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

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Chapter 3

Improved transplant survival and long-term disease outcome in children with MHC class II deficiency

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Abstract

Major histocompatibility complex (MHC) class II deficiency is a rare but life-threatening primary combined immunodeficiency. Haematopoietic cell transplantation (HCT) remains the only curative treatment for this condition, but transplant survival in the published series was poor. We analysed the outcome of 25 such patients undergoing first HCT at our centre between 1995 and 2018. Median age at diagnosis was 6.5 months (range, birth to 7.5 years). Median age at transplant was 21.4 months (range, 0.1-7.8 years). Donors were matched family donors (MFD) (n=6), unrelated donors (UD) (n=12) and haploidentical donors (HID) (n=7). PBSC was the stem cell source in 68% of patients. Conditioning was treosulfan-based in 84% of patients; 84% received either alemtuzumab (n=14) or ATG (n=8) as serotherapy. With a 2.9-year median follow-up, overall survival (OS) improved from 33% (95%CI, 46-68%) for the children transplanted before 2008 (n=6) to 94% (95% CI, 66-99%) for children transplanted after 2008 (n=19) (p=0.003). For HCT after 2008, the OS according to donor was 100% for MFD, 100% for UD and 85% for HID (p=0.40). None had grade III-IV acute or chronic GvHD. Latest median donor myeloid and lymphocyte chimerism was 100% (range, 0-100%) and 100% (range, 64-100%) respectively. Latest $CD4^+$ T-lymphocyte number was significantly lower in transplant survivors (n=14) compared to post-transplant disease controls (p=0.01). All survivors were off immunoglobulin replacement and had protective vaccine responses to tetanus and *Haemophilus influenza*. None had any significant infection or autoimmunity. Changing transplant strategy in our center has significantly improved outcomes for MHC class II deficiency.

Introduction

Major histocompatibility complex (MHC) class II deficiency is a rare autosomal recessive combined immunodeficiency.(15) The MHC class II genes are located on chromosome 6 - expression is largely restricted to activated T-lymphocytes, thymic epithelial cells and antigen presenting cells [APC] (dendritic cells, macrophages and B-lymphocytes). In patients with MHC class II deficiency, the MHC locus itself is intact but transcriptionally silent owing to loss-of-function mutations in one of four genes encoding the key regulatory factors, *CIITA* (class II transactivator), *RFX5* (regulatory factor 5), *RFXAP* (RFX-associated protein) and *RFXANK* (RFX-associated ankyrin-containing protein). MHC class II molecules are pivotal for the adaptive immune system and guide the development and function of CD4+ T-lymphocytes. The immunologic hallmark of the disease is the absence of constitutive and inducible expression of MHC class II molecules on all cell types which leads to impaired antigen presentation by HLA-DR, HLA-DQ, and HLA-DP molecules on APC.(16) The lack of MHC class II expression on thymic epithelium leads to delayed and incomplete maturation of CD4+ T-lymphocyte population. Overall, MHC class II deficiency leads to combined immunodeficiency with defective CD4+ T-lymphocyte maturation and activation and a lack of T helper lymphocyte-dependent antibody production by B-lymphocytes, resulting in significant susceptibility to severe infections(17).

Haematopoietic cell transplantation (HCT) is the only curative therapy for children with MHC class II deficiency. The natural history of non-transplanted patients is dismal and the main cause of death is overwhelming viral infection.(18) Very few children survive into adulthood. (19) HCT for MHC class II deficiency is challenging as many children have significant co-morbidities at the time of HCT, increasing their susceptibility to regimen-related toxicities, serious infections, graft rejection and graft-versus-host disease (GvHD). Historically the use of HCT has been limited due to the high risk of transplant-related morbidity and mortality. Reported transplant survival was poor compared to those seen in children with classical severe combined immunodeficiency (SCID), with a survival rate of 50% or less(17, 20-23). In the light of significant improvements in transplant care for children with primary immunodeficiency (PID) over time, the present retrospective study aimed to examine the transplant survival and long-term disease outcomes of children with MHC class II deficiency transplanted at a single national centre.

Methods

From January 1995 to December 2018, a total of 25 children with MHC class II deficiency underwent first HCT at Great North Children's hospital, Newcastle Upon Tyne, United

Kingdom. The diagnosis of MHC class II deficiency was confirmed by absence of DR expression on monocytes, B lymphocytes and T lymphocytes. The clinical and laboratory data were retrieved from the transplantation database, patients' medical files and laboratory records. Long-term follow-up outcomes from overseas patients were collected from respective referral hospitals. Written informed consent was obtained from the parents or legal guardians of the patients as per institutional practice for HCT.

Donor selection, stem cell source, conditioning regimen and GvHD prophylaxis

The donor selection hierarchy was as follows: (i) matched family donor, (ii) matched unrelated donor, (iii) single antigen mismatched unrelated donor, (iv) haploidentical donor. For unrelated donor grafts, donor-recipient matching was by allele-level typing at HLA-A, -B, -C, -DQ and –DR. Prior to 2009, the conditioning regimen was busulfan or treosulfan, cyclophosphamide with or without serotherapy. From 2009, the conditioning regimen was switched to a myeloablative reduced toxicity regimen (RTC) using fludarabine, treosulfan and alemtuzumab for matched family and unrelated donors while fludarabine, treosulfan, thiotepa, ATG (Grafalon®) and rituximab were used for TCRαβ/CD19-depleted parental grafts. Marrow was the conventional preferred stem cell source but PBSC was preferred for patients who received RTC with matched donors or TCRαβ/CD19-depleted parental grafts. Before 2008, GvHD prophylaxis regimens were variable but consisted primarily of ciclosporin alone (CSA) or in combination with methotrexate (MTX). From 2008, GvHD prophylaxis shifted almost exclusively to CSA and mycophenolate mofetil (MMF). From 2017, the recipients of TCRαβ/CD19-depleted parental graft did not receive any post-transplant GvHD prophylaxis.

Supportive care

Prior to transplant, all patients were screened by PCR for viremia, gut viruses and respiratory viruses from bronchoalveolar lavage samples. Surveillance for cytomegalovirus (CMV), adenovirus, Epstein Barr virus (EBV), human herpes virus type 6 (HHV6) viraemia, respiratory and gut viruses was performed weekly since 2000. All patients received antimicrobial prophylaxis against fungi, *Pneumocystis jiroveci* (PCP), and human herpesvirus reactivation. All patients received immunoglobulin replacement until normal IgM was made. Donor haematopoietic chimerism monitoring was monitored by molecular techniques.

Definition and endpoints

The main outcomes of interest were overall survival (OS) and event-free survival (EFS). OS was defined as survival from first HCT to last follow-up or death. An event was defined as death, graft failure or second procedures for slipping chimerism. Other endpoints assessed were as follows: (i) time to neutrophil recovery (first day of achieving a neutrophil count ≥ 0.5 × 10^9/L for three consecutive days); (ii) time to platelet recovery (platelet > 20 × 10^9/L without transfusions for 7 days); (iii) incidences of transplant-related complications as defined and graded according to existing institutional guidelines at the time of HSCT, including infections, hepatic veno-occlusive disease (VOD) and GvHD; (iv) degree of donor haematopoietic chimerism at the most recent assessment; (v) immune reconstitution.

Statistical analysis

Quantitative variables were described with median and range while categorical variables were reported with counts and percentages. Chi-square test was used to compare categorical variables. Probabilities of OS and EFS were calculated using the Kaplan-Meier estimate. Log-rank test was used to compare predictors on OS. Variables analysed included age at transplant, year of transplant (1995 to 2008 vs 2009 to 2018), donor type, stem cell source and stem cell dose. For immune reconstitution kinetics for first 12 months and latest lymphocyte subset results, a nested matched case-control study was performed in which each MHC class II-deficient patient was matched with 2 disease controls for the following variables: age (difference < 2 years at transplant for immune reconstitution kinetics and at last review for latest lymphocyte subset), donor type, stem cell source, conditioning regimen, serotherapy, GvHD prophylaxis and CMV reactivation. Disease controls (n=36) were patients who were transplanted for alternative PID diagnoses: severe combined immunodeficiency (SCID) (n=10), CD40 ligand deficiency (n=2), Wiskott-Aldrich syndrome (n=7) and chronic granulomatous disease (n=13) and other PID (n=4; 1 natural killer cell deficiency; 1 inducible co-stimulator deficiency; 1 hemophagocytic lymphohistiocytosis; 1 combined immunodeficiency. Lymphocyte subset at last review was available for 14 of 19 survivors (1 on active treatment for GvHD, 1 was less than one year post-HCT at the time of analysis, 3 overseas patients were uncontactable). Multilevel mixed effects modelling was performed for the longitudinal analysis of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, NK cells and HLA-DR. Wilcoxon rank-sum test was used to compare latest lymphocyte subsets between patients and disease controls as above. All *p*-values quoted are two-sided, with a level of significance of 0.05. Statistical analyses were performed using STATA 14.2.

Results

Patient characteristics

Patient clinical and immunological features at presentation are shown in tables 1 and 2. The median age at diagnosis was 6.5 months (range, at birth to 7.5 years). Immunological evaluation performed at referral showed that 14 (56%) had lymphocytopenia, 10 (40%) normal lymphocyte count and one had lymphocytosis. CD4+ lymphocytopenia was present in 19 patients (76%) and 6 (24%) had a normal CD4+ lymphocyte count. Six (24%) had CD19 lymphocytopenia. All except 4 patients who were diagnosed at birth had multiple infections. Ten (40%) had a history of *Pneumocystis jiroveci* pneumonia*,* 13 (53%) had viral enteropathy and 14 (56%) had growth failure at transplant. Two patients had autoimmune phenomena (1 polyarticular juvenile idiopathic arthritis (JIA); 1 probable autoimmune hepatitis). Two siblings were affected by macrophage activation syndrome (MAS) prior to transplant (patients 8 and 9). Patient 8 was diagnosed with polyarticular JIA at 14 months of age and treated with etanercept, infliximab, methotrexate, local and systemic steroids. The diagnosis of MHC class II deficiency was made following immunological evaluation for prolonged fever at the 7 years of age. Three months prior to HCT, she developed MAS with persistent fever, cytopenia, high serum ferritin (highest 38,348mg/L), elevated triglycerides, and low fibrinogen. She responded to steroid and ciclosporin. There were no clinical features and no laboratory evidence of MAS reactivation during HCT. Patient 9 was diagnosed at 22 months of age after the diagnosis of MHC class II deficiency was made in patient 8. Patient 9 had chronic diarrhoea since birth but did not have history of JIA. Similarly, patient 9 developed MAS while waiting for HCT, of which she responded well to steroid and ciclosporin. Three patients (Patients 15, 19, 21) had novel homozygous *RFXANK* mutation c.477C>A (p.Ser159Arg) and Patient 7 had novel homozygous *CIITA* mutation c.3003C>G (p.D1001E).

Transplantation characteristics

Transplantation characteristics are summarized in tables 2 and 3. The median age at transplant was 21.4 months (range, 0.9 months to 7.8 years) and the median interval between diagnosis and transplant was 9.2 months (range, 0.9 months to 6 years). Six (24%) had matched family donors, 12 (48%) had unrelated donors and 7 (28%) had haploidentical donors. Unmanipulated PBSC was the stem cell source in 10 (40%) patients and 7 (28%) received TCRαβ/CD19-depleted parental PBSC. Only one patient received a cord blood transplant. Treosulfan-based reduced toxicity conditioning was used in 84% of patients (n=21). Twenty-one patients (84%) received either alemtuzumab (n=14) or ATG (n=8) as serotherapy.

Engraftment and transplant-related complications (Table 3)

The median day to neutrophil and platelet engraftment was 15 days (range, 8 to 22 days), and 16 days (range, 11 to 42 days) respectively. Thirteen (52%) patients had acute GvHD, of whom 4 (16%) had grade II acute cutaneous GvHD and none had visceral GvHD. None had grade III-IV acute GvHD or chronic GvHD. All five patients who had pre-transplant parenteral-nutritional dependent enteropathy were able to establish full enteral nutrition post-transplant. The parenteral nutrition was discontinued at a median day of 135 post-HCT (range, 67 to 641 days) in these patients. Sixteen (64%) patients developed new onset viremia during transplant. Five of six deaths were due to transplant-related complications (4 pneumonitis; 1 cerebral haemorrhage). Patient 5 had congenital pelvic ureteric junction obstruction and died of urosepsis after two successful matched family donor transplants and good immune reconstitution.

Overall and event-free survival

The median duration of follow-up of surviving patients was 2.9 years (range, 0.7 to 7.3 years). The 3-year OS was 78% (95% CI, 56-91%), rising to 94% (95% CI, 66-99%) for children transplanted after 2008 (n=19) versus 33% (95%CI, 46-68%) for the children transplanted between 1995 and 2008 (n=6) (p=0.003) (Figure 1a and 1b). For transplants after 2008, the OS was comparable between matched family (100%), unrelated donor transplants (100%) and haploidentical donor (83%, 95% CI, 27 to 97%) (p=0.40) (Figure1c). Age at transplant (p=0.59), stem cell source (p=0.50), total nucleated cell dose (p=0.59) and CD34 cell dose (p=0.59) and stem cell doses were not associated with OS in patients who were transplanted between 2009 to 2018.

The 3-year EFS for the cohort was 78% (95% CI 56-91%). Of 3 patients (patient 2, 5 and 22) who had graft failure, two died (patients 2 and 22) during second transplant (1 cerebral haemorrhage; 1 pneumonitis). Patient 5 underwent a successful second transplant with good immune reconstitution but died of urosepsis 3.8 years after a second transplant.

Donor chimerism, immune reconstitution and long-term disease outcome

Long-term follow-up data, available for 14 transplant survivors, are shown in table 4. The median age of long-term survivors was 5.3 years (range, 2.2 to 14 years) with median duration of follow-up of 3.5 years (range, 1.1-7.3). The median donor myeloid and lymphocyte chimerism at last follow-up (n=19) was 100% (range, 0-100%) and 100% (range, 64 to 100%) respectively. Immune-reconstitution kinetics within first 12 months was available in 18 of 19 survivors (one patient was excluded for on-going active GvHD treatment) and is illustrated

in Figure 2. By comparison with disease controls, patients transplanted for MHC class II deficiency had significantly lower $CD4^+$ T-lymphocyte counts at months 5 (p=0.06), 6 (p=0.01) and 12 (p=0.002) post-transplant and at latest follow-up (p=0.01) (Figure 3). While seven survivors (50%) had low or low normal CD4+ T-lymphocytes at last review, the remainder had CD4+ T lymphocyte counts in the normal range. There was no significant difference in circulating $CD3^+$ (p=0.61), $CD8^+$ (p=0.59), $CD19^+$ (p=0.90), NK cells (p=0.22) or % activated T-lymphocytes (HLA-DR) (p=0.66) between patients and controls at any time point post-transplant. Donor type had no impact on immune reconstitution kinetics (supplemental figure 1). All long-term survivors were well with no end organ damage (Table 4). Patient 14 had good neurological recovery after enterovirus meningitis with communicating hydrocephalus. All survivors were off immunoglobulin replacement and had protective vaccine responses. None had any significant infection or autoimmunity.

Discussion

This is one of the largest haematopoietic cell transplantation series for MHC class II deficiency from a single transplant center over the past two decades. A number of important observations emerge from this report. Whilst the majority of patients present with classical clinical and immunological features, some patients can have non-infectious manifestations and an unusual pattern of laboratory results. The overall survival has improved significantly and no patient had either severe acute GvHD or chronic GvHD. Despite numerically decreased CD4+ T-lymphocyte reconstitution, all transplant survivors had good immunological outcome.

Children with MHC class II deficiency usually present with a clinical phenotype that is very similar to SCID and generally have severe CD4+ T-lymphocytopenia, hypogammaglobulinemia and lack of antigen-specific antibody responses. The CD4+ lymphocytopenia reflects the abnormal CD4+ thymocyte development due to defective MHC class II expression in the thymus. CD8+ T-lymphocyte counts may be normal or raised, leading to an inverted CD4/CD8 ratio. The diagnosis is confirmed by absence or very low HLA-DR expression on lymphocytes which is the immunological hallmark of the disease. In our cohort, 6 (24%) had a normal CD4+ T-lymphocyte, 4 (16%) had a high CD8+ T-lymphocyte count and 8 (32%) had a normal CD4:CD8 ratio. Autoimmunity was the first presentation in patient 8, who was treated with multiple biologic agents for refractory arthritis before the diagnosis of MHC class II deficiency was made. She and her younger sibling (patient 9), both of whom had homozygous *CIITA* mutation, developed macrophage activation syndrome prior to transplant, which has not been reported previously. Although disseminated BCGitis is not expected in these patients because of the presence of residual immunity in the form of $CD8^+$ T-lymphocytes and natural killer cells, one patient in our cohort had disseminated BCGosis prior to transplant.

We have shown that the 3-year OS has improved to 94% for children with MHC class II deficiency transplanted since 2008, comparable to HCT for children with severe combined immunodeficiency.(17, 20-24) Small *et al.* (2013) reported transplant survival of 69% in 16 children with MHC class II deficiency from five transplant centers in the Europe and the United States. Al-Mousa *et al.* (2010) reported a similar transplant survival of 66% in a cohort of 30 patients from a single transplant center in Saudi Arabia. Many factors have contributed to this improvement in our center including a detailed graft selection hierarchy, superior HLA matching technology, improved methods for graft manipulation, improved supportive care, vigilant infection surveillance and pre-emptive treatment, more effective anti-microbial therapy and a multidisciplinary team approach prior to and after HCT. In our center, we have used reduced toxicity conditioning with fludarabine, treosulfan, and alemtuzumab for family and unrelated donor transplant since 2009. Our haploidentical transplant strategy in this cohort includes conditioning with fludarabine, treosulfan, thiotepa, ATG (Grafalon®) and rituximab and graft manipulation by TCRαβ/CD19 depletion of peripheral blood stem cells. The OS following haploidentical transplant in our cohort was comparable with family and unrelated donor transplants, with a single fatality. Despite being diagnosed at 6 months of age, the deceased patient was referred for transplant at 6 years, by which age he had multiple viral infections, gut failure and chronic lung disease. The use of alemtuzumab and graft engineering has reduced the incidence of acute GvHD in our cohort compared to high reported incidence ranging 47-73%.(23, 25, 26) A high incidence of acute GvHD in previous reports may be due to inadequate in-vivo T-lymphocyte depletion and chronic viral infection. Despite pre-HCT enteropathy is common in our study cohort, none had gut GvHD.

The long-term disease outcome in our cohort is excellent with good immunological outcomes, infection-free and recovery of previous organ damage. In contrast to Klein *et al.'s* observation *of* persistent CD4+ lymphopenia in all eight transplant survivors in his series of 19 transplants, 50% (n=7) of the survivors have normal CD4+ lymphocytes post-transplant. (21) Godthelp *et al.* described impaired immune repertoire in two patients with partial engraftment post-HCT.(27) The persistent CD4+ T-lymphopenia in some patients might be explained by impaired thymocyte maturation caused by defective MHC class II expression on thymic epithelia. Our cohort with the largest series of transplant survivors showed that despite low CD4+ T-lymphocyte numbers, transplanted patients show normalization of antigen-specific T-lymphocyte stimulation and antibody production in response to immunization antigens. However, it is unclear what the long-term impact of decreased CD4+ T-lymphocyte reconstitution may be, particularly the risks of autoimmunity and premature immunosenescence.

In conclusion, HCT is a safe curative therapy for children with MHC class II deficiency. Unrelated and parental haploidentical donors are associated with excellent survival at our center, comparable to matched family donors. As young age at HCT is associated with favourable outcome in children with MHC class II deficiency, HCT should be performed as early as possible after the diagnosis, before the onset of disease-related organ damage. (21) Family screening plays an important role in advancing the transplant care for children with MHC class II deficiency. There is a need for a multi-center study using registry data to delineate the predictors of transplant survival and long-term disease outcomes in children with MHC class II deficiency. Advances in graft manipulation, additional cellular therapy and patient-tailored conditioning regimens may enable precise personalised transplant care and further improve the transplant survival while reducing late effects such as infertility. In addition, it is conceivable that gene therapy may in future offer a therapeutic alternative for children with MHC class II deficiency.

Table 1: Genetics and immunological features at diagnosis (N=25)

No/ Year of HCT	Country of origin (ethnicity)	Age at dx (mo)	Genetics	Lymphocyte subset (cells/L) at presentation/prior to HCT (abnormal result according to age based reference range)									DR expression (%)			Ig (g/L)			Auto anti-bodies[1]
				Lymp	CD3	CD19	CD56	CD4	CD8	CD4: CD8 ratio	Total naïve T cells	CD27+ IgM-IgD- (%)	T	B	M	G	A	M	
1*/1995	England (Pakistan)	4.9	Homozygous *CIITA* mutation	5796	4750	640	460	640 (↓)	4120 (↑)	0.15 (↓)	ND	ND	0	0	0	1.64	0.11	1.76	ND
2*/1997	England (Pakistan)	At birth	Homozygous *CIITA* mutation	4200	1905	368 (↓)	368	500 (↓)	1395	0.35 (↓)	ND	ND	0	0	0	6.16[#]	0.02	0.18	ND
3/1997	England (Bangladesh)	4.0	Homozygous *CIITA* mutation	1950 (↓)	858 (↓)	839	215	332 (↓)	585	0.57 (↓)	ND	ND	0	0	0	0.77	<0.13	0.08	ND
4/2003	England (Pakistan)	4.7	Homozygous *RFX5* c.1198C>T (p.R400X)	2876 (↓)	1409 (↓)	1167	252	249 (↓)	1109	0.22 (↓)	73	ND	0	0	0	0.76	<0.07	0.10	ND
5/2008	Scotland	8.4	Compound heterozygous *CIITA* mutation c. 2582T>A (p.L861Q); c. 2888+1G>A	1856 (↓)	1194 (↓)	400 (↓)	139	135 (↓)	1030	0.13 (↓)	ND	ND	0	0	0	0	0	0	ND
6/2008	England	15.3	Homozygous *RFX5* c.1198G>T (p.E413X)	20838 (↑)	17780 (↑)	2253	805	1259	14434 (↑	0.09 (↓)	711	ND	0	0	0	1.6	<0.07	<1.1	ND
7/2011	England	7.8	Homozygous *CIITA* mutation[§] c. 3003C>G (p.D1001E)	952 (↓)	331 (↓)	443 (↓)	164	106 (↓)	222(↓)	0.48 (↓)	162	<1	0	0	0	2.3	0.53	0.96	ND
8/2012	Scotland (Pakistan)	89.9	Homozygous *CIITA* mutation c. 1595T>C (p.532P)	5446	3851	956	611	519 (↓)	3203 (↑)	0.16 (↓)	616	4	0	0	0	14.9	<0.04	1.30	Negative
9/2012	Scotland (Pakistan)	22.7	Homozygous *CIITA* mutation c. 1595T>C (p.L532P)	8066	4478	3009	425	989	3520 (↑)	0.28 (↓)	1165	<1	0	0	0	NA	NA	NA	Negative
10/2015	Saudi	8.0	Homozygous *RFXANK* mutation c.362A>T (p.D121V)	2842 (↓)	1402	1056	329	777 (↓)	553	1.40	686	0	0	0	0	<1.0	0	0	ND
11/2015	Saudi	6.0	Homozygous *RFAXNK* mutation c.362A>T (p.R121V)	2988(↓)	1059	1163	685	453 (↓)	209 (↓)	2.17	ND	ND	0	0	0	2.3	0	0	ND
12*/2015	Saudi	7.6	Homozygous *RFAXNK* mutation c.362A>T (p.R121V)	6011	1987	3563	382	1581	300(↓)	5.27	596	0	0	0	0	<1.0	0.05	0.18	ND
13*/2015	Saudi	6.6	Homozygous *RFAXNK* mutation c.362A>T (p.R121V)	3161	1015	1621	436	695 (↓)	209	3.32	233	0	0	0	0	NA	NA	NA	ND
14/2015	Saudi	6.4	Homozygous *RFAXNK* mutation c.362A>T (p.R121V)	3361	1474	1529	304	1097	277	3.96	575	0	0	0	0	<3.2	0.43	<0.25	Negative
15/2016	Saudi	At birth	Homozygous *RFXANK* mutation[§] c.477C>A (p.S159R)	6939	5963(↑)	722	217	1077	4009 (↑)	0.27 (↓)	358	0	0	0	0	NA	NA	NA	ND

No/ Year of HCT	Country of origin (ethnicity)	Age at dx (mo)	Genetics	Lymphocyte subset (cells/L) at presentation/prior to HCT (abnormal result according to age based reference range)									DR expression (%)			Ig (g/L)			Auto anti-bodies[1]
				Lymp	CD3	CD19	CD56	CD4	CD8	CD4: CD8 ratio	Total naïve T cells	CD27+ IgM-IgD- (%)	T	B	M	G	A	M	
16/2016	Kuwait	At birth	Homozygous *RFXANK* mutation c.271+1G>C (IVS4+1G>c)	5679	4327	939	311	3126	1106	2.83	2652	0	0	0	0	NA	NA	NA	ND
17/2017	Saudi	17.0	Homozygous *RFXANK* mutation c.362A>T (p.D121V)	1126 (↓)	744 (↓)	198 (↓)	172	265 (↓)	390	0.68 (↓)	ND	ND	0	0	0	NA	NA	NA	ND
18/2017	Saudi	6.0	Homozygous *RFXANK* mutation c.271+1G>C (IVS4+1G>c)	1004 (↓)	755 (↓)	123 (↓)	106	374 (↓)	445	0.84 (↓)	8	0	0	0	0	NA	NA	NA	ND
19*/2017	Saudi	13.3	Homozygous *RFXANK* mutation[§] c.477C>A (p.S159R)	1928 (↓)	1555	323	32 (↓)	378 (↓)	648	0.58 (↓)	218	<1	0	0	0	NA	NA	NA	ND
20/2017	Kuwait	4.6	Homozygous *RFXANK* mutation c.271+1delGinsTCAC	2615 (↓)	1551(↓)	797	227	849 (↓)	530	1.60	ND	ND	0	0	0	0.66	0.07	0.3	ND
21*/2017	Saudi	At birth	Homozygous *RFXANK* mutation[§] c.477C>A (p.S159R)	1196 (↓)	483 (↓)	603	94 (↓)	158 (↓	266 (↓)	0.59 (↓)	ND	ND	0	0	0	NA	NA	NA	ND
22/2017	Saudi	6	Homozygous *RFXANK* mutation c.271+1G>TCAC (IVS4+1G>TCAC)	1761 (↓)	1372	365	12 (↓)	509 (↓)	684	0.74 (↓)	ND	ND	0	0	0	NA	NA	NA	ND
23/2018	Saudi	6	Homozygous *RFXANK* mutation c.362A>T (p.D121V)	2010 (↓)	850 (↓)	971	151	414 (↓)	322 (↓)	1.28	206	0	0	0	0	NA	NA	NA	ND
24/2018	Saudi	60	Homozygous *RFAXNK* mutation c.362A>T (p.R121V)	918 (↓)	626 (↓)	193 (↓)	87 (↓)	190 (↓)	292 (↓)	0.65 (↓)	ND	ND	0	0	0	0.3	0.25	0.17	ND
25/2018	Bulgaria	6	Homozygous *RFXANK* mutation p.R78*C>T	4246	2287	1530	388	759 (↓)	1311	0.58 (↓)	297	ND	0	0	0	3.8	0.08	0.26	ND

*siblings; [§]Novel variant
#IgG level was done on Day 12 of life (normal level due to maternal transplacental IgG transfer)
[1]Autoantibodies: antinuclear antibody (ANA), double-stranded DNA (dsDNA), rheumatoid factor (RF), anti-gastric parietal cell ab, anti-Mitochondrial antibody, anti-smooth muscle antibody, centromere antibody, tissue transglutaminase ab, ENA RNP Sm, Sm Ab, Ro, anti-La antibody, Scl-70 antibody, Jo-1 antibody.
Lymph: lymphocyte; B: B cells; T: T cells; M; monocytes
NA: not available; ND: not done

Table 2: Detailed patient and transplant characteristics (N=25)

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
1/1995	4.9	7.0	2.1	Disseminated CMV infection (blood, liver, stools, NPS, urine) Poliovirus and rotavirus (stools) *Haemophilus influenzae* (NPS) Maternal T cell engraftment (skin biopsy proven)	MFD (paternal uncle)	BM	Oral Bu 16mg/kg (Bu AUC 1837mmolxmin) Cy 200mg/kg Alemtuzumab 1mg/kg CSA	None	Pneumonitis required mechanical ventilation AKI, seizures, toxic epidermal necrolysis (skin biopsy proven)	Died of CMV pneumonitis (post-mortem) at D+17
2/1997	At birth	0.9	0.8	***Staphylococcus aureus*** septicaemia	MFD (great grand uncle)	BM	Oral Bu 16mg/kg (no Bu pK) Cy 200mg/kg Rabbit ATG 2.2mg/kg CSA/MTX	None	Engraftment pneumonitis required mechanical ventilation and responded IST (treated with neb budesonide, neb immunoglobulin, systemic steroid, ATG, anti-TNF monoclonal antibody) Severe hypertension with seizures CMV viraemia *Staphylococcus epidermidis* bacteraemia Diagnosed to pyloric stenosis at D+56 and underwent pyloromyotomy on D+69	Secondary autologous reconstitution at D+76. Second HCT using a different 7/8 MMFD at 5.6 mos of age. Died of interstitial pneumonitis at D+88 post second HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
3/1997	4.0	5.8	1.8	PCP pneumonia (BAL) Candida perianal ulcers Congenital retinal dystrophy	MSD	BM	Oral Bu 16mg/kg (no Bu pK) Cy 200mg/kg No serotherapy CSA/MTX	None	Worsening pre-transplant respiratory dysfunction from D+9 and required mechanical ventilation from Day +17. NPS was positive for Parainfluenza 3 and rhinovirus. Treated with neb budesonide, neb IVIg, systemic steroid, ATG, neb and IV ribavirin	Died of Parainfluenza 3 pneumonitis at D+19
4/2003	4.7	6.2	1.5	PCP pneumonia (BAL) Fungal pneumonia (hyphae on BAL) Poliovirus (gut and NPS)	7/8 MMUD	CB	Flu 150mg/m^2 Melph 140mg/m^2 Alemtuzumab 1mg/kg CSA/steroid	None	*Staphylococcus epidermidis* bacteraemia CMV pneumonitis Rotavirus	Alive
5/2008	8.4	11.3	2.9	PCP pneumonia (BAL) Norovirus type 2 enteropathy HHV6 viraemia Maternal T cell engraftment Seizure with neurodevelopmental delay and absent corpus collusum Operated gut malrotation	MFD	BM	Treo 36mg/m^2 Cy 200mg/kg No serotherapy CSA/MMF	Grade I skin	Pneumonitis Capillary leakage syndrome Diagnosed to have pelvic ureteric junction obstruction at 2 years of age following urosepsis. Had stenting done Had sterile liver nodules 2.8 years of age Required ventricular peritoneal shunt at 3 years of age	Cryopreserved marrow top-up at D+54 for slipping chimerism Second conditioned HCT using the same donor at D+186 Died of urosepsis at 3.8 years post second-HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
6/2008	15.3	17.7	2.4	CMV (NPS) Norovirus type 2 enteropathy RSV and Parainfluenza 3 (NPS) Probable autoimmune hepatitis (isolated raised hepatic transaminases)	MSD	BM	Treo 42mg/m^2 Cy 200mg/kg No serotherapy CSA/MMF	Grade I skin	CMV viraemia Adenovirus (stools) Developed respiratory failure required mechanical ventilation at 8 mos post-HCT	Died of pneumonitis with multi-organ failure at Day +261. Post-mortem showed positive HHV6 in lung and liver.
7/2011	7.8	9.8	2.0	PCP pneumonia Chronic diarrhoea (normal gut biopsy and negative pathogens)	MRD	PBSC	Treo 36g/m^2 Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade I skin	Engraftment pneumonitis (treated with neb budesonide)	Alive
8 */2012	89.9	93.3	3.5	Polyarticular juvenile idiopathic arthritis since 14 mo of age, treated with steroid, etanercept and infliximab Macrophage activating syndrome at 7 years of age, treated with steroid and CSA Mycobacterium avium intracellulare (sputum and BAL)	MUD	PBSC	Treo 42g/m^2 Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade I skin	Disseminated adenovirus (blood, stools and NPS) CMV viraemia *Micrococcus luteus* bacteraemia	Alive

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
				Haemophilus influenzae (BAL) CMV (BAL) HHV7 (blood) Norovirus and sapovirus (stools) Right middle lobe bronchiectasis						
9*/2012	22.7	27.4	4.8	Macrophage activating syndrome at 2 year old, treated with steroid and CSA Bronchiectasis Norovirus enteropathy CMV (BAL)	9/10 A-MMUD	PBSC	Treo 42g/m^2 Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade II skin	Disseminated adenovirus (blood, gut, NPS) CMV viraemia	Alive
10/2015	8.0	16.2	8.2	PCP pneumonia ***Haemophilus influenzae*** (BAL) Pseudomonas aeruginosa (BAL)	9/10 C-MMUD	BM	Treo 42g/m^2 Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade I skin	***Staphylococcus epidermidis*** bacteraemia Immune reconstitution BCG abscess at D+141	Alive

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
11/2015	6.0	29.4	23.4	Diagnosed at 6 months as older sibling died at 6 years old Disseminated adenovirus (BAL, blood stools) Multiple gut viruses (Norovirus and adenovirus)	Maternal HIT	TCR αβ/ CD19 de-pleted PBSC	Treo 42g/m^2, Flu 150mg/m^2 Thio 10mg/kg ATG 15mg/kg RTX 200mg/mg CSA	Grade II skin	Disseminated adenovirus (blood, stool, NPS); received adenovirus CTL HHV6 viraemia Parainfluenza 1 *E.coli* urinary tract infection	Alive
12* /2015	7.6	15.7	8.1	Enterovirus hepatitis (biopsy proven) PN dependent enteropathy with multiple gut viruses (enterovirus and sapovirus) *Streptococcus oralis* bacteraemia	9/10 DQ-MMUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade II skin	None	Alive Stopped PN at Day +135 post-HCT
13* /2015	6.6	16.4	9.8	PCP pneumonia Enterovirus viraemia and hepatitis (biopsy proven) PN dependent enteropathy with multiple gut viruses (enterovirus and sapovirus)	9/10 DQ-MMUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade I skin	none	Alive Stopped PN at Day +110 post-HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
				E. coli and alpha haemolytic streptococcus bacteraemia						
14 /2015	6.5	21.4	14.9	PCP pneumonia Multiple gut viruses (norovirus, enterovirus)	Paternal HIT	TCR αβ/ CD19 depleted PBSC	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg CSA	None	Enterovirus meningitis with communicating hydrocephalus on Day+56	Alive
15 /2016	At birth	47.7	47.7	CMV viraemia Disseminated parechovirus (blood, stool) Sapovirus (stool) Non-tuberculous mycobacteria of lung (biopsy proven) *E.coli* urinary tract infection Osteopenic fracture of right tibia and fibula	MUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	None	Disseminated adenovirus (blood, eye swab) CMV viraemia	Alive

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
16 /2016	At birth	9.6	9.6	Parainfluenza III (BAL) *Streptococcus pneumoniae* (BAL) Enterovirus (stool)	MUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	None	None	Alive
17 /2017	17	31	14	Multiple gut viruses (adenovirus, sapovirus, enterovirus, norovirus) Multiple respiratory viruses (Parainfluenza 3, adenovirus, RSV) HHV6 viraemia *Streptococcus pneumoniae* (BAL)	Maternal HIT	TCR αβ/ CD19 de-pleted PBSC Add-back T-cells	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg No GvHD prophylaxis	Grade I skin	Disseminated adenovirus with pericardial effusion requiring pericardial window	Alive
18 /2017	6	60.7	54.7	PCP pneumonia Severe malnutrition PN dependent enteropathy with multiple gut viruses (norovirus, adenovirus, enterovirus) HHV6 viraemia Norovirus and adenovirus enteropathy	Paternal HIT	TCR αβ/ CD19 de-pleted PBSC Add-back T cells	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg No GvHD prophylaxis	Grade I skin	Disseminated adenovirus (blood, stool, NPS)	Alive Stopped PN at Day +67 post-HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
				Presumed fungal splenic abscess Multiple osteopenic fractures secondary to vitamin D deficiency						
19 */2017	13.2	81.7	68.4	PCP pneumonia Norovirus (gut) HHV6 viraemia	MUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg	None	Disseminated adenovirus (blood, stool, NPS)	Alive
20 /2017	4.6	12.3	7.7	HHV 6 (blood and CSF) Adenovirus (blood and stool) Coxsackie A type 6 (stool, NPS) Norovirus (stool) RSV, Parainfluenza 2 and 3 on NPS	Paternal HIT	TCR αβ/ CD19 depleted PBSC	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg No GvHD prophylaxis	Grade I skin	Disseminated adenovirus (blood, stool) HHV6 viraemia Encephalopathy of unknown aetiology (normal CSF and MRI brain). Full neurological recovery	Alive
21 */2017	At birth	62.7	62.7	Severe malnutrition PN dependent enteropathy with multiple gut viruses (norovirus, parechovirus, Parainfluenza 4 HHV6 viremia	MUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	None	HHV6 viraemia	Alive Stopped PN at Day +59 post-HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
22 /2017	24	73.6	49.6	Severe malnutrition Disseminated adenovirus (blood, BAL, stools) Disseminated CMV (blood, BAL) EBV in BAL HHV6 viraemia RSV (NPS) Multiple gut viruses (adenovirus, enterovirus, sapovirus, astrovirus, norovirus)	Paternal HIT	TCR αβ/ CD19 depleted PBSC Add-back T cell	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg No GvHD prophylaxis	Grade I skin	PN dependent gut failure Disseminated adenovirus RSV pneumonia HHV 6 viraemia	Received second HCT for secondary aplasia Died of cerebral haemorrhage post second HCT
23 /2018	6.0	22.2	16.7	HHV6 viraemia Norovirus (stool) RSV and Parainfluenza 1 (NPS)	MUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	None	HHV6 viraemia	Alive
24 /2018	5.0	78.8	73.8	PCP pneumonia RSV pneumonia PN dependent enteropathy with multiple gut viruses (adenovirus, norovirus, astrovirus) Candida oesophagitis Disseminated BCG at 3 years of age	9/10 A-MMUD	PBSC	Treo 42g/m^2, Flu 150mg/m^2 Alemtuzumab 1mg/kg CSA/MMF	Grade II skin	HHV6 viraemia PN-dependent viral enteropathy on gut biopsy; no evidence of gut GvHD on gut biopsy Slow immune reconstitution secondary steroid dependent skin acute GvHD	Alive Stopped PN at Day +641 post-HCT

No/ year of HCT	Age at dx (mo)	Age at HCT (mo)	Interval between dx and HCT (mo)	Pre-transplant infections and medical issues	Donor	Stem cell source	Conditioning and GvHD prophylaxis	Acute GvHD	Significant complications during and after HCT	Outcome
25 /2018	6.0	22.8	16.8	PCP pneumonia Chronic diarrhoea (*Salmonella sp* and norovirus)	Maternal HIT	TCR αβ/ CD19 depleted PBSC	Treo 42g/m^2, Flu 150mg/m^2, Thio 10mg/kg, RTX 200mg/m^2 ATG 15mg/kg No GvHD prophylaxis	Grade II skin	None	Alive

AKI: acute kidney injury; ATG: Anti-thymocyte globulin (Grafalon); AUC: area under the curve; BAL: Bronchoalveolar lavage; BM: bone marrow; Bu pK: Busulfan pharmacokinetics; CB; cord blood; CSA: ciclosporin; Cy: cyclophosphamide; GvHD: graft-versus-host disease; HCT: haematopoietic cell transplantation; HIT: haploidentical donor; HPT: hypertension; IST: immunosuppressive therapy; IV: intravenous, MAS: macrophage activating syndrome; MFD: matched family donor; MMFD: mismatched family donor; MSD: matched sibling donor; MTX; methotrexate; mo: months; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; NA: not available; neb: nebulize; NPS: nasopharyngeal specimen; PBSC: peripheral blood stem cell; PN: parenteral nutrition; RTX: Rituximab; TCD: T cell depleted marrow; Thio: Thiotepa; Treo: Treosulfan

Table 3: Patient and transplantation characteristics and outcome after HCT in children with MHC class II expression deficiency (N=25)

Patient characteristics	
Year of transplant	
1998-2007, n (%)	6 (24)
2008-2018, n (%)	19 (76)
Male, n (%)	15 (60)
Age at diagnosis, months, median (range)	6.5 (at birth – 89.6)
Age at transplant, months, median (range)	21.4 (0.9-93.3)
Interval between diagnosis and HCT, months, median (range)	9.2 (0.9 – 71.5)
Newborn MHC class II expression deficiency, n (%)	4 (16)
Positive family history, n (%)	12 (48)
Consanguineous parents, n (%)	22 (88)
BCG vaccination, n (%)	21 (84)
History of PCP pneumonia, n (%)	10 (40)
Pre-transplant chronic diarrhoea, n (%)	13 (52)
Growth failure at HCT (<9th centile), n (%)	14 (56)
Pre-transplant autoimmune disease, n (%)	2 (8)
Donor characteristics	
Type of donor	
Matched family donor, n (%)	6 (24)
Matched unrelated donor, n (%)	6 (24)
Mismatched unrelated donor^{+}, n (%)	6 (24)
Parental haploidentical donor*, n (%)	7 (28)
Stem cell source	
Marrow, n (%)	7 (28)
Unmanipulated PB, n (%)	10 (40)
TCR αβ/CD19 depleted PBSC, n (%)	7 (28)
CB, n (%)	1 (4)
Graft details	
Marrow	
TNC, x 10^8/kg, median (range)	4.1 (2.5-11.5)
CD34, x 10^6/kg, median (range)	3.4 (3.1-5.9)
CD3, x10^8/kg	0.61 (0.37-1.0)
CD19, x10^7/kg	3.85 (1.6-6.1)
Unmanipulated PB	
TNC, x 10^8/kg, median (range)	16.8 (13.4-42.7)
CD34, x 10^6/kg, median (range)	12.5 (6.3-28.6)
CD3, x10^8/kg	4.95 (3.4-9.6)
CD19, x10^7/kg	9.6 (1.6-22.0)
TCR αβ/CD19 depleted PBCS	
TNC, x 10^8/kg, median (range)	12.7 (7.6-28.0)
CD34, x 10^6/kg, median (range)	26.1 (6.9-56.6)
CD3, x107/kg	4.2 (1.4-45)
CD19, x10^5/kg	6.0 (3.6-12.0)
TCR αβ , x104/kg	5.0 (2.7-7.0)
NK cells x10^7/kg	5.5 (1.8-11.0)

Transplant characteristics	
Conditioning regimen	
Myeloablative conditioning (MAC)	
Busulfan-Cyclophosphamide, n (%)	3 (12)
Treosulfan-Cyclosphosphamide, n (%)	2 (8)
Fludarabine-Treosulfan-Thiotepa, n (%)	7 (28)
Reduced toxicity conditioning (RTC)	
Treosulfan-fludarabine, n (%)	12 (48)
Fludarabine-melphalan, n (%)	1 (4)
Serotherapy	
None, n (%)	4 (12)
ATG[#], n (%)	8 (32)
Alemtuzumab, n (%)	14 (56)
GVHD prophylaxis	
None, n (%)	5 (20)
CSA alone, n (%)	3 (12)
CSA + MTX, n (%)	2 (8)
CSA + MMF, n (%)	14 (56)
CSA + steroid (for CB), n (%)	1 (4)
Haematopoietic recovery	
Days to neutrophil recovery, median (range)	15 (8-22)
Days to platelet recovery, media (range)	16 (11-42)
Transplant-related complications	
Acute GvHD	13 (52)
Grade II-IV, n (%)	4 (16)
Grade III-IV, n (%)	0
Chronic GvHD[2]	0
Veno-occlusive disease, n (%)	0
CMV viraemia, n (%)	7 (28)
Adenoviraemia, n (%)	9 (36)
HHV6 viraemia, n (%)	7 (28)
EBV viraemia, n (%)	1 (4)
Number of patients required parenteral nutrition, n (%)	17(68)
Number of patients with graft failure, n (%)	3 (12)
Secondary autologous reconstitution, n (%)	2 (8)
Secondary aplasia, n (%)	1 (4)
Cause of death (n=6)	
Pneumonitis	4 (16)
Cerebral haemorrhage	1 (4)
Infection	1 (4)

[+]6 had 9/10 mismatched unrelated donor transplant; *3 received add-back T cells; [#]6 had ATG (Grafalon) and 1 had thymoglobulin

ATG: anti-thymocyte globulin (Grafalon) for haploidentical transplant; CB: cord blood; CSA: ciclosporin; MMF: mycophenolate mofetil; MTX: methotrexate; PBSC: peripheral blood stem cells; TNC: total nucleated cell dose

Table 4: Immunological features and donor chimerism of long-term transplant survivors at last follow-up (> 1 year post-HCT)

No/year of	Age at follow-up (years)	Time post-HCT (years)	Clinical status	Donor chimerism	Lymphocyte subset (cells/μL) at last follow-up						DR expression (%)			Stop IVIg replacement (time post-HCT)	Ig (g/L)			Vaccine response	
						CD3	CD19	CD4	CD8	CD4: CD8 ratio	Total naïve T cells	T*	B	M		G	A	M	Tet	Hib
4/2003	5.5	4.0	Well	CD15 85% CD3 98% CD15 60%	Pre-HCT	1409	1167	249	1109	0.22	ND	0	0	0	Yes (9 mo)	13.0	1.05	1.06	0.81	>9.0
					Post-HCT	1884	332	**310**	1156	0.26	NA	23	ND	ND						
7/2011	8.1	7.3	Well	CD15 42% CD3 81% CD19 76%	Pre-HCT	331 (↓)	443	106	222	0.48	162	0	0	0	Yes (9 mo)	11.9	1.66	0.58	>7.0	5.4
					Post-HCT	1660	545	**399**	1079	0.37	697	10	BD	ND						
8*/2012	14	7.2	Well. Unassisted menarche	CD15 94% CD3 69% CD19 95%	Pre-HCT	3851	956	519	3203	0.16	616	0	0	0	Yes (9 mo)	11.1	1.73	1.09	0.32	6.4
					Post-HCT	1106	327	**201**	829	0.24	353	16	92	95						
9*/2012	9.5	7.1	Well	CD15 8% CD3 8% CD19 59%	Pre-HCT	4478	3009	989	3520	0.28	1165	0	0	0	Yes (13 mo)	12.0	<0.04	1.15	4.00	>9.0
					Post-HCT	2375	770	**503**	1669	0.30	1093	11	9	2						
10/2015	4.4	3.0	Well	CD15: 0% CD3: 64%	Pre-HCT	1402	1056	777	553	1.40	686	0	0	0	Yes (12 mo)	7.4	0.81	0.61	1.68	ND
					Post-HCT	1127	340	**326**	680	0.48	315	ND	29	1						
11/2015	6.2	3.8	well	WB 100%	Pre-HCT	1059	1163	453	209	2.17	ND	0	0	0	Yes (10 mo)	8.4	1.1	1.1	ND	ND
					Post-HCT	3700	650	1900	1260	1.51	ND	ND	100	ND						
12*/2015	4.8	3.7	well	WB 100% at 6 mo post BMT	Pre-HCT	6011	1987	1581	300	5.27	300	0	0	0	Yes (13 mo)	NA	NA	NA	ND	ND
					Post-HCT	2232	298	804	952	0.84	NA	ND	ND	ND						
13*/2015	4.8	3.6	well	WB 100% at 6mo post HCT	Pre-HCT	1015	1621	695	209	3.32	233	0	0	0	Yes (12 mo)	NA	NA	NA	0.76	ND
					Post-HCT	2413	340	959	1114	0.85	ND	ND	ND	ND						
14/2015	5.1	3.4	Good neurological recovery but speech delay	WB 100% at 9 mo post HCT	Pre-HCT	1474	1529	1097	277	3.96	575	0	0	0	Yes (15 mo)	14.2	<0.05	1.28	0.56	ND
					Post-HCT	1956	460	863	604	1.43	ND	19	ND	ND						

No/year of	Age at follow-up (years)	Time post-HCT (years)	Clinical status	Donor chimerism	Lymphocyte subset (cells/μL) at last follow-up						DR expression (%)			Stop IVIg replacement (time post-HCT)	Ig (g/L)			Vaccine response	
					CD3	CD19	CD4	CD8	CD4: CD8 ratio	Total naïve T cells	T*	B	M		G	A	M	Tet	Hib
16/2016	2.8	2.0	Well	WB 100% at 4 mo post HCT	Pre-HCT 4327	939	3126	1106	2.83	2682	16	ND	62	Yes (12 mo)	5.74	0.41	0.32	3.77	ND
					Post0HCT 2398	371	1238	1373	0.90	1077									
17/2017	4.2	1.6	Well	CD15 100% CD3 100%	Pre-HCT 744	198	172	265	0.68	NA	0	0	0	Yes (14 mo)	7.7	0.66	0.97	2.23	ND
					Post HCT 2251	894	704	844	0.83	ND	ND	ND	ND						
19/2017	8.1	1.3	Well	WB 100% at 5 mo post HCT	Pre-HCT 1555	324	378	648	0.58	218	0	0	0	Yes (16 mo)	NA	NA	NA	3.04	ND
					Post-HCT 1357	847	**516**	649	0.80	NA	ND	ND	ND						
20/2017	2.2	1.2	Well	WB 100% at 6 mo post HCT	Pre-HCT 1551 (↓)	797	849	530	1.60	ND	0	0	0	Yes (12 mo)	5.5	0.25	0.49	ND	ND
					Post-HCT 2227	292	1092	710	1.53	ND	30	ND	70						
21*/2017	6.3	1.1	Well	WB 100% at 5 mo post-HCT	Pre-HCT 207 (↓)	58 (↓)	71	117	0.60	49	0	0	0	Yes (11 mo)	NA	NA	NA	0.09	ND
					Post-HCT 2337	947	**516**	649	0.80	NA	ND	ND	ND						

*T cells were not activated in DR expression; Red bold: below normal reference range for age; NA: not available; ND: not done; tet: tetanus; HCT: Haematopoietic cell transplantation; WB: whole blood

Normal tetatus antibody reference range: 0.01-10IU/μL; Normal haemophilus B antibodies (Hib): 1.0-20 mg/L

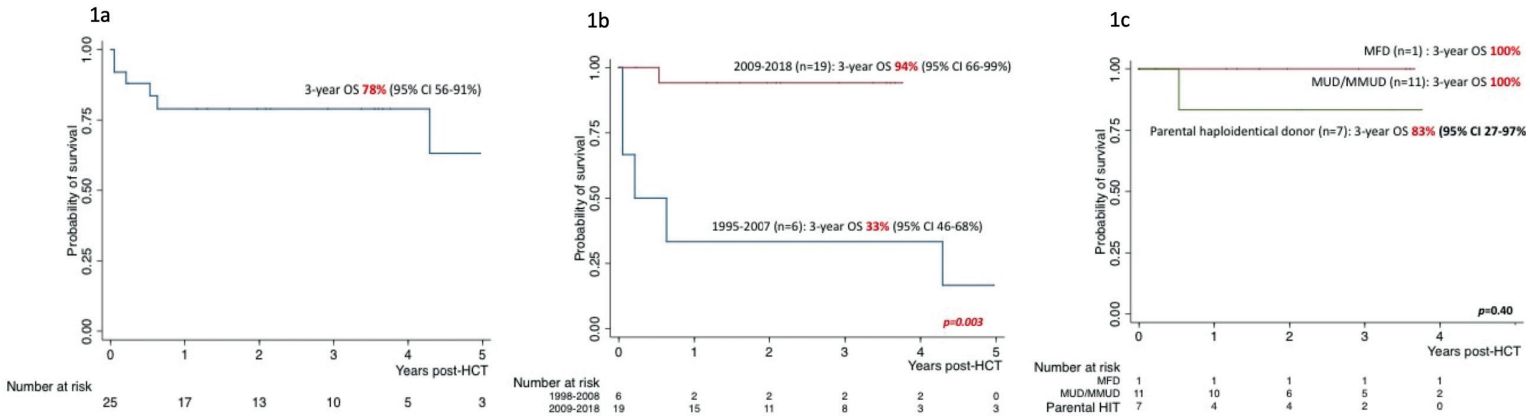


Figure 1: Transplant survival in children with MHC class II deficiency. 1a) Overall survival (OS) for entire cohort (n=25); 1b) OS according to years of transplant; 1c) OS according to donor type in patients transplanted between 2008 to 2018 (n=19)

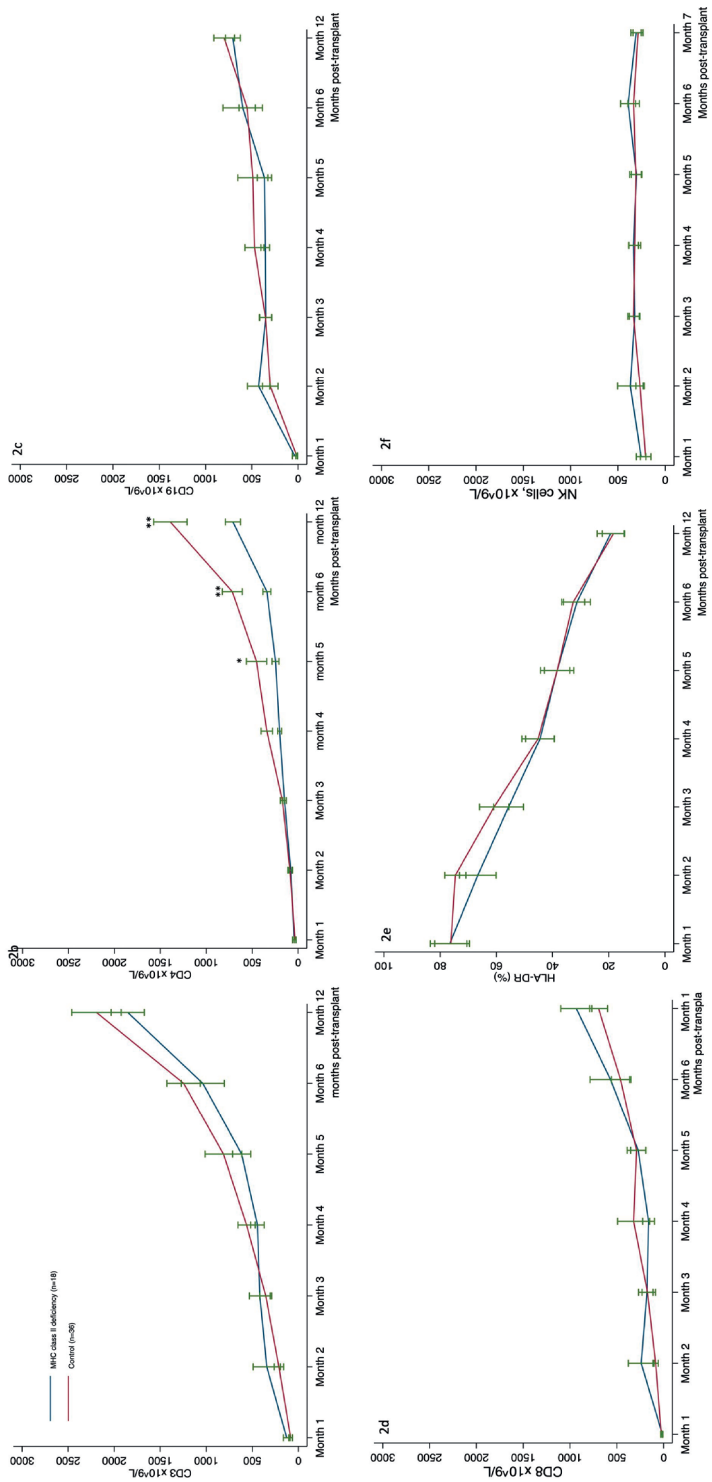


Figure 2: Immune reconstitution kinetics post transplant. Means CD3+ (2a), means CD4+ (2b), means CD8 (2c), means CD19 (2d), means activated T cells (2e) and means NK cells (2f) measured at different time points post-transplant. Disease controls (n=36) included were patients who were transplanted for severe combined immunodeficiency (SCID) (n=10), CD40 ligand (CD40L) deficiency (n=2), Wiskott-Aldrich syndrome (WAS) (n=7) and chronic granulomatous disease (CGD) (n=13) and other PID (n=4; 1 natural killer cell deficiency (NK deficiency); 1 inducible co-stimulator deficiency (ICOS deficiency); 1 haemophagocytic lymphohistiocytosis (HLH); 1 combined immunodeficiency (CID)). *p=0.06; **p<0.05

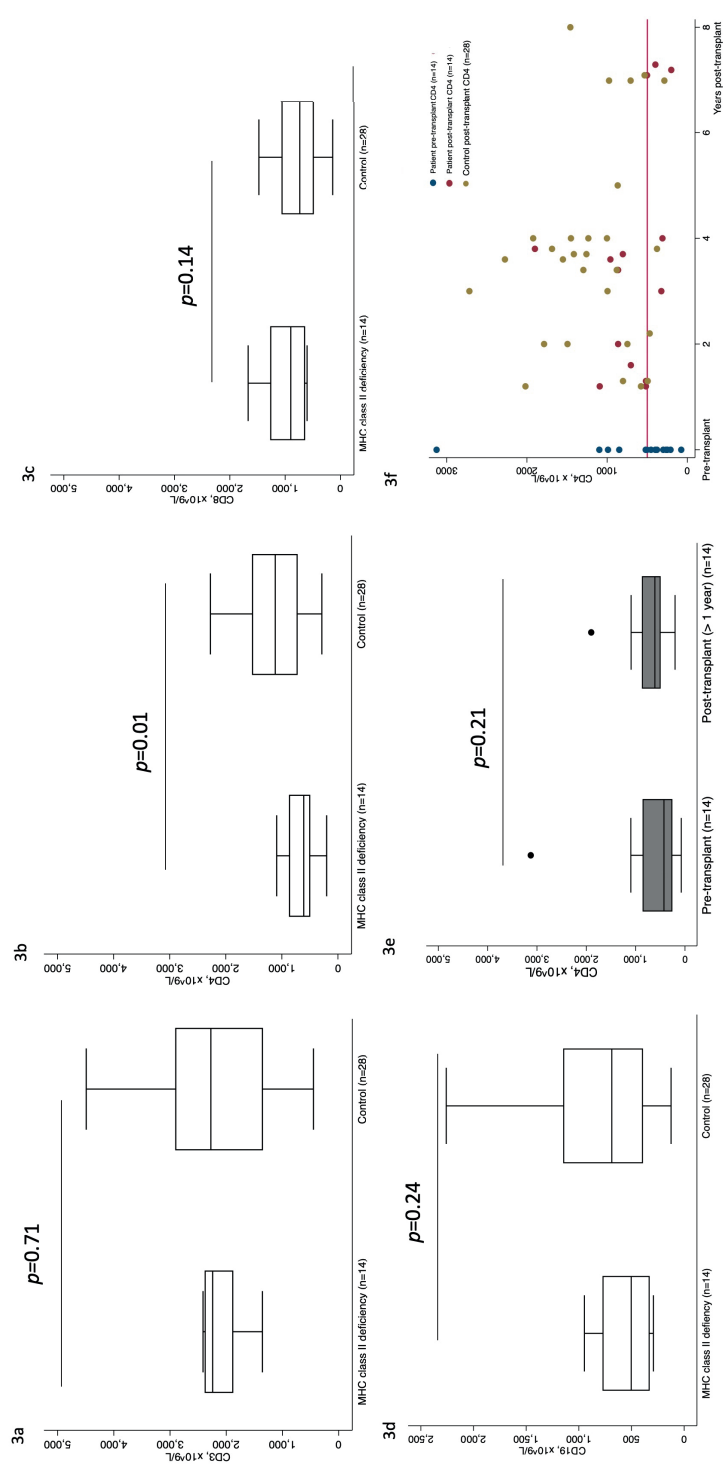
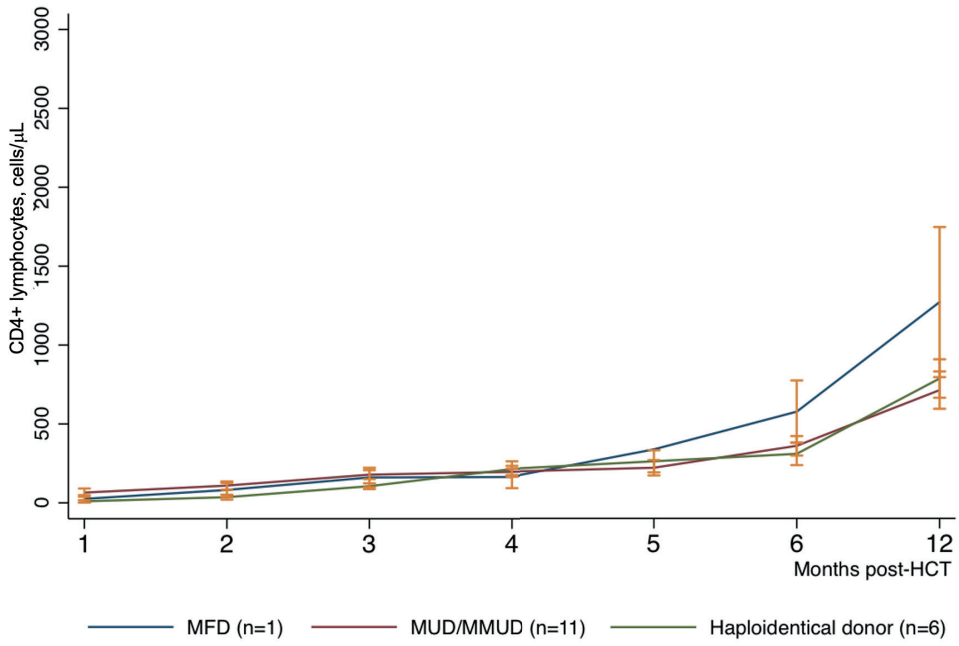


Figure 3: Lymphocyte subset at latest follow-up (> 1 year post-transplant). CD3+ cells (figure 3a), CD8+ cells (figure 3c) and CD19+ (figure 3d) cells were comparable between patients MHC class II deficiency (n=14) and controls (controls). CD4+ cells was significantly lower in in patients with MHC class II deficiency compared to controls (p=0.01) (figure 3b). There was no significant difference in CD4+ cells before and after transplant in patients with MHC class II deficiency. Figure 3f showed pre- and post-transplant CD4+ cells in individual patients and controls.



Supplemental figure 1: CD4+ lymphocyte reconstitution kinetics according to donor types

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