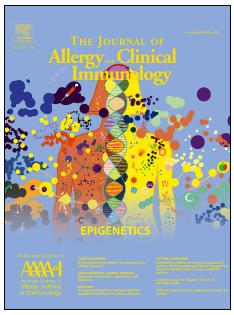
Accepted Manuscript



New graft manipulation strategies improved outcome of mismatched stem cell transplantation in children with primary immunodeficiencies

Reem Elfeky, MD, Ravi M. Shah, MD, Mohamed NM. Unni, MD, Giorgio Ottaviano, MD, Kanchan Rao, MRCPH, MNAMS, Robert Chiesa, MD, Persis Amrolia, PhD, Austen Worth, PhD, Terry Flood, MD, Mario Abinun, MD, Sophie Hambleton, PhD, Andrew J. Cant, PhD, Kimberly Gilmour, PhD, Stuart Adams, PhD, Gul Ahsan, PhD, Dawn Barge, PhD, Andrew R. Gennery, PhD, Waseem Qasim, MBBS, PhD, Mary Slatter, MD, Paul Veys, FRCP, FRCPath

PII: S0091-6749(19)30187-3

DOI: https://doi.org/10.1016/j.jaci.2019.01.030

Reference: YMAI 13873

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 10 June 2018

Revised Date: 11 January 2019

Accepted Date: 17 January 2019

Please cite this article as: Elfeky R, Shah RM, Unni MN, Ottaviano G, Rao K, Chiesa R, Amrolia P, Worth A, Flood T, Abinun M, Hambleton S, Cant AJ, Gilmour K, Adams S, Ahsan G, Barge D, Gennery AR, Qasim W, Slatter M, Veys P, New graft manipulation strategies improved outcome of mismatched stem cell transplantation in children with primary immunodeficiencies, *Journal of Allergy and Clinical Immunology* (2019), doi: https://doi.org/10.1016/j.jaci.2019.01.030.

New graft manipulation strategies improved outcome of mismatched stem cell transplantation in children with primary immunodeficiencies

Authors full names: Reem Elfeky[1,2] , MD, Ravi M Shah[3,6], MD, Mohamed NM Unni[4] ,MD, Giorgio Ottaviano[5], MD, Kanchan Rao[3], MRCPH, MNAMS, Robert Chiesa[3], MD, Persis Amrolia[1,3], PhD, Austen Worth[3], PhD Terry Flood[4], MD, Mario Abinun[4], MD, Sophie Hambleton[4], PhD, Andrew J Cant[4], PhD, Kimberly Gilmour[3], PhD , Stuart Adams[3], PhD, Gul Ahsan[3], PhD, Dawn Barge[4], PhD, Andrew R Gennery[4], PhD, Waseem Qasim[1], MBBS, PhD, Mary Slatter[4], MD, Paul Veys[1,3], FRCP, FRCPath.

1. Molecular and Cellular Immunology Unit, University College London (UCL) Great Ormond Street Institute of Child Health, London, United Kingdom.
2. Department of Paediatric Allergy and Immunology, Ain Shams University, Egypt.
3. Blood and Bone marrow transplant Unit, Great Ormond Street Hospital, London, UK.
4. Host Defence Unit, The Great North Children's Hospital, Newcastle Upon Tyne, UK.
5. Department of Paediatrics, Fondazione MBBM University of Milan-Bicocca, Monza, Italy.
6. Department of Paediatric Oncology and BMT, Alberta Children's Hospital, Calgary, Canada

COI: The authors have nothing to disclose in relation to the published manuscript.

Abstract:

Background: Mismatched stem cell transplantation is associated with high risk of graft loss, graft versus host disease (GvHD) and transplant related mortality (TRM). Alternative graft manipulation strategies have been employed over the last 11 years to reduce these risks.

Objective: We investigated the outcome of using different graft manipulation strategies among children with primary immunodeficiency (PID).

Methods: Between 2006-2017, 147 PID patients received 155 mismatched grafts; 30 TCRαβ/CD19 depleted, 43 cords (72% with no serotherapy), 17 CD34+ selection with T cell add-back and 65 unmanipulated grafts.

Results: The estimated 8-year survival of the entire cohort was 79%, TRM was 21.7% and graft failure rate was 6.7%. Post-transplant viral reactivation, aGvHD grades II-IV and chronic GvHD complicated 49.6%, 35% and 15% transplants, respectively. The use of TCR αβ/CD19 depletion was associated with a significantly lower incidence of grade II-IV aGvHD (11.5%) and cGvHD (0%) however with a higher incidence of viral reactivation (70%) in comparison to other grafts. T cell immune reconstitution was robust among cord transplants however with a high incidence of aGvHD grade II-IV 56.7%. Stable full donor engraftment was significantly higher at 80% among TCRαβ$^+$/CD19$^+$depleted and cord transplants versus 40-60% among the other groups.

Conclusions: Rapidly accessible cord and haploidentical grafts are suitable alternatives for patients with no HLA matched donor. Cord transplantation without serotherapy and TCRαβ$^+$/CD19$^+$depleted grafts produced comparable survival rates of around 80% albeit with a high rate of aGvHD with the former and high risk of viral reactivation with the latter that need to be addressed.

Keywords: Mismatched stem cell transplantation, GvHD, Cord, TCRαβ/CD19, Immune reconstitution.

List of abbreviations:

GvHD: Graft versus host disease.

TRM: Transplant related mortality.

PID: primary immune deficiency.

CD34+/T cell add-back: CD34 positive selection with T cell add-back.

HSCT: Haematopoietic stem cell transplantation.

SCETIDE: The European Registry for stem cell transplantation in primary immunodeficiency.

SCID: Severe combined immune deficiency.

OS: Overall survival.

PID: Primary immune deficiency.

RIC: Reduced intensity conditioning.

MAC: Myeloablative conditioning.

MIC: Minimal intensity conditioning.

Treo: Treosulfan.

Flu: Fludarabine.

TT: Thiotepa.

Bu: Busulphan.

Mel: Melphalan.

Cyc: Cyclophosphamide.

CB: Cord blood.

PBSCs: Peripheral blood stem cells.

BM: Bone marrow.

NPA: Nasopharyngeal aspirate.

TPN: Total parental nutrition.

rATG: rabbit anti-thymocyte globulin.

Alem: Alemtuzumab.

CSA: Ciclosporin A.

MMF: Mycophenolate mofetil.

MP: Methylprednisolone.

EBV_PTLD: EBV induced post-transplant lymphoproliferative disease.

ECP: Extracorporeal photopheresis.

VOD: Veno-occlusive disease.

TMA: Thrombotic microangiopathy.

Rag: Recombinase activating genes.

ADA: Adenosine deaminase.

PNP: Purine nucleoside phosphorylase.

CGD: chronic granulomatous disease.

CHH: cartilage hair hypoplasia.

LAD: leukocyte adhesion defect.

CID: combined immune deficiency

HLH: Haemophagocytic lymphohistiocytosis.

XLP: X-linked lymphoproliferative disease.

WAS: Wiskott Aldrich syndrome

TBI: Total body irradiation.

Figure legends:

Figure 1: Overall survival among different graft manipulations

1a) 8-year overall survival among all PID was 78.1%

1b) 8-year overall survival among SCID was 73.3%

1c) 8-year overall survival among Non-SCID was 80.3%

Figure 2: Effect of conditioning on overall survival among unmanipulated grafts

Figure 3: Effect of post-transplant viraemia on TRM

Figure 4: Effect of aGvHD on TRM

Figure 5: T cell immune reconstitution across the different graft manipulations

5a) Robust CD3 recovery at 3 months post-transplant among Cord grafts

5b) CD4 recovery at 3 months post-transplant among different graft manipulations

5C) Naïve CD4 counts at 6 months post-transplant among different graft manipulations

Table legends:

Table 1: Diagnoses (n=155)

Table 2: Patients' characteristics

Table 3: Analysis of factors affecting outcome among PID receiving a mismatched graft.

Table 4: Patients who required a second transplant or an unconditioned stem cell boost (n=10)

Table 5: Engraftment and immune recovery post-transplant across different graft manipulations

Table E1: Cause of deaths among the different graft manipulations (n=34)

Table E2: Characteristics of patients who developed TMA (n=7)

Table E3: Analysis of factors affecting outcome among SCID

Capsule summary:

This study demonstrated improved overall survival among mismatched grafts over the last 11 years; 22% TRM. cord transplant without serotherapy and TCRαβ/CD19 depleted grafts produced comparable survival rates of 80% and exhibited stable full donor engraftment.

Key messages:

1. Improved overall survival among mismatched grafts over the last 11 years with a TRM of 22% and a graft rejection rate of 6.5%.
2. Rapidly accessible cord and haploidentical grafts are suitable alternatives for patients with no HLA matched donor.
3. Cord transplantation without serotherapy allowed early T cell recovery with high level donor engraftment but high grades of aGvHD.
4. TCRαβ$^+$/CD19$^+$depleted grafts produced survival rates of 80% and exhibited high level donor chimerism together with a lower risk of acute and chronic GvHD but high risks of viral reactivation.
5. Mismatched grafts can be an effective alternative for patients with MHC class II, CGD and WAS.

Introduction:

Primary immunodeficiencies (PID) arise from genetic defects that lead to qualitative or quantitative abnormalities in cells involved in mediating immune function. Partial or complete replacement of the defective cell lineage by allogenic haematopoietic stem cell transplantation (HSCT) from HLA-matched related or unrelated donors remains the curative treatment for most patients (1). However, depending on ethnicity, 30%-80% of patients lack a 10/10 HLA-matched donor (2,3). Although mismatched transplantation (less than 10/10 HLA matched) from related or unrelated stem cells or cord blood donors can be used in this scenario, such approaches are associated with a higher risk of morbidity and mortality compared to HLA-matched transplantation, due to the higher rates of graft rejection, severe Graft versus Host Disease (GvHD) and delayed immune reconstitution. The European Registry for stem cell transplantation in primary immunodeficiency (SCETIDE) has shown similar outcomes for severe combined immunodeficiency (SCID) using either a matched sibling or a matched unrelated donor with a 10 year overall survival (OS) of 82%, however, significantly inferior outcomes were achieved with mismatched unrelated donors or haploidentical grafts during the same period with an OS of 62% and 58%, respectively (4).

Gennery et al (5) conducted a multicentre European study analysing the outcome of patients with SCID and non-SCID PID treated during 1968-2005.Between the year 2000-2005, 181 SCID patients and 267 non-SCID patients were included. Data revealed a poor outcome with the use of mismatched related grafts for SCID (n=96) and non-SCID (n=47) patients with a 3-year survival being 66% and 55%, respectively in contrast to 83% and 76% with the use of a matched related donor transplant.

In more recent years, several groups have developed promising strategies to address the problems of mismatched transplantation. Chiesa et al (2012) (6) reported successful outcome with the use of mismatched cord blood transplantation for a group of non-malignant diseases including PID, achieving full donor engraftment in 86% of the 30 patients studied. Omission of serotherapy in the conditioning regimen in this cohort led to a very rapid CD4+ T-cell immune reconstitution, with early control of viral infections, although there was an increased incidence of aGvHD (6).

Multiple centres in the USA and some centres in Europe have adopted the use of unmanipulated haploidentical transplantation with the use of post-transplant cyclophosphamide as GvHD prophylaxis (7,8,9). Despite encouraging reports in adult patients with malignant disease, there are only few cases reported in children especially with

non-malignant diseases including PID. One of the potential drawbacks of this approach in children has been a high incidence of severe aGvHD among patients less than 10 years of age, possibly reflecting the escape of alloreactive T-cells from post-HSCT cyclophosphamide because of variable metabolism of the drug amongst this age group (9).

Different centres in Europe have moved from CD34+ positive selection with a 3-4 log depletion of T-cells (10,11) to a T-cell receptor (TCR) alpha beta and B-cell depletion strategy of haploidentical and mismatched unrelated grafts to alleviate the risk of GvHD through depletion of GvHD causing T-cells while promoting the transfer of natural killer (NK) cells (12), gamma delta (γδ) T-cells and haematopoietic progenitor cells , to facilitate engraftment and immune recovery. Overall survival has improved with this approach ranging between 83.9% and 91.1% (13,14,15).

To address the impact of these different approaches in mismatched transplantation, we have analysed the outcome of consecutive mismatched donor transplantation in PID patients performed over the last 11 years in the 2 supra-regional centres in the UK.

Methods

Patients

Records of patients with PID who underwent mismatched related or unrelated donor transplantation at the two supra-regional UK centers: Great Ormond Street Hospital for Children, London and The Great North Children's Hospital, Newcastle between January 2006– May 2017 were analyzed. Pre-HSCT data included patient demographics, type of PID, presence of infection and/or autoimmunity, donor-recipient HLA matching, conditioning regimen and graft manipulation. Post-transplant data included count recovery, immune reconstitution, lineage specific chimerism, and occurrence of GvHD, infection and autoimmunity. Informed consent was obtained from the parents of all children.

Donor source, HLA typing, conditioning protocol and graft manipulation.

Bone marrow (BM), peripheral blood stem cells (PBSCs) and cord blood were used as stem cell sources. High resolution typing was performed by molecular typing (at allele level) for HLA-A, -B-C, -DR, -DQ loci. Unrelated donors (including cord blood) were matched for between 5/10 and 9/10 HLA antigens. Preparative regimens were defined as: reduced intensity conditioning (RIC) protocols including Treosulfan/Fludarabine (Treo/Flu) or Fludarabine/Melphalan (Flu/Mel) or RIC Busulphan/Fludarabine (Bu/Flu) targeting Bu AUC45-65mg*hr/L. Myeloablative protocols included myeloablative Bu/Flu (Targeted Bu AUC>70 mg*hr/L) or Treo/Flu/Thiotepa (Treo/Flu/TT). Graft manipulation strategies employed:1) CD34+ selection (16) with add-back of 1-3 X 10*8/Kg CD3+ T-cells [CD34+/T cell add-back], 2) TCR alpha beta and B-cell depletion (17) [TCRαβ/B depletion], 3) unmanipulated cord blood [CB]and 4) unmanipulated bone marrow [BM]or peripheral blood stem cells [PBSC]. Details on the selection of the conditioning regimen , graft manipulation strategy and T cell add-back dose among CD34+ selected grafts are shown in the online repository.

Supportive care:

All patients were nursed in single rooms with laminar flow. Supportive therapy included antimicrobial prophylaxis as per institutional practice (co-trimoxazole prophylaxis was given in both centers in addition to ciprofloxacin in London). Co-trimoxazole was given throughout the transplant in Newcastle while discontinued in D-1 in London to be restarted once absolute neutrophil counts were ≥1000 cells/ul (usually around D+28). In both centers, co-trimoxazole

was completely stopped once the patient was off Cyclosporine and had a CD4 count >300 cells/ul. In London, ciprofloxacin in a dose of 10mg/Kg was given twice daily until absolute neutrophil counts were ≥ 1000 cells/ul. Based on the primary diagnosis, patients received immunoglobulin replacement until B-cell function recovery and ursodeoxycholic acid until D+28. All patients received acyclovir prophylaxis that was discontinued once the patient was off cyclosporine with a CD4≥300 cells/ul (until at least 1-year post-HSCT). The presence of virus detected by PCR in blood (CMV, EBV, Adenovirus in both centres and HHV-6 in Newcastle), nasopharyngeal aspirate (NPA) and stool were recorded weekly from D-10 onwards. Cord transplant patients in London had empirical gut rest and received total parenteral nutrition (TPN) from day -10 until engraftment, to prevent engraftment syndrome, cord colitis and gut GvHD. In addition, they received vancomycin prophylaxis (400 mg/m2) twice daily from day +1, until neutrophil count ≥ 0.2 x 10^9/l) (18).

GvHD

Grading of acute GvHD (aGvHD) was performed according to Seattle criteria (19). Chronic GvHD (cGvHD) was assessed and scored according to the National Institute of Health (NIH) criteria (20).

Engraftment, graft failure and chimerism:

Engraftment was defined as the first of 3 consecutive days with ANC≥500 cells/μL. Primary graft failure was defined as failure to achieve ANC ≥ 500/μL after 28 days of transplant and absence of donor engraftment. Lineage specific chimerism was assessed by polymerase chain reaction amplification of specific polymorphic DNA sequences (short tandem repeats) in circulating lymphoid and myeloid cells.

Immune reconstitution :

T-, B-, NK-cell enumeration used standard flow cytometry markers; CD3, CD4, CD8, CD19, CD56+CD16+. T cell proliferation to mitogen and serological vaccine response to tetanus and pneumococcal antigen were assessed where indicated.

Statistical Analysis:

Statistical analysis was performed using SPSS version 24. Descriptive analyses were performed using the median, mean, minimum and maximum. Parametric data were analyzed using one-way ANOVA and post hoc test. Survival and transplant related mortality (TRM) were analyzed using Kaplan Meier estimates and log rank test. A comparison with 2-sided P < .05 was statistically significant. Variables reaching P < .10 in univariate analysis for overall survival estimations were included in Cox proportional hazard regression models using a backward stepwise selection. GraphPad Prism 7 was used for plotting of T-cell immune reconstitution amongst different methods of graft manipulation. The threshold for statistical significance for all tests was set to P values<0.05.

Results:

Patient characteristics:

There were 147 patients with PID who underwent 155 mismatched related or mismatched unrelated donor transplants at the two centres during this 11years and 4 months period: London (n=91), Newcastle (n=64). 34 patients have been previously reported (15, 21, 22). Among the 155 grafts, 38 had SCID and 117 had non-SCID PID. Table 1 shows a full list of patients' diagnoses. Median age at transplant for the entire cohort was 23 months (range: 1.13-202.9 m) with the median time from diagnosis to transplant being 8 months (range: 0.5-156). Younger age at transplant was seen among patients who either received a CB or a TCR αβ/CD19 depleted graft; worth mentioning that 30/38 (78.9%) SCID patients had received either one of these grafts.

Conditioning &GvHD prophylaxis (table 2)

Reduced intensity conditioning approach [Treo/Flu (n=67) or Flu/Mel (n=26), or Fludarabine/Cyclophosphamide (Flu/Cyc) 120mg/Kg (n=1) or RIC Bu/Flu (n=12)] were mainly used in 106/155 transplants (68.3%). In vivo T-cell depletion using rabbit anti-thymocyte globulin (rATG):6 to 15 mg/kg or Alemtuzumab (Alem):0.3 to 1 mg/kg was employed in the conditioning regimen of 120 HSCTs. The majority (72%) of CB transplants were performed without serotherapy. Five SCID cases (2 δ chain, 1 Rag2, 1ADA, 1 unidentified T-B+NK+ SCID) received an unconditioned transplant including three TCR αβ/CD19 depleted haploidentical infusions and 2 CB grafts (both CB were matched for 9/10 HLA antigens).

Acute (a)GvHD prophylaxis was used in 149/155 transplants [cyclosporine A (CSA) (n=12), CSA+ mycophenolate mofetil (MMF) (n= 126), CSA+ methylprednisolone (MP) (n= 4), or MMF+ steroids (n=4), methotrexate/CSA (n=1), MMF +sirolimus or tacrolimus (n=2)]. Six did not receive any GvHD prophylaxis and were all recipients of the TCRαβ/CD19 depleted grafts as shown in table 2.

Graft Manipulation and HLA matching

Among the 155 grafts, CD34 selection/T-cell addback was employed in 17 transplants (82% were 9/10 HLA matched), TCR αβ/B cell depletion in 30 transplants (90% 5/10 matched) and unmanipulated grafts in 65 (89% were 9/10 HLA matched) and CB in 43 transplants (53% were ≤8/10 HLA matched; a single mismatch at DQ locus being recorded in only 2 cases among CB grafts).

Most of the SCID patients received either a CB (n=20) or a TCR αβ/CD19 depleted graft (n=10) with a median age at transplant of 8.7 and 8.8 months, respectively. The non-SCID cohort received either an unmanipulated BM/PBSC graft (n=61), CB graft (n=23), TCR αβ/B cell depleted graft (n=20) or CD34+/T cell add-back (n=13). Table 2 summarizes the patients' characteristics across different graft manipulations.

Transplant related toxicities

Mucositis grade I-III was recorded among 79 transplants with significantly higher rates of mucositis among unmanipulated grafts: 46/65 (70.7%) versus 17/43 (39.5%) CB, 5/17 (29.4%) CD34+/T-cell add-back and 11/30 (36.6%) after TCR αβ/B-cell depletion($p<0.001$).

CSA induced posterior reversible encephalopathy syndrome (PRES) complicated 2 cords, 2 TCR αβ/CD19 depleted grafts and 1 unmanipulated graft. All had CSA discontinued with subsequent resolution of PRES.

Survival:

The median follow-up for the whole group was 42 months (m) post-HSCT (0.96-139.5m). OS at 8 years was 78.1%:73.3% amongst the SCID cohort and 80.3% amongst the non-SCID cohort. Different graft manipulations did not influence survival: 76.7%, 74.4%, 70.6% and 83.1% among TCR αβ/CD19 depleted grafts, CB grafts, CD34+/T-cell add-back and unmanipulated grafts, respectively ($p=0.579$) (table 3, figure 1).

100-day TRM was 15% (24/155) and overall TRM was 21.9% (34/155). Median time to death was1.8m (range: 0.06-60.3 m). Most deaths were associated with infection and /or GvHD. Table E1 online repository summarizes the cause of deaths among the different graft manipulations. Of note aGvHD with or without viral infection contributed to 4 out of 11 deaths among unmanipulated BM/PBSC grafts. Another 2 patients died of EBV-driven post-transplant lymphoproliferative disease. Viral pneumonitis was the main cause of death among CB grafts: 7 out of 11 deaths. Five had positive respiratory virus detection in NPA at D0. Respiratory failure with or without pulmonary hypertension was the main cause of death among patients who received TCR αβ/CD19 depleted grafts; 5/7 deaths. Interestingly, 4/5 cases had active co-morbid condition at the time of transplant (on methylprednisolone therapy for Omenn syndrome (P29, P31) and active pneumonitis (P30, P32).

Disseminated viral infection contributed to 2/5 deaths among recipients of CD34+ /T-cell add-back grafts. One patient died from veno-occlusive disease (VOD) post-Flu/Mel/Alem conditioning for Artemis SCID (P27). Severe pericardial effusion with respiratory compromise as a complication of GvHD was responsible for the death of one patient (P23). The fifth case died out of respiratory failure and pulmonary hypertension at 1-month post-transplant. This case developed active shingles at the time of conditioning (P25).

Late death beyond 100 days post-transplant was recorded among 10 patients. Median time to late death was 14.6m (range:8-60.3m).; 6 received unmanipulated BM/PBSC grafts (P1, P2, P3, P8, P19, and P21). Three died from active GvHD with or without viral infection (P1, P2, P3) and 2 died from EBV PTLD (P8, P19). Another 2 patients died at 8m and 9m post-TCR αβ/CD19 depleted transplant from disseminated Aspergillus infection (P28) and GvHD/TMA induced Multisystem organ failure (MOF) (P34). P5 died from MOF and sepsis in the context of prolonged immune suppression 5years post CB transplant and P23 died at 42 months post-CD34+ /T cell add-back from aGvHD. Detailed description on the cause of death and factors

influencing survival among mismatched grafts are discussed in detail below and shown in table E1 online repository and table 3.

Effect of conditioning on overall survival:

The use of MAC versus RIC conditioning did not influence OS as shown in table 3. There was however an effect of conditioning within different grafts manipulations. The use of MAC conditioning with unmanipulated BM/PBSC grafts was found to have a negative impact on survival; OS of 66.7% compared to 86.2%; (p=0.01) with the use of RIC conditioning protocols (figure 2).

Post-transplant infections and TRM:

Viral reactivation- mainly occurred in the first 100 days post-transplant- including one or more of CMV, HHV6, EBV, adenovirus, or enteroviral infection were reported among 49.6% (77/155),with a trend to a higher frequency of post-transplant viraemia among TCR αβ/CD19 depleted grafts 70% (21/30) versus other grafts: 37.2% (16/43) CB, 47% (8/17) CD34+/T cell add-back, 49.5% (32/65) unmanipulated grafts (p=0.05). 25/155 (16%) of the patients had active viraemia at time of transplant (D-10-D-1) and 22 of them developed post-transplant viral reactivation.

EBV reactivation was recorded among 14 cases; 4 of which developed EBV-PTLD. All 4 received Alem 1mg/kg in the conditioning regimen; 3 of the 4 died (P8, P19, P21), EBV PTLD being responsible for the death in two. Noticeably, all 4 patients had received prolonged immune suppression for treatment of aGvHD (n=3) or cGvHD (n=1).

Viral reactivation had a negative impact on the outcome. Presence of viraemia between D-10 to D-1 had a negative impact on the outcome with a rise of TRM from 17.6% in absence of viraemia to 44% in the presence of active infection (p=0.004). Moreover, post-transplant viraemia was associated with a rise in TRM from 17.9% in absence of post-transplant viraemia to 26% in presence of post-transplant viraemia however this rise was not statistically significant (table 3, figure 3).

Post-transplant aGvHD/cGvHD and TRM

The cumulative incidence of aGvHD grade I-IV and grade II –IV by 180 days post-transplant was 62.5% (85/136 evaluable cases) and 35.2% (48/136 evaluable patients) respectively. aGvHD grades II-IV was significantly more frequent among CB grafts (56.7%), CD34+/T cell add-back (40%), and unmanipulated grafts (31%) while only few recipients of TCRαβ/CD19 depleted grafts experienced significant aGvHD (11.5%); p=0.002. Liver and gut GvHD were noticeably low among TCR αβ/CD19 depleted grafts (3.4%) in comparison to other grafts; 18.9 % among unmanipulated grafts, 20% among CD34+/T cell add-back and 29.7% among CB grafts (p=0.06).

Patients were treated with steroids either alone or in combination with monoclonal antibodies; daclizumab/infliximab (n=18), Alem (n=1), extracorporeal photopheresis (ECP) (n=4) or mesenchymal stem cells (MSC) (n=2).

aGvHD grade II-IV was associated with a significant rise of TRM from 2.3% in patients with grade 0-I to 31.4% among patients with grades II-IV; p<0.001. Data are shown in table 3 and figure 4.

One-year cumulative incidence of cGvHD was 15.9% (18 out of 113 evaluable patients). cGvHD was not recorded among any recipient of TCR αβ/CD depleted grafts (0/18) versus 21.8% (7/32), 12% (6/50) and 38.4% (5/13) amongst CB, unmanipulated BM/PBSC grafts and CD34+/T cell add-back respectively (p<0.001). 7/18 patients did not receive any serotherapy; all 7 received CB grafts.

Extensive cGvHD was recorded among 8 out of the 18 patients including lung(n=2), gut (n=4), pericardial (n=1) or extensive polyarticular arthritis (n=1). Only 2 out of the eight cases are still on immunosuppressive medications to control either lung or gut/skin cGvHD-both are recipients of CB graft with no serotherapy. The remaining 10 cases had limited skin cGvHD that is currently under control.

Post-transplant autoimmunity :

Data on post-transplant autoimmunity (AI) was available for 126 grafts who survived at least 6 months post-transplant. Nineteen grafts were associated with post-transplant AI; occurring at a median of 7 months post-transplant (range: 1-24). 16 developed either autoimmune haemolytic anaemia (AIHA), autoimmune thrombocytopenia (ITP) or autoimmune neutropenia (AIN) that responded to either one or a combination of prednisolone, rituximab and high dose intravenous immunoglobulin (IVIG). Other forms of AI included oligoarticular juvenile idiopathic arthritis at 30 months post-unconditioned CB transplant for ADA SCID, SLE-like picture with the nephrotic syndrome at 4.36 months post-Treo/Flu/Alem unmanipulated BM for IFKB GOF mutation and Guillian Barre syndrome (GBS) at 16 months post RIC Bu/Flu/Alem unmanipulated BM for XL-CGD.

Pre-transplant autoimmunity was recorded in 2/19 patients who developed an autoimmune process post-transplant. One had IPEX syndrome complicated with autoimmune enteropathy and insulin dependent diabetes mellitus (with positive anti-enterocyte antibodies and anti-insulin antibodies) whose enteropathy settled at 4 months post-HSCT however, he developed AIHA and AIN at 5 months post-HSCT that required a combination of prednisolone and rituximab therapy. The second patient was a WAS patient who had autoimmune neutropenia and developed post-transplant autoimmune thrombocytopenia requiring prolonged immunosuppression. All patients were in remission at the time of last follow-up. Diagnosis (SCID versus non-SCID), conditioning (MAC versus RIC), use of serotherapy, graft manipulation, presence or absence of aGvHD grade II-IV, presence or absence of cGvHD, post-transplant viral infection, donor engraftment (full versus mixed) did not influence the occurrence of post-transplant AI; p=0.46, p=0.514,p=0.89,p=0.24, p=0.9 and p=0.5, p=0.75

respectively. Post-transplant autoimmunity did not influence overall survival as shown in table 3.

Endothelial toxicities

Veno-occlusive disease (VOD) was seen following 6 grafts between D+6 and D+90. All patients received CSA based GvHD prophylaxis. None received a Bu- based conditioning. Three received Treo/Flu, two Flu/Mel and one had a Treo/Flu/TT conditioned transplant. Three of the six patients died; VOD was the cause of death in only one of them (P27).

TMA was seen among 7 cases. All received a TCR αβ/CD19 depleted haploidentical (n=5) or 8/10 mMUD (n=2) transplant. All patients had aGvHD grade I-III and 6/7 had concurrent systemic viral infections/reactivations. In three cases TMA developed after a second conditioned mismatched transplant procedure. Active co-morbid condition at time of transplant was also present in 3/7 cases; active aGvHD at time of transplant (P35) and lung disease (P32, P34). 4/7 patients died but only one directly due to TMA (TMA involving lung, with adenoviraemia and MOF (P32). Table E2 online repository summarizes the characteristics of patients who developed TMA. Of note, P35 had a confirmed mutation in CD46 gene that codes for type I membrane protein known to play a regulatory role in the complement system.

Factors affecting overall survival among mismatched grafts:

Based on data from both univariate and multivariate analysis (detailed in table 3), the occurrence of aGvHD ≥II (HR:14.9; p<0.001) occurrence of TMA (HR:8.2; p:0.001) were the main factors associated with poor outcome among mismatched grafts while other factors including diagnosis (SCID versus non-SCID), HLA typing (9/10 versus 5/10-8/10 HLA matched donor), stem cell source (BM versus PBSCs versus CB), graft manipulation ,conditioning (MAC versus RIC) , the use of serotherapy (yes versus no), type of serotherapy (rATG versus Alem), the use of aGvHD prophylaxis agents (yes versus no), Pre-transplant viremia (D-10-D-1 (yes versus no), blood viral reactivation infection (yes versus no), post-transplant respiratory viral infection (yes versus no), post-transplant autoimmunity (yes versus no) and donor engraftment (full versus mixed) did not influence overall survival (table 3).

Engraftment (data given in tables 4 and 5):

Seven patients died early before D+28; thus, were excluded from the analysis. 10 patients (10/148; 6.7%) had either primary graft loss (failure to achieve a neutrophil count ≥500 cells/ul within 28 days of HSCT) or low-level donor chimerism requiring intervention with a second mismatched graft or an unconditioned stem cell boost. Eight of 10 had received a RIC conditioned graft either Flu/Mel (n=1), Treo/Flu (n=5), RIC Bu Flu (n=2). Two patients died post-intervention, one developed hyperacute GvHD post-PBSC stem cell boost for combined immune deficiency and another developed idiopathic pneumonitis post- 2nd transplant for CGD.

More rapid neutrophil and platelet engraftment were achieved in recipients of TCR αβ/CD19 depleted grafts without using G-CSF versus other grafts (table 2). Among individual groups; the rate of neutrophil recovery was significantly quicker among TCRαβ/CD19 versus CB; (p=0.001) and versus CD34+/T cell add-back (p=0.05) while no difference was seen in relation to unmanipulated grafts (p=1). Platelet recovery was significantly quicker among TCRαβ/CD19 depleted grafts versus all other grafts; CB, unmanipulated and CD34+/T cell add-back; p=0.001, p=0.007, p=0.03. There was no difference recorded in the rate of platelet and neutrophil recovery between unmanipulated and CD34+ selection/T cell add-back; p=1, p=1 respectively.

Data on donor engraftment were available for 140 grafts. Full donor chimerism was achieved more readily among recipients of either TCR αβ/CD19 depleted or CB grafts compared to CD34+ /T cell add-back and unmanipulated BM/PBSC grafts; 78.5%, 81.5% vs 41.1%, 47.3%, respectively (p=0.028). Full donor engraftment was more frequently achieved among recipients of MAC conditioning (83%; 31.37) versus either RIC or MIC conditioning (66.6%; 66/99); p=0.013. Five patients received an unconditioned graft; data were available for 4 patients, all had mixed donor engraftment. The degree of donor engraftment (full versus mixed) did not influence OS as shown in table 3.

Immune reconstitution :

At one-year post-transplant (data available for 97 grafts), CD3≥1000 cells/ul, CD4≥ 300cells/ul and CD8≥500 cells/ul was achieved by 68/97 (70%), 78/97 (80%) and 56/97 (57.7%) of the survivors.

Robust CD3+T-cell recovery was observed as early as 3 months amongst recipients of CB grafts, significantly faster than other groups (p<0.0001). CD4+ T-cell counts ≥ 300cells/ul was achieved amongst 109 (70.3%) recipients of mismatched grafts: at a median of 2.5 m for CB grafts versus 5 months for TCR αβ/CD19 depleted grafts and 7 months for both the CD34+/T cell add-back and unmanipulated BM/PBSC grafts (p=0.007); Table 5 and figure 5. This difference in the speed of CD4 recovery was significant between CB versus unmanipulated and CD34+/T cell add-back; p=0.006, p=0.05 while non-significant between CB versus TCRαβ/CD19 grafts (p=0.4) and between unmanipulated versus CD34+/T cell add-back grafts (p=1).

At one-year post-transplant, 71/82 (86.5%) survivors (who were on regular IVIG pre-transplant) were able to discontinue immunoglobulin replacement therapy; 14/17 (82%) TCRαβ/CD19 depleted graft, 22/28 (78.5%) for CB, 7/8 (87.5%) for CD34+/T cell add-back grafts and 29/30 (96.6%) for unmanipulated BM/PBSCs grafts (p=0.206).

Outcome of mismatched transplantation among patients with SCID/Omenn phenotype:

Thirty-eight patients with SCID/Omenn syndrome received 38 mismatched grafts. Details on diagnoses was shown in table 1; 68% had T- B- SCID (mainly with either RAG 1, RAG 2 mutation or combined RAG1 and RAG2) while 32% had a T-B+ SCID (mainly common γ chain and IL7 receptor α defect). 27/38 (71%) patients had developed at least one severe

infectious episode before going for HSCT, 7/38 (18%) patients had active viraemia at D-10-D-1. 49% of the patients were transplanted before the age of 6 months while 38% had their transplant after their first birthday with a median age at transplant of 8.5 months. 30 patients received either a mismatched CB (n=20; 60% are 9/10 HLA matched) or a TCRαβ/CD19 depleted grafts (n=10; all 5/10 HLA matched related donors). Treo/Flu was the main conditioning protocol among CB (13/20; 65%) while recipients of TCRαβ/CD19 depleted grafts mainly received Treo/Flu/TT (6/10; 60%). Serotherapy was included in the conditioning protocol of 5/20 CB; 25% (rATG (n=1), Alem (0.3-1mg/kg (n=4)) while 80% of recipients of TCRαβ/CD19 depleted grafts received serotherapy in the form of rATG 15 mg/kg; n=5 or Alem 1mg/kg; n=3.

Overall survival was 71 %. Previous severe infection and T+B- SCID were associated with unfavourable outcome with OS of 66.6%, 65% versus 88.8% and 83% in absence of any reported infection and B+SCID, respectively; however, the difference was not statistically significant; p=0.09, p=0.21. Possibility reflecting the small sample size.

Post-transplant viral reactivation, aGvHD grade ≥ II, cGvHD, graft loss was reported among 39% 40%, 18.5% and 0% among evaluable cases (table E3 online repository).

Based on data from both univariate and multivariate analysis (detailed in table E3 online repository), the occurrence of aGvHD ≥II (HR: 20.3 $p<0.001$) was the main factor associated with poor outcome among mismatched grafts while HLA typing (9/10 versus 5-8/10 HLA matched donor), stem cell source (PBSCs versus CB), graft manipulation ,conditioning (MAC versus RIC) , the use of serotherapy (yes versus no), type of serotherapy (rATG versus Alem), pre-transplant viraemia (D-10-D-1 (yes versus no), post-transplant viral reactivation (yes versus no), post-transplant respiratory viral infection (yes versus no), post-transplant AI (yes versus no) and donor engraftment (full versus mixed) did not influence OS (table E 3).

Five patients had unconditioned stem cell transplant; 3 of them had an active respiratory infection at time of transplant. Unfortunately, 2 of the patients died; P14 and P31 (table E1 online repository). The remaining 3 patients (ADA SCID, T-B+ SCID and a common γ chain SCID are alive and well with stable high- level donor T cell engraftment at last follow-up.

Outcome of mismatched transplantation within specific non-SCID diseases:

CGD

17 patients with chronic granulomatous disease (CGD) received 19 transplants:15 unmanipulated BM/PBSC grafts, 1 CB graft, 1 CD34+ /T cell add-back and, 2 TCR αβ/CD19 depleted grafts. Eight (50%) received RIC Bu/Flu conditioning, 1 had MAC Bu based conditioning while the remainder received a Treo-based conditioning. Overall survival was 94.7% with a median time to neutrophil recovery of 15 days and high- level donor engraftment above 85% amongst all survivors at a median follow-up of 31.7 months.

MHC class II

Ten patients received 10 HSCT transplant for MHC class II; 4 Treo/Flu/Alem 9/10 unmanipulated grafts, 4 Treo/Flu CB grafts with no serotherapy and 2 Treo/Flu/TT/rATG conditioned TCR αβ/CD19 depleted grafts. All were alive at a median follow-up of 16.28 (6-64.8m).9 /10 patients achieved CD4 counts above 300 cells/ul at a median of 4m post-HSCT (range: 3-12m).

Wiskott Aldrich syndrome (WAS)

Ten patients received 10 mismatched transplants for XLT (n=1) and WAS (n=9); 3 Treo/Flu/Alem unmanipulated grafts, 3 Treo/Flu CB grafts with no serotherapy and 4 TCR αβ/CD19 depleted grafts conditioned with Treo/Flu/TT/rATG conditioning (n=3) or Bu (MAC)/Flu/TT/rATG. All patients were alive at a median follow-up of 52.3m post-HSCT with platelet counts above 100 $X10^9$/L and a median time to CD4 recovery of 6m. 9/10 were off immunoglobulin replacement at last follow-up.

Primary Haemophagocytic Lymphohistiocytosis (HLH)

Twenty-two cases received 23 transplants; 9unmanipulated grafts, 7CB, 4 CD34+ /Tcell add-back and 3 TCR αβ/CD19 depleted grafts. Overall survival was 69.9% at a median follow up of 33 m (range: 0.23-120.3m); being lowest among cases with non-genetically defined HLH (57%; 4/7) versus 83.3% (5/6) with Perforin mutations, 80% (4/5) with XLP, 75% (3/4) with Munc 13-4 or Syntaxin mutations (p=0.43). 15 patients survived transplant with disease amelioration at 56m post-transplant (6-120.36m).

Discussion:

This study directly compared the outcome of mismatched HSCT in PID using different graft sources and different types of graft manipulation. The data clearly showed an improvement in outcomes among both SCID and non-SCID PID patients who received mismatched grafts during this recent period, with a drop in TRM from 40-50% (4,5) to 22% in the current study. While it can be argued that more than half of the grafts were 9/10 HLA matched (59%) and this might have influenced the outcome, it is clear from the current data that single antigen mismatches (9/10) was not associated with a better survival in comparison to 5/10-8/10 mismatches (73%.9% vs 84.1%, respectively); p=0.131 .

Comparable rates of survival were recorded among different graft manipulation strategies., however there were differing advantages and disadvantages between the different approaches. In SCID, the use of rapidly available graft sources namely TCR αβ/CD19 depleted haploidentical grafts or CB grafts was associated with an overall survival of 73% which is better than previous reports (from Europe) but still suboptimal in comparison to matched sibling donor transplantation. However, it is important to highlight that the median age of transplant of these patients, was around 8 months, with some patients being diagnosed relatively late in the absence of neonatal screening, some waiting for unrelated matched donor search results and 24/30 (80%) patients had already acquired significant pre-transplant infections. All these factors have negatively influenced the success rate. The Primary Immune

Deficiency Treatment Consortium (PIDTC) recently published data on a prospective study including 100 SCID patients where the 2-year OS was 90%. Most patients in this study were in US centres and many diagnosed by neonatal screening. While this study clearly illustrated that the type of donor did not influence survival, TRM was increased in those patients with infection at the time of transplant: OS was 95% for those infection-free at HSCT vs. 81% for those with active infection (p=0.009) (23). Both studies therefore advocate proceeding to HSCT prior to the development of infection. Prolonged waits for the outcome of unrelated donor searches may be counterproductive particularly in SCID patients.

T-B-SCID constituted 70% of our studied SCID cohort and was associated with a dismal outcome versus T-B+SCID with survival rates of 66% vs 83%; respectively. Our results are equivalent to previous report from Gennery et al, 2010 who reported a reduced 10-year overall survival of 50% among T-B-_SCID versus 70% survival among T-B+ SCID. Consequently, our results clearly demonstrated improved overall survival with the use of mismatched grafts amongst SCID patients, including more challenging SCID subtypes, using new modalities of graft manipulation: TCRαβ/CD19 depletion and CB with no serotherapy.

The use of TCR αβ/CD19 depletion was associated with low rates of severe (grade II-IV) aGvHD (11.5%) and absence of cGvHD. One drawback of TCR αβ/CD19 depleted HSCT was the increased incidence of post-transplant viraemia reaching 70% versus 37%-49% among other graft manipulations. γδT cells and NK cells in TCR αβ/CD19 grafts were thought to provide some protection against viral reactivation, however, it seems that the degree of TCR αβ depletion that abrogated the incidence of aGvHD and cGvHD might have limited the capacity of the graft in managing early post-transplant viral infection. Further strategies are therefore required to promote immune recovery after TCR αβ/CD19 depleted grafts. In this respect, Algeri et al, recently reported data on 46 patients with PID given TCR αβ/CD19 depleted grafts followed by the adoptive transfer of genetically modified donor T-cells transduced with inducible caspase 9 suicide gene (icas9). Two-year overall survival was 95% with improved T cell recovery; the mean number of CD3+ cells at 1, 3, 6, 12 and 24 months after HSCT was 377, 690, 1563, 3096 and 3300/µl with few patients having significant problems with post-transplant viraemia (24).

Another recognised complication of mismatched grafts was TMA. This was recorded amongst 7 cases in our study, all of whom received TCR αβ/CD19 depleted grafts (7/30 = 24%). Though this incidence is equivalent to that reported in the literature among matched related and unrelated grafts 20-30% (25), it is interesting to understand why TMA was not seen among the other graft manipulation strategies. One possible explanation is that TMA might have been missed or misdiagnosed as aGvHD especially in transplants performed before 2014 when Jodele et al (26) published the latest diagnostic criteria for post-HSCT TMA. In a larger cohort of 57 TCR αβ/CD19 depleted grafts (including patients who received adoptive transfer of genetically modified T cells with icas9) performed in patients at both centres for PID (n=48) or malignant disease (n=9), 18 % of patients developed TMA. In multivariate analysis, the only 2 risk factors for the development of TMA were the presence of aGvHD grade II-IV (OR: 10.4; p=0.01) and active comorbid condition at time of transplant (OR: 6.5; p=0.06) (personal communication). Looking at the 7 cases that

developed TMA in this paper, 4 had active comorbid condition at transplant, 3 had developed TMA after a second conditioned graft and all experienced aGvHD. All these factors might have contributed to endothelial stress and the development of TMA in our studied cohort.

Another readily accessible stem cell source is CB from the expanding number of CB banks worldwide. The London group have previously reported encouraging results in children who underwent mismatched CB transplant without serotherapy for malignant and non-malignant diseases with a TRM of 3.5% and early T cell recovery with a median time to achieve CD4+T cells $\geq$ 300 of 30 days due to the peripheral expansion of adoptively transferred naïve T cells (6). The same results were extrapolated among 6 patients with MHC class II deficiency who received a cord graft without serotherapy where all patients were alive at a median follow-up of 25 months post-HSCT. Though this approach secured high rates of donor engraftment and rapid immune reconstitution, there was an increased risk of significant acute and chronic GvHD (16). In the whole cohort of CB transplants, 72% received a T cell replete graft and despite the low incidence of viral infections associated with early CD4 recovery, there was a high incidence of aGvHD grade II-IV and visceral (gut) aGvHD: 56.7% and 29.7% respectively. These patients required prolonged immunosuppressive therapy beyond 1-year post-HSCT until their GvHD resolved. Investigators are now looking at the use of targeted ATG based on patient weight and lymphocyte count to alleviate the risk of GvHD while preserving prompt immune reconstitution (27). The Newcastle group has also published promising data using low dose Alem 0.3-0.6 mg/Kg with matched and mismatched cord transplants. Interestingly, low dose Alem allowed rapid T cell reconstitution as early as 4 months post-transplant with comparable rates of aGvHD and cGvHD between recipients of low versus high dose Alem (21).

One of the main problems with mismatched grafts is a high rate of graft rejection. Here, we observed a significantly low rate of graft rejection of 6.5%. Though, there was no difference in engraftment among the different graft manipulations, both TCR αβ/CD19 depleted and CB grafts showed superiority over other graft manipulations in achieving full donor chimerism: 80% of the patients versus 40% among unmanipulated BM/PBSC grafts and CD34+/T cell add-back grafts. While omission of serotherapy has probably allowed high levels of donor engraftment among CB grafts, it is not clear why TCR αβ/CD19 depleted grafts showed the same finding. One possible explanation might be the use of a myeloablative conditioning among recipients of this type of graft while RIC conditioning was given to most of the recipients of unmanipulated BM/PBSC or CD34+ selection/T cell add-back grafts. Another possibility might be related to the constituents of the graft with the infusion of mega dose of CD34+ cells accompanied by γδT-cells, dendritic cells and NK cells acting as engraftment facilitators (12).

There was a centre preference in the selection of the best mismatched graft. The London team preferred to use mismatched cords with no serotherapy while the Newcastle team preferred to use a TCRαβ/CD19 parental haploidentical transplant in the absence of a 9/10 or 10/10 HLA matched donor. Currently, the Newcastle team use TCRαβ/CD19 depletion for any 9/10 matches instead of using an unmanipulated bone marrow or peripheral blood stem cell graft.

In conclusion, this study presented a detailed analysis of the outcomes of HLA-mismatched HSCT in 147 PID patients at 2 supra-regional UK paediatric centres. Importantly, these are the patients that have frequently been most challenging to manage, and some developing comorbidities while waiting for HSCT with some centres electing to delay transplantation or pursue gene therapy, if available. OS of the cohort was 79%, which is markedly better than the survival in some of the large historical cohorts. Impressively, there was only a 6.7% incidence of graft failure. Disappointingly, a high percentage of viral reactivation (70% with TCR αβ/CD19 depletion) and grade II-IV aGvHD (56.7% with CB HSCT without serotherapy) was observed. Stable full donor engraftment was >80% in TCRαβ/CD19 depletion and CB compared to only 40-60% in other groups, probably reflecting the differential conditioning regimens.

This study described in detail the pattern of immune reconstitution after mismatched grafts where immune reconstitution was most rapid after CB, followed by TCR αβ/CD19 depletion, while reconstitution for CD34+ selection/T cell add-back and unmanipulated grafts was slower.

Finally, one of the important findings in this analysis is the excellent outcome of mismatched grafts among specific diseases, in particular MHC class II deficiency, CGD and WAS. Although the numbers are relatively small, these outcomes are equivalent to that from matched donor sources and this offers significant hope of cure in these patients who do not have matched donors available. Unfortunately, outcome in HLH remains poor and requires further improvement.

Based on our results, we would recommend 1) the use of a mismatched grafts without delay in patients with PID lacking a matched donor or when an urgent HSCT is indicated, 2) consider using a targeted ATG dose or low dose Alem with mismatched CB grafts, and 3) investigating the possibility of increasing the TCRαβ dose given in TCRαβ/CD19 depleted parental grafts or the adoptive transfer of genetically modified T cells with a suicide gene to allow earlier immune recovery with better control of viral reactivation and without increasing the risks of aGvHD or cGvHD.

References:

1. Steno S, Boelens JJ. Advances in unrelated and alternative donor hematopoietic cell transplantation for nonmalignant disorders. Curr Opin Pediatr. 2015 Feb;27(1):9-17.
2. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014 Jul 24;371(4):339-48.
3. Tiercy JM. How to select the best available related or unrelated donor of hematopoietic stem cells? Haematologica. 2016 Jun; 101(6): 680–687.

4. Booth C, Silva J, Veys P. Stem cell transplantation for the treatment of immunodeficiency in children: current status and hopes for the future. Expert Rev Clin Immunol. 2016 Jul;12(7):713-23.

5. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P et al. 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 Sep; 126(3):602-610 e601-611.

6. Chiesa R, Gilmour K, Qasim W, Adams S, Worth AJ, Zhan H, et al. Omission of in vivo T-cell depletion promotes rapid expansion of naive CD4+ cord blood lymphocytes and restores adaptive immunity within 2 months after unrelated cord blood transplant. British journal of haematology. 2012; 156(5):656-66.

7. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and post-transplantation cyclophosphamide. Blood. 2001 Dec 1; 98(12):3456-64.

8. O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using post transplantation cyclophosphamide. Biol Blood Marrow Transplant. 2002; 8(7):377-86.

9. Jaiswal SR, Chakrabarti A, Chatterjee S, Ray K, Chakrabarti S. Haploidentical transplantation in children with unmanipulated peripheral blood stem cell graft: The need to look beyond post-transplantation cyclophosphamide in younger children. Pediatr Transplant. 2016 Aug;20(5):675-82.

10. Finke J, Brugger W, Bertz H, Behringer D, Kunzmann R, Weber-Nordt RM, et al. Allogeneic transplantation of positively selected peripheral blood CD34+ progenitor cells from matched related donors. Bone Marrow Transplant. 1996 Dec; 18(6):1081-6.

11. Handgretinger R, Klingebiel T, Lang P, Schumm M, Neu S, Geiselhart A, et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplant. 2001 Apr;27(8):777-83.

12. Escobedo-Cousin M, Jackson N, Laza-Briviesca R, Ariza-McNaughton L, Luevano M, Derniame S, et al. Natural Killer Cells Improve Hematopoietic Stem Cell Engraftment by Increasing Stem Cell Clonogenicity In Vitro and in a Humanized Mouse Model. PLoS One. 2015 Oct 14;10(10): e0138623.

13. Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R, et al. HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with non-malignant disorders. Blood. 2014 Jul 31;124(5):822-6.

14. Balashov D, Shcherbina A, Maschan M, Trakhtman P, Skvortsova Y, Shelikhova L, et al. Single-Center Experience of Unrelated and Haploidentical Stem Cell Transplantation with TCRαβ and CD19 Depletion in Children with Primary

Immunodeficiency Syndromes. Biol Blood Marrow Transplant. 2015 Nov;21(11):1955-62.

15. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, et al. T-cell receptor αβ+ and CD19+ cell-depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J Allergy Clin Immunol. 2018 Apr; 141(4):1417-1426.

16. Lang P, Schumm M, Taylor G, Klingebiel T, Neu S, Geiselhart A, et al. Clinical scale isolation of highly purified peripheral CD34+progenitors for autologous and allogeneic transplantation in children. Bone Marrow Transplant.1999;24:583-589.

17. Bremm M, Cappel C, Erben S, Jarisch A, Schumm M, Arendt A, et al. Generation and flow cytometric quality control of clinical-scale TCRαβ/CD19-depleted grafts. Cytometry B Clin Cytom. 2017 Mar;92(2):126-135.

18. Kurt B, Flynn P, Shenep JL, Pounds S, Lensing S, Ribeiro RC, et al. Prophylactic antibiotics reduce morbidity due to septicemia during intensive treatment for pediatric acute myeloid leukemia. Cancer. 2008 Jul 15;113(2):376-82.

19. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974; 18(4):295-304.

20. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. 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: journal of the American Society for Blood and Marrow Transplantation. 2005;11(12):945-56.

21. Lane JP, Evans PTG, Nademi Z, Barge D, Jackson A, Hambleton S, et al. Low dose serotherapy improves early immune reconstitution after cord blood transplantation for primary immunodeficiencies. Biol Blood Marrow Transplant. 2014; 243-249.

22. Elfeky R, Furtado-Silva JM, Chiesa R, Rao K, Lucchini G, Amrolia P, et al. Umbilical cord blood transplantation without in vivo T-cell depletion for children with MHC class II deficiency. J Allergy Clin Immunol. 2018 Jan 31.

23. Heimall J, Logan BR, Cowan MJ, Notarangelo LD, Griffith LM, Puck JM, et al. Immune reconstitution and survival of 100 SCID patients post-hematopoietic cell transplant: a PIDTC natural history study. Blood. 2017 Dec 21; 130(25):2718-2727.

24. Algeri M, Slatter M, Qasim W, Bertaina V, Pagliara D, Galaverna F, et al. Outcomes of children with primary immunodeficiencies receiving alpha/beta T cell depleted HLA-haplo-HSCT followed by infusion of lymphocytes transduced with inducible caspase 9 (IC9) suicide gene. Oral presentation; EBMT 2018.

25. Laskin BL, Goebel J, Davies SM, Jodele S. Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Blood; 2011 118; 1452-1462.

26. Jodele S, Davies SM, Lane A, Khoury J, Dandoy C, Goebel J, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood. 2014 Jul 24; 124(4):645-53.

27. Admiraal, R, van Kesteren, C, Jol-van der Zijde, CM , Lankester AC, Bierings MB, Egberts TC et al. Association between anti-thymocyteglobulin exposure and CD4+ immune reconstitution in paediatric haemopoietic cell transplantation: a multicentre, retrospective pharmacodynamic cohort analysis. Lancet Haematol. 2015; S2352-3026(15): 45-9

Table 1: Diagnoses (n=155)

DIAGNOSIS	NUMBER
SCID	**38**
T-B+SCID	Total:12
IL7R defect	3
Jak3	1
Common δ chain	4
LAT SCID	1
Non-genetically identified	3
T-B-SCID	Total:26
ADA	4
PNP	1
RAG 1	7
RAG2	2
Combined RAG1 and RAG2	2
Artemis	3
DNA ligase IV	1
Non-genetically identified	6
CGD	19
AR CGD	3
XL CGD	6
Not mentioned	10
DOCK8	5
CD40L	4
NEMO	2
CHH	5
Cernunnos	1
ICF	1

PI3Kinase	1
DNA repair defect	1
Other CID	10
MHC CLASS II	10
WAS	10
HLH	23
Perforin HLH	6
XLP	4
XIAP	1
Munc 13-4	3
Syntaxin	1
Non-genetically identified	7
IPEX	3
Crohn's like IBD	1
STAT3 GOF	1
LAD I	2
Severe Congenital neutropenia	5
CINCA like syndrome	1
Chediak Higashi	1
LAD III	1
GATA2 mutation	1
IFKB GOF mutation	2

Abbreviations: SCID: severe combined immune deficiency, IL7R: IL7 receptor defect, RAG: recombinase activating gene, ADA: adenosine deaminase, PNP: purine nucleoside phosphorylase CGD: chronic granulomatous disease, CD40L: CD40 Ligand, CHH: Cartilage hair hypoplasia, ICF: immune deficiency centromeric instability facial dysmorhism syndrome, CID: combined immune deficiency, WAS: Wiskott Aldrich syndrome, HLH: Haemophagocytic lymphohistiocytosis, XLP: X-linked lymphoproliferative disease, XIAP: X-linked inhibitor of apoptosis, IPEX: immune dysregulation polyendocrinopathy X-linked disease, GOF: gain of

function, LAD: leukocyte infusion defect, CINCA: chronic infantile neurological cutaneous articular syndrome, IFKB: interferon kappa beta.

Table 2: Patients' characteristics

Type of graft	TCR αβ/CD19 dep N=30	Cords N=43	CD34+selection/ Tcell addback N=17	Unmanipulated BM/PBSC graft N=65
Diagnosis				
SCID (n=38)	10/30	20/43	4/17	4/65
Non-SCID (n=117)	20/30	23/43	13/17	61/65
Age at HSCT Median (range) (m)	20.4 (3.36-146)	11.76 (1.13-93.5)	42.4 (5.76-180.5)	53.6 (5-202.7)
Time from Diagnosis to HSCT Median (range) (m)	4 (0.5-16)	5.5 (1-48)	8 (3-84)	14 (2-156)
HLA typing				
9/10	0/30	20/43	14/17	58/65
8/10	3/30	14/43	3/17	7/65
5/10 to 7/10	27/30	9/43	0/17	0/65
Graft				
BM	0/30	0/43	1/17	33/65
Cord	0/30	43/43	16/17	0/65
PBSC	30/30	0/43	0/17	32/65
Conditioning				
MAC	25/30	10 /43	0/17	6/65
Given protocol	Treo/Flu/TT(n=24) Bu/Flu /TT(n=1)	Treo/Flu/TT(n=2) Treo/Cyc200(n=7) Bu(MAC)/Flu(n=1)	NA	Treo/Flu/TT (n=2) Treo/Cyc200 (n=2) Bu/Cyc (n=2)
RIC	1/30	31/43	16/17	58/65
Given protocol	Treo/Flu	Treo/Flu	Treo/Flu (n=6)	Treo/Flu (n=29)

			Flu/Mel (n=10)	Bu/Flu (n=11) Bu/Mel/Cyc (n=1) Flu/Mel (n=16) Flu/Cyc20mg/kg (n=1)
MIC	1/30	0/43	1/17	1/65
Given protocol	Cyc/TBI 3Gy/Flu	NA	Cyc1500mg/m^2/Flu 150/antiCD45 1600ug/kg	Cyc/TBI 3Gy/Flu
UC	3/30	2/43	0/17	0/65
Serotherapy				
(N, %)	27/30; 90%	12/43; 27.9%	17/17; 100%	64/65; 98.4%
Serotherapy used				
rATG	n=22	n=4	n=0	n=0
Alem	n=5	n=8	n=17	n=64
(N)				
GvHD prophylaxis				
(N, %)	24/30 (80%)	43/43 100%	17/17 100%	65/65 100%
CSA only (N)	12/30	0/43	0/17	0/65
2 agents (N)	12/30	43/43	17/17	65/65
CD34 Cell dose				
(X10^6/kg)	17.6	0.37	18.5	6
Median range	(4-50.9)	(0.1-1.53)	(3.55-63.85)	(0.75-50.19)
CD3 Cell dose				
(X10^6/kg)	17	6.4	300	89
Median range	(2-45)	(0.68-100)	(45-636)	(1.22-2047)
Included HSCT (N)	16/30 δ	41/43 δ	17/17	59/65 δ

Abbreviations: MAC: myeloablative conditioning, RIC: reduced intensity conditioning, MIC: minimal intensity conditioning, Treo: Treosulfan, Flu: Fludarabine, TT: Thiotepa, Cyc: Cyclophosphamide, Cyc 200: Cyclophosphamide 200mg/Kg, TBI: total body irradiation, UC: unconditioned, rATG: rabbit anti-thymocyte globulin, Alem: Alemtuzumab, m: months. N: number, %: percentage, NA: not applicable.

δ data on CD3+ cell dose was only available for 16 TCRαβ/CD19 grafts, 41 CB and 56 unmanipulated grafts.

For TCRαβ/CD19 depletion, TCRαβ dose was calculated in all grafts with a median of 2.9 x10^4/Kg (range: 0.08-5.2 x10^4/Kg).

Table 3 : Analysis of independent factors affecting overall survival among mismatched grafts

Univariate analysis					**Multivariate analysisΩ**	
Outcome factors	Absolute number of patients	Absolute number of deaths	2 year overall survival	P-value	Hazard ratio (95% CI)	P-value
Diagnosis						
SCID	38	11	71%	0.229		
Non-SCID	117	23	80.3%			
HLA						
9/10	92	24	73.9%	0.131		
5-8/10	63	10	84.1%			
Stem cell source						
BM	34	5	85.2%	0.489		
PBSCs	78	18	76.9%			
Cords	43	11	74.4%			
Graft manipulation						
	30	7	76.7%	0.579		
TCRαβ/CD19 dep	43	11	74.4%			
Cord CD34+/T cell add-back	17	5	70.6%			
Unmanipulated grafts	65	11	83.1%			
Conditioning						
MAC	41	9	78%	0.607		
Others	109	23	78.8%			
No conditioning	5	2	60%			
Serotherapy included						
Yes	120	26	78.3%	0.881		
No	35	8	77.1%			
Type of serotherapy used						
rATG	26	6	76.9%	0.844		
Alemtuzumab	94	20	78.7%			
Use of GvHD prophylaxis						
Yes	149	31	79.1%	0.09	1.9 (0.4-10)	0.6
No	6	3	50%		1	
Presence of pre-transplant viraemia						

(D-10-D-1)						
Yes	25	11	56%	0.004	2.24 (0.76-6.5)	0.14
No	130	23	82.3%		1	
Post-transplant viraemia						
Yes	77	20	74%	0.25		
No	78	14	82%			
Post-transplant respiratory infection						
Yes	28	10	64.2%	0.052	3(0.9-9.9)	0.065
No	127	24	81.8%		1	
aGvHD						
	48				14.9(3.4-66.1)	
	88				1	
Grade II-IV		15	68.7%	0.001		<0.001
Grade 0-I		2	97.7%			
TMA						
Yes	7	4	42.8%	0.021	8.2 (2.3-29.5)	0.001
No	148	30	79.7%		1	
cGvHD						
Yes	18	2	88.8%	0.231		
No	95	4	95.7%			
Post-transplant autoimmunity						
Yes	19	0	100%	0.139		
No	105	11	89.5%			
Donor chimerism						
	97	22	77.3%	0.106		
Full donor (≥90%)	43	6	86%			
Mixed donor						

Abbreviations: SCID: severe combined immune deficiency, BM: bone marrow, PBSCs: peripheral blood stem cells, rATG: rabbit anti-thymocyte globulin, TMA: thrombotic microangiopathy, aGvHD: acute GvHD, cGvHD: chronic GvHD, CI: confidence interval.

Ω Variables reaching a P value < .10 in univariate analysis for overall survival estimations were included in Cox proportional hazard regression models using a backward stepwise selection (multivariate analysis)

Table 4: Patients who required a second transplant or an unconditioned stem cell boost (n=10):

Diagnosis	1st graft	Time to Graft loss	Cause	2nd graft	Outcome/Last-follow-up or time to death (m)
CID-Immuno-osseous dysplasia **(P2) ¥**	Flu/Mel/ Alem 9/10 1C UM PBSCs	8m	Mixed chimerism Donor M7%, T: 83%, B:13%	Stem cell boast PBSCs	Deceased 100% engrafted. Severe aGvHD 12m
CGD	Bu(RIC)/Flu/ Alem 0.5mg/kg 9/10 1A UM BM	2m	Primary graft loss	Treo/Flu/ Alem1mg/kg 9/101A UM PBSCs	A/W 100% engrafted Off Ig 89.9m
CHH	Treo/Flu/ Alem 1mg/kg 9/10 1A UM PBSCs	9m	Lost myeloid engraftment with repeated E-Coli sepsis requiring ICU admission	Stem cell boost PBSCs	A/W 100% engrafted Off Ig 86.3m
IFK GOF mutation	Treo/Flu/ Alem 1mg/Kg 9/10 1A UM BM	28m	Mixed engraftment Donor T=38%, M=0%	Treo/Flu/TT/ rATG 15mg/Kg 5/10 TCR αβ /CD19 dep	A/W 100% 6m
CHH	Treo/Flu 8/10 1A,1C Cord	16m	Immune mediated rejection; hi fever/rash D+9 Donor 5% WB 60% T 0% M Complete graft loss D+32	Flu/Mep/ Alem 1mg/kg 1A 9/10 CD34+ /T cell add back PBSCs.	A/W 100% WB Off Ig 60m
LAD1	Treo/Flu rATG 10mg/Kg 7/101A,1C,1DQ Cord	7years	Progressive loss of donor engraftment (3% WB)	Flu/Mel/ Alem 1mg/Kg 8/101C, 1DQ CD34+/T cell addback PBSCs	A/W On Ig (post-Rituximab for AIN at 24m) 72% WB (84%CD3, 66% CD15). 31m
IPEX	Treo/Flu/ rATG 10mg/Kg	10m	Primary graft loss	Treo/Flu/TT rATG 15mg/kg	A/W 100%

	7/10 2A, 1B Cord			Paternal haplo- TCR αβ/CD19 dep PBSCs	Off Ig 12m
XLP	Bu (MAC)/Cyc Combined mMUD+MSD (brother who had the same donor before) 1C BM.	6m	Primary graft loss	Flu/Mel/ Alem1mg/Kg 9/10 1C UM PBSCs (same donor)	A/W 100% WB Off Ig 63m
CGD **(P22) ¥**	Bu (RIC)/Flu/ Alem 0.6mg/Kg 1A BM	2m	100% engraftment followed by immune mediated rejection; Donor WB :0% at D+25	Cyc/TBI 3Gy/Flu / Alem 1mg/kg 9/10 UM BM	Deceased 100% WB Idiopathic pneumonia syndrome 3m
ELANE Congenital neutropenia/MDS	Treo/Flu/TT/rATG 5/10 TCR αβ /CD19 dep PBSCs	1m	Primary graft loss with recorded HLA antibodies	TBI 3Gy/Flu/ TT/ rATG 6mg/Kg 8/10 1DRB1, 1DQB1 TCR αβ/CD19 dep PBSCs	A/W 100% Off Ig 36 m

Abbreviation: Ig: immunoglobulin, A/W: alive and well, XLP: X linked lymphoproliferative disease, CGD: chronic granulomatous disease, CHH: cartilage hair hypoplasia, LAD: leukocyte adhesion defect, CID: combined immune deficiency, MAC: myeloablative conditioning, RIC: reduced intensity conditioning, Bu: Busulphan, Treo: Treosulfan, TT: Thiotepa, Flu: Fludarabine, TBI: Total body irradiation, rATG: rabbit anti-thymocyte globulin,Mel: Melphalan,Alem: Alemtuzumab, Cyc: Cyclophosphamide, mMUD: mismatched unrelated donor, MSD: matched sibling donor, WB: whole blood, T: T cell, M: myeloid, unmanipulated: unmanipulated, BM: bone marrow, PBSCs: peripheral blood stem cells, m: months, IPEX:immune dysregulation polyendrinopathy enteropathy X-linked disease, MDS: myelodysplasia, ICU: intensive care unit admission,

9/10 1C represents 1 mismatch being at the C locus, 9/10 1A means 1 mismatch being at the A locus, etc.

¥: For P2 and P22, please refer to table E1 online repository.

Table 5: Engraftment and immune recovery post-transplant across different graft manipulations

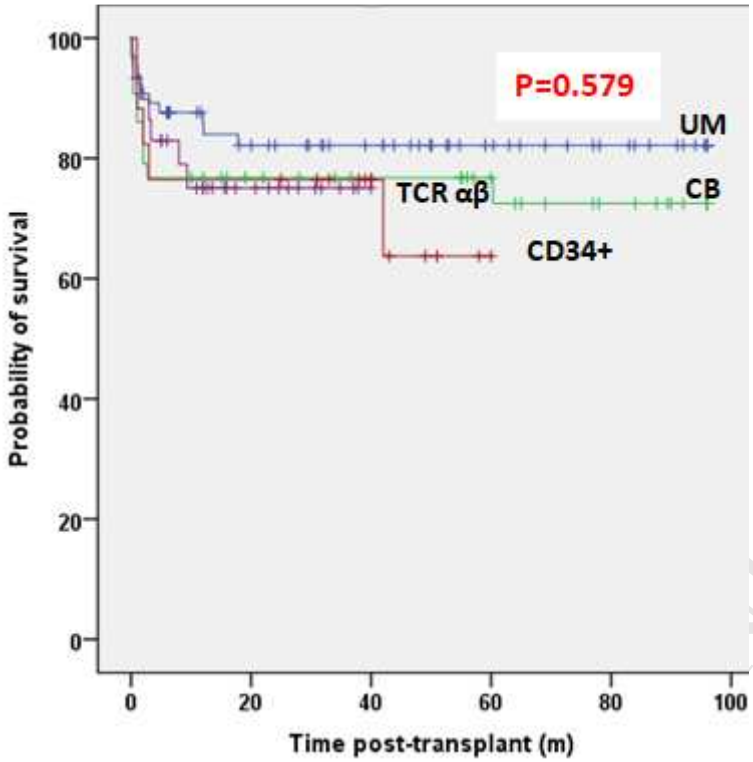
Type of graft	TCR αβ/ CD19 dep	CB	CD34+/ T cell addback	UM grafts	P value
Median days to NT recovery	14	23	18	14.5	P<0.001
Median days to PLT recovery	8	29	11	14	P<0.001
Median CD4 counts at 3m	73	430	50	184.5	P<0.001
Median CD4 counts at 6 m	494	690	455	276	P<0.001
Median Naïve CD4 at 6m	172	357.5	275	68.5	P=0.056
Median Time to CD4 ≥ 300 cells/ul	5	2.5	7	7	P=0.006
Full donor Chimerism (%)¥	22/28; 78.5%	31/38; 81.5%	7/17; 41.1%	27/57; 47.3%	P=-0.02

Abbreviations: NT: neutrophil, PLT: platelet, CB: cord blood, UM: unmanipulated grafts.

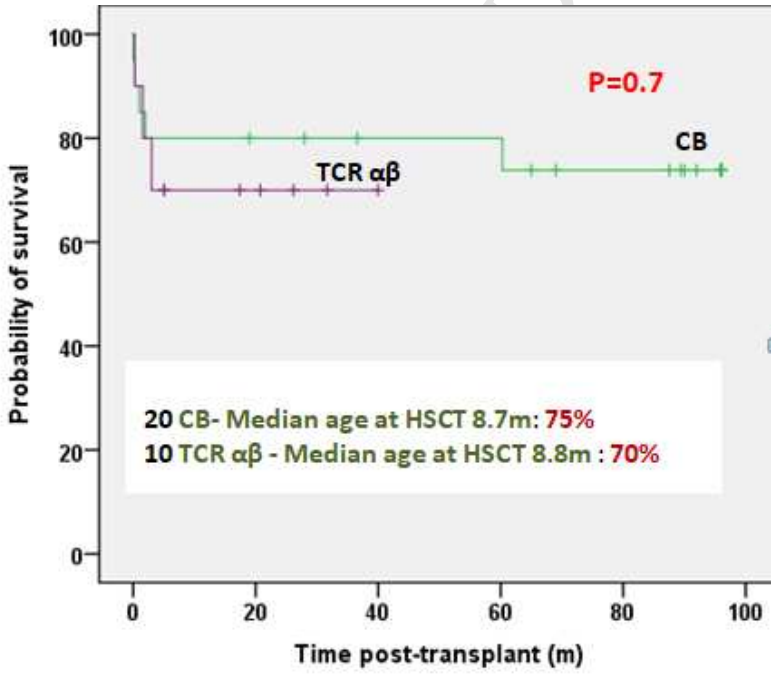
¥ Molecular assessment for donor engraftment was not available for 15 grafts.

Figure 1: Overall survival among different graft manipulations:

1a) 8- year overall survival among all PID was 78.1%



1b) 8-year overall survival among SCID was 73.3%



1c) 8-year overall survival among Non-SCID was 80.3%

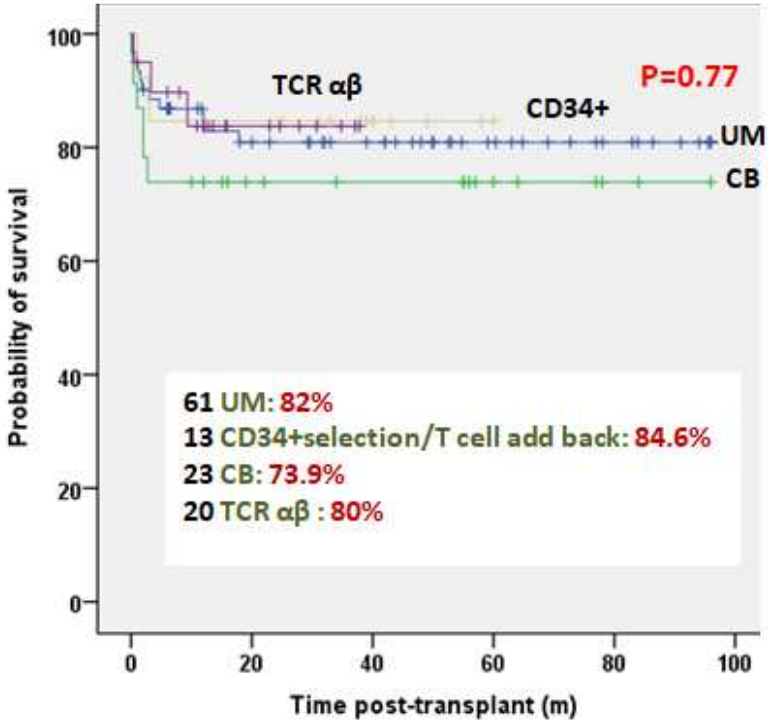


Figure 2: Effect of conditioning on overall survival among unmanipulated grafts

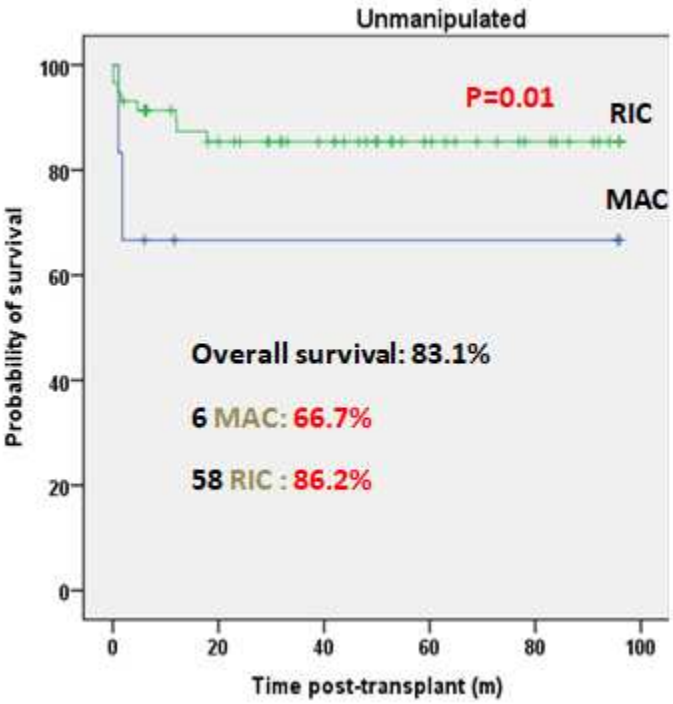


Figure 3: Effect of post-transplant viraemia on TRM

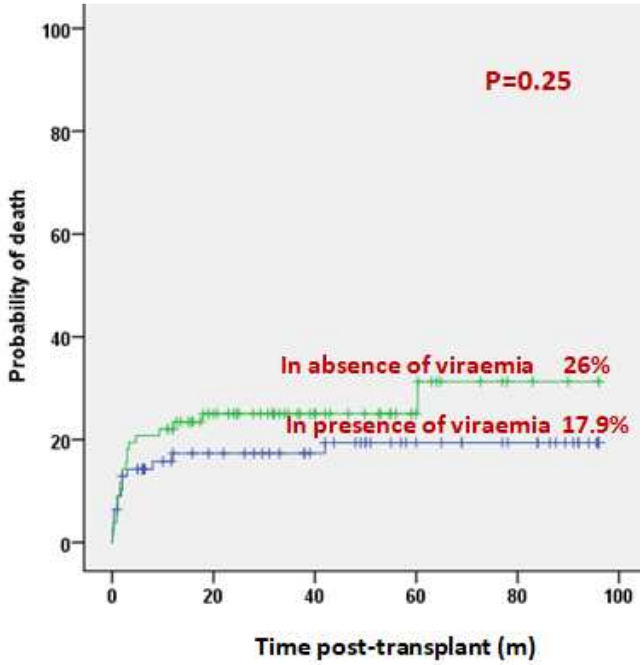


Figure 4: Effect of aGvHD on TRM

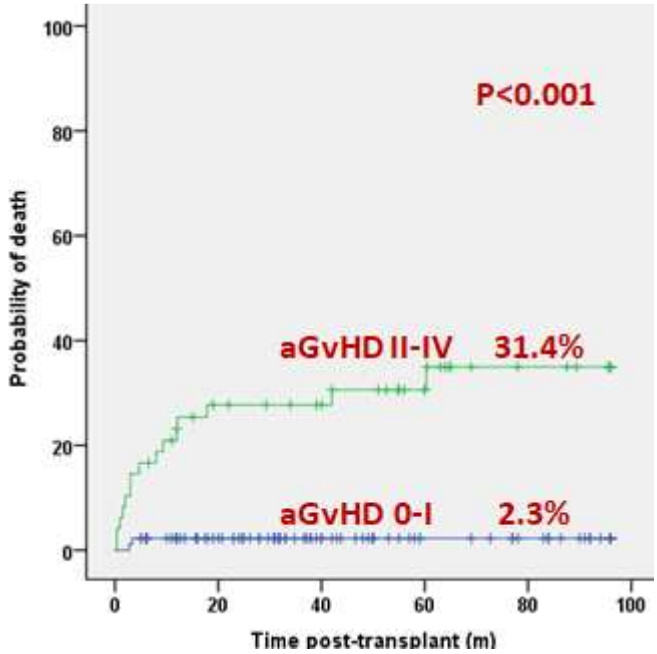
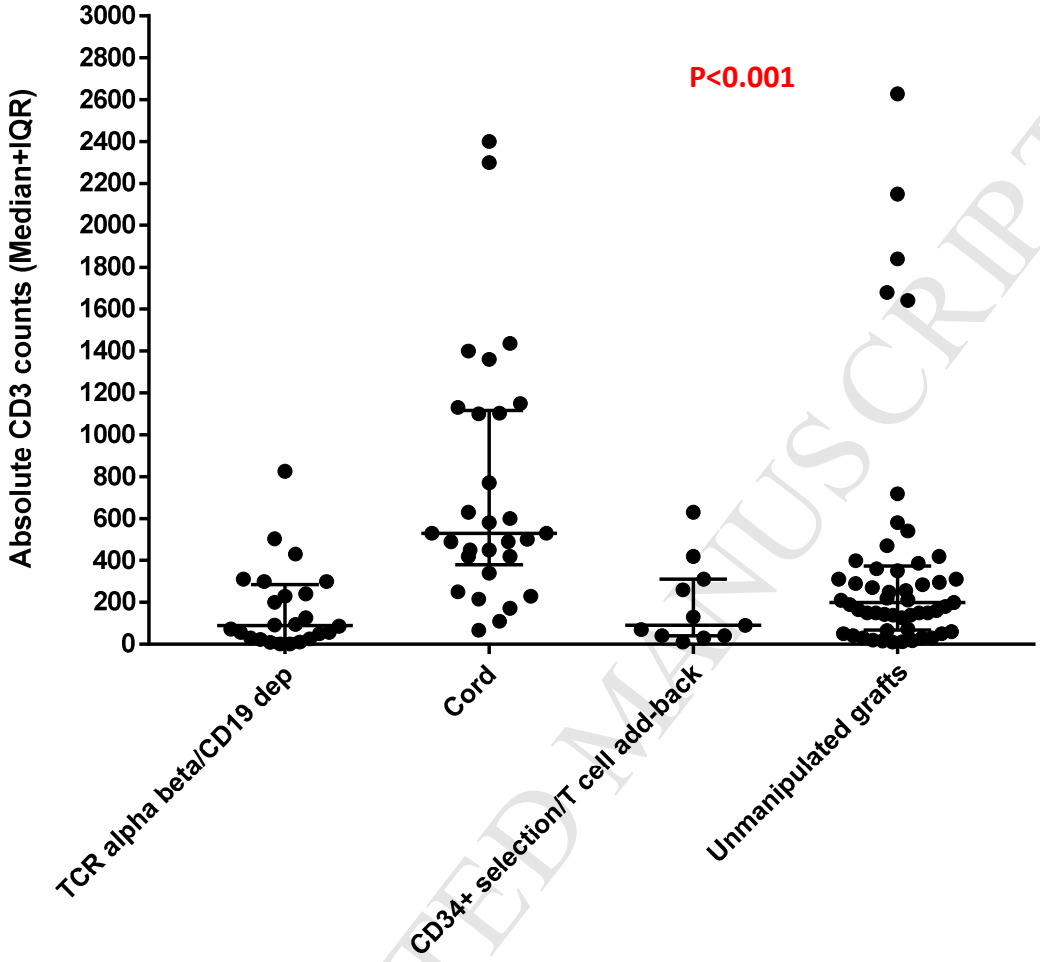
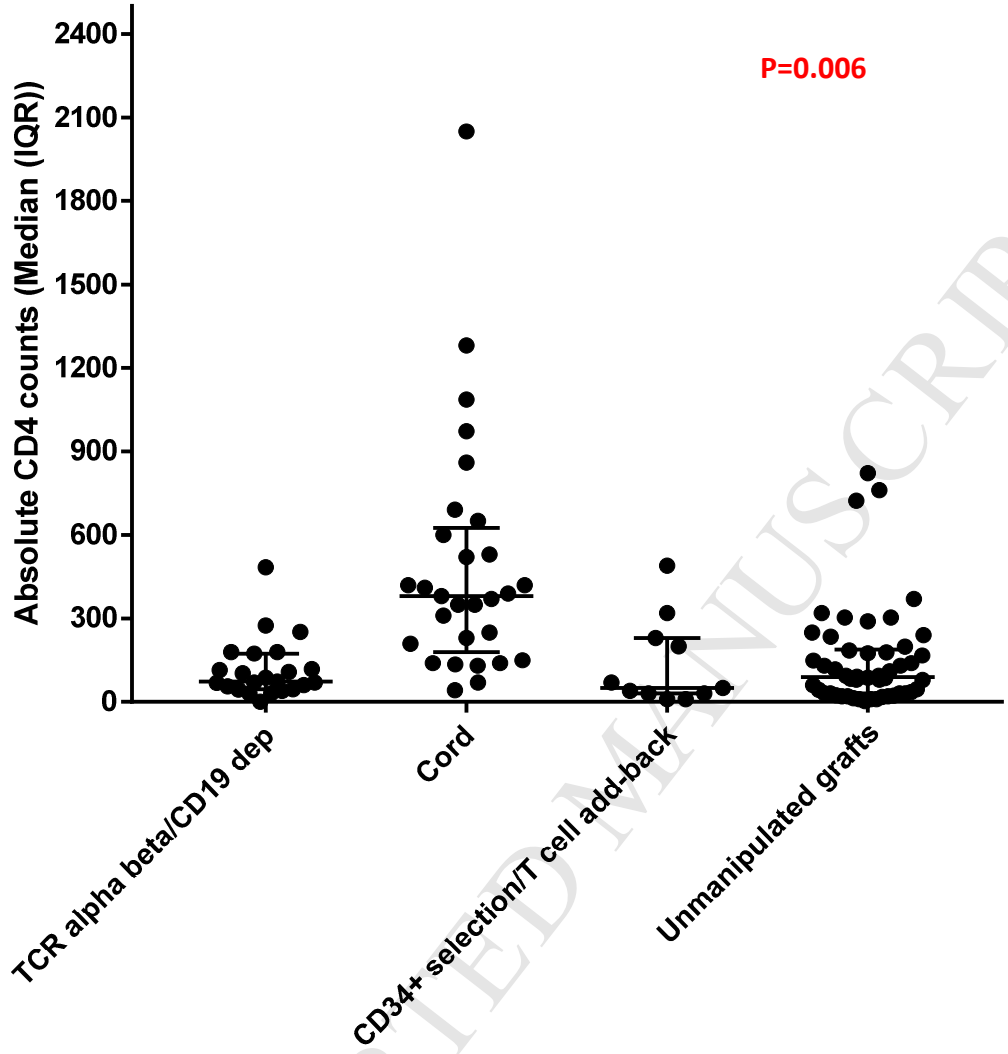


Figure 5: T cell immune reconstitution across the different graft manipulations

5a) Robust CD3 recovery at 3 months post-transplant among Cord grafts



5b) CD4 recovery at 3 months post-transplant among different graft manipulations



5C) Naïve CD4 counts at 6 months post-transplant among different graft manipulations

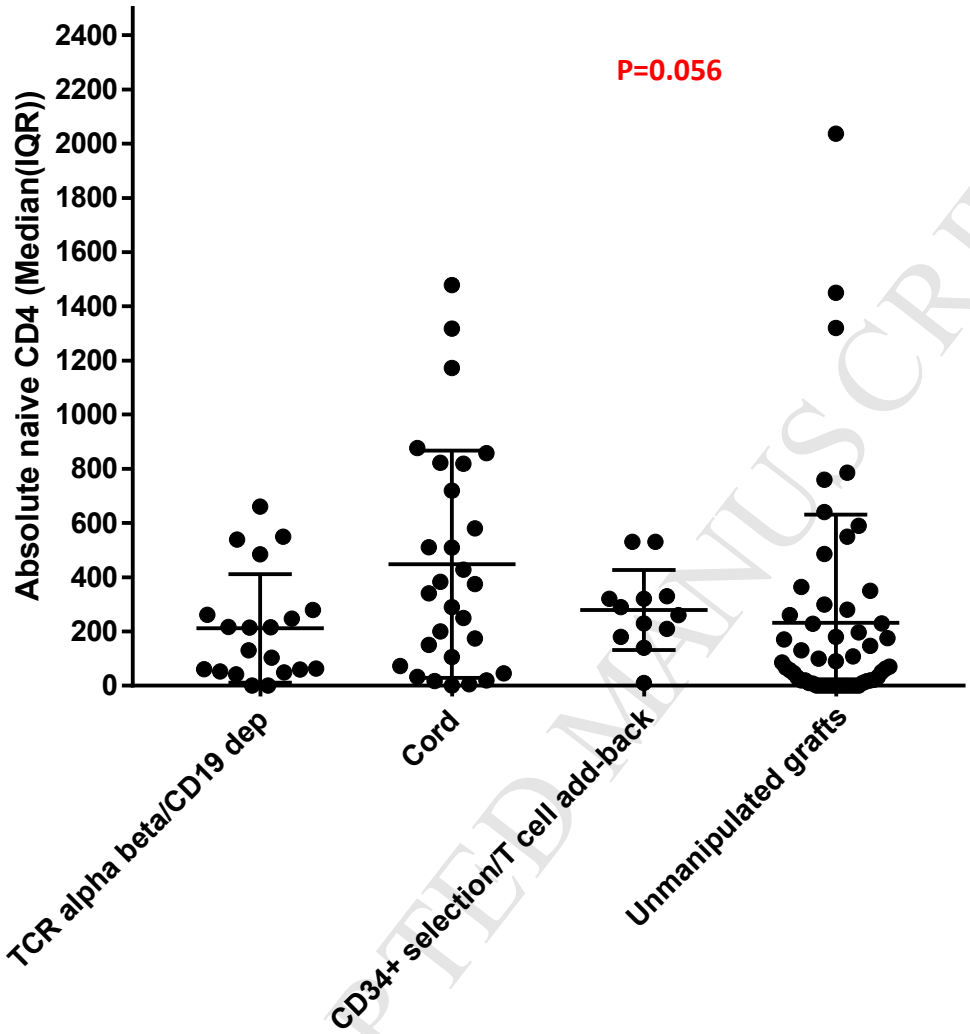


Table E1: Cause of deaths among the different graft manipulations (n=34)

P	Diagnosis	Morbidities	Infection at time of transplant D-10-D0	Age at HSCT (m) Year of HSCT	HLA match	Graft manipulation Stem cell source	Conditioning/ serotherapy	Post-transplant complications Viral VOD/TMA GvHD	Cause of death/Timing
P1	AI enteropathy with hypo-gammaglobinaemia	None	None	21.9 2007	9/10 1C	Unmanipulated PBSC	Flu/Mel/ Alem (1mg/kg)	VOD aGvHD IV (skin/liver/gut)	aGvHD inducing intestinal failure 11.96m
P2	CID-Immune osteodysplasia	Cryptosporidium enteropathy. PCP pneumonitis Top-up at 8m post-HSCT	None	53.6m 2008	9/10 1C	Unmanipulated PBSC	Flu/Mel/ Alem(1mg/kg)	CMV aGvHD III (skin, gut)	aGvHD 12.1 m
P3	Cerunnos CID	Disseminated CMV at time of transplant. Microcephaly	None	35.7m 2008	9/10 1C	Unmanipulated BM	Flu/Cyc 20mg/kg/ Alem (0.6mg/kg)	Adeno/HHV6 aGvHD III (skin/gut)	aGvHD inducing intestinal failure. Disseminated CMV/HHV6 viraemia. 17.83m
P4	CHH CID	Chronic lung disease.	Disseminated CMV/EBV	43.2m 2009	9/10 1A	Unmanipulated BM	Flu/Cyc 200mg/kg	Adeno/CMV	Capillary leak syndrome 1m
P6	Severe immune dysregulation	Mycobacterial avium of lung	None	157	9/10	Unmanipulated	Treo/Flu/TT/ Alem (1mg/Kg)	None	MOF with sepsis/encephal

		Rt upper lobe bronchiectasis Polyarticular JIA		2011	1A	PBSC			opathy 1.8m
P8	DNA repair defect	Wide spread bronchiectasis	None	49.1 2013	9/10 1DQ	Unmanipulated BM	Treo/Flu/ Alem(1mg/kg)	HHV6/adeno viraemia EBV PTLD Grade II (skin)	EBV PTLD with respiratory failure 4.7m
P9	AI enteropathy (TTC37 defect)	None	None	17.5 2013	9/10 1A	Unmanipulated BM	Treo/Flu/ Alem(1mg/kg)	Adeno (Blood, NPA). aGvHD II-III (skin/gut)	Pulmonary haemorrhage due to adenoviral pneumonitis Ongoing active gut GvHD 1.5m
P19	IPEX	Congenital myopathy. FTT. HSV duedenitis/ pancolitis/ mucosal prolapse refractory to steroids/CSA/mabs). Multiple bacterial and fungal blood infection.	None	58.3 2010	9/10 1A	Unmanipulated PBSC	Treo/Flu/ Alem(1mg/kg)	CMV viraemia, and retinitis Adenoviral conjunctivitis EBV PTLD Extensive cGvHD; skin/gut	MOF due to EBV PTLD.

		Piperacillin/ Tazobactam anaphylaxis							11m
P20	CID	Recurrent chest infections. Recurrent AIN Candidal line infections. AI enteropathy	None	27.26 2007	9/10 1A	Unmanipulated PBSC	Flu/Mel/ Alem(1mg/kg)	None	MOF/bacterial sepsis 0.06m
P21	CINCA-like	Chronic lung disease Several PICU for respiratory support. Hypertension FTT and GORD. Recurrent aspiration pneumonia. Global development delay.	None	25.73 2007	9/10 1A	Unmanipulated PBSC	Flu/Mel/ Alem(1mg/kg)	EBV PTLD aGvHD IV (skin/liver/gut)	Chronic lung disease/Renal failure/ pseudomonas septicaemia 12m
P22	AR p67 CGD	TB. Multiple cerebral lesions (Aspergillus). Large pericardial effusion. G6PD.	None	126.6 2016	9/10 1A	Unmanipulated BM	TBI 3cGy/ Cyclo120mg/Kg /Flu Alem (1mg/Kg)	NA	Idiopathic Pneumonia syndrome (PM: non-specific)

		Failed first transplant (No HLA antibodies).							3m
P5	T low B-NK- SCID	Disseminated adenoviraemia	Disseminated adenoviraemia	2.9m 2010	9/10 1A	Cord	Treo/Flu	Adeno aGvHD II (skin)	Bacterial infection secondary to prolonged immune suppression due to gut dysregulation 60.3m
P7	Severe AI enteropathy	Myopathic facies. Cerebral atrophy(MRI)	None	10.16 2013	9/10 1DQ	Cord	Treo/Flu/ Alem(1mg/kg)	HHV6 viraemia Grade I (Skin)	HHV6 pneumonitis, MOF 2.73m
P10	Unidentified HLH	CNS HLH	None	4.2 2010	7/10 1DRB1 1DQB1 1B	Cord	Treo/Flu/ Alem (1mg/kg)	None	Unidentified respiratory failure 2m
P11	T-B-NK+SCID-Multiple intestinal atresias	Positive FH of sib death of the same condition. Perinatal diagnosis of intestinal atresias(operated) Klebsiella line sepsis.	Paraflu 2 NPA (no pneumonitis)	8.93 2008	9/10 1B	Cord	Treo/Flu/ rATG (10mg/kg)	Paraflu 2 pneumonitis	Paraflu 2 pneumonitis and haemoptysis Pseudomonas Sepsis 1m

P12	Unidentified HLH	CNS HLH	Paraflu 3 NPA (no pneumonitis)	21.9 2010	9/10 1B	Cord	Treo/Flu/ Alem(1mg/kg)	Paraflu pneumonitis	3	Respiratory failure due to paraflu3 pneumonitis. D+7
P13	T-B+NK+SCID	Previous sib with SCID. Recurrent conjunctivitis.	Paraflu 3 NPA (no pneumonitis)	3.6 2009	9/10 1C	Cord	Treo/Cyc 200mg/kg	Paraflu pneumonitis	3	Paraflu 3 pneumonitis D+2
P14	Common gamma chain SCID	PCP, Influenza B pneumonitis. Rota enteropathy. FTT. Encephalitis (no known pathogens).	None	10.43 2011	9/10 GVH 1A (5/6, 9/10); HvG 1A, 1DQB1 (5/6, 8/10)	Cord	None	aGvHD III (skin/gut)		Meningitis (PM brain biopsy: T cell infiltration-no viral particles) 0.33m
P15	Unidentified CID-Probable mitochondrial disease	Entrovirus encephalitis. PCP/CMV pneumonitis (MV) and P++ CMV haemorrhagic cystitis. AIHA, AI ITP, Developmental	None	17.43 2012	7/10 1B, 2C	Cord	Treo/Flu	CMV viraemia		Encephalopathy and Renal failure with evidence of vasculopathy on renal biopsy and respiratory compromise (CSA toxicity)

		delay.							2m
P16	Unidentified Primary HLH	HLH. Paraflu 3 pneumonitis. Pneumatosis Intestinalis.	Paraflu 3 NPA (no pneumonitis)	19.46 2010	9/10 1A	Cord	Treo/Flu	Paraflu 3 pneumonitis	Paraflu 3 pneumonitis. 1m
P17	Perforin HLH	CNS HLH	None	6.86 2010	6/10 1A,1B,1C, 1DQB1	Cord	Treo/Flu	RSV pneumonitis. Engraftment Syndrome (fever/rash). Systemic HTN (ventricular hypertrophy) aGvHD IV (skin/gut)	RSV pneumonitis Grade IV aGvHD (? Lung involvement). 0.33m
P18	RAG 1-Omenn	Omenn syndrome. Rhino/Paraflu 3 NPA no pneumonitis. CMV viraemia.	CMV viraemia (low copies)/NPA	16.5 2012	9/10 1A	Cord	Treo/Flu	CMV reactivation. Paraflu 3/CMV pneumonitis Engraftment syndrome. aGvHD II (skin)	Paraflu 3/CMV pneumonitis. 2m
P23	ADA-SCID	Recurrent infections. FTT. Failed gene therapy (x2) at with aplastic marrow	None	119.6 2011	9/10 1C	CD34+/T cell add-back	Flu/Mel/ Alem(1mg/kg)	aGvHD II (skin). cGvHD (skin/? Lung)	Pericardial effusion/ pulmonary compromise MOF (? Lung GvHD no PM biopsy)

										42m
P24	DOCK8-CID	Bronchiectasis. Sort stature (GH deficiency). Cryptosporidium sclerosing cholangitis. Aortic dilatation. CMV viraemia	CMV/Rubella encephalitis	180.56 2014	9/10 1A	CD34+/T cell add back		Flu/Mel/ Alem (1mg/kg)	Rubella /CMV encephalitis. aGvHD II-III (gut)	Rubella/CMV encephalitis 3m
P25	XIAP	EBV-HLH. Recurrent line infections.	Adeno/ Shingles at conditioning	42.43 2014	9/10 1DQB1	CD34+/T cell add back		Flu /Cyc 1500 mg/m2/Anti-CD45 1600ug/Kg	Shingles at time of deterioration. Probable Lung aGvHD	Respiratory failure. (PB lung biopsy: vasculopathy - no viral inclusion). 1m
P26	RAG1/RAG2 SCID	Adenoviraemia	Adeno	88.7 2015	9/10	CD34+/T cell add back		Flu/Mel/ Alem (1mg/kg)	Adeno reactivation. HSV stomatitis. RSV pneumonitis.	Adeno LCF with intracranial haemorrhage. 1m
P27	Artemis -SCID	None	None	14.57 2013	9/10	CD34+/T cell add back		Flu/Mel/ Alem(1mg/kg)	VOD	VOD/MOF 2m
P28	CID	Multi-drug resistant CMV.Renal Tubulopathy	CMV viraemia	13.2 2013	Haplo-HSCT (P)	TCR αβ/CD19		Treo/Flu/TT	VOD CMV viraemia aGvHD II (skin)	Prolonged IS/Aspergillosis

									9.36m
P29	RAG1 -Omenn	Sickle cell trait. Adenoviremia. Fungal lung nodule. Cardiac dysfunction. Omenn syndrome (MP 1mg/Kg)	Adeno viraemia	9.6 2013	Haplo-HSCT (P)	TCR αβ/CD19	Treo/Flu/TT/ rATG 15mg/Kg	Adenoviraemia	P++ and acute lung injury 1.56
P30	HLH MUNC13-4; c817c>tpR273x)	Respiratory distress RR at D0 ranges between 60-90 breaths/min	CMV viraemia	3.96 2014	Haplo-HSCT (P)	TCR αβ/CD19	Treo/Flu/TT/ rATG 15mg/Kg	CMV viraemia	P++ 0.36m
P31	RAG2 -SCID	Omenn-like syndrome with fever/rash and T cell clonal expansion pre-transplant. On HFO at D0	CMV viraemia Pneumonitis/ encephalitis	4.8 2015	Haplo-HSCT (P)	TCR αβ/CD19	Alem(1mg/kg)	CMV	Respiratory failure PM lung biopsy: evidence of T cell clonal expansion. 0.24m
P32	DNA Ligase IV SCID	Mild pneumonitis (oxygen therapy at D0)	None	8m 2017	Haplo-HSCT (P)	TCR αβ/CD19	Treo/Flu/TT/ rATG 15mg/Kg	Adenoviraemia. aGvHD II (skin) TMA	Adenoviraemia with MOF 3m
P33	DOCK8 CID	Multiple warts. Recurrent chest	None	156 2016	Haplo HSCT (m)	TCR αβ/CD19	Treo/Flu/TT/ rATG 15mg/kg	Adenoviraemia Fungal pneumonia	Adenoviral driven lung TMA.

		infection. Food allergy.						aGvHD I TMA	3.3m
P34	XLP	CNS HLH. Previous paraflu2 pneumonitis. Persistent lung nodules at D0	None	18m 2017	mMUD 1A, 1DQ	TCR αβ/CD19	Treo/Flu/TT/ rATG 15mg/Kg	aGvHD II-III (gut) TMA	MOF XLP (HLH/TMA/ GvHD) 8m

Abbreviations:, FH: family history, HLH: Haemophagocytic lymphohistiocytosis, CNS: cerebral nervous system, NPA: nasopharyngeal aspirate, Alem: Alemtuzumab, rATG: rabbit anti-thymocyte globulin, Treo: Treosulfan, Flu: Fludarabine, TT: Thiotepa, Mel: Melphalan, Cyc: cyclophosphamide, SCID: severe combined immune deficiency, RAG: recombinase activating gene , PCP: Pneumocystis pneumonia, PM: post-mortem, HvG: host versus graft, GvH: graft versus host, MV: mechanical ventilation, P++: pulmonary hypertension, AIHA: auto-immune haemolytic anaemia, AI ITP: auto immune idiopathic thrombocytopenic purpura, mabs: monoclonal antibodies, AIN: autoimmune neutropenia, GH: growth hormone, LFT: liver cell failure, Adeno: adenovirus, EBV: Ebstein Barr virus, CMV: cytomegalovirus, HSV: herpes simplex virus, CID:combined immune deficiency, ADA: adenosine deaminase, Rag: recombinase activation gene, MOF: multisystem organ failure, IS: immune suppressed, haplo: haploidentical, P: paternal, m: maternal, TMA: Thrombotic microangiopathy, HFO: high frequency oscillation, GvHD: graft versus host disease, VOD: veno-occlusive disease, PTLD: post-transplant lymphoproliferative disease, m: months.

Table E2: Characteristics of patients who developed TMA (n=7)

Diagnosis	Ethnicity	Conditioning/timing of TMA	GvHD prophylaxis	GvHD	Viral reactivation	Outcome/last follow-up or time to death (m)
Artemis SCID ***2nd Transplant for aGvHD***	Caucasian	Treo/Flu/TT/Alem 1m	CSA/MMF	Grade II skin	Adeno	A/W 39.6m
CID **(P28) ¥**	Portuguese	Treo/Flu/TT 2.9m	CSA/MMF	Grade II skin	CMV	Deceased Aspergillus sepsis due to prolonged IS

						9.36m
IPEX ***2nd transplant for graft loss***	Middle East	Treo/Flu/TT/rATG 9m	None	Grade I skin	Late onset EBV	A/W 12m
Congenital neutropenia ***2nd transplant for graft loss***	Caucasian	TBI 3Gy/Flu/TT/ rATG 13m	CSA/MMF	Grade II skin	CMV	A/W 36m
DNA Ligase IV Defect (P32) ¥	Caucasian	Treo/Flu/TT/rATG 0.96m	None	Grade II skin	Adeno	Deceased Adeno/MSOF 3m
DOCK8 (P33)	Middle East	Treo/Flu/TT/rATG 0.93m	None	Grade II skin	Adeno	Deceased Sepsis/MSOF 3.3m
XLP (P34) ¥	Middle East	Treo/Flu/TT/rATG 5m	CSA	Grade III; skin/gut	None	Deceased MSOF (HLH/GvHD/TMA) 8m

Abbreviations: A/W: alive and well, XLP: X-linked lymphoproliferative disease. Treo: Treosulfan, Flu: Fludarabine, TT: Thiotepa, TBI: Total body irradiation, CSA: Cyclosporine A, MMF: Mycophenolate mofetil, MSOF: multi-system organ failure, GvHD: graft versus host disease, TMA: thrombotic microangiopathy IS: immunosuppressive therapy, m: months. ¥ : For P28, P32, P34, please refer to table E1 online repository

Table E3: Analysis of independent factors affecting overall survival among the SCID cohort

	Univariate analysis				Multivariate analysisΩ	
Outcome factors	Absolute number of patients	Absolute number of deaths	3-year overall survival	P-value	Hazard ratio (95% CI)	P-value
Type of SCID						
B+	12	2	83%	0.25		

B-	26	9	65%			
Age at HSCT						
<6m	12	3	75%	0.715		
≥6m	26	8	69%			
Age at HSCT						
<6m	13	3	76.9%	0.774		
6-12	11	4	63.6%			
>12m	14	4	71.4%			
Pre-transplant viraemia (D-10-D-1)						
Yes	7	5	71.4%	0.001	2.2(0.5-8.9)	0.27
No	31	6	80.6%		1	
Pre-transplant respiratory infection D-10-D-1						
Yes	5	3	40%	0.1		
No	33	8	75.7%			
Previous infection δ						
Yes	27	9	66.6%	0.09	0.6 (0.09-4.3)	0.6
No	11	2	81.8%		1	
HLA						
				0.113		
9/10	20	8	60%			
5-8/10	18	3	83.3%			
Stem cell source						
BM	0	NA	NA			
PBSCs	18	5	72.2%	0.57		
Cords	20	6	70%			
Graft manipulation						

TCRαβ/CD19 dep	10	3	70%	
Cord	20	5	75%	0.116
CD34+/T cell add-back	4	3	25%	
Unmanipulated grafts	4	0	100%	
Conditioning				
MAC	11	3	72.7%	0.84
Others	22	6	72.7%	
No conditioning	5	2	60%	
Serotherapy included				
Yes	21	7	66.6%	0.5
No	17	4	76.4%	
Type of serotherapy used				
rATG	6	3	50%	0.3
Alemtuzumab	15	4	73.3%	
Use of GvHD prophylaxis				
Yes				0.12
No	37	10	72.9%	
	1	1	0%	
Post-transplant viraemia				
Yes				
No	15	6	60%	0.225
	23	5	78.2%	
Post-transplant respiratory infection				
Yes	8	4	50%	0.139

No	30	7	76.6%			
aGvHD €					20.3 (3.7-110.9)	
					1	
Grade II-IV						
Grade 0-I	13	5	61.5%	0.003		0.001
	19	0	100%			
TMA						
Yes	2	1	50%	0.5		
No	36	10	72.2%			
cGvHD ¥						
Yes	5	1	80%	0.23		
No	22	1	95.4%			
Post-transplant autoimmunity©				0.54		
Yes	3	0	100%			
No	27	3	88.8%			
Donor chimerism						
Full donor (≥90%)	27	8	77.7%	0.1		
Mixed donor	9	1	88.8%			

Abbreviations: SCID: severe combined immune deficiency, BM: bone marrow, PBSCs: peripheral blood stem cells, rATG: rabbit anti-thymocyte globulin, TMA: thrombotic microangiopathy, aGvHD: acute GvHD, cGvHD: chronic GvHD, CI: confidence interval.

Ω Variables reaching P < .10 in univariate analysis for overall survival estimations were included in Cox proportional hazard regression models using a backward stepwise selection (multivariate analysis).

δ: means occurrence of at least one episode of severe infection pre-HSCT.

€: Data on aGvHD were available for 32 transplants.

¥: Data on cGvHD were available for 27 transplants.

©: Data on post-transplant autoimmunity were available for 30 transplants.

Selection of conditioning protocol

The transplant experience in this cohort extends over 11 years. After reports of mixed chimerism especially with Flu/Mel conditioning, and since 2008, both UK centres moved from using Flu/Mel or Flu/Cyc to the use of Treo/Flu which is considered a reduced toxicity but a more myeloablative conditioning than Flu/Mel and thus can allow high level donor engraftment. Since 2014, Thiotepa was added to Treo/Flu for the conditioning of PID patients who receive a TCR αβ/CD19 MMUD/haploidentical transplant has been described by Bertaina et al; Blood. 2014 Jul 31; 124(5):822-6) and again to support better engraftment.

There was a discrepancy in the conditioning protocol used for CGD cases where London centre mainly used targeted Bu (AUC=45-65 mg*hr/L))/Flu as been described by Güngör T et al; Lancet. 2014 Feb 1; 383(9915):436-48) while the Newcastle team preferred to use Treo/Flu conditioning as they have previously published by Morillo-Gutierrez; Blood. 2016 Jul 21; 128(3):440-8. Currently, both centres are looking retrospectively on the differences between both conditioning protocols on the final outcome in patients with CGD. Preliminary results showed a high incidence of post-transplant autoimmunity post- targeted Bu/Flu conditioning in contrast to Treo/Flu. Final results should be available soon.

Selection of graft manipulation strategies:

In both centres, BM was the preferred stem cell source for an unmanipulated 9/10 or 8/10 HLA matched grafts. However, if the donor preferred to donate PBSCs then a graft manipulation was sought. Due to the promising results of TCRαβ/CD19 depletion in terms of engraftment and low risk of GvHD, both centres moved from the usage of a CD34+/T cell add-back to a TCRαβ/CD19 depletion with any ≤ 8/10 HLA matched graft and currently Newcastle are using a TCRαβ/CD19 depletion even for 9/10 matched donors.

In addition, there was a centre preference in selection of a mismatched graft where London team preferred to use more mismatched cords with no serotherapy while Newcastle team preferred to use a TCRαβ/CD19 paternal haploidentical transplant in the absence of a 9/10 or 10/10 HLA matched donor. Nowadays, Newcastle team even uses TCRαβ/CD19 depletion with any 9/10 instead of using an unmanipulated bone marrow with very promising results. Both approaches have been discussed in details and both had comparable outcome.

CD34 positive selection followed by T cell add-back

The dose of T cell add-back that was given here was 2-3 log higher than what others have used (1,2).

In haplo-HSCT, a CD3 dose of 5X10*4/Kg in combination with CD34 positive selection was our rationale as been reported by Veys et al, 1998 (3). In patients who had either 1 or 2 antigen HLA mismatched donor (8-9/10 HLA match), the London group proposed the usage with a high T cell add-back of 1-3X10*8/kg with CD34+ selected PBSCs in combination with reduced intensity conditioning to improve competition for the stem cell niche and thus boost high level donor engraftment with limited toxicity. In our current study, 17 cases had a CD34+ selection with T cell add-back 1-3X10*8/Kg. These patients were either 8/10 (3/17 patients) or 9/10 (14/17 patients) HLA matched. None had a haplo-HSCT. Though toxicity was limited post-RIC conditioning, however high rates of aGvHD (40%) and cGvHD (38%) complicated the use of this high dose of T cell add-back.

1. Handgretinger R, Klingebiel T, Lang P , Schumm M, Neu S, Geiselhart A et al. Megadose transplantation of purified peripheral blood CD34+progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplantation volume 2001, pp :777– 783.
2. Geyer MB1, Ricci AM, Jacobson JS, Majzner R, Duffy D, Van de Ven C, Ayello J, Bhatia M, Garvin JH Jr, George D, Satwani P, Harrison L, Morris E, Semidei-Pomales M, Schwartz J, Alobeid B, Baxter-Lowe LA, Cairo MS. T cell depletion utilizing CD34(+) stem cell selection and CD3(+) addback from unrelated adult donors in paediatric allogeneic stem cell transplantation recipients. Br J Haematol. 2012 Apr;157(2):205-19.
3. Veys PA, Meral A, Hassan A, Goulden N, Webb D, Davies G. Haploidentical related transplants and unrelated donor transplants with T cell addback. Bone Marrow Transplant. 1998 Apr;21 Suppl 2:S42-4.