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

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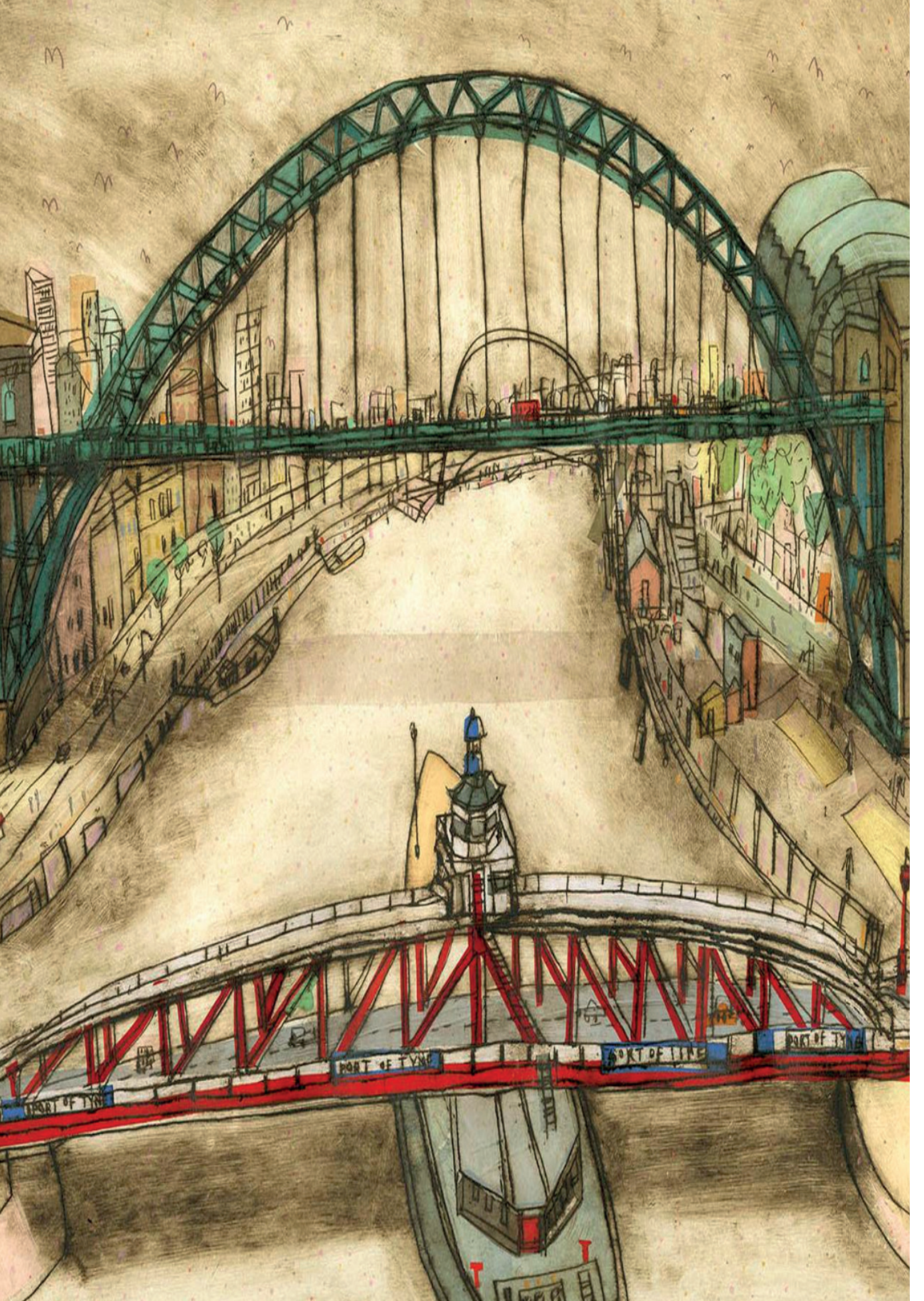
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Chapter 6

T-replete HLA-matched grafts versus T-depleted HLA-mismatched grafts in inborn errors of immunity: Where are we now?

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Abstract

Haematopoietic cell transplantation (HCT) has become standard of care for an increasing number of inborn errors of immunity (IEI). This is the first report to compare the transplant outcomes according to T-replete HLA-matched grafts using alemtuzumab (n=117) and T-depleted HLA-mismatched grafts using TCR αβ/CD19 depletion (n=47) in children with IEI who underwent first HCT between 2014 and 2019. All patients received treosulfan-based conditioning except patients with DNA repair disorders. For T-replete grafts, the stem cell source was marrow in 25 (21%) patients, PBSC in 85 (73%) and CB in 7 (6%). TCR αβ/CD19 depletion was performed on PBSC from 45 haploidentical parental donors and 2 mismatched unrelated donors. The 3-year OS and EFS for the entire cohort were 85% (77-90%) and 79% (69-86%) respectively. Analysis by age at transplant revealed a comparable 3-year OS between T-replete grafts (88%, 76-94%) and T-depleted grafts (87%, 64-96%) in younger patients (< 5 years of age at HCT). For older patients more than 5 years of age, the OS was significantly lower in T-depleted grafts (55%, 23-78%), compared to T-replete grafts (87%, 68-95%) (p=0.03). Grade III-IV aGvHD was observed in 8% of T-replete marrow, 7% of T-replete PBSC, 14% of T-replete CB and 2% of T-depleted PBSC (*p*=0.73). Higher incidence of viraemia (p<0.001) and delayed CD3 reconstitution (p=0.003) were observed after T-depleted graft HCT. These data indicate that mismatched donor transplant after TCR αβ and CD19 depletion represents an excellent alternative for younger children with IEI in need of an allograft.

Introduction

Inborn errors of immunity (IEI) encompass a group of more than 400 inherited disorders. (1). IEI lead to severe problems and may be fatal in severe phenotypes. Haematopoietic cell transplantation (HCT) is now an established curative treatment for an increasing number of IEI. For many IEI such as chronic granulomatous disease, studies have demonstrated that long-term survival, disease outcomes and quality of life are better in transplanted patients compared with non-transplanted patients who were treated with conventional therapy using antimicrobial prophylaxis. (2) In recent years, HCT has developed from a treatment of last resort into the standard of care that corrects the defects of immunity. Many studies have also shown that younger age at transplant is significantly associated with better survival and outcomes. (3) However, approximately 25-60% of eligible paediatric patients for HCT do not have a suitably well-matched donor. (4) An alternative is to use a mismatched related or unrelated donor and deplete the T-lymphocytes in the graft prior to infusion. Historically, the major challenges of using such mismatched donors for HCT are graft-versus-host disease (GvHD), graft failure, and high transplant-related mortality. In the absence of a suitably matched donor, physicians are reluctant to offer a curative transplant procedure to children with IEI, leading to prolonged periods of illness, poor quality of life, significant psychosocial problems and reduced life expectancy.

A new graft selection method has now enabled selective depletion of the T-lymphocyte receptor-ab-bearing (TCRαβ+) cells which cause GvHD and CD19+ (B) cells, decreasing the risk of EBV driven post-transplant lymphoproliferative disease. In this method, the cellular product contains CD34+ progenitors, TCR-γδ+ T-lymphocytes, innate lymphocytes including natural killer cells, dendritic cells and graft-facilitating cells, which enhance engraftment and early immune reconstitution, with a low incidence of graft-versus-disease (GvHD) in children with malignant and non-malignant disorders. To address the questions of whether transplant outcomes after a TCR αβ/CD19 depleted graft are comparable to a matched family or unrelated donor, we have analysed the outcome of consecutive transplants in children with IEI over the past six years in Newcastle upon Tyne, United Kingdom.

Methods

Patients and Methods

Between January 2014 to December 2019, 184 children with IEI underwent their first allograft at the Great North Children's Hospital. Sixteen patients who received an unmanipulated T-replete mismatched graft and four patients with SCID who received stem cell infusion

without conditioning or serotherapy, were excluded from this study. A total number of 164 patients were included in the final analysis. The clinical and laboratory data were retrieved from the transplantation database, patients' medical files and laboratory records. 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

Donor selection was based on allele level high resolution HLA-typing for HLA-A, B, C, DQ and DR loci. The donor hierarchy for SCID was i) matched family donor ii) matched unrelated cord blood iii) haploidentical donor while the donor hierarchy for non-SCID IEI was: i) matched family donor ii) matched unrelated donor iii) mismatched family/unrelated donor iv) haploidentical donor. From 2018 onwards, a haploidentical donor was preferred to a mismatched unrelated donor except if parents were not suitable as donors. The donor selection criteria for a haploidentical donor was as follows: (1) non-carrier donor for X-linked diseases (2) maternal donor for a patient with SCID with maternal fetal engraftment (3) donor with a better HLA match. In our centre, peripheral blood stem cell (PBSC) has been the first choice of stem cell source with 10/10 HLA matched donors since 2011 except when using young sibling donors aged less than 16 years. (5) From 2011 onwards, the CD3+ cell dose in T-replete PBSC was capped at 5 $x10^8$/kg and the maximum dose of CD34+ cells was given within this dose of CD3+ cells. All patients received fludarabine-treosulfan (Flu-Treo) -based chemotherapy except three patients with DNA repair disorders who received fludarabine and low dose cyclophosphamide, and one patient with X-linked lymphoproliferative disease who received Fludarabine-Melphalan (Flu-Mel). Additional thiotepa was added for patients with diseases which were associated with a high risk of graft failure. In T-replete HLA-matched grafts, alemtuzumab 1mg/kg was given for all PBSC recipients while alemtuzumab 0.6mg/kg was given for family donor marrow and unrelated cord blood (CB) recipients. Post-transplant graft-versus-host disease (GvHD) prophylaxis was ciclosporin and mycophenolate mofetil (MMF). For T-depleted mismatched grafts, patients received standardized conditioning which consisted of fludarabine, treosulfan, ATG (Grafalon) and 1 dose of rituximab (200mg/m^2) for SCID. Thiotepa was added for non-SCID PID. One T-depleted graft recipient received alemtuzumab (1mg/kg). The graft manipulation strategy was TCRαβ and CD19 depletion as previously described.(6) Thirteen patients received a gene modified T-lymphocyte add-back as part of a phase I/II clinical trial. For TCR αβ/CD19 depleted grafts, no GvHD prophylaxis was given since 2015 in patients receiving TCR αβ cells of < 5 $x10^4$/kg.

Supportive care

Surveillance for cytomegalovirus (CMV), adenovirus, Epstein Barr virus (EBV), human herpes virus type 6 (HHV6) viraemia, respiratory and gut viruses was performed weekly. All patients received antimicrobial prophylaxis against fungi, *Pneumocystis jiroveci* (PCP), and human herpesvirus reactivation. All patients received immunoglobulin replacement until normal IgM levels were evident. Donor haematopoietic chimerism was monitored by molecular techniques.

Definition and endpoints

The main outcomes of interest were overall survival (OS) and event-free survival (EFS) and cumulative incidence of GvHD. 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 $\geq 0.5 \times 10^9$/L for three consecutive days); (ii) incidence of transplant-related complications as defined and graded according to existing institutional guidelines at the time of HCT (iii) immune reconstitution; (iv) degree of donor haematopoietic chimerism at the most recent assessment. A matched-pair analysis of CD3+ lymphocyte reconstitution was carried out in T-depleted mismatched graft survivors (n=28) who did not receive add-back T-lymphocytes and T-replete PBSC graft survivors (n=28). The two-patient series were matched for age at HCT and diagnosis.

For pre-transplant patient-related risk factors, the following factors were included: 1) active infection (viraemia, cryptosporidium, PCP, disseminated BCG, mycobacteria, serious bacterial infection requiring intravenous antibiotics) within 100 days prior to or at transplant (2) chronic diarrhoea (3) autoimmunity/autoinflammatory (4) lung damage (abnormal lung function test or bronchiectasis or oxygen dependency at HCT) (5) liver damage (proven fibrosis/sclerosing cholangitis on liver biopsy or deranged liver function test within 100 days prior to HCT) (6) renal impairment within 100 days prior to HCT (7) neurological disorders secondary to infection, bleeding, vasculitis or encephalitis (disease-related congenital malformation or developmental delay was not included) (8) growth failure (9) pre-transplant malignancy.

Statistical analysis

Quantitative variables were described with median and range while categorical variables were reported with counts and percentages. The association between variables was assessed with the use of Kruskal Wallis test for continuous variables and the chi-square test for categorical

variables. Subgroup differences in OS and EFS were evaluated by log-rank test. Competing risks methods were used for the cumulative incidence (CNI) of acute GvHD (aGvHD) and viraemia, with death as a competing event. Subgroup differences in aGvHD and viraemia were evaluated by Gray's test. All estimates were reported with 95% confidence intervals. A matched-pair analysis of CD3 reconstitution was carried out in T-replete HLA-matched PBSC survivors (n=28) and T-depleted mismatched survivors. The two-patient series were matched for age at transplant and diagnosis. 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 and transplantation characteristics

Patient and transplantation characteristics are summarized in Table 1 and table S1. There were no significant differences in age at diagnosis and age at transplant between T-replete HLA-matched graft and T-depleted HLA-mismatched graft recipients. The median age at transplant for the entire cohort was 2.5 years (range 0.14 to 18.1 years). For pre-transplant risk factors, there was no significant difference in number of pre-transplant risk factors between T-replete HLA-matched graft and T-depleted mismatched graft recipients for both severe combined immunodeficiency (SCID) and non-SCID IEI. T-depleted HLA-mismatched graft recipients (n=29/47, 62%) had a significantly higher rate of active infection within 100 days of HCT compared to T-replete HLA-matched family (13/37, 35%) or unrelated donor (n = 28/80, 35%) recipients (p=0.008). There was a greater proportion of patients with chronic diarrhoea in T-depleted HLA-mismatched graft (n=17/47, 36%) and T-replete HLA-matched unrelated graft (n=31/80, 39%) recipients compared to T-replete HLA-matched family graft (n=6/37, n=16%) recipients (p=0.04). The proportions of patients with autoimmunity/ autoinflammation, lung damage, liver damage, renal impairment, neurological disorders, growth failure, or pre-transplant malignancy were comparable between T-replete HLA matched graft and T-depleted HLA-mismatched graft recipients (Table S1 and S2). There was no significant difference in the proportion of patients with pre-transplant history of viraemia (CMV, EBV, adenovirus and HHV6) between T-replete HLA-matched graft (n=24/117, 21%) and T-depleted HLA-mismatched graft recipients (n=13/47, 28%) (p=0.41) (Table S3).

For T-replete grafts, the stem cell source was marrow in 25 (21%) patients, PBSC in 84 (72%) and CB in 7 (6%). TCR αβ/CD19 depletion was performed on PBSC from 45 haploidentical parental donors and 2 mismatched unrelated donors. Of 47 T-depleted graft recipients, 33 (70%) had no post HCT GvHD prophylaxis and 14 (%) received GvHD prophylaxis (8 CSA

only; 6 CSA/MMF). For T-replete graft recipients, 93 received Flu-Treo-alemtuzumab, 17 Flu-Treo-Thiotepa-alemtuzumab, 3 Flu-Cy-alemtuzumab (1 DNA ligase IV deficiency; 2 Nijmegen Breakage syndrome), 1 Flu-Mel-alemtuzumab and 3 patients with SCID received alemtuzumab only. For T-depleted graft recipients, 11 with SCID received Flu-Treo-ATG-Rituximab and 36 with non-SCID PID had Flu-Treo-Thiotepa-ATG-Rituximab, except one patient who received alemtuzumab as serotherapy.

The graft composition is shown in Figure 1. Between T-replete PBSC and T-depleted PBSC, total nucleated cell dose was significantly higher in T-replete PBSC (p= 0.002) but the CD34+ cell dose was comparable between both T-replete PBSC and T-deplete PBSC (Figure 1A and 1B). CD3+ cells (p<0.001) and CD19+ cells (p<0.001) were lowest in T-depleted PBSC (Figure 1C and 1D).

Engraftment and transplant-related complications

The engraftment kinetics and transplant-related complications are summarized in Table 2. The rates of neutrophil and platelet engraftment were comparable between T-depleted PBSC and T-replete PBSC and significantly earlier compared to T-replete marrow and T-replete CB (Figure 1E and 1F).

The incidence of grade II-IV aGVHD was 18% (95% CI 7-48%) in T-replete marrow, 26% (16-41%) in T-replete PSBC, 14% in T-replete CB and 18% in T-depleted PBSC (p=0.73) (Figure 2A). Grade III-IV aGvHD was observed in 8% of T-replete marrow, 7% of T-replete PBSC, 14% of T-replete CB and 2% of T-depleted PBSC (p=0.73) (Figure 2B). Only one T-depleted graft recipient who did not receive ATG due to concerns about drug-resistant CMV disease, developed grade III-IV aGvHD. None had chronic GvHD in the entire cohort.

With routine surveillance for CMV, adenovirus, EBV and HHV6, the cumulative incidence of any reactivation of viraemia at 6 months after transplant was significantly higher in T-depleted grafts (80%, 70-99%) compared to T-replete grafts (55%, 46-64%) (p<0.001) (Figure 2C). Comparing to T-replete graft recipients, a greater proportion of T-depleted graft recipients had adenoviraemia (p=0.004) and HHV6 viraemia (p=0.002) (Table 2). All CB recipients were patients with SCID and only one had new onset viraemia after transplant.

A greater proportion of T-replete marrow (n=16, 64%) and T-replete PBSC (n=57, 67%) recipients developed acute kidney injury during transplant, compared to T-replete CB recipients (n=2, 28%) and T-depleted graft recipients (n=11, 23%) (p=0.001). Of 86 patients with acute kidney injury, seven (8%; 4 T-replete PBSC, 1 T-replete CB and 2 T-depleted PBSC) required renal replacement therapy. There was no significant difference in occurrence of

transplant associated microangiopathy between T-replete graft recipients (marrow, 0; PBSC, n=4 (5%); CB, n=1 (14%)) and T-depleted graft recipients (n=4, 9%) (*p*=0.33). Only one patient developed macrophage activation syndrome after T-replete PBSC for juvenile idiopathic arthritis.

Transplant survival

The 3-year OS and EFS for the entire cohort were 85% (77-90%) and 79% (69-86%) respectively. Analysis by age at transplant revealed a comparable 3-year OS between T-replete grafts (88%, 76-94%) and T-depleted grafts (87%, 64-96%) in patients who were less than 5 years of age at transplant. For older patients, more than 5 years of age, the OS was significantly lower in T-depleted grafts (55%, 23-78%), compared to T-replete grafts (87%, 68-95%). (*p*=0.006). A similar pattern was observed in EFS (Figure 2E). Regarding the pre-transplant risk factors, there were no significant differences between T-replete HLA-matched graft and T-depleted HLA-mismatched graft recipients in patients who were aged > 5 years at HCT (tables S4 and S5), and between patients aged < 5 years and patients aged > 5 years after T-depleted HLA-mismatched graft HCT (tables S6 and S7). In patients who were older than 5 years of age and received T-depleted grafts, a greater proportion of deceased recipients (n=4/5, 80%) had more than two risk factors compared to the survivors (n=4/8, 50%), but the number was too small to reach statistical significance (table S8).

A trend towards lower survival was observed in T-depleted graft recipients with viraemia (75%, 54-88%), compared to T-depleted graft recipients without viraemia (88%, 41-98%), and in T-replete graft recipients with viraemia (89%, 75-95%) and without viraemia (86%, 72-94%) (*p*=0.38) (Figure 2F). All deceased T-depleted recipients died of infection and its associated complications (table S9).

Six (5%) grafts failed in the entire cohort, 4 (5%; all secondary autologous reconstitution) after T-replete PBSC and 2 (4%; 1 secondary aplasia; 1 secondary reconstitution) after T-depleted PBSC. All received a second HCT: 5 received a TCR αβ/CD19 depleted parental graft and one received T-replete PBSC from an HLA-matched unrelated donor. All survived except one patient, who sadly died of cerebral haemorrhage before engraftment.

Hospital stay and parenteral nutritional support

The median duration of hospitalization was 46 days (range, 13 to 187 days) for T-replete MFD, 60 days (range, 14 to 365 days) for T-replete MUD and 62 days (range, 27 to 365 days) for T-depleted mismatched donor recipients (*p*=0.15*)* (Figure 3A). The proportion of patients requiring intensive care was 11% (n=4/37) for T-replete MFD, 11% (n=9/80) for T-replete

MUD and 17% (n=8/47) for T-depleted mismatched donor recipients (p=0.62). The proportion of patients requiring parenteral nutrition support was significantly higher in T-depleted mismatched donor transplants (77%, n =36/47), compared to T-replete MFD (41%, n=15/37) and T-replete MUD (51%, n=41/80) (p=0.003). There was no significant difference in duration of parenteral nutrition between T-depleted mismatched donor (median 53 days, range 17-196 days), T-replete MFD (median 41 days, range 9-172 days) and T-replete MUD (median 60 days, range 11-189 days) (p=0.52; Figure 3B).

Immune reconstitution and donor chimerism

To compare the CD3 reconstitution, a matched pair analysis was performed in survivors of T-depleted grafts (n=28, received ATG) and T-depleted PBSC (n=28, received alemtuzumab 1mg/kg). The median day to CD3 >200 cells/μL was 66 (range, 33-133 days) in T-replete graft patients and 89 days (range, 34 to 397 days) in T-depleted graft patients (p=0.003) (Figure 4A). Patients receiving a T-depleted graft had significantly lower CD3+ lymphocyte counts at month 2 (p=0.002, Figure 4B) and month 3 (p=0.05, Figure 4C) after transplant, compared to T-replete graft recipients.

The median follow-up of surviving patients (n=146) was 2.8 years (range 0.5 to 6.5 years). Data on latest donor chimerism after first successful HCT were available in 140 patients. The median donor myeloid chimerism at last follow-up was 100% (range, 4-100%) in T-depleted graft and 96% (range, 0 to 100%) in T-replete graft recipients (p=0.013) (Figure 5A). The median donor T-lymphocyte chimerism was 100% (range, 47-100%) in T-depleted graft and 95% (range, 10 to 100%) in T-replete graft recipients (p=0.001) (Figure 5B).

Discussion

This study reports the first series comparing transplant outcomes between T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts in children with IEI who were transplanted between 2014 and 2019. Treosulfan-based conditioning was used for both T-replete and T-depleted grafts. Thiotepa was given for all non-SCID IEI in T-depleted grafts but in a subset of non-SCID IEI with a high risk of graft rejection in T-replete grafts. The survival after T-depleted grafts was comparable to T-replete grafts in younger children with IEI, but inferior in older children with IEI. We observed a significantly higher incidence of adenoviraemia and HHV6 viraemia after T-depleted grafts, but a similar incidence of CMV viraemia compared to T-replete graft recipients. Delayed CD3 reconstitution remains a significant issue after T-depleted grafts. We have also demonstrated T-replete PBSC using alemtuzumab was not significantly associated with increased aGvHD, compared to T-replete

marrow and CB, this is consistent with Shaw *et al's*. report in patients with leukaemia. (7) In our cohort, no recipients of T-replete PBSC had chronic GvHD.

HCT has become a curative option for a variety of otherwise incurable diseases such as high-risk leukaemia and many non-malignant conditions. It has been increasingly used as standard of care for an increasing number of IEI. Unfortunately, only about one in four patients who need an HCT will have a HLA-matched sibling available to become a stem cell donor. (8) The likelihood of finding an optimal unrelated donor varies among racial and ethnic groups, as high as 75% in whites of European descent to as low as 16% among blacks of South or Central American descent. (4) Transplantation from a haploidentical donor has some unrivalled advantages, including highly motivated and readily available donors and, in the setting of malignancy, a haploidentical donor allows the selection of a donor with the highest degree of mismatch in their natural killer cell immunoglobulin-like receipt (KIR) repertoire, which provides a better graft-versus-tumour effect. (9) Historically, the major challenges of using such mismatched haploidentical donors for HCT are GvHD, graft failure, and high transplant-related mortality. In the absence of a suitably matched donor, physicians are reluctant to offer a curative transplant procedure to children with IEI, leading to prolonged periods of illness, poor quality of life, significant psychosocial problems and reduced life expectancy. Locatelli *et al.* reported comparable risks of non-relapse mortality and relapse in children with acute leukaemia between CD3+ TCR αβ/CD19 depleted haploidentical donor graft and matched family and unrelated donor recipients. (10) In this report, we demonstrated comparable outcomes between T-depleted HLA-mismatched grafts and T-replete HLA-matched grafts in younger children with IEI.

The current strategy using TCR αβ/CD19 depletion has a comparable incidence of GvHD to T-replete HLA-matched grafts, but the immune reconstitution and the generation of a broad TCR repertoire, which are required for normal immunity, are delayed. (11) This is reflected in a significant rate of viral infections in our cohort, especially adenoviraemia and HHV6 viraemia. Of note, the incidence of acute kidney injury was significantly lower in T-depleted graft patients, this is likely due to the fact that the majority of these patients did not receive any calcineurin inhibitors for GvHD prophylaxis. In long-term survivors, the donor myeloid and T-lymphocyte chimerism were better in the T-depleted graft group. One possible explanation for this is that additional thiotepa was given to patients with non-SCID IEI for T-depleted grafts to prevent graft rejection, which is more myeloablative compared to Fludarabine-Treosulfan based conditioning. Although Leiper *et al* showed that treosulfan-based conditioning conferred a more favourable outcome for gonadal reserve (12), the long-term impact of additional thiotepa on fertility function is unknown.

Whilst the introduction of CD3+ TCR αβ/CD19 depletion has transformed our practice for children with IEI, improved strategies are required to accelerate immune recovery and reduce viral infection. A number of strategies are emerging such as the generation of donor-derived cytotoxic T-lymphocytes against multiple viruses, Rivo-cel (genetically modified αβ T-lymphocyte receptor bearing cells with an added caspase suicide gene) and photodynamic purging. (13, 14) Whilst these approaches might be promising, they are complex, time-consuming, and additional harvests from donors are required. Depletion of naïve (TCRαβ+CD45RA+) T-lymphocytes has been developed as a new method of graft selection with the potential to confer improved reactivity to pathogens by memory T-lymphocytes (TCRαβ+CD45RO+), without conferring an increased risk of GvHD. Recent trials in children with malignancy show positive benefits from CD45RO+ memory T-lymphocyte add-back with improvement in immune reconstitution, reduction in incidence and severity of viral infection, and lower transplant related mortality. (15-17) To date, there is no prospective trial in children with IEI.

In conclusion, our data indicate that, through a more advanced strategy of graft selection, mismatched related or unrelated donor transplant allows the development of universal donors and offers the opportunity to transplant virtually every child with IEI in need of an allograft, with an expected outcome comparable to that obtained with an HLA-matched donor in younger children. We are now setting up a new prospective trial, based on CD45RO+ memory T-lymphocyte add-back, with the aim of accelerating recovery of adaptive immunity and reducing the incidence of viral infection in patients who receive in vitro T-depleted grafts. Demonstration of efficacy and safety would allow physicians to refer IEI patients earlier to transplant; avoiding many IEI related complications as well as transplant-related morbidity and mortality which rise with each year of delay. For infants who are diagnosed with IEI through newborn and family screening, transplant can be performed as soon as possible before any infection or organ damage. Every child with IEI deserves a cure with transplant: the major obstacle of "no suitable donor" will be eliminated if this clinical trial demonstrates promising outcomes.

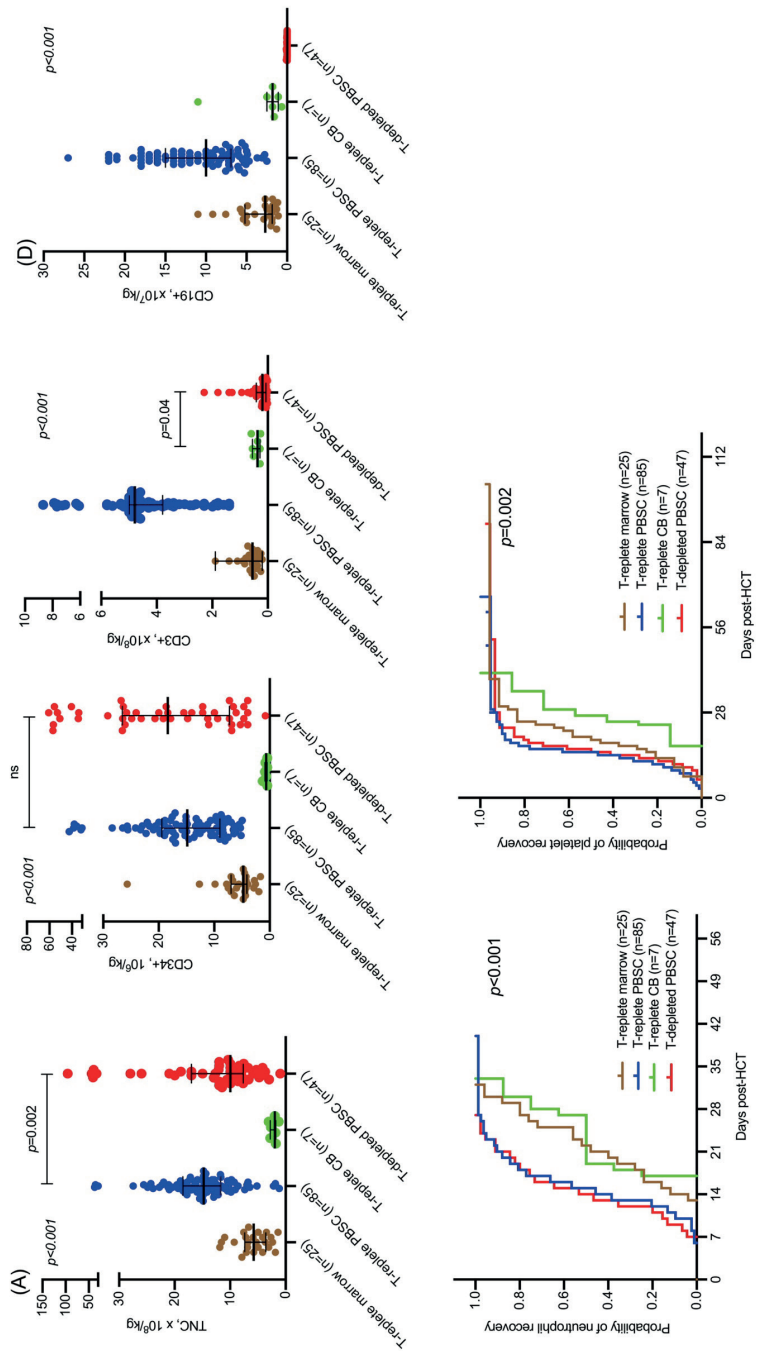

Figure 1: Graft composition and engraftment kinetics according to T-replete marrow, T-replete PBSC, T-replete CB and T-depleted PBSC. (A) Total nucleated cell dose, x108/kg (B) CD34+ cells, x106/kg (C) CD3+ cells, x108/kg (D) CD19+ cells, x107/kg (E) Neutrophil recovery (F) Platelet recovery
Abbreviations: CB: cord blood; PBSC: peripheral blood stem cells

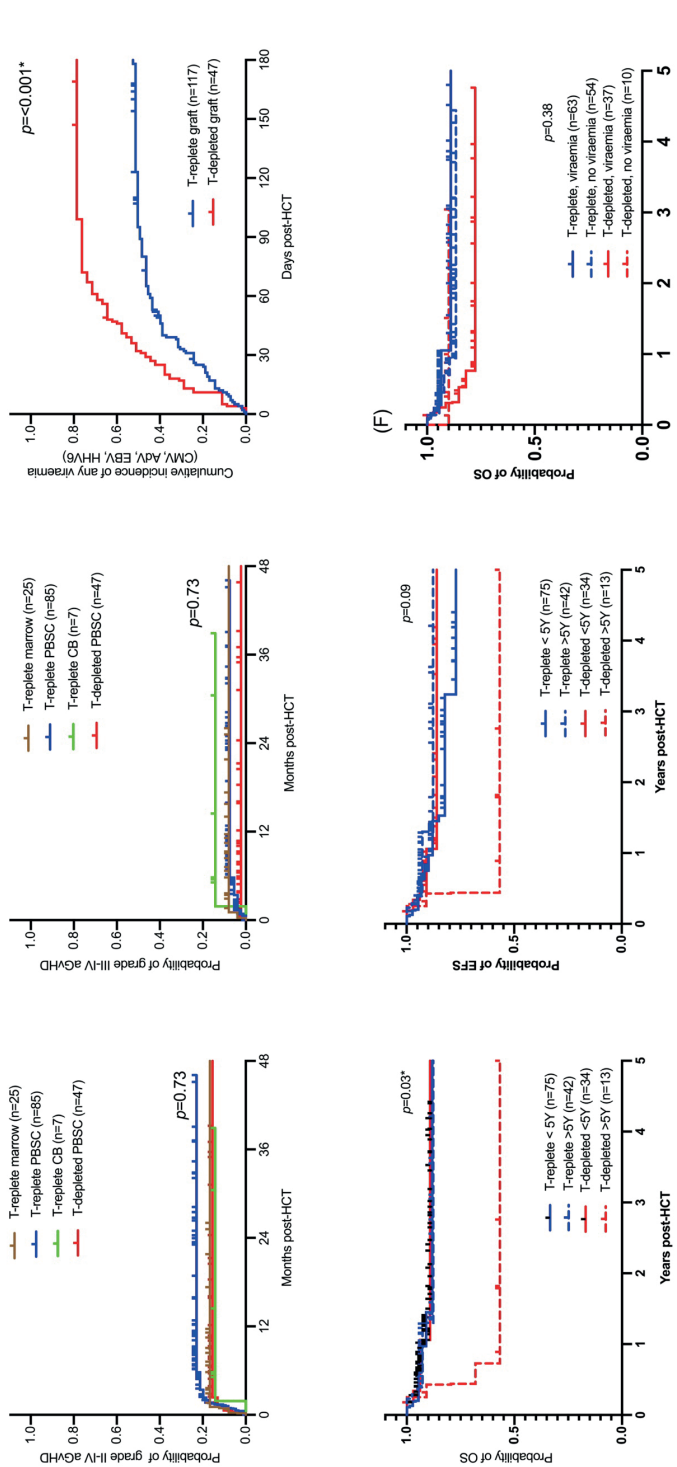

Figure 2: Cumulative incidence of aGvHD and viraemia, and survival according to T-replete and T-depleted grafts: (A) Grade II-IV aGVHD (B) Grade III-IV aGvHD (C) Viraemia (CMV, AdV, EBV and HHV6) (D) OS according to age at transplant (E) EFS according to age at transplant (F) Impact of viraemia on OS

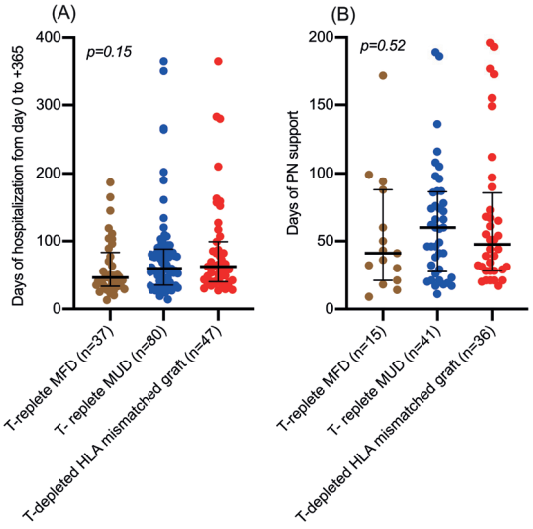

Figure 3: Length of hospital stay and parenteral support (PN) according to donor type and ex-vivo T cell depletion. (A) Days of hospitalization from day 0 to +365 (B) Days of parenteral support (PN) during transplant

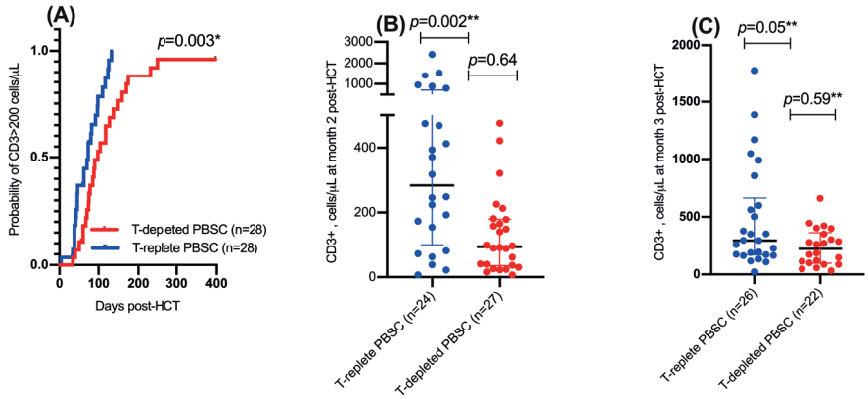

Figure 4: A matched pair analysis for CD3+ lymphocyte reconstitution in T-replete PBSC and T-depleted PBSC. (A) Time to CD3+ lymphocyte > 200 cells/µL (B) CD3+ lymphocyte count at month 2 after transplant (C) CD3+ lymphocyte count at month 3 after transplant

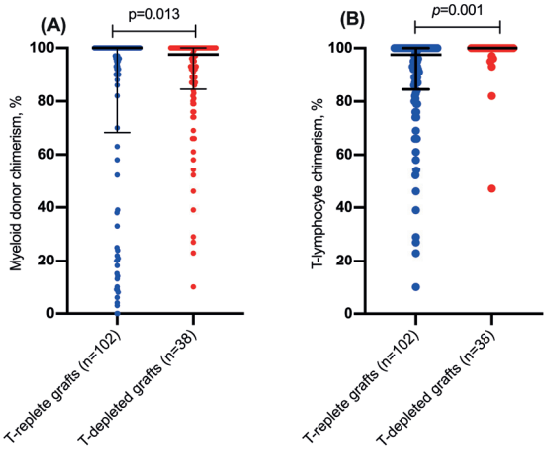

Figure 5: The lastet donor chimerism in T-replete and T-depleted grafts (A) Myeloid donor chimerism (B) T-lymphocyte chimerism

Table 1: Patient and transplantation characteristics according donor type (n=164)

	All	T-replete HLA-matched graft		T-depleted HLA-mismatched graft	*p*-value
		MFD	MUD		
Number	164	37	80	47	
Male, n (%)	97 (59)	16 (16)	52 (54)	29 (30)	0.08
Diagnosis, n (%)					0.46
SCID	35 (21)	10 (27)	14 (18)	11 (23)	
Non-SCID IEI	129 (79)	27 (73)	66 (83)	36 (77)	
Median age at diagnosis (range), years					
SCID	0.2 (at birth-1.0)	0.13 (at birth-0.7)	0.3 (at birth-0.8)	0.3 (at birth – 1.04)	0.38
Non-SCID IEI	1.9 (at birth-17.1)	2.1 (at birth -12.8)	1.5 (at birth -14.3)	2.0 (0.1-17.1)	0.37
Median age at transplant (range), years					
SCID	0.4 (0.14-1.4)	0.3 (0.2-0.8)	0.54 (0.14-1.4)	0.65 (0.14-1.4)	0.27
Non-SCID IEI	4.1 (0.2-18.1)	4.32 (0.2-16.8)	4.7 (0.3-17.2)	3.1 (0.2-18.1)	0.93
Median interval between diagnosis and transplant (range), years					
SCID	0.2 (0.02-1.2)	0.16 (0.11-0.32)	0.24 (0.02-1.2)	0.22 (0.13-0.8)	0.23
Non-SCID IEI	1.2 (0.1-17.2)	0.8 (0.2-12.8)	1.3 (0.1-17.2)	1.2 (0.1-4.6)	0.91
Median number of pre-transplant risk factors (range)					
SCID	2 (0-4)	1 (0-3)	2 (0-4)	2 (0-3)	0.11
Non-SCID IEI	2 (0-6)	1 (0-5)	2 (0-6)	2 (0-5)	0.10
Graft composition					
Median TNC (range), $x10^8$/kg	12.0 (1.0-96.0)	7.6 (1.4-22.8)	13.9 (1.2 – 37.9)	10 (1.0-96.0)	<0.0001
Median CD34 (range), $x10^6$/kg	12.4 (0.5-60.9)	6.7 (1.7-25.7)	13.5 (0.5-8.7)	18.4 (0.8-60.9)	<0.0001
CD3 (range), $x10^8$/kg	1.9 (0.02-14.4)	0.9 (0.2-7.7)	4.6 (0.3-8.7)	0.2 (0.02-14.4)	<0.0001
CD19 (range), $x10^7$/kg	5.25 (0.0008-27.0)	5.0 (1.1-21.0)	9.4 (0.7-27.0)	0.007 (0.008-0.11)	<0.0001

CB: cord blood; CSA: ciclosporin; IEI: inborn errors of immunity; MFD: matched family donor; MMF: mycophenolate mofetil; MMFD: mismatched family donor; MMUD; mismatched unrelated donor; MUD; matched unrelated donor; PBSC: peripheral blood stem cell; SCID: severe combined immunodeficiency; WAS: Wiskott-Aldrich syndrome

Table 2 Engraftment kinetics and transplant-related complications according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts

	T-replete HLA-matched graft			T-deplete HLA-mismatched graft	*p*-value
	Marrow	PBSC	CB		
	25	85	7	47	
Engraftment kinetics					
Median day to neutrophil recovery (range)	22 (13-32)	15 (6-40)	27 (17-33)	14 (0-27)	<0.001
Median day to platelet recovery (range)	19.5 (7-103)	15 (3-66)	27 (17-41)	15 (6-90)	0.002
Acute GvHD, % (95% CI)					
1-year CIN grade II-IV aGVHD	18 (7-48)	26 (16-41)	14 (2-79)	18 (9-39)	0.73
1-year CIN grade III-IV aGVHD	8 (2-3)	7 (3-17)	14 (2-79)	2 (3-17)	0.73
Chronic GvHD, n (%)	0	0	0	0	
New onset of viraemia after HCT, n (%)					
CMV viraemia	6 (24)	17 (20)	0	14 (30)	0.59
Adenoviraemia	5 (20)	19 (22)	0	22 (47)	0.004
HHV 6 viraemia	4 (16)	24 (28)	0	24 (51)	0.002
EBV viraemia	5 (20)	11 (13)	1 (14)	8 (17)	0.79
TMA, n (%)	0	4 (5)	1 (14)	4 (9)	0.33
Acute kidney injury, n (%)	16 (64)	57 (67)	2 (28)	11 (23)	<0.001
Graft failure, n (%)	0	4 (5)	0	2 (4)	0.80
Number of deaths, n (%)	1 (4)	9 (10)	1 (14)	8 (17)	0.34
Cause of death					
GvHD	0	1	0	0	
Multi-organ failure	1	2	0	3	
Infection	0	3	0	3	
Others	0	3[1]	1[2]	2[3]	

[1]neuromyelitis optica spectrum disorder, n=1; [2]encephalopathy, n=1; [2]post-transplant lymphoproliferative disease, n=1
[2]pulmonary TMA, n=1
[3]encephalopathy, n=1; cerebral haemorrhage, n=1
aGvHD: acute GvHD; CMV: cytomegalovirus; EBV: Epstein Barr Virus; GvHD: graft-versus-host disease; TMA: transplant associated microangiopathy

Table S1: Pre-transplant risk factors according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts

	All (N=164)	T-replete HLA-matched graft		T-depleted HLA-mismatched graft (n=47)	*p*-value
		MFD (n=37)	MUD (n=80)		
Active infection within 100 days of HCT, n (%)	74 (45)	13 (35)[1]	28 (35)[2]	29 (62)[3]	0.008
Chronic diarrhoea, n (%)	54 (33)	6 (16)	31 (39)	17 (36)	0.04
Autoimmunity/autoinflammation, n (%)	34 (21)	6 (16)	22 (28)	6 (13)	0.12
Lung damage, n (%)	47 (29)	9 (24)	26 (33)	35 (26)	0.60
Liver damage, n (%)	20 (12)	4 (11)	12 (15)	4 (9)	0.55
Renal impairment, n (%)	8 (5)	1 (3)	7 (9)	0	0.07
Neurological disorders, n (%)	9 (6)	1 (3)	6 (8)	2 (4)	0.67
Growth failure, n (%)	52 (32)	10 (27)	26 (33)	16 (34)	0.77
Pre-transplant malignancy, n (%)	3 (2)	0	2 (3)	1 (2)	0.63

[1] achromobacter xylosoxidans sepsis, n=1; aspergillosis, n=2; adeno, n=1; CMV+adeno, n=1; CMV +HHV6, n=2; cryptosporidium, n =1; disseminated BCG, n=2; HHV6, n =1; PJP + adeno, n =1; Burkoderia cepacia, n=1
[2]CMV+adeno+cryptosporidium, n=1; CMV+ disseminated BCG, n=1; CMV + PJP, n=1; CMV+ HHV6, n=1; EBV + PJP, n=1; EBV, n=1; adeno+ HHV6, n=1; HHV6, n=3; PCP, n=8; disseminated BCG + multifocal salmonella, n=1; disseminated BCG, n=1; aspergillosis, n =2; Influenzae A + RSV, n=1; non-tuberculous mycobacteria of lungs, n =1
[3]CMV, n=2; CMV+cryptosporidium, n=1; adeno, n=1; EBV, n=2; EBV+HHV6, n =2; HHV6+ PCP, n=1; fungal splenic abscess, n =1; PCP, n =7; disseminated BCG, n=5; multifocal abscess, n=1; Para3+RSV+ adeno, n =1; CMV+ENV, n =1; CMV + EBV + Hep B & C, n=1; HHV6, n=2; E.coli bacteraemia, n=1

Table S2: Pre-transplant number of risk factors according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts

	All (N=164)	T-replete HLA-matched graft		T-depleted HLA-mismatched graft (n=47)	*p*-value
		MFD (n=37)	MUD (n=80)		
Number of risk factors					0.17
0	34 (21)	12 (32)	12 (15)	10 (21)	
1	38 (23)	11 (30)	18 (23)	9 (19)	
2	40 (24)	8 (22)	19 (24)	13 (28)	
≥3	52 (32)	6 (16)	31 (39)	15 (32)	

Table S3: Pre-transplant history of viraemia to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts

History of pre-transplant viral infection	T-replete HLA-matched graft (n=117)	T-depleted HLA-mismatched graft (n=47)	*p*-value
Any viraemia	24 (21)	13 (28)	0.41
CMV	12 (10)	7 (14)	0.40
Adenovirus	5 (4)	3 (6)	0.57
EBV	5 (4)	5 (11)	0.12
HHV6	7 (6)	3 (6)	0.92

Table S4: Pre-transplant risk factors according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts in patients > 5 years at HCT

	T-replete HLA-matched graft (n=42)	T-depleted HLA-mismatched graft (n=13)	*p*-value
Active infection within 100 days of HCT, n (%)	13 (31)	7 (54)	0.13
Chronic diarrhoea, n (%)	18 (43)	5 (39)	0.79
Autoimmunity/autoinflammation, n (%)	18 (43)	5 (39)	0.78
Lung damage, n (%)	18 (43)	5 (39)	0.78
Liver damage, n (%)	6 (14)	1 (8)	0.53
Renal impairment, n (%)	3 (7)	0	0.32
Neurological disorders, n (%)	3 (7)	1 (8)	0.95
Growth failure, n (%)	13 (31)	4 (31)	0.99
Pre-transplant malignancy, n (%)	2 (5)	0	0.42

Table S5: Pre-transplant number of risk factors according to T-replete HLA-matched grafts and T-depleted HLA-mismatched grafts in patients > 5 years at HCT

	T-replete HLA-matched graft (n=42)	T-depleted HLA-mismatched graft (n=13)	*p*-value
Median number of pre-transplant risk factors (range)	2 (0-3)	2 (0-4)	0.68
Number of risk factors			0.39
0	3 (7)	3 (23)	
1	11 (26)	2 (15)	
2	9 (21)	2 (15)	
≥3	19 (45)	6 (46)	

Table S6: Pre-transplant number of risk factors according to age group in T-depleted HLA-mismatched graft recipients

	T-depleted HLA-mismatched graft (n=47)		*p*-value
	< 5 years (n=34)	>5 years (n=13)	
Median number of pre-transplant risk factors (range)	2 (0-5)	2 (0-4)	0.37
Number of risk factors			0.80
0-1	14 (41)	5 (38)	
2-4	19 (56)	8 (62)	
>4	1 (3)	0	

Table S7: Pre-transplant risk factors according to age group in patients with PID who received T-depleted HLA-mismatched grafts

	T-depleted HLA-mismatched graft (n=47)		*p*-value
	< 5 years (n=34)	>5 years (n=13)	
Median number of pre-transplant risk factors (range)	2 (0-5)	2 (0-4)	0.37
Active infection within 100 days of HCT, n (%)	22 (65)	7 (54)	0.49
Chronic diarrhoea, n (%)	12 (35)	5 (38)	0.84
Autoimmunity/autoinflammation, n (%)	1 (3)	5 (38)	0.001
Lung damage, n (%)	7 (21)	5 (38)	0.21
Liver damage, n (%)	3 (9)	1 (8)	0.90
Renal impairment, n (%)	0	0	NA
Neurological disorders, n (%)	1 (3)	1 (8)	0.47
Growth failure, n (%)	12 (35)	4 (31)	0.77
Pre-transplant malignancy, n (%)	1 (3)	0	0.53

Table S8: Pre-transplant number of risk factors according to survival status in T-depleted HLA-mismatched graft recipients who is > 5 years of age

	T-depleted HLA-mismatched graft, > 5 years of age (n=13)		*p*-value
	Alive (n=8)	Death (5)	
Number of risk factors			0.56
0-1	4 (50)	1 (20)	
2-4	4 (50)	4 (80)	

Table S9 Characteristics of decreased patients after T-depleted HLA-mismatched graft

No/ Year	Diagnosis	Risk factors	Age at dx (years)	Age at tx (years)	donor	conditioning	GvHD prophylaxis	GvHD	Viraemia	HCT complications	Cause of death
1/2014	CID with CD4 lymphopenia and abnormal apoptosis	Multi-resistant CMV infection	1.1	0.3	6/10 mother	FTT Rituximab No serotherapy	CSA/MMF	Grade 3	Persistent pre-transplant CMV viraemia EBV viraemia	VOD AKI	Day +281 progressive aspergillus chest infection, drug resistant disseminated CMV infection
2/2016	DOCK 8 deficiency	none	12.0	13.0	6/10 mother	FTT ATG Rituximab	CSA	no	CMV Disseminated adenovirus HHV6 viraemia	Fungal pneumonia Pericardial effusion	Day +117 Multi-organ failure
3/2017	DNA ligase IV defect (initial dx T-B-NK+ SCID)	none	0.3	0.7	5/10 father	FTT ATG Rituximab	none	Grade 2	Disseminated adenovirus	TMA Pericardial effusion	Day +91 Multi-organ failure
4/2017	Complex autoimmune disease with enteropathy	Chronic diarrhoea Disseminated varicella with pneumonitis Lung damage Autoimmune hypothyroidism	17.1	18.1	6/10 father	FTT ATG Rituximab	none	none	CMV	none	Day +1 Sepsis with multi-organ failure

No/ Year	Diagnosis	Risk factors	Age at dx (years)	Age at tx (years)	donor	conditioning	GvHD prophylaxis	GvHD	Viraemia	HCT complications	Cause of death
5/2017	Wiskott Aldrich syndrome	CMV, EBV, hepatitis B&C Osteomyelitis Chronic diarrhoea Autoimmune cytopenia	0.2	12.6	7/10 father	FTT ATG Rituximab	none	none	CMV Disseminated adenovirus HHV6	Encephalopathy	Day +113 Encephalopathy
6/2017	MHC class II deficiency with osteogenesis imperfecta	CMV Chronic diarrhoea with viral enteropathy (norovirus, enterovirus, astrovirus, sapovirus) Growth failure Bronchiectasis	2.0	6.1	5/10 father	FTT ATG Rituximab	none	Grade 2	CMV Disseminated adenovirus EBV HHV6 RSV	Secondary graft failure	Day +193 Cerebral haemorrhage
7/2018	DOCK 8 deficiency	CMV Cryptosporidium Bronchiectasis Sclerosing cholangitis	8.0	10.5	5/10 mother	FTT ATG Rituximab	none	Grade 2	CMV Disseminated adenovirus	Pneumonia	Day +66 Astrovirus (VA1/HMO-C) encephalitis
8/2019	STAT1 deficiency	Disseminated BCG cholangitis	0.5	1.0	7/1 father	FTT ATG Rituximab	none	none	CMV	none	Day +51 Disseminated CMV disease

AKI: acute kidney injury; CID: Combined immunodeficiency; CSA: ciclosporin; FTT: Fludarabine, treosulfan, thiotepa; MMF: mycophenolate mofetil; TMA: transient associated microangiopathy;

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