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

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Part 3

Alternative donors in haematopoietic cell transplantation for inborn errors of immunity

Chapter 5

Impact of different ex vivo T-cell depletion strategies on outcomes following haematopoietic cell transplantation for children with primary immunodeficiency

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Abstract

Haematopoietic cell transplantation (HCT) is the curative therapy to restore normal immunity in patients with primary immunodeficiency (PID). For patients without a matched donor, an ex-vivo T cell depleted (TCD) graft from a mismatched donor is an alternative. This is the first report to compare the transplant outcomes according to TCD methods in 105 PID children who underwent first HCT between 1987 and 2019. Different TCD methods were used including CAMPATH-1M TCD marrow (n=34), CD34+ selected marrow (CD34-S, n=34), CD3/CD19 TCD PBSC (n=7), and CD3+TCRαβ/CD19 TCD PBSC (ab-TCD, n=30). Donors were haploidentical (n=88), MUD/MFD (n=9) and MMFD/MMUD (n=6). 94% received conditioning. 3 had grade III-IV acute GvHD. None had cGvHD. Analysis by TCD methods revealed a 5-year OS of 58% for CAMPATH-1M, 68% for CD34-S, 69% for CD3/CD19 TCD and 83% for ab-TCD (p=0.24). The corresponding EFS was 46% for CAMPATH-1M, 47% for CD34-S, 69% (for CD3/CD19 TCD and 83% for ab-TCD (p=0.04). The CI of graft failure reduced significantly, from 29% (14-61%) for CAMPATH-1M, to 19% for CD34-S, 17% (for CD3/CD19 TCD and none had graft failure in the ab-TCD (p=0.002). The median donor myeloid chimerism at last follow-up was higher in the newer TCD methods; 100% for ab-TCD, 93% for CD3/CD 19 depletion, 6% for CD34-S, 20% for CAMPATH-1M (p<0.001). There was no significant difference in donor T-lymphocyte chimerism between TCD methods. Outcomes after CD3+TCRαβ/CD19+ depletion are superior to previously used TCD methods. The result has led to an evolution of the donor hierarchy in our centre

Introduction

Haematopoietic cell transplantation (HCT) is a successful curative treatment for an increasing number of primary immunodeficiencies (PIDs). Following the first successful HCTs for severe combined immunodeficiency (SCID) and Wiskott-Aldrich syndrome (WAS) in 1968, HCT has undergone a major evolution due to superior HLA matching technology, greater availability of alternative donors, cord blood banking, reduced toxicity conditioning regimens, vigilant infection surveillance and more effective antimicrobial therapy.[1,2] Precise molecular diagnosis and an understanding of the natural history of PID enable early identification of suitable candidates for HCT before organ damage is present. There are now over 400 molecularly defined inborn errors of immunity and the underlying phenotypes encompass infection, malignancy, allergy, auto-immunity and autoinflammation. By 2008, more than 2500 children had received a HCT for PID in Europe and North America. [3,4] Transplant survival has improved to 90% for SCID with a matched family donor and 70% for an unrelated donor transplant. [5,6] Similar transplant survival has been reported in non-SCID PID recently, and younger age of transplant has been consistently shown to be a significant positive predictor of transplant outcome. [7,8] For patients with non-SCID PID such as chronic granulomatous disease and CD40 ligand deficiency, many studies have demonstrated that long-term survival, disease outcome and quality of life are better in transplanted patients compared with non-transplanted patients who were treated with conventional therapy using antimicrobial prophylaxis and immunoglobulin replacement.[9,10] In many non-SCID PID, HCT has developed from a last resort into a standard of care that corrects the defects of immunity.

Whilst international donor registries and cord blood banking provide increased availability of alternative donors, some patients, particularly from ethnic minorities, do not have suitable HLA-matched donors. The United States registry data has shown that the probability of an eight out of eight HLA-matched donor varies with ethnic group; 69% for Caucasian patients, 32% for African American, 41% for Asian/Pacific Islanders and 47% for Hispanics.[11] An alternative is to use a haploidentical related donor and deplete the T-cells in the graft prior to infusion. Historically, E-rosette lectin and ex-vivo CAMPATH-1M anti-CD52 antibodies were the main methods used in the 1980s.[12-14] CD34+ stem cell selection became the preferred method in the 1990s, where an organic iron bead attached to an anti-CD34 antibody isolates purified CD34+ stem cells from the other cells when the graft is passed through a magnetic column, the most widely used method being the Miltenyi Clini-MACS system (Miltenyi Biotec, Surrey, UK)[15] Whilst these methods are able to achieve profound T-cell depletion, which is essential to prevent fatal graft-versus-host disease, engraftment failure and delayed immune reconstitution render the patients at risk of potentially life threatening viral infection. A promising step forward in improving immune reconstitution comes with graft manipulation

to selectively deplete CD3/CD19 and T-cell receptor (TCR) αβ/CD19 lymphocytes. In these two newer methods, the cellular products contain CD34+ progenitors, natural killer cells, dendritic cells and graft-facilitating cells, which enhance engraftment and early immune reconstitution. In TCR αβ/CD19 depleted grafts, additional TCR αβ T-cells in the graft have been shown to provide rapid and sustained engraftment, rapid immune reconstitution and a low incidence of graft-versus-disease (GVHD) in children with malignant and non-malignant disorders. [16-19]

The first ex-vivo T-cell depleted haploidentical allograft at our center was performed in 1987. Since then, different T-cell depletion methods have been used for children with PID, including CAMPATH-1M anti-CD52 antibodies, CD34+ stem cell selection, CD3/CD19 depletion, and CD3 TCRαβ/CD19 depletion. In the light of significant developments in the ex-vivo T-cell depletion strategy over the past three decades, this retrospective study aimed to examine transplant survival and graft outcome according to different graft manipulation methods in children with PID.

Methods

Patients and Methods

Between January 1987 to March 2019, 118 children with PID underwent their first *ex vivo* T-cell depleted allograft at the Great North Children's Hospital. Thirteen patients who received additional gene-modified add-back T-cells were excluded from this study and 105 were included in the final analysis. The clinical and laboratory data were retrieved from the transplantation database, patients' medical files and laboratory records. Written informed consent was obtained from the parents or legal guardians of the patients as per institutional practice for HCT.

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

The majority of ex-vivo T-cell depletions in this study were performed in haploidentical parental donors (n=88, 84%). The donor selection criteria for a haploidentical donor was as follows: (i) donor with a better HLA match (ii) non-carrier donor for X-linked diseases (iii) maternal donor for a patient with SCID with maternal fetal engraftment. Marrow was used for CAMPATH-1M depletion and CD34 selection while peripheral blood stem cells (PBSC) were used for CD3/CD19 and TCR αβ/CD19 depletions. Prior to 2009, various conditioning regimens were used with the majority of patients (n=59) undergoing conditioning with

busulfan and cyclophosphamide. Other conditioning regimens were busulfan (n=1), cyclophosphamide (n=3), fludarabine/melphalan (n=1), treosulfan/cyclophosphamide (n=2), and busulfan/fludarabine/thiotepa (n=1). Patients received serotherapy according to the institutional guideline at the time of HCT, including none, alemtuzumab or ATG. From 2009, the conditioning regimen was switched to a myeloablative reduced toxicity regimen (RTC) using fludarabine, treosulfan, ATG (Grafalon) and rituximab for SCID (n=10) and thiotepa was added for non-SCID PID (n=22). Seven patients with SCID did not receive conditioning due to severe co-morbidities. Post-transplant graft-versus-host disease prophylaxis (GvHD) prophylaxis varied according to T-cell depletion methods at the time of transplant, including no GvHD prophylaxis, ciclosporin, ciclosporin/methotrexate or ciclosporin/mycophenolate mofetil. No GvHD prophylaxis was given since 2015 except in patients who received grafts with a TCR αβ content of more than 5 x10^4/kg.

Supportive care

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

Definition and endpoints

The main outcomes of interest were overall survival (OS), event-free survival (EFS) and cumulative incidence (CI) of graft failure. OS was defined as survival from first HCT to last follow-up or death. An event was defined as death, graft failure or second procedures for slipping chimerism. Other endpoints assessed were as follows: (i) time to neutrophil recovery (first day of achieving a neutrophil count ≥ 0.5 × 10^9/L for three consecutive days); (ii) incidences of transplant-related complications as defined and graded according to existing institutional guidelines at the time of HCT, including hepatic veno-occlusive disease (VOD) and GvHD; (iii) new autoimmunity post-HCT; (iv) immune reconstitution; (v) degree of donor haematopoietic chimerism at the most recent assessment. For immune reconstitution kinetics for the first 12 months, data on CD3+, CD4+, CD8+, CD19+, and natural killer cell (NK) numbers were collected. For patients who had an event, either death or second procedure, the lymphocyte subset results prior to the event were included for analysis. The intensity of the conditioning regimens in this manuscript has been classified as myeloablative conditioning (MAC),

reduced toxicity conditioning (RTC), and reduced intensity conditioning (RIC). MAC referred to Busulfan (16mg/kg)-Cyclophosphamide (200mg/kg) (Bu16-Cy). RTC included Treosulfan-Cyclophosphamide (Treo-Cy), and Treosulfan-Fludarabine (Treo-Flu) with or without thiotepa (Treo-Flu-Thio). RIC regimens were Fludarabine-Melphalan (Flu-Melp), Busulfan (8mg/kg)-Cyclophosphamide (200mg/kg) (Bu8-Cy), busulfan monotherapy and cyclophosphamide monotherapy

Statistical analysis

Quantitative variables were described with median and range while categorical variables were reported with counts and percentages. The association between variables was assessed with the use of Kruskal Wallis test for continuous variables and the chi-square test for categorical variables. Probabilities of OS and EFS were calculated using the Kaplan-Meier estimate. Cox proportional hazards regression model were used to perform univariate analyses of predictors on OS and EFS. The selected variables were: age at transplant, diagnosis (SCID versus non-SCID PID), T cell depletion methods (Campath-1M versus CD34 selection versus CD3/CD19 depletion versus TCR αβ/CD19 depletion), conditioning regimen (MAC versus RTC versus RIC versus none), serotherapy (none versus Alemtuzumab versus ATG), total nucleated cell dose (TNC) and CD34 cell dose. All factors associated with a *p*-value <0.10 by univariate analysis were included into multivariate analysis. Cumulative incidence of graft failure, neutrophil engraftment and new post-HCT autoimmunity was calculated using a competing risk analysis, considering death as a competing event. Gray's test was used for univariate comparison. Multilevel mixed effects modelling was performed for the longitudinal analysis of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and NK cells and CAMPATH-1M was used as a reference group. All *p*-values quoted are two-sided, with a level of significance of 0.05. Statistical analyses were performed using STATA 14.2.

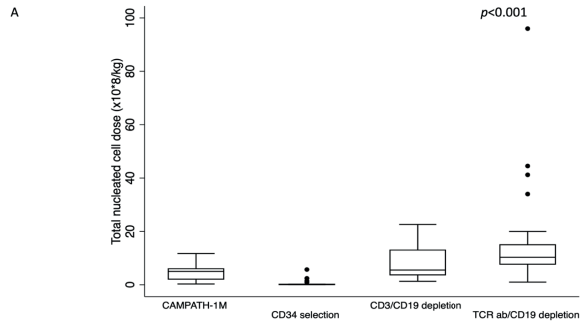
Results

Patient and transplantation characteristics

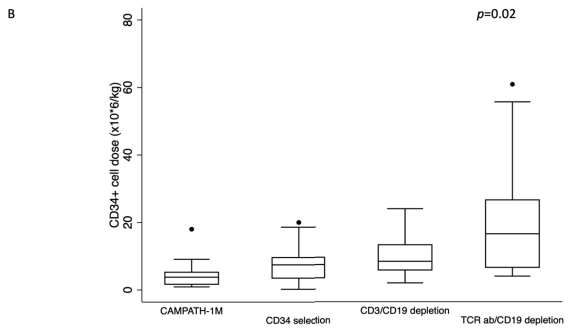
Patient and transplantation characteristics are summarized in Table 1. The proportion of patients who received ex-vivo T-cell depleted grafts changed significantly, from 50% during 1987 to 1998, to 27% during 1999 to 2006, 6% during 2007 to 2011, and 14% during 2012 to 2019 ($p<0.001$). The indication for an ex-vivo T-cell depleted graft transplant has changed significantly, from predominantly SCID to non-SCID from 1987 to 2019 ($p<0.001$). There was no significant difference in the age at diagnosis and the age at transplant for SCID and

non-SCID patients between these methods. The interval between diagnosis and transplant was significantly longer in newer methods of ex-vivo T-cell depletion grafts in SCID patients (p=0.001), but there was no significant difference in non-SCID PID patients (p=0.68). Parents (35/37, 95%) were the main donors for CD3/CD19 depletion and TCR αβ/CD19 depletion. CAMPATH-1M and CD34 selection were performed in 6 and 3 matched family or matched unrelated donors respectively in order to minimise the risk of GVHD at the time. Two patients received a TCR αβ/CD19 depleted MMUD due to unsuitability of their parents.

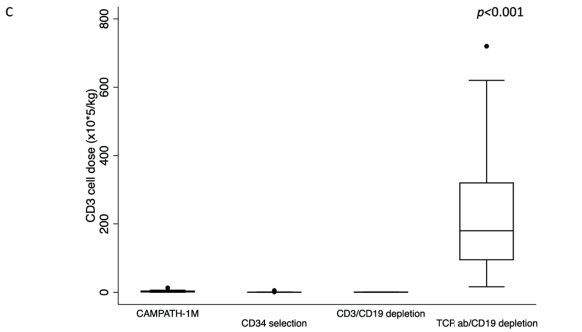
No serotherapy was given in 27 (79%) of CAMPATH-1M, 19 (56%) of CD34 selected, 3 (43%) of CD3/CD19 depleted and 3 (10%) of TCR αβ/CD19 depleted recipients (p<0.001). Post-transplant GvHD prophylaxis was used in 3 (9%) of CAMPATH-1M, 27 (79%) of CD34 selected, 8 (86%) of CD3/CD19 depleted and 17 (57%) of TCR αβ/CD19 depleted recipients (p<0.001). The graft composition improved significantly with higher total nucleated cell dose (p<0.001, figure 1A), CD34+ cell dose (p=0.02, figure 1B) and CD3+ cell dose (p<0.001, figure 1C) in newer T-cell depletion methods.



Number	34	34	7	30
Median TNC ($x10^8$/kg)	5	0.1	5.5	10.3
Range	0.3-11.7	0.05-5.7	1.3-22.6	1.0-96



Number	14*	34	7	30
Median CD34+ cells ($x10^6$/kg)	3.8	7.5	8.4	16.5
Range	0.9-18	0.2-20.0	2.0 -24.0	4.0-60.9



Number	30	34	7	30
Median CD3+ cells ($x10^5$/kg)	2.6	0.18	0.29	180
Range	0.1-12.8	0.01-5.0	0-1.0	16-720

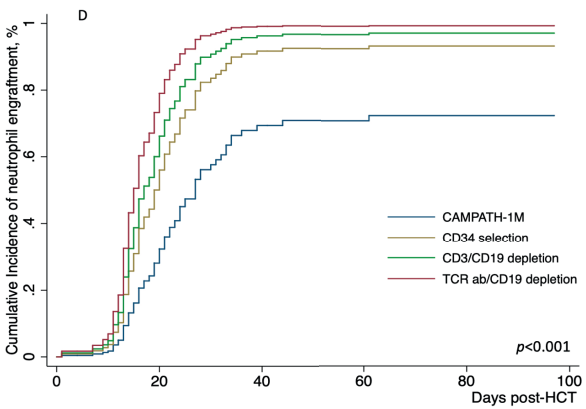


Figure 1: Graph composition and engraftment kinetics according to ex-vivo T cell depletion methods. Total nucleated cell dose (A), CD3+ cell dose (C) were significantly higher in patients who received TCR αβ/CD19 depletion. Neutrophil engraftment kinectics (D) was significantly faster in newer methods.

*** CD34+ cell dose was not in 20 patients who had CAMPATH-1M depleted marrow.**

The engraftment and transplant-related complications are summarized in Table 2. The engraftment kinetics were significantly different between these methods (figure 1D) with earlier neutrophil recovery demonstrated in CD3/CD19 depleted and TCR αβ/CD19 depleted PBSC. Thirteen (12%) patients had acute GvHD, of whom 3 (3%) grade III-IV. None had chronic GvHD.

With routine surveillance for CMV, adenovirus, EBV and HHV6 after 2000, the incidence of any viraemia was 26% (n=6/23) following CD34 selection, 50% (n=3/6) for CD3/CD19 depletion and 47% (n=14/30) for TCR αβ/CD19 depletion (p=0.27). Adenoviraemia was significantly higher in TCR αβ/CD19 depletion (37%, n=11/37), compared to CD34 selection (none had adenoviraemia) and CD3/CD19 depletion (17%, n-1/6) (p=0.001). There were no significant differences in CMV viraemia (p=0.52), HHV6 viraemia (p=0.67) and EBV viraemia (p=0.71) between these methods.

Infection-related death remained a significant problem, 72% (18/25 deaths) in CAMPATH-1M and CD34 selection and 66% (4/6 deaths) in CD3/CD19 and TCR αβ/CD19 depletions. Three of four deceased patients who received TCR αβ/CD19 graft had active viraemia (1 adenovirus, 1 CMV and 1 adenovirus and HHV6) during HCT.

Cumulative incidence of new autoimmunity post-HCT was 6% (95% CI, 2-15%) and 9% (95% CI, 4-21%) at one year and five years post-HCT. Of 9 patients who developed new autoimmunity, four (4/34, 12%) had CAMPATH-1M depleted marrow, 3 (3/34, 9%) had CD34 selected marrow, 1 received CD3/CD19 depleted PBSC, and 1 (1/30, 3%) had TCR αβ/CD19 depleted PBSC. Autoimmune cytopenia (AIC, n=7) was the most common type of autoimmunity post-transplant; 3 autoimmune haemolytic anaemia (AIHA), 2 AIHD + immune cytopenia, and 1 red cell aplasia. One patient who had AIC post CAMPATH-1M depleted parental graft developed Guillain Barre syndrome after second transplant using CD34 selected parental graft. One patient had multiple endocrinopathy (hypothyroidism and diabetes mellitus) and one had colitis.

Transplant survival and graft failure

The 5-year OS and EFS for the entire cohort were 68% (95% CI, 58-67%) and 55% (95% CI 44-64%) respectively. Analysis by ex-vivo T-cell depletion methods revealed a 5-year OS of 58% (95% CI, 40-73%) for CAMPATH-1M, 68% (95% CI, 49-81%) for CD34 selection, 69% (95%CI, 23-91%) for CD3/CD19 depletion and 83% (95% CI, 40-37%, p=0.05) for TCR αβ/CD19 depletion (Figure 2A). On univariate analysis, TCR αβ/CD19 depletion was the only factor associated with OS (Table 3). Age at transplant, diagnosis, conditioning, serotherapy, and stem cell doses were not associated with OS.

EFS was 46% (95% CI, 29-62%) for CAMPATH-1M, 47% (95% CI, 30-62%) for CD34 selection, 69% (95% CI, 22-91%) for CD3/CD19 depletion and 83% (95% CI, 61-93%) for TCR αβ/CD19 depletion (p=0.04) (Figure 2B). On univariate analysis, reduced toxicity conditioning (p=0.01), TCR αβ/CD19 depletion (p=0.01) and CD34 cell doses were significantly associated with improved EFS. On multivariate analysis, none of these factors was independently associated with EFS (conditioning, p=0.71, TCD methods, p=0.42 and CD34 cell dose, p=0.16).

The cumulative incidence of graft failure reduced significantly, from 29% (95% CI, 14-61%) for CAMPATH-1M, to 19% (95%CI, 8-45%) for CD34 selection, 17% (95% CI 2-18%) for CD3/CD19 depletion and none had graft failure in TCR αβ/CD19 depletion (p=0.002) (figure 2C). Sixteen (24%) patients who had CAMPATH-1M and CD34 selection received second procedures (7 second HCT and 7 unconditioned stem cell boost). None of CD3/CD19 and TCR αβ/CD19 methods required second procedures.

On subgroup analysis, similar transplant outcomes were observed in SCID and non-SCID PID (supplemental figure 1 and 2). For SCID patients, both OS and EFS were 100% and none had graft failure. For non-SCID PID, TCR αβ/CD19 depletion was also associated with superior OS (p=0.05) and EFS (p=0.01) and none had graft failure with this method (p=0.02).

Immune reconstitution, donor chimerism and new autoimmunity

Immune-reconstitution kinetics within the first 12 months post-HCT according to ex-vivo T-cell depletion methods are shown in figure 3 and supplemental figure 3. CD34 selected recipients had significantly higher CD3+ lymphocyte counts at months 2 (p=0.048), 3 (p=0.03), 4 (p=0.02), 5 (p=0.004), 6 (p=0.002) and 12 (p=0.03) post-HCT compared to CAMPATH-1M. (supplemental figure 3). A similar pattern of CD3+ lymphocyte recovery was observed in TCR αβ/CD19 depletion at months 3 (p=0.02), 4 (p=0.006), 5 (p=0.07) and 6 (p=0.02) post-HCT compared to CAMPATH-1M. When comparing between CD34 selection and TCR αβ/CD19 depletion, there was no significant difference in CD3+ lymphocyte recovery at any time point post-transplant. CD4+ lymphocyte counts were significantly higher post CD34 selection at months 4 (p=0.02), 5 (p=0.01), and 6 (p=0.006) post-HCT and at month 4 (p=0.04) post-HCT in TCR αβ/CD19 depletion, when compared to CAMPATH-1M (Figure 3). There was no significant difference in circulating $CD8^+$, $CD19^+$ and NK cells between ex-vivo T-cell depletion methods.

The median donor myeloid chimerism at last follow-up was higher following newer T-cell depletion methods; 100% (range, 0-100%) for TCR αβ/CD19 depletion, 93% (range, 0-100%) for CD3/CD 19 depletion, 6% (range, 0 -49%) for CD34 selection, 20% (range, 0-100%) for CAMPATH-1M (p<0.001) (Figure 4A). There was no significant difference in donor T-lymphocyte chimerism between T-cell depletion methods (Figure 4B).

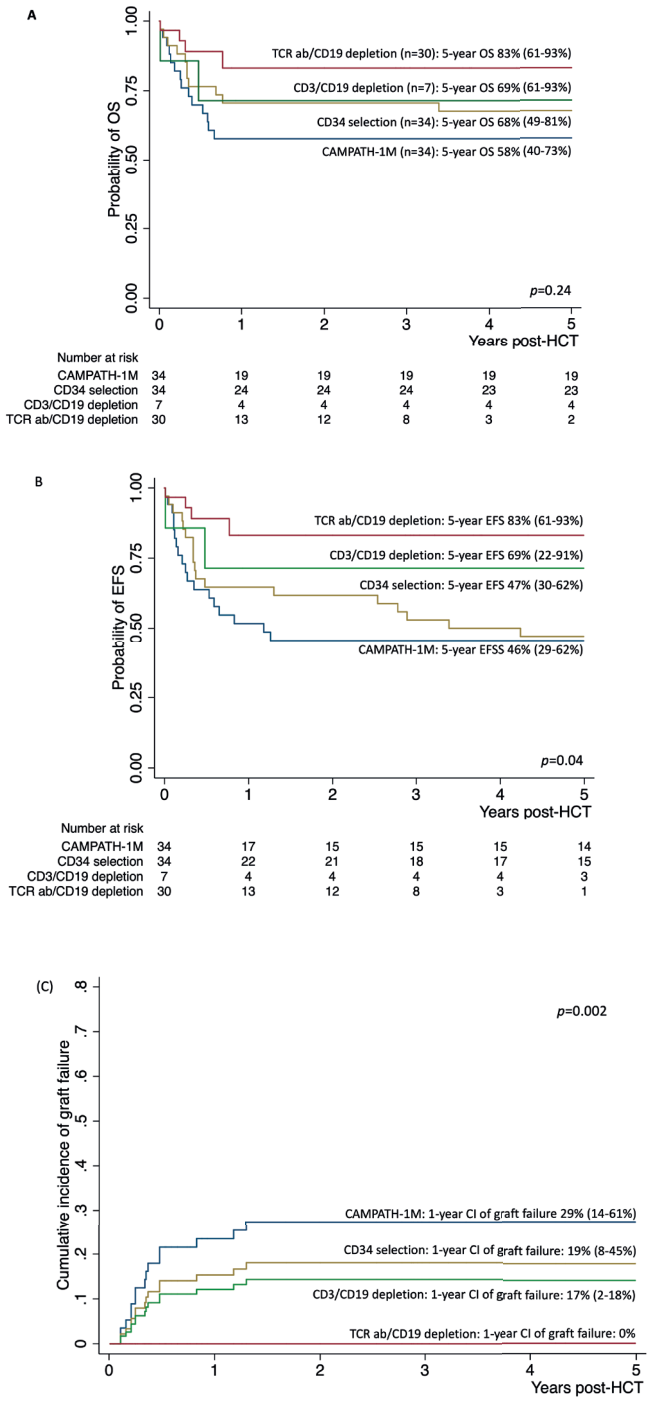
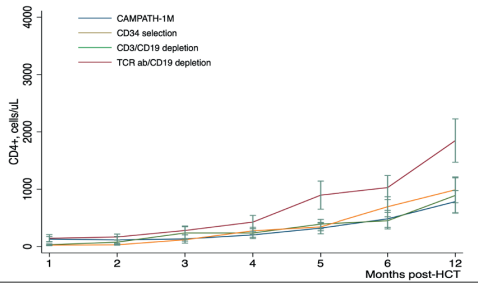


Figure 2: Overall survival (A), event-survival survival (B), and cumulative incidence of graft failure (C) according to ex-vivo T-cell depletion methods

(A)



Ex-vivo T-cell depletion method		M1	M2	M3	M4	M5	M6	M12
CAMPATH-1M (n=34)	n	22	20	19	17	16	11	5
	CD4+, mean±SE	67±42	30±9	52±19	162±48	273±178	487±245	782±194
CD34 selection (n=34)	n	27	24	20	14	12	15	11
	CD4+, mean±SE	294±181	375±120	279±82	495±135*	762±237*	952±260*	1854±495
CD3/CD19 depletion (n=7)	n	6	6	6	5	4	4	4
	CD4+, mean±SE	50±27	93±51	100±52	267±162	348±127	480±198	1074±350
TCR ab/CD19 depletion (n=30)	n	29	28	24	19	16	13	8
	CD4+, mean±SE	10±6	24±18	154±49	270±36	356±19	607±66	718±303

(B)

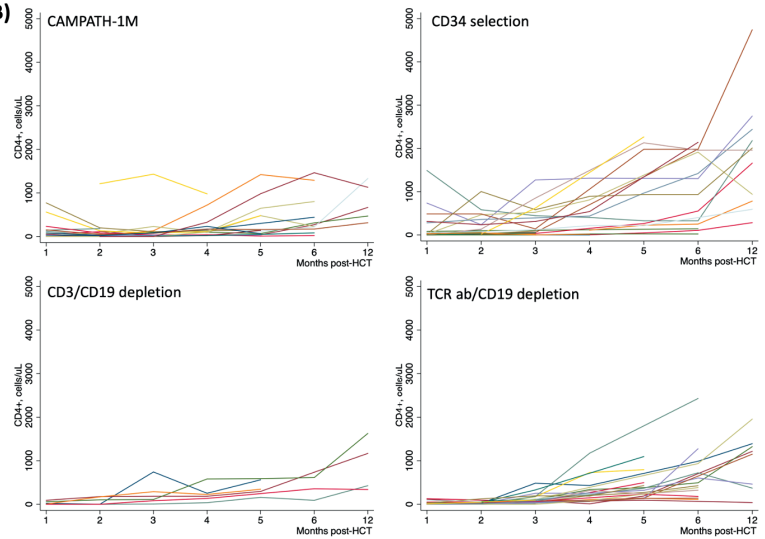
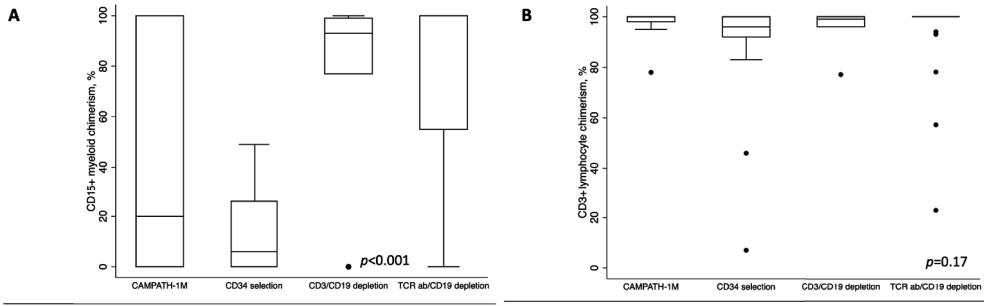


Figure 3: Immune reconstitution kinetics post-HCT: Means and standard errors (SE) for CD4+ (A) lymphocytes measured at different time points post-HCT. Individual patient immune reconstitution kinetics according to ex-vivo T cell depletion methods are shown in (B) for CD4+ lymphocytes according to campath-1M, CD34 selection, CD3/CD19 depletion and TCR ab/CD19 depletion.* indicates $p<0.05$



	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR ab/CD19 depletion
Number	15	15	5	26
Median, %	20	6	93	100
Range	0-100	0-49	0-100	0-100

	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR ab/CD19 depletion
Number	15	15	5	26
Median, %	100	96	99	100
Range	0-100	7-100	77-100	23-100

Figure 4: Latest donor chimerism after first successful HCT. CD15+ myeloid chimerism (A) was higher in CD3/CD19 depletion and TCR αβ/CD19 depletion ($p<0.001$). There was no significant difference in CD3+ lymphocyte chimerism between ex-vivo T cell depletion methods (p=0.17)

Discussion

HCT is curative for an increasing number of PIDs. Despite an increase in the availability of potential matched donors worldwide including the use of banked cord bloods, there are a large number of patients for whom no matched donor can be found. Various techniques for T cell depleting mismatched donors have been used over the years to minimise the risks of graft rejection and GvHD.

This study reports the first and largest series comparing transplant outcomes according to TCD method in children with PID over the last 30 years. Historically poor survival following MMRD transplants for non SCID PID using the previous techniques is well known. Gennery *et al*. reported only 46% survival in 47 such patients transplanted in Europe.[3] This report clearly demonstrates the improvement in OS and EFS with more recent techniques and the successful use of CD3+ TCR αβ/CD19 depletion in patients with a diverse range of non SCID PID. Importantly for many conditions we have also demonstrated better myeloid chimerism. We have shown low rates of aGvHD and no cGvHD even in patients not given any post-transplant GvHD prophylaxis, which for example, removes the renal toxicity associated with calcineurin inhibitors. Despite other changes in care over the last 30 years for these patients such as changing chemotherapy for conditioning, better surveillance and treatment of infections, on univariate analysis TCR αβ/CD19 depletion was the only factor associated with significantly improved OS.

The early CD4 recovery seen in recipients of CD34+ selected grafts is surprising and may be related to the absence of serotherapy in the majority of these patients prior to transplant. The improved outcome in the newer techniques is most likely related to the presence of other cells such as NK cells and graft enhancing stromal components.

The introduction of CD3+ TCR αβ/CD19 depletion has transformed our practice in the last 7 years so all patients with any form of PID now have a donor. However further improvements are necessary to decrease morbidity and mortality associated with viral infections. A number of techniques are emerging such as re-infusion of genetically modified αβ T lymphocyte receptor bearing cells with an added caspase suicide gene. The addition of these cells may confer additional anti-viral properties, but if aGvHD occurs, the cells can be removed by administrating an inert investigational compound which activates the suicide gene. An alternative is to remove naive T lymphocytes bearing the CD45RA marker, most likely to cause aGvHD, but retain CD45RO-bearing T lymphocytes which are more likely to confer antiviral activity.[20-22] These cells could be given as an add-back in single or multiple doses to improve immune reconstitution.

Changing the dose and timing of serotherapy effects the residual T cells in the graft. Measurement of serotherapy levels may enable us to improve the speed of immune reconstitution without an increase in GvHD[23,24]. An attractive alternative approach to TCD is to infuse replete HLA-haplo-identical product followed by two doses of cyclophosphamide a few days after transplant. Rapidly proliferating cells are preferentially targeted by the cyclophosphamide so alloreactive donor T lymphocytes are selectively deleted, thus minimising the risk of GvHD and leaving viral specific T lymphocytes and lymphocyte precursor cells.[25,26] An international multicentre study is being undertaken to compare outcomes using this method with CD3+ TCR αβ/CD19 depletion in patients with PID.

In this study the time to transplant was not shortened using newer techniques for TCD compared to old because a haploidentical donor was not the first choice. However for a patient with SCID our current practice is to choose a haploidentical donor if no family donor is available thus avoiding the time and expense taken to look for an unrelated donor. For patients with non-SCID PID we would choose a haploidentical donor if no 10/10 HLA matched unrelated donor can be found. In time this donor hierarchy may further evolve such that haploidentical donors may replace unrelated donors.

Of note, the donor myeloid chimerism was higher in CD3/CD19 depletion and CD3+ TCR αβ/CD19 depletion. This could be due to higher stem cell doses infused. Our center has shown that donor myeloid chimerism has a strong correlation with long-term B cell function and freedom from immunoglobulin replacement in children with SCID.[27]

In conclusion this study demonstrates the change in practice of TCD methods and the successful application of CD3+ TCR αβ/CD19 depletion of HLA mismatched donors for patients with a wide variety of PIDs who historically had a very poor outcome from haploidentical HCT. Reduced toxicity conditioning and vigilant supportive care are very important in complementing TCD haploidentical donor transplantation.

Table 1: Patient and transplantation characteristics according ex-vivo T-cell depletion methods (N=105)

	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR αβ/CD19 depletion	*p*-value
Number	34	34	7	30[1]	
Year of transplant	1987-1998	1999-2006	2007-2011	2012-2019	
Proportion of first HCT during the study period, n (%)	34/68 (50)	34/125 (27)	7/121 (6)	30[1]/215 (14)	<0.001
Male, n (%)	23 (68)	22 (65)	4 (57)	17 (57)	0.81
Diagnosis					<0.001
SCID, n (%)	31 (91)	24 (71)	5 (71)	9 (30)	
Non-SCID PID, n (%)	3 (9)	10 (29)	2 (29)	21 (70)	
CD40 ligand deficiency	1	5	0	1	
CGD	0	0	0	2	
MHC class II deficiency	0	0	0	3	
Others	2[2]	5[3]	2[4]	15[5]	
Median age at diagnosis (range), years					
SCID	0.4 (birth-0.9)	0.2 (birth-1.5)	0.5 (0.2-0.7)	0.2 (birth-0.2)	0.25
Non-SCID PID	0.9 (0.4-2.9)	1.1 (0.3-9.8)	0.7 (0.6-0.7)	0.8 (birth – 17.1)	0.92
Median age at transplant (range), years					
SCID	0.5 (0.1-1.0)	0.2 (0.1-1.7)	0.8 (0.3-0.9)	0.5 (0.1-1.7)	0.26
Non-SCID PID	2.9 (1.1 -5.3)	3.1 (0.5-12.4)	1.1 (0.7-1.5)	2.7 (0.2-17.5)	0.92
Median interval between diagnosis and transplant (range), years					
SCID	0.1 (0.03-0.3)	0.1 (0.07-0.2)	0.2 (0.2-0.4)	0.2 (0.05-0.8)	0.001
Non-SCID PID	2.5 (0.2-2.5)	0.7 (0.1-6.6)	0.6 (0.5-0.8)	1.3 (0.1-4.3)	0.68
Donor, n (%)					0.23
MUD/MFD	6 (18)	3 (9)	0	0	
MMFD/MMUD (1 to 2 loci MM)	2 (6)	4 (12)	0	2 (7)	
Haploidentical donor (≥3 loci MM)	26 (76)	27 (79)	7 (100)	28 (93)	
Mother	4	11	6	13	
Father	24	16	1	15	

	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR αβ/CD19 depletion	*p*-value
Stem cell source					<0.001
Marrow, n (%)	34 (100)	34 (100)	0	0	
PBSC, n (%)	0	0	7 (100)	30 (100)	
Conditioning regimen					<0.001
None	2 (6)	3 (8)	0	2 (3)	
MAC	11 (32)	21 (62)	0	1 (3)	
RTC	0	0	7 (100)	27 (90)	
RIC	21 (62)	10 (29)	0	0	
In vivo serotherapy					<0.001
None, n (%)	27 (79)	19 (56)	3 (43)	3 (10)	
ATG, n (%)	3 (9)	2 (6)	4 (57)	25 (83)	
Alemtuzumab, n (%)	4 (12)	13 (38)	0	2 (7)	
GVHD prophylaxis					<0.001
None, n (%)	31 (91)	7 (21)	1 (14)	13 (43)	
CSA±MTX/MMF, n (%)	3 (9)	27 (79)	6 (86)	17 (57)	

[1]13 were excluded for add-back T-cell (CGD, 2; DOCK8 deficiency, 2; hyperIgD syndrome, 1; MHC class II deficiency, 4; SCID, 3; WAS, 1.
Proportion of ex-vivo T-depleted allograft was 20% from 2012-2019
[2]HyperIgM syndrome, 1; ZAP70 deficiency, 1; [3]Cartilage hair hypoplasia, 1; CHARGE syndrome, 1; CID, 1; WAS, 1; ZAP70 deficiency, 1; [4]ALPS, 1; HLH, 1
[5]CID, 1; autoimmune enteropathy, 1; DOCK 8 deficiency, 2; HLH, 1; ICOS, 1; IFGR2, 2; IRF8, 1; PI3 kinase deficiency, 1; reticular dysgenesis, 1; RIPK1, 1; STAT3 gain of function, 1; WAS, 2
ALPS: autoimmune lymphoproliferative disease; CID: combined immunodeficiency; HLH: haemophagocytic lymphohistiocytosis; MAC: myeloablative conditioning; MFD: matched family donor; MM; mismatched; MMFD: mismatched family donor; MMUD; mismatched unrelated donor; MUD; matched unrelated donor; PID: primary immunodeficiency; RIC: reduced intensity conditioning; RTC: reduced toxicity conditioning; SCID: severe combined immunodeficiency; WAS: Wiskott-Aldrich syndrome

Table 2: Haematopoietic recovery and transplant-related complications according ex-vivo T-cell depletion methods (N=105)

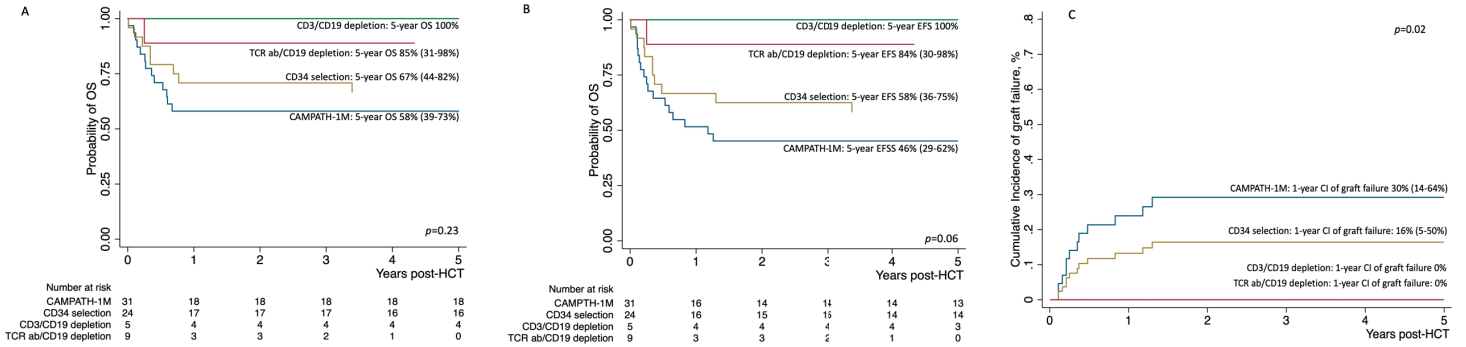
	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR αb/CD19 depletion	*p*-value
Number	34	34	7	30[1]	
Year of transplant	1987-1998	1999-2006	2007-2011	2012-2019	
Median duration of follow-up of surviving patients (range), year	21 (5.0-30.5)	16 (5.0-19.3)	7.6 (5.0-9.2)	1.3 (0.6-5.1)	
Number of observed neutrophil engraftment/conditioned patients (%)	[2]25/32 (78)	[3]29/31 (94)	[4]6/7	[5]27/30	
Median day to neutrophil recovery, (range)	27 (14-61)	19 (11-44)	14 (10-19)	15 (7-27)	<0.001
Acute GvHD, n (%)					0.15
Grade II-IV	8 (23)	2 (6)	0	3 (10)	
Grade III-IV	0	2 (6)	0	1 (3)[6]	
Chronic GvHD, n (%)	0	0	0	0	ND
VOD, n (%)	4 (12)	3 (9)	1 (14)	1 (3)	0.5
Viraemia	NA[7]	23[8]	6	30	
Any viraemia	NA[7]	6 (26)	3 (50)	14 (47)	0.27
CMV viraemia	NA[7]	2 (9)	1 (17)	6 (20)	0.52
Adenoviraemia	NA[7]	0	1 (17)	11 (37)	0.001
EBV viraemia	NA[7]	2 (9)	0	2 (7)	0.71
HHV6 viraemia	NA[7]	2 (9)	1 (17)	5 (17)	0.67

	CAMPATH-1M	CD34 selection	CD3/CD19 depletion	TCR αb/CD19 depletion	*p*-value
Second procedure, n (%)	8 (24)	8 (24)	0	0	0.008
Stem cell boost	0	7	0	0	
Second HCT	6	1	0	0	
Stem cell boost + second HCT	2	0	0	0	
Death, n (%)	14 (41)	11 (32)	2 (29)	4 (13)[9]	
GvHD	0	1	0	1	
VOD	0	2	0	0	
Infection	12	6	0	0	
Non-infectious respiratory failure	1	2	1	0	
Multi-organ failure	0	0	1	3	
Others	1	0	0	0	

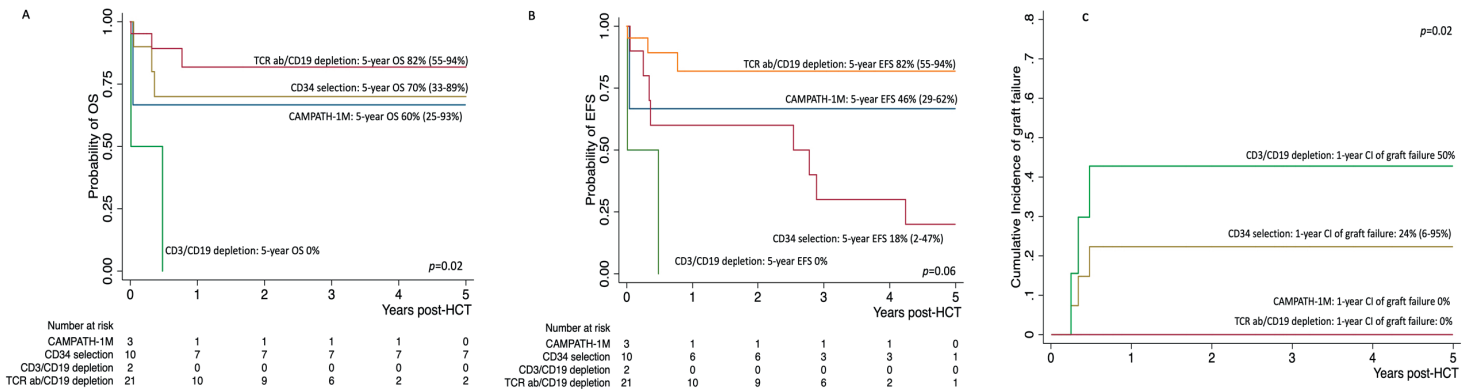
[1]13 were excluded for add-back T-cell (CGD, 2; DOCK8 deficiency, 2; hyperIgD syndrome, 1; MHC class II deficiency, 4; SCID, 3; WAS. Proportion of ex-vivo T-depleted allograft was 20% from 2012-2019
[2]3 died before Day+42; [3]2 died before Day +42; [4]1 died before Day +42; [5]1 died before Day +42
[6]The patient did not receive in-vivo serotherapy for pre-transplant CMV viraemia and persistent CMV viraemia during HCT
[7]NA: surveillance for viral infection was not done during this period
[8]Of 4 deaths, one had adenoviraemia, 1 CMV viraemia, 1 adenoviraemia and 1 HHV6 viraemia, one had no viral infection.

Table 3: Univariate analysis for OS and EFS

	OS		EFS	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**
Age at transplant	1.07 (0.97-1.18)	0.83	1.07 (0.98-1.17)	0.11
SCID vs non-SCID PID	0.90(0.41-1.95)	0.79	1.21 (0.65-2.28)	0.55
T-cell depletion methods				
Campath-1M	1		1	
CD34 selection	0.70 (0.32-1.54)	0.37	0.88 (0.47-1.68)	0.71
CD3/CD19 depletion	0.64 (0.14-2.80)	0.55	0.46 (0.11-1.99)	0.30
TCR αβ/CD19 depletion	0.33 (0.11-1.01)	0.05	0.25 (0.08-0.74)	0.01
Conditioning				
MAC	1		1	
RTC	0.87 (0.25-3.04)	0.82	0.32 (0.13-0.79)	0.01
RIC	0.41 (0.10-1.64)	0.21	0.63 (0.32-1.26)	0.20
None	0.57 (0.15-2.11)	0.40	1.09 (0.37-3.19)	0.88
Serotherapy				
None	1		1	
Alemtuzumab	1.66 (0.60-4.57)	0.30	1.67 (0.67-4.16)	0.27
ATG	1.03 (0.47-2.25)	0.08	1.19 (0.62-2.27)	0.60
Infused TNC	1.01 (0.97-1.04)	0.60	0.99 (0.96-1.03)	0.75
Infused CD34	0.94 (0.88-1.01)	0.08	0.92 (0.86-0.98)	0.01

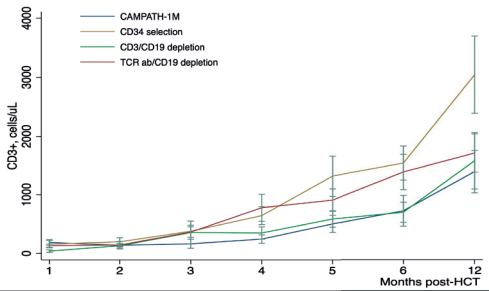


Supplemental figure 1: Overall survival (A), event-free survival (B) and cumulative incidence (C) of graft failure for SCID according to ex-vivo T-cell depletion methods.



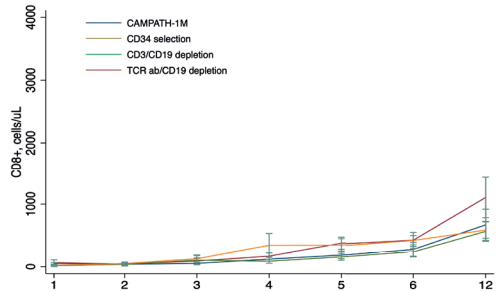
Supplemental figure 2: Overall survival (A), event-free survival (B) and cumulative incidence (C) of graft failure for non-SCID PID according to ex-vivo T-cell depletion methods.

S3A



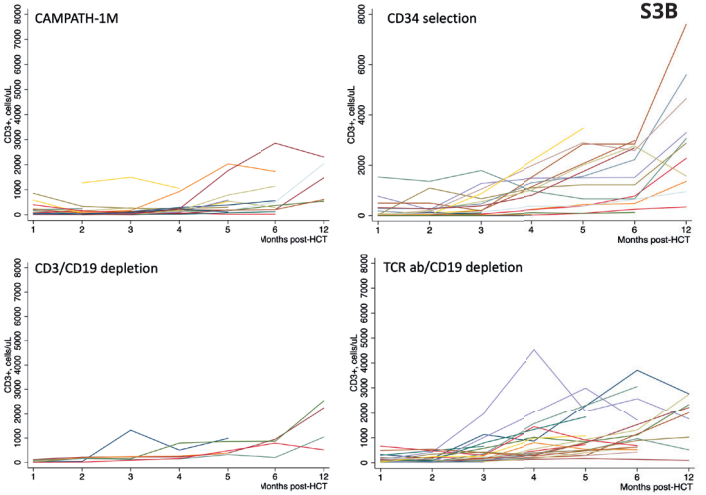
Ex-vivo T-cell depletion method		M1	M2	M3	M4	M5	M6	M12
CAMPATH-1M (n=34)	n	22	20	19	17	16	11	5
	CD3+, mean±SE	114±54	40±13	117±14	156±26	174±7	358±85	1063±489
CD34 selection (n=34)	n	27	24	20	14	12	15	11
	CD3+, mean±SE	304±185	447±180*	483±205*	802±191*	1124±337*	1407±366*	3022±864*
CD3/CD19 depletion (n=7)	n	6	6	6	5	4	4	4
	CD3+, mean±SE	68±36	183±15*	162±33	388±204	521±171	674±237	1934±454
TCR ab/CD19 depletion (n=30)	n	29	28	24	19	16	13	8
	CD3+, mean±SE	52±25	176±89	710±437*	1579±1005*	958±378	1368±406*	1392±406

S3C



Ex-vivo T-cell depletion method		M1	M2	M3	M4	M5	M6	M12
CAMPATH-1M (n=34)	n	22	20	19	17	15	11	5
	CD8+, mean±SE	35±14	19±12	55±37	131±64	217±178	487±245	708±321
CD34 selection (n=34)	n	27	24	20	14	12	15	11
	CD8+, mean±SE	9±6	107±95	141±119	234±84	312±87	391±109	1067±392
CD3/CD19 depletion (n=7)	n	6	6	6	5	4	4	4
	CD8+, mean±SE	27±27	70±32	41±15	91±41	137±48	163±41	712±114
TCR ab/CD19 depletion (n=30)	n	29	28	24	19	15	13	8
	CD8+, mean±SE	9±9	131±87	587±432	1389±1147	593±400	657±420	573±277

S3B



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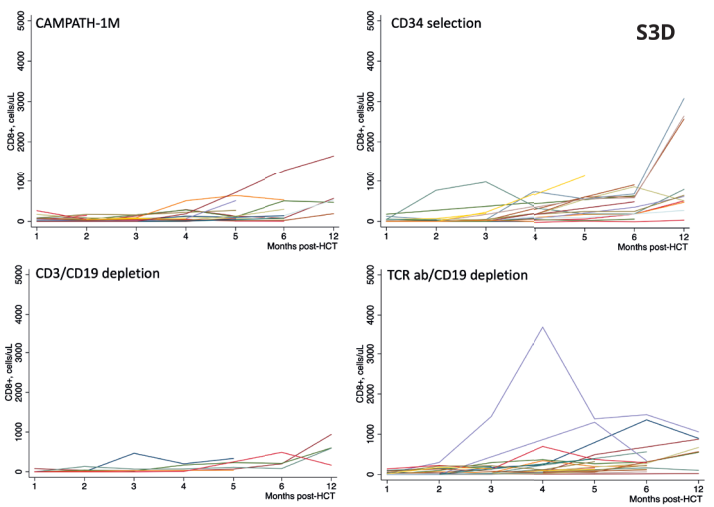

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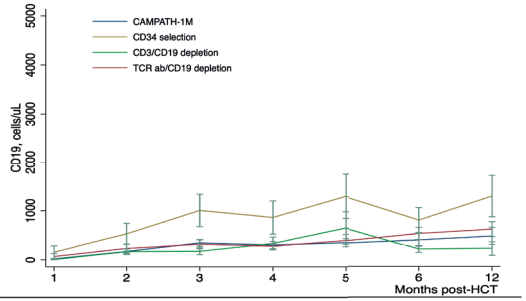
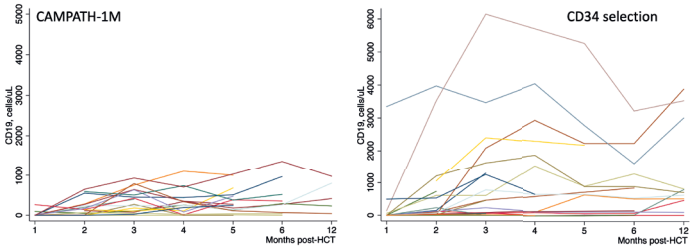
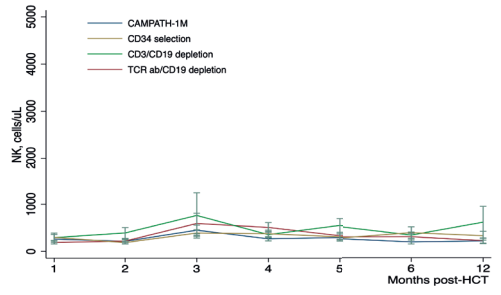


Ex-vivo T-cell depletion method		M1	M2	M3	M4	M5	M6	M12
CAMPATH-1M (n=34)	n	22	20	19	17	15	11	5
	CD19+, mean±SE	27±21	235±146	513±203	386±107	353±225	495±293	511±22
CD34 selection (n=34)	n	27	24	20	14	12	15	11
	CD19+, mean±SE	428±417	782±476	1086±434*	1381±526*	938±364	827±287	1287±481
CD3/CD19 depletion (n=7)	n	6	6	6	5	4	4	4
	CD19+, mean±SE	4±2	254±61	163±90	263±112	299±111	279±46	304±176
TCR ab/CD19 depletion (n=30)	n	29	28	24	19	15	13	8
	CD19+, mean±SE	433±428	620±544	592±456	312±173	204±43	572±320	357±52

S3F

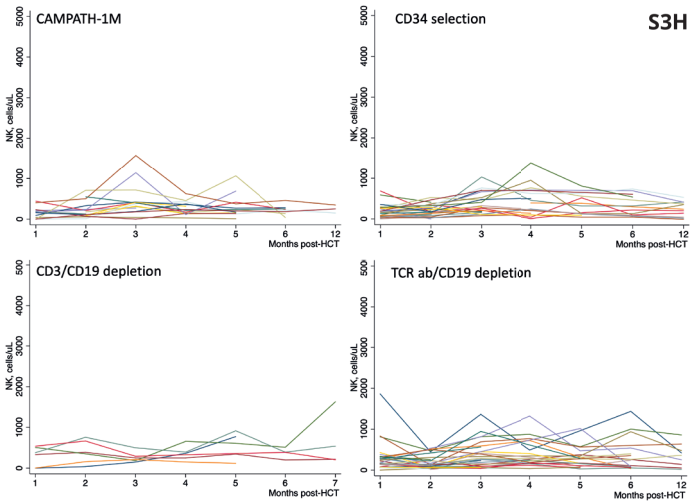


S3G



Ex-vivo T-cell depletion method		M1	M2	M3	M4	M5	M6	M12
CAMPATH-1M (n=34)	n	22	20	19	17	15	11	5
	NK cells, mean±SE	134±134	207±150	711±436	430±113	236±73	289±67	249±57
CD34 selection (n=34)	n	27	24	20	14	12	15	11
	NK cells, mean±SE	227±78	241±64	432±118	407±126	317±81	268±84	261±69
CD3/CD19 depletion (n=7)	n	6	6	6	5	4	4	4
	NK cells, mean±SE	405±52	498±130	310±96	432±119	623±166	367±91	794±427
TCR ab/CD19 depletion (n=30)	n	29	28	24	19	15	13	8
	NK cells, mean±SE	390±144	350±99	513±181	676±263	355±118	635±218	402±179

S3H



Supplemental Figure 3: Means and standard errors (SE) for CD3+ (A), CD8+ (C), CD19+ (E), and natural killer cells (NK) (G) were measured at different time points post-HCT. Individual patient immune reconstitution kinetics according ex-vivo T cell depletion methods are shown in (B) for CD3+, (D) for CD8+, (F) for CD 19+, (H) for NK cells. * indicates $p<0.05$

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