Successful outcome following allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency.

Running title: HSCT for adults with primary immunodeficiency

Thomas A Fox MB ChB[1,2], Ronjon Chakraverty MB ChB, PhD[2,5,6], Siobhan Burns MB BCh, PhD[1,2,5], Benjamin Carpenter MB BS, PhD[2,3], Kirsty Thomson MB ChB[2,6], David Lowe MB BChir, PhD[1,4], Adele Fielding MB BS, PhD[2,5,6], Karl Peggs MB BCh[2,6], Panagiotis Kottaridis MD PhD[2,5], Benjamin Uttenthal MB BS, PhD[7], Venetia Bigley MB BChir, PhD[8], Matthew Buckland MB BS, PhD[1,4], Victoria Grandage MB ChB, PhD[3], Shari Denovan RN[2,3,5], Sarah Grace RN[2,3,5], Julia Dahlstrom RN[2,3], Sarita Workman RN[4], Andrew Symes RN[4], Stephen Mackinnon MB ChB, MD[2,5,6], Rachael Hough MB BS[3], and Emma Morris MB BChir, PhD[1,2,4].

Author Affiliations:

[1]Institute of Immunity and Transplantation, UCL, London, United Kingdom; [2]BMT Programme, University College London Hospital NHS FT, London, United Kingdom; [3]TYA BMT Programme, University College London Hospital NHS FT, London, United Kingdom; [4]Department of Immunology, Royal Free London NHS FT, London, United Kingdom; [5]Department of Haematology, Royal Free London NHS FT, London, United Kingdom; [6]UCL Cancer Institute, Department of Haematology, University College London, London, United Kingdom, [7]Department of Haematology, Addenbrookes' Hospital, Cambridge, United Kingdom, [8]Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom.

Text word Count: 3440 (excluding abstract, tables, figures, acknowledgements and references)

Abstract word count: 243

Figures: 4

Tables: 2

Refs: 39

Corresponding Author:

Professor Emma C Morris, Professor of Clinical Cell & Gene Therapy, Consultant Haematologist, UCL Institute of Immunity & Transplantation, 2nd Floor, Royal Free London Hospital, Rowland Hill Street, London, NW3 2PF, United Kingdom.

Email: e.morris@ucl.ac.uk; Tel: +44 (0) 207 794 0500 ext 22475.

KEY POINTS

1. Allogeneic HSCT with reduced intensity conditioning is safe and effective in younger adults with severe PID.
2. Referral triggers should include severe infections, autoimmunity, malignancy and disease progression despite conservative management.

ABSTRACT

Introduction The primary immunodeficiencies (PID), rare inherited diseases characterised by severe dysfunction of immunity have been successfully treated by allogeneic hematopoietic stem cell transplantation (Allo-HSCT) in childhood. Controversy exists regarding optimal timing and use of Allo-HSCT in adults, due to lack of experience and previous poor outcomes.

Materials and methods 29 **c**onsecutive adult patients, with a mean age at transplant of 24 years (range 17-50) underwent Allo-HSCT. Reduced intensity conditioning included Flu/Mel/Alemtuzumab (n=20), Flu/Bu/Alemtuzumab (n=8) and Flu/Bu/ATG (n=1). Stem cell donors were matched or mismatched unrelated (MUD/MMUD) (n=18) and matched related donors (MRD) (n=11). Overall survival, event free survival, transplant related mortality, acute and chronic GVHD incidence and severity, time to engraftment, lineage specific chimerism, immune reconstitution and discontinuation of immunoglobulin replacement therapy were recorded.

Results Overall survival (OS) at 3 years for the whole cohort was 85.2%. The rarer, non-CGD PID patients achieved an OS at 3 years of 88.9% (n=18), compared to 81.8% for CGD patients (n=11). Transplant related mortality (TRM) was low with only four deaths observed at a median follow-up of 3.5 years. There were no cases of early or late rejection. In all surviving patients either stable mixed chimerism or full donor chimerism were observed. At last follow-up 87% of the surviving patients had no evidence of persistent or recurrent infections.

Conclusion Allo-HSCT is safe and effective in young adult patients with severe PID and should be considered the treatment of choice where an appropriate donor is available.

INTRODUCTION

The primary immunodeficiencies (PID) are a rare group of inherited diseases characterised by severe dysfunction of adaptive and/or innate immunity, typically arising from genetic mutations in hematopoietic stem cells. Nearly 300 distinct immunodeficiencies have now been described, with 20 accounting for over 90% of cases. Excluding common variable immunodeficiency (CVID), three of the most common are severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome (WAS) and chronic granulomatous disease (CGD). Children with severe PIDs have been successfully treated by allogeneic hematopoietic stem cell transplantation (Allo-HSCT), which has been the major therapeutic option for inherited cellular immunodeficiency disorders since 1968[1-6]. However, almost all series published on outcomes for Allo-HSCT have focussed on pediatric patients. The median age at transplant of patients described in the largest published series are < 1 year for SCID[7], 2.8 years for WAS[8] and 12.7 years for CGD, utilising reduced intensity conditioning (RIC)[9, 10].

Significant advances in hematopoietic transplantation over the past 20 years including refinement of HLA-tissue typing, adoption of RIC regimens, increased availability of alternative stem cell sources, improved methods of graft-versus-host disease (GvHD) prophylaxis and improved supportive care have translated into better outcomes today compared to the early experience[5, 11].

Early Allo-HSCT is important in infants or children presenting with serious or life-threatening infections as without definitive treatment, patients with severe PID, such as SCID, rarely survive beyond 1 year of age. In addition, younger patients have less end-organ damage from repeated or severe infections[11, 12]. Indeed, overall survival has been shown to fall from 95% in SCID patients transplanted at ages less than 3.5 months to 76% in older children[7, 13].

Whilst early Allo-HSCT is preferred for PID, this is often not possible. An initial milder clinical phenotype, delayed diagnosis, late presentation, lack of a genetic diagnosis or an inability to identify a suitable stem cell donor may result in patients surviving to adolescence and early adulthood without having undergone Allo-HSCT. Furthermore, in non-SCID forms of PID, the clinical phenotype can be very heterogeneous and 'atypical', largely due to the high number of genetic and functional defects affecting T, B and NK cells, neutrophils and/or antigen presentation. For these patients, diagnosis can be delayed or difficult and the natural history of exceptionally rare underlying diseases is often unclear. In several PIDs, controversy surrounding optimum timing of Allo-HSCT remains, due to rarity of disease, lack of experience, emerging gene therapies[14-17] and infrequent published outcome data due to the very low numbers in any one centre.

One such disorder where optimum timing remains unclear is CGD, which is characterised by impairment of the phagocyte NADPH-oxidase complex. Patients with absent NADPH activity are one group thought to benefit from early transplant[18] and criteria have been devised for Allo-HSCT in children. These are: life-threatening infection, non-compliance with antimicrobial prophylaxis or steroid-dependent auto-inflammatory disease[19]. In adolescents and adults, criteria are more difficult to apply due to higher rates of organ dysfunction and reported increased mortality from Allo-HSCT[20]. The largest published case series reported a cohort of 70 CGD patients with excellent overall survival of 91.4% at median follow up (34 months), but this cohort had a median age of 8.9 years (interquartile range 3.8 to 19.3 years)[10.] Similarly, the second largest series of 56 CGD patients also had an excellent overall survival of 93% at median follow up (21 months), but again the median age was young at 12.5 years[9] with only 14 patients aged ≥ 17 years of age.

There is even less experience of Allo-HSCT in rarer PIDs and the literature is confined to pediatric series or adult case reports. In the lethal genetic PID caused by GATA2 mutations, HSCT has resulted in reversal of the hematologic, immunologic and clinical phenotype in small series including adults[21, 22]. In addition, in XIAP deficiency, poor outcomes post Allo-HSCT have been reported, with high rates of transplant related mortality (TRM)[23], although a recent paper has described excellent outcome for 10 Japanese patients (all < 17 years) undergoing RIC conditioned HSCT[24].

Given the reduced toxicity of RIC regimens, these appear to be better tolerated in patients with PID who often have significant organ dysfunction and associated elevated haematopoietic cell transplant-comorbidity index (HCT-CI) scores[25] pre-transplant. Elsewhere others have used low toxicity myeloablative conditioning regimens incorporating targeted treosulfan with excellent results in children[26, 27].

We report here the outcome of 29 consecutive adult patients (≥ 17 years) who underwent Allo-HSCT for a variety of PIDs in the Allo-HSCT programmes of University College London Hospitals NHS Foundation Trust and the Royal Free London Hospital NHS Foundation Trust, UCL Centre for Immunodeficiency, July 2004 - November 2016. Prior to transplant all patients had developed complications that necessitated definitive treatment with curative intent. During the study period, 5 additional adult patients were referred for consideration of Allo-HSCT who did not proceed to transplant. Referral was triggered by life-threatening infection, malignancy, autoimmune or inflammatory phenomena, new genetic diagnosis or new donor availability.

METHODS

Study Design and Participants

All patients provided written consent as per institutional practice for Allo-HSCT. The mean age at transplant was 24 years (range 17-50 years). Of these, 11 patients had CGD and 18 had various other PID. Detailed patient demographics and transplant characteristics are shown in Table 1. Primary immunodeficiencies were diagnosed using international criteria. Patients with severe dysfunction of adaptive and/or innate immunity can present with recurrent infections, autoimmunity and/or malignancy. 19 patients had a genetic diagnosis and 10 had a combination of clinical phenotype with a corresponding functional defect. Secondary causes of immunodeficiency were excluded. Data on donor status, conditioning regimen, clinical condition during and after transplant and at last follow up was collected retrospectively from the medical notes and our Allo-HSCT database. The hematopoietic cell transplantation-comorbidity index (HCT-CI) is a comorbidity tool, which captures the prevalence, magnitude and severity of various organ impairments before Allo-HSCT in order to predict risk of transplant related mortality (TRM). HCT-CI scores pre-transplant were calculated for all patients[22]. All patients had a score of ≥1 whilst 12 (41%) had a score of ≥3. HCT-CI scores were higher in the PID cohort (50% scored ≥3) compared to the CGD cohort (27% scored ≥3), which was not statistically significant (p=0.206), but may suggest an increased risk of TRM.

Stem Cell Source

11 patients had matched related donors (10 siblings, 1 10/10 matched paternal donor) and 18 had matched unrelated donors, including 5 with 1 antigen mismatched unrelated donors (4 A antigen mismatch, 1 C antigen mismatch). Patients and donors were matched for human leucocyte antigen (HLA) A, B, C, DRB1 and DQB1 by intermediate or high-resolution DNA-typing as appropriate.

Mobilised peripheral blood stem cells (PBSC) were the stem cell source in 79% of the patients (n=23, including all non-CGD patients) whilst bone marrow was used in the other 21% (n=7, all CGD patients).

Allo-HSCT Conditioning Regimen

All patients were transplanted using previously described T cell-depleted (in vivo alemtuzumab or antithymocyte globulin, ATG) reduced intensity conditioning regimens[9,28,29]. All non-CGD PID patients received fludarabine (30mg/m^2 daily for 5 days), melphalan 140mg/m^2 and alemtuzumab (100mg for MUD recipients delivered as 20mg OD, days -7 to day -3 and 30mg single dose on day -1 for MRD recipients after May 2010). Two of the early MRD patients also received 100mg alemtuzumab delivered as 20mg OD, days -7

to day -3. Conditioning for CGD patients consisted of fludarabine (30mg/m^2 daily for 5 days), busulfan (1.6mg/kg twice daily for 3 days) or melphalan (140mg/m^2 total), together with alemtuzumab (0.2mg/kg daily for 3 days) for 10 patients or, in one patient, rabbit anti-thymocyte globulin (rATG) (2.5mg/kg daily for 3 days). The mean busulfan cumulative AUC was 56.05 (umol/L)(min) (range 44.34-62.66, n=7). Details are shown in Table 1.

In addition to antibody-mediated in vivo T cell depletion, GVHD prophylaxis with cyclosporine was used in all patients. Acute and chronic GVHD were graded as previously reported[30,31].

Supportive Care

Reverse isolation, antimicrobial and antifungal prophylaxis were used to reduce the risk of infectious complications. All patients received prophylaxis against *Pneumocystis jiroveci* and acyclovir prophylaxis against varicella zoster virus reactivation. Surveillance for cytomegalovirus (CMV), adenovirus (ADV) and Epstein Barr virus (EBV) infection was performed by weekly polymerase chain reaction or antigenemia testing, and pre-emptive treatment was administered according to institutional guidelines.

In patients who were receiving immunoglobulin replacement therapy prior to transplant this was continued until normal trough levels were demonstrated post transplant and the risk of further infectious complications was minimal.

Chimerism analysis

Chimerism samples were processed at 3-monthly intervals until 1 year post transplant and then 6 monthly or when indicated clinically (cytopenias, persistent infections, ongoing mixed chimerism). In patients with a sex-mismatched donor, chimerism was analysed by fluorescence in situ hybridization. In those with sex-matched donors, polymerase chain reaction of short tandem repeats was used. Lineage specific chimerism was performed on PBMC, T cell, B cell and granulocyte compartments. The laboratory reports results as follows; 'donor' where donor DNA ≥ 97%, 'mixed' where donor DNA ≥ 50% and <97%, 'very mixed' where there is more recipient than donor DNA (donor DNA ≥ 1% <50%) and 'recipient' where no donor DNA can be detected.

Study endpoints and statistical analysis

Data were analysed in April 2017, in accordance with published guidelines[32, 33]. Primary outcome measures were overall survival (OS), event free survival (EFS), transplant related mortality (TRM), neutrophil engraftment and platelet engraftment. OS was defined as the time from transplant to death from any cause. EFS was defined as the time from transplant to graft failure, graft rejection or death from any cause.

Probabilities of OS and EFS were calculated using the Kaplan-Meier method with SPSS 22.0 statistical package (SPSS, Chicago, IL, USA). Comparison of survival curves was made using the log-rank method.
Cumulative incidence estimates were used to calculate TRM and engraftment of neutrophils ($\geq 0.5 \times 10^9$/L) and platelets ($\geq 50 \times 10^9$/L) was calculated using.

RESULTS

Survival

Overall survival of the whole cohort was 89.2% at 1 year and 85.2% at 3 years, with a mean follow up of 3.5 years (range 4 months – 12 years). Overall survival at 1 year was 90.9% and 77.9% at 3 years for MRD transplants (n=11) and 94.4% at 1 year and 88.9% at 3 years for M/MUD transplants, p=0.51 (n=18). Overall survival was 90.9% at 1 year and 81.8% at 3 years for CGD patients (n=11) and 94.4% at 1 year and 88.9% at 3 years for other PIDs, p=0.75 (n=18) (Figures 1A-1C).
Event-free survival for the whole cohort was 89.7% at 1 and 3 years (n=29), with no significant difference observed for EFS between MRD transplants and M/MUD transplants; 90.9% vs 88.9% respectively (p=0.73). There was no significant difference in EFS in patients with CGD and other PID; 90.9% vs 88.9% at 1 and 3 years (p=0.65) (Figures 1D-1F). One late death occurred in a patient over 9 years post transplant due to respiratory complications in the context of progression of pre-existing bronchiectasis.

Engraftment

There were no episodes of graft rejection or graft failure. For the whole cohort, neutrophil engraftment occurred after a median 12 days (mean 14, interquartile range 11-17) and median platelet engraftment was 14 days (mean 17, interquartile range 11-20). Comparing MRD to MUD/MMUD, median time to neutrophil engraftment was 11 (IQR 11-12) versus 15 (IQR 12-21) days respectively (Figure 2A). Comparing the two diagnostic groups, time to neutrophil engraftment was longer in patients with CGD (n=11), median 16 days (IQR 12-22) versus 11 days (IQR 11-14) in patients with other PIDs (n=18) (Figure 2B). The observed longer time to engraftment in the CGD group reflects the fact that 7 of 11 CGD patients had BM as the stem cell source compared to the non-CGD PID patients who all received PBSC.
Median time to platelet engraftment in MRD transplants was 12 days (IQR 11-21) and 18 days (IQR 12-21) in MUD/MMUD transplants (Figure 2C). Longer platelet engraftment was also seen in CGD patients, median 19 days (IQR 14-20) compared to 16 days (IQR 11-21) in patients with other PIDs (Figure 2D). None of the observed differences were statistically significant. One patient had a $CD34^+$ selected stem cell top-up due to

persistent cytopenias, with good effect.

Transplant related mortality

Conditioning was generally well tolerated with no cases of interstitial pneumonitis, veno-occlusive disease or severe mucositis (grade III-IV). Transplant related mortality was low with only four deaths observed at a median follow-up of 31 months for the whole cohort (n=29) (Figures 3A-3C). Two patients died during the neutropenic phase, prior to engraftment, of multi organ failure secondary to sepsis (one had no active infection immediately prior to transplant and the other had chronic cholangiopathy and was on antimicrobial prophylaxis), one at 7 months post-transplant of granulomatous meningoencephalitis and a further patient at 28 months post transplant secondary to sepsis in the context of chronic extensive GVHD.

Graft versus host disease

13 patients developed grades I-II acute GVHD (10 skin only, 1 skin and gut, 1 gut only and 1 liver only) and one patient developed grade III aGVHD of the liver. Of these, 7 progressed to limited (single organ) chronic GVHD (4 skin only, 2 gut only and 1 pulmonary). A further patient developed steroid refractory extensive cGVHD. At last follow-up no patients had ongoing active cGVHD requiring systemic immune suppression.

Infectious Complications

A large proportion of patients had a high infectious burden pre-transplant as expected in patients with PID. Of the 29 patients, 24 (82%) had prior recurrent or severe infections, including recurrent bacterial infection (n=8), recurrent viral infection (n=7), both bacterial and systemic fungal infections (n=3), systemic fungal infection (n=2), both viral and bacterial infection (n=2), atypical mycobacterial infection (n=1) and combined viral and atypical mycobacterial infection (n=1). One patient had active pulmonary aspergilloma and three patients had ongoing HPV infection at the time of transplant (indicated in Table 1). Despite this, there was a distinct absence of major infection issues both intra and immediately post Allo-HSCT in our cohort. No patients suffered serious fungal infection post transplant, although azole prophylaxis was continued until off CSA in patients with pre-existing fungal infection (Table 2). No patients required granulocyte infusions.

CMV reactivation was only observed in 6 patients (35% of at risk patients, defined as +/+, -/+ or +/- recipient/donor pairs) and in all cases these responded to standard antiviral therapy. EBV reactivation with evidence of PTLD was observed in 4 patients all of whom responded completely to rituximab. An additional patient was treated with donor lymphocyte infusions (DLI) for presumed PTLD where no biopsy was available (n=1). No patient died of CMV, EBV or adenoviral infection.

Five patients had persistent viral warts pre-transplant with complete resolution or ongoing resolution of warts observed at last follow-up in two patients. In three patients, no improvement in warts was observed,

despite full donor T cell chimerism in all, including the two patients transplanted for DCML deficiency, who had pre-existing extensive perineal HPV-related intraepithelial neoplasia (VIN/CIN or AIN). A further patient with extensive confluent warts pre-transplant (NK deficiency) had persistent warts despite full reconstitution of T, B and NK cells.

At last follow-up the remaining 21 patients had no evidence of persistent or recurrent infections.

Immune Reconstitution

Lymphocyte subset analysis was performed on all the PID patients pre-transplant. Lymphocyte subset analysis was performed on all patients surviving beyond 3 months post-transplant (n=27) within the first 12 months post-transplant. Of the non-CGD PID patients (n=18), 13 of the 16 surviving PID patients had lymphocyte subset results at 12 months (3 had not reached 12 months follow up at the time of data collection). 70% of those with subset analysis performed (n=13) had achieved a normal lymphocyte count (1.0-2.8 x 10^9/L), 62% had normal absolute CD3+ cell counts (0.7-2.1 x 10^9/L), 62% normal CD4+ cell counts (0.3-1.4 x10^9/L) and 77% normal CD8+ cell counts (0.2-0.9 x10^9/L).

All CGD patients had normal neutrophil function tests post transplant (data not shown).

Of the 9 surviving patients who had been receiving monthly immunoglobulin replacement therapy pre-transplant, 89% were immunoglobulin-free at last follow up (Table 2).

Chimerism

Chimerism data was available for 23 of the 24 surviving patients at last follow up. At the time of analysis, 21 patients had results available at 12 months post transplant and 85% had full donor chimerism in unfractionated PBMCs. In the T cell fraction 52% of patients tested had achieved full donor chimerism at 12 months (Figure 4).

Multilineage full donor chimerism was observed in 10 (48%) of the patients with the rest showing mixed chimerism in at least one of the cell lineages tested. A trend to increasing chimerism stability with time was observed in our cohort. Chimerism was more robust in the B and myeloid cells compared to the T cell fraction. No correlation was found between mixed donor chimerism and age of patient, underlying diagnosis or donor type.

2 patients received DLI for persistent mixed chimerism (1 in the context of presumed PTLD with rising B cell numbers and FDG-avid lymphadenopathy on PET/CT scan). One achieved full donor chimerism in B cells and granulocytes with T cells remaining mixed and the other converted to full donor chimerism.

There were no cases of graft rejection and in all surviving patients either stable mixed chimerism or full donor chimerism were observed.

PID-associated Colitis

Of the 10 patients with moderate-severe inflammatory bowel disease pre-transplant (6 CGD, 1 XIAP, 1 CVID, 1 ALPS and 1 DCML deficiency), two died of TRM (both with CGD, patients 21 and 23) and the colitis has resolved in all 8 surviving patients, including the XIAP patient. No increased incidence of gut GVHD was observed in these patients.

DISCUSSION

We have demonstrated that in 29 young adult patients with high-risk primary immune deficiencies, reduced-intensity, *in vivo* T cell depleted Allo-HSCT was both effective and safe, with an overall survival of 85.2% at 3 years and a mean follow-up of 3.5 years (41 months). There was no significant difference in outcome between those undergoing matched related donor transplants and matched, or 1 antigen mismatched unrelated donor transplants (p=0.51). As predicted using our previously described T cell-depleting conditioning regimens, the observed cumulative incidence of severe acute GVHD incidence (grades III-IV) was very low at 6.5% and only 31% of patients developed chronic GVHD, symptoms which resolved allowing withdrawal of systemic immune suppression from 3 months post transplant in all but one of these patients. Full multilineage donor chimerism was achieved in 42% of patients, and all others achieved stable mixed chimerism. Larger studies are required to determine the degree of donor chimerism required post transplant for some of the rarer PIDs in order to achieve a functional cure. Good functional immune reconstitution was observed in all but one of the patients permitting the withdrawal of immunoglobulin replacement therapy post-transplant in 89% of patients. At last follow up 92% of surviving patients (n=24) were off immune suppression and the remaining two were in the process of weaning.

The overall and event free survival observed in this series of adult patients is comparable or better than that seen in published series of Allo-HSCT outcomes for pediatric and adolescent patients with PID and CGD[9, 23, 34-36].

The patients in this study had not undergone Allo-HSCT earlier in life due to a variety of reasons including mild-moderate clinical phenotype in childhood therefore not precipitating referral, delay in diagnosis until adolescence/adulthood, late presentation, and/or lack of a suitable donor. Patients had subsequently developed complications that necessitated definitive treatment in the form of Allo-HSCT. Triggers for referral included life-threatening infection, malignancy, autoimmune or inflammatory phenomena, newly confirmed genetic diagnosis or new donor availability. Due to pre-existing organ dysfunction, ongoing infectious or

inflammatory phenomena all patients in this cohort had a HCT-CI of at least 1 whilst 12 had a score of 3 or greater predicting a higher than observed TRM[25, 37]. Both the HCT-CI score or European Group for Blood and Bone Marrow Transplantation (EBMT) score have been validated in patients with haematological malignancies and in paediatric populations[38, 39]. Neither score has been validated in patients with PID or specifically CGD however it provides validated information on the clinical condition of patients pre-transplant. Patients with cellular immunodeficiency have been shown to have a higher HCT-CI and this score is used most frequently in other published studies of HSCT in PID[25].

These promising results suggest that Allo-HSCT is safe when delivered in a specialist centre and should be considered as a potentially curative option for younger adult PID patients with an appropriate donor and a sufficiently severe clinical picture. We recommend proceeding to Allo-HSCT for adult patients with a known genetic diagnosis amenable to correction by transplantation, and where conservative management results in a shortened life expectancy and ongoing morbidity. The current widespread use of next generation sequencing is expected to facilitate earlier referral of eligible adults.

AUTHOR CONTRIBUTIONS

TF, SOB, KJT, SG and ECM collected the data and wrote the manuscript. All other authors provided clinical care for the patients described. All authors had access to the clinical and laboratory data. ECM had final responsibility for the decision to submit for publication.

ACKNOWLEDGEMENTS

ECM, KT and SOB are supported by the UCLH NIHR Biomedical Research Centre. A number of co-authors receive funding from the National Institute of Health Research (NIHR), the NIHR UCLH/UCL Biomedical Research Centre, Bloodwise, Wellcome Trust, Medical Research Council, CRUK, The CRUK Experimental Cancer Medicine Centre, Teenage Cancer Trust and UK Primary Immunodeficiency Network. We thank Stuart Ings and Fiona O'Brien for their assistance in collecting data on CD34 cell doses.

CONFLICT OF INTEREST DISCLOSURE

No author has a relevant conflict of interest to declare.

REFERENCES

1. Bach, F., Albertini, R., Joo, P., Anderson, J., Bortin, M. (1968) Bone-marrow transplantation in a patient with the wiskott-aldrich syndrome. *Lancet* 292 (2), 1364-1366.
2. Gati, R., Meuwissen, H., Allen, h., Hong, R., Good, R. (1968) Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 292 (2), 1366-1369.
3. Fischer, A., Landais, P., Friedrich, W., Morgan, G., Gerritsen, B., Fasth, A., Porta, F., Griscelli, C., Goldman, S., Levinsky, R., Vossen, J. (1990) European experience of bone-marrow transplantation for severe combined immunodeficiency. *Lancet* 336, 850-854.
4. Fischer, A., Landais, P., Friedrich, W., Gerritsen, B., Fasth, A., Porta, F., Vellodi, A., Benkerrou, M., Jais, J., Cavazzana-Calvo, M. (1994) Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. *Blood* 83 (3), 1149-1154.
5. Slatter, M., Cant, A. (2011) Hematopoietic stem cell transplantation for primary immunodeficiency diseases. *Ann. N.Y. Acad. Sci.* 1238, 122-131.
6. Booth C, Silva J, Veys P. Stem cell transplantation for the treatment of immunodeficiency in children: current status and hopes for the future (2016). *Expert Rev Clin Immunol.* 12 (7), 713-23.
7. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, Kapoor N, Hanson IC, Filipovich AH, Jyonouchi S, Sullivan KE, Small TN, Burroughs L, Skoda-Smith S, Haight AE, Grizzle A, Pulsipher MA, Chan KW, Fuleihan RL, Haddad E, Loechelt B, Aquino VM, Gillio A, Davis J, Knutsen A, Smith AR, Moore TB, Schroeder ML, Goldman FD, Connelly JA, Porteus MH, Xiang Q, Shearer WT, Fleisher TA, Kohn DB, Puck JM, Notarangelo LD, Cowan MJ, O'Reilly RJ (2014). Transplantation outcomes for severe combined immunodeficiency, 2000-2009. *N Engl J Med* 371(5), 434-46.
8. Moratto D, Giliani S, Bonfim C, Mazzolari E, Fischer A, Ochs HD, Cant AJ, Thrasher AJ, Cowan MJ, Albert MH, Small T, Pai SY, Haddad E, Lisa A, Hambleton S, Slatter M, Cavazzana-Calvo M, Mahlaoui N, Picard C, Torgerson TR, Burroughs L, Koliski A, Neto JZ, Porta F, Qasim W, Veys P, Kavanau K, Hönig M, Schulz A, Friedrich W, Notarangelo LD. Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980-2009: an international collaborative study. *Blood.* 2011 Aug 11;118(6):1675-84.

9. Gungor, T., Teira, P., Slatter, M., Stussi, G., Stepensky, P., Moshous, D., Vermont, C., Ahmad, I, Shaw, P., Telles da Cunha, J., Schlegel, P., Hough, R., Fasth, A., Kentouche, K., Gruhn, B., Fernandes, J., Lachance, S., Bredius, R., Resnick, I., Belohradsky, B., Gennery, A., Fisher, A., Gaspar, H., Schanz, U., Segar, R., Rentsch, K., Veys, P., Haddad, E., Albert. M., Hassan, M. (2014) Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. *Lancet* 383, 436-448.
10. Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, Gibson B, Sedlacek P, Krol L, Strahm B, Zaidman I, Kalwak K, Talano JA, Woolfrey A, Fraser C, Meyts I, Müller I, Wachowiak J, Bernardo ME, Veys P, Sykora KW, Gennery AR, Slatter M (2016). Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience. *Blood.* 128(3):440-8.
11. Slatter, M., Gennery, A. (2013) Advances in hematopoetic stem cell transplantation for primary immunodeficiency. *Expert Rev. Clin. Immunol.* 9 (10), 991-999.
12. Bortoletto, P., Lyman, K., Camacho, A., Fricchione, M., Khanolkar, A., Katz, B. (2015) Chronic granulomatous disease. A large, single-centre US experience. *Pediatr Infect Dis J* 34 (10), 1110-1114.
13. Buckley, R., Schiff, S., Schiff, R., Markert, L., Williams, L., Roberts, J., Myers, L., Ward, F. (1999) Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 18, 340 (7), 508-516.
14. Siler U, Paruzynski A, Holtgreve-Grez H, Kuzmenko E, Koehl U, Renner ED, Alhan C, de Loosdrecht AA, Schwäble J, Pfluger T, Tchinda J, Schmugge M, Jauch A, Naundorf S, Kühlcke K, Notheis G, Güngor T, Kalle CV, Schmidt M, Grez M, Seger R, Reichenbach J (2105). Successful Combination of Sequential Gene Therapy and Rescue Allo-HSCT in Two Children with X-CGD - Importance of Timing. *Curr Gene Ther.* 15 (4), 416-27.
15. Hacein-Bey Abina S, Gaspar HB, Blondeau J, Caccavelli L, Charrier S, Buckland K, Picard C, Six E, Himoudi N, Gilmour K, McNicol AM, Hara H, Xu-Bayford J, Rivat C, Touzot F, Mavilio F, Lim A, Treluyer JM, Héritier S, Lefrère F, Magalon J, Pengue-Koyi I, Honnet G, Blanche S, Sherman EA, Male F, Berry C, Malani N, Bushman FD, Fischer A, Thrasher AJ, Galy A, Cavazzana M. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. *JAMA.* 2015 Apr 21;313(15):1550-63.
16. Morris EC, Fox T, Chakraverty R, Tendeiro R, Snell K, Rivat C, Grace S, Gilmour K, Workman S, Buckland K, Butler K, Chee R, Salama AD, Ibrahim H, Hara H, Duret C, Mavilio F, Male F, Bushman FD, Galy A, Burns SO, Gaspar HB, Thrasher AJ. Gene therapy for Wiskott-Aldrich syndrome in a severely affected adult. *Blood.* 2017 Sep 14;130(11):1327-1335.

17. Thrasher AJ, Williams DA. Evolving Gene Therapy in Primary Immunodeficiency. *Mol Ther.* 2017 May 3;25(5):1132-1141.
18. Seger, R. (2011) Advances in the diagnosis and treatment of chronic granulomatous disease. *Curr Op Haem*. 18 (1), 36-41.
19. Horwitz, M., Barrett, A., Brown, M., Carter,C., Childs, R., Holland, S., Linton, G., Miller, J., Read, E., Malech, H. (2001) Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. *N Engl J Med* 344 (12), 881-888.
20. Seger, R., Gungor, T., Belohradsky, B., Blanche, S., Bordigoni, P., Di Bartolomeo, P., Flood, T., Landais, P., Muller, S., Ozsahin, H., Passwell, J., Porta, F., Slavin, S., Wulffraat, N., Zintl., F., Nagler, A., Cant. A., Fischer, A. (2002) Treatment of chronic granulomatous disease with myeloablative conditioning with an unmodified hemopoietic allograft: a survey of the European experience, 1985-2000. *Blood* 100 (13), 4344-4350.
21. Cuellar-Rodriguez, J., Gea-Banacloche, J., Freeman, A., Hsu, A., Zerbe, C., Calvo, K., Wilder, J., Kurlander, R., Olivier, K., Holland, S., Hickstein, D. (2011) Successful allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Blood* 118 (13), 3715-3720.
22. Spinner, M., Sanchez, L., Hsu, A., Shaw, P., Zerbe, C., Calvo, K., Arthur, D., Gu, W., Gould, C., Brewer, C., Cowen, E., Freeman, A., Olivier, K., Uzel, G., Zelazney, A., Daub, J., Spalding, C., Claypool, r., Giri, N. Alter, B., Mace, E., Orange, J., Cuellar-Rodriguez, J., Hickstein, D., Holland, S. (2014) GATA2 deficiency: a protean disorder of hematopoiesis. *Blood* 123 (6) 809-821.
23. Marsh RA, Rao K, Satwani P, Lehmberg K, Müller I, Li D, Kim MO, Fischer A, Latour S, Sedlacek P, Barlogis V, Hamamoto K, Kanegane H, Milanovich S, Margolis DA, Dimmock D, Casper J, Douglas DN, Amrolia PJ, Veys P, Kumar AR, Jordan MB, Bleesing JJ, Filipovich AH (2013). Allogeneic hematopoietic cell transplantation for XIAP deficiency: an international survey reveals poor outcomes. *Blood.* 121 (6), 877-83.
24. Ono S, Okano T, Hoshino A, Yanagimachi M, Hamamoto K, Nakazawa Y, Imamura T, Onuma M, Niizuma H, Sasahara Y, Tsujimoto H, Wada T, Kunisaki R, Takagi M, Imai K, Morio T, Kanegane H (2017). Hematopoietic Stem Cell Transplantation for XIAP Deficiency in Japan. *J Clin Immunol.* 37(1):85-91.
25. Sorror, M., Maris, M., Storb, R., Baron, F., Sandmaier, B., Maloney, D., Storer, B. (2005) Hematopoietic stem cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessement before allogeneic HCT. *Blood* 106 (8), 2912-2919.
26. Slatter MA, Boztug H, Pötschger U, Sykora KW, Lankester A, Yaniv I, Sedlacek P, Glogova E, Veys P, Gennery AR, Peters C; EBMT Inborn Errors and Paediatric Diseases Working Parties. (2015). Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell

transplantation in children with non-malignant diseases. *Bone Marrow Transplant.* 2015 Dec; 50(12):1536-41.

27. Slatter MA, Rao K, Amrolia P, Flood T, Abinun M, Hambleton S, Nademi Z, Goulden N, Davies G, Qasim W, Gaspar HB, Cant A, Gennery AR, Veys P. (2011). Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. *Blood.* 2011 Apr 21;117(16):4367-75.
28. Chakraverty R, Peggs K, Chopra R, Milligan DW, Kottaridis PD, Verfuerth S, Geary J, Thuraisundaram D, Branson K, Chakrabarti S, Mahendra P, Craddock C, Parker A, Hunter A, Hale G, Waldmann H, Williams CD, Yong K, Linch DC, Goldstone AH, Mackinnon S (2002). Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood*. 99 (3), 1071-8.
29. Morris EC, Rebello P, Thomson KJ, Peggs KS, Kyriakou C, Goldstone AH, Mackinnon S and Hale G (2003). Pharmacokinetics of Alemtuzumab (CAMPATH-1H) Used for in vivo and in vitro T cell Depletion in Allogeneic Transplants: Relevance for Early Adoptive Immunotherapy and Infectious Complications. *Blood* 102 (1), 404-406.
30. Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H., Beatty, P., Hows, J., Thomas, E. (1994) Consensus conference on acute GVHD grading. *Bone Marrow Transplantation* 15 (6), 825-828.
31. Akpek, G., Lee, S., Flowers, M., Pavletic, S., Arora, M., Lee, S., Piantadosi, S., Guthrie, K., Lynch, J., Takatu, A., Horowitz, M., Antin, J., Weisdorf, D., Martin, P., Vogelsang, G. (1995) Performance of a new clinical grading system for chronic graft-versus-host disease: a multicentre study. *Blood* 102 (3), 802-809.
32. Klein, J., Rizzo, J., Zhang, M., Keiding, N. (2001) Statistical methods for the analysis and presentation of the results of bone marrow transplants. *Bone Marrow Transplantation* 28 (10), 909-915.
33. Klein, J., Rizzo, J., Zhang, M., Keiding, N. (2001) Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part II: regression modelling. *Bone Marrow Transplantation* 28 (11), 1001-10.
34. Satwani, P., Cooper, N., Rao, K., Veys, P., Amrolia, P. (2008) Reduced intensity conditioning and allogeneic stem cell transplantation in childhood malignant and nonmalignant diseases. *Bone Marrow Transplantation* 41, 173-182.
35. Wehr C, Gennery AR, Lindemans C, Schulz A, Hoenig M, Marks R, Recher M, Gruhn B, Holbro A, Heijnen I, Meyer D, Grigoleit G, Einsele H, Baumann U, Witte T, Sykora KW, Goldacker S, Regairaz L, Aksoylar S, Ardeniz Ö, Zecca M, Zdziarski P, Meyts I, Matthes-Martin S, Imai K, Kamae C, Fielding A, Seneviratne S, Mahlaoui N, Slatter MA, Güngör T, Arkwright PD, van Montfrans J, Sullivan KE, Grimbacher B, Cant A, Peter HH, Finke J, Gaspar HB, Warnatz K, Rizzi M; Inborn Errors Working

Party of the European Society for Blood and Marrow Transplantation and the European Society for Immunodeficiency (2015). Multicenter experience in hematopoietic stem cell transplantation for serious complications of common variable immunodeficiency. *J Allergy Clin Immunol*. 135 (4), 988-97.

36. Marsh RA, Rao MB, Gefen A, Bellman D, Mehta PA, Khandelwal P, Chandra S, Jodele S, Myers KC, Grimley M, Dandoy C, El-Bietar J, Kumar AR, Leemhuis T, Zhang K, Bleesing JJ, Jordan MB, Filipovich AH, Davies SM (2015). Experience with Alemtuzumab, Fludarabine, and Melphalan Reduced-Intensity Conditioning Hematopoietic Cell Transplantation in Patients with Nonmalignant Diseases Reveals Good Outcomes and That the Risk of Mixed Chimerism Depends on Underlying Disease, Stem Cell Source, and Alemtuzumab Regimen. *Biol Blood Marrow Transplant.* 21 (8), 1460-70.
37. Bokhari SW, Watson L, Nagra S, Cook M, Byrne JL, Craddock C, Russell NH (2012). Role of HCT-comorbidity index, age and disease status at transplantation in predicting survival and non-relapse mortality in patients with myelodysplasia and leukemia undergoing reduced-intensity-conditioning hemopoeitic progenitor cell transplantation. *Bone Marrow Transplant.* 47 (4), 528-34.
38. Smith AR, Majhail NS, MacMillan ML, DeFor TE, Jodele S, Lehmann LE, Krance R, Davies SM (2011). Hematopoietic cell transplantation comorbidity index predicts transplant outcomes in pediatric patients. *Blood* 117 (9), 2728-34.
39. ElSawy M, Storer BE, Sorror ML (2014). "To Combine or Not to Combine:" Optimizing Risk Assessment before Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* 20, 1455-1458.

FIGURE TITLES AND LEGENDS

Figure 1. Probabilities of overall survival (OS) and event-free survival (EFS)

(A) OS for the whole cohort was 89.2% at 12 months and 85.2% at 3 years (B) OS for matched related donors (MRD) 90.9% and 77.9% at 1 and 3 years respectively. OS for matched unrelated donors/mis-matched unrelated donors (MUD/MMUD) transplants was 94.4% and 88.9% at 1 and 3 years respectively, p=0.51. (C) OS for PID patients 94.4% and 88.9% at 1 and 3 years respectively. OS for adults for CGD patients was 90.9% and 81.8% at 1 and 3 years, p=0.75 (D) EFS 89.7% at 1 and 3 years. (E) EFS for MRD transplants 90.9% at both

1 and 3 years. EFS MUD/MMUD transplants 88.9% at 1 and 3 years, p=0.73 (F) EFS for PID patients 88.9%, EFS for CGD patients 90.9% at both 1 and 3 years, p=0.65.

Figure 2. Hematopoietic engraftment kinetics.

(A) Cumulative incidence of neutrophil engraftment (defined as $>0.5x10^9$/L for 3 consecutive days). Median time to neutrophil engraftment was 11 days in matched related donor (MRD) transplants and 15 days in MUD/MMUD transplants. (B) Cumulative incidence of neutrophil engraftment in the two main diagnostic groups. Median time to neutrophil engraftment was 11 days in non-CGD PIDs and 16 days in CGD patients. (C) Cumulative incidence of platelet engraftment (defined as $>50x10^9$/L for 3 consecutive days). Median time to platelet engraftment was 12 days in MRD transplants and 18 days in MUD/MMUD transplants. (D) Cumulative incidence of platelet engraftment in the two main diagnostic groups. Median time to platelet engraftment was 16 days in non-CGD PIDs and 19 days in CGD patients.

Figure 3. Cumulative incidence of transplant related mortality (TRM).

(A) Transplant related mortality (TRM) for all patients was 11% (n=29) at 1 year and 15% at 3 years. (B) TRM for patients with matched related donors (MRD) was 10% at 1 year and 22% at 3 years (n=11) vs 11% at 1 year and 3 years for MUD/MMUD transplants (n= 18), p=0.536 (C) TRM for patients with CGD was 10% at 1 year and at 3 years (n=11) vs other PID 12% at 1 year and 3 years (n=18), p=0.73.

Figure 4: Peripheral blood chimerism post transplant.

(A) PBMC chimerism results at 3, 6 and 12 months (n = 20, 17 and 19 respectively): Full donor chimerism (≥97% donor DNA) was achieved in PBMC fraction in 71% at 3 months, 83% at 6 months and 85% at 12 months. (B) T cell chimerism results: full donor chimerism was achieved in the T cell fraction in 30% at 3 months, 41% at 6 months and 52% at 12 months. (C) B cell chimerism: Full donor chimerism was achieved in the B cell fraction in 65% at 3 months, 82% at 6 months and 69% at 12 months. (D) Chimerism in the granulocyte fraction. Full donor chimerism was seen in 83% patients at 3 months, 67% at 6 months and 67% at 12 months.

	Diagnosis	Genetic Mutation	Age at Diagnosis	Age at HSCT/Sex	Medical Complications Prior to Transplant	HCT-CI Score*	Donor (HLA mis-match)	Stem cell source	CD34 dose x 10^6/kg	CMV Status (R/D)	Conditioning Regimen	In vivo T-cell depletion (dose)	GVHD Prophylaxis
	CVID	Not done.	20	31/F	Red cell aplasia.	2	MRD	PBSC	10.8	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	Granulomatous CVID	No mutation in TNFSF5. (Normal T cell numbers at presentation and no T cell related infections).	15	30/F	Inflammatory lung disease, sclerosing cholangitis, prior splenectomy, pulmonary fibrosis, pulmonary aspergilloma*, osteopenia.	≥3	MUD	PBSC	15.3	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	APDS2	Splice donor mutation in PIK3R1 (predicting exon skipping).	10	50/M	Severe lymphatic pan-colitis, previous mycoplasma arthritis, bronchiectasis, previous peripheral CD8+ T cell NHL, pulmonary hypertension.	≥3	MUD	PBSC	5.1	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	Autoimmune LPD	Heterozygous TNFRSF 6 gene encoding Fas (c.785T>C (I232T).	3	32/M	Refractory thrombocytopenia, previous splenectomy, hypertension.	2	MRD	PBSC	3.7	+/-	Flu Mel	Alemtuzumab (100mg)	CSA
	Autoimmune LPD	No mutation identified, including in Fas, SH2D1A. Functional apoptosis defect.	32	34/M	Indolent lymphoma (NHL), autoimmune neutropenia, MGUS with IgG paraprotein, previous splenectomy, Factor XI deficiency.	1	MUD	PBSC	6.7	-/-	Flu Mel	Alemtuzumab (100mg)	CSA
	Cγ-chain SCID	Confirmed by sequencing.	1	26M	Failed haploidentical allo and gene therapy. Sclerosing cholangitis. Recurrent infections. Bronchiectasis and pneumocele. Brachio-cephalic thrombus. Hepatomegaly with chronic cholangiopathy. Refractory facial planar warts. Depression.	≥3	MUD	PBSC	12.6	+/+	Flu Mel	Alemuzumab (100mg)	CSA
	Absolute NK deficiency, Hypogammaglobulinaemia	No mutation identified. Whole genome sequencing pending.	14	27/M	Refractory planar warts (HPV2)[5], recurrent infections, bronchiectasis, sarcoid arthropathy, IgG 1 and 2 subclass deficiency.	≥3	MRD	PBSC	7.8	+/+	Flu Mel	Alemtuzumab (30mg)	CSA
	DCML deficiency	Gata2 wild type; Whole exome sequencing Pending.	24	27/F	Trilineage MDS, bronchiectasis, Crohn's colitis, recurrent viral warts, Mycobacterium avium complex, anal and vulval intraepithelial neoplasia (HPV)[5].	≥3	MMUD (C Ag)	PBSC	6.3	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	AR IL12Recβ deficiency	Confirmed by sequencing.	19	29/M	Recurrent salmonella sepsis, salmonella pericarditis with cardiac tamponade, mild asthma, recurrent otitis externa.	≥3	MMUD (A Ag)	PBSC	7.0	-/-	Flu Mel	Alemtuzumab (100mg)	CSA
	Rag2/red cell aplasia	RAG2 (c.104G>T (Gly35Val)/het and c.814G>A (Val72Ile)/het andc.965T>C(Met 322Thr)Het.	16	20/M	Red cell aplasia, granulomatous skin lesions, high transfusion requirement and iron overload, chronic clonal NK cell proliferation, EBV viraemia.	≥3	MUD	PBSC	5.4	-/-	Flu Mel	Alemtuzumab (100mg)	CSA
	X-linked LPD	Confirmed by sequencing.	3	18/M	B cell NHL, hypogammglobulinaemia.	1	MRD	PBSC	11.0	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	Undefined CID	c.205del heterozygote in CD27 gene. Heterozygous mutations in CD27, LRBA, LYST and PRKDC.	11	22/M	EBV viraemia, atypical stage 4B T cell rich B cell NHL, LP HL (R-CHOP x6, high dose MTX).	2	MUD	PBSC	4.0	+/+	Flu Mel	Alemtuzumab (100mg)	CSA
	CD27 deficiency	CD27 (c.251_252insT (C71Lfs*4) and SNP in exon 3 37176138 7).	13	18/M	Nodular sclerosing HL, EBV+ Stage IV Diffuse Large B cell Lymphoma.	1	MMUD (A Ag)	PBSC	6.4	-/-	Flu Mel	Alemtuzumab (100mg)	CSA
	Gata2 deficiency	735-736insC Amino Acid Subs P245fs	20	22/F	MDS, persistent viral warts, mild IgG hypogammaglobulinaemia, CD19, CD56 Lymphopenia, HPV VIN3[5].	1	MUD	PBSC	6.3	+/-	Flu Mel	Alemtuzumab (100mg)	CSA
	XIAP deficiency	XIAP - absent by FACs (2 centres); No mutation identified.	19	21/M	HLH, Crohn's (requiring pan-colectomy as a child).	≥3	MRD	PBSC	4.7	-/+	Flu Mel	Alemtuzumab (30mg)	CSA
	Autoimmune LPD	I246T Fas Mutation	2	21/F	Relapsed Hodgkin's lymphoma, autoimmune haemolysis, genital HSV, previous splenectomy, juvenile inflammatory arthropathy, patchy colonic lymphocytic infiltration.	2	MRD	PBSC	2.1	+/+	Flu Mel	Alemtuzumab (30mg)	CSA
	Gata 2 mutation	Frame duplication of 6 nucleotides not previously reported.	19	22/F	MDS with profound monocytopenia, prolonged severe EBV infection with hepatitis and meningoencephalitis.	≥3	MRD	PBSC	2.0	-/-	Flu Mel	Alemtuzumab (30mg)	CSA
	XIAP deficiency	Sequence variant c.497G>A in exon 2 of the XIAP gene. Not previously reported.	4	24/M	EBV lymphoproliferative disease x2 episodes, hypogammaglobulinaemia, splenomegaly, granulomatous lymphocytic inflammatory lung disease with bronchiectasis.	2	MRD	PBSC	5.5	+/-	Flu Mel	Alemtuzumab (30mg)	CSA
	AR-CGD	P47 deficiency identified on FACs analysis.	12	18/M	Recurrent infections including staphylococcal abscesses.	1	Father 10/10	BM	3.7	+/+	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	AR-CGD	p47 deficiency identified on FACs analysis.	18	19/M	Recurrent infections , chronic relapsing multifocal osteomyelitis, mould pulmonary infection, dyslexia.	2	MUD	BM	2.1	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA

	AR-CGD	NCF-1 (P47 deficiency by FACS. Homozygous for c.579g>A (Trp193X).	3	27/F	Recurrent infections including presumed nocardia meningitis. Severe inflammatory bowel disease necessitating subtotal colectomy and ileostomy formation. Colonised with resistant pseudomonas.	≥3	MUD	BM	6.3	+/+	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	Variant CGD	NCF-1 (Fusion of NCF-1 and pseudo NCF-1 with crossover between exon 4 and exon 16).	24	28/F	Severe Crohn's disease with perineal and perianal disease requiring colectomy and proctectomy with ileostomy.	1	MUD	PBSC	14.5	-/-	Flu Bu	rATG (7.5mg/kg)	CSA
	X-linked CGD	CYBB (c.G764A(Trp251*))	1	27/M	Recurrent infections, severe colitis.	2	MRD	PBSC	5.0	+/-	Flu Mel	Alemtuzumab (100mg)	CSA
	X-linked CGD	p47 deficiency identified on FACs analysis.	2	18/M	Pan-colitis, previous hemicolectomy for stricture of ascending colon, recurrent infections, renal impairment.	≥3	MUD	BM	4.2	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	X-linked CGD	CYBB (TC342/343>AT (His115Tyr)).	4	19/M	Recurrent fungal chest infections, extensive granulomas, growth failure, previous gene therapy (August 2007) with transient engraftment.	≥3	MMUD (A Ag)	PBSC	32.3	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	X-linked CGD	GP91 intron 6 gtg>atg	1	19/M	Recurrent infections including osteomyelitis and fungal chest infection (aspergillus nidulans).	1	MMUD (A Ag)	BM	0.3	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	X-linked CGD	GP91phox 20kb deletion leading to complete absence of CYBB and Kell genes (McLeod Phenotype).	At birth	17/M	Pan-colitis, recurrent infections, McLeod phenotype.	2	MUD	BM	1.4	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA
	X-linked CGD	NCF1 (c.579G>A (TRP193*).	16	23/M	Recurrent infections including staphylococcal skin abscesses, progressive granulomas.	2	MRD	PBSC	4.1	+/-	Flu Mel	Alemtuzumab (100mg)	CSA
	X-linked CGD	Gp91absent by FACs.	2 months	17/M	Progressive colitis, multiple infective complications.	2	MUD	BM	1.9	-/-	Flu Bu	Alemtuzumab (0.6mg/kg)	CSA

Table 1: Patient demographics and transplant characteristics.

CVID common variable immunodeficiency; LPD lymphoproliferative disease; SCID severe combined immunodeficiency; NK natural killer cell; DCML dendritic cell, monocyte, B lymphocyte, and natural killer lymphocyte deficiency; AR autosomal recessive; CID combined immunodeficiency; XIAP X-linked inhibitor of apoptosis protein deficiency; CGD chronic granulomatous disease; APDS2 Activated PI3Kδ syndrome type 2; MUD matched unrelated donor; MMUD mismatched unrelated donor; MRD matched related donor; PBSC peripheral blood stem cells; BM bone marrow; CSA cyclosporin A; Flu fludarabine; Bu busulphan; Mel melphalan, rATG rabbit anti-thymocyte globulin; FACs fluorescence-activated cell sorting; HCT-CI hematopoietic cell transplant comorbidity index; R/D recipient/donor CMV sero status; *active infection at transplant; $^{\$}$unresolved viral warts at transplant.

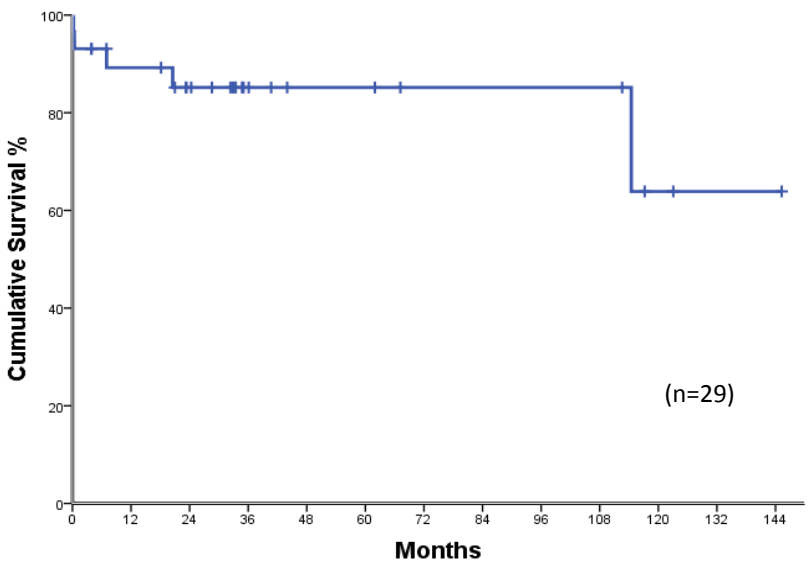
	Diagnosis	Acute GVHD (grade)	Chronic GVHD	CMV Status (R/D)	Infectious Complications	Other complications	Days FU	Present Status	Immuno-Suppression at last FU (Y/N)	Immunoglobulin replacement (Continues, Off or N/A)	Peripheral Blood Chimerism at last F/U (PBMC)*	Outcome
1	CVID	N/A	N/A	+/+	Sepsis.	Multi organ failure.	12	Dead.	Y	N/A	N/A.	Died TRM (sepsis).
2	CVID	None.	Probable Pulmonary.	+/+	CMV reactivation. Recurrent bacterial chest infection.	ITP. Progressive pulmonary fibrosis/bronchiolitis obliterans.	3434	Dead.	Y	Continued until death.	Full donor chimerism	Died (progressive respiratory failure secondary to pre-existing pulmonary fibrosis +/- pulmonary GVHD).
3	APDS2	Grade 1 gut	Limited (gut).	+/+	Intermittent respiratory tract infections.	Papillary renal cell carcinoma (unrelated)	730	Alive.	N	Off	Full donor chimerism	Well.
4	Autoimmune LPD	None.	Limited post DLI.	+/-	None.	Oesophageal stricture secondary to peptic ulceration. DLI x 3 for mixed chimerism.	4358	Alive.	N	N/A	Full donor chimerism	Well.
5	Autoimmune LPD	None.	None.	-/-	None.	CSA-induced neurotoxicity DLI x 4 for presumed PTLD (no biopsy, FDG-avid lymphadenopathy on CT/PET scan).	3378	Alive.	N	Off	Full donor chimerism	Well. Neurotoxicity resolved.
6	Cγ-chain SCID	N/A	N/A	+/+	Sepsis.	Multi organ failure.	7	Dead.	Y	N/A	N/A	Died TRM (sepsis).
7	Absolute NK deficiency	Grade 1 skin.	None.	+/+	Persistent planar warts (HPV2).	None.	2015	Alive.	N	Off	Full donor chimerism	Persistent extensive warts.
8	DCML deficiency	Grade 2 skin & gut.	None.	+/+	CMV reactivation (resolved). Radial excision for persistent HPV associated AIN and VIN.	Acute thyroiditis (antibody negative). Ovarian failure.	1221	Alive.	N	N/A	Full donor chimerism	Well. Thyroiditis resolved. GVHD resolved.
9	AR IL12Recβ deficiency	Grade 1 skin.	Limited (gut).	-/-	EBV reactivation treated with rituximab.	None.	1083	Alive.	N	Off	Full donor chimerism	Well.
10	Rag2/red cell aplasia	None.	None.	-/-	None.	Delayed engraftment.	700	Alive.	N	Off	Stable mixed chimerism (full donor in B cell fraction, mixed in other lineages).	Well. Resolution of granulomatous skin lesions on shins.
11	X-linked LPD	None.	None.	+/+	BK virus cystitis.	None.	3692	Alive.	N	Off	Stable mixed chimerism (mixed all lineages).	Well. Normal spermatozoa.
12	Combined immune deficiency	Grade 2 skin.	None.	+/+	CMV reactivation.	Intermittent neutropenia.	1042	Alive.	N	Continues	Stable mixed chimerism (mixed all lineages).	Well.
13	CD27 deficiency	Grade 2 skin.	Limited (skin).	-/-	Rhinovirus. EBV reactivation treated with rituximab.	None.	544	Alive.	N	Continues	Full donor chimerism	Well. CT/PET ongoing remission.
14	Gata2 deficiency	None.	None.	+/-	Persistent perineal HPV with VIN3.	DLI x 3 for MC. Fibromyalgia. Chronic fatigue. Thyrotoxicosis	1051	Alive.	N	N/A	Full donor chimerism	Well. Resolution of warts on hands & feet.
15	XIAP deficiency	None.	None.	-/+	Warts left foot and right index finger resolving.	None.	629	Alive.	N	N/A	Stable mixed chimerism (mixed all lineages).	Well. No further colitis.
16	Autoimmune LPD	Grade 2 skin.	None.	+/+	CMV reactivation x 1.	Biopsy proven EBV PTLD treated with 4 cycles rituximab.	118	Alive.	N	N/A	Chimerism pending	Well. No further CMV or EBV reactivations.
17	Gata 2 mutation	Grade 1 skin.	None.	-/-	Low level EBV reactivation not requiring intervention.	None.	209	Alive.	Y	N/A	Full donor chimerism	Well. Weaning cyclosporin.
18	XIAP deficiency	None.	None.	+/-	None.	Slow recovery of counts and persistent splenomegaly.	115	Alive.	Y	Off	Full donor chimerism	Well. Weaning cyclosporin. Trial off Immunoglobulin as IgG in normal range at 4m post.
19	AR-CGD	None.	None.	+/+	CMV reactivation.	Iron and vitamin D deficiency.	983	Alive.	N	N/A	Stable mixed chimerism (mixed all lineages).	Well. Viable sperm.
20	AR-CGD	None.	None.	-/-	None.	None.	971	Alive.	N	N/A	Stable mixed chimerism (full donor in PBMCs and B cells, mixed in T cell and granulocyte fractions)	Well.
21	AR-CGD	None.	None.	+/+	Bilateral lower lobe consolidation (no organisms identified)	Haemophagocytosis and prolonged cytopenias; Granulomatous meningitis (no pathogen identified); Acute hepatic failure.	210	Dead.	Y	N/A	Full donor chimerism	Died TRM (multi-organ failure).
22	Variant CGD	Grade 1 skin.	None.	-/-	None.	Premature ovarian insufficiency.	992	Alive.	N	N/A	Stable mixed chimerism (full donor in B cell fraction, mixed	Well. Resolution of granulomatous colitis.

											in other lineages).	
23	X-linked CGD	Grade 3 liver.	Steroid refractory, extensive.	+/-	Multiple infective complications associated with immune suppression for GVHD.		616	Dead.	Y	N/A	Full donor chimerism	Died TRM (steroid refractory extensive cGVHD).
24	X-linked CGD	None.	None.	-/-	None.	Mild renal impairment.	1858	Alive.	N	N/A	Stable mixed chimerism (mixed in all lineages).	Well. Renal impairment resolved.
25	X-linked CGD	Grade 1 skin.	None.	-/-	Adenoviraemia.	EBV PTLD treated with 4 cycles rituximab.	1003	Alive.	N	N/A	Full donor chimerism	Well.
26	X-linked CGD	Grade 1 skin.	Limited (skin).	-/-	Pulmonary aspergillosis.	Renal impairment (resolved), CD34+ top up for prolonged cytopenias.	1319	Alive.	N	N/A	Full donor chimerism	Well. Some viable sperm suitable for ICSI.
27	X-linked CGD	Grade 1 skin.	Limited (skin).	-/-	None.	None.	857	Alive.	N	N/A	Stable mixed chimerism (full donor in PBMC and B cell fractions, mixed in T cell and granulocyte fractions).	Well. Viable sperm on testing, suitable for ICSI.
28	X-linked CGD	None.	None.	+/-	None.	DLI x 4 for mixed chimerism.	3516	Alive.	N	N/A	Stable mixed chimerism (mixed in all lineages)	Well. Normal full blood count. Father of 2 children.
29	X-linked CGD	None.	None.	-/-	Rotavirus diarrhoea.	Depression.	697	Alive.	N	N/A	Stable mixed chimerism (full donor in PBMC and B cell fractions, mixed in T cell and granulocyte fractions).	Well.

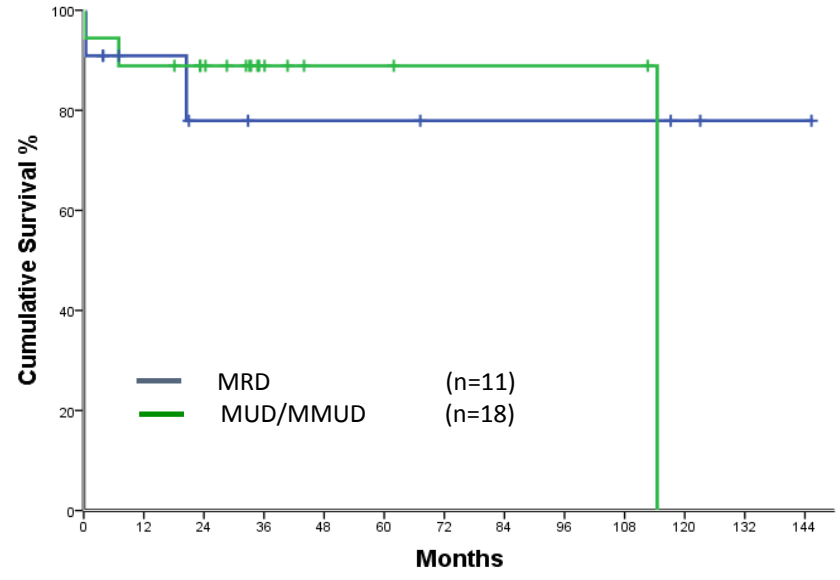
Table 2: Transplant outcome and resolution of PID-related complications.

GVHD graft versus host disease; R/D recipient/donor CMV sero status; FU follow up; TRM transplant related mortality; ITP idiopathic thrombocytopenic purpura; DLI donor lymphocyte infusion; PTLD post transplant lymphoproliferative disease; HPV human papilloma virus; AIN/VIN/CIN anal/vaginal/cervical intraepithelial neoplasia; EBV Epstein Barr virus; MC mixed chimerism; ICSI intracytoplasmic sperm injection; N/A Not applicable; Full donor ≥97% donor DNA; Mixed chimerism ≥ 50% < 97% donor DNA; * See Figure 4 for lineage specific chimerism.

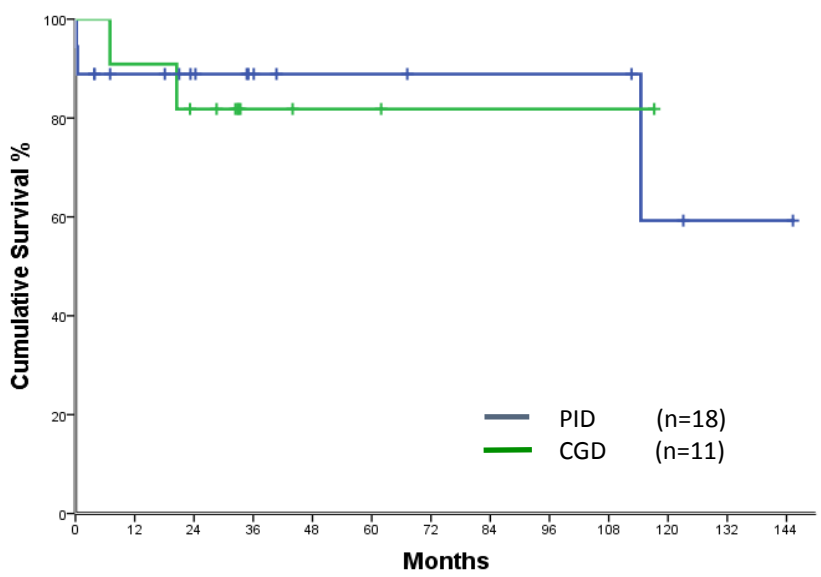
A OS



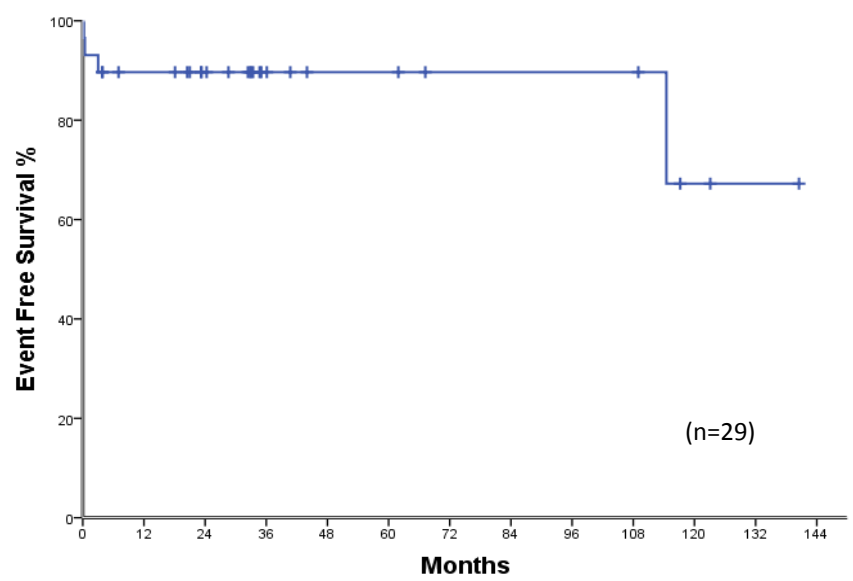
B OS by donor



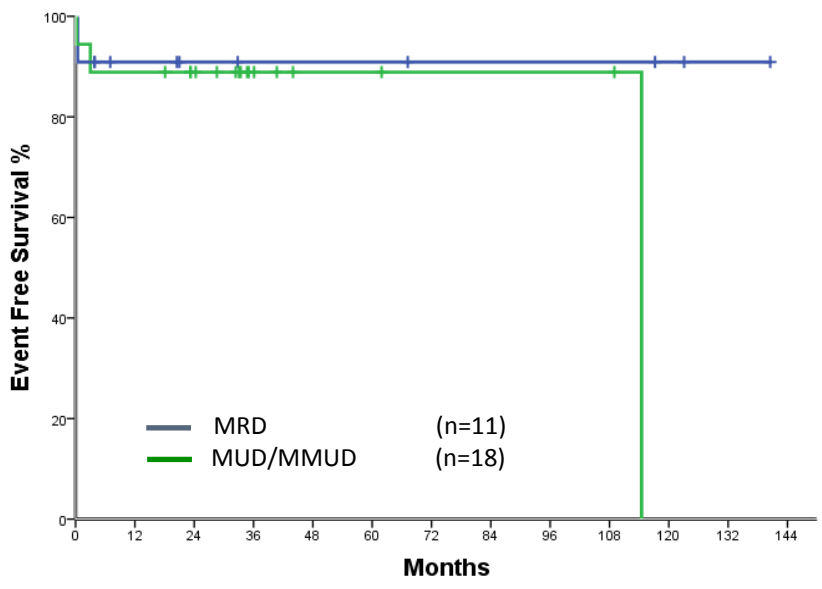
C OS by diagnostic group



D EFS



E EFS by donor



F EFS by diagnostic group

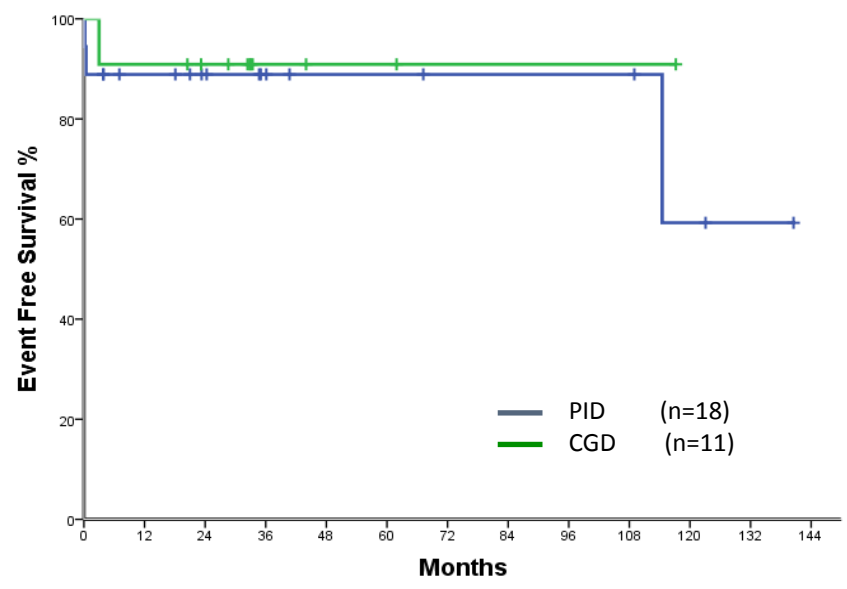
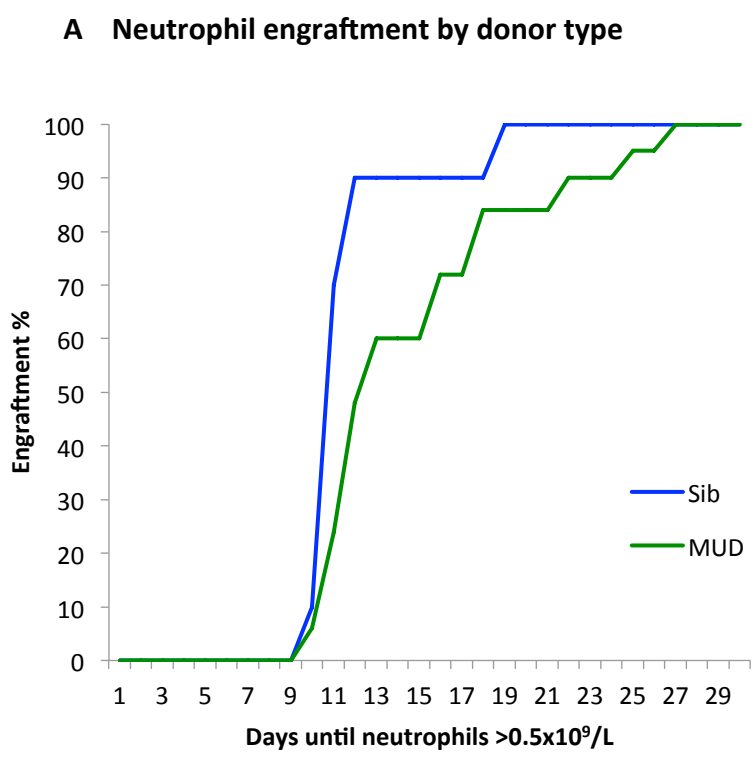
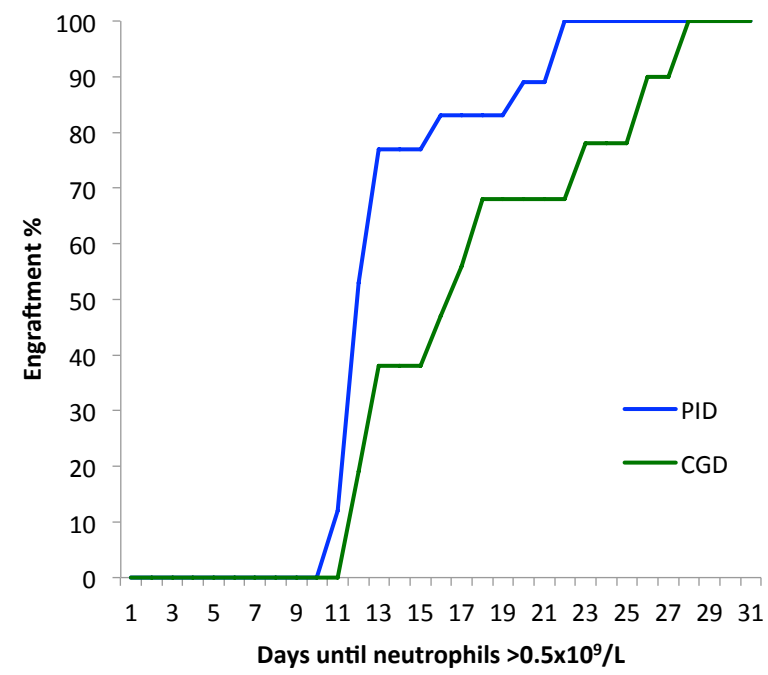
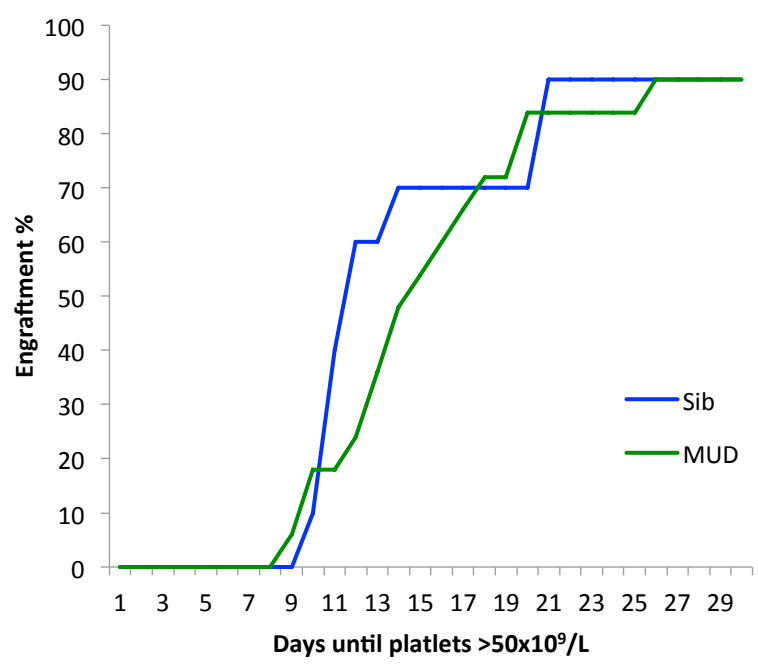


Figure 1. Probabilities of overall survival (OS) and event-free survival (EFS).







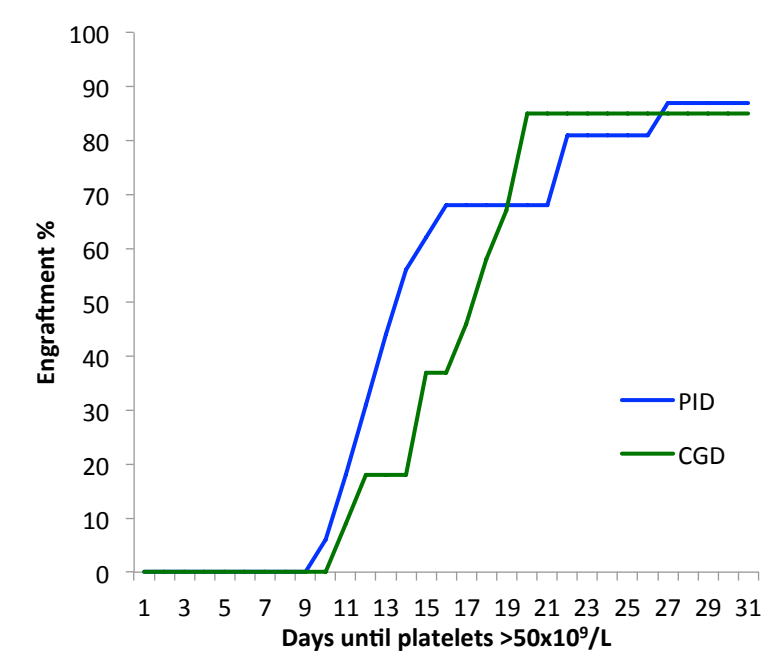
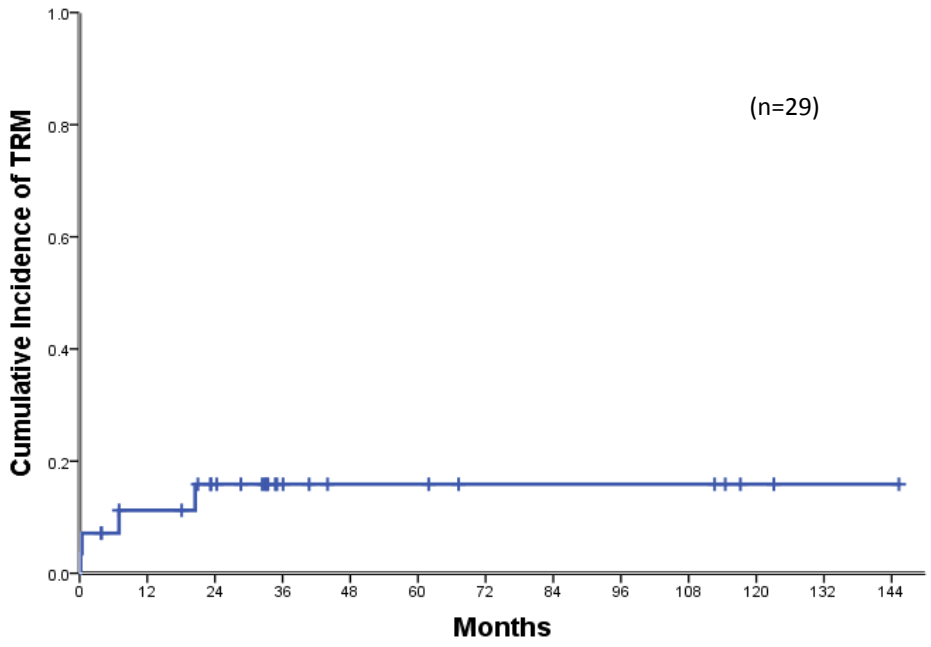
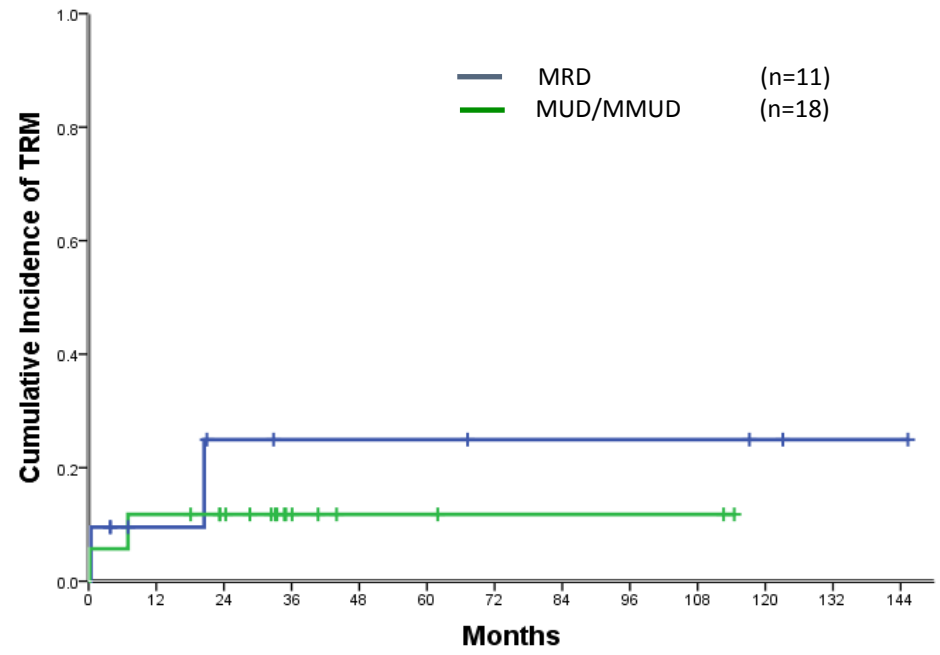


Figure 2. Haematopoietic engraftment kinetics.

A Cumulative incidence of TRM (all patients)



B Cumulative incidence of TRM by donor



C Cumulative incidence of TRM by diagnostic group

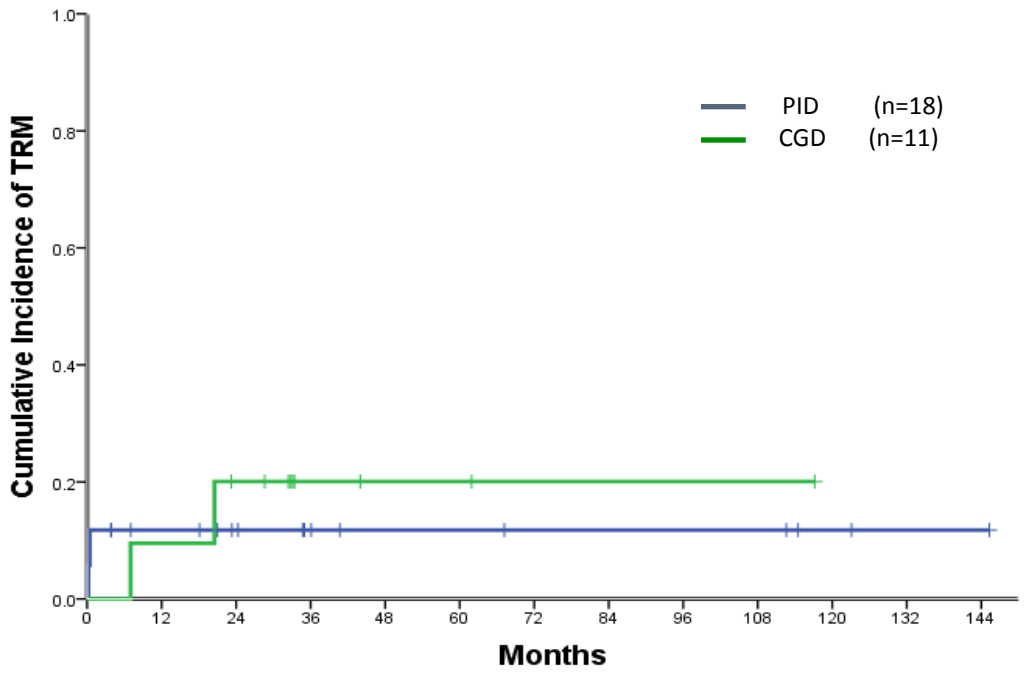


Figure 3. Cumulative incidence of transplant related mortality (TRM).

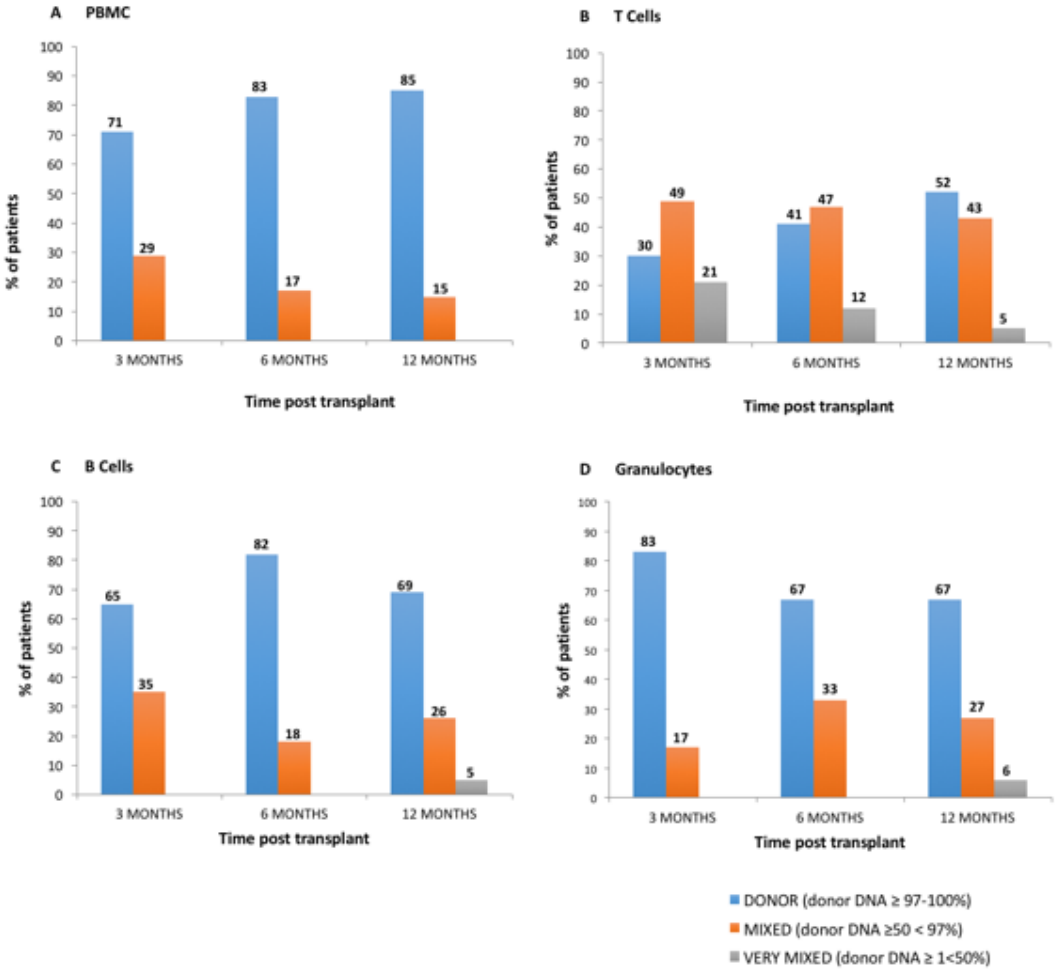


Figure 4: Chimerism Kinetics