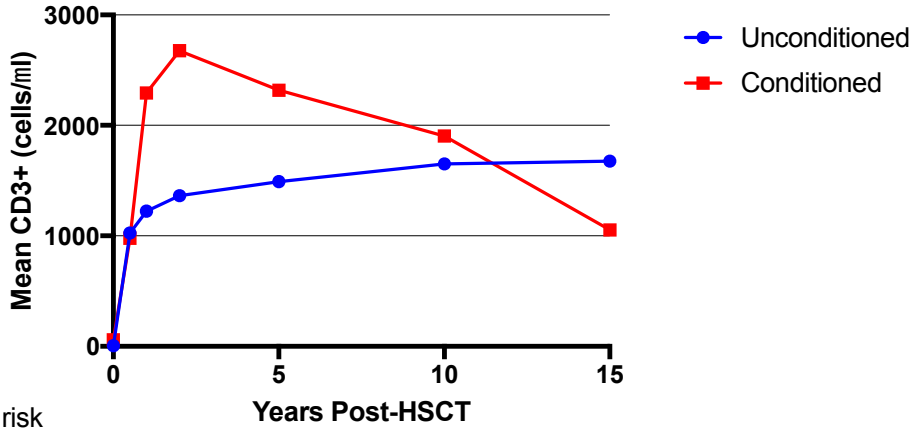
R^2 = 100%
CI indicates confidence interval
p value < 0.05 was considered significant

8.6 Long-term immune reconstitution post-HSCT

8.6.1 Longitudinal analysis of CD3+ lymphocyte reconstitution post-HSCT

The conditioned recipients had a non-significantly higher CD3+ lymphocyte output over time post-transplant, except from 10 years post-transplant when it started to decrease in value. Longitudinal output of CD3+ lymphocyte remained stable after 2 years post-transplant for unconditioned recipients. There was no significant difference in the overall trend of CD3+ lymphocytes changes with time post-transplant between conditioned and unconditioned recipients, (p = 0.28) (Figure 8.6 and Table 8.5).

Figure 8.6 Longitudinal analysis of CD3+ lymphocyte output for ADA SCID patients post-HSCT according to conditioned and unconditioned recipients.



Number at risk				
Unconditioned	11	5	4	2
Conditioned	8	6	4	2

Table 8.5 Multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-HSCT for ADA SCID patients.

Time (Years)	Contrast	SE	p value
0	195.1	264.3	0.46
0.5	300.9	236.7	0.20
1	406.8	261.9	0.12
2	512.6	327.8	0.11
5	618.4	415.6	0.13
10	724.3	514.2	0.15
Overall trend			0.28

SE indicates standard error.
p value < 0.05 is considered significant.

8.6.2 Longitudinal analysis of CD4+ Naïve lymphocyte reconstitution post-HSCT

The conditioned recipients showed a significantly higher overall trend for CD4+ naïve lymphocyte count with time post-transplant, compared to unconditioned ADA SCID recipients, ($p = 0.04$) (Figure 8.7 and Table 8.6). The observed value of CD4+ naïve lymphocytes in unconditioned recipients was persistently less than 500 cells/µl at all time points post-transplant (Figure 8.7).

Figure 8.7 Longitudinal analysis of CD4+ naive lymphocyte output for ADA SCID patients according to unconditioned versus conditioned recipients.

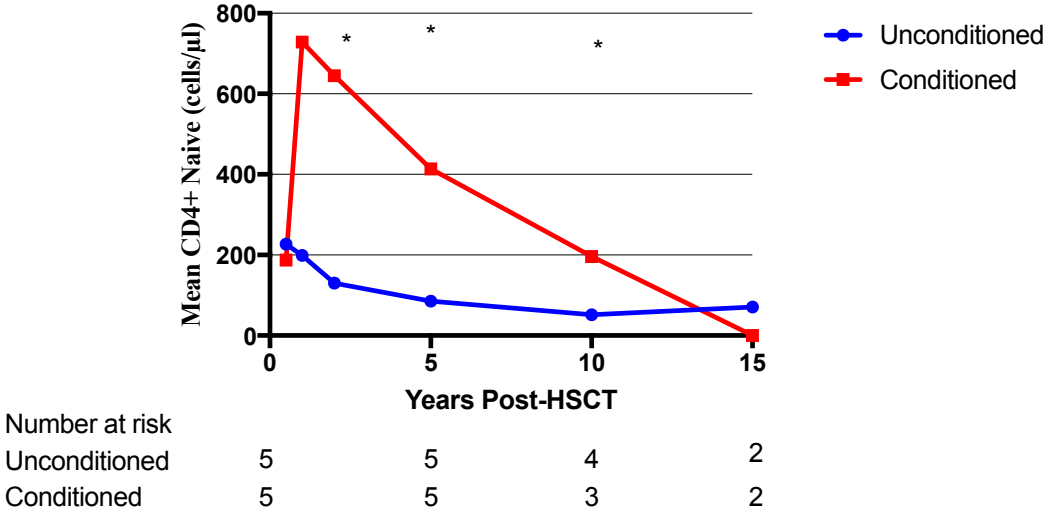


Table 8.6 Multi-level mixed effect model analysis of conditioning on CD4+ Naive cells output with time post-HSCT for ADA SCID patients.

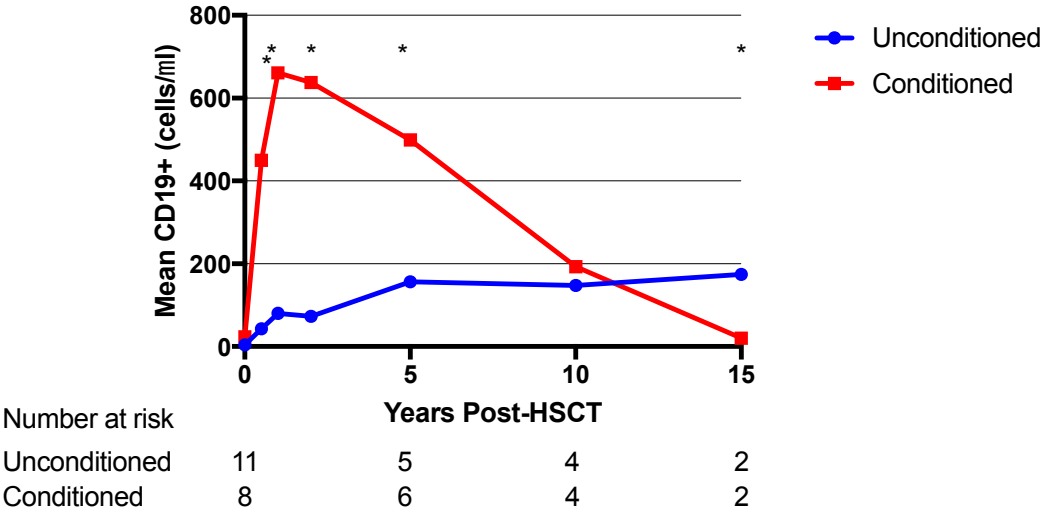
Time (Years)	Contrast	SE	p-value
0.5	218.6	184.3	0.23
1	227.9	146.0	0.11
2	237.1	115.5	0.04
5	246.3	100.0	0.01
10	255.5	106.4	0.01
15	264.7	131.5	0.04
Overall trend			0.04

SE indicates standard error.
p value < 0.05 is considered significant.

8.6.3 Longitudinal analysis of CD19+ lymphocyte reconstitution post-HSCT

The conditioned recipients had a significantly higher CD19+ lymphocyte with time post-transplant, compared to unconditioned recipients, ($p < 0.0001$) (Figure 8.8 and Table 8.7). Again, a similar trend was observed in unconditioned recipients, where the CD19+ lymphocyte output was stable with time post-transplant.

Figure 8.8 Longitudinal analysis of CD19+ lymphocyte output for ADA SCID patients according to unconditioned versus conditioned recipients.



* indicates $p < 0.05$

Table 8.7 Multi-level mixed effect model analysis of conditioning on CD19+ lymphocyte output for ADA SCID patients according to unconditioned versus conditioned recipients.

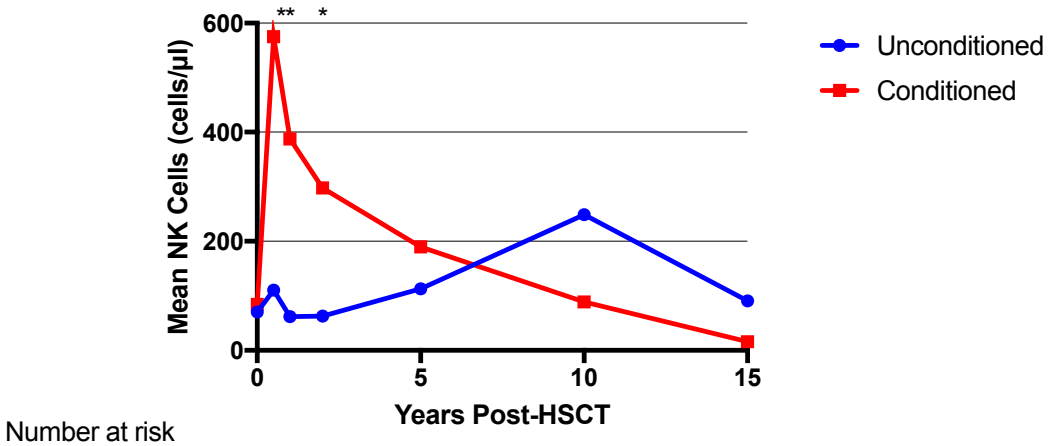
Time (Years)	Contrast	SE	p-value
0	231.2	61.9	0.0002
0.5	278.7	55.1	< 0.0001
1	326.1	72.1	< 0.0001
2	373.6	101.8	0.0002
5	421.1	136.0	0.002
10	468.5	172.1	0.006
15	516.0	209.1	0.01
Overall trend			< 0.0001

SE indicates standard error. p value < 0.05 is considered significant.

8.6.4 Longitudinal analysis of NK cell reconstitution post-HSCT

The NK cell count was significantly higher in conditioned compared to unconditioned recipients during the first two years post-transplant. The NK cell count changed 5 years post-transplant in that unconditioned recipients had a non-significantly higher value. However, from the multi-level mixed effect model analysis, there was a significant difference in overall trend of NK cells between both groups, p = 0.001 (Figure 8.9 and Table 8.8).

Figure 8.9 Longitudinal analysis of NK cell output for ADA SCID patients according to unconditioned versus conditioned recipients.



Number at risk				
Unconditioned	11	5	4	2
Conditioned	7	6	4	2

* indicates p < 0.05

Table 8.8 Multi-level mixed effect model analysis of conditioning on NK cell output with time post-HSCT for ADA SCID patients.

Time (Years)	Contrast	SE	p-value
0	270.7	75.6	0.0003
0.5	223.8	61.2	0.0003
1	176.8	53.7	0.001
2	129.9	55.9	0.02
5	82.9	66.7	0.21
10	36.0	83.0	0.66
15	-10.9	102.1	0.91
Overall trend			0.001

SE indicates standard error. p value < 0.05 is considered significant.

8.7 Quality of Life post-HSCT

A total of 12 out of 16 (75%) patients and families answered the PedsQL questionnaires. The median age of responders was 12.5 years (range 3 – 25). Only 6 out of 12 responders answered the child report part of the PedsQL questionnaire. The reasons for not answering the child report were; age less than 4 years (2 patients), parent/caretaker considered child as incapable of understanding (3 patients) and refusal (1 patient). All comparisons of the mean scores were performed against published normal values for the UK [112].

Both parents and ADA SCID patients reported significantly lower PedsQL mean scores in four domains compared to the UK's normal published values (total, psychosocial, physical, social and school, Table 8.9). The School domain had the lowest mean score recorded for both parent and child reports (Parent report 53.8, $p = 0.007$ and Child report 38.3, $p = 0.008$). However, there was no significant difference in mean score in the emotional domain observed (Parent report 77.1, $p = 0.88$ and Child report 62.5, $p = 0.16$).

Further subgroup comparison was not performed due to an imbalance in the subgroup members (only one responder was categorized as without on-going medical issues as opposed to 11 responders, and all 12 responders were free from immunoglobulin replacement therapy).

Table 8.9 Mean PedsQL Scores for ADA SCID patients' post-HSCT (Parent and Child Report)

	UK Norms[112] Mean	ADA SCID Mean (p-value)
Parent Report		**N = 12**
Total	84.6	**67.9 (0.02)**
Psychosocial	82.2	**67.4 (0.02)**
Physical	89.1	**71.1 (0.04)**
Emotional	78.3	77.1 (0.88)
Social	86.8	**71.3 (0.04)**
School	81.5	**53.8 (0.007)**
Child Report		**N = 6**
Total	83.9	**55.1 (0.05)**
Psychosocial	81.8	**52.5 (0.04)**
Physical	88.5	59.9 (0.07)
Emotional	78.5	62.5 (0.16)
Social	87.7	56.7 (0.07)
School	78.9	**38.3 (0.008)**

Bold indicates p value < 0.05 and is considered significant
All comparisons were made to UK published normal value using one sample T-test.

8.8 Summary of ADA SCID long-term outcome post-transplantation

The following summarizes the long-term outcome post-transplantation for ADA SCID. Unconditioned recipients of ADA SCID had a significantly faster recovery of neutrophil and CD3+ lymphocyte than conditioned recipients. Only one patient developed Grade III Acute GVHD (Unconditioned MUD), but 8 patients had acute GVHD Grade II. There was no significant difference in 10 year survival outcome between ADA SCID vs. other SCID genotypes and between conditioned vs. unconditioned recipients of ADA SCID.

The majority of patients (87%), had on-going medical issues, which were neurocognitive issues (44%), short stature (47%), hearing loss (40%), hyperpigmented skin rash (19%), endocrine issues (12%), dental issues and autoimmune hypothyroidism (both 6%). ADA SCID was significantly associated with neurocognitive issues and hearing loss. There was a high proportion of ADA SCID patients who were free from immunoglobulin replacement therapy (13 out of 16 patients, 81%).

Myeloid and B-lymphocyte donor chimerism was significantly correlated. Low toxicity MAC and MAC conditioning was significantly predictive for a higher percentage of myeloid chimerism at the last follow up compared to no conditioning, and after controlling for other independent factors such as stem cell doses, graft source and donor type. The long-term thymic output (CD4+ naïve lymphocyte) was significantly better in conditioned than unconditioned ADA recipients but by 10 to 15 years post-HSCT this significance is lost. Conditioned ADA SCID patients had significantly better long-term CD4+ Naïve lymphocytes, CD19+ lymphocytes and NK cells than unconditioned recipients.

For ADA SCID patients, both parents and children reported a significantly lower quality of life compared to UK normal published values. The lowest reported mean score was for the school domain. However, the mean score for the emotional domain was normal in both parent and child reports.

Important Findings:

- ADA SCID is significantly associated with neurocognitive issues and hearing loss.

- Conditioned ADA SCID patients had significantly better long-term CD4+ Naïve lymphocytes, CD19+ lymphocytes and NK cells than unconditioned recipients.

- For ADA SCID patients, both parents and children reported a significantly lower quality of life compared to UK normal published values.

Chapter 9 Long-term Outcome for Newborn SCID Post-HSCT

The survival of newborn SCID have been shown to be superior compared to those who were diagnosed later; but the clinical long term outcome, immune reconstitution and the quality of life of the newborn SCID patients were still not fully elucidated [3, 44, 119, 120]. Hence, the analysis and results of the investigation into the long-term outcome of those diagnosed as newborn SCID across all genotypes in Newcastle was performed and presented in this chapter. This is a retrospective cohort as all patients were identified either from positive family history or by symptoms. They all had their HSCT and subsequent follow up in the BMT clinic in Newcastle. None of the patients were detected through newborn screening as it is not implemented yet in the UK.

9.1 Cohort Characteristics

A total of 49 out of 120 patients (40%) in Newcastle from 1987 - 2012 were diagnosed during the neonatal period and categorized as newborn SCID. Forty patients were identified early due to positive family history (previous affected siblings with SCID). Other reasons for early detection were investigation of dysmorphic features (1 patient), presence of lymphopenia in initial full blood count (4 patients) and infection (4 patients). Forty-three out of 49 patients (87%) were alive at the last follow-up. The median age at the last follow-up in January 2015 was 12 years (range 2 – 26). The median age at first transplant was 8 weeks, range 1 – 44.

With regard to SCID genotypes, the majority had IL2RG SCID (9 patients), IL7Rα SCID (9 patients) and ADA SCID (8 patients). Other SCID genotypes were RAG1/2 SCID (6 patients), Artemis SCID (4 patients), JAK3 SCID (5 patients), undefined SCID (5 patients), reticular dysgenesis (2 patients) and CHARGE syndrome (1 patient). Table 9.2 summarises those who were alive in 2015 according to specific SCID genotypes.

Thirteen patients did not receive any conditioning prior to their transplant. Of those who were conditioned, 24 patients received MAC, 11 patients received low toxicity MAC and one patient had RIC (Table 8.1). Serotherapy was administered

in 15 patients, rATG (2 patient) and Campath 1H (13 patients). Most received bone marrow (35 patients) as their graft source, followed by umbilical cord blood (13 patients) and peripheral blood stem cells (1 patient).

Table 9.1 Newborn SCID according to the SCID genotypes.

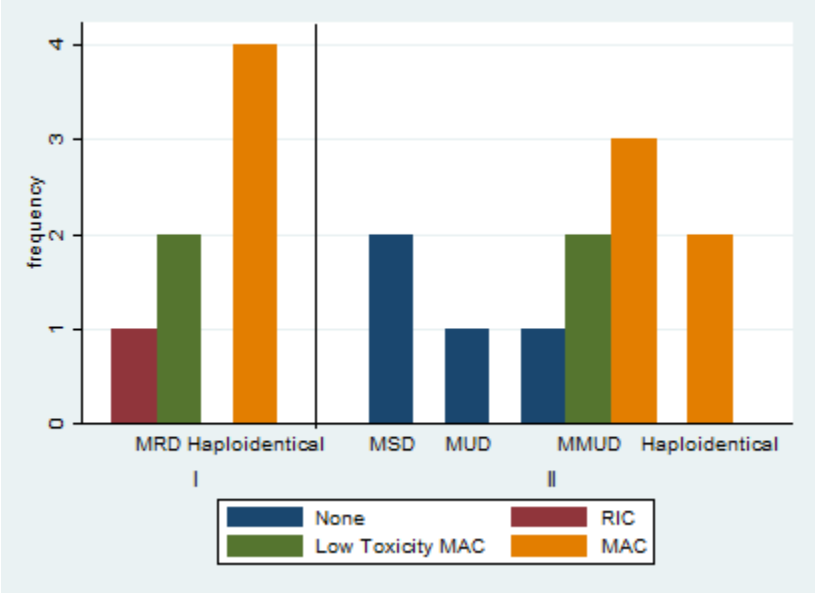
SCID genotypes	Total number	Died by end of follow-up
IL2RG SCID	9	0
IL7Rα SCID	9	1
ADA SCID	8	0
Artemis	4	0
RAG 1/2 SCID	6	1
JAK3 SCID	5	1
Undefined SCID	5	1
Reticular Dysgenesis	2	1
CHARGE Syndrome	1	1

Table 9.2 Conditioning regimen and donor type for all Newborn SCID patients in Newcastle cohort.

Conditioning	MSD	MRD	MUD	MMUD	Haploidentical
Unconditioned	5	3	2	2	1
RIC	0	1	0	0	0
Low Toxicity MAC	1	5	1	4	0
MAC	0	1	3	3	17

Thirty-one out of 49 patients (63%) developed acute GVHD during the immediate post-transplant period. The majority developed acute GVHD Grade II (11 patients) and 7 had acute GVHD Grade I (Figure 9.1). There was no Grade III/IV acute GVHD noted in this cohort. There was an almost significant association between severity of acute GVHD and preparative regimen (p = 0.07).

Figure 9.1 Number of newborn SCID patients with acute GVHD (Grade I and II) after the first HSCT according to donor type and conditioning regimen.



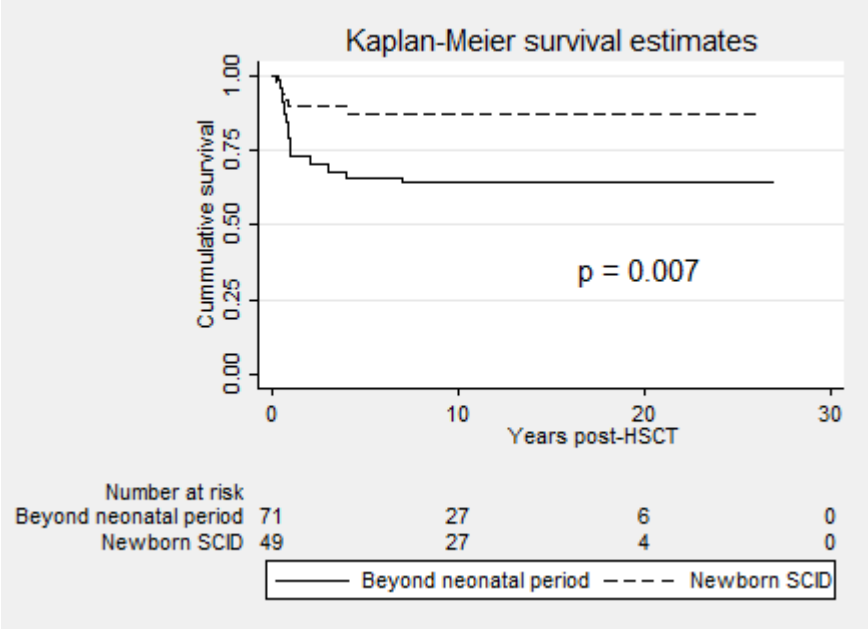
9.2 Survival

Ten year survival for the newborn SCID cohort was 87.4% (95% CI: 74.1 – 94.1%) with a TRM of 12.6%. Six deaths were recorded, all but one during the first year post-transplant. The causes of death were veno-occlusive disease (2 patients, both received busulfan 16mg/kg), pneumonitis with pulmonary hypertension (2 patients, one received busulfan 16mg/kg and another received treosulfan/cyclophosphamide) and renal failure with CHARGE Syndrome (1 patient - unconditioned).

One late death (at 4 years post-HSCT) in this cohort was an Undefined SCID patient who received MMUD donor and low toxicity MAC conditioning, cause of death = haemophilus influenza infection..

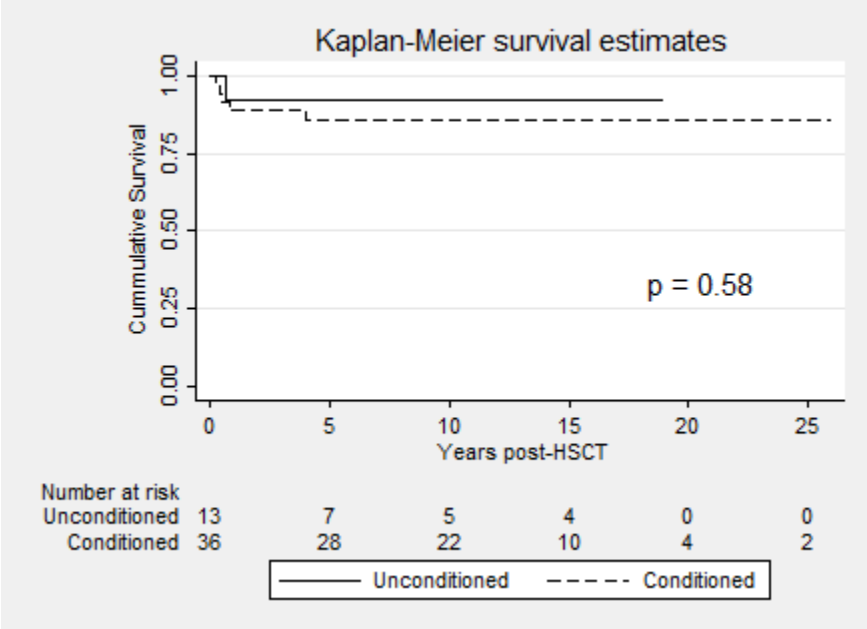
As a comparison, the newborn SCIDs had significantly better 10 year survival than those who were diagnosed later, 87.4% (95% CI: 74.1 – 94.1%) vs. 64.0% (95% CI: 51.4 – 74.1%), p = 0.007 (Figure 9.2).

Figure 9.2 Survival outcome comparison between newborn SCID and those who were diagnosed beyond neonatal period.



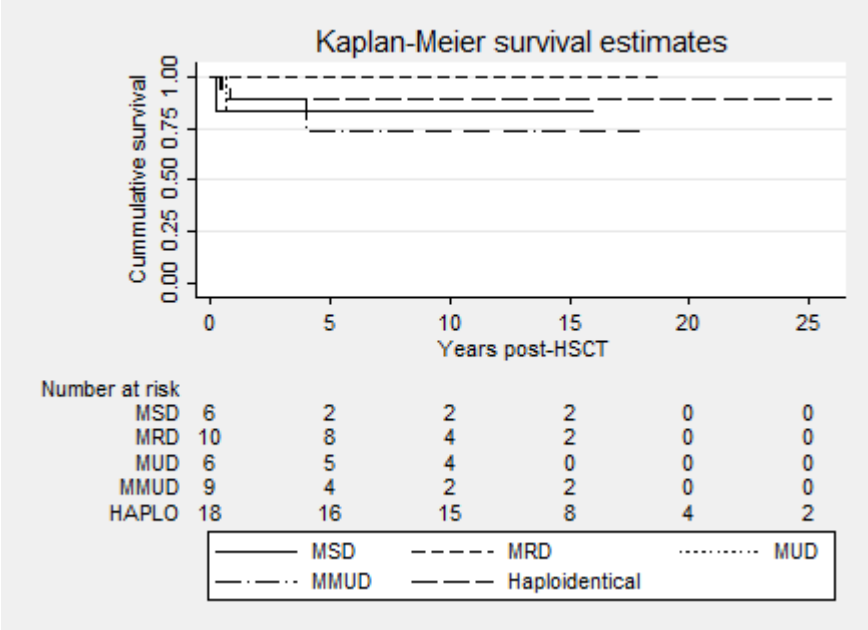
There was no significant difference in 10 year survival between conditioned [85.8% (95% CI: 69.1 – 93.8%)] and unconditioned [92.3% (95% CI: 56.6 – 98.8%), p = 0.58] recipients (Figure 9.3).

Figure 9.3 Comparison of survival outcome among the unconditioned and conditioned recipients of newborn SCID.



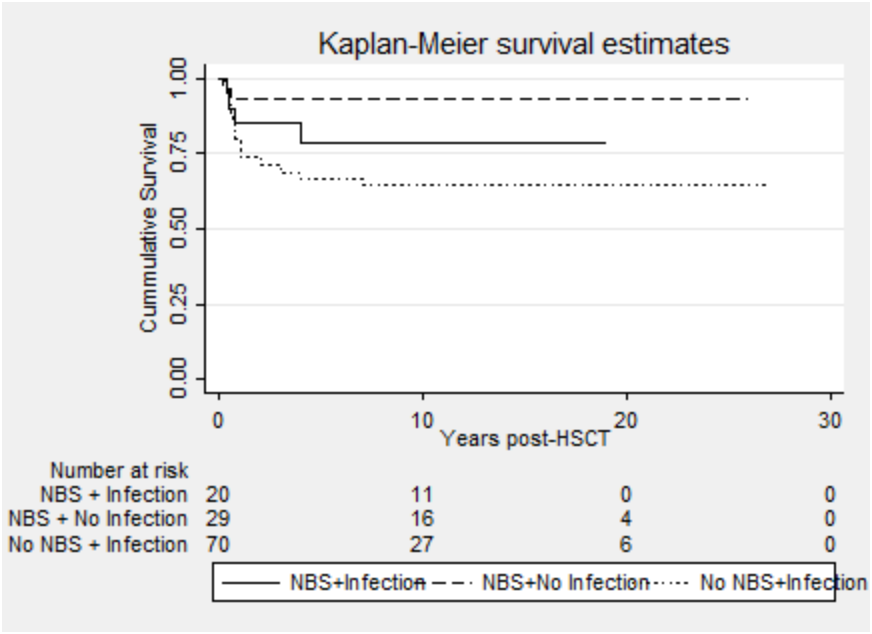
There was no significant difference in survival of newborn SCID according to different donor groups; MSD 83.3% (95% CI 27.3 – 97.4), MRD 100%, MUD 83.3% (95% CI 27.3 – 97.4), MMUD 74.0% (95% CI 28.9 – 93%) and haploidentical 88.8% (95% CI 62.4 – 97.1%), p = 0.66 (Figure 9.4).

Figure 9.4 Survival of newborn SCID according to different donor groups.



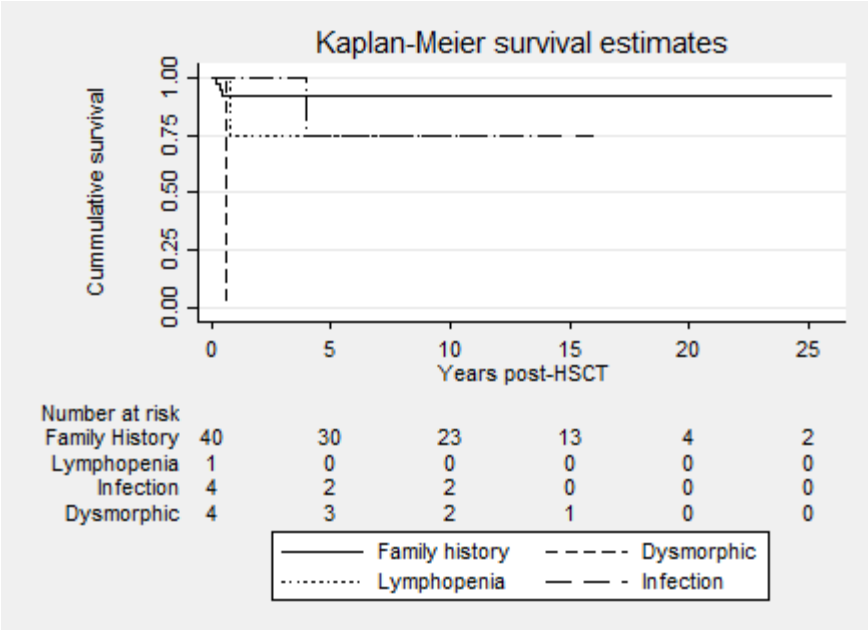
Ten year survival was significantly better for newborn SCID without infection prior to HSCT = 93.1% (95% CI 75.1 – 98.2%) compared to newborn SCID with infection = 78.9% (95% CI 52.8 – 91.5). Those who were diagnosed later and had infection prior to HSCT had the lowest survival = 64.9% (95% CI 52.3 – 75.1%), log rank test p = 0.02 (Figure 9.5).

Figure 9.5 Survival outcome of newborn SCID according to the infection status pre-HSCT.



Newborn SCIDs who were detected through positive family history had significantly better survival compared to those who presented with symptoms, p = 0.008 (Figure 9.6). Ten year survival according to methods of detection were for 92.5% (95% CI 78.5 – 97.5) family history, 75% (95% CI 12.7 – 96%) lymphopenia and 75% (95% CI 12.7 – 96%) infection. None of those who were dysmorphic survived 10 years post-HSCT (CHARGE Syndrome).

Figure 9.6 Survival outcome of newborn SCID according to methods of detection/diagnostic triggers.



9.3 Long-term Clinical Outcome

9.3.1 Clinical Outcome

A total of 43 out of 49 newborn SCID patients were alive at last follow up in January 2015, but one patient was lost to follow up. Thirty-one patients out of 42 newborn SCID patients had on-going medical issues at the last follow-up in January 2015. Table 9.3 summarises these on-going medical issues. There was no association between on-going medical issues and conditioning regimen, (unconditioned recipients=11/12 patients vs conditioned recipients=20/30 patients, p = 0.09). Further subgroup comparison showed low toxicity MAC

recipients have significantly less on-going medical issues compared to MAC recipients (2/9 patients vs 18/20 patients, $p < 0.001$).

Short stature (Height $\leq$ 2SD) was found in 30% of the cohort. There was a significant association between short stature and preparative regimen, $p = 0.05$. All except for one patient with short stature were conditioned recipients. There was no significant association between short stature and SCID genotype; $p = 0.47$.

Dermatological issues were the second most common medical on-going problem in the newborn SCID cohort, seen in 11 out of 40 patients (27%). The issues include warts (5 patients), dry skin (2 patients), vitiligo (1 patient), skin rash (1 patient), psoriasis (1 patient) and molluscum contagiosum (1 patient).

Neurocognitive issues were seen in 5 out of 40 patients (12%). Further characterisation of the neurocognitive issues showed learning difficulties (2 patients), attention deficit hyperactive disorder (2 patients) and autism spectrum disorder (1 patient).

Four of the newborn SCID cohort developed hearing loss. It was seen in ADA SCID (2 patients), Artemis SCID (1 patient) and unidentified SCID (1 patient).

Five patients had endocrine issues which were autoimmune hypothyroidism (2 patient), central precocious puberty (1 patient), diabetes type II (1 patient) and hypogonadism (1 patient).

Lung function testing was available for 7 patients and it was normal, except for one patient with a major restrictive lung defect. All patients aged more than 13 years old had achieved puberty. There were two pregnancies reported in this cohort (Unidentified SCID – 1 patient received Busulfan 16mg/kg and IL7Rα SCID – 1 patient received Busulfan 8mg/kg).

Generally, there was no significant difference between incidence of specific medical issues at last follow up between newborn SCID and those who were diagnosed later (Table 9.4).

Table 9.3 Long-term clinical outcome of newborn SCID post-transplant.

Clinical Outcome	Newborn SCID % (n/N)
10 years survival	87.4% (43/49)
On-going medical issues	74% (31/42)
On-going immunoglobulin replacement therapy	24% (10/42)
Bronchiectasis	5% (2/40)
Neurocognitive issues	12% (5/40)
Short stature	30% (12/40)
Hearing loss	10% (4/40)
Dermatological issues	27% (11/40)
Endocrine issues	12% (5/40)
Dental issues	15% (6/40)
Normal lung function test	85% (6/7)

Table 9.4 Comparison of clinical outcome between NBS and those diagnosed beyond neonatal period.

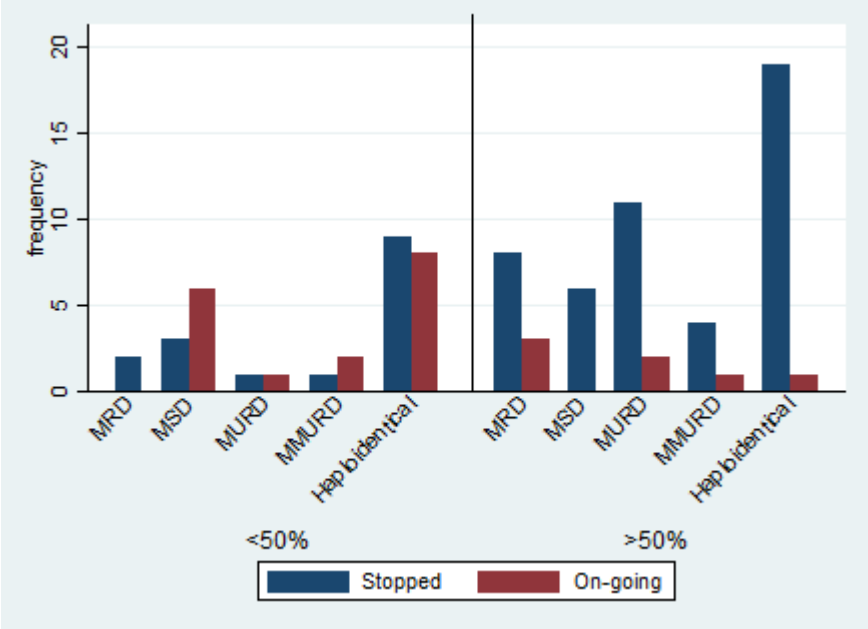
Parameter	NBS % (n/N)	Diagnosed later % (N/N)	p value
10 years survival	87.4% (43/49)	64.0% (46/71)	0.007
On-going medical issues	73% (31/42)	74% (34/46)	0.99
Stopped immunoglobulin	76% (32/42)	69% (32/46)	0.48
Bronchiectasis	4% (2/41)	9% (4/44)	0.44
Neurocognitive issues	12% (5/40)	11% (5/45)	0.84
Puberty (aged >13 years old)	90% (19/21)	82% (18/22)	0.41
Short stature	30% (12/40)	27% (11/41)	0.75
Hearing loss	10% (4/40)	9% (4/44)	0.61
Dental issues	15% (6/40)	5% (2/43)	0.11
Warts	12% (5/40)	16% (7/45)	0.68
Autoimmune hemolytic anemia	4% (2/42)	17% (8/46)	0.06
Endocrine issues	12% (5/40)	4% (2/44)	0.18

p value < 0.05 was considered significant

9.3.2 Immunoglobulin replacement therapy at last follow up

Thirty-two of the surviving patients were free from immunoglobulin replacement therapy at the last review in January 2015. The main feature observed in this cohort is that most of the patients with more than 50% B-lymphocyte donor chimerism were able to discontinue immunoglobulin replacement (Figure 9.7). A total of 27 out of 30 conditioned newborn SCIDs were able to stop immunoglobulin replacement therapy as compared to 5 out of 12 unconditioned newborn SCID. There was a significant association between preparative regimen and freedom from immunoglobulin replacement therapy (p = 0.001).

Figure 9.7 Number of newborn SCID with immunoglobulin replacement therapy status at last follow up according to the donor type, conditioning regimen and B-lymphocyte donor chimerism.



9.4 Long-term Immunological reconstitution

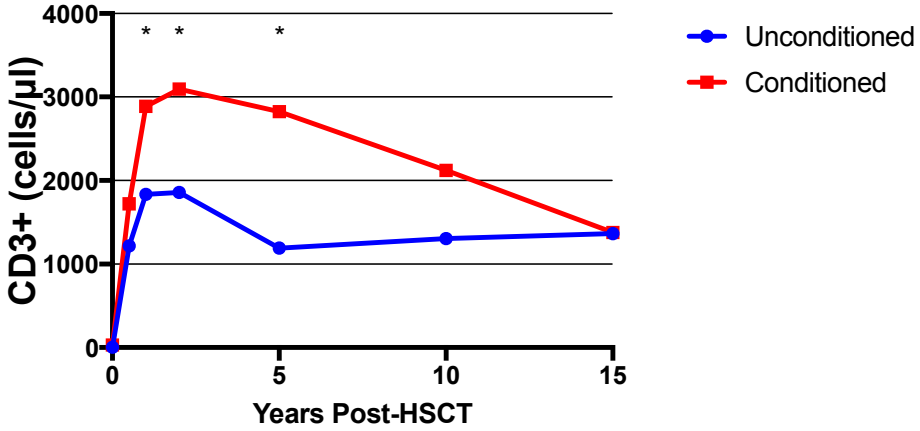
Longitudinal analysis of CD3+ and CD4+ naïve lymphocyte numbers was performed to assess the trend of changes with time for both markers and comparison was performed between conditioned versus unconditioned newborn SCID and newborn SCID versus those who were diagnosed later (not NBS).

As described before the degree of differences in immune parameters' mean values between comparison groups at each time point are presented as contrast. SE indicates standard error and it serves as an indicator for precision of mean values as population parameters [117].

9.4.1 Comparison of CD3+ lymphocyte between conditioned and unconditioned newborn SCID

From the multi-level mixed modelling analysis, CD3+ lymphocytes were significantly higher in the conditioned newborn SCID compared to unconditioned SCID, p 0.03 (Figure 9.8, Table 9.5).

Figure 9.8 Longitudinal analysis of CD3+ lymphocyte output for newborn SCID patients according to unconditioned versus conditioned recipients.



Number at risk				
Unconditioned	13	7	5	3
Conditioned	36	25	19	7

* indicates $p < 0.05$

Table 9.5 Results of the multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-HSCT for newborn SCID patients.

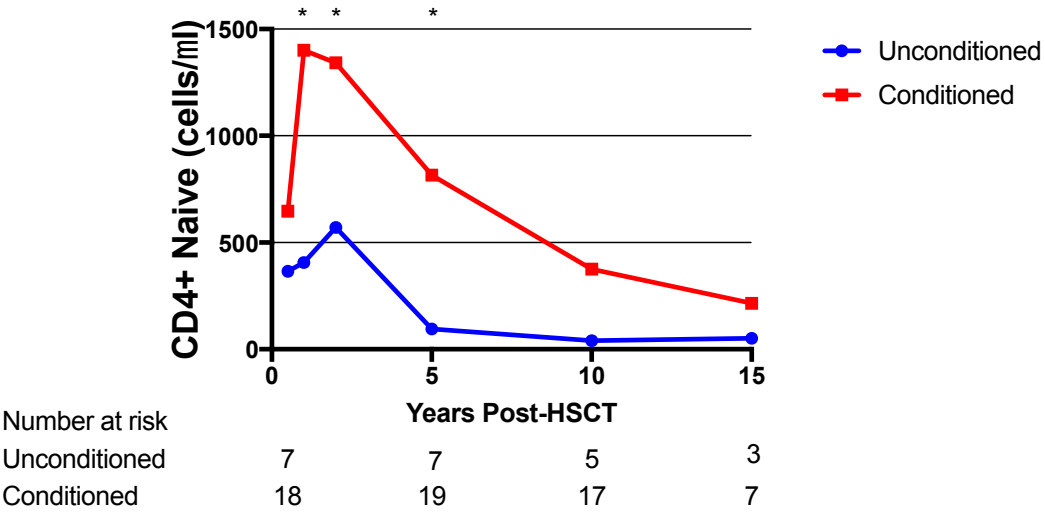
Time	Contrast	SE	P value
0	26.1	412.2	0.95
0.5	556.9	449.1	0.21
1	1095.5	451.6	0.01
2	1191.6	443.7	0.007
5	1297.1	505.7	0.01
10	439.1	571.9	0.44
15	310.9	741.4	0.67
Overall trend			0.03

SE indicates standard error.
p value < 0.05 is considered significant.

9.4.2 Comparison of CD4+ naive lymphocyte between conditioned and unconditioned newborn SCID

Conditioned newborn SCID had significantly higher trend of CD4+ naïve lymphocytes compared to unconditioned newborn SCID, p = 0.002 over time post-HSCT (Figure 9.9, Table 9.6).

Figure 9.9 Longitudinal analysis of CD4+ naive lymphocyte output for newborn SCID patients according to unconditioned versus conditioned recipients.



* indicates p < 0.05

Table 9.6 Results of the multi-level mixed effect model analysis of conditioning on CD4+ naive lymphocyte output with time post-HSCT for newborn SCID patients.

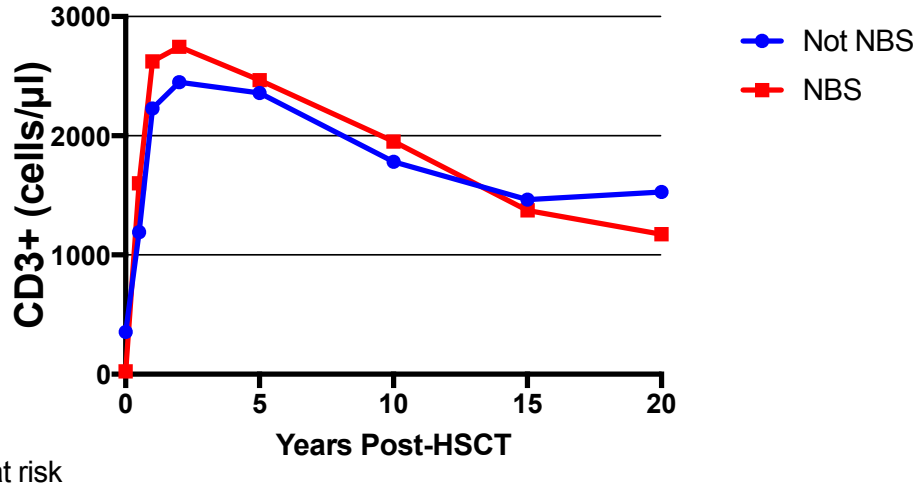
Time	Contrast	SE	p value
0.5	249.1	161.2	0.12
1	641.1	157.4	<0.001
2	418.9	155.4	0.007
5	413.1	156.7	0.008
10	242.6	169.1	0.15
15	48.5	245.9	0.84
Overall trend			0.002

SE indicates standard error. p value < 0.05 is considered significant.

9.4.3 Comparison of CD3+ lymphocyte between newborn SCID and those who were diagnosed later.

There was no significant difference in trend of CD3+ lymphocytes across time post-HSCT between newborn SCID and those who were diagnosed later, p = 0.45 (Figure 9.10, Table 9.7).

Figure 9.10 Longitudinal analysis of CD3+ lymphocyte output for SCID patients according to newborn SCID versus those who were diagnosed later.



Number at risk					
Not NBS	70	36	25	13	4
NBS	49	32	24	10	3

Table 9.7 Multi-level mixed effect model analysis of conditioning on CD3+ lymphocyte output with time post-HSCT for newborn SCID patients.

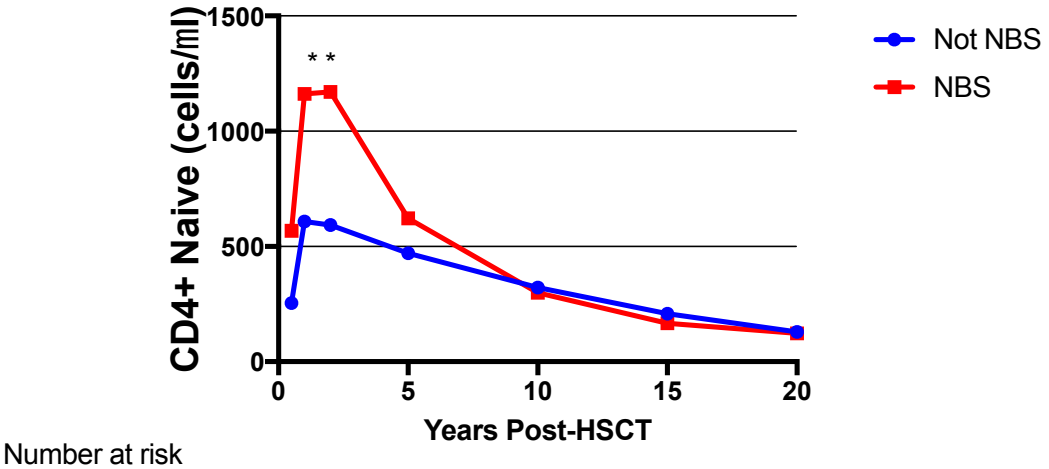
Time	Contrast	SE	P value
0	-331.3	286.2	0.24
0.5	413.4	311.4	0.18
1	382.1	327.9	0.24
2	364.6	328.5	0.26
5	185.8	357.9	0.60
10	473.3	410.5	0.24
15	350.7	579.9	0.54
20	-64.6	1024.3	0.95
Overall trend			0.45

SE indicates standard error. p value < 0.05 is considered significant.

9.4.4 Comparison of CD4+ naive lymphocyte between newborn SCID and those who were diagnosed later.

There was a significant difference in trend of CD4+ naive lymphocyte between newborn SCID and those who were diagnosed later especially during the first 5 years post-HSCT, p = 0.002 (Figure 9.11, Table 9.8).

Figure 9.11 Longitudinal analysis of CD4+ naive lymphocyte output for SCID patients according to newborn SCID versus those who were diagnosed later.



Number at risk					
Not NBS	26	26	23	12	4
NBS	25	26	22	10	3

* indicates p < 0.05

Table 9.8 Results of the multi-level mixed effect model analysis of conditioning on CD4+ naive lymphocyte output with time post-HSCT for newborn SCID patients.

Time	Contrast	SE	p value
0.5	263.2	148.1	0.07
1	502.3	145.0	0.001
2	527.5	140.9	<0.001
5	78.6	145.2	0.58
10	-15.6	154.7	0.91
15	130.3	219.4	0.55
20	50.7	382.4	0.89
Overall trend			0.002

SE indicates standard error. p value < 0.05 is considered significant.

9.4.5 Cross-sectional analysis of immune reconstitution

The analysis was performed as a cross-sectional analysis, with comparison of latest immune parameters between newborn SCID and those who were diagnosed and had their HSCT later according to the specific SCID genotypes whenever permissible.

With regards to latest CD3+ lymphocyte, only newborn IL2RG/JAK3 SCID had significantly higher counts when compared to those who were diagnosed and had their transplant later, p = 0.0008 (Table 9.9). There was no significant difference in CD3+ lymphocyte values seen for IL7Rα SCID (p = 0.65), Artemis and RAG 1/2 SCID (p = 0.20) and ADA SCID (p = 0.94) (Table 9.9).

Similar patterns were seen in for the CD4+ naïve lymphocyte counts at the latest follow up. The newborn IL2RG/JAK3 SCIDs had significantly higher CD4+ naïve lymphocyte counts compared to those who were diagnosed later, p = 0.01 (Table 9.10). No significant difference in CD4+ naïve lymphocyte counts was seen for other SCID genotypes, IL7Rα SCID (p = 0.68), Artemis and RAG 1/2 SCID (p = 0.20) and ADA SCID (p = 0.13) (Table 9.10).

Table 9.9 Comparison of latest CD3+ lymphocyte between newborn SCID and those who were diagnosed later.

SCID genotypes	Diagnosed during neonatal period N Mean (SD)	Diagnosed beyond neonatal period N Mean (SD)	p value
IL2RG/JAK3	N = 10 3260.6 (1359.2)	N = 19 1874.2 (627.7)	0.0008
IL7Rα	N = 8 1749.1 (1040.8)	N = 7 1524.0 (838.4)	0.65
Artemis & RAG 1/2	N = 9 2027.5 (1001.8)	N = 6 1450.8 (423.4)	0.20
ADA	N = 8 1675.0 (709.4)	N = 8 1650.5 (589.4)	0.94

Data were presented as mean value due to normally distributed and comparison performed using T-test.. SD = standard deviation. p value < 0.05 was considered significant

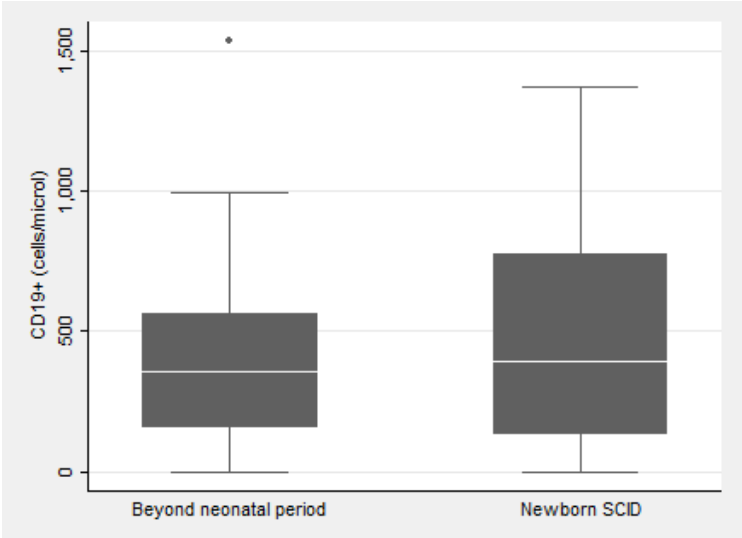
Table 9.10 Comparison of latest CD4+ naïve lymphocyte between newborn SCID and those diagnosed later.

SCID genotypes	Diagnosed during neonatal period N Mean (SD)	Diagnosed later N Mean (SD)	p value
IL2RG/JAK3	N = 10	N = 18	0.01
	890.3 (777.4)	387.5 (281.2)	
IL7Rα	N = 8	N = 7	0.68
	365.2 (267.3)	309.5 (245.9)	
Artemis & RAG 1/2	N = 9	N = 4	0.20
	357.2 (309.0)	137.2 (139.9)	
ADA	N = 7	N = 8	0.13
	84 (79.3)	188.3 (158.2)	

Data were presented as mean value due to normally distributed and comparison performed using T-test.. SD = standard deviation. p value < 0.05 was considered significant

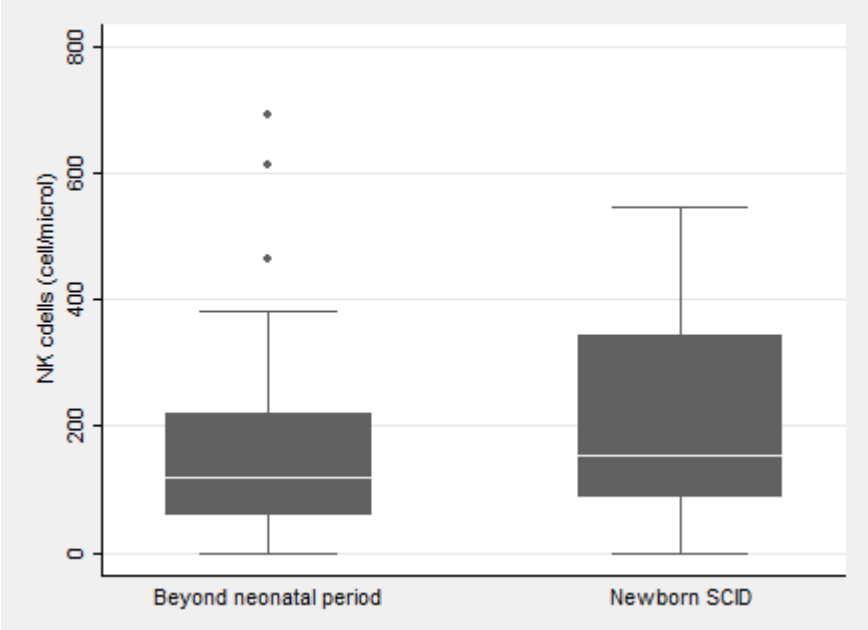
There was no significant difference in CD19+ lymphocyte mean values between newborn SCID and diagnosed beyond neonatal period, p = 0.53 (Figure 9.12).

Figure 9.12 Boxplot of CD19+ lymphocyte counts at last follow up according to whether a newborn or later SCID diagnosis.



There was no significant difference in NK cell mean values between newborn SCID and those diagnosed beyond neonatal period, p = 0.32 (Figure 9.13).

Figure 9.13 Boxplot of NK cell counts at last follow up according to newborn SCID and those diagnosed later.



9.5 Quality of Life post-HSCT

A total of 20 out of 43 (47%) patients and families answered the PedsQL questionnaires. The median age of responders was 11 years (range 2 – 26). Mean scores were performed against published normal values for the UK [112].

Parents reported no significant difference in PedsQL mean scores across all domains, except for the school domain. Newborn SCID patients reported significantly lower PedsQL mean scores in four domains compared to the UK's normal published values (total, psychosocial, social and school, Table 9.11). The school domain had the lowest mean score recorded for both parent and child reports. However, there was no significant difference for the emotional domain for either parents or patients.

Parent/carers of those who were diagnosed later reported lower quality of life in all domains compared to the UK normal population, which were worse than parents of newborn SCID report. Patients who were diagnosed later reported lower quality of life in all domains, except emotional and social.

Table 9.11 The result of the mean PedsQL Scores for newborn SCID patients' post-HSCT compared to UK norms (Parent and Child Report)

	UK Norms [112] Mean	Newborn SCID Mean (p value)*	Those diagnosed later Mean (p value)*
Parent Report		**N = 21**	**N = 28**
Total	84.6	76.1 (0.08)	**67.2 (0.0002)**
Psychosocial	82.2	73.1 (0.10)	**64.5 (0.0002)**
Physical	89.1	85.8 (0.47)	**73.6 (0.004)**
Emotional	78.3	80.2 (0.73)	**69.0 (0.04)**
Social	86.8	81.1 (0.30)	**74.4 (0.009)**
School	81.5	**64.5 (0.009)**	**62.2 (0.001)**
Child Report		**N = 20**	**N = 25**
Total	83.9	72.8 (0.06)	**73.8 (0.02)**
Psychosocial	81.8	**70.2 (0.05)**	**71.4 (0.03)**
Physical	88.5	79.9 (0.13)	**77.0 (0.01)**
Emotional	78.5	79.9 (0.80)	69.2 (0.09)
Social	87.7	**735. (0.04)**	79.2 (0.08)
School	78.9	**59.5 (0.002)**	**66 (0.02)**

Bold indicates p value < 0.05 and is considered significant.
All comparisons were made to UK published normal value using one sample T-test.

9.5.1 Quality of Life according to specific SCID genotypes

Further subgroup comparison between newborn SCID and those diagnosed later was performed according to specific SCID genotypes (Table 9.12). There were no significant differences in mean PedsQL scores of either parent or child report for IL2RG/JAK3 SCID, IL7R SCID and Artemis & RAG1/2 SCID, comparing newborn SCID and those who were diagnosed later.

However, parents of newborn ADA SCID reported better mean PedsQL scores across all domains except school domains compared to parents of ADA SCID who were diagnosed later. It was difficult to ascertain the validity of child report for ADA SCID due to small numbers (newborn ADA SCID = 4 patients and ADA diagnosed later = 2 patients).

Table 9.12 Comparison of PedsQL report between newborn SCID and those diagnosed beyond neonatal period according to specific genotypes (Parent and child report).

SCID genotypes	IL2RG/JAK3 SCID			IL7Rα SCID			Artemis & RAG 1/2 SCID			ADA SCID		
PedsQL	**NBS**	**No**	**p value**	**NBS**	**No**	**p value**	**NBS**	**No**	**p value**	**NBS**	**No**	**p value**
	Mean (SD)	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**	
Parent Report	**N = 8**	**N = 11**		**N = 3**	**N = 4**		**N = 3**	**N = 4**		**N = 6**	**N = 6**	
Total	72.1 (23.5)	69.9 (17.3)	0.82	77.1 (32.0)	67.9 (18.5)	0.64	81.8 (14.7)	65.2 (22.5)	0.32	**83.6 (10.0)**	**52.4 (26.7)**	**0.02**
Psychosocial	67.9 (28.5)	65.4 (18.5)	0.82	76.7 (34.6)	64.2 (34.6)	0.57	82.2 (8.4)	60.8 (24.5)	0.21	**80.8 (15.3)**	**53.8 (23.9)**	**0.04**
Physical	83.2 (24.9)	81.8 (19.5)	0.89	78.1 (27.7)	75.0 (25.5)	0.88	81.2 (27.2)	73.4 (19.1)	0.67	**93.3 (6.6)**	**48.9 (36.2)**	**0.01**
Emotional	75.0 (30.8)	71.3 (19.1)	0.75	76.6 (40.4)	61.2 (20.1)	0.53	81.6 (16.1)	62.5 (25.0)	0.30	**93.3 (7.5)**	**60.8 (32.2)**	**0.03**
Social	78.1 (29.1)	76.8 (24.2)	0.91	78.3 (33.2)	82.5 (28.7)	0.86	91.6 (14.4)	73.7 (18.9)	0.23	**86.6 (18.6)**	**55.8 (18.8)**	**0.01**
School	61.2 (29.7)	65.2 (30.2)	0.79	75.0 (30.4)	68.3 (15.2)	0.75	73.3 (2.8)	61.6 (22.5)	0.42	62.5 (30.7)	45.0 (11.4)	0.16
Child report	**N = 5**	**N = 10**		**N = 4**	**N = 6**		**N = 5**	**N = 5**		**N = 4**	**N = 2**	
Total	80.2 (18.3)	76.5 (20.0)	0.73	73.6 (25.2)	76.8 (18.0)	0.82	80.6 (20.1)	63.5 (15.8)	0.21	60.1 (38.3)	45.1 (42.2)	0.68
Psychosocial	74.9 (21.7)	73.6 (21.1)	0.91	71.6 (26.4)	73.3 (26.5)	0.92	80.3 (17.7)	65.0 (16.5)	0.22	56.2 (36.1)	45.0 (42.0)	0.74
Physical	90.0 (12.9)	81.8 (21.9)	0.46	77.3 (24.1)	83.3 (7.3)	0.57	83.7 (20.1)	60.9 (18.1)	0.86	67.1 (46.1)	45.3 (41.9)	0.60
Emotional	92.0 (13.0)	73.0 (26.4)	0.15	67.5 (29.5)	62.5 (34.0)	0.81	**91.5 (8.6)**	**62.5 (18.4)**	**0.01**	67.5 (42.5)	52.5 (24.7)	0.67
Social	74.0 (37.1)	76.5 (19.8)	0.86	77.5 (22.2)	88.3 (13.6)	0.36	82.0 (28.4)	76.2 (17.9)	0.73	60.0 (38.2)	50.0 (70.7)	0.82
School	59.0 (21.9)	71.5 (24.2)	0.35	70.0 (28.5)	69.2 (36.8)	0.97	67.6 (18.5)	56.2 (19.3)	0.39	41.2 (31.1)	32.5 (31.8)	0.76

Bold indicates p value < 0.05 and is considered significant.
All comparisons were performed between NBS and those who were diagnosed beyond neonatal period using T-test.

9.6 Summary of Newborn SCID long-term outcome post-transplantation

The following summarises the long-term outcome post-transplantation for newborn SCID. Newborn SCID showed better survival compared to those who were diagnosed later. With regard to newborn SCID, those who were detected by positive family history and those without infection prior to HSCT had better survival. There was no difference in survival according to preparative regimen, donor groups and SCID genotypes.

The majority of patients (72%), had on-going medical issues, which were mainly short stature (30%) dermatological issues (27%), and dental issues (15%). Low toxicity MAC recipients have significantly less on-going medical issues compared to MAC recipients. There were 2 pregnancies recorded in this cohort and both were conditioned recipients.

There was a significant association between preparative regimen and freedom from immunoglobulin replacement therapy.

The longitudinal analysis demonstrated that conditioned newborn SCID patients had significantly better CD3+ lymphocytes and CD4+ naïve lymphocyte than those who were unconditioned. There was no significant difference in CD3+ lymphocyte between newborn SCID and those who were diagnosed later.

Newborn IL2RG/JAK3 SCID patients had better CD3+ and CD4+ naïve lymphocyte counts at the time of the last follow up compared to those IL2RG/JAK3 SCID patients who were diagnosed and had their transplant later.

For newborn SCID patients, both parents and children reported no significant difference in quality of life compared to UK normal published values, except for the lower QoL in the school domain.

Important Findings:

- Low toxicity MAC recipients have significantly less on-going medical issues compared to MAC recipients.

- Conditioned newborn SCID patients had significantly better thymopoiesis and freedom from immunoglobulin replacement therapy compared to unconditioned newborn SCID.

- For newborn SCID patients, both parents and children reported no significant difference in quality of life compared to UK normal published values, except for the school domain.

Chapter 10 More than 20 years SCID HSCT outcome in UK.

This chapter presents the results of very long-term outcomes of SCID patients post-HSCT in 2 centres in the UK (Newcastle and London). The very long-term outcome was defined as more than 20 years after the first HSCT was performed. All SCID genotypes were considered in the analysis, including undefined SCID. Undefined SCID is categorised as those with an immune-phenotype of SCID but without any specific gene mutation identified. All patients identified from the database had their HSCT performed either in Newcastle or GOSH, London and were followed up at the GNCH, Newcastle and Royal Free Hospital, London. The end of the follow up time for both centres was the end of January 2015.

10.1 Cohort characteristics

A total of 74 patients with a SCID diagnosis who had undergone HSCT were identified from databases in both centres (London and Newcastle). Thirty-five patients had died (46.6%). Out of the 39 surviving patients, 6 were unidentified (as the research identification from the database was not able to be decoded) and 5 identifiable patients were lost to follow up, hence, were excluded from the analysis of clinical and immune reconstitution outcome post-HSCT (Figure 10.1).

Figure 10.1 Flow chart of patients in the cohort of more than 20 years post-HSCT.

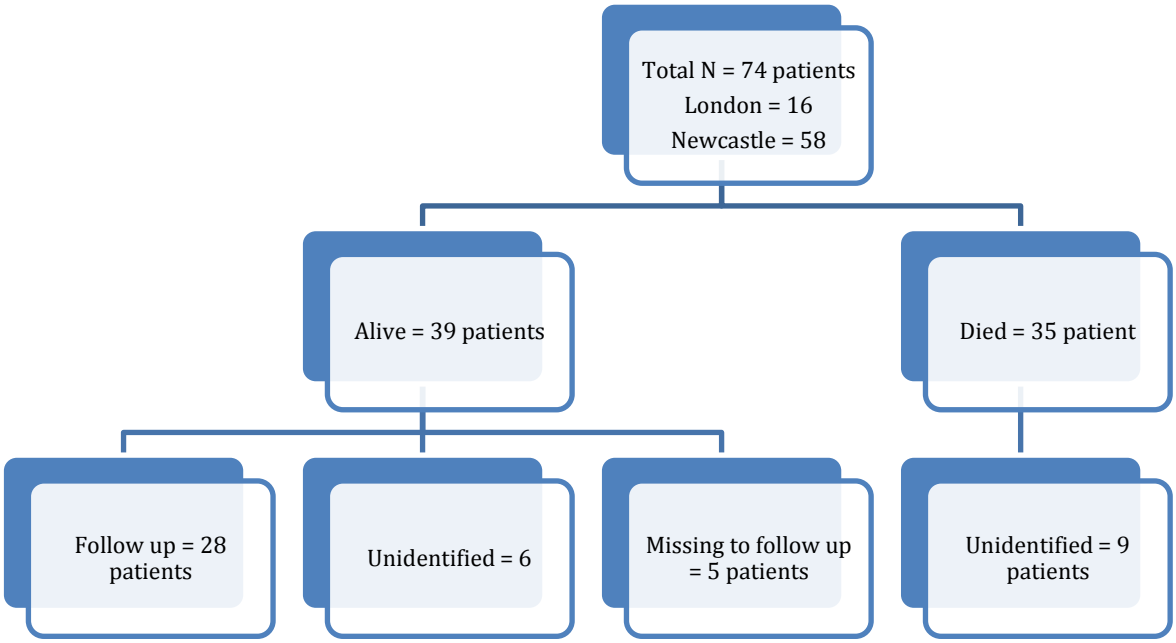


Table 10.1 demonstrates further the distribution of SCID genotypes' diagnosis in this cohort. The majority of patients did not have any identifiable mutation, which accounts for 47% (35 out of 74 patients). Most of the patients that were labelled as Undefined SCID had either died or were lost to follow up, so were unable to be included in the advanced analysis for mutational genotypes identification. Of all with SCID genotypes identified, ADA SCID was the highest proportion with 12 out of 74 patients (16%), followed by IL2RG SCID (11 patients, 15%), IL7Rα SCID (5 patients, 7%), RAG 1 and 2 SCID (4 patients, 5%), JAK3 SCID (3 patients, 4%) and both reticular dysgenesis and Artemis SCID (2 patients, 3%) respectively. Most of the diagnoses were made long after the transplant procedure.

Table 10.1 List of patients according to their SCID genotypes diagnosis.

Diagnosis	Number of patients	%
Undefined SCID	35	47
ADA SCID	12	16
IL2RG SCID	11	15
IL7Rα SCID	5	7
RAG 1/2 SCID	4	5
JAK3 SCID	3	4
Reticular dysgenesis	2	3
Artemis SCID	2	3

Just over half of the patients (56%) in this cohort had their first HSCT between 1980 and 1989. Further details of donor type, graft source and conditioning regimen are given in Table 10.2. The majority received a haploidentical donor (60%) with only 24% of patients receiving an MSD donor. Almost all received bone marrow grafts. However, there were no data available for the graft source in 24% of patients.

The conditioning regimen available at this time was categorized to 3 groups (Unconditioned, MAC and NMA). A total of 38 patients received MAC conditioning as part of their preparative regimen (14 patients received busulfan ≥ 16mg/kg/cyclophosphamide, 15 patients received busulfan ≤ 8mg/kg/cyclophosphamide and 9 patients with unknown busulfan dose/cyclophosphamide). Only three patients had NMA conditioning and 33 patients were unconditioned.

Table 10.2 Table of donor type, graft source and conditioning regimen received during first HSCT for all patients in the cohort.

Transplantation characteristic/group	Number of patients	%
Year of HSCT		
<1980	8	10.7
1980 – 1984	18	24.3
1985 – 1989	23	31.1
1990 – 1994	25	33.7
Donor type		
MSD	18	24
MRD	7	9
MUD	3	4
MMUD	2	3
Haploidentical	44	60
Graft Source		
BM	55	75
PBSC	1	1
Missing	18	24
Conditioning regimen		
Unconditioned	33	45
MAC	38	51
NMA	3	4

Comparisons of profile characteristics between known and those who were unidentified or missing, were performed in order to identify any potential bias in reporting the outcome of this cohort (Table 10.3). Those whom were unidentified or missing were significantly older than the known patients ($p = 0.01$) and none of them had definitive SCID genotype mutations identified.

Table 10.3 Comparison of profile between those who remained in follow up versus unidentified or missing to follow up.

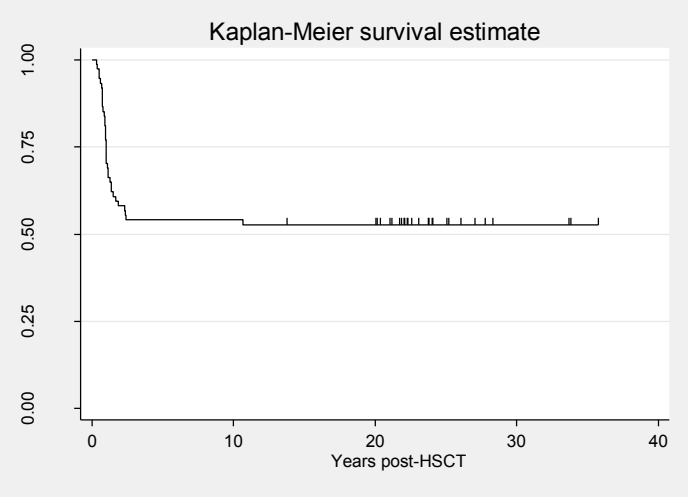
Characteristics	Known Median (range) n = 28	Unidentified/Missing Median (range) n = 11	p value*
Age at first HSCT (weeks)	25 (2 – 58)	29 (7 – 50)	0.07
Age at January 2015 (year)	25.3 (20 – 35.5)	31.5 (25 – 44)	0.01
Interval of follow up from first HSCT	25.3 (20 – 35.2)	31.2 (24.3 – 43.7)	0.02

*Median test due to data not normally distributed
p value < 0.05 was considered significant

10.2 Survival outcome and factors influencing survival outcome

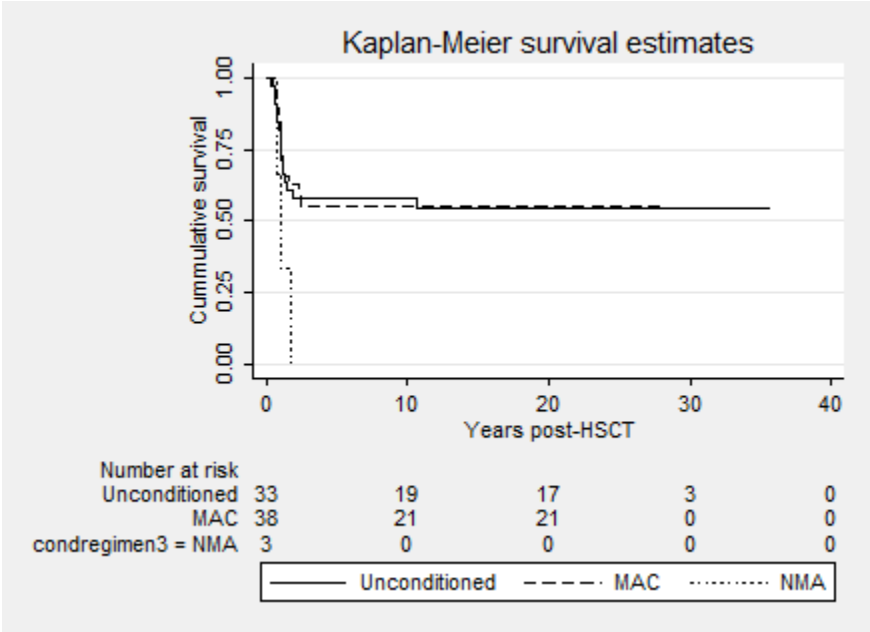
Twenty year survival for this cohort was 52.7% (95% CI 40.7 – 63.3%) (Figure 9.2). The transplant-related mortality was 42.6%. Most deaths occurred within 2 years post-HSCT and only one 'late' death occurred at 10.6 years post-HSCT (Undefined SCID, unconditioned MSD with cause of death documented as infection). The main cause of death was infection (22 out of 35 patients) followed by acute GVHD (7 patients), others (3 patients) and one each for capillary leak, toxicity and giant cell hepatitis.

Figure 10.2 Kaplan Meier survival analysis of the cohort.



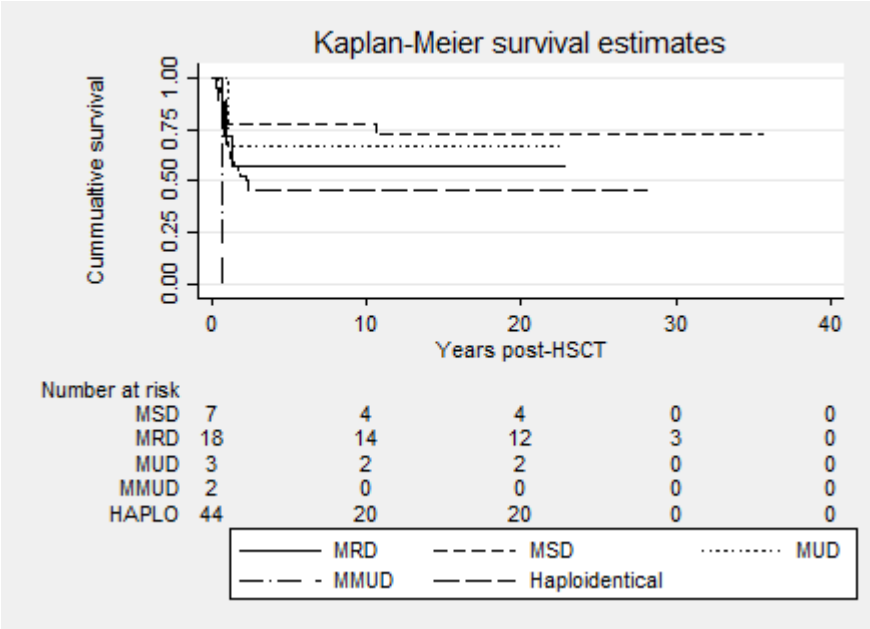
There was no significant difference in survival outcome between unconditioned recipients [54.5% (95% CI 36.3 – 69.5%)] and MAC recipients [55.2% (95% CI 38.2 – 69.3%)], p = 0.11. None of the NMA recipients survived more than 2 years post-transplantation (Figure 9.3).

Figure 10.3 Kaplan Meier survival analysis of the cohort according to different conditioning regimen.



There was a significant difference in survival between the donor groups, p = 0.0009 (Figure 9.4). The best survival was seen for MSD, 72.2% (95% CI 45.6 – 87.3%). MUD survival was 66.6% (95% CI 5.4 – 94.5%) and MRD was 57.1% (95% CI 17.1 – 83.7%). Haploidentical recipients' survival outcome was 45.4% (95% CI 30.4 – 59.3%) and none of the MMUD recipients survived more than 2 years post-transplantation.

Figure 10.4 Kaplan Meier survival analysis of the cohort according to different donor types.



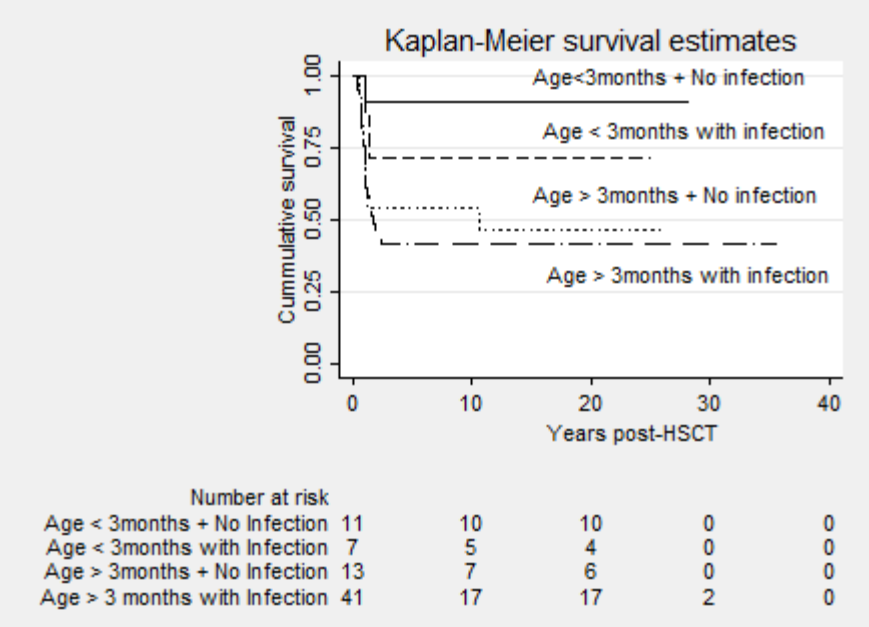
The influence of SCID genotypes on the survival of patients post-transplantation, was approaching significance, p = 0.06. However, most of the genotype diagnoses were made after the transplantation procedure had been completed, except for some of the IL2RG SCID and ADA SCID. All patients with a diagnosis of RAG1 and 2 SCID in this cohort survived. The 20 year survival probability according to the SCID genotypes is described in Table 9.4.

Table 10.4 The 20 year survival according to SCID genotypes.

SCID genotypes	20 year survival	95% CI
IL7Rα SCID	80.0%	20.3 – 96.9%
IL2RG and JAK3 SCID	78.5%	47.2 – 92.5%
ADA SCID	50%	20.8 – 73.6%
Artemis	50%	6 – 91.0%
Reticular dysgenesis	50%	6 – 91%
Undefined SCID	34.2% (95% CI 19.3 – 49.7%).	19.3 – 49.7%

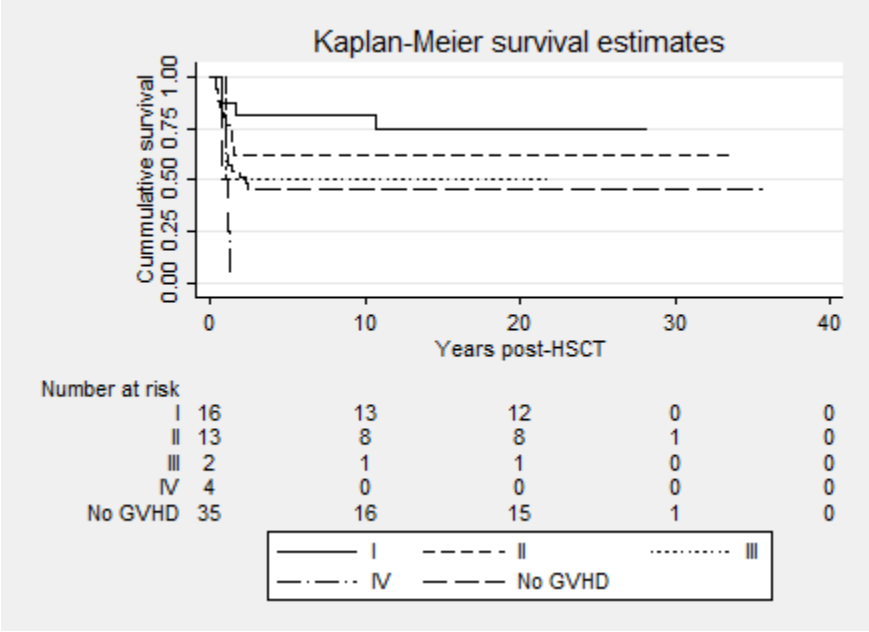
Survival was noted to be significantly influenced by age at transplantation and infection status prior to HSCT, p = 0.03 (Figure 9.6). The best survival was seen in those who had their first HSCT before the age of 3 months old and did not have any infection prior to HSCT, 90.9% (95% CI 50.8 – 98.6%). The worst survival was seen in those transplanted after the age of 3 months old with an underlying infection prior to HSCT, 41.4% (95% CI 26.4 – 55.8%). Those who had an infection but were transplanted earlier than 3 months old fared better than those who were transplanted later than 3 months old but did not have an infection prior to HSCT; survival 71.4% (95% CI 25.8 – 91.9%) versus 46.1% (95% CI 19.2 – 69.6%), respectively.

Figure 10.5 Kaplan Meier survival analysis according to age at HSCT and status of infection prior to HSCT.



Another significant risk factor influencing the survival was grading of acute GVHD, p = 0.01 (Figure 9.7). Following are the percentage of patients with acute GVHD in the UK SCID cohort: Grade I = 23%, Grade II = 18%, Grade III = 3%, Grade IV = 6% and 50% of the cohort did not had acute GVHD. None of those who had acute GVHD Grade IV survived more than 2 years post-HSCT. Among those with acute GVHD, Grade I had the highest survival, 75% (95% CI 46.3 – 89.8%) followed by Grade II 61.5% (95%CI 30.8 – 81.8%) and Grade III 50% (95% CI 6 – 91%). Interestingly, those without acute GVHD had a survival of 45.7% (95% CI 28.9 – 61.1%).

Figure 10.6 Kaplan Meier survival analysis according to grade of acute GVHD post-HSCT.



10.3 Clinical outcome

Clinical outcome data were only available for 28 out of 39 patients. A summary of the clinical outcome for this cohort is listed in Table 10.5. A further detail of clinical outcome distribution according to SCID genotypes and conditioning regimens are listed in Table 10.6 and Table 10.7, respectively.

A majority of the cohort (93%) had on-going medical issues at the last follow up. However, only one patient needed on-going immunological replacement. This information could be confounded by missing data on a number of patients from this cohort. The most prevalent issue in this cohort is dermatology, which affected 13 out of 27 patients (48%), mainly cutaneous warts (12 patients), vitiligo (1 patient) and suspected dermatofibrosarcoma protuberans (1 patient). There was a significant association between SCID genotypes and incidence of warts, which were only found in those with IL2RG, JAK3 SCID, IL7Rα SCID, Reticular dysgenesis and not the other SCID genotypes $p = 0.008$ (Table 10.6).

The second highest on-going medical issue was dental problems. Eleven out of 28 patients (38%) had dental issues and almost of all of them were MAC recipients except for one unconditioned recipient, $p = 0.11$ (Table 10.7). Dental issues included hypodontia in 7 patients, and multiple absent teeth, widely spaced teeth and microdontia (one patient each).

Two patients had respiratory issues (one with bronchiectasis, one with bronchiolitis obliterans) and both were unconditioned recipients. The explanation could be due to unconditioned recipients displaying lower myeloid chimerism levels at the last follow up, which in turn increased their susceptibility for recurrent respiratory tract infections leading to bronchiectasis. No significant association was noted between incidence of respiratory issues and SCID genotypes, $p = 0.26$.

From the analysis, a significant association between SCID genotypes and presence of neurocognitive issues was noted, $p = 0.006$ (Table 10.6). Five patients had neurocognitive issues at the last follow up and all of them were ADA SCID, except for one with RAG1/2 SCID. The neurocognitive issues were;

autism, behavioural issues, learning difficulties, low mood and short term memory loss (one patient for each diagnosis).

Four patients experienced endocrine issues, which were; absent sperm count, primary ovarian failure, diabetes mellitus and one patient was being investigated for polycystic ovarian syndrome. However, there was no significant association between SCID genotypes ($p = 0.37$), conditioning regimen ($p = 0.10$) and incidence of endocrine issues. This could be related to the small sample size.

Four patients had height less than 2 SD at the last follow up and all of them were MAC recipients. Hearing loss was documented in 4 patients, with an equal proportion between unconditioned (2 patients) and MAC recipients (2 patients).

There were 4 successful pregnancies documented in this cohort (2 male patients and 2 female patients). Further description of those with successful pregnancy were: Undefined SCID received busulfan 16mg/kg (1 patient), IL2RG SCID received busulfan 16mg/kg (1 patient), unconditioned IL2RG SCID (1 patient) and IL7Rα SCID received busulfan 8mg/kg (1 patient). No further information was available regarding whether the pregnancies were assisted or spontaneous conception.

Only one patient still had on-going immunoglobulin replacement therapy and he was an unconditioned IL2RG SCID. One case of secondary malignancy was noted in this cohort. A patient with IL2RG SCID had squamous cell carcinoma of the scalp 8 years after his second HSCT. He has had busulfan 16mg/kg and cyclophosphamide regimen from the first HSCT and haploidentical donor. However, details about the second HSCT were not available. This patient experienced chronic GVHD of the scalp and severe cutaneous warts prior to the diagnosis of squamous cell carcinoma. He underwent surgical removal of the lesion and there was no secondary metastasis noted during the surveillance.

Table 10.5 Clinical outcome for cohort of very long-term post-HSCT SCID Newcastle and London.

Clinical Outcome	Number of patients % (n/N)
Ongoing medical issues	93% (26/28)
Ongoing immunoglobulin replacement	4% (1/28)
Respiratory issues	7% (2/27)
Neurocognitive issues	18% (5/28)
Endocrine issues	22% (4/18)
Height <= 2SD	25% (4/16)
Pregnancy/Fertility	14% (4/28)
Hearing loss	14% (4/28)
Dental issues	36% (10/28)
Dermatology issues	48 (13/27)
Secondary malignancy	5% (1/19)

Table 10.6 Distribution of clinical outcome according to SCID genotypes.

Clinical outcome	IL2RG & JAK3 N = 11	IL7Rα N = 4	ADA N = 5	RAG1/2 N = 4	Reticular Dysgenesis N = 1	Artemis N = 1	Undefined SCID N = 2
On-going medical issues	10	4	5	4	1	1	1
On-going IVIG	1	0	0	0	0	0	0
Respiratory issues	1	0	0	0	1	0	0
Neurocognitive issues	0	0	4	1	0	0	0
Endocrine issues	1	0	1	2	-	-	0
Short stature	2	0	1	0	0	0	1
Hearing loss	1	0	2	0	0	0	1
Dental issues	2	1	1	3	1	1	1
Warts+	8	2	0	0	1	0	0

Table 10.7 Distribution of clinical outcome according to conditioning regimen for HSCT.

Clinical condition	Unconditioned N = 8	MAC N = 20	p value
On-going medical issues	8	18	0.35
On-going IVIG	1	0	0.10
Respiratory issues	2	0	0.08
Neurocognitive issues	2	3	0.44
Endocrine issues	2	2	0.10
Short stature	0	4	0.55
Hearing loss	2	2	0.32
Dental issues	1	9	0.11
Warts+	4	7	0.45

Pearson chi square
p value < 0.05 was considered significant

10.4 Long-term immune-reconstitution

Sustained production of CD3+ lymphocytes and CD4+ lymphocytes is demonstrated in Figure 9.8 and Figure 9.9. Missing data remains a major problem due to the retrospective nature of the study. Hence, multi-level mixed modelling for the analysis of longitudinal immune reconstitution could not be undertaken.

Figure 10.7 Individual CD3+ lymphocyte cell counts in SCID patients with follow up of 20 years or longer post-transplantation.

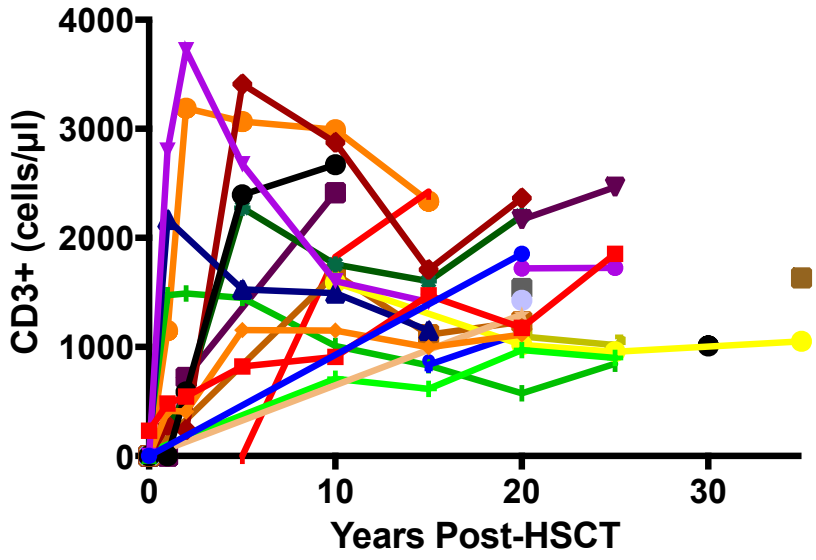
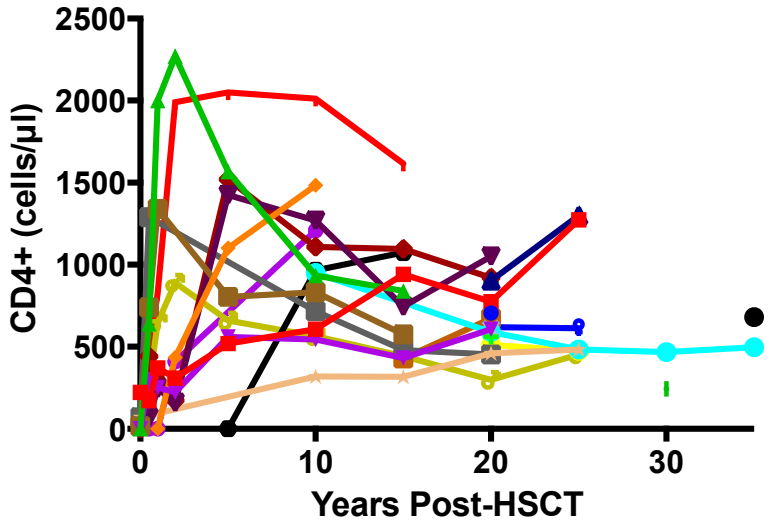
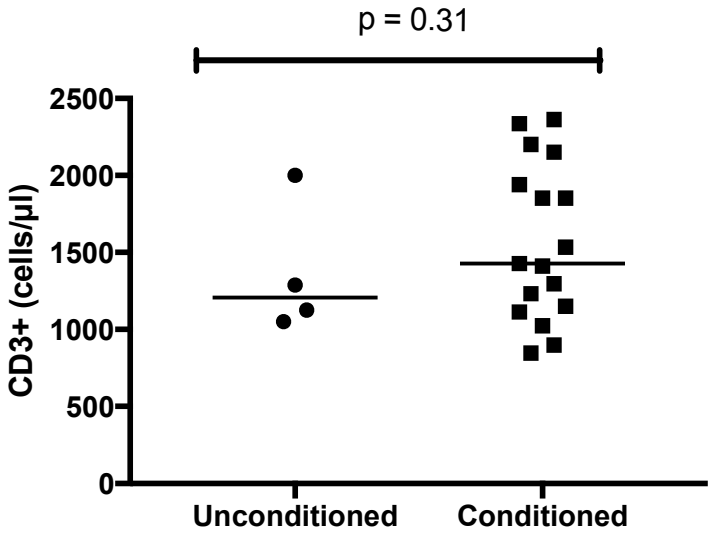


Figure 10.8 Individual CD4+ lymphocyte cell counts in SCID patients with follow up of 20 years or longer post-transplantation.



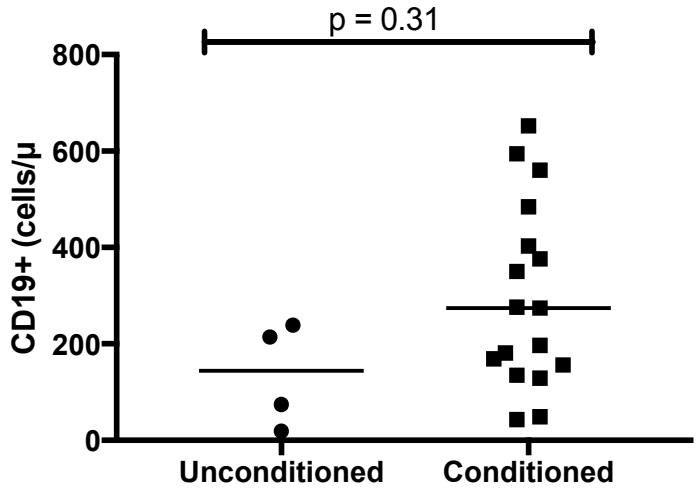
Median tests were performed to identify any significant differences between median values of immunological parameters at the last follow up according to preparative regimens (due to non-normally distributed data). Results of CD3+, CD19+, CD4+ lymphocytes and NK cells count at the last follow up was available for 21 out of 28 patients (Unconditioned = 4 patients, Conditioned = 17 patients). There was no significant difference of CD3+ median values between preparative regimen, p = 0.31 (Figure 9.10).

Figure 10.9 Relation between individual CD3+ lymphocyte at last follow up and the preparative HSCT regimen received.



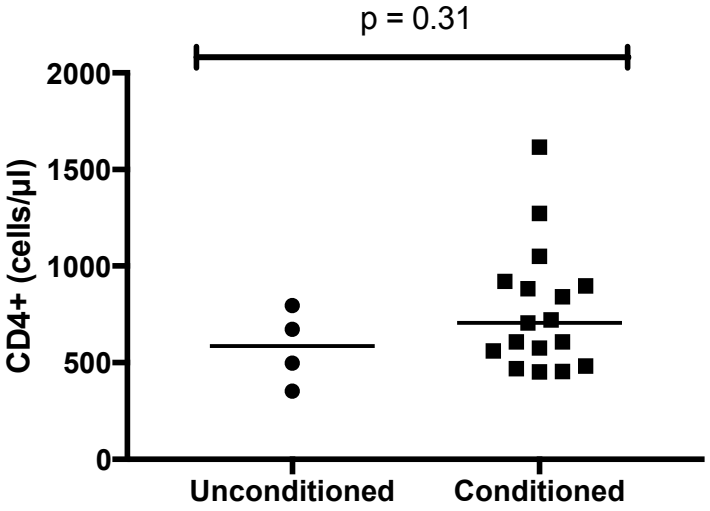
Median of CD19+ lymphocyte was non-significantly higher in those who received conditioning compared to unconditioned recipients, p = 0.31 (Figure 9.11).

Figure 10.10 Relation between individual CD19+ lymphocyte at last follow up and the preparative HSCT regimen received.



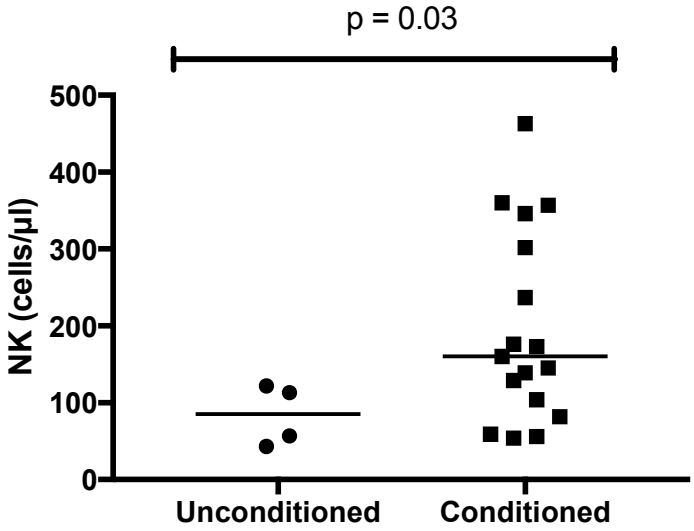
There was also no significant difference between different preparative regimens in the median values of CD4+ lymphocyte (p = 0.31) at the last follow up (Figure 9.12).

Figure 10.11 Relation between individual CD4+ lymphocyte at last follow up and the preparative HSCT regimen received.



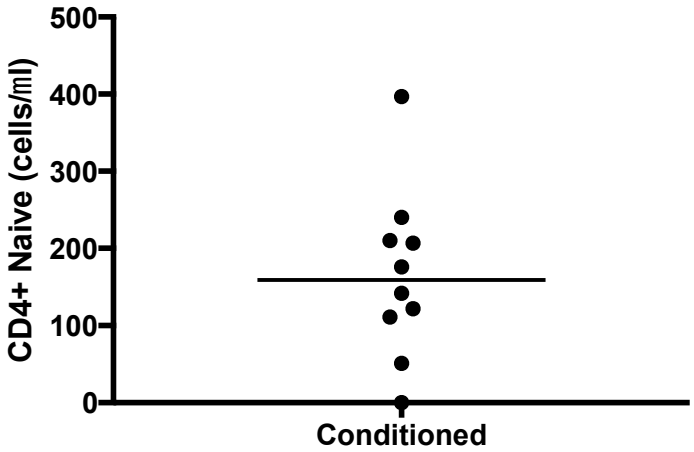
The median of NK cells values were significantly higher in conditioned recipients compared to unconditioned recipients, p = 0.03 (Figure 9.13).

Figure 10.12 Relation between individual NK cells at last follow up and the preparative HSCT regimen received.



Data for CD4+ naïve lymphocyte counts at the last follow up were only available for 10 out of 28 patients (Figure 9.14). Subgroup comparison was omitted as all measurements were from conditioned recipients.

Figure 10.13 Individual CD4+ naive cell counts at last follow up for SCID survivors 20 years or longer post-transplantation.

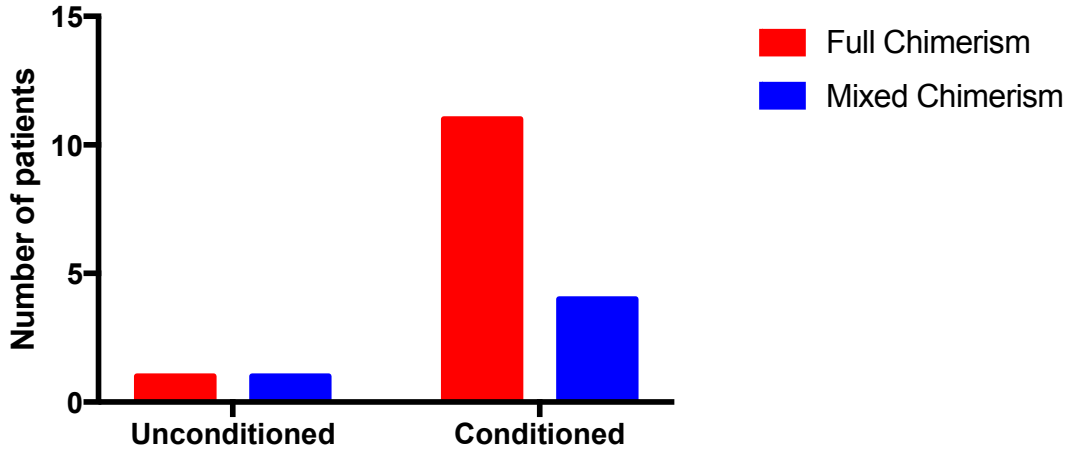


10.5 Cross-sectional analysis of whole blood donor chimerism

Results of whole blood donor chimerism were only available for 17 out of 28 patients (60%). Donor chimerism data are defined as full donor (more than 95% donor chimerism), mixed chimerism (between 20% and 94% donor chimerism) and recipient chimerism (less than 20% donor cell chimerism).

A total of 12 patients had full donor chimerism and 5 patients had mixed chimerism at the last follow up (Figure 9.15). Out of 12 patients with full donor chimerism, 6 patients received busulfan 16mg/kg, 5 patients received busulfan 8mg/kg and only 1 patient was unconditioned. There was no association between latest donor chimerism more than 95% and preparative regimen prior to HSCT, p = 0.51 (Fisher exact test).

Figure 10.14 Bar chart showing number of patients with donor chimerism according to the preparative regimen received prior to HSCT.



10.6 Summary of very long-term outcome for Newcastle and London SCID cohort post-HSCT

A 20 year survival for this cohort was 52.7% (95% CI 40.7 – 63.3%) and TRM was 42.6%. Significant influencing factors of survival outcome in this cohort was donor groups ($p = 0.0009$), early age at HSCT and infection free status pre-HSCT ($p = 0.03$), and grading of acute GVHD ($p = 0.01$). A significant number of patients was missing or unidentified and this may influenced the result of this cohort.

The majority of those who are more than 20 years post-HSCT have on-going medical issues (26 out of 28 patients). The main clinical issues observed were dermatological issues (48%) and dental issues (36%). Only one patient remained on immunoglobulin replacement therapy. Four successful pregnancies have been documented in this cohort. Significant association is noted between types of SCID genotypes and the incidence of warts ($p = 0.004$) and neurocognitive issues ($p = 0.006$).

Sustained CD3+ and CD4+ lymphocyte production continues until the third decade of life. There was no significant difference noted between all immune parameters at the last follow up and preparative regimen; except for NK cells, which were significantly higher in conditioned recipients ($p = 0.03$). There was no association between latest donor chimerism more than 95% and the preparative regimen prior to HSCT, $p = 0.51$.

Important findings:

- Only one late death was documented in this cohort.
- Significant influencing factors of survival outcome were donor groups, early age at HSCT and infection free status pre-HSCT, and severity of acute GVHD.
- Sustained CD3+ and CD4+ lymphocyte production continues until the third decade of life.

Chapter 11 Discussion

In this chapter findings of the study will be discussed, specifically focusing on four major themes; clinical outcome, longitudinal immune reconstitution, chimerism at last follow up, and quality of life in reference to specific SCID genotypes. The result of newborn SCID transplants and outcome of SCID patients more than 20 years post-HSCT of Newcastle and London cohorts will also be discussed as separate subtopics.

Two new major findings were identified from this study which were the determination of impact of conditioning on the long-term immune reconstitution and characterization of the quality of life according to specific SCID genotypes.

The principal findings of this study are that many SCID survivors' post-HSCT experience on-going medical issues long after their transplant, and that conditioning was associated with better long-term immune reconstitution, donor B-lymphocyte chimerism, a greater chance of freedom from immunoglobulin replacement therapy and normal quality of life.

Apart from the descriptions of the clinical long-term outcome of SCID post-transplantation, detailed outcomes of long-term immune reconstitution, factors influencing the outcome of donor chimerism and importantly, objective assessment of quality of life for the SCID survivors according to SCID genotypes, have also been identified. This serves as an important landmark as it demonstrate that different SCID genotypes have different characteristics, which may influence transplantation outcome in the long-term.

11.1 Discussion on the findings of the Newcastle SCID cohort

11.1.1 Survival outcome

The ten-year survival outcome for the described SCID genotypes in the Newcastle cohort ranged between 71 – 84%. This is comparable with the published survival rates from multiple established centres around the world [2, 43, 44]. Importantly, most of the deaths in this cohort occurred within the first year post-HSCT and were mainly due to infections. Similar results have been found in the published

American multi-centre outcome, suggesting that transplant-related mortality remains the biggest concern for patients post-HSCT [44].

Despite the concerns of the preparative regimen prior to HSCT, there were no significant differences in survival between unconditioned and conditioned recipients in all 8 SCID genotypes from this cohort. This reflects similar observations in European multi-centre reports [2] but differs from the American multi-centre report of poorer survival in conditioned recipients [44]. An alternative explanation would be that varying centre's expertise may influence the outcome. Further prospective research is necessary to establish a clearer relationship between conditioning and survival, with comparison between specific types of conditioning regimens.

11.1.2 Long-term clinical outcome

On-going medical concerns remain a significant issue among the SCID survivors post-HSCT. The proportion documented was 55% - 87% depending on the specific SCID genotype. There are several long-term clinical conditions identified that occur specifically in some SCID genotypes, but not in others.

Firstly, ADA SCID survivors had the highest percentage of on-going medical issues at the last follow up (87%), with a significant increase of neurocognitive problems and hearing loss. These findings support previous evidence on the association of cognitive/behavioural abnormalities and ADA SCID [69, 89, 121, 122]. A possible explanation for this observation is the ubiquitous nature of the ADA gene causing non-immunologic systemic manifestations which may not be corrected by HSCT [123]. A study had showed that despite the systemic detoxification achieved by HSCT or gene therapy, full correction of metabolic alteration in the brain remains a significant issue [124]. Furthermore, a recent update by the Italian group has reported persistence of neurological deficits even after gene therapy [125]. At the same time, the availability of newborn screening enables early detection of SCID during neonatal period [1, 126-128]. It would be of interest to see whether treatment at birth (HSCT) would ameliorate the neurocognitive defect in ADA SCID.

Secondly, this study's findings concur with published reports on the incidence of warts in IL2RG/JAK3 SCID survivors [43, 65-68, 121]. The present study

identified a 24% incidence of warts in the IL2RG/JAK3 SCID cohort. However, no association was found between the incidence of warts and low NK cell count or preparative regimen. Additionally, warts were reported in 5 patients of the IL7Rα SCID cohort, which has been described in few studies [43, 121]. From personal observations, warts in the IL7Rα SCID cohort were less severe in terms of clinical disease manifestations and resolved completely as opposed to those in the IL2RG/JAK3 cohort, which tended to have a chronic and debilitating course. This view is supported by a previous report in the Italian cohort, where a similar pattern of severe warts was observed in JAK3 patients, but not in IL7Rα SCID [121]. A future prospective study with a larger number of patients is needed to confirm this observation.

Thirdly, Artemis SCID survivors experienced more on-going medical issues than RAG 1/2 SCID, (85% and 55%, respectively). Notably, all conditioned Artemis SCID patients in this cohort experienced on-going medical issues, which supports a single previously published research that conditioning may lead to a higher risk of long-term issues (such as poor growth, dental abnormalities and endocrine late effect) [54].

With regards to growth, short stature was identified in all described SCID genotypes with ADA SCID and Artemis SCID having the most affected numbers of patients post-transplantation. This finding confirms the previously published observation of growth retardation in Artemis SCID recipients conditioned with alkylating agents, even though no association was noted between short stature and conditioning regimen in either genotype in this study, although this could be due to the small number of patients available for analysis [54, 129].

From the study, dental issues were identified in conditioned recipients of Artemis and ADA SCID. This observation is supported by earlier work suggesting an association between preparative regimen and dental developmental anomalies post-HSCT [54, 130]. Possibly the DNA repair defects in Artemis SCID with combination of damage from the myeloablative conditioning makes them more susceptible to dental damage post-HSCT [54].

11.1.3 Chimerism at last follow up

There was a high correlation between B-lymphocyte donor chimerism and myeloid donor chimerism post-transplant in IL2RG/JAK3 SCID, IL7Rα SCID, ADA SCID, Artemis and RAG 1/2 SCID. This provides objective evidence of a strong association between B-lymphocyte donor chimerism and myeloid donor chimerism post-transplant, irrespective of B+ SCID phenotype or B- SCID phenotype.

Importantly, further analysis demonstrated that conditioning was highly predictive for improved myeloid donor chimerism at the last follow up. This supports the hypothesis that conditioning prior to HSCT is vital in achieving better myeloid and B-lymphocyte engraftment post-transplantation and is consistent with previous published reports [40, 43, 54]. From the multivariate analysis, low toxicity MAC regimen (treosulfan/fludarabine) is superior when compared to MAC regimen (busulfan) for better myeloid donor chimerism at last follow up. This finding has an important clinical implication in which it added the advantages of low toxicity MAC regimen in SCID HSCT planning, as low toxicity MAC also showed to have lower toxicity profile compared to MAC regimen in previous publications [131, 132].

The translation of B-lymphocyte donor chimerism status and immunoglobulin replacement therapy has been further investigated. Those with immunoglobulin independence have more than 50% B-lymphocyte donor chimerism in IL2RG/JAK3 SCID patients. This evidence supports the theory that donor B-lymphocyte engraftment is essential for functioning B-lymphocyte reconstitution in the presence of an intrinsic defect of host's B-lymphoycte in IL2RG/JAK3 SCID [75, 133].

IL7Rα SCID survivors post-HSCT have the highest proportion of patients free from immunoglobulin replacement therapy; in comparison to other SCID genotypes [IL7Rα (93%), ADA SCID (81%), RAG 1 and RAG 2 (77%), Artemis SCID (57%), IL2RG/JAK3 SCID (55%)]. Almost all IL7Rα SCID patients were free from immunoglobulin replacement therapy, except for one patient with chronic lung disease. The normal functioning host B-lymphocytes seen in IL7Rα

SCID provides a possible explanation to the high percentage of freedom from immunoglobulin replacement therapy [63].

My results demonstrated that more conditioned Artemis and RAG 1/2 SCID recipients were able to be free from immunoglobulin replacement therapy. This supports the notion that conditioning is vital for better B-lymphocyte reconstitution, chimerism and chances of freedom from immunoglobulin replacement therapy after HSCT in Artemis and RAG 1/2 SCID [54, 63]. As Artemis and RAG 1/2 SCID patients do not have B-lymphocyte, preparative regimen prior to HSCT is vital to ensure better donor' myeloid and B-lymphocyte engraftment. However, medical on-going issues is a major issues for conditioned Artemis SCID patients during long term follow up post-HSCT. This necessitate the search for conditioning regimen with high safety profile, low toxicity and allowing durable engraftment simultaneously.

11.1.4 Longitudinal immune reconstitution post-HSCT

As well as confirming findings from other published studies, this study has demonstrated several findings that differ from other studies. Importantly, this study showed that conditioned groups had significantly higher CD4+ naïve lymphocyte in ADA SCID, Artemis and RAG1/2 SCID patients and a borderline significance in IL2RG/JAK3 SCID patients. The thymopoeisis was non-significantly higher in conditioned IL7Rα SCID patients compared to unconditioned recipients.

Another important finding from the longitudinal analysis of the immune reconstitution parameters is the downward trends of CD3+ and CD4+ naïve lymphocytes over time. This observation conforms to the normal downward trend of thymopoiesis seen in normal aging population due to the effect of time on thymus involution [49, 134]. All conditioned recipients in individual SCID genotypes and newborn SCID in the Newcastle cohort have mean values of CD4+ naïve lymphocyte more than 200cells/μl at 10 years post-HSCT which was comparable to normal adult population value [43].

A possible explanation may be that in conditioned patients, the thymic niche is consistently re-seeded from bone marrow-derived donor stem cells leading to on-going thymopoiesis, whereas for unconditioned recipients, initial seeding of the thymic niche at time of infusion is not generally followed by re-seeding, as donor stem cell engraftment does not consistently occur in the bone marrow, and thymic seeding may have a finite lifetime, leading eventually to thymic exhaustion [73, 106, 135]. The finding of better thymopoiesis in the conditioned Artemis, RAG1/2 SCID and IL7Rα SCID patients also supports the hypothesis of association between host natural killer cells and permissive condition in the marrow niche and conditioning is a pre-requisite for better engraftment post-HSCT in NK+ SCID phenotypes [61].

Furthermore, this study demonstrated sustained production of CD3+, CD4+ and CD4+ naïve lymphocytes; even until the third decade post-HSCT in some of the 8 described SCID genotypes (ADA SCID, IL2RG/JAK3 SCID, IL7Rα, Artemis and RAG1/2 SCID). This confirmed previous findings showing that T-lymphocyte production persists until adulthood [73, 74, 104, 106].

However, it was not so straightforward in the longitudinal analysis of CD19+ and NK cells. The findings varied with each SCID genotype. The longitudinal trend of CD 19+ lymphocytes was significantly higher in conditioned ADA SCID patients and non-significantly better in conditioned Artemis and RAG 1/2 SCID patients. There were no significant differences in the longitudinal trend of CD19+ lymphocytes between conditioned and unconditioned recipients in IL2RG/JAK3 SCID, IL7Rα SCID. Importantly, B-lymphocyte counts alone were inadequate for the assessment of B-lymphocyte functions. More detail assessments of B-lymphocyte function require the quantification of serum immunoglobulin levels and donor B-lymphocyte and myeloid chimerism.

With regards to the longitudinal trend of NK cells post-HSCT, higher trends were seen in conditioned ADA SCID patients, unconditioned Artemis and RAG 1/2 SCID patients and unconditioned IL7Rα SCID patients. No difference was noted for IL2RG/JAK3 SCID patients.

As mentioned in Chapter 2, conditioning has a role in emptying the marrow niche and this facilitates engraftment of the donor's progenitor stem cells. Theoretically, the longitudinal trend of NK cells was expected to be higher in conditioned Artemis and RAG1/2 SCID, but my study showed a contradictory result. However, there are limited specific publications discussing the long-term reconstitution of CD19+ lymphocyte and NK cells for each specific SCID genotype, and they were mainly cross-sectional rather than longitudinal making direct comparisons between studies difficult [54, 63, 70]. Hence, it is unclear whether this observation is accurate, possibly the small sample size coupled with missing data may be a possible explanation for the contradictory findings. A prospective study involving a larger number of patients would be the next step forward as an attempt to further characterize the long-term immune reconstitution post-transplant.

11.1.5 Quality of Life

One of the most important outcome measure in this study was the objective measurement of quality of life. This is the only study that has examined the quality of life of survivors post-HSCT according to specific SCID genotypes. The principal findings in this study are that, for IL2RG/JAK3 SCID, RAG1/2 SCID, Artemis SCID and ADA SCID parents/carers, reported a lower quality of life in their child in comparison to the UK's normal population. This is comparable to prior published finding where parents/carers of SCID patients post-HSCT reported worse emotional and behavioural difficulties compared to a normal population [89]. In view of this finding, it is important for SCID patients post-HSCT to be follow up lifelong so that early identification of any issues can be guaranteed.

However, parents and patients of IL2RG/JAK3 SCID who are free from immunoglobulin replacement therapy reported no significant difference in quality of life to the normal UK population. Furthermore, 93% of IL7Rα SCID survivors in this cohort were free from immunoglobulin therapy and they reported a normal quality of life. These findings suggest freedom from immunoglobulin replacement therapy is associated with normal quality of life. It also provides evidence that immunoglobulin replacement therapy does influence patients'

quality of life, even though the methods of immunoglobulin administration have substantially improved from intravenous administration that requires hospital or daycare attendance, to subcutaneous immunoglobulin delivery that can be provided at home. This finding was also consistent with the lower quality of life reported in the study of the cohort of the UK's Primary Antibody Deficiency patients, who are on lifelong immunoglobulin replacement therapy [93]. For this reason, future development in improving methods of immunoglobulin administration is important as a way of improving the quality of life of patients.

Furthermore, ensuring SCID patients become immunoglobulin independent should be the aim of our clinical management as this study shows that immunoglobulin independence contributes to normal quality of life in SCID patients post-HSCT. Conditioning with low toxicity MAC generally restores B-lymphocyte function, but is associated with possible long-term effects such as infertility. The use of non-toxic conditioning such as anti-cKit or anti CD45 antibodies that create marrow niche space may have a better safety profile with reduced side effects and improved B-lymphocyte reconstitution [131, 132].

Freedom from on-going medical issues was associated with normal quality of life as reported by both parents and patients of Artemis and RAG 1/2 SCID. Thus, normal quality of life for SCID survivors post-HSCT can be achieved if they have no on-going medical issues and are immunoglobulin independent.

Another interesting finding was that parents tend to give lower scores than their children. This is similar to a few other published reports [90, 92]. A possible explanation could be due to disruption in the family socio-dynamic and daily routine activity, especially concerning those with on-going medical issues and/or immunoglobulin dependence. Parents also tend to have a higher expectation of their child's health outcome when the immune defect has been corrected via HSCT. Future studies including qualitative components are needed to explore the cause for discrepancy in parent/child QoL reports and to improve understanding of what influences the long-term quality of life for SCID survivors.

As a summary, those with higher disease burden are more likely to report lower quality of life compared to those who did not have on-going medical issues and

are free from immunoglobulin replacement therapy, who have a normal quality of life.

11.2 Discussion of the long-term outcome of newborn SCID in Newcastle cohort

This study highlighted the superior survival noted in those with newborn diagnosis of SCID compared to those who were diagnosed later. Infection status prior to HSCT remains the most important factor influencing the survival as reported in previous published studies [3, 44, 120]. These findings added strength to current body of evidence that earlier detection with infection free status leads to better chances of survival post-HSCT and poses strong support for newborn screening for SCID. The earlier detection of SCID patients enables earlier institution of protective isolation, antibiotic prophylaxis, nutritional rehabilitation and importantly, avoidance of live vaccinations that could lead to major problems during HSCT such as disseminated BCG infections and vaccine-related rotavirus gastroenteritis.

Four very important findings were established from the newborn cohort study in Newcastle. Firstly, improved thymopoiesis was observed in the conditioned newborn SCID patients compared to unconditioned recipients. Further comparison showed better CD4+ naïve lymphocytes counts trend over time in the newborn SCID compared to those who were diagnosed beyond the neonatal period, but not in the longitudinal trend of CD3+ lymphocytes. In a way, these findings support a previous finding of superior thymopoiesis and survival seen in those transplanted in neonatal period compared to later but not completely [119]. Thus, it may suggest a possibility that the preparative regimens have a major influence in determination of long-term thymopoiesis compared to timing of HSCT. However, this hypothesis needs to be confirmed with further prospective study of long-term thymopoiesis involving newborn SCID detected through a newborn screening program.

Secondly, newborn SCID patients who received low toxicity MAC have significantly less on-going medical issues at last follow up compared to MAC recipients. This finding is new and interesting as it suggest a low toxicity MAC

has better long term safety profile, especially considering the early timing of HSCT for newborn SCID, which poses the highest risk of long term side effects. However, it is worth noting that the low toxicity MAC recipients were younger than MAC recipients cohort. This warrants a longer follow up to fully ascertain the side effects of different preparative regimens and the effect on the long term outcome post-HSCT. Only one available published report on the long term outcome for neonatal SCID HSCT, which showed superior survival and thymopoeisis for those who had transplantation during neonatal period [119]. However, all patients in that cohort were unconditioned recipients and there was no description of the on-going medical issues for the survivors at last follow up.

Thirdly, the likelihood of freedom from immunoglobulin replacement therapy was significantly higher in the conditioned newborn SCID patients. Although conditioning is associated with on-going medical issues at last follow up, it remains the most significant predictive factor for freedom from immunoglobulin replacement therapy. What is important now is to find a conditioning regimen with a low toxicity but at the same time is adequately myeloablative to ensure maximal engraftment.

Finally, both parents of and children with newborn SCID reported normal QoL compared to those who were diagnosed later, except for the social and school domains. This finding is new. Further comparison showed those who were diagnosed beyond neonatal period reported worse QoL compared to UK normal population. This is interesting as there were no significant differences in incidence of on-going medical issues between newborn SCID and those who were diagnosed later in the Newcastle cohort to explain this discrepancy. A possible explanation could be that the experience of undergoing HSCT possibly differs between parents of newborn SCID as opposed to parents of older children. Thus, this result supported the idea of screening for newborn SCID with the aim of earlier detection and earlier timing of HSCT, which could lead to better survival and improved long-term quality of life.

11.3 Discussion of the more than 20 years long-term outcome of Newcastle and London cohort

This section of the study is very important as it describes the very long-term outcome of SCID patients post-HSCT. Only 24% of the cohort were MSD recipients and the majority of the patients received haploidentical HSCT. Due to the historical timeline, only three types of conditioning were practiced, busulfan (myeloablative), NMA (non-myeloablative) or no conditioning. As expected, the number of HSCTs done increased throughout the decades, which reflects the increased development of HSCT units in the UK over time. The proportion of conditioned and unconditioned recipients was similar, with only a minority receiving NMA.

Twenty year survival for this cohort was 52.7%, which is comparable with previous publications from the same timeframe [20, 60]. Importantly, there was only one death after 2 years post-HSCT. This finding suggest that if patients survive the immediate post-HSCT period, they may have a normal life expectancy. Almost all causes of early deaths were related to the transplant procedures. Furthermore, none of those with acute GVHD grade IV survived more than 2 years post-HSCT. This finding also emphasized the importance of careful planning for HSCT with the aim of avoiding immediate post-transplant complications such as acute GVHD, capillary leak and pneumonitis.

There were no significant differences between conditioned and unconditioned recipients, but none of the NMA recipients survived more than 2 years post-HSCT. Clearly, NMA was not an attractive regimen, hence explaining why it is not currently used. Another possible explanation of worse outcome in NMA recipients could be that these patients were already too sick for a more intense preparative regimen but were not suitable for infusion of stem cell. From the limited available information, all three NMA recipients were diagnosed after 3 months old, had on-going infections prior to their HSCT and 2 patients received haploidentical donor and one patient received MRD HSCT.

The late death was an unconditioned recipient with infection as the cause of death. It would be interesting to get further details on this patient immunoglobulin replacement status and the immune reconstitution, as these may support the hypothesis that conditioning gives better immune reconstitution in the long run, and subsequently offers better protection from infections [43].

Even though this research demonstrated that MSD recipients had the best survival (at 72.2%) among all donor categories; importantly, those who were transplanted early with no pre-HSCT infection were shown to have a better survival rate of 90.9%. These findings are impressive considering the HSCT timing was before 1994 and the results are comparable to those from a current modern American multicentre cohort report of 2000 until 2009 [44]. This has highlighted the importance of the early timing of HSCT and infection free status for SCID patients for a better survival outcome post-HSCT [3, 44]. Concurrently, this provides strong evidence to support and promote SCID's newborn screening development programme in the UK and globally.

A few medical issues were seen in specific SCID genotypes as shown in previous publications, such as viral cutaneous warts (noted only in IL2RG, JAK3, IL7Rα SCID and reticular dysgenesis patients) and neurocognitive issues, mainly observed in ADA SCID survivors [43, 67, 70, 77]. Further analysis of other medical issues and SCID genotypes was not done due to the limited sample size. Ten out of eleven patients with dental issues had received a conditioning regimen prior to their HSCT. Of note, four successful pregnancies were recorded, with three patients being conditioned recipients, and all except for one IL2RG SCID were free from immunoglobulin replacement therapy. The sustained production of CD3+ and CD4+ lymphocytes even up until thirty years post-HSCT has clearly been demonstrated. However, further detailed analysis was not performed due to missing data.

11.4 Strengths of the study

The main strength of this study is in the detailed nature of the data focusing on specific SCID genotypes. This enables us to develop a better evidence base and identify prognostic factors useful for counselling of parents and patients for

specific genotypes, rather than for the SCID cohort as a whole which may be misleading, given the reported genotype differences.

As the long-term outcome for specific SCID genotypes (IL2RG/JAK3, IL7Rα SCID, ADA SCID, Artemis and RAG 1/2 SCID) is a single centre study, this eliminates the potential bias seen in multicentre studies (from centre effects) as the protocols, quality of medical care, quality of the stem cell grafts and the managing team remains constant.

All data collection, clinical record retrieval and data analyses were performed solely by myself. This ensures consistency and also minimised the risk of an error that could have happened if data retrieval was done by several investigators. Measures of checking for data errors and cleaning the data sheet were undertaken by the primary researcher.

The longitudinal analysis performed for the long-term immune-reconstitution enables the assessment of change over time [114]. This is to answer the research questions of how immune parameter changed after transplantation and can we predict changes of immune parameter post-HSCT according to the preparative therapy prior to HSCT. Longitudinal analysis is more accurate in addressing that research question in comparison to cross-sectional analysis, as it involves analysis of a serial data measurements of same patients over a period of time [136]. Furthermore, analysis using the multi-level mixed modelling handles the random missing data efficiently as it is not influenced by the missing data [137].

11.5 Limitation of the study

11.5.1 Research methodology

The main limitation was missing data. This is unavoidable due to the retrospective nature of this study. Measures have been taken to improve data collection both for Newcastle and London centres, such as extensive trawling of information from various storage formats (hardcopy medical notes, clinic letters, electronic databases and digital data storage). It was beyond the scope of the research for data retrieval in the external sources such as general practitioner records.

.

11.5.2 Statistical analysis

Due to the rarity of the disease, the number of patients in the study was small, especially with consideration of sub-group analysis and comparisons. As mentioned before, multiple testing giving chance significant findings is another possibility to consider.

11.5.3 Potential Bias

Potential bias identified from the very long-term UK cohort was that those who were unidentified or missing from follow up were significantly older than the known patients. This could cause bias in reporting the clinical outcome as it is not clear whether they are still alive and well, or have on-going medical issues, therefore it is crucial to acknowledge this potential bias. Detailed analysis of immune reconstitution was deferred in view of missing data.

Another limitation is the selection bias that could arise from the respondents of PedsQL questionnaires. The respondent rates were not 100%, and the majority of the non-respondents were also follow up defaulters. This may lead to bias in reporting QoL as it is not possible to identify whether those who did not turn up to follow up neglected to do so because they are healthy and decided not to come for medical follow up, or whether it was because they may no longer be alive. Measures such as postal questionnaires were undertaken to improve the response rate, with a focus on reaching the defaulters.

Ideally, a future study would be best as mixed method, including both qualitative and quantitative components, with focus on exploring the risk factors associated with quality of life for SCID survivors post-transplant. However, time was limited, and this opens the possibility for future research.

11.6 Future perspectives

11.6.1 Gene Therapy

Gene therapy offers promising hope for SCID patients. This intervention was created as a means of finding safer methods for the cure of SCID for those lacking

a suitable donor, other than hematopoietic stem cell transplantation. In principal, gene therapy involves a vector as a carrier for gene material which is introduced to the patient cells for the correction of the defect of the disease. Work on gene therapy for ADA SCID and Common γ chain SCID has been explored since the 1990s [138-140]. Five clinical trials on the ex-vivo gene therapy with γ-retroviral vectors were published between 1999 and 2009, involving 20 classic Common γ chain SCID and 27 ADA SCID patients [139-143]. The results were impressive as 85% of Common γ chain SCID patients and 70% of ADA SCID were considered cured of their disease with almost complete immune-reconstitution. However, this success was hampered by the occurrence of serious side effects, in which five of the twenty Common γ chain SCID patients developed T lymphocyte leukemic disease within 15 years of the procedure. One died and the rest received chemotherapy plus a booster transplant and survived. As a result, the clinical trials were suspended temporarily. It has since been found that integration of the retroviral vector in the gene region causes deregulated expression of LMO2 T-lymphocyte oncogenes and multiple hits in addition to other genes leading to uncontrolled clonal proliferation of T lymphocytes [144].

New lentiviral vectors have been formulated and shown to be effective with better safety profiles in trials [145, 146]. Currently, gene therapy is recommended as the next therapeutic option for ADA SCID patients without a matched sibling donor [128]. What is exciting would be a comparison of the long-term immune reconstitution between gene therapy and HSCT recipients. It would be also interesting to see whether gene therapy is able to ameroliate the neurocognitive behavioural defects associated with ADA SCID.

11.6.2 Newborn Screening

Data from multiple observational studies supports the notion that early diagnosis, and early transplantation improves the survival outcome and quality of life of SCID patients [3, 44]. The idea began to materialize after the ability to recognize and quantify T-lymphocyte receptor excision circles (TRECSs) from dried bloodspots became available in 2005 [147]. TRECS is useful as an indicator of intra-thymic T lymphopoeisis. They are produced during the V(D)J re-arrangement of T lymphocyte in the thymus and cut-out from the naïve T lymphocyte upon activation in the peripheral circulation. The absence or

reduction of TRECSs does correlate with poor thymic output of T-lymphocytes, thus indicating profound T-lymphocyte deficiency which is a universal feature in SCID, irrespective of the genotype and phenotype characteristics [148].

Several steps have been taken by immunologists in the United States of America including pilot projects and systemic reviews in assessing the feasibility of population-based newborn screening for SCID [147, 149-151]. Finally, population-based newborn screening for SCID via measuring TRECSS in dried bloodspots was approved by the Secretary's Advisory Committee on Heritable Disorders of Newborn and Children in 2010 [152]. Up until 2011, it was practiced in 6 states and 1 territory in the USA; and out of 961, 925 newborn screened, 14 cases of classical SCID and 40 cases of T lymphopenia that were not related to SCID have been identified [153]. Latest publication by an American institution proposed a systematic guideline for the management of newborn identified with SCID during neonatal screening [126].

Newborn screening has had several major impacts on SCID patients. The latest result from the newborn screening project demonstrated higher prevalence of SCID, 1 in 58000 births [1]. This is suggestive that SCID might not be as rare as previously thought. Furthermore, implementation of newborn SCID screening in US showed that more autosomal recessive SCID have been detected than X-linked SCID. This is suggestive that the actual incidence of SCID according to genotypes may differ between the observational reported cases and those detected by unbiased neonatal screening [127].

From my study and the previous published studies, it is clearly shown that earlier transplant age coupled with infection free status predicts the best survival and clinical outcome [2, 44]. The availability of newborn screening enables early detection of SCID patients during neonatal period with absence of infectious disease burden and earlier potential for treatment, and this give better chance of survival after HSCT [154].

11.6.3 New HSCT techniques

Encouraging data have been published in identifying new efforts to improve HSCT techniques. Improved manipulation of the stem cell graft by TCRαβ and CD19 depletion has shown to speed up the T-lymphocyte immune reconstitution

of the haploidentical recipients [81-83]. This has successfully met the major shortcoming of delayed immune reconstitution found in haploidentical HSCT, and offers improved viral clearance.

Another improvement is the development of adoptive transfer of *ex vivo* selected donor derived T lymphocytes in combination with the suicide gene, which offers the option of controlling the viral infection during the immediate post-HSCT period before thymus-derived T-lymphocyte immune recovery occurs [84]. The availability of suicide genes and administration of prodrugs enables recognition and apoptosis of the donor T-lymphocyte if acute GVHD occurs; thus avoiding risk of GVHD and concurrently offering viral protection. Currently, it is still in clinical trial phase I-II and preliminary results have been promising [85].

Further studies are needed in assessing the quality of immune reconstitution post-HSCT. Apart from quantification of thymopoiesis marker, new techniques in assessing the repertoire of T lymphocytes have been introduced such as CDR3 spectratyping. CDR3 spectratyping enables the assessment of diversity and versatility of T lymphocyte repertoires by characterizing length distribution of third complementarity determining region (CDR3) in beta variable (TRBV) subfamilies [155].

Chapter 12 Conclusion

12.1 General

Hematopoietic stem cell transplantation offers a curative option for severe combined immunodeficiency patients. Even though HSCT posed a significant risk, survival has improved as the options of better HSCT techniques, donor options, improved specialised care are available now.

12.2 Clinical Implication

Early timing of HSCT and infection status pre-HSCT remains vital in ensuring better survival outcome of patients. There was no significant difference of survival between conditioned and unconditioned recipients.

A high awareness of specific medical issues that may occur in specific SCID genotypes may aid clinicians during the long-term follow up care so that interventions such as psychosocial supports and cognitive behaviour therapy for ADA SCID patients may be offered.

This study demonstrated that conditioning is crucial in HSCT for SCID to achieve better engraftment, sustained thymopoiesis, donor chimerism and a higher chance of immunoglobulin independence.

Low toxicity MAC regimen was shown to have better safety profile and promotes better myeloid engraftment post-HSCT compared to MAC regimen.

There is a significant correlation between myeloid donor chimerism and B-lymphocyte donor chimerism during post-HSCT period.

Freedom from immunoglobulin replacement therapy and on-going medical issues contribute significantly to a normal quality of life for SCID survivors post-HSCT.

Lifelong follow up should be offered to all SCID survivors so that any medical issues can be identified leading to early intervention.

12.3 Research Implication

A prospective study assessing the immune reconstitution profile post-HSCT with focus on conditioning regimen, donor types and SCID genotypes is needed.

The development of a conditioning regimen with a better safety profile, but at the same time one which is myeloablative enough to ensure optimal engraftment at the risk of minimum side effects, is needed.

A mixed-methods study assessing the long-term quality of life would facilitates our understanding of the factors influencing QoL of SCID patients post-HSCT.

Chapter 13 Appendices

13.1 Appendix A: Permission to use PedsQL

Mapi Research Trust

User agreement

Special Terms

Mapi Research Trust, a non-for-profit organisation subject to the terms of the French law of 1st July 1901, registered in Carpentras under number 453 979 346, whose business address is 27 rue de la Villette, 69003 Lyon, France, hereafter referred to as "Mapi" and the User, as defined herein, (each referred to singularly as a "Party" and/or collectively as the "Parties"), do hereby agree to the following User Agreement Special and General Terms:

Mapi Research Trust
Information Support Unit
27 rue de la Villette
69003 Lyon
France
Telephone: +33 (0)4 72 13 65 75
Fax: +33 (0)4 72 13 66 82
Email: PROInformation@mapi-trust.org

Recitals

The User acknowledges that it is subject to these Special Terms and to the General Terms of the Agreement, which are included in Appendix 1 to these Special Terms and fully incorporated herein by reference. Under the Agreement, the Questionnaire referenced herein is licensed, not sold, to the User by Mapi for use only in accordance with the terms and conditions defined herein. Mapi reserves all rights not expressly granted to the User.

The Parties, in these Special Terms, intend to detail the special conditions of their partnership.

The Parties intend that all capitalized terms in the Special Terms have the same definitions as those given in article 1 of the General Terms included in Appendix 1.

In this respect, the Parties have agreed as follows:

Article 1. Conditions Specific to the User

Section 1.01 Identification of the User

User name	INTAN JULIANA ABD HAMID
Legal Form	INSTITUTE OF CELLULAR MEDICINE, NEWCASTLE UNIVERSITY
Address	7, CLOVERDALE GARDENS, NEWCASTLE UPON TYNE, NE7 7QJ
Country	United Kingdom

Name of the contact in charge of the Agreement	DR INTAN JULIANA ABD HAMID
Telephone number	+447931764079
Fax number	
Email address	I.j.abd-hamid@newcastle.ac.uk
If different:	
Legal Form	
Address	
Country	

Section 1.02 Identification of the Questionnaire

Title	Pediatric Quality of Life Inventory™ (PedsQL™)
Author(s)	Varni James W, PhD
Owner	Varni James W, PhD

Copyright	Copyright © 1998 JW Varni, Ph.D. All rights reserved
Original bibliograhic references	See Appendix 2

Article 2. Rights to Use

Section 2.01 Context of the Use of the Questionnaire

The User undertakes to only use the Questionnaire in the context of the Study as defined hereafter.

Context of use	Clinical project or study
Title	long-term Survival Outcome of Severe Combined Immunodeficiency Disorder (SCID) patients who underwent Hematopoietic Stem Cell Transplantation in Newcastle, UK from 1987 till 2012.
Disease or condition	Severe Combined Immunodeficiency Disorder
Type of research	Other: Prospective Cross-sectional Research
Number of patient expected	200
Number of submission to the Questionnaire for each patient	2
Term of clinical follow-up for each patient	Yearly follow up
Mode of administration	Paper

Section 2.02 Conditions for Use

The User undertakes to use the Questionnaire in accordance with the conditions for use defined hereafter.

(a) Rights transferred
Acting in the Author's name, Mapi transfers the following limited, non-exclusive rights, to the User (the "Limited Rights")
(i) to use the Questionnaire, only as part of the Study; this right is made up exclusively of the right to communicate it to the Beneficiaries only, free of charge, by any means of communication and by any means of remote distribution known or unknown to date, subject to respecting the conditions for use described hereafter; and

(ii) to reproduce the Questionnaire, only as part of the Study; this right is made up exclusively of the right to physically establish the Questionnaire or to have it physically established, on any paper, electronic, analog or digital medium, and in particular documents, articles, studies, observations, medical publications, websites whether or not protected by restricted access, CD, DVD, CD-ROM, hard disk, USB flash drive, for the Beneficiaries only and subject to respecting the conditions for use described hereafter; and

(iii) Should the Questionnaire not already have been translated into the language requested, the User is entitled to translate the Questionnaire or have it translated in this language, subject to informing Mapi of the same beforehand by the signature of a Translation Agreement and to providing a copy of the translation thus obtained as soon as possible to Mapi.

The User acknowledges and accepts that it is not entitled to amend, condense, adapt, reorganise the Questionnaire on any medium whatsoever, in any way whatsoever, even minor, without Mapi's prior specific written consent.

(b) Specific conditions for the Author

The Author has intended to transfer a part of the copyright on the Questionnaire and/or the Documentation to Mapi in order to enable Mapi to make it available to the User for the purpose of the Study, subject to the User respecting the following conditions:

User shall not modify, abridge, condense, translate, adapt, recast or transform the Questionnaire in any manner or form, including but not limited to any minor or significant change in wordings or organisation in the Questionnaire, without the prior written agreement of the Author. If permission is granted, any improvements, modifications, or enhancements to the Questionnaire which may be conceived or developed, including translations and modules, shall become the property of the Author.

The User therefore undertakes to respect these special terms.

(c) Specific conditions for the Questionnaire

- Use in individual clinical practice or Research study / project

The User undertakes never to duplicate, transfer or publish the Questionnaire without indicating the Copyright Notice.

- In the case of use of an electronic version of the Questionnaire, the User undertakes to respect the following special obligations:

- Not modify the questionnaire (items and response scales, including the response scale numbers from 0-4)
- Cite the reference publications
- Insert the copyright notice on all pages/screens on which the Questionnaire will be presented and insert the Trademark information: PedsQL™, Copyright © 1998 JW Varni, Ph.D. All rights reserved.
- Mention the following information: "PedsQL™ contact information and permission to use: Mapi Research Trust, Lyon, France. E-mail: PROinformation@mapi-trust.org – internet: www.proqolid.org and www.pedsql.org/index.html "

- Submit the screenshots of the US English original version of all the Pages where the Questionnaire appears to the Author, through Mapi Research Trust, before implementation in the translated versions and before release for approval and to check that the above-mentioned requirements have been respected.

* Use in a publication:

In the case of a publication, article, study or observation on paper or electronic format of the Questionnaire, the User undertakes to respect the following special obligations:

- not to include any full copy of the Questionnaire, but a version with the indication "sample copy, do not use without permission"
- to indicate the name and copyright notice of the author
- to include the reference publications of the Questionnaire
- to indicate the details of Mapi Research Trust for any information on the Questionnaire as follows: contact information and permission to use: Mapi Research Trust, Lyon, France. E-mail: PROInformation@mapi-trust.org – Internet: www.proqolid.org and www.pedsql.org
- to provide Mapi, as soon as possible, with a copy of any publication regarding the Questionnaire, for information purposes.

* Use for dissemination or marketing:

In the case of use in a dissemination/marketing context:

- On a website with unrestricted access:

In the case of publication on a website with unrestricted access, the User undertakes only to include a copy of the Questionnaire that cannot be amended, including the watermark on all pages or screens indicating "Sample copy – do not use without permission" along with the copyright notice and Mapi Research Trust's contact information.

- On a website with restricted access:

In the case of publication on a website with restricted access, the User may include a version of the Questionnaire that may be amended, subject to this version being protected by a sufficiently secure access to only allow the Beneficiaries to access it.

Article 3. Term

Mapi transfers the Limited Rights to use the Questionnaire as from the date of delivery of the Questionnaire to the User and for the whole period of the Study.

Article 4. Beneficiaries

The Parties agree that the User may communicate the Questionnaire in accordance with the conditions defined above to the Beneficiaries involved in the Study only, in relation to the Study defined in section 2.01.

Article 5. Territories and Languages

Mapi transfers the Limited Rights to use the Questionnaire on the following territories and in the languages indicated in the table below:

Language
English for the USA

Modules			
PedsQL™ Cognitive Functioning Scale	PedsQL™ Family Impact module	PedsQL™ Generic Core Scales	PedsQL™ Transplant module

Article 6. Price and Payment Terms

The User undertakes in relation to Mapi to pay the price owed in return for the availability of the Questionnaire, according to the prices set out below, depending on the languages requested and the costs of using the Questionnaire, in accordance with the terms and conditions described in section 6.02 of the General Terms included in Appendix 1.

Access to the Questionnaire in non-funded academic research and individual clinical practice is free of charge.

Agreed and acknowledged by

User's name: INTAN JULIANA ABD HAMID

Date:
05/02/2014

Mapi Research Trust

Appendix 1 to the Special Terms: User Agreement General Terms

User has read and accepted the Mapi's General Terms of the Agreement, which are available on Mapi Research Trust website (http://www.mapi-trust.org/services/questionnairelicensing/catalog-questionnaires)

Appendix 2 to the Special Terms: References

Generic Core Scales:

- Varni JW, et al. The PedsQL™: Measurement Model for the Pediatric Quality of Life Inventory. *Medical Care*, 1999; 37(2):126-139

- Varni, J.W., et al. The PedsQL™ 4.0: Reliability and validity of the Pediatric Quality of Life Inventory™ Version 4.0 Generic Core Scales in healthy and patient populations. *Medical Care*, 2001; 39(8): 800-812.

- Varni, J.W., et al., (2002). The PedsQL™ 4.0 Generic Core Scales: Sensitivity, responsiveness, and impact on clinical decision-making. *Journal of Behavioral Medicine*, 25, 175-193.

- Varni, J.W., et al. (2003). The PedsQL™ 4.0 as a pediatric population health measure: Feasibility, reliability, and validity. *Ambulatory Pediatrics*, 3, 329-341.

- Chan, K.S., Manglone-Smith, R., Burwinkle, T.M., Rosen, M., & Varni, J.W. (2005). The PedsQL™: Reliability and validity of the Short-Form Generic Core Scales and Asthma Module. *Medical Care*, 43, 256-265.

- Varni, J.W., & Limbers, C.A. (2009). The PedsQL™ 4.0 Generic Core Scales Young Adult Version: Feasibility, reliability and validity in a university student population. *Journal of Health Psychology*; 14, 611-622.

Asthma Module:

- Varni, J.W., Burwinkle, T.M., Rapoff, M.A., Kamps, J.L., & Olson, N. The PedsQL™ in pediatric asthma: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales and Asthma Module. *Journal of Behavioral Medicine*, 2004; 27:297-318.

- Chan, K.S., Manglone-Smith, R., Burwinkle, T.M, Rosen, M., & Varni, J.W. (2005). The PedsQL™: Reliability and validity of the Short-Form Generic Core Scales and Asthma Module. *Medical Care*, 43, 256-265.

Brain Tumor Module:

- Palmer, S.N., Meeske, K.A., Katz, E.R., Burwinke, T.M., & Varni, J.W. (2007). The PedsQL™ Brain Tumor Module: Initial reliability and validity. *Pediatric Blood and Cancer*, 49, 287-293.

Cancer Module:

- Varni, J.W., Burwinkle, T.M., Katz, E.R., Meeske, K., & Dickinson, P. The PedsQL™ in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer*, 2002; 94: 2090-2106.

- Robert RS, Paxton RJ, Palla SL, Yang G, Askins MA, Joy SE, Ater JL. Feasibility, reliability, and validity of the pediatric quality of life inventory™ generic core scales, cancer module, and multidimensional fatigue scale in long-term adult survivors of pediatric cancer. *Pediatric Blood & Cancer* 2012;59:703–707.

Cerebral Palsy Module:

- Varni JW, Burwinkle TM, Berrin SJ, Sherman SA, Artavia K, Malcarne VL, Chambers HG (2006). The PedsQL™ in Pediatric Cerebral Palsy: Reliability, Validity, and Sensitivity of the Generic Core Scales and Cerebral Palsy Module. *Developmental Medicine and Child Neurology*, 48: 442-449.

Cardiac Module:

- Uzark, K., Jones, K., Burwinkle, T.M., & Varni, J.W. The Pediatric Quality of Life Inventory™ in children with heart disease.Progress in Pediatric Cardiology, 2003; 18:141-148.

- Uzark, K., Jones, K., Slusher, J., Limbers, C.A., Burwinkle, T.M., & Varni, J.W. (2008). Quality of life in children with heart disease as perceived by children and parents. *Pediatrics*, 121, e1060-e1067.

Cognitive Functioning Scale:

- McCarthy, M.L., MacKenzie, E.J., Durbin, D.R., Aitken, M.E., Jaffe, K.M., Paidas, C.N. et al. (2005). The Pediatric Quality of Life Inventory: An evaluation of its reliability and validity for children with traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, 86, 1901-1909.

- Varni, J.W., Burwinkle, T.M., Katz, E.R., Meeske, K., & Dickinson, P. (2002). The PedsQL™ in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer*, 94, 2090-2106.

- Varni, J.W., Limbers, C.A., Sorensen, L.G., Neighbors, K., Martz, K., Bucuvalas, J.C., & Alonso, E.M. (2011). PedsQL™ Cognitive Functioning Scale in pediatric liver transplant recipients: Feasibility, reliability and validity. *Quality of Life Research*, 20, 913–921.

Diabetes Module:

- Varni, J.W., Curtis, B.H., Abetz, L.N., Lasch, K.E., Plault, E.C., & Zeytoonjian, A.A. (In press). Content validity of the PedsQL™ 3.2 Diabetes Module in newly diagnosed patients with Type 1 Diabetes Mellitus ages 8-45. *Quality of Life Research*.

- Varni, J.W., Burwinkle, T.M., Jacobs, J.R., Gottschalk, M., Kaufman, F., & Jones, K.L. The PedsQL™ in Type 1 and Type 2 diabetes: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales and Type 1 Diabetes Module. *Diabetes Care*, 2003; 26: 631-637.

- Nansel, T.R., Weisberg-Benchell, J., Wysocki, T., Laffel, L. & Anderson, B. (2008). Quality of life in children with Type 1 diabetes: A comparison of general and disease-specific measures and support for a unitary diabetes quality of life construct. *Diabetic Medicine*, 25, 1316-1323.

- Naughton, M.J., Ruggiero, A.M., Lawrence, J.M., Imperatore, G., Klingensmith, G.J. Waltzfelder, B., McKeown, R.E., Standiford, D.A., Liese, A.D., & Loots, B. (2008). Health-related quality of life of children and adolescents with type 1 or type 2 diabetes mellitus: SEARCH for Diabetes in Youth Study. *Archives of Pediatrics and Adolescent Medicine*, 162, 649-657.

- Hilliard, M.E., Lawrence, J.M., Modi, A.C., Anderson, A., Crume, T., Dolan, L.M., Merchant, A.T., Yi-Frazier, J.P., & Hood, K.K. (2013). Identification of minimal clinically important difference scores of the Pediatric Quality of Life Inventory in children, adolescents, and young adults with Type 1 and Type 2 diabetes. *Diabetes Care*, 36, 1891–1897.

Duchenne Muscular Dystrophy Module:

- Uzark, K., King, E., Cripe, L., Spicer, R., Sage, J., Kinnett, K., Wong, B., Pratt, J., & Varni, J.W. (2012). Health-related quality of life in children and adolescents with Duchenne Muscular Dystrophy. *Pediatrics*, 130, e1559-e1566.

End Stage Renal Disease Module:

- Goldstein, S.L., Graham, N., Warady, B.A., Selkaly, M., McDonald, R., Burwinkle, T.M., Limbers, C.A., & Varni, J.W. (2008). Measuring health-related quality of life in children with ESRD: Performance of the Generic and ESRD-Specific Instrument of the Pediatric Quality of Life Inventory™ (PedsQL™). *American Journal of Kidney Diseases*, 51, 285-297.

Eosinophilic Esophagitis:

- Franciosi, J.P., Hommel, K.A., Bendo, C.B., King, E.C., Collins, M.H., Eby, M.D., Marsolo, K., Abonia, J.P., von Tiehl, K.F., Putnam, P.E., Greenler, A.J., Greenberg, A.B., Bryson, R.A., Davis, C.M., Olive, A.P., Gupta, S.K., Erwin, E.A., Klinnert, M.D., Spergel, J.M., Denham, J.M., Furuta, G.T., Rothenberg, M.E., & Varni, J.W. (2013). PedsQL™ Eosinophilic Esophagitis Module: Feasibility, reliability and validity. *Journal of Pediatric Gastroenterology & Nutrition*, 57, 57-66.

- Franciosi, J.P., Hommel, K.A., Greenberg, A.B., Debrosse, C.W., Greenler, A.J., Abonia, J.P., Rothenberg, M.E., & Varni, J.W. (2012). Development of the Pediatric Quality of Life Inventory™ Eosinophilic Esophagitis Module items: Qualitative methods. *BMC Gastroenterology*, 12:135, 1-8.

- Franciosi J.P., Hommel, K.A., Debrosse, C.W., Greenberg, A.B., Greenler, A.J., Abonia, J.P., Rothenberg, M.E., & Varni, J.W. (2012). Quality of life in paediatric eosinophilic oesophagitis: What is important to patients? *Child: Care, Health and Development*, 38, 477–483.

Family Impact Module:

- Varni, J.W., Sherman, S.A., Burwinkle, T.M., Dickinson, P.E., & Dixon, P. (2004). The PedsQL™ Family Impact Module: Preliminary reliability and validity. *Health and Quality of Life Outcomes*; 2 (55), 1-6.

- Medrano, G.R., Berlin, K.S., & Davies, W.H. (In press). Utility of the PedsQL™ Family Impact Module: Assessing the psychometric properties in a community sample. *Quality of Life Research*.

- Jiang, X., Sun, L., Wang, B., Yang, X., Shang, L., & Zhang, Y. (2013). Health-related quality of life among children with recurrent respiratory tract infections in Xi'an, China. *PLoS One*, 8(2): e56945.

- Mano, K.E., Khan, K.A., Ladwig, R.J., & Weisman, S.J. (2011). The impact of pediatric chronic pain on parents' health-related quality of life and family functioning: Reliability and validity of the PedsQL 4.0 Family Impact Module. *Journal of Pediatric Psychology*, 36, 517-527.

Gastrointestinal Symptom Scale:

- Varni, J.W., Lane, M.M., Burwinkle, T.M., Fontaine, E.N., Youssef, N.N., Schwimmer, J.B., Pardee, P.E., Pohl, J.F., & Easley, D.J. (2006). Health-related quality of life in pediatric patients with irritable bowel syndrome: A comparative analysis. *Journal of Developmental and Behavioral Pediatrics*,27, 451-458.

General Well-Being Scale:

- Varni, J.W., Seid, M., & Kurtin, P.S. (1999). Pediatric health-related quality of life measurement technology: A guide for health care decision makes. *Journal of Clinical Outcomes Management*, 6, 33-40.

- Hallstrand, T.S., Curtis, J.R., Aitken, M.L., & Sullivan, S.D. (2003). Quality of life in adolescents with mild asthma. *Pediatric Pulmonology*, 36, 536-543.

Healthcare Satisfaction Generic Module:

- Varni, J.W., Burwinkle, T.M., Dickinson, P., Sherman, S.A., Dixon, P., Ervice, J.A., Leyden, P.A. & Sadler, B.L. (2004). Evaluation of the built environment at a Children's Convalescent Hospital: Development of the Pediatric Quality of Life Inventory™ Parent and Staff Satisfaction Measures for pediatric health care facilities. *Journal of Developmental and Behavioral Pediatrics*, 2004; 25:10-25.

Health Care Satisfaction Module specific for Hematology/Oncology:

- Varni, J.W., Quiggins, D.J.L., & Ayala, G.X. (2000). Development of the Pediatric Hematology/Oncology Parent Satisfaction survey. *Children's Health Care*, 29, 243-255.

Infant Scales:

- Varni, J.W., Limbers, C.A., Neighbors, K., Schulz, K., Lieu, J.E.C., Heffer, R.W., Tuzinkiewicz, K., Mangione-Smith, R., Zimmerman, J.J., & Alonso, E.M. (2011). The PedsQL™ Infant Scales: Feasibility, internal consistency reliability and validity in healthy and ill infants. *Quality of Life Research*, 20, 45-55.

Multidimensional Fatigue Scale:

- Varni, J.W., Burwinkle, T.M., Katz, E.R., Meeske, K., & Dickinson, P. (2002). The PedsQL™ in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer*, 94, 2090-2106.

- Varni, J. W., Beaujean, A., & Limbers, C. A. (In press). Factorial invariance of pediatric patient self-reported fatigue across age and gender: A multigroup confirmatory factor analysis approach utilizing the PedsQL™ Multidimensional Fatigue Scale. *Quality of Life Research*.

- Varni, J.W., Burwinkle, T.M., & Szer, I.S. (2004). The PedsQL™ Multidimensional Fatigue Scale in pediatric rheumatology: Reliability and validity. *Journal of Rheumatology*, 31, 2494-2500.

- Varni, J.W., & Limbers, C.A. (2008). The PedsQL™ Multidimensional Fatigue Scale in young adults: Feasibility, reliability and validity in a university student population. *Quality of Life Research*, 17, 105-114.

Neurofibromatosis Type 1 Module:

- Nutakki, K., Hingtgen, C.M., Monahan, P., Varni, J.W., & Swigonski, N.L. (2013). Development of the adult PedsQL™ Neurofibromatosis Type 1 Module: Initial feasibility, reliability and validity. *Health and Quality of Life Outcomes*, 11:21, 1-9

Neuromuscular Module:

- Iannaccone, S.T., Hynan, L.S., Morton, A., Buchanan, R., Limbers, C.A., & Varni, J.W. (2009). The PedsQL™ in pediatric patients with Spinal Muscular Atrophy: Feasibility, reliability, and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales and Neuromuscular Module. *Neuromuscular Disorders*, 19, 805-812.

- Davis, S.E., Hynan, L.S., Limbers, C.A., Andersen, C.M., Greene, M.C., Varni, J.W., & Iannaccone, S.T. (2010). The PedsQL™ in pediatric patients with Duchenne Muscular Dystrophy: Feasibility, reliability, and validity of the Pediatric Quality of Life Inventory™ Neuromuscular Module and Generic Core Scales. *Journal of Clinical Neuromuscular Disease*, 11, 97-109.

Oral Health Scale:

- Steele, M.M., Steele, R.G., & Varni, J.W. (2009). Reliability and validity of the PedsQL™ Oral Health Scale: Measuring the relationship between child oral health and health-related quality of life. *Children's Health Care*, 38, 228-224.

Pediatric Pain Coping Inventory™:

- Varni, J.W., Waldron, S.A., Gragg, R.A., Rapoff, M.A., Bernstein, B.H., Lindsley, C.B., & Newcomb, M.D (1996). Development of the Waldron/Varni Pediatric Pain Coping Inventory. *Pain*, 67, 141-150.

Pediatric Pain Questionnaire:

- Varni, J.W., Thompson, K.L., & Hanson, V. (1987). The Varni/Thompson Pediatric Pain Questionnaire: I. Chronic musculoskeletal pain in juvenile rheumatoid arthritis. *Pain*, 28, 27-38.

Present Functioning Visual Analogue Scales:

- Sherman, S.A., Eisen, S., Burwinkle, T.M., & Varni, J.W. (2006). The PedsQL™ Present Functioning Visual Analogue Scales: Preliminary reliability and validity. *Health and Quality of Life Outcomes*, 4:75, 1-10.

Sickle Cell Disease Module:

- Panepinto, J.A., Torres, S., Bendo, C.B., McCavit, T.L., Dinu, B., Sherman-Bien, S., Bemrich-Stolz, C., & Varni, J.W. (2013). PedsQL™ Sickle Cell Disease Module: Feasibility, reliability and validity. *Pediatric Blood & Cancer*, 60, 1338–1344.

- Panepinto, J.A., Torres, S., & Varni. J.W. (2012). Development of the PedsQL™ Sickle Cell Disease Module items: Qualitative methods. *Quality of Life Research*, 21, 341-357.

Rheumatology Module:

- Varni, J.W., Seid, M., Knight, T.S., Burwinkle, T.M., Brown, J., & Szer, I.S. (2002). The PedsQL™ in pediatric rheumatology: Reliability, validity, and responsiveness of the Pediatric Quality of Life Inventory™ Generic Core Scales and Rheumatology Module. *Arthritis and Rheumatism*, 2002; 46: 714-725.

Transplant Module:

- Weissberg-Benchell, J., Zielinski, T.E., Rodgers, S., Greenley, R.N., Askenazi, D., Goldstein, S.L., Fredericks, E.M., McDiarmid, S., Williams, L., Limbers, C.A., Tuzinkiewicz, K., Lerret, S., Alonso, E.M., & Varni, J.W. (2010). Pediatric health-related quality of life: Feasibility, reliability and validity of the PedsQL™ Transplant Module. *American Journal of Transplantation*, 10, 1677-1685.

13.2 Appendix B: PedsQL Quality of Life version 4.0 Questionnaires (Parent Report)

13.2.1 PedsQL Quality of Life version 4.0 Questionnaires for Age 2 – 4 years old (Parent Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **TODDLERS** (ages **2-4**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking Text	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in active play or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Bathing	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Playing with other children	0	1	2	3	4
2. Other kids not wanting to play with him or her	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

Please complete this section if your child attends school or daycare

SCHOOL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Doing the same school activities as peers	0	1	2	3	4
2. Missing school/daycare because of not feeling well	0	1	2	3	4
3. Missing school/daycare to go to the doctor or hospital	0	1	2	3	4

13.2.2 PedsQL Quality of Life version 4.0 Questionnaires for Age 5 - 7 years old (Parent Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **YOUNG CHILDREN** (ages **5-7**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores, like picking up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with school activities	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

13.2.3 PedsQL Quality of Life version 4.0 Questionnaires for Age 8 - 12 years old (Parent Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

13.2.4 PedsQL Quality of Life version 4.0 Questionnaires for Age 13 - 18 years old (Parent Report)

ID#_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **TEENS** (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your teen**. Please tell us **how much of a problem** each one has been for **your teen** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your teen had with …*

PHYSICAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other teens	0	1	2	3	4
2. Other teens not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other teens	0	1	2	3	4
4. Not able to do things that other teens his or her age can do	0	1	2	3	4
5. Keeping up with other teens	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

13.2.5 PedsQL Quality of Life version 4.0 Questionnaires for Age 18 -25 years old (Parent Report)

ID#_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **YOUNG ADULTS** (ages **18-25**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other young adults	0	1	2	3	4
2. Other young adults not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other young adults	0	1	2	3	4
4. Not able to do things that others his or her age can do	0	1	2	3	4
5. Keeping up with other young adults	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention at work or school	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with work or studies	0	1	2	3	4
4. Missing work or school because of not feeling well	0	1	2	3	4
5. Missing work or school to go to the doctor or hospital	0	1	2	3	4

13.2.6 PedsQL Quality of Life version 4.0 Questionnaires for Adult (Parent Report)

ID#_____

Date:_____

PedsQL™

Adult Quality of Life Inventory

Version 4.0

PARENT REPORT for ADULTS

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other adults	0	1	2	3	4
2. Other adults not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other adults	0	1	2	3	4
4. Not able to do things that others his or her age can do	0	1	2	3	4
5. Keeping up with other adults	0	1	2	3	4

WORK/STUDIES FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention at work or school	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with work or studies	0	1	2	3	4
4. Missing work or school because of not feeling well	0	1	2	3	4
5. Missing work or school to go to the doctor or hospital	0	1	2	3	4

13.3 Appendix C: PedsQL Quality of Life version 4.0 Questionnaires (Child Report)

13.3.1 PedsQL Quality of Life version 4.0 Questionnaires for Age 5 - 7 years old (Child Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

YOUNG CHILD REPORT (ages 5-7)

Instructions for interviewer:

I am going to ask you some questions about things that might be a problem for some children. I want to know how much of a problem any of these things might be for you.

Show the child the template and point to the responses as you read.

If it is <u>not at all</u> a problem for you, point to the smiling face

If it is <u>sometimes</u> a problem for you, point to the middle face

If it is a problem for you <u>a lot</u>, point to the frowning face

I will read each question. Point to the pictures to show me how much of a problem it is for you. Let's try a practice one first.

	Not at all	Sometimes	A lot
Is it hard for you to snap your fingers	☺	😐	☹

Ask the child to demonstrate snapping his or her fingers to determine whether or not the question was answered correctly. Repeat the question if the child demonstrates a response that is different from his or her action.

Think about how you have been doing for the last few weeks. Please listen carefully to each sentence and tell me how much of a problem this is for you.

After reading the item, gesture to the template. If the child hesitates or does not seem to understand how to answer, read the response options while pointing at the faces.

PHYSICAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Is it hard for you to walk	0	2	4
2. Is it hard for you to run	0	2	4
3. Is it hard for you to play sports or exercise	0	2	4
4. Is it hard for you to pick up big things	0	2	4
5. Is it hard for you to take a bath or shower	0	2	4
6. Is it hard for you to do chores (like pick up your toys)	0	2	4
7. Do you have hurts or aches (***Where?***)	0	2	4
8. Do you ever feel too tired to play	0	2	4

Remember, tell me how much of a problem this has been for you for the last few weeks.

EMOTIONAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Do you feel scared	0	2	4
2. Do you feel sad	0	2	4
3. Do you feel mad	0	2	4
4. Do you have trouble sleeping	0	2	4
5. Do you worry about what will happen to you	0	2	4

SOCIAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Is it hard for you to get along with other kids	0	2	4
2. Do other kids say they do not want to play with you	0	2	4
3. Do other kids tease you	0	2	4
4. Can other kids do things that you cannot do	0	2	4
5. Is it hard for you to keep up when you play with other kids	0	2	4

SCHOOL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Is it hard for you to pay attention in school	0	2	4
2. Do you forget things	0	2	4
3. Is it hard to keep up with schoolwork	0	2	4
4. Do you miss school because of not feeling good	0	2	4
5. Do you miss school because you have to go to the doctor's or hospital	0	2	4

How much of a problem is this for you?

Not at all	Sometimes	A lot
		

13.3.2 PedsQL Quality of Life version 4.0 Questionnaires for Age 8 - 12 years old (Child Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

CHILD REPORT (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting along with other kids	0	1	2	3	4
2. Other kids do not want to be my friend	0	1	2	3	4
3. Other kids tease me	0	1	2	3	4
4. I cannot do things that other kids my age can do	0	1	2	3	4
5. It is hard to keep up when I play with other kids	0	1	2	3	4

ABOUT SCHOOL *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

13.3.3 PedsQL Quality of Life version 4.0 Questionnaires for Age 13 - 18 years old (Child Report)

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

TEEN REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has this been for you …*

About My Health and Activities *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

About My Feelings *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

How I Get Along with Others *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting along with other teens	0	1	2	3	4
2. Other teens do not want to be my friend	0	1	2	3	4
3. Other teens tease me	0	1	2	3	4
4. I cannot do things that other teens my age can do	0	1	2	3	4
5. It is hard to keep up with my peers	0	1	2	3	4

About School *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

13.3.4 PedsQL Quality of Life version 4.0 Questionnaires for Age 18 - 25 years old (Young Adult Report)

ID# _____

Date:_____

PedsQL™

Young Adult Quality of Life Inventory

Version 4.0

YOUNG ADULT REPORT (ages **18-25**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or feel pain	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting along with other young adults	0	1	2	3	4
2. Other young adults do not want to be my friend	0	1	2	3	4
3. Other young adults tease me	0	1	2	3	4
4. I cannot do things that others my age can do	0	1	2	3	4
5. It is hard to keep up with my peers	0	1	2	3	4

ABOUT MY WORK/STUDIES *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention at work or school	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my work or studies	0	1	2	3	4
4. I miss work or school because of not feeling well	0	1	2	3	4
5. I miss work or school to go to the doctor or hospital	0	1	2	3	4

13.3.5 PedsQL Quality of Life version 4.0 Questionnaires for Adult (Adult Report)

ID# _____

Date:_____

PedsQL™

Adult Quality of Life Inventory

Version 4.0

ADULT REPORT

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting along with other adults	0	1	2	3	4
2. Other adults do not want to be my friend	0	1	2	3	4
3. Other adults tease me	0	1	2	3	4
4. I cannot do things that others my age can do	0	1	2	3	4
5. It is hard to keep up with my peers	0	1	2	3	4

ABOUT MY WORK/STUDIES *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention at work or school	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my work or studies	0	1	2	3	4
4. I miss work or school because of not feeling well	0	1	2	3	4
5. I miss work or school to go to the doctor or hospital	0	1	2	3	4

13.4 Appendix D: Publication

13.4.1 Brief Report - Blood

Abd Hamid, I. J., Slatter, M. A., McKendrick, F., Pearce, M. S., & Gennery, A. R. (2017). Long-term outcome of hematopoietic stem cell transplantation for IL2RG/JAK3 SCID-: a cohort report. Blood, (), blood-2016-11-748616. Accessed April 26, 2017. https://doi.org/10.1182/blood-2016-11-748616

Brief Report

blood

TRANSPLANTATION

Long-term outcome of hematopoietic stem cell transplantation for IL2RG/JAK3 SCID: a cohort report

Intan Juliana Abd Hamid,[1-3] Mary A. Slatter,[1,2] Fiona McKendrick,[4] Mark S. Pearce,[5] and Andrew R. Gennery[1,2]

[1]Pediatric Immunodeficiency Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; [2]Department of Paediatric Immunology and Hematopoietic Stem Cell Transplantation, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom; [3]Regenerative Medicine Cluster, Institut Perubatan and Pergigian Termaju, Universiti Sains Malaysia, Kepala Batas, Malaysia; [4]Department of Health Psychology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; and [5]Institute of Health and Society, Newcastle University, Newcastle upon Tyne, United Kingdom

Key Points

- Conditioning is associated with better thymopoiesis, donor B-lymphocyte chimerism, cessation of immunoglobulin therapy, and normal QoL.

Hematopoietic stem cell transplantation (HSCT) cures the T-lymphocyte, B-lymphocyte, and natural killer (NK)–cell differentiation defect in interleukin-2 γ-chain receptor (IL2RG)/JAK3 severe combined immunodeficiency (SCID). We evaluated long-term clinical features, longitudinal immunoreconstitution, donor chimerism, and quality of life (QoL) of IL2RG/JAK3 SCID patients >2 years post-HSCT at our center. Clinical data were collated and patients/families answered PedsQL Generic Core Scale v4.0 questionnaires. We performed longitudinal analyses of $CD3^+$, $CD4^+$ naive T-lymphocyte, $CD19^+$, and NK-cell numbers from pretransplant until 15 years posttransplant. Thirty-one of 43 patients (72%) survived. Median age at last follow-up was 10 years (range, 2-25 years). Twenty-one (68%) had persistent medical issues, mainly ongoing immunoglobulin replacement (14; 45%), cutaneous viral warts (7; 24%), short stature (4; 14%), limb lymphoedema (3; 10%), and bronchiectasis (2; 7%). Lung function was available and normal for 6 patients. Longitudinal analysis demonstrated sustained $CD3^+$, $CD19^+$, and NK-cell output 15 years post-HSCT. $CD4^+$ naive lymphocyte numbers were better in conditioned vs unconditioned recipients (*P*, .06). B-lymphocyte and myeloid chimerism were highly correlated (ρ, 0.98; $P < .001$). Low-toxicity myeloablative conditioning recipients have better B-lymphocyte/myeloid chimerism and are free from immunoglobulin replacement therapy. IL2RG/JAK3 SCID survivors free from immunoglobulin replacement have normal QoL. (*Blood*. 2017;129(15):2198-2201)

Introduction

Severe combined immunodeficiencies (SCIDs) are due to defective T development or function, with variable effects on B- and natural killer (NK)–lymphocyte differentiation and development. Defects in the interleukin-2 γ-chain receptor (IL2RG) are most common.[1] Less common defects in JAK3 are downstream of IL2RG signaling: both forms present with a T-lymphocyte–negative, B-lymphocyte–positive, NK-cell–negative immunophenotype. B lymphocytes are present, but intrinsic signaling defects render them nonfunctional.[2,3] Since the first hematopoietic stem cell transplant (HSCT) to correct the immunodeficiency in SCID in 1968, incremental improvements in techniques and supportive care have led to better survival.[4,5] However, most publications described long-term outcome of the entire SCID cohort, irrespective of the genotypic and phenotypic diversity.[4-9]

Detailed description of single-genotype cohorts is important because different donor sources and conditioning regimens, or use of hematopoietic stem cell infusion alone, may result in different outcomes, depending on the immunophenotype and genotype.[9,10] T-lymphocyte donor chimerism and reconstitution are generally good despite various types of conditioning and donor selection. However, poor B-lymphocyte and myeloid chimerism are noted, particularly in the absence of chemotherapy conditioning.[11] One study suggested that donor B-lymphocyte chimerism is required for establishment of functioning B lymphocytes in IL2RG, JAK3, and VDJ-recombinant defect SCID genotypes.[12] Sustained thymopoiesis and donor B-lymphocyte chimerism may require administration of myeloablative preparative regimens[7,13,14] and modified T-lymphocyte depletion techniques.[15] Long-term immune function may impact on subsequent health-related quality of life (QoL) and presence or absence of ongoing medical issues, which may be dependent on prior use of a preparative regimen. Therefore, we aimed to assess the long-term immune function, health outcome, and QoL in a single-center cohort of IL2RG/JAK3 SCID patients post-HSCT.

Study design

Forty-three patients received 49 transplants between 1987 and 2012. Procedures were performed based on the European Inborn Errors Working Party guidelines current at time of transplant. Patients and families consented to data collection at time of transplant. Patients were invited to answer the previously validated PedsQL Generic Core Scale Quality of Life v4.0[16] as part of their routine psychological health assessment.

Submitted 31 October 2016; accepted 12 February 2017. Prepublished online as *Blood* First Edition paper, 16 February 2017; DOI 10.1182/blood-2016-11-748616.

The online version of this article contains a data supplement.

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

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

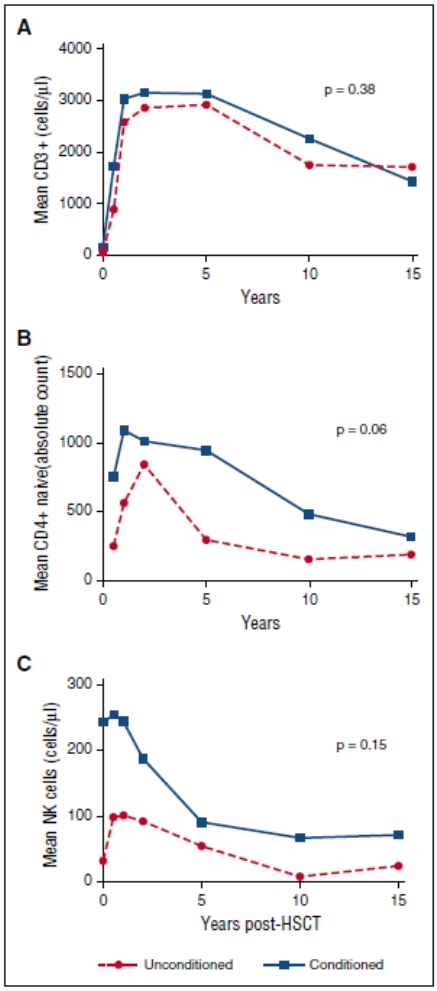


Figure 1. The longitudinal analysis of immune parameters posttransplant according to unconditioned vs conditioned recipients of IL2RG/JAK3 SCID. (A) Mean $CD3^+$ cells measured at different time points posttransplant. There was no significant difference in trend of circulating $CD3^+$ numbers between conditioned vs unconditioned IL2RG/JAK3 SCID patients ($P = .38$) and at each time point posttransplant. (B) Mean $CD4^+$ naive cells measured at different time points posttransplant. Naive $CD4^+$ cell ($CD3^+CD4^+CD45RA^+CD27^+$) measurement was used as an indicator of the thymic output posttransplantation. Conditioned recipients demonstrated borderline higher overall trend of $CD4^+$ naive output compared with unconditioned recipients (*P*, .06). Conditioned recipients had a better $CD4^+$ naive output at early time points after transplant compared with unconditioned recipients. The data are in the format of contrast, which shows the difference between the mean of both groups and standard error (SE): 0.5 years posttransplant (contrast, 611.1; SE, 286.1; *P*, .03), 1 year posttransplant (contrast, 513.1; SE, 224.8; *P*, .02), 2 years posttransplant (contrast, 415.1; SE, 178.0; *P*, .01), 5 years posttransplant (contrast, 317.0; SE, 159.0; *P*, .04), respectively. (C) Mean NK cells measured at different time points posttransplant. The conditioned recipients had a nonsignificantly higher overall NK-cell number compared with unconditioned recipients (*P*, .15). However, conditioned recipients did have significantly higher NK-cell numbers compared with unconditioned recipients at time point of baseline (contrast, 126.5; SE, 66.1; *P*, .05) and 0.5 years posttransplant (contrast, 112.1; SE, 59.3; *P*, .05). Numbers of patients available for longitudinal data analysis of immunoreconstitution ($CD3^+$, $CD4^+$ naive, and NK cells) are shown in supplemental Table 4.

In the earlier patients, conditioning consisted of busulphan (8 or 16 mg/kg) and cyclophosphamide (200 mg/kg). Low-toxicity myeloablative conditioning (MAC) consisted of treosulphan and fludarabine (150 mg/m^2) or cyclophosphamide (200 mg/kg). Reduced-intensity conditioning (RIC) consisted of fludarabine (150 mg/m^2) and melphalan (140 mg/m^2). Further details about donor types, conditioning regimens, graft sources, and serotherapy for each patient are summarized in supplemental Table 1 (available on the *Blood* Web site).

CD3, CD4, CD8, CD19, CD27/CD45RA,[15] and CD16/56 enumeration and serum immunoglobulin G (IgG), IgA, and IgM levels were measured routinely. Donor cell chimerism was based on polymerase chain reaction amplification of short tandem repeats.

All data analysis was performed using STATA version 14.1. Multilevel mixed-effect modeling was performed for longitudinal analysis of $CD3^+$, $CD19^+$, NK cells, and $CD4^+$ naive output posttransplant.

Results and discussion

Thirty-one patients survived to August 2015. Median age at last follow-up was 10 years (range, 2-25 years). Overall survival at 10 years post-HSCT was 71.9%. Transplant-related mortality (TRM) was 23.3% (1 patient died of nontransplant-related causes). There was no significant difference in 10-year survival outcome comparing unconditioned vs conditioned recipients considering first HSCT characteristics (69.8% vs 72.4%; $P = .91$). Details of those who received a second procedure are given in supplemental Table 2.

Longitudinal immune-reconstitution results were available for 29 of 31 patients. There was no significant difference in $CD3^+$ numbers between conditioned vs unconditioned patients ($P = .38$) (Figure 1A). Conditioned recipients trended toward more sustained thymopoiesis, compared with unconditioned recipients ($P = .06$) (Figure 1B). Longitudinal NK-cell numbers were nonsignificantly higher in conditioned recipients compared with unconditioned recipients (*P*, .15) (Figure 1C). There was no significant difference in mean values of NK cells at latest follow-up between those with or without verrucosis (84.4 cells per microliter [standard deviation (SD), 61.2] vs 87.19 cells per microliter [SD, 83.1]; $P = .53$), or after comparing numbers of patients with verrucosis in conditioned vs unconditioned recipients (5 of 19 vs 2 of 10; $P = .14$).

Donor chimerism was available for 29 patients. B-lymphocyte and myeloid cell donor chimerism were highly correlated (Spearman ρ, 0.98; $P < .001$) (supplemental Figure 1). Low-toxicity MAC recipients had significantly better myeloid donor chimerism at last follow-up, compared with unconditioned or other conditioning recipients ($P < .001$) (supplemental Figure 2).

Seventeen patients (55%) with normal B-lymphocyte function were free from immunoglobulin replacement therapy. All low-toxicity MAC survivors (7 of 8) were free from immunoglobulin replacement therapy with >50% donor B-lymphocyte chimerism irrespective of donor. Of unconditioned survivors, 4 of 10 (and of survivors conditioned with 8 mg/kg busulphan, 6 of 12) did not require immunoglobulin replacement (supplemental Table 3). There was a significant association between >50% donor B-lymphocyte chimerism and the ability to cease immunoglobulin replacement therapy ($P = .0001$) (supplemental Figure 3).

Table 1. Mean PedsQL scores for IL2RG/JAK3 SCID patients post-transplantation (parent and children report)

	UK norm	IL2RG/JAK3 SCID, mean (P)	Ongoing immunoglobulin replacement, mean (P)	No immunoglobulin replacement, mean (P)	Ongoing medical issues (P)	No medical issues (P)
Parent report		N = 19	N = 8	N = 11	N = 12	N = 7
Total	84.6	70.9 (.006)	66.9 (.02)	75.0 (.06)	70.0 (.02)	72.9 (.04)
Psychosocial	82.2	66.5 (.007)	58.5 (.01)	73.0 (.06)	64.7 (.01)	69.5 (.06)
Physical	88.1	82.4 (.13)	77.7 (.13)	85.8 (.36)	79.2 (.10)	86.6 (.97)
Emotional	78.9	72.9 (.34)	60.0 (.08)	82.9 (.81)	69.6 (.15)	78.6 (.55)
Social	86.9	77.4 (.15)	70.0 (.06)	82.7 (.24)	77.5 (.15)	77.1 (.12)
School	81.5	69.5 (.03)	55.9 (.01)	80.0 (.09)	58.9 (.01)	70.2 (.19)
Child report		N = 15	N = 5	N = 10	N = 8	N = 7
Total	83.9	77.8 (.29)	71.7 (.16)	80.8 (.39)	77.6 (.29)	77.9 (.17)
Psychosocial	81.9	74.1 (.17)	67.9 (.15)	77.5 (.29)	75.2 (.29)	72.9 (.12)
Physical	88.5	84.8 (.45)	80.0 (.29)	86.9 (.39)	82.0 (.24)	87.5 (.46)
Emotional	78.5	73.9 (.66)	67.0 (.24)	85.5 (.66)	75.0 (.37)	84.2 (.73)
Social	87.7	75.7 (.09)	77.0 (.17)	75.0 (.09)	81.9 (.21)	69.9 (.09)
School	78.9	67.9 (.09)	58.0 (.05)	72.0 (.19)	69.4 (.15)	65.0 (.09)

All mean comparisons were made against UK norms[17] using the 1-sample t test.

QoL assessments were available for 20 of 31 patients (65%) and comparisons performed with published UK normal values.[17] Parents reported significantly lower QoL in total, psychosocial, and school domains, but there were no significant differences between self-reporting of patients and UK published norms (Table 1). Subgroup analysis revealed that patients and parents of patients not requiring immunoglobulin replacement therapy reported no significant difference in QoL from normal, compared with those who were receiving weekly subcutaneous immunoglobulin infusions at home.

A number of key novel findings arise from this study. Durability of T-lymphocyte levels is confirmed, but of interest is the difference in long-term thymic output between those that received conditioning and unconditioned recipients. The difference between groups did not quite reach statistical significance, but given the small sample size, the observation was striking and likely to be real. A biological explanation may be that in conditioned patients, the thymic niche is consistently seeded from bone marrow–derived donor stem cells leading to ongoing thymopoiesis, whereas for unconditioned recipients, initial seeding of the thymic niche at time of infusion is not generally followed by reseeding, as donor stem cell engraftment does not consistently occur in the bone marrow, and thymic seeding may have a finite lifetime, leading eventually to thymic exhaustion.[6]

The strong association of a preparative regimen with donor myeloid and B-lymphocyte chimerism and function is confirmed. A busulphan dose of 8 mg/kg, in combination with cyclophosphamide may not be myeloablative enough to reliably ensure donor stem cell engraftment with donor B lymphopoiesis, despite a historical view that it was adequate.[12] Higher doses of busulphan are associated with increased toxicity particularly in infants. The use of busulfan-based regimens has previously been reported in patients with primary immunodeficiency with few significant short-term toxicities.[9,13] It is encouraging to find that busulfan-containing low-toxicity myeloablative regimens confer improved donor chimerism. In these patients, the goal of any conditioning regimen should be to achieve >50% donor B-lymphocyte chimerism to reliably cease immunoglobulin replacement.

Finally, we assessed health-related QoL. A previous study found decreased QoL in transplanted SCID patients.[22] However, this was a heterogeneous cohort with different primary immunodeficiency disorders, some of which may intrinsically affect QoL. Although our initial results confirmed these findings, a subgroup analysis showed that patients who discontinued immunoglobulin substitution appeared to have normal health-related QoL compared with normal controls, whereas those who remained on immunoglobulin had significantly worse results.

In conclusion, we have demonstrated, in a single-center cohort of IL2RG/JAK3 SCID patients, that thymopoiesis is durable over time, but better in those who received conditioning. Low-toxicity myeloablative regimens achieve better donor stem cell engraftment, with few significant short-term toxicities, although long-term follow-up will be required to assess late effects. Freedom of immunoglobulin replacement leads to normal life quality, and is most associated with preparative chemotherapy. The debate about the use of chemotherapy vs infusion is likely to continue, and we should continue to strive for safer, nontoxic regimens.

Acknowledgments

The authors acknowledge permission for the use of PedsQL 3.1 A.H. acknowledges Universiti Sains Malaysia and Ministry of Higher Education of Malaysia for study funding in Newcastle University, United Kingdom.

Authorship

Contribution: J.I.A.H. designed the research project, collected the data and questionnaires, performed the statistical analysis and interpretation of the data, and wrote the manuscript; M.A.S., F.M., M.S.P., and A.R.G. contributed equally to the conceptualization of the research, statistical analysis, data interpretation, manuscript writing, and critical review at every level of the research stages.

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

ORCID profiles: J.I.A.H., 0000-0001-6349-1498.

Correspondence: Intan Juliana Abd Hamid, Pediatric Immunodeficiency Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom, e-mail: i.j.abd-hamid@newcastle.ac.uk or intanjh@usm.my.

References

1. Kwan A, Abraham RS, Currier R, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. [published correction appears in JAMA. 2014; 312(20):2163]. JAMA. 2014;312(7):729-738.
2. Fischer N, Siegrist CA, Avery DT, et al. IL-21 is the primary common γ chain-binding cytokine required for human B-cell differentiation in vivo. Blood. 2011;118(25):6624-6635.
3. Wada H, Thrasher A, Veys P, Kinnon C, Gaspar HB. Intrinsic defects of B cell function in X-linked severe combined immunodeficiency. Eur J Immunol. 2000;30(9):2708-2717.
4. Fischer A, Griscelli C, Friedrich W, et al. Bone-marrow transplantation for immunodeficiencies and osteopetrosis: European survey, 1968-1985. Lancet. 1986;2(8512):1080-1084.
5. Antoine C, Müller S, Cant A, et al; European Group for Blood and Marrow Transplantation; European Society for Immunodeficiency. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet. 2003; 361(9357):553-560.
6. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: long-term outcomes. Immunol Res. 2011;49(1-3):25-49.
7. Mazzolari E, Forino C, Razzari C, et al. Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. Blood. 2009;113(17):4114-4124.
8. Pai S-Y, Logan BR, Griffith LM, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. N Engl J Med. 2014;371(5):434-446.
9. Gennery AR, Slatter MA, Grandin L, et al; Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation; European Society for Immunodeficiency. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? J Allergy Clin Immunol. 2010;126(3):602-610.e1-11.
10. Bertrand Y, Landais P, Friedrich W, et al. Influence of severe combined immunodeficiency phenotype on the outcome of HLA non-identical, T-cell-depleted bone marrow transplantation: a retrospective European survey from the European group for bone marrow transplantation and the european society for immunodeficiency. J Pediatr. 1999;134(6):740-748.
11. Haddad E, Leroy S, Buckley RH. B-cell reconstitution for SCIDs: should a conditioning regimen be used in SCID treatment? J Allergy Clin Immunol. 2006;118(6):1388-1393.
12. Buckley RH, Win CM, Moser BK, Parrott RE, Sajaroff E, Sarzotti-Kelsoe M. Post-transplantation B cell function in different molecular types of SCID. J Clin Immunol. 2013; 33(1):26-110.
13. Borghans JA, Bindra RG, Hissenberg ND, et al. Early determinants of long-term T-cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency. Blood. 2000;100(5):1758-1763.
14. Cavazzana-Calvo M, Carlier F, Le Deist F, et al. Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. Blood. 2000;95(10): 4585-4591.
15. Slatter MA, Brigham K, Dokal I, Abinun M, et al. Long-term immune reconstitution after anti-CD52-treated or anti-CD34-treated hematopoietic stem cell transplantation for severe T-lymphocyte immunodeficiency. J Allergy Clin Immunol. 2008; 121(2):361-367.
16. Varni JW, Seid M, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Med Care. 2001; 39(8):800-812.
17. Upton P, Eiser C, Cheung I, et al. Measurement properties of the UK-English version of the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales. Health Qual Life Outcomes. 2005;3(1):22.
18. Thrasher AJ, Hacein-Bey-Abina S, Gaspar HB, et al. Failure of SCID-X1 gene therapy in older patients. Blood. 2005;105(11):4255-4257.
19. Slatter MA, Rao K, Amrolia P, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. Blood. 2011;117(16):4367-4375.
20. Slatter MA, Boztug H, Potschger U, et al; EBMT Inborn Errors and Paediatric Diseases Working Parties. Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases. Bone Marrow Transplant. 2015;50(12): 1556-1561.
21. Morillo-Gutierrez B, Slatter M, Rao K, et al. Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience. Blood. 2016; 128(26):3140-3148.
22. Tiroan P, Pink E, Slusak E, et al. Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies. Blood. 2008; 112(3):2987-2913

13.4.2 IL7Rα SCID paper

Engelhardt, K.R., Yaobo Xu, Angela Grainger, Mila G. C. Germani Batacchi, David J. Swan, Joseph D. P. Willet, Intan J. Abd Hamid, Philipp Agyeman, Dawn Barge, Shahnaz Bibi, Lucy Jenkins, Terence J. Flood, Mario Abinun, Mary A. Slatter, Andrew R. Gennery, Andrew J. Cant, Mauro Santibanez Koref, Kimberly Gilmour, Sophie Hambleton, *Identification of Heterozygous Single- and Multi-exon Deletions in IL7R by Whole Exome Sequencing.* J Clin Immunol, 2017. **37**(1): p. 42-50.

J Clin Immunol (2017) 37:42–50
DOI 10.1007/s10875-016-0343-9

ORIGINAL ARTICLE

Identification of Heterozygous Single- and Multi-exon Deletions in *IL7R* by Whole Exome Sequencing

Karin R. Engelhardt[1] · Yaobo Xu[2] · Angela Grainger[1] · Mila G. C. Germani Batacchi[1] · David J. Swan[1] · Joseph D. P. Willet[1] · Intan J. Abd Hamid[1,3] · Philipp Agyeman[3] · Dawn Barge[3] · Shahnaz Bibi[4] · Lucy Jenkins[4] · Terence J. Flood[3] · Mario Abinun[1,3] · Mary A. Slatter[1,3] · Andrew R. Gennery[1,3] · Andrew J. Cant[1,3] · Mauro Santibanez Koref[2] · Kimberly Gilmour[5] · Sophie Hambleton[1,3]

Received: 16 December 2015 / Accepted: 10 October 2016 / Published online: 2 November 2016

Abstract
Purpose We aimed to achieve a retrospective molecular diagnosis by applying state-of-the-art genomic sequencing methods to past patients with T-B+NK+ severe combined immunodeficiency (SCID). We included identification of copy number variations (CNVs) by whole exome sequencing (WES) using the CNV calling method ExomeDepth to detect gene alterations for which routine Sanger sequencing analysis is not suitable, such as large heterozygous deletions.
Methods Of a total of 12 undiagnosed patients with T-B+NK+ SCID, we analyzed eight probands by WES, using GATK to detect single nucleotide variants (SNVs) and small insertions and deletions (INDELs) and ExomeDepth to detect CNVs.
Results We found heterozygous single- or multi-exon deletions in *IL7R*, a known disease gene for autosomal recessive T-B+NK+ SCID, in four families (seven patients). In three families (five patients), these deletions coexisted with a heterozygous splice site or nonsense mutation elsewhere in the same gene, consistent with compound heterozygosity. In our cohort, about a quarter of T-B+NK+ SCID patients (26%) had such compound heterozygous *IL7R* deletions.
Conclusions We show that heterozygous *IL7R* exon deletions are common in T-B+NK+ SCID and are detectable by WES. They should be considered if Sanger sequencing fails to detect homozygous or compound heterozygous *IL7R* SNVs or INDELs.

Keywords IL7R · copy number variation · compound heterozygous · SCID · whole exome sequencing

Introduction

Whole exome sequencing (WES) is a powerful tool for discovering pathogenic variants in rare genetic diseases, detecting both single nucleotide variants (SNVs) and small insertions/deletions in known disease genes with relative efficiency. WES has not been seen as offering equivalent advantages for the detection of copy number variations (CNVs), which instead may be sought by chromosomal microarrays or multiplex ligation-dependent probe amplification (MLPA). However, recent computational advances have also made it possible to identify CNVs by WES [1, 2]. This is of great advantage as limitations of array-based CNV detection methods, such as noisy signal and low resolution, make detection of small CNVs difficult, and large heterozygous deletions can only be detected by conventional PCR-based sequencing methods using genomic DNA if the breakpoints of the deletion are known. Therefore, routine Sanger sequencing analysis is not suitable for the detection of such gene alterations.

Electronic supplementary material The online version of this article (doi:10.1007/s10875-016-0343-9) contains supplementary material, which is available to authorized users.

✉ Karin R. Engelhardt
karin.engelhardt@newcastle.ac.uk

[1] Primary Immunodeficiency Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
[2] Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
[3] Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK
[4] NE Thames Regional Genetics Service, Great Ormond Street Hospital NHS Foundation Trust, London, UK
[5] Immunology, Great Ormond Street Hospital NHS Foundation Trust, London, UK

In our center, we have treated a cohort of 19 patients with T-B+NK+ severe combined immunodeficiency (SCID), characterized by early symptoms of immunodeficiency (such as failure to thrive and recurrent infections), and/or a positive family history of T-B+NK+ SCID, together with profound T lymphocytopenia, low serum immunoglobulin levels and impaired lymphocyte proliferation to mitogens. Autosomal recessive (AR) mutations in several genes have been reported to cause T-B+NK+ SCID. Amongst these are *CD3E*, *CD3D* and *CD3Z*, all of which encode subunits of the CD3/T cell receptor complex [3, 4], as well as *IL7R* [5], which encodes IL7Rα, commonly known as IL7R, the unique alpha chain of the heterodimeric receptor for interleukin-7 (IL-7). IL-7 is necessary for T lymphocyte development in the thymus and for proliferation and survival of T lymphocytes in the periphery [6, 7]. Coronin-1A deficiency due to AR mutations in *CORO1A* can cause T-B+NK+ SCID through impaired actin cytoskeleton regulation [8–10]. Coronin-1A is an actin-binding protein required for lymphocyte migration and thymic egress. The human Nude/T-B+NK+ SCID phenotype is caused by mutations in the gene *FOXN1*, which encodes a transcription factor crucial for thymus development [11, 12]. An autosomal dominant T cell differentiation defect due to congenital absence of the thymus can be found in 'complete' chromosome 22q11.2 deletion syndrome (DiGeorge syndrome), in which the immunodeficiency is usually part of a clinical triad also including congenital cardiac and parathyroid gland defects [13].

IL7R deficiency had been diagnosed in seven patients of our cohort of non-syndromic T-B+NK+ SCID by conventional methods (Fig. 1). The remaining 12 patients from eight families were screened by WES to identify disease-causing mutations, resulting in the molecular diagnosis of ten patients.

Here, we show successful detection by WES of heterozygous single- or multi-exon deletions in the gene *IL7R*, invisible by conventional sequencing techniques, in patients with T-B+NK+ SCID.

Methods

Study Subjects

Patients and their relatives provided written informed consent to participate in research protocols approved by the Newcastle and North Tyneside 1 Research Ethics Committee. Whole blood samples, buccal samples or dermal fibroblast cultures were obtained from these individuals, and genomic DNA was isolated using the DNeasy or QIAamp DNA mini kit (Qiagen).

PCR and Sequencing Analysis

Specific primers were designed in Primer3web version 4.0.0 (http://bioinfo.ut.ee/primer3/). Primer sequences are available on request. Capillary sequencing was performed according to standard methods. Sequences were aligned with the consensus coding sequence (human genome assembly 37 in nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/blast/). ChromasLite Version 2.1.1 was used for visualization of the sequences.

WES and CNV Analysis

Whole exomes of the patients were enriched with the Agilent SureSelect Human All Exon V5 kit (Santa Clara, CA, USA) and subsequently sequenced using the Illumina HiSeq 2500 sequencing system (San Diego, CA, USA) by AROS (Applied Biotechnology AS, Denmark). Sequencing reads were analyzed using the following workflow to identify variants in patients. Firstly, the quality of sequencing reads was checked with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Duplicated reads were removed with FastUniq [14]. The remaining reads were mapped to the human reference genome GRCh37 with BWA [15]. The alignments were refined with tools of the GATK suite [16]. Variants were called according to GATK Best Practice recommendations [17, 18], including recalibration, as well as using Freebayes [19]. The variants called by Freebayes with total coverage ≥5, minor allele coverage ≥5 and variant call quality ≥20 were added to those identified by GATK. Non-synonymous exonic variants were subsequently filtered by quality and minor-allele frequency (MAF) reported in the 1000 Genomes project (2012 Feb release) [20] and ESP6500 [21]. Variants with MAF >0.05 were excluded. Annovar was used for annotations and prediction of functional consequences [22]. Copy number variants (CNVs) were called using ExomeDepth from the GATK refined alignments [1].

Lymphocyte Proliferation

Lymphocyte proliferation in vitro to mitogen phytohaemagglutinin (PHA) was assayed by tritiated thymidine incorporation in an accredited clinical laboratory using standard techniques.

IL-7Rα Expression

Five microliters of anti-human CD127 PE (IL7Rα) (BD Biosciences) or matched isotype control (BD Biosciences) was added to 100 μl of blood or 10^6 PBMCs, incubated for 10 min at room temperature. Cells were lysed with FACsLyse (BD Biosciences) for 10 min, and the remaining cells were washed with Cell Wash (BD Biosciences) before being fixed (FACsFix, BD Bioscience). Ten thousand lymphocytes were

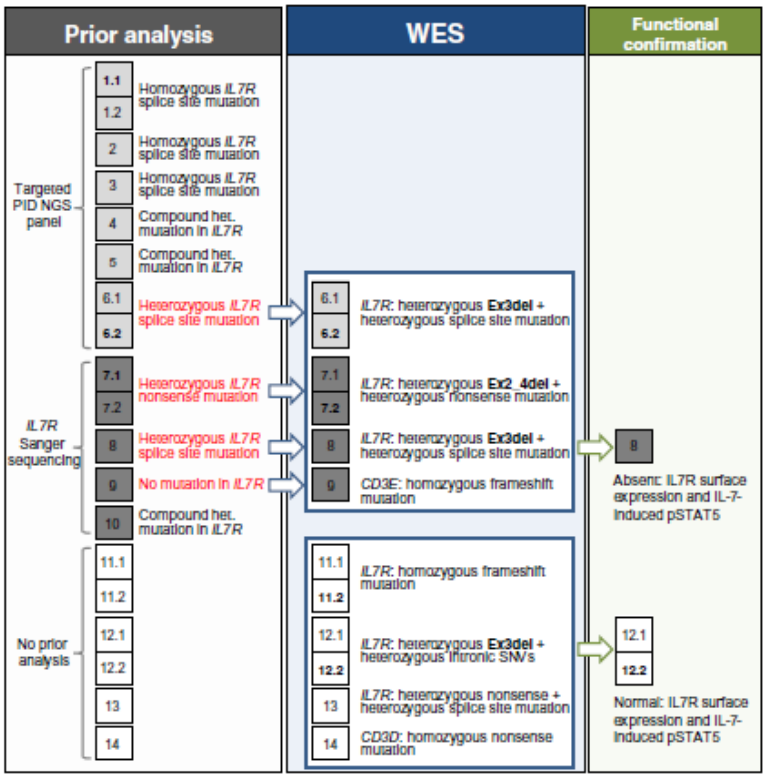


Fig. 1 Workflow and findings of patient analysis. The *first column* shows molecular analysis before whole exome sequencing (WES) for 19 patients from 14 families. *Rectangles* contain either single patients (x) or siblings (x.1, x.2). If in a family only one sibling was analyzed, his or her number is shown in *bold*. Patients who had either not been previously analyzed or for whom no causative mutation had been found (findings shown in *red*) were subjected to WES (*second column*). For two patients, cryopreserved PBMCs were available for functional testing (*third column*). *Het.* heterozygous; *Ex3del* deletion of exon 3; *Ex2_4del* deletion of exons 2–4

acquired (FACsCalibur, BD Biosciences) and analyzed (Cell Quest Pro, BD Biosciences).

STAT5 Phosphorylation Assay

1×10^4 IU/ml IL-2 (Chiron), 50 ng/ml IL-15 (R&D systems) or 10 ng/ml IL-7 (R&D Systems) were added to 100 ml of whole blood or 10^6 PBMCs and placed at 37°C for 10 min to stimulate cells. Two milliliters of prewarmed FACs Lyse/Fix (BD Biosciences) were then added to the cells, mixed and placed at 37°C for 10 min. The cells were pelleted and washed once with STAT wash (phosphate-buffered saline containing 1 % fetal calf serum). The cells were resuspended in cold Perm Buffer III (BD Biosciences) and placed at 4°C for 30 min. The cells were then washed once with STAT wash before 5 ml of antibodies (STAT5 ptyr, and CD4 PerCP (BD Biosciences) were added, and the cells were incubated at room temperature for 30 min in the dark, washed with STAT wash and fixed (FACsFix, BD Biosciences). Ten thousand lymphocyte events were acquired (FACsCalibur, BD Biosciences) and analyzed using CellQuest software (BD Biosciences).

Results

Case Summaries

Patient 6.1 presented at age 3 months with diarrhea and failure to thrive (Table 1). Diagnosis was made at the age of 10 months, when he additionally presented with superficial candidiasis and lymphocytopenia with absent T lymphocytes. Patient 6.2 is the sister of patient 6.1 and was diagnosed shortly after birth because of family history, low lymphocyte numbers and weak proliferative responses to mitogen. Patient 7.1 presented with recurrent febrile episodes from the age of 1 month. At 7 months, persistent rotavirus gastroenteritis and weight loss were documented. At 8 months, he was admitted to hospital with pneumocystis jirovecii pneumonia and the diagnosis of SCID was made. Patient 7.2 is the brother of patient 7.1 and was diagnosed shortly after birth because of family history, absent T cells and low lymphocyte proliferation. Patient 8 presented at the age of 5 months with progressive cough, vomiting, poor appetite and then failure to thrive. Diagnosis was made at age of 8 months, when she was admitted to the hospital with pneumocystis jirovecii pneumonia and

Table 1 Patients' clinical and laboratory characteristics

	Family 6		Family 7		Family 8	Family 12	
Patient	6.1	6.2	7.1	7.2	8	12.1	12.2
Gender	M	F	M	M	F	M	M
Consanguinity	No	No	No	No	No	No	No
Age of first presentation (months)	3	NA	NA	6–7	8	3	NA
Age at diagnosis (months)	10	0	0	8	8	8	0
Diagnostic trigger	Failure to thrive	Family history	Family history	Recurrent infections	Failure to thrive, respiratory infection, lymphopenia	Recurrent infections, failure to thrive	Family history
Infections pre diagnosis	Candida, viral gastroenteritis	None	None	RSV bronchio-litis, persistent rotavirus, candida, PJP	RSV, PJP	Recurrent URTI, para-influenza type 1, persistent candida	None
Other problems	Persistent diarrhea	None	None	Failure to thrive, encephalopathy	None	Possible encephalitis	None
Age at BMT (months)	12	1	2	NA	11	8 and 12	2
Status	Alive	Alive	Alive	Died pre transplant	Alive	Died 115 days post second transplant	Alive
Serum immunoglobulins at diagnosis (g/l) (normal ranges according to the Harriet Lane Handbook 19th edition)							
IgG	8.1 (2.9–10.7)	10.4 (6.4–16.1)	8.7 (6.4–16.1)	3 (2.2–9.0)	<0.4 (2.2–9.0)	<0.33 (2.2–9.0)	10.5 (6.4–16.1)
IgA	0.23 (0.16–0.84)	<0.01 (0.01–0.04)	<0.3 (0.01–0.04)	0.12 (0.11–0.90)	<0.3 (0.11–0.90)	<0.07 (0.11–0.90)	<0.07 (0.01–0.04)
IgM	1.71 (0.41–1.49)	0.18 (0.06–0.25)	<0.22 (0.06–0.25)	1.15 (0.34–1.26)	<0.19 (0.34–1.26)	<0.2 (0.34–1.26)	0.17 (0.06–0.25)
Lymphocyte subpopulations (cells/mm3) (normal ranges according to Comans-Bitter, J Pediatr 1997)							
CD3	185 (1600–6700)	0 (600–5000)	0 (600–5000)	0 (2400–6900)	0 (2400–6900)	10 (2400–6900)	0 (600–5000)
CD4	42 (1000–4600)	0 (400–3500)	0 (400–3500)	0 (1400–5100)	0 (1400–5100)	0 (1400–5100)	0 (400–3500)
CD8	144 (400–2100)	0 (200–1900)	0 (200–1900)	0 (600–2200)	0 (600–2200)	0 (600–2200)	0 (200–1900)
CD19	227 (600–2700)	24 (40–1100)	39 (40–1100)	1395 (700–2500)	362 (700–2500)	250 (700–2500)	627 (40–1100)
NK	52 (200–1200)	23 (100–1900)	832 (100–1900)	71 (100–1000)	161 (100–1000)	ND	697 (100–1900)
Lymphocyte proliferation (cpm) [SI]							
Patient PHA (control)	No response (ND)	217 [−] (49370 [117x])	ND	2336 [1x] (95381 [521x])	ND	138 [0.9x] (32003 [89x])	511 [0.3x] (95069 [211x])
ConA	97 (1824)	ND	ND	ND	ND	ND	ND
PWM	131 (4337)	ND	ND	ND	ND	ND	ND
Genetic analysis							
Prior to WES	No	Targeted PID panel	*IL7R* Sanger sequencing	No	*IL7R* Sanger sequencing	No	No
Results	NA	Het. *IL7R* c.221+2T>G	Het. IL-7R p.Q26X	NA	Het. *IL7R* c.221+2T>G	NA	NA
WES	No	Yes	No	Yes	Yes	No	Yes
Results	NA	Het. Ex3del + het. c.221+2T>G	NA	Het. Ex2_4del + het. p.Q26X	Het. Ex3del + het. c.221+2T>G	NA	Het. Ex3del + intronic SNVs

ConA concanavalin A, *Het.* heterozygous, *NA* not applicable, *ND* not determined, *PHA* phytohemagglutinin, *PJP* pneumocystis jiroveci pneumonia, *PWM* poke weed mitogen, *RSV* respiratory syncytial virus, *SI* stimulation index (cpm of stimulated/cpm of unstimulated cells), *URTI* upper respiratory tract infection

respiratory syncytial virus pneumonitis. Patient 12.1 presented at the age of 3 months with recurrent chest infections, oral candidiasis and failure to thrive. He was diagnosed as having SCID at the age of 8 months. Patient 12.2 is the brother of patient 12.1 and was diagnosed shortly after birth because of family history, absent T cells and low lymphocyte proliferation.

Whole Exome Sequencing Results

In two patients, we found homozygous loss-of-function mutations in CD3 chains. In patient 9, the mutation was in *CD3E* (c.424delG; p.G142fsX162), and in patient 14 in *CD3D* (c.202C>T; p.R68X). Two siblings had a homozygous frameshift deletion in *IL7R* (c.493delC; p.H165fsX167; family 11) (data not shown). These homozygous variants are predicted to be disease-causing in each case. Patient 13 had a compound heterozygous mutation in *IL7R* (heterozygous c.221+2T>G and heterozygous c.76C>T, p.Q26X) (data not shown). Several splice site prediction programs predict disruption of the exon 2 splice donor site due to the c.221+2T>G mutation (Table S1). Furthermore, a similar mutation (heterozygous c.221+2T>A) together with a heterozygous missense mutation in *IL7R* was found by Lee et al. in a patient with T-B+ SCID [23]. Thus, compound heterozygosity for these variants could be considered causative.

WES sequencing of patients 6.2, 7.2, 8 and 12.2 from the remaining four kindreds revealed a previously undetected heterozygous deletion of one or three exons of *IL7R*, coexisting with heterozygous SNVs elsewhere in the same gene (Fig. 2). Patients 6.2 and 8 had a heterozygous deletion of exon 3 (Ex3del) together with a heterozygous exon 2 splice donor site mutation (c.221+2T>G). Patient 7.2 had a heterozygous deletion of exons 2-4 (Ex2_4del) together with a nonsense mutation in exon 1 (c.76C>A; p.Q26X). Patient 12.2 had a heterozygous exon 3 deletion (Ex3del) together with heterozygous SNVs at positions +6, +12 and +15 in the exon 7 splice donor site (c.876+6T>G; c.876+12T>G; c.876+15T>G). The breakpoints in patients 6.2 and 7.2 were determined by Sanger sequencing (Figure S1), confirming the WES finding of exon 3 or exon 2-4 hemizygosity, respectively. Unfortunately, we were unable to define the exon 3 deletion breakpoints in patients 8 and 12.2, which suggests that they are different from that of family 6. Each of these exonic deletions implies a frameshift with a premature stop codon: if mRNA were to escape nonsense-mediated decay, any protein product would be severely truncated (84–95 %) with only a small part of the extracellular domain left. Hence, all are predicted to be loss of function alleles.

In patients 6.2, 7.2 and 8, the heterozygous exonic deletion we identified by WES accompanied a heterozygous SNV in *IL7R* already identified by conventional diagnostic means and predicted to be deleterious. We could not confirm compound heterozygosity, as parental DNA was not available, but the apposite phenotypes of the patients imply that the predicted pathogenic *IL7R* mutations found here are biallelic. In the case of patient 8, cryopreserved PBMCs were available for functional testing that confirmed absence of IL7R expression and failure of STAT5 phosphorylation in response to IL-7 (Fig. 3c). This confirms the pathogenic nature of each allele, i.e. both the heterozygous exon 3 deletion and the heterozygous exon 2 splice donor site mutation c.221+2T>G produce loss of function (Fig. 3b).

Of the three SNVs in the exon 7 splice donor site of patient 12.2, c.876+6T>G is predicted to have the strongest effect, with Human Splicing Finder suggesting a broken donor site, and BDGP detecting the splice donor site with a score of 0.46, which is just above the threshold level of 0.4. The confidence of NetGene2 that a splice donor site is present drops from 0.81 for the wild-type sequence to 0.62 for the region with all three SNVs. However, the patient expressed IL7R at similar levels to a healthy control (67 % patient vs. 78 % control) (Fig. 3b) and sustained normal IL-7-induced STAT5 phosphorylation (39 % in both patient and control) (Fig. 3c). Thus, either the SNVs do not affect splicing or they are on the same allele as the exon 3 deletion. Our molecular findings for kindred 12 do not therefore explain the patients' phenotype and emphasize the importance of confirmatory functional testing for variants of unknown significance.

Interestingly, in all patients that had a heterozygous *IL7R* mutation detected by prior analysis, we found a complementary heterozygous *IL7R* exon(s) deletion (Fig. 1), emphasizing the importance of looking for CNVs in *IL7R* in such cases. In total, about a quarter of our patients (5/19, 26 %) had such compound heterozygous *IL7R* deletions, with an additional patient (12.2) being a carrier. In our cohort, the exon 2 splice donor site mutation (c.221+2T>G) was most frequent, being present in six alleles, followed by p.Q26X and Ex3del, which were present in three alleles each.

Discussion

In recent years, the discovery of PID-causing genes has accelerated markedly due to advanced sequencing technologies. Achieving a molecular diagnosis for individual patients with PID can, however, still prove difficult even if the disease is well defined and disease-causing genes are known. In our center, one such disease was T-B+NK+ SCID. New patients were directly analyzed by WES. Some patients were screened by a targeted PID NGS panel that had not yet incorporated the detection of CNVs. Older patients, for whom these technologies were not available at the time of presentation, were only analyzed by Sanger sequencing. Of these, five patients did not obtain a molecular diagnosis because only a single heterozygous nonsense or splice site mutation in *IL7R* was found. Only

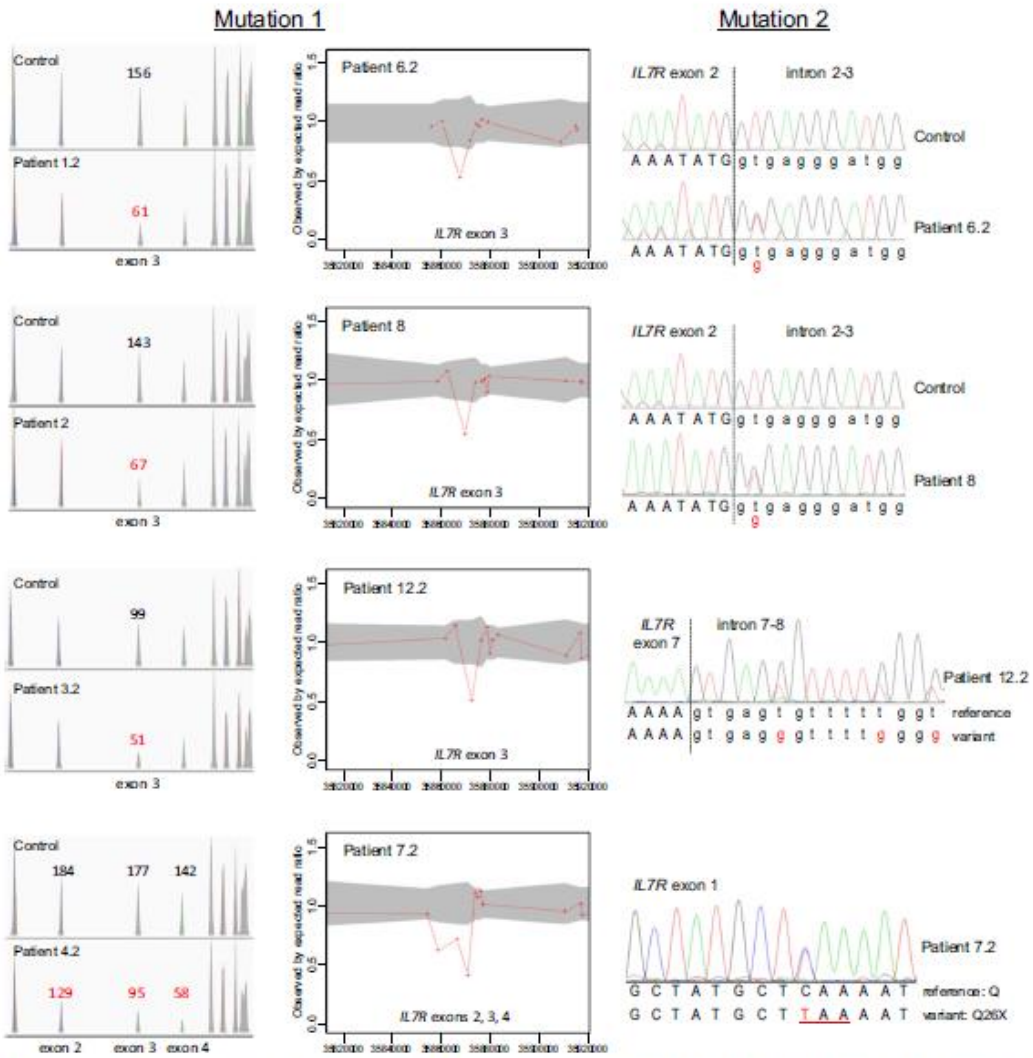


Fig. 2 Compound heterozygous *IL7R* mutations. Mutation 1—hemizygosity of exon 3 or exons 2-4 demonstrated by ExomeDepth. Shown for each exon are peaks representing read depth (*left*) and the observed to expected ratio of reads (*right*). *Gray area* expected value range. Affected exons have fewer reads than those of other samples of the same batch. Mutation 2—Sanger sequencing of heterozygous SNVs (indicated in *red*)

when we applied WES with CNV detection by ExomeDepth were we able to identify the cause of the disease in these five patients, through detection of a heterozygous single- or multi-exon deletion in *IL7R* as the second hit. Recently, Bayer et al. applied WES together with custom-designed chromosomal microarray to detect compound heterozygous mutations in *IL7R*, which also included a heterozygous deletion of exon 3 [24]. We found three kindreds (at least two alleles) with a deletion of exon 3, and one with a deletion of exons 2-4, suggesting a deletion hotspot involving exon 3.

At both deletion breakpoints that we mapped, we detected a microhomology of 2 and 3 bp, respectively (Figure S1 and Table S2). Short stretches of 2–30 bp of microhomology at the breakpoint sites are common in CNVs, with 70–80 % of

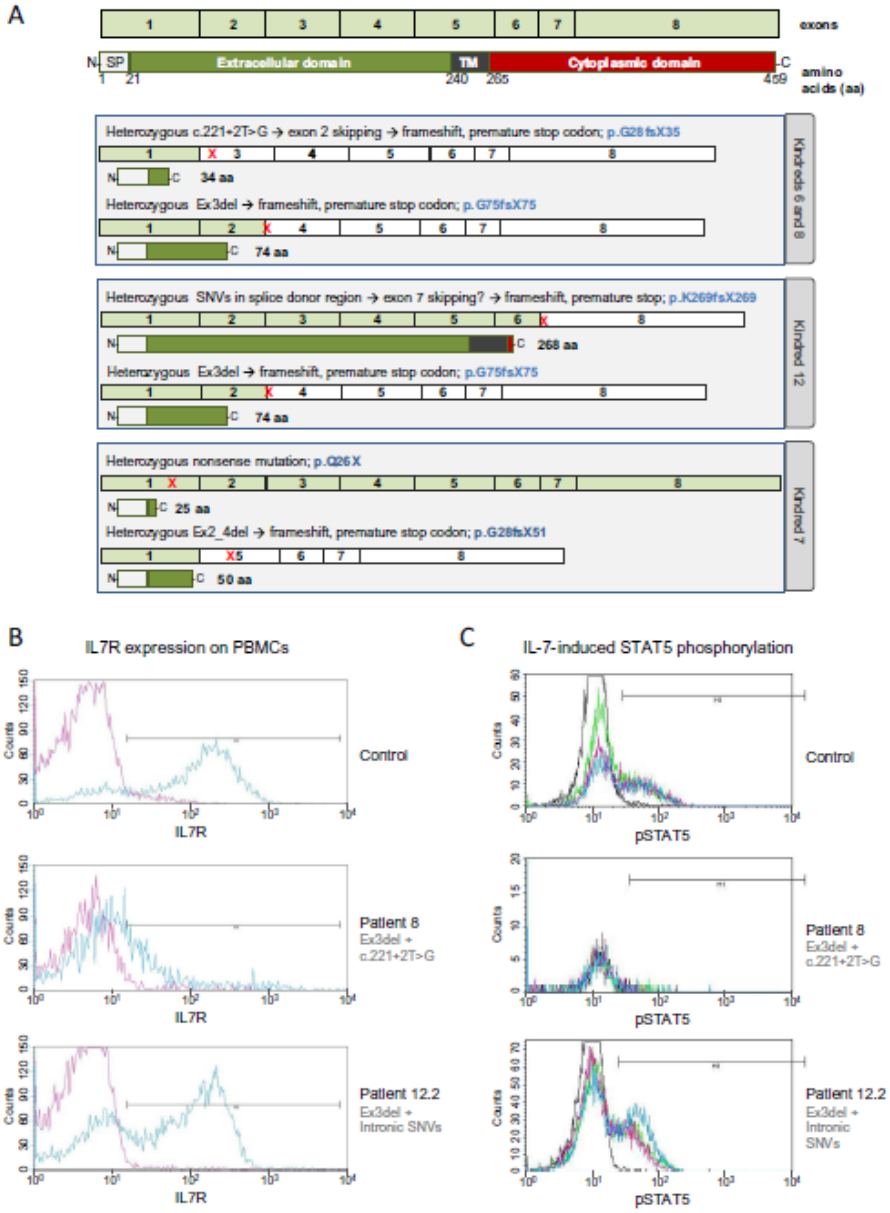


Fig. 3 The impact of the mutations on IL7R expression and IL-7 signaling. **a** Schematic showing expected effect of the mutations on protein expression. If, as the phenotype suggests, the mutations are in a compound heterozygous setting, no patient would express full-length IL7R. **b** IL7R expression was measured by flow cytometry on PBMCs from a healthy control, patient 8 and patient 12.2. **c** STAT5 phosphorylation after stimulation with IL-7 (*red*), IL-2 (*green*) and IL-15 (*blue*) for 10 min was assessed by intracellular staining using whole blood or PBMCs from a healthy control, patient 8 and patient 12.2

human deletion CNVs showing microhomology [25, 26]. Interestingly, a third of microhomology stretches found in rare pathogenic microdeletions were 2 or 3 bp long [26]. Formation of microhomology-associated microdeletions is thought to be mediated by replication-based mechanisms such as non-homologous end-joining (NHEJ), microhomology-mediated end-joining (MMEJ) and fork stalling and template switching (FoSTeS) [26]. In addition to the microhomology, 83 % of deletion CNVs have at least one breakpoint within a known repetitive element [26]. The analysis of the four breakpoint regions in our patients showed this to be true for our deletion breakpoints too (Table S2). Breakpoint region 1 of the exon 3 deletion is within a long interspersed nuclear element/L1 repeat, whereas breakpoint region 2 of the exon 2-4 deletion is within a small interspersed nuclear element/Alu repeat. The local genomic architecture in parts of the *IL7R* gene may thus render it particularly susceptible to deletion CNVs.

We found that single- or multi-exon deletions in *IL7R* are relatively frequent disease alleles in autosomal recessive T-B+ NK+ SCID and can be detected by CNV analysis. Without the application of methods specifically to scrutinize CNVs, these disease alleles would be missed in the heterozygous state. Our cohort suggests that CNV analysis would be required to reach a molecular diagnosis in about a quarter of patients. Especially for centers that already use WES or targeted gene panels, we suggest they extend their analysis to include CNV detection. Various techniques are already established so can be applied without much difficulty. Coverage of *IL7R* by available whole exome platforms is sufficient to call such variants using either ExomeDepth or one of the other CNV calling methods summarized in Tan et al. [2]. The same applies for targeted gene panels that include *IL7R*; at Great Ormond Street Hospital, we now use ExomeDepth for CNV detection in samples analyzed with the targeted primary immunodeficiency gene panel. Alternatively, MLPA analysis could be employed, as is routinely done in screening for Artemis deficiency [27].

Acknowledgments We thank clinical and laboratory colleagues and the patients and their families for their participation. This work was funded by the Sir Jules Thorn Charitable Trust (grant 12/JTA) and the Bubble Foundation.

Authorship Contributions KRE and SH designed the study and wrote the paper; KRE, AG and MGCGB performed PCR and sequencing analysis; YX and MSK did bioinformatic filtering of the WES data; KRE, DJS, JDPW and SH analyzed the variants; IJAH and PA provided clinical information; DB performed lymphocyte proliferation assays; KG, SB and LJ performed conventional genetic analysis, IL7R expression and IL-7-induced STAT5 phosphorylation assays; and TJF, MA, MAS, ARG, AJC and SH diagnosed and cared for the patients.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics. 2012;28(21):2747-54. Pubmed Central PMCID: 3476336.
2. Tan R, Wang Y, Kleinstein SE, Liu Y, Zhu X, Guo H, et al. An evaluation of copy number variation detection tools from whole-exome sequencing data. Hum Mutat. 2014;35(7):899–907.
3. de Saint Basile G, Geissmann F, Flori E, Uring-Lambert B, Soudais C, Cavazzana-Calvo M, et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. The Journal of clinical investigation. 2004;114(10):1512-7. Pubmed Central PMCID: 525745.
4. Roberts JL, Lauritsen JP, Cooney M, Parrott RE, Sajaroff EO, Win CM, et al. T-B+NK+ severe combined immunodeficiency caused by complete deficiency of the CD3zeta subunit of the T-cell antigen receptor complex. Blood. 2007;109(8):3198-206. Pubmed Central PMCID: 1852234.
5. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat Genet. 1998;20(4):394–7.
6. Hong C, Luckey MA, Park JH. Intrathymic IL-7: the where, when, and why of IL-7 signaling during T cell development. Seminars in immunology. 2012;24(3):151-8. Pubmed Central PMCID: 3358706.
7. Huang HY, Luther SA. Expression and function of interleukin-7 in secondary and tertiary lymphoid organs. Semin Immunol. 2012;24(3):175–89.
8. Shiow LR, Paris K, Akana MC, Cyster JG, Sorensen RU, Puck JM. Severe combined immunodeficiency (SCID) and attention deficit hyperactivity disorder (ADHD) associated with a Coronin-1A mutation and a chromosome 16p11.2 deletion. Clinical immunology. 2009;131(1):24-30. Pubmed Central PMCID: 2692687.
9. Shiow LR, Roadcap DW, Paris K, Watson SR, Grigorova IL, Lebet T, et al. The actin regulator coronin 1A is mutant in a thymic egress-deficient mouse strain and in a patient with severe combined immunodeficiency. Nature immunology. 2008;9(11):1307-15. Pubmed Central PMCID: 2672406.
10. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies Coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. The Journal of allergy and clinical immunology. 2013;131(6):1594-603. Pubmed Central PMCID: 3824285.
11. Frank J, Pignata C, Panteleyev AA, Prowse DM, Baden H, Weiner L, et al. Exposing the human nude phenotype. Nature. 1999;398(6727):473–4.
12. Vigliano I, Gorrese M, Fusco A, Vitiello L, Amorosi S, Panico L, et al. FOXN1 mutation abrogates prenatal T-cell development in humans. J Med Genet. 2011;48(6):413–6.
13. McLean-Tooke A, Spickett GP, Gennery AR. Immunodeficiency and autoimmunity in 22q11.2 deletion syndrome. Scand J Immunol. 2007;66(1):1–7.
14. Xu H, Luo X, Qian J, Pang X, Song J, Qian G, et al. FastUniq: a fast de novo duplicates removal tool for paired short reads. PloS one. 2012;7(12):e52249. Pubmed Central PMCID: 3527383.

13.5 Appendix E: Oral Presentation

13.5.1 Clinical Immunology Society (CIS) Annual Meeting: Immune Deficiency & Dysregulation North American Conference, Boston, USA, April 2016

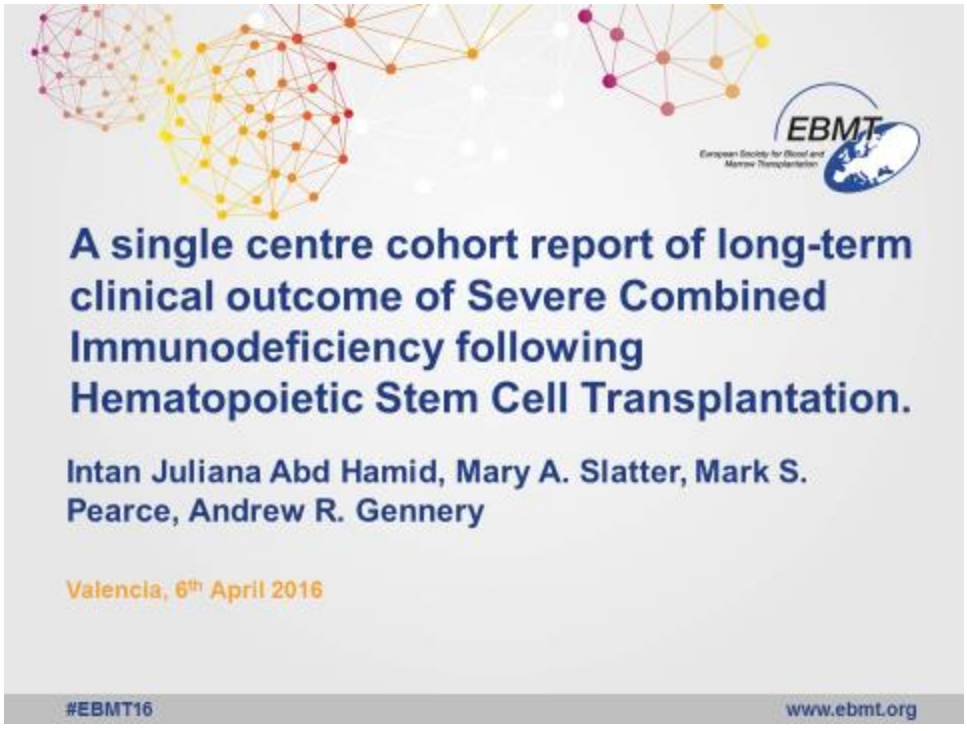
Title: A Single Centre Cohort Report of Long-term Clinical Outcome of Severe Combined Immunodeficiency Following hematopoietic stem cell transplantation.

Recording available at:

https://cis.confex.com/cis/2016/webprogram/Session1282.html

13.5.2 European Society for Blood and Bone Marrow Transplantation (EBMT) Annual Meeting, Valencia, April 2016

Title: A Single Centre Cohort Report of Long-term Clinical Outcome of Severe Combined Immunodeficiency Following hematopoietic stem cell transplantation.



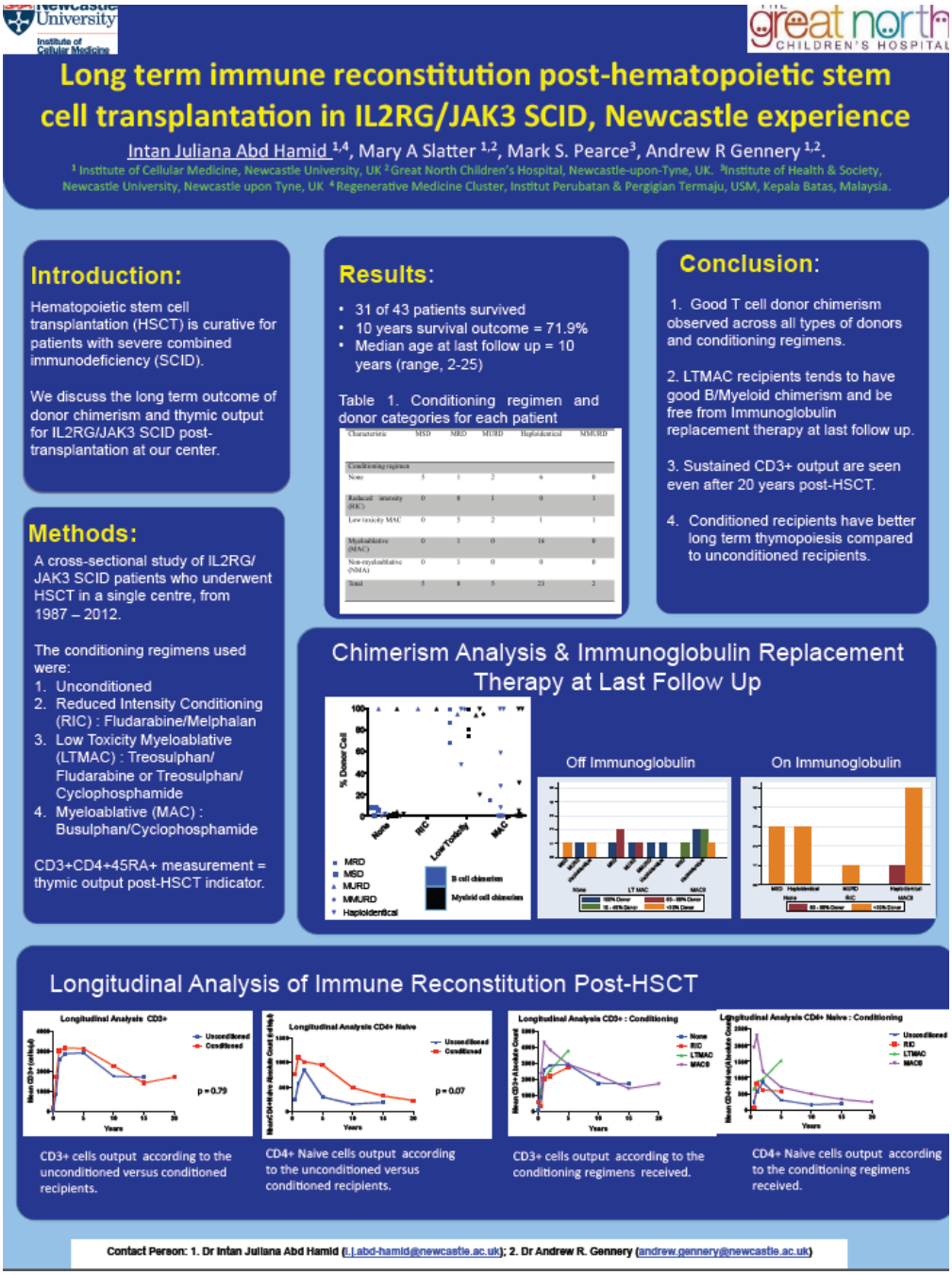
13.5.3 Late Effects after Pediatric HSCT: State of the Science, Future Directions. Pediatric Blood and Marrow Transplant Consortium. Minneapolis, MN, USA May 10 – 11, 2016.

Title: A Single Centre Cohort Report of Long-term Clinical Outcome of Severe Combined Immunodeficiency Following hematopoietic stem cell transplantation.

13.6 Appendix F: E-Poster Presentation

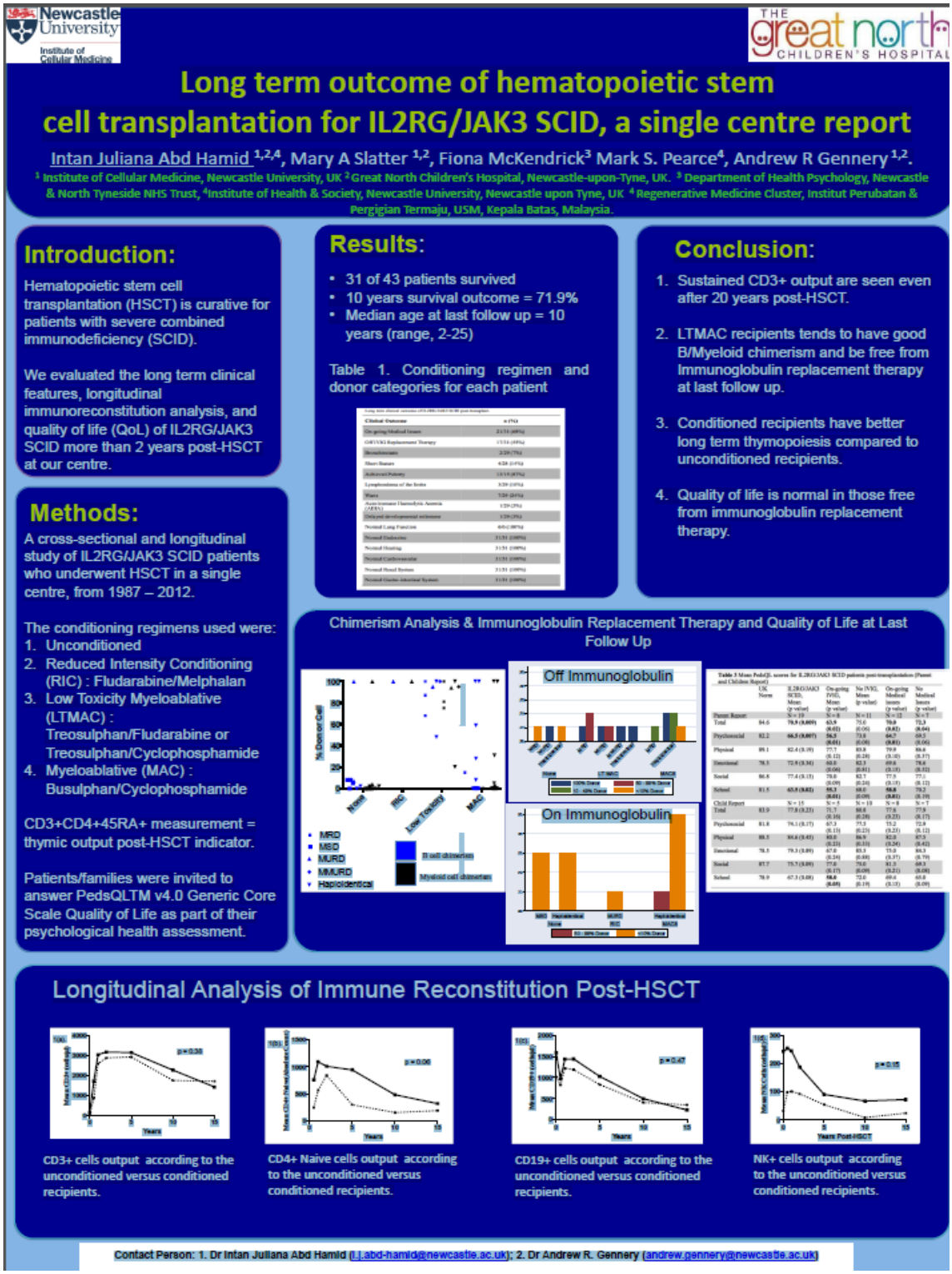
13.6.1 Clinical Immunology Society (CIS) Annual Meeting: Immune Deficiency & Dysregulation North American Conference, Boston, USA, April 2016

Title: Long-term immune reconstitution post-hematopoietic stem cell transplantation in IL2RG/JAK3 SCID, Newcastle experience



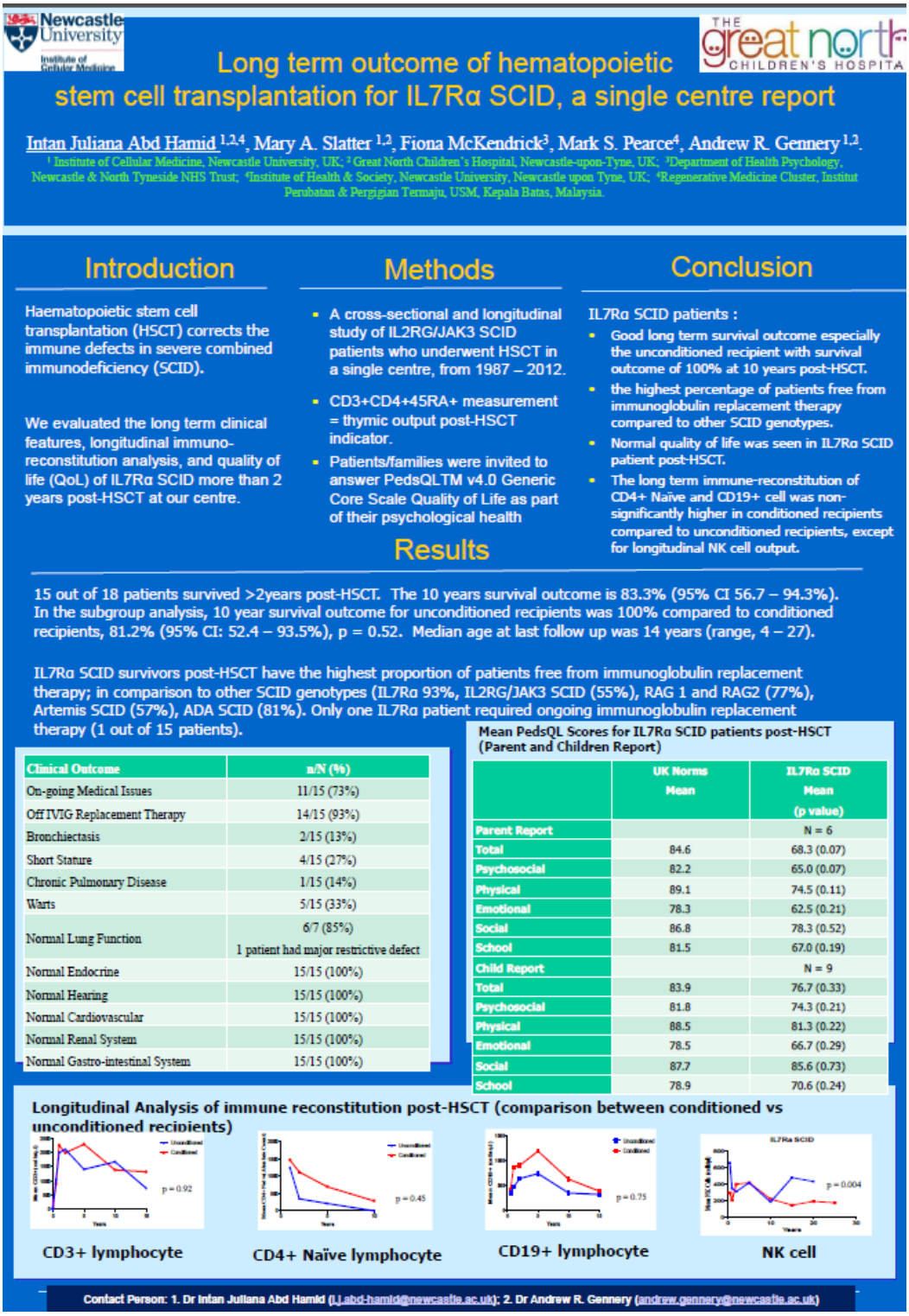
13.6.2 17th Biennial Meeting of the European Society for Immunodeficiencies, Barcelona, September 2016.

ESID6-0137



13.6.3 17th Biennial Meeting of the European Society for Immunodeficiencies, Barcelona, September 2016.

ESID6-0406



Newcastle University Institute of Cellular Medicine

THE great north CHILDREN'S HOSPITAL

Long term outcome of hematopoietic stem cell transplantation for IL7Rα SCID, a single centre report

Intan Juliana Abd Hamid [1,2,4], Mary A. Slatter [1,2], Fiona McKendrick[3], Mark S. Pearce[4], Andrew R. Gennery [1,2].

[1] Institute of Cellular Medicine, Newcastle University, UK; [2] Great North Children's Hospital, Newcastle-upon-Tyne, UK; [3]Department of Health Psychology, Newcastle & North Tyneside NHS Trust; [4]Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK; [4]Regenerative Medicine Cluster, Institut Perubatan & Pergigian Termaju, USM, Kepala Batas, Malaysia.

Introduction

Haematopoietic stem cell transplantation (HSCT) corrects the immune defects in severe combined immunodeficiency (SCID).

We evaluated the long term clinical features, longitudinal immuno-reconstitution analysis, and quality of life (QoL) of IL7Rα SCID more than 2 years post-HSCT at our centre.

Methods

- A cross-sectional and longitudinal study of IL2RG/JAK3 SCID patients who underwent HSCT in a single centre, from 1987 – 2012.
- CD3+CD4+45RA+ measurement = thymic output post-HSCT indicator.
- Patients/families were invited to answer PedsQLTM v4.0 Generic Core Scale Quality of Life as part of their psychological health

Conclusion

IL7Rα SCID patients :

- Good long term survival outcome especially the unconditioned recipient with survival outcome of 100% at 10 years post-HSCT.
- the highest percentage of patients free from immunoglobulin replacement therapy compared to other SCID genotypes.
- Normal quality of life was seen in IL7Rα SCID patient post-HSCT.
- The long term immune-reconstitution of CD4+ Naïve and CD19+ cell was non-significantly higher in conditioned recipients compared to unconditioned recipients, except for longitudinal NK cell output.

Results

15 out of 18 patients survived >2years post-HSCT. The 10 years survival outcome is 83.3% (95% CI 56.7 – 94.3%). In the subgroup analysis, 10 year survival outcome for unconditioned recipients was 100% compared to conditioned recipients, 81.2% (95% CI: 52.4 – 93.5%), p = 0.52. Median age at last follow up was 14 years (range, 4 – 27).

IL7Rα SCID survivors post-HSCT have the highest proportion of patients free from immunoglobulin replacement therapy; in comparison to other SCID genotypes (IL7Rα 93%, IL2RG/JAK3 SCID (55%), RAG 1 and RAG2 (77%), Artemis SCID (57%), ADA SCID (81%). Only one IL7Rα patient required ongoing immunoglobulin replacement therapy (1 out of 15 patients).

Clinical Outcome	n/N (%)
On-going Medical Issues	11/15 (73%)
Off IVIG Replacement Therapy	14/15 (93%)
Bronchiectasis	2/15 (13%)
Short Stature	4/15 (27%)
Chronic Pulmonary Disease	1/15 (14%)
Warts	5/15 (33%)
Normal Lung Function	6/7 (85%) 1 patient had major restrictive defect
Normal Endocrine	15/15 (100%)
Normal Hearing	15/15 (100%)
Normal Cardiovascular	15/15 (100%)
Normal Renal System	15/15 (100%)
Normal Gastro-intestinal System	15/15 (100%)

Mean PedsQL Scores for IL7Rα SCID patients post-HSCT (Parent and Children Report)

	UK Norms Mean	IL7Rα SCID Mean (p value)
Parent Report		N = 6
Total	84.6	68.3 (0.07)
Psychosocial	82.2	65.0 (0.07)
Physical	89.1	74.5 (0.11)
Emotional	78.3	62.5 (0.21)
Social	86.8	78.3 (0.52)
School	81.5	67.0 (0.19)
Child Report		N = 9
Total	83.9	76.7 (0.33)
Psychosocial	81.8	74.3 (0.21)
Physical	88.5	81.3 (0.22)
Emotional	78.5	66.7 (0.29)
Social	87.7	85.6 (0.73)
School	78.9	70.6 (0.24)

Longitudinal Analysis of immune reconstitution post-HSCT (comparison between conditioned vs unconditioned recipients)

CD3+ lymphocyte | CD4+ Naïve lymphocyte | CD19+ lymphocyte | NK cell

Contact Person: 1. Dr Intan Juliana Abd Hamid (I.J.abd-hamid@newcastle.ac.uk); 2. Dr Andrew R. Gennery (andrew.gennery@newcastle.ac.uk)

13.7 Appendix G: Poster presentations

European Bone Marrow Transplantation (EBMT), April 2016, Valencia

Long term immune reconstitution post-hematopoietic stem cell transplantation in IL2RG/JAK3 SCID, Newcastle experience

Intan Juliana Abd Hamid [1,4], Mary A Slatter [1,2], Mark S. Pearce[3], Andrew R Gennery [1,2].

[1] Institute of Cellular Medicine, Newcastle University, UK [2] Great North Children's Hospital, Newcastle-upon-Tyne, UK. [3] Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK [4] Regenerative Medicine Cluster, Institut Perubatan & Pergigian Termaju, USM, Kepala Batas, Malaysia.

Introduction:

Hematopoietic stem cell transplantation (HSCT) is curative for patients with severe combined immunodeficiency (SCID).

We discuss the long term outcome of donor chimerism and thymic output for IL2RG/JAK3 SCID post-transplantation at our center.

Methods:

A cross-sectional study of IL2RG/ JAK3 SCID patients who underwent HSCT in a single centre, from 1987 – 2012.

The conditioning regimens used were:

1. Unconditioned
2. Reduced Intensity Conditioning (RIC) : Fludarabine/Melphalan
3. Low Toxicity Myeloablative (LTMAC) : Treosulphan/ Fludarabine or Treosulphan/ Cyclophosphamide
4. Myeloablative (MAC) : Busulphan/Cyclophosphamide

CD3+CD4+45RA+ measurement = thymic output post-HSCT indicator.

Results:

- 31 of 43 patients survived
- 10 years survival outcome = 71.9%
- Median age at last follow up = 10 years (range, 2-25)

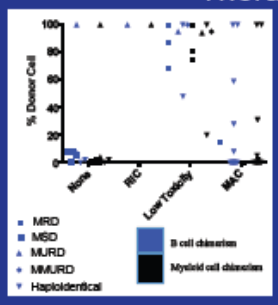
Table 1. Conditioning regimen and donor categories for each patient

Characteristic	MSD	MRD	MURD	Haploidentical	MMURD
Conditioning regimen					
None	5	1	2	6	0
Reduced intensity (RIC)	0	8	1	0	1
Low toxicity MAC	0	5	2	1	1
Myeloablative (MAC)	0	1	0	16	0
Non-myeloablative (NMA)	0	1	0	0	0
Total	5	8	5	23	2

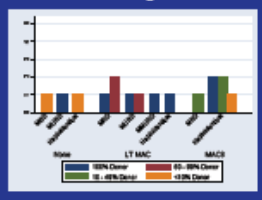
Conclusion:

1. Good T cell donor chimerism observed across all types of donors and conditioning regimens.
2. LTMAC recipients tends to have good B/Myeloid chimerism and be free from Immunoglobulin replacement therapy at last follow up.
3. Sustained CD3+ output are seen even after 20 years post-HSCT.
4. Conditioned recipients have better long term thymopoiesis compared to unconditioned recipients.

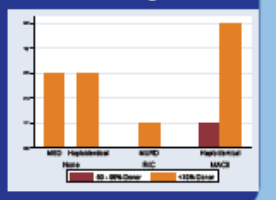
Chimerism Analysis & Immunoglobulin Replacement Therapy at Last Follow Up



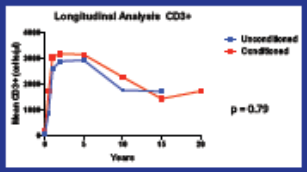
Off Immunoglobulin



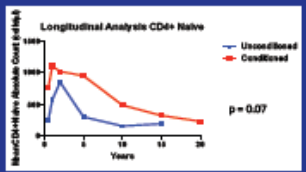
On Immunoglobulin



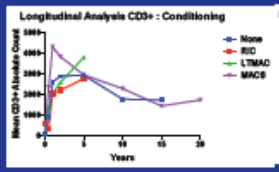
Longitudinal Analysis of Immune Reconstitution Post-HSCT



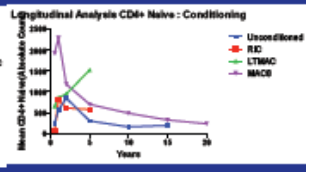
CD3+ cells output according to the unconditioned versus conditioned recipients.



CD4+ Naive cells output according to the unconditioned versus conditioned recipients.



CD3+ cells output according to the conditioning regimens received.



CD4+ Naive cells output according to the conditioning regimens received.

Contact Person: 1. Dr Intan Juliana Abd Hamid (I.Labd-hamid@newcastle.ac.uk); 2. Dr Andrew R. Gennery (andrew.gennery@newcastle.ac.uk)

Predictors of survival among individuals with severe combined immunodeficiency disorder undergoing hematopoeitic stem cell transplantation

Intan Juliana Abd Hamid [1,4], Mary A. Slatter [1,2], Mark S. Pearce[3], Andrew R. Gennery [1,2].

[1] Institute of Cellular Medicine, Newcastle University, UK [2] Great North Children's Hospital, Newcastle-upon-Tyne, UK [3] Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK [4] Regenerative Medicine Cluster, Institut Perubatan & Pergigian Termaju, USM, Kepala Batas, Malaysia.

Introduction

Haematopoietic stem cell transplantation (HSCT) corrects the immune defects in severe combined immunodeficiency (SCID).

We aimed to identify factors that influence survival outcome of transplanted SCID patients, to improve the future management of HSCT for SCID.

Methods

- A retrospective review of SCID patients undergoing HSCT in a single centre, from 1987 – 2012.

Conclusion

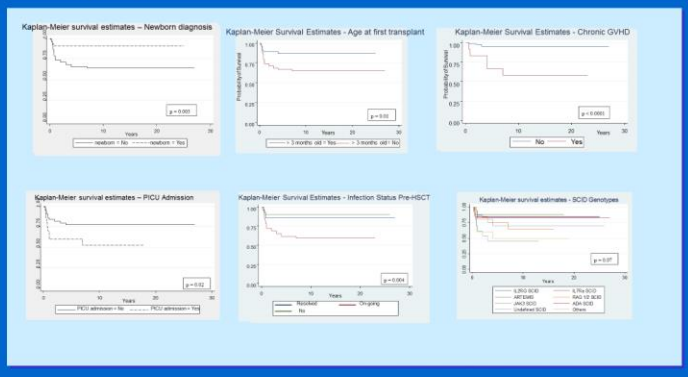
- These data emphasise the importance of early diagnosis and transplant in SCID, which contribute significantly towards superior survival outcome following transplantation.

Results

96 out of 120 patients survived >2years post-HSCT. The ten years survival outcome is 73.5%. Median time of death after HSCT was 124 days (range, 3 – 2473). 65% of deaths occurred during the first 6 months post-HSCT, mainly due to viral infection and acute GVHD. There is significantly more viral death for SCID HSCT prior to year 2000 compared to after 2000 (64% vs 29%, p 0.05).

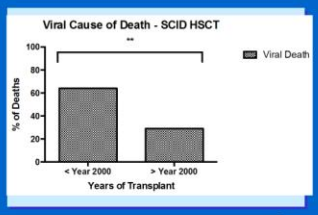
Six out of 32 deaths are late death. Late deaths are defined as occurring >2years post-HSCT. The cause for late death varied, including malignancy (RAG2 SCID patient with recipient Acute B Lymphoblastic Leukemia and a CHARGE Syndrome patient with Lymphoma), Haemophilus influenza infection, EBV PTLD, inflammatory pneumonitis and sudden death.

The best 10 years survival are seen in ARTEMIS SCID (87.5%), ADA and IL2RG SCID (both 84%). Survival was significantly better in those diagnosed early (<4weeks old) (90.7% vs 64.1%, p 0.003), those transplanted before the age of 3 months old (86.9% vs 65.1%, p 0.01), those without chronic GVHD (95.2% vs 58.3%, p<0.0001), whom are free from infection prior to transplant (90.0% vs 68.1%, p 0.004) and those not requiring PICU admission (77.6% vs 52.5%, p 0.02).



Ten Years Survival Outcome According to Genetic Diagnosis

Gene Diagnosis	Total Number of Patients, N	Survival Function (%)	95% Confidence Interval
ARTEMIS SCID	8	87.50	38.7 – 98.14
ADA SCID	19	84.21	58.65 – 94.62
IL2RG SCID	30	83.33	64.50 – 92.70
IL7Ra SCID	17	82.35	54.71 – 93.94
Undefined SCID	10	70.00	32.87 – 89.19
RAG 1/2 SCID	14	64.47	29.78 – 85.29
Others	10	50.00	18.36 – 75.32
JAK3 SCID	13	44.87	17.70 – 69.02



Contact Person: 1. Dr Intan Juliana Abd Hamid (i.j.abd-hamid@newcastle.ac.uk); 2. Dr Andrew R. Gennery (andrew.gennery@newcastle.ac.uk)

Post-transplant health-related quality of life for different Severe Combined Immunodeficiency Genotypes

Intan Juliana Abd Hamid [1,5], Mary A Slatter [1,2], Fiona McKendrick [3], Mark S. Pearce[4], Andrew Gennery [1,2].

[1] Institute of Cellular Medicine, Newcastle University, UK [2] Great North Children's Hospital, Newcastle-upon-Tyne, UK. [3] Department of Health Psychology, Newcastle & North Tyneside NHS Trust. [4] Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK [5] Regenerative Medicine Cluster, Institut Perubatan & Pergigian Termaju, USM, Kepala Batas, Malaysia.

Introduction:

Survival outcome has improved following hematopoietic stem cell transplantation (HSCT) for patients with severe combined immunodeficiency (SCID). Many studies have demonstrated evolution of immune-reconstitution, but there are scarce data considering long-term quality of life (QoL), with one study suggesting poor function compared to healthy controls (1).

We objectively assessed QoL of SCID survivors at our centre according to their genetic diagnosis.

Results:

- 59 of 88 (67%) patients responded
- 14 children aged < 5 years old were excluded from child questionnaires
- 28 patients not contactable, 3 patients refused.
- Median interval post-HSCT: 11 years (range, 2-27)
- Proportion of Responder:
  - IL2RG/JAK3 (65%)
  - IL7Ra (71%)
  - ADA (75%)
  - ARTEMIS (71%)
- ADA SCID reported lower QoL (both parent & patient report)

Conclusion:

1. In contrast to a previous report (1), a number of SCID genotypes were associated with normal QoL (IL7Ra and ARTEMIS SCID).

2. Particular risk factors include ADA SCID and need for on-going medication. Factors postulated as causing reduced QoL include prolonged hospitalization, parental consanguinity and on-going medical issues, but most parameters we examined were normal.

3. Larger qualitative studies are needed to clarify QoL differences in SCID survivors.

Methods:

All SCID patients more than 2 years post-HSCT attending the HSCT Clinic follow up were invited to answer the Pediatric Quality of Life Inventory (PedsQL), Generic Score Scale v4.0 questionnaires as part of the routine psychology assessment.

The PedsQL questionnaires consist of parent and patient reports, both have 6 domains (physical, emotional, social, school, psychosocial and total).

Table. Mean PedsQL Scores according to different SCID Genotypes (Parents and Children)

	UK Norms Mean	IL-2Rγ/ JAK3, Mean (p value)	IL-7Rα , Mean (p value)	Artemis, Mean (p value)	ADA, Mean (p value)	RAG 1/2, Mean (p value)
Parent Report		N = 19	N = 6	N = 2	N = 12	N = 5
Total	84.6	**70.9 (0.009)**	68.3 (0.07)	68.5 (0.32)	**67.9 (0.02)**	73.9 (0.19)
Psychosocial	82.2	**66.5 (0.007)**	65.0 (0.07)	65.8 (0.33)	**67.4 (0.02)**	71.7 (0.21)
Physical	89.1	82.4 (0.19)	74.5 (0.11)	73.4 (0.31)	**71.1 (0.04)**	78.1 (0.28)
Emotional	78.3	72.9 (0.34)	62.5 (0.21)	75.0 (0.92)	77.1 (0.88)	69.0 (0.37)
Social	86.8	77.4 (0.13)	78.3 (0.52)	80.0 (0.79)	**71.3 (0.04)**	82.0 (0.57)
School	81.5	**63.5 (0.02)**	67.0 (0.19)	85.0 (-)	**53.8 (0.007)**	**64.0 (0.03)**
Child Report		N = 15	N = 9	N = 4	N = 6	N = 5
Total	83.9	77.8 (0.23)	76.7 (0.33)	73.6 (0.19)	**55.1 (0.05)**	72.6 (0.30)
Psychosocial	81.8	74.1 (0.17)	74.3 (0.21)	76.3 (0.29)	**52.5 (0.04)**	71.3 (0.30)
Physical	88.5	84.6 (0.45)	81.3 (0.22)	71.9 (0.16)	**59.9 (0.07)**	75.0 (0.31)
Emotional	78.5	79.3 (0.89)	66.7 (0.29)	83.8 (0.41)	62.5 (0.16)	74.5 (0.74)
Social	87.7	75.7 (0.09)	85.6 (0.73)	77.5 (0.53)	**56.7 (0.07)**	81.0 (0.51)
School	78.9	67.3 (0.08)	70.6 (0.24)	67.5 (0.32)	**38.3 (0.008)**	**58.7 (0.03)**

T-test, p value derived from comparison between each SCID Genotype versus UK Norm[(2)] (**Bold = significant**)

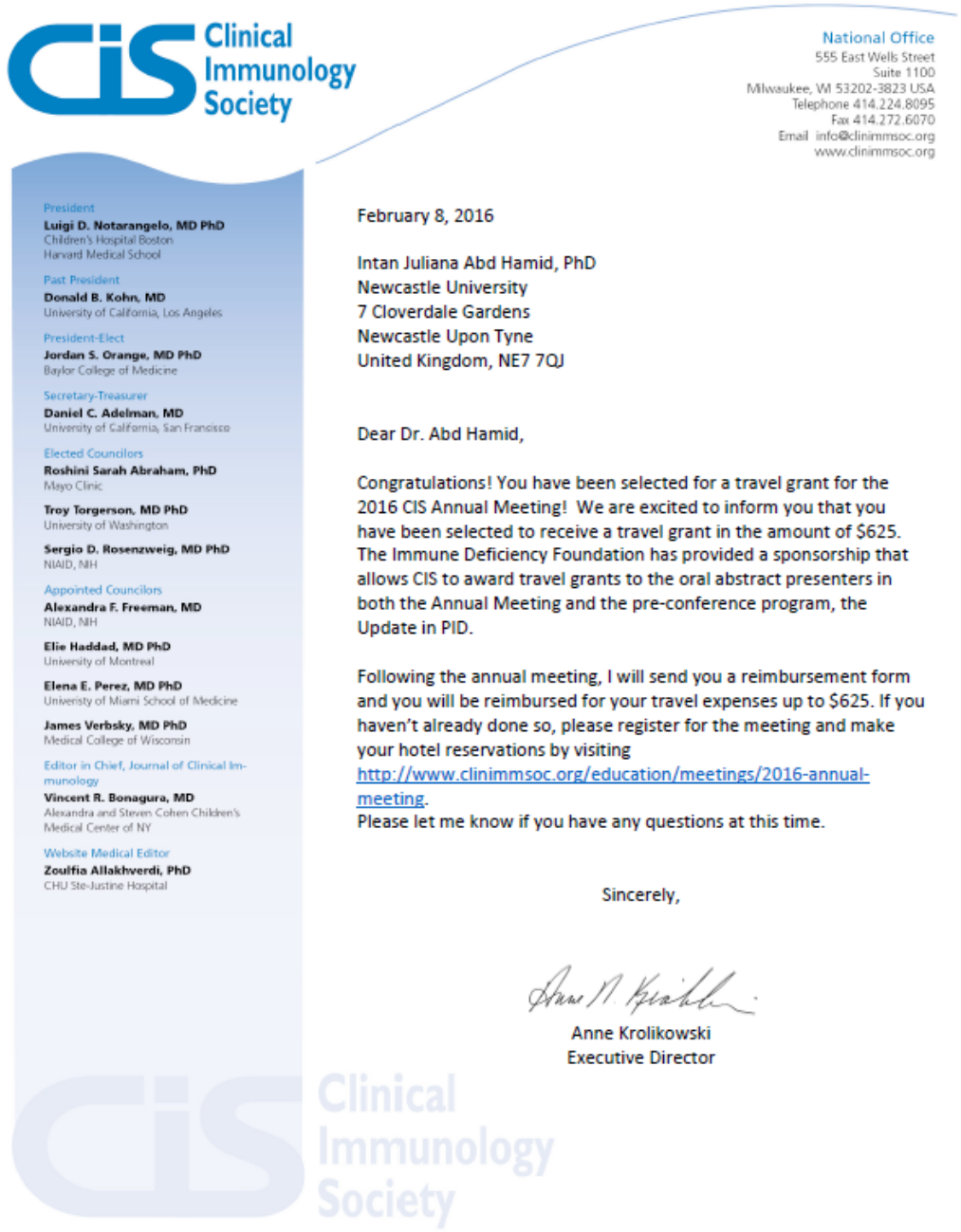
Reference:

1. Titman, P., Pink, E., Skucek, E., O'Hanlon, K., Cole, T.J., Gaspar, J., Xu-Bayford, J., Jones, A., Thrasher, A.J., Davies, E.G., Veys, P.A. and Gaspar, H.B. (2008) 'Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies', *Blood*, 112(9), pp. 3907-3913.
2. Upton, P., Eiser, C., Cheung, I., Hutchings, H., Jenney, M., Maddocks, A., Russell, I. and Williams, J. (2005) 'Measurement properties of the UK-English version of the Pediatric Quality of Life InventoryTM 4.0 (PedsQLTM) generic core scales', *Health and Quality of Life Outcomes*, 3(1), p. 22.

Contact Person: 1. Dr Intan Juliana Abd Hamid (i.j.abd-hamid@newcastle.ac.uk); 2. Dr Andrew R. Gennery (andrew.gennery@newcastle.ac.uk)

13.8 Appendix H: Prizes

13.8.1 Travel Grant Award for Clinical Immunology Society (CIS) Annual Meeting, Boston USA 2016.



Clinical Immunology Society

National Office
555 East Wells Street
Suite 1100
Milwaukee, WI 53202-3823 USA
Telephone 414.224.8095
Fax 414.272.6070
Email info@clinimmsoc.org
www.clinimmsoc.org

February 8, 2016

Intan Juliana Abd Hamid, PhD
Newcastle University
7 Cloverdale Gardens
Newcastle Upon Tyne
United Kingdom, NE7 7QJ

Dear Dr. Abd Hamid,

Congratulations! You have been selected for a travel grant for the 2016 CIS Annual Meeting! We are excited to inform you that you have been selected to receive a travel grant in the amount of $625. The Immune Deficiency Foundation has provided a sponsorship that allows CIS to award travel grants to the oral abstract presenters in both the Annual Meeting and the pre-conference program, the Update in PID.

Following the annual meeting, I will send you a reimbursement form and you will be reimbursed for your travel expenses up to $625. If you haven't already done so, please register for the meeting and make your hotel reservations by visiting http://www.clinimmsoc.org/education/meetings/2016-annual-meeting.
Please let me know if you have any questions at this time.

Sincerely,

Anne Krolikowski
Executive Director

Chapter 14 References

1. Kwan, A., D. Hu, M. Song, H. Gomes, D.R. Brown, T. Bourque, D. Gonzalez-Espinosa, Z. Lin, M.J. Cowan, and J.M. Puck, *Successful newborn screening for SCID in the Navajo Nation.* Clin Immunol, 2015. **158**(1): p. 29-34.
2. Gennery, A.R., M.A. Slatter, L. Grandin, P. Taupin, A.J. Cant, P. Veys, P.J. Amrolia, H.B. Gaspar, E.G. Davies, W. Friedrich, M. Hoenig, L.D. Notarangelo, E. Mazzolari, F. Porta, R.G. Bredius, A.C. Lankester, N.M. Wulffraat, R. Seger, T. Gungor, A. Fasth, P. Sedlacek, B. Neven, S. Blanche, A. Fischer, M. Cavazzana-Calvo, P. Landais, B. Inborn Errors Working Party of the European Group for, T. Marrow, and I. European Society for, *Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better?* J Allergy Clin Immunol, 2010. **126**(3): p. 602-10 e1-11.
3. Brown, L., J. Xu-Bayford, Z. Allwood, M. Slatter, A. Cant, E.G. Davies, P. Veys, A.R. Gennery, and H.B. Gaspar, *Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening.* Blood, 2011. **117**(11): p. 3243-6.
4. Puck, J.M., *Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles.* Journal of Allergy & Clinical Immunology, 2012. **129**(3): p. 607-16.
5. Tsuji, Y., K. Imai, M. Kajiwara, Y. Aoki, T. Isoda, D. Tomizawa, M. Imai, S. Ito, H. Maeda, Y. Minegishi, H. Ohkawa, J. Yata, N. Sasaki, K. Kogawa, M. Nagasawa, T. Morio, S. Nonoyama, and S. Mizutani, *Hematopoietic stem cell transplantation for 30 patients with primary immunodeficiency diseases: 20 years experience of a single team.* Bone Marrow Transplant, 2006. **37**(5): p. 469-477.
6. Lee, W.-I., J.-L. Huang, T.-H. Jaing, S.-D. Shyur, K.D. Yang, Y.-H. Chien, B.-L. Chiang, W.-J. Soong, S.-S. Chiou, C.-C. Shieh, S.-J. Lin, K.-W. Yeh, L.-C. Chen, L.-S. Ou, T.-C. Yao, T.-Y. Lin, C.-H. Chiu, Y.-C. Huang, K.-H. Wu, C.-Y. Lin, H.-H. Yu, Y.-H. Yang, H.-R. Yu, H.-J. Yen, M.-Y. Hsieh, M.-L. Kuo, W.-L. Hwu, Y.-C. Tsai, H.-C. Kuo, Y.-L. Lin, Y.-F. Shih, and K.-W. Chang, *Distribution, clinical features and treatment in Taiwanese patients with symptomatic primary immunodeficiency diseases (PIDs) in a nationwide population-based study during 1985–2010.* Immunobiology, 2011. **216**(12): p. 1286-1294.
7. Amayiri, N., A. Al-Zaben, L. Ghatasheh, H. Frangoul, and A.A. Hussein, *Hematopoietic stem cell transplantation for children with primary immunodeficiency diseases: single center experience in Jordan.* Pediatr Transplant, 2013. **17**(4): p. 394-402.
8. Gaspar, H.B., W. Qasim, E.G. Davies, K. Rao, P.J. Amrolia, and P. Veys, *How I treat severe combined immunodeficiency.* Blood, 2013. **122**(23): p. 3749-58.

9. Buckley, R.H., R.I. Schiff, S.E. Schiff, M.L. Markert, L.W. Williams, T.O. Harville, J.L. Roberts, and J.M. Puck, *Human severe combined immunodeficiency: Genetic, phenotypic, and functional diversity in one hundred eight infants.* J Pediatr, 1997. **130**(3): p. 378-387.
10. McWilliams, L.M., M.D. Railey, and R.H. Buckley, *Positive family history, infection, low absolute lymphocyte count (ALC), and absent thymic shadow: Diagnostic clues for all molecular forms of severe combined immunodeficiency (SCID).* Journal of Allergy and Clinical Immunology: In Practice, 2015. **3**(4): p. 585-591.
11. Rivers, L. and H.B. Gaspar, *Severe combined immunodeficiency: recent developments and guidance on clinical management.* Archives of Disease in Childhood, 2015. **100**(7): p. 667-72.
12. Bruton OC, *Agammaglobulinemia.* Pediatrics, 1952. **9**: p. 722-728.
13. Ochsa, H.D. and W.H. Hitzigb, *History of primary immunodeficiency diseases.* Current Opinion in Allergy and Clinical Immunology, 2012. **12**(6): p. 577-587.
14. Bruton OC, *The discovery of agammglobulinemia*, in *Immunologic deficiency disease in man*, B. D, Editor. 1968, The National Foundation: New York. p. 2-6.
15. Glanzmann E and Riniker P, *Essentialle Lymphozytophthise: Ein neues Krankheitsbild der Sauglingspathologie.* Ann Peaediatr, 1950. **175**: p. 1-32.
16. Hitzig WH and Willi H., *Hereditary lymphoplasmocytic dysgenesis ("Alymphocytose mit Agammaglobulinemia").* Schweiz Med Wschr, 1961. **91**: p. 1625-1633.
17. Fudenberg HH, Good RA, and Hitzig W, *Classification of the primary immune deficiencies: WHO recommendation.* New England Journal of Medicine, 1970. **283**: p. 656-657.
18. Stiehm, E.R. and R.B. Johnston, *A History of Pediatric Immunology.* Pediatr Res, 2005. **57**(3): p. 458-467.
19. Gatti, R.A., Allen HD, Meuwissen HJ, Hong R., and Good RA., *Immunological reconstitution of sex-linked lymphopenic immunological deficiency.* Lancet, 1968. **292**(7583): p. 1366-1369.
20. Fischer A., Friedrich W., Levinsky R., Vossen J., Griscelli C., Kubanek B., Morgan G., Wagemaker G., and Landais P., *BONE-MARROW TRANSPLANTATION FOR IMMUNODEFICIENCIES AND OSTEOPETROSIS: EUROPEAN SURVEY, 1968-1985.* The Lancet, 1986. **328**(8515): p. 1080-1084.
21. Aloj, G., G. Giardino, L. Valentino, F. Maio, V. Gallo, T. Esposito, R. Naddei, E. Cirillo, and C. Pignata, *Severe combined immunodeficiences: new and old scenarios.* Int Rev Immunol, 2012. **31**(1): p. 43-65.
22. Picard, C., W. Al-Herz, A. Bousfiha, J.L. Casanova, T. Chatila, M.E. Conley, C. Cunningham-Rundles, A. Etzioni, S.M. Holland, C. Klein, S. Nonoyama, H.D. Ochs, E. Oksenhendler, J.M. Puck, K.E. Sullivan, M.L. Tang, J.L. Franco, and H.B. Gaspar, *Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015.* J Clin Immunol, 2015. **35**(8): p. 696-726.
23. Bonilla, F.A., I.L. Bernstein, D.A. Khan, Z.K. Ballas, J. Chinen, M.M. Frank, L.J. Kobrynski, A.I. Levinson, B. Mazer, R.P. Nelson Jr, J.S. Orange, J.M. Routes, W.T. Shearer, and R.U. Sorensen, *Practice parameter for the diagnosis and management of primary*

immunodeficiency. Annals of Allergy, Asthma & Immunology, 2005. **94**(5, Supplement 1): p. S1-S63.
24. Bousfiha, A., L. Jeddane, W. Al-Herz, F. Ailal, J.L. Casanova, T. Chatila, M.E. Conley, C. Cunningham-Rundles, A. Etzioni, J.L. Franco, H.B. Gaspar, S.M. Holland, C. Klein, S. Nonoyama, H.D. Ochs, E. Oksenhendler, C. Picard, J.M. Puck, K.E. Sullivan, and M.L. Tang, *The 2015 IUIS Phenotypic Classification for Primary Immunodeficiencies.* J Clin Immunol, 2015. **35**(8): p. 727-38.
25. Villa, A., D. Moshous, J.-P. de Villartay, L.D. Notarangelo, and F. Candotti, *Chapter 4 - Severe Combined Immunodeficiencies*, in *Stiehm's Immune Deficiencies*. 2014, Academic Press: Amsterdam. p. 87-141.
26. Recher, M., L.J. Berglund, D.T. Avery, M.J. Cowan, A.R. Gennery, J. Smart, J. Peake, M. Wong, S.-Y. Pai, S. Baxi, J.E. Walter, U. Palendira, G.A. Tangye, M. Rice, S. Brothers, W. Al-Herz, H. Oettgen, H. Eibel, J.M. Puck, F. Cattaneo, J.B. Ziegler, S. Giliani, S.G. Tangye, and L.D. Notarangelo, *IL-21 is the primary common gamma chain-binding cytokine required for human B-cell differentiation in vivo.* Blood, 2011. **118**(26): p. 6824-35.
27. Conley, M.E., R.H. Buckley, R. Hong, C. Guerra-Hanson, C.M. Roifman, J.A. Brochstein, S. Pahwa, and J.M. Puck, *X-linked severe combined immunodeficiency. Diagnosis in males with sporadic severe combined immunodeficiency and clarification of clinical findings.* J Clin Invest, 1990. **85**(5): p. 1548-54.
28. O'Shea, J.J., M. Husa, D. Li, S.R. Hofmann, W. Watford, J.L. Roberts, R.H. Buckley, P. Changelian, and F. Candotti, *Jak3 and the pathogenesis of severe combined immunodeficiency.* Molecular Immunology, 2004. **41**(6–7): p. 727-737.
29. Giliani, S., L. Mori, G. de Saint Basile, F. Le Deist, C. Rodriguez-Perez, C. Forino, E. Mazzolari, S. Dupuis, R. Elhasid, A. Kessel, C. Galambrun, J. Gil, A. Fischer, A. Etzioni, and L.D. Notarangelo, *Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients.* Immunological Reviews, 2005. **203**: p. 110-26.
30. Hershfield, M.S., *Genotype is an important determinant of phenotype in adenosine deaminase deficiency.* Current Opinion in Immunology, 2003. **15**(5): p. 571-577.
31. Ljungman, P., A. Urbano-Ispizua, M. Cavazzana-Calvo, T. Demirer, G. Dini, H. Einsele, A. Gratwohl, A. Madrigal, D. Niederwieser, J. Passweg, V. Rocha, R. Saccardi, H. Schouten, N. Schmitz, G. Socie, A. Sureda, J. Apperley, B. European Group for, and Marrow, *Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: definitions and current practice in Europe.* Bone Marrow Transplant, 2006. **37**(5): p. 439-49.
32. Buckley, R., *Advances in the understanding and treatment of human severe combined immunodeficiency.* Immunol Res, 2000. **22**(2-3): p. 237-251.
33. Reisner, Y., A. Ravid, and N. Sharon, *Use of soybean agglutinin for the separation of mouse B and T lymphocytes.* Biochemical and Biophysical Research Communications, 1976. **72**(4): p. 1585-1591.
34. Friedrich, W., U. Vetter, B. Heymer, Y. Reisner, S.F. Goldmann, T.M. Fliedner, H.H. Peter, and E. Kleihauer, *IMMUNORECONSTITUTION IN SEVERE COMBINED IMMUNODEFICIENCY AFTER*

TRANSPLANTATION OF HLA-HAPLOIDENTICAL, T-CELL-DEPLETED BONE MARROW. The Lancet, 1984. **323**(8380): p. 761-764.
35. Gennery, A.R., A.M. Dickinson, K. Brigham, D. Barge, G.P. Spickett, A. Curtis, V. Spencer, A. Jackson, G. Cavanagh, V. Carter, P. Palmer, T.J. Flood, A.J. Cant, and M. Abinun, *CAMPATH-1M T-cell depleted BMT for SCID: long-term follow-up of 19 children treated 1987-98 in a single center.* Cytotherapy, 2001. **3**(3): p. 221-32.
36. *Bone Marrow Donors Worldwide*. 2014 29/04/2014]; Available from: http://www.bmdw.org/.
37. Wilson, A. and A. Trumpp, *Bone-marrow haematopoietic-stem-cell niches.* Nat Rev Immunol, 2006. **6**(2): p. 93-106.
38. Wodnar-Filipowicz, A., *Biological properties of haematopoietic stem cells*, in *Haematopoietic Stem Cell Transplantation*, J. Apperley, et al., Editors. 2012, forum service editore: Genoa, Italy. p. 57-73.
39. Yu, V.W. and D.T. Scadden, *Hematopoietic Stem Cell and Its Bone Marrow Niche.* Curr Top Dev Biol, 2016. **118**: p. 21-44.
40. Gennery, A.R. and M.A. Slatter, *Chemotherapy-free conditioning: one step closer*. Vol. 124. 2014. 838-840.
41. Howell, W.M., V. Carter, and B. Clark, *The HLA system: immunobiology, HLA typing, antibody screening and crossmatching techniques.* J Clin Pathol, 2010. **63**(5): p. 387-90.
42. Klein, J. and A. Sato, *The HLA system. First of two parts.* The New England journal of medicine, 2000. **343**(10): p. 702-9.
43. Neven, B., S. Leroy, H. Decaluwe, F. Le Deist, C. Picard, D. Moshous, N. Mahlaoui, M. Debré, J.-L. Casanova, L. Dal Cortivo, Y. Madec, S. Hacein-Bey-Abina, G. de Saint Basile, J.-P. de Villartay, S. Blanche, M. Cavazzana-Calvo, and A. Fischer, *Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency.* Blood, 2009. **113**(17): p. 4114-4124.
44. Pai, S.-Y., B.R. Logan, L.M. Griffith, R.H. Buckley, R.E. Parrott, C.C. Dvorak, N. Kapoor, I.C. Hanson, A.H. Filipovich, S. Jyonouchi, K.E. Sullivan, T.N. Small, L. Burroughs, S. Skoda-Smith, A.E. Haight, A. Grizzle, M.A. Pulsipher, K.W. Chan, R.L. Fuleihan, E. Haddad, B. Loechelt, V.M. Aquino, A. Gillio, J. Davis, A. Knutsen, A.R. Smith, T.B. Moore, M.L. Schroeder, F.D. Goldman, J.A. Connelly, M.H. Porteus, Q. Xiang, W.T. Shearer, T.A. Fleisher, D.B. Kohn, J.M. Puck, L.D. Notarangelo, M.J. Cowan, and R.J. O'Reilly, *Transplantation Outcomes for Severe Combined Immunodeficiency, 2000–2009.* New England Journal of Medicine, 2014. **371**(5): p. 434-446.
45. Lee, S.J., J. Klein, M. Haagenson, L.A. Baxter-Lowe, D.L. Confer, M. Eapen, M. Fernandez-Vina, N. Flomenberg, M. Horowitz, C.K. Hurley, H. Noreen, M. Oudshoorn, E. Petersdorf, M. Setterholm, S. Spellman, D. Weisdorf, T.M. Williams, and C. Anasetti, *High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation.* Blood, 2007. **110**(13): p. 4576-83.
46. Petersdorf, E.W., M. Malkki, K. Hsu, P. Bardy, A. Cesbron, A. Dickinson, V. Dubois, K. Fleischhauer, T. Kawase, A. Madrigal, Y. Morishima, B. Shaw, S. Spellman, E. Spierings, M. Stern, J.M. Tiercy, A. Velardi, T. Gooley, and T. International Histocompatibility Working Group in Hematopoietic Cell, *16th IHIW: international histocompatibility working group in hematopoietic cell transplantation.* Int J Immunogenet, 2013. **40**(1): p. 2-10.

47. Gluckman, E., *Choice of donor according to HLA typing and stem cell source*, in *The EBMT Handbook: Haematopoietic Stem Cell Transplantation*, J. Apperley, et al., Editors. 2012, forum service editore: Genoa, Italy. p. 91 - 107.
48. Neven, B., M. Cavazanna-Calvo, and A. Fischer, *Late immunologic and clinical outcomes for children with SCID.* Biol Blood Marrow Transplant, 2008. **14**(1 Suppl 1): p. 76-8.
49. Muller, S.M., T. Kohn, A.S. Schulz, K.M. Debatin, and W. Friedrich, *Similar pattern of thymic-dependent T-cell reconstitution in infants with severe combined immunodeficiency after human leukocyte antigen (HLA)-identical and HLA-nonidentical stem cell transplantation.* Blood. **96**(13): p. 4344-9.
50. Ciurea, S.O. and B.S. Andersson, *Busulfan in hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2009. **15**(5): p. 523-36.
51. Guidelines, E.E. *EBMT/ESID GUIDELINES FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PRIMARY IMMUNODEFICIENCIES* 2011 [cited 2014 1/04/2014]; Available from: http://www.ebmt.org/Contents/About-EBMT/Who-We-Are/ScientificCouncil/Documents/EBMT_ESID%20GUIDELINES%20FOR%20INBORN%20ERRORS%20FINAL%202011.pdf?Mobile=1.
52. Slatter, M.A. and A.J. Cant, *Hematopoietic stem cell transplantation for primary immunodeficiency diseases.* Ann N Y Acad Sci, 2011. **1238**: p. 122-31.
53. Bacigalupo, A., *Third EBMT//AMGEN Workshop on reduced-intensity conditioning allogeneic haemopoietic stem cell transplants (RIC-HSCT), and panel consensus.* Bone Marrow Transplant, 2004. **33**(7): p. 691-696.
54. Schuetz, C., B. Neven, C.C. Dvorak, S. Leroy, M.J. Ege, U. Pannicke, K. Schwarz, A.S. Schulz, M. Hoenig, M. Sparber-Sauer, S.A. Gatz, C. Denzer, S. Blanche, D. Moshous, C. Picard, B.N. Horn, J.P. de Villartay, M. Cavazzana, K.M. Debatin, W. Friedrich, A. Fischer, and M.J. Cowan, *SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID.* Blood, 2014. **123**(2): p. 281-9.
55. *Guidance to bone marrow and peripheral blood stem cell teams and accredited assessors*. May 2015 [cited 2017 05/02/2017]; Available from: hta.gov.uk/sites/default/Guidance%20to%20Bone%20Marrow%20And%20Peripheral%20Blood%20Stem%20Cell%20Transplant%20Teams%20and%20Accredited%20Accessors.pdf.
56. Shaw, B.E., P. Veys, A. Pagliuca, J. Addada, G. Cook, C.F. Craddock, A.R. Gennery, J. Goldman, S. Mackinnon, J.A. Madrigal, D.I. Marks, C. Navarrete, M.N. Potter, S. Querol, F. Regan, N.H. Russell, and R.E. Hough, *Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: conditioning protocols and donor selection algorithms.* Bone Marrow Transplant, 2009. **44**(1): p. 7-12.
57. Buckley, R.H., S.E. Schiff, R.I. Schiff, M.L. Markert, L.W. Williams, J.L. Roberts, L.A. Myers, and F.E. Ward, *Hematopoietic Stem-Cell Transplantation for the Treatment of Severe Combined Immunodeficiency.* New England Journal of Medicine, 1999. **340**(7): p. 508-516.
58. Gennery, A.R. and A.J. Cant, *Advances in Hematopoietic Stem Cell Transplantation for Primary Immunodeficiency.* Immunology and Allergy Clinics of North America, 2008. **28**(2): p. 439-456.

59. Griffith, L.M., M.J. Cowan, D.B. Kohn, L.D. Notarangelo, J.M. Puck, K.R. Schultz, R.H. Buckley, M. Eapen, N.R. Kamani, R.J. O'Reilly, R. Parkman, C.M. Roifman, K.E. Sullivan, A.H. Filipovich, T.A. Fleisher, and W.T. Shearer, *Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: current status and critical needs.* J Allergy Clin Immunol, 2008. **122**(6): p. 1087-96.
60. Antoine C., Müller S., Cant A., Cavazzana-Calvo M., Veys P., Vossen J., Fasth A., Heilmann C., Wulffraat N., Seger R., Blanche S., Friedrich W., Abinun M., Davies G., Bredius R., Schulz A., Landais P., and Fischer A., *Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968–99.* The Lancet, 2003. **361**(9357): p. 553-560.
61. Hassan, A., P. Lee, P. Maggina, J.H. Xu, D. Moreira, M. Slatter, Z. Nademi, A. Worth, S. Adams, A. Jones, C. Cale, Z. Allwood, K. Rao, R. Chiesa, P. Amrolia, H. Gaspar, E.G. Davies, P. Veys, A. Gennery, and W. Qasim, *Host natural killer immunity is a key indicator of permissiveness for donor cell engraftment in patients with severe combined immunodeficiency.* Journal of Allergy and Clinical Immunology, 2014. **133**(6): p. 1660-1666.
62. Cavazzana-Calvo, M., I. Andre-Schmutz, and A. Fischer, *Haematopoietic stem cell transplantation for SCID patients: where do we stand?* Br J Haematol, 2013. **160**(2): p. 146-52.
63. Buckley, R.H., C.M. Win, B.K. Moser, R.E. Parrott, E. Sajaroff, and M. Sarzotti-Kelsoe, *Post-transplantation B cell function in different molecular types of SCID.* J Clin Immunol, 2013. **33**(1): p. 96-110.
64. Haddad, E., F.L. Deist, P. Aucouturier, M. Cavazzana-Calvo, S. Blanche, G.D.S. Basile, and A. Fischer, *Long-Term Chimerism and B-Cell Function After Bone Marrow Transplantation in Patients With Severe Combined Immunodeficiency With B Cells: A Single-Center Study of 22 Patients.* Blood, 1999. **94**(8): p. 2923-2930.
65. Laffort C., Le Deist F., and Favre M., *Severe cutaneous papillomavirus disease after haematopoeitic stem-cell transplantation in patients with severe combined immunodeficiency cauesd by common gamma cytokine receptor subunit or JAK-3 deficiency.* Lancet, 2004(363): p. 2051-4.
66. Leiding, J.W. and S.M. Holland, *Warts and all: Human papillomavirus in primary immunodeficiencies.* Journal of Allergy and Clinical Immunology, 2012. **130**(5): p. 1030-1048.
67. Gaspar, H.B., C. Harwood, I. Leigh, and A.J. Thrasher, *Severe cutaneous papillomavirus disease after haematopoietic stem-cell transplantation in patients with severe combined immunodeficiency.* Br J Haematol, 2004. **127**(2): p. 232-3.
68. Kamili, Q.-u.-A., F.O. Seeborg, K. Saxena, S.K. Nicholas, P.P. Banerjee, L.S. Angelo, E.M. Mace, L.R. Forbes, C. Martinez, T.S. Wright, J.S. Orange, and I.C. Hanson, *Severe cutaneous human papilloma virus infection associated with Natural Killer cell deficiency following stem cell transplantation for severe combined immunodeficiency.* J Allergy Clin Immunol, 2014. **134**(6): p. 1451-1453.e1.
69. Rogers, M.H., R. Lwin, L. Fairbanks, B. Gerritsen, and H.B. Gaspar, *Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency.* Journal of Pediatrics, 2001. **139**(1): p. 44-50.

70. Hassan, A., C. Booth, A. Brightwell, Z. Allwood, P. Veys, K. Rao, M. Hönig, W. Friedrich, A. Gennery, M. Slatter, R. Bredius, A. Finocchi, C. Cancrini, A. Aiuti, F. Porta, A. Lanfranchi, M. Ridella, C. Steward, A. Filipovich, R. Marsh, V. Bordon, S. Al-Muhsen, H. Al-Mousa, Z. Alsum, H. Al-Dhekri, A. Al Ghonaium, C. Speckmann, A. Fischer, N. Mahlaoui, K.E. Nichols, E. Grunebaum, D. Al Zahrani, C.M. Roifman, J. Boelens, E.G. Davies, M. Cavazzana-Calvo, L. Notarangelo, and H.B. Gaspar, *Outcome of hematopoietic stem cell transplantation for adenosine deaminase–deficient severe combined immunodeficiency.* Blood, 2012. **120**(17): p. 3615-3624.
71. Kesserwan, C., R. Sokolic, E.W. Cowen, E. Garabedian, K. Heselmeyer-Haddad, C.C. Lee, S. Pittaluga, C. Ortiz, K. Baird, D. Lopez-Terrada, J. Bridge, A.S. Wayne, and F. Candotti, *Multicentric dermatofibrosarcoma protuberans in patients with adenosine deaminase-deficient severe combined immune deficiency.* J Allergy Clin Immunol, 2012. **129**(3): p. 762-769 e1.
72. Buckley, R.H., *Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: longterm outcomes.* Immunol Res, 2011. **49**(1-3): p. 25-43.
73. Borghans, J.A., R.G. Bredius, M.D. Hazenberg, H. Roelofs, E.C. Jol-van der Zijde, J. Heidt, S.A. Otto, T.W. Kuijpers, W.E. Fibbe, J.M. Vossen, F. Miedema, and M.J. van Tol, *Early determinants of long-term T-cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency.* Blood, 2006. **108**(2): p. 763-9.
74. Sarzotti-Kelsoe, M., C.M. Win, R.E. Parrott, M. Cooney, B.K. Moser, J.L. Roberts, G.D. Sempowski, and R.H. Buckley, *Thymic output, T-cell diversity, and T-cell function in long-term human SCID chimeras*. Vol. 114. 2009. 1445-1453.
75. Buckley, R.H., *B-cell function in severe combined immunodeficiency after stem cell or gene therapy: a review.* Journal of Allergy & Clinical Immunology, 2010. **125**(4): p. 790-7.
76. Haddad, E., S. Leroy, and R.H. Buckley, *B-cell reconstitution for SCID: Should a conditioning regimen be used in SCID treatment?* Journal of Allergy and Clinical Immunology, 2013. **131**(4): p. 994-1000.
77. Mazzolari, E., D. de Martiis, C. Forino, A. Lanfranchi, S. Giliani, R. Marzollo, P. Airo, L. Imberti, F. Porta, and L.D. Notarangelo, *Single-center analysis of long-term outcome after hematopoietic cell transplantation in children with congenital severe T cell immunodeficiency.* Immunol Res, 2009. **44**(1-3): p. 4-17.
78. Dvorak, C.C., A. Hassan, M.A. Slatter, M. Hönig, A.C. Lankester, R.H. Buckley, M.A. Pulsipher, J.H. Davis, T. Güngör, M. Gabriel, J.H. Bleesing, N. Bunin, P. Sedlacek, J.A. Connelly, D.F. Crawford, L.D. Notarangelo, S.-Y. Pai, J. Hassid, P. Veys, A.R. Gennery, and M.J. Cowan, *Comparison of outcomes of hematopoietic stem cell transplantation without chemotherapy conditioning by using matched sibling and unrelated donors for treatment of severe combined immunodeficiency.* Journal of Allergy and Clinical Immunology, 2014. **134**(4): p. 935-943.e15.
79. Dvorak, C.C., G.-Y. Hung, B. Horn, E. Dunn, C.-Y. Oon, and M.J. Cowan, *Megadose CD34+ Cell Grafts Improve Recovery of T Cell Engraftment but not B Cell Immunity in Patients with Severe Combined Immunodeficiency Disease Undergoing Haplocompatible*

Nonmyeloablative Transplantation. Biology of Blood and Marrow Transplantation, 2008. **14**(10): p. 1125-1133.
80. Fernandes, J.F., V. Rocha, M. Labopin, B. Neven, D. Moshous, A.R. Gennery, W. Friedrich, F. Porta, C. Diaz de Heredia, D. Wall, Y. Bertrand, P. Veys, M. Slatter, A. Schulz, K.W. Chan, M. Grimley, M. Ayas, T. Gungor, W. Ebell, C. Bonfim, K. Kalwak, P. Taupin, S. Blanche, H.B. Gaspar, P. Landais, A. Fischer, E. Gluckman, and M. Cavazzana-Calvo, *Transplantation in patients with SCID: mismatched related stem cells or unrelated cord blood?* Blood, 2012. **119**(12): p. 2949-55.
81. Balashov, D., A. Shcherbina, M. Maschan, P. Trakhtman, Y. Skvortsova, L. Shelikhova, A. Laberko, A. Livshits, G. Novichkova, and A. Maschan, *Single-Center Experience of Unrelated and Haploidentical Stem Cell Transplantation with TCRαβ and CD19 Depletion in Children with Primary Immunodeficiency Syndromes.* Biology of Blood and Marrow Transplantation, 2015. **21**(11): p. 1955-1962.
82. Bertaina, A., P. Merli, S. Rutella, D. Pagliara, M.E. Bernardo, R. Masetti, D. Pende, M. Falco, R. Handgretinger, F. Moretta, B. Lucarelli, L.P. Brescia, G. Li Pira, M. Testi, C. Cancrini, N. Kabbara, R. Carsetti, A. Finocchi, A. Moretta, L. Moretta, and F. Locatelli, *HLA-haploidentical stem cell transplantation after removal of αβ⁺ T and B-cells in children with non-malignant disorders.* Blood, 2014.
83. Slatter, M., Z. Nademi, T.R. Leahy, B. Morillo-Gutierrez, J. Dunn, D. Barge, R. Skinner, C. Ryan, S. Hambleton, M. Abinun, T. Flood, A. Cant, and A. Gennery, *Haploidentical CD3 TCRalphabeta and CD19-depleted second stem cell transplant for steroid-resistant acute skin graft versus host disease.* J Allergy Clin Immunol, 2016. **138**(2): p. 603-605 e1.
84. Reis, M., J. Ogonek, M. Qesari, N.M. Borges, L. Nicholson, L. Preußner, A.M. Dickinson, X.-n. Wang, E.M. Weissinger, and A. Richter, *Recent Developments in Cellular Immunotherapy for HSCT-Associated Complications.* Front Immunol, 2016. **7**: p. 500.
85. Kapoor, N., A. Bertaina, P. Merli, S. Naik, W. Qasim, M. Slatter, R. Elfeky, B. Lucarelli, M. Smogorzewska, G. Li Pira, V. Bertaina, A. Moseley, and F. Locatelli, *Outcome of Children with Primary Immune-Deficiencies (PIDs) Enrolled in a Phase I-II Trial Based on the Infusion of BPX-501 Donor T Cells Genetically Modified with a Novel Suicide Gene (inducible Caspase 9, iC9) after T-Cell Depleted HLA-Haploidentical Allogeneic Stem Cell Transplantation (haplo-HSCT).* Blood, 2016. **128**(22): p. 72-72.
86. Bonichini, S., M. Tremolada, M. Pillon, D. Aloisio, and C. Messina, *Medical treatment late effects, quality of life and psychosocial sequelae in paediatric patients after hematopoietic stem cell transplantation*. 2012. p. 119-176.
87. Brice, L., R. Weiss, Y. Wei, P. Satwani, M. Bhatia, D. George, J. Garvin, E. Morris, L. Harrison, M.S. Cairo, and S.A. Sands, *Health-related quality of life (HRQoL): the impact of medical and demographic variables upon pediatric recipients of hematopoietic stem cell transplantation.* Pediatr Blood Cancer, 2011. **57**(7): p. 1179-85.
88. Sirilla, J. and J. Overcash, *Quality of life (QOL), supportive care, and spirituality in hematopoietic stem cell transplant (HSCT) patients.* Support Care Cancer, 2013. **21**(4): p. 1137-44.
89. Titman, P., E. Pink, E. Skucek, K. O'Hanlon, T.J. Cole, J. Gaspar, J. Xu-Bayford, A. Jones, A.J. Thrasher, E.G. Davies, P.A. Veys, and H.B.

Gaspar, *Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies.* Blood, 2008. **112**(9): p. 3907-3913.
90. Skucek, E., S. Butler, H.B. Gaspar, and P. Titman, *Social outcome in children treated by haematopoietic cell transplant for congenital immunodeficiency.* Bone Marrow Transplant, 2011. **46**(10): p. 1314-20.
91. Lin, M., K. Epport, C. Azen, R. Parkman, D. Kohn, and A. Shah, *Long-Term Neurocognitive Function of Pediatric Patients with Severe Combined Immune Deficiency (SCID): Pre- and Post-Hematopoietic Stem Cell Transplant (HSCT).* J Clin Immunol, 2009. **29**(2): p. 231-237.
92. Cole, T., F. McKendrick, P. Titman, A.J. Cant, M.S. Pearce, C.M. Cale, D. Goldblatt, and A.R. Gennery, *Health related quality of life and emotional health in children with chronic granulomatous disease: a comparison of those managed conservatively with those that have undergone haematopoietic stem cell transplant.* J Clin Immunol, 2013. **33**(1): p. 8-13.
93. Titman, P., Z. Allwood, C. Gilmour, C. Malcolmson, C. Duran-Persson, C. Cale, G. Davies, H. Gaspar, and A. Jones, *Quality of life in children with primary antibody deficiency.* J Clin Immunol, 2014. **34**(7): p. 844-52.
94. Barlogis, V., N. Mahlaoui, P. Auquier, I. Pellier, F. Fouyssac, C. Vercasson, M. Allouche, C.B. De Azevedo, F. Suarez, D. Moshous, B. Neven, M. Pasquet, E. Jeziorski, N. Aladjidi, N. Schleinitz, C. Thomas, V. Gandemer, F. Mazingue, P. Lutz, O. Hermine, C. Picard, S. Blanche, G. Michel, and A. Fischer, *Physical health conditions and quality of life in adults with primary immunodeficiency diagnosed during childhood: A French Reference Center for PIDs (CEREDIH) study.* Journal of Allergy and Clinical Immunology, 2017. **139**(4): p. 1275-1281.e7.
95. Eapen, M., K.W. Ahn, P.J. Orchard, M.J. Cowan, S.M. Davies, A. Fasth, A. Hassebroek, M. Ayas, C. Bonfim, T.A. O'Brien, T.G. Gross, M. Horwitz, E. Horwitz, N. Kapoor, J. Kurtzberg, N. Majhail, O. Ringden, P. Szabolcs, P. Veys, and K.S. Baker, *Long-Term Survival and Late Deaths after Hematopoietic Cell Transplantation for Primary Immunodeficiency Diseases and Inborn Errors of Metabolism.* Biology of Blood and Marrow Transplantation, 2012. **18**(9): p. 1438-1445.
96. *Bone Marrow Transplant Unit, Great Ormond Street Hospital*. 2016 [cited 2016 27/07/2016]; Available from: http://www.gosh.nhs.uk/medical-information/clinical-specialties/bone-marrow-transplant-bmt-information-parents-and-visitors/bone-marrow-transplant-unit.
97. LeMaistre, C.F., S. Farnia, S. Crawford, J. McGuirk, R.T. Maziarz, J. Coates, D. Irwin, P. Martin, and J.L. Gajewski, *Standardization of Terminology for Episodes of Hematopoietic Stem Cell Patient Transplant Care.* Biology of Blood and Marrow Transplantation, 2013. **19**(6): p. 851-857.
98. Apperley J. and Masszi T., *Graft-versus-host disease*, in *The EBMT Handbook : Haematopoeitic Stem Cell Transplantation*, Apperly J, et al., Editors. 2012, Forum service editore: France
99. Gennery, A.R. and A.J. Cant, *Diagnosis of severe combined immunodeficiency.* Journal of Clinical Pathology, 2001. **54**(3): p. 191-195.
100. Filipovich AH, Daniel Weisdorf, Steven Pavletic, Gerard Socie, John R. Wingard, Stephanie J. Lee, Paul Martin, Jason Chien, Donna Przepiorka, Daniel Couriel, Edward W. Cowen, Patricia Dinndorf, Ann Farrell, Robert Hartzman, Jean Henslee-Downey, David Jacobsohn, George McDonald,

Barbara Mittleman, J. Douglas Rizzo, Michael Robinson, Mark Schubert, Kirk Schultz, Howard Shulman, Maria Turner, Georgia Vogelsang, and M.E.D. Flowers, *National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report.* Biology of Blood and Marrow Transplantation, 2005. **11**(12): p. 945-956.

101. Cromer, B., *Menstrual Problems*, in *Nelson Textbook Of Pediatrics*, R.M. Kliegman, et al., Editors. 2011, Elsevier Saunders: Philadelphia, USA. p. 685 - 688.
102. Keane, V., *Assessment of Growth*, in *Nelson Textbook of Pediatrics*, R.M. Kliegman, et al., Editors. 2011, Elsevier Saunders: Philadelphia, USA. p. e13-0 - e13-5.
103. Johnson, J.D. and W.M. Theurer, *A stepwise approach to the interpretation of pulmonary function tests.* Am Fam Physician, 2014. **89**(5): p. 359-66.
104. Cavazzana-Calvo, M., F. Carlier, F. Le Deist, E. Morillon, P. Taupin, D. Gautier, I. Radford-Weiss, S. Caillat-Zucman, B. Neven, S. Blanche, R. Cheynier, A. Fischer, and S. Hacein-Bey-Abina, *Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype.* Blood, 2007. **109**(10): p. 4575-4581.
105. Douek, D.C., R.D. McFarland, P.H. Keiser, E.A. Gage, J.M. Massey, B.F. Haynes, M.A. Polis, A.T. Haase, M.B. Feinberg, J.L. Sullivan, B.D. Jamieson, J.A. Zack, L.J. Picker, and R.A. Koup, *Changes in thymic function with age and during the treatment of HIV infection.* Nature, 1998. **396**(6712): p. 690-695.
106. Patel, D.D., M.E. Gooding, R.E. Parrott, K.M. Curtis, B.F. Haynes, and R.H. Buckley, *Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency.* New England Journal of Medicine, 2000. **342**(18): p. 1325-32.
107. Bader, P., D. Niethammer, A. Willasch, H. Kreyenberg, and T. Klingebiel, *How and when should we monitor chimerism after allogeneic stem cell transplantation?* Bone Marrow Transplant, 2005. **35**(2): p. 107-19.
108. McCann, S.R., M. Crampe, K. Molloy, and M. Lawler, *Hemopoietic chimerism following stem cell transplantation.* Transfus Apher Sci, 2005. **32**(1): p. 55-61.
109. WHO, *Study protocol for the World Health Organization project to develop a Quality of Life assessment instrument (WHOQOL).* Qual Life Res, 1993. **2**(2): p. 153-9.
110. Wallander, J.L. and H.M. Koot, *Quality of life in children: A critical examination of concepts, approaches, issues, and future directions.* Clin Psychol Rev, 2016. **45**: p. 131-43.
111. Varni, J.W., T.M. Burwinkle, M. Seid, and D. Skarr, *The PedsQLTM 4.0 as a pediatric population health measure: Feasibility, reliability, and validity.* Ambul Pediatr, 2003. **3**: p. 329 - 341.
112. Upton, P., C. Eiser, I. Cheung, H. Hutchings, M. Jenney, A. Maddocks, I. Russell, and J. Williams, *Measurement properties of the UK-English version of the Pediatric Quality of Life InventoryTM 4.0 (PedsQLTM) generic core scales.* Health and Quality of Life Outcomes, 2005. **3**(1): p. 22.

113. Liu, P., Y. Zhao, J.W. Xiao, C. Zhang, and X.D. Zhao, *[Respiratory syncytial virus infection in hematopoietic stem cell transplantation recipients with primary immunodeficiencies]*. Zhonghua Erke Zazhi. **49**(7): p. 489-94.
114. Singer, J.D. and J.B. Willet, *Applied longitudinal data analysis : modelling change and event*. 2003, New York: Oxford University Press, Inc.
115. Rabe-Hesketh, S. and S. A., *Multilevel and longitudinal modelling using Stata, Volume II: Categorical responses, counts and survival.* . 2012, College Station, TX: Stata Press.
116. JPT, H., *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*, G. S, Editor. 2011, The Cochrane Collaboration.
117. Sedgwick, P., *Standard deviation or the standard error of the mean.* BMJ (Clinical research ed), 2015. **350**: p. h831.
118. Kesserwan, C., R. Sokolic, E.W. Cowen, E. Garabedian, K. Heselmeyer-Haddad, C.-C.R. Lee, S. Pittaluga, C. Ortiz, K. Baird, D. Lopez-Terrada, J. Bridge, A.S. Wayne, and F. Candotti, *Multicentric dermatofibrosarcoma protuberans in patients with adenosine deaminase-deficient severe combined immune deficiency.* J Allergy Clin Immunol, 2012. **129**(3): p. 762-769.e1.
119. Myers, L.A., D.D. Patel, J.M. Puck, and R.H. Buckley, *Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival.* Blood, 2002. **99**(3): p. 872-8.
120. Kane, L., A.R. Gennery, B.N. Crooks, T.J. Flood, M. Abinun, and A.J. Cant, *Neonatal bone marrow transplantation for severe combined immunodeficiency.* Arch Dis Child Fetal Neonatal Ed, 2001. **85**(2): p. F110-3.
121. Mazzolari, E., C. Forino, S. Guerci, L. Imberti, A. Lanfranchi, F. Porta, and L.D. Notarangelo, *Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency.* Journal of Allergy and Clinical Immunology, 2007. **120**(4): p. 892-899.
122. Honig, M., M.H. Albert, A. Schulz, M. Sparber-Sauer, C. Schutz, B. Belohradsky, T. Gungor, M.T. Rojewski, H. Bode, U. Pannicke, D. Lippold, K. Schwarz, K.M. Debatin, M.S. Hershfield, and W. Friedrich, *Patients with adenosine deaminase deficiency surviving after hematopoietic stem cell transplantation are at high risk of CNS complications.* Blood, 2007. **109**(8): p. 3595-3602.
123. Whitmore, K.V. and H.B. Gaspar, *Adenosine deaminase deficiency - more than just an immunodeficiency.* Front Immunol, 2016. **7**(AUG).
124. Sauer, A.V., R.J. Hernandez, F. Fumagalli, V. Bianchi, P.L. Poliani, C. Dallatomasina, E. Riboni, L.S. Politi, A. Tabucchi, F. Carlucci, M. Casiraghi, N. Carriglio, M. Cominelli, C.A. Forcellini, F. Barzaghi, F. Ferrua, F. Minicucci, S. Medaglini, L. Leocani, G. la Marca, L.D. Notarangelo, C. Azzari, G. Comi, C. Baldoli, S. Canale, M. Sessa, P. D'Adamo, and A. Aiuti, *Alterations in the brain adenosine metabolism cause behavioral and neurological impairment in ADA-deficient mice and patients.* Sci Rep, 2017. **7**: p. 40136.
125. Cicalese, M.P., F. Ferrua, L. Castagnaro, R. Pajno, F. Barzaghi, S. Giannelli, F. Dionisio, I. Brigida, M. Bonopane, M. Casiraghi, A. Tabucchi, F. Carlucci, E. Grunebaum, M. Adeli, R.G. Bredius, J.M. Puck,

P. Stepensky, I. Tezcan, K. Rolfe, E. De Boever, R.R. Reinhardt, J. Appleby, F. Ciceri, M.G. Roncarolo, and A. Aiuti, *Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency.* Blood, 2016. **128**(1): p. 45-54.
126. Dorsey, M.J., C.C. Dvorak, M.J. Cowan, and J.M. Puck, *Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening.* Journal of Allergy and Clinical Immunology, 2017. **139**(3): p. 733-742.
127. Kwan, A. and J.M. Puck, *History and current status of newborn screening for severe combined immunodeficiency.* Semin Perinatol, 2015. **39**(3): p. 194-205.
128. Kohn, D.B. and H.B. Gaspar, *How We Manage Adenosine Deaminase-Deficient Severe Combined Immune Deficiency (ADA SCID).* J Clin Immunol, 2017: p. 1-6.
129. O'Marcaigh, A.S., K. DeSantes, D. Hu, H. Pabst, B. Horn, L. Li, and M.J. Cowan, *Bone marrow transplantation for T-B- severe combined immunodeficiency disease in Athabascan-speaking native Americans.* Bone Marrow Transplant, 2001. **27**(7): p. 703-9.
130. Cole, B.O., R.R. Welbury, E. Bond, and M. Abinun, *Dental manifestations in severe combined immunodeficiency following bone marrow transplantation.* Bone Marrow Transplant, 2000. **25**(9): p. 1007-9.
131. Slatter, M.A., K. Rao, P. Amrolia, T. Flood, M. Abinun, S. Hambleton, Z. Nademi, N. Goulden, G. Davies, W. Qasim, H.B. Gaspar, A. Cant, A.R. Gennery, and P. Veys, *Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience.* Blood, 2011. **117**(16): p. 4367-75.
132. Slatter, M.A., H. Boztug, U. Potschger, K.W. Sykora, A. Lankester, I. Yaniv, P. Sedlacek, E. Glogova, P. Veys, A.R. Gennery, C. Peters, E.I. Errors, and P. Paediatric Diseases Working, *Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases.* Bone Marrow Transplant, 2015. **50**(12): p. 1536-41.
133. White, H., A. Thrasher, P. Veys, C. Kinnon, and H.B. Gaspar, *Intrinsic defects of B cell function in X-linked severe combined immunodeficiency.* European journal of immunology, 2000. **30**(3): p. 732-7.
134. Lynch, H.E., G.L. Goldberg, A. Chidgey, M.R.M. Van den Brink, R. Boyd, and G.D. Sempowski, *Thymic involution and immune reconstitution.* Trends in Immunology, 2009. **30**(7): p. 366-373.
135. Thrasher, A.J., S. Hacein-Bey-Abina, H.B. Gaspar, S. Blanche, E.G. Davies, K. Parsley, K. Gilmour, D. King, S. Howe, J. Sinclair, C. Hue, F. Carlier, C. von Kalle, G. de Saint Basile, F. le Deist, A. Fischer, and M. Cavazzana-Calvo, *Failure of SCID-X1 gene therapy in older patients.* Blood, 2005. **105**(11): p. 4255-7.
136. Rabe-Hesketh, S. and S. A., *Multilevel and longitudinal modelling using Stata, Volume II: Categorical responses, counts, and survival.* . 2012, College Station, TX: Stata Press.
137. Xanthakis, V., L.M. Sullivan, and R.S. Vasan, *Multilevel modeling versus cross-sectional analysis for assessing the longitudinal tracking of cardiovascular risk factors over time.* Statistics in Medicine, 2013. **32**(28): p. 5028-5038.

138. Hacein-Bey-Abina, S., A. Fischer, and M. Cavazzana-Calvo, *Gene therapy of X-linked severe combined immunodeficiency.* International Journal of Hematology. **76**(4): p. 295-8.
139. Cavazzana-Calvo, M., S. Hacein-Bey, G. de Saint Basile, F. Gross, E. Yvon, P. Nusbaum, F. Selz, C. Hue, S. Certain, J.L. Casanova, P. Bousso, F.L. Deist, and A. Fischer, *Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease.* Science (New York, N Y), 2000. **288**(5466): p. 669-72.
140. Aiuti, A., S. Slavin, M. Aker, F. Ficara, S. Deola, A. Mortellaro, S. Morecki, G. Andolfi, A. Tabucchi, F. Carlucci, E. Marinello, F. Cattaneo, S. Vai, P. Servida, R. Miniero, M.G. Roncarolo, and C. Bordignon, *Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning.* Science (New York, N Y), 2002. **296**(5577): p. 2410-3.
141. Hacein-Bey-Abina, S., F. Le Deist, F. Carlier, C. Bouneaud, C. Hue, J.-P. De Villartay, A.J. Thrasher, N. Wulffraat, R. Sorensen, S. Dupuis-Girod, A. Fischer, E.G. Davies, W. Kuis, L. Leiva, and M. Cavazzana-Calvo, *Sustained Correction of X-Linked Severe Combined Immunodeficiency by ex Vivo Gene Therapy.* New England Journal of Medicine, 2002. **346**(16): p. 1185-1193.
142. Gaspar, H.B., K.L. Parsley, S. Howe, D. King, K.C. Gilmour, J. Sinclair, G. Brouns, M. Schmidt, C. Von Kalle, T. Barington, M.A. Jakobsen, H.O. Christensen, A. Al Ghonaium, H.N. White, J.L. Smith, R.J. Levinsky, R.R. Ali, C. Kinnon, and A.J. Thrasher, *Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector.* Lancet (London, England), 2004. **364**(9452): p. 2181-7.
143. Aiuti, A., F. Cattaneo, S. Galimberti, U. Benninghoff, B. Cassani, L. Callegaro, S. Scaramuzza, G. Andolfi, M. Mirolo, I. Brigida, A. Tabucchi, F. Carlucci, M. Eibl, M. Aker, S. Slavin, H. Al-Mousa, A. Al Ghonaium, A. Ferster, A. Duppenthaler, L. Notarangelo, U. Wintergerst, R.H. Buckley, M. Bregni, S. Marktel, M.G. Valsecchi, P. Rossi, F. Ciceri, R. Miniero, C. Bordignon, and M.-G. Roncarolo, *Gene therapy for immunodeficiency due to adenosine deaminase deficiency.* The New England journal of medicine, 2009. **360**(5): p. 447-58.
144. Boudes, P.F., *Gene therapy as a new treatment option for inherited monogenic diseases.* Eur J Intern Med, 2014. **25**(1): p. 31-6.
145. Candotti, F., K.L. Shaw, L. Muul, D. Carbonaro, R. Sokolic, C. Choi, S.H. Schurman, E. Garabedian, C. Kesserwan, G.J. Jagadeesh, P.-Y. Fu, E. Gschweng, A. Cooper, J.F. Tisdale, K.I. Weinberg, G.M. Crooks, N. Kapoor, A. Shah, H. Abdel-Azim, X.-J. Yu, M. Smogorzewska, A.S. Wayne, H.M. Rosenblatt, C.M. Davis, C. Hanson, R.G. Rishi, X. Wang, D. Gjertson, O.O. Yang, A. Balamurugan, G. Bauer, J.A. Ireland, B.C. Engel, G.M. Podsakoff, M.S. Hershfield, R.M. Blaese, R. Parkman, and D.B. Kohn, *Gene therapy for adenosine deaminase–deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans.* Blood, 2012. **120**(18): p. 3635-3646.
146. Hacein-Bey-Abina, S., S.-Y. Pai, H.B. Gaspar, M. Armant, C.C. Berry, S. Blanche, J. Bleesing, J. Blondeau, H. de Boer, K.F. Buckland, L. Caccavelli, G. Cros, S. De Oliveira, K.S. Fernández, D. Guo, C.E. Harris, G. Hopkins, L.E. Lehmann, A. Lim, W.B. London, J.C.M. van der Loo, N. Malani, F. Male, P. Malik, M.A. Marinovic, A.-M. McNicol, D. Moshous, B. Neven, M. Oleastro, C. Picard, J. Ritz, C. Rivat, A. Schambach, K.L.

Shaw, E.A. Sherman, L.E. Silberstein, E. Six, F. Touzot, A. Tsytsykova, J. Xu-Bayford, C. Baum, F.D. Bushman, A. Fischer, D.B. Kohn, A.H. Filipovich, L.D. Notarangelo, M. Cavazzana, D.A. Williams, and A.J. Thrasher, *A Modified γ-Retrovirus Vector for X-Linked Severe Combined Immunodeficiency.* New England Journal of Medicine, 2014. **371**(15): p. 1407-1417.

147. Chan, K. and J.M. Puck, *Development of population-based newborn screening for severe combined immunodeficiency.* Journal of Allergy and Clinical Immunology, 2005. **115**(2): p. 391-398.
148. Puck, J.M., *Neonatal screening for severe combined immune deficiency.* Curr Opin Allergy Clin Immunol, 2007. **7**(6): p. 522-7.
149. Puck, J.M., *Population-based newborn screening for severe combined immunodeficiency.* Biol Blood Marrow Transplant, 2008. **14**(1 Suppl 1): p. 78-80.
150. Puck, J.M. and S.N.S.W. Group, *Population-based newborn screening for severe combined immunodeficiency: steps toward implementation.* J Allergy Clin Immunol, 2007. **120**(4): p. 760-8.
151. Ellen A. Lipstein, Sienna Vorona BA, Nancy S. Green, Alex R. Kemper, Alixandra A. Knapp, Lisa A. Prosser, and J.M. Perrin, *Systematic Evidence Review of Newborn Screening and Treatment of Severe Combined Immunodeficiency.* Pediatrics, 2010. **125**(5): p. e1226-e1235.
152. Kwan, A., R.S. Abraham, R. Currier, and et al., *Newborn screening for severe combined immunodeficiency in 11 screening programs in the united states.* JAMA, 2014. **312**(7): p. 729-738.
153. Buckley, R.H., *The long quest for neonatal screening for severe combined immunodeficiency.* Journal of Allergy and Clinical Immunology, 2012. **129**(3): p. 597-604.
154. Buelow, B.J., J.W. Verbsky, and J.M. Routes, *Newborn screening for SCID: lessons learned.* Expert Rev Hematol, 2016. **9**(6): p. 579-84.
155. Fozza, C., F. Barraqueddu, G. Corda, S. Contini, P. Virdis, F. Dore, S. Bonfigli, and M. Longinotti, *Study of the T-cell receptor repertoire by CDR3 spectratyping.* Journal of Immunological Methods, 2017. **440**: p. 1-11.