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Immunotherapy with CD25/CD71-allodepleted T cells to improve T-cell reconstitution after matched unrelated donor hematopoietic stem cell transplant: a randomized trial

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ABSTRACT

Background aims: Delayed immune reconstitution is a major challenge after matched unrelated donor (MUD) stem cell transplant (SCT). In this randomized phase 2 multi-center trial, Adoptive Immunotherapy with CD25/71 allodepleted donor T cells to improve immunity after unrelated donor stem cell transplant (NCT01827579), the authors tested whether allodepleted donor T cells (ADTs) can safely be used to improve immune reconstitution after alemtuzumab-based MUD SCT for hematological malignancies.
Methods: Patients received standard of care or up to three escalating doses of ADTs generated through CD25+/CD71+ immunomagnetic depletion. The primary endpoint of the study was circulating CD3+ T-cell count at 4 months post-SCT. Twenty-one patients were treated, 13 in the ADT arm and eight in the control arm.
Results: The authors observed a trend toward improved CD3+ T-cell count at 4 months in the ADT arm versus the control arm (230/μL versus 145/μL, $P = 0.18$), and three ADT patients achieved normal CD3+ T-cell count at 4 months (>700/μL). The rates of significant graft-versus-host disease (GVHD) were comparable in both cohorts, with grade ≥2 acute GVHD in seven of 13 and four of eight patients and chronic GVHD in three of 13 and three of eight patients in the ADT and control arms, respectively.
Conclusions: These data suggest that adoptive transfer of ADTs is safe, but that in the MUD setting the benefit in terms of T-cell reconstitution is limited. This approach may be of more use in the context of more rigorous T-cell depletion.

Introduction

Graft-versus-host disease (GVHD) after allogeneic haematopoetic stem cell transplant (HSCT or SCT) is mediated by alloreactive donor T cells infused in the graft that recognize host antigens. Alemtuzumab, a monoclonal anti-CD52 antibody, reduces the incidence of GVHD after unrelated donor SCT through *in vivo* T-cell depletion

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[1–5]. However, non-selective T-cell depletion results in profound immunosuppression, leading to high morbidity/mortality from viral infections [6–8].

To circumvent this, a number of strategies have been developed to improve immune reconstitution after T-cell-depleted transplant. A number of groups have used donor lymphocytes transduced with a safety "suicide" gene that is activated in the event of GVHD [9,10], but it would be clearly preferable to prevent rather than switch off GVHD. *Ex vivo*-generated donor/third-party virus-specific T cells with specificity for one or multiple viruses show promise, but to date their use has largely been restricted to major academic centers, and randomized studies demonstrating efficacy are lacking [11–17]. Additional approaches for accelerating immune reconstitution [18] include infusion of T cells depleted of either CD8+ [19–21] or naive [22,23] T cells or induction of T-cell anergy [24,25].

Selective *ex vivo* allodepletion represents a logical approach to improving T-cell recovery post-SCT without causing GVHD. This strategy seeks to remove only those donor T cells that are reactive against the host while preserving T-cell responses to pathogens. Donor T cells are co-cultured with host antigen-presenting cells, following which alloreactive T cells can be identified by expression of surface activation markers such as CD25, CD71 or CD69 or retention of photoactive dyes. They can then be depleted by a variety of methods, including immunotoxins, immunomagnetic separation, chemotherapy, flow cytometry sorting and photodynamic elimination [26–31]. Clinical studies of this approach have demonstrated the feasibility and safety of this strategy [26,32–35], and the authors' previous study [33] demonstrated accelerated T-cell recovery and cytomegalovirus (CMV)/Epstein–Barr virus (EBV)-specific T-cell responses when patients were infused with a higher dose (10^5/kg) of allodepleted donor T cells (ADTs). However, the majority of these studies were in the haploidentical setting. Unrelated donor bone marrow/peripheral blood SCT is much more frequently used, and the authors wished to determine whether allodepletion could be used to improve immune reconstitution in this setting.

The authors developed a methodology for selective immunomagnetic depletion of alloreactive T cells upregulating CD25 and CD71 after activation with host dendritic cells (DCs) and showed that ADTs retain anti-viral responses with minimal host alloreactivity *in vitro* [30]. The authors have now tested this approach in a randomized phase 2 multi-center clinical study, Individualized Cancer Therapy (NCT01827579), to determine whether CD25/CD71 ADTs can be safely used to improve immune reconstitution after alemtuzumab-conditioned matched unrelated donor (MUD) SCT for hematological malignancies.

Methods

Study population

The study was open to adult patients undergoing alemtuzumab-conditioned 9/10 or 10/10 HLA MUD peripheral blood SCT for hematological malignancies at three participating sites in the UK.

Study design

Eligible patients were randomized 2:1 to an intervention arm (ADT immunotherapy) or standard-of-care MUD peripheral blood SCT. Patients in the ADT cohort received up to three escalating doses of allodepleted T cells post-transplant (0.1×10^6/kg at day 30, 0.3×10^6/kg at day 60 and 1×10^6/kg at day 90) provided they had engrafted and there were no severe intercurrent infections, severe hepatopathy (bilirubin >50 μM or alanine transaminase >200 U/L), oxygen requirement or grade >1 acute GVHD and circulating CD3+ T cells were less than 700/μL (lower limit of normal for adults).

The study was approved by the UK South Central Oxford A Research Ethics Committee and the Medicines and Healthcare Products Regulatory Agency as well as by the relevant unrelated donor registries (Anthony Nolan in the UK, German Bone Marrow Donor Center in Germany and National Marrow Donor Program in the US). The study was managed by Cancer Research UK and the University College London Cancer Trials Center. Informed consent was obtained from all patients as well as donors.

Study objectives

The primary endpoint of the study was circulating CD3+ T-cell count at 4 months post-hematopoietic SCT (HSCT). Secondary endpoints included incidence of grade 2–4 acute or chronic GVHD; time to recovery of normal T cells and CD4+ cells (>700/μL and >300/μL, respectively) and normal T cell receptor (TCR) diversity, as assessed by Vβ spectratyping; *in vitro* anti-viral responses of circulating peripheral blood mononuclear cells (PBMCs), as assessed by interferon gamma (IFN-γ) ELISpot; and treatment-related mortality (TRM) and disease-free survival (DFS) at 1 year post-HSCT.

Transplantation

All patients received alemtuzumab as part of their conditioning. Alemtuzumab dosing and conditioning regimens were not predefined by the trial protocol, but rather were in accordance with institutional protocols. Details of the different conditioning regimens are specified in supplementary Table 1.

Peripheral blood was the stem cell source for all patients, and the graft was not further manipulated beyond *ex vivo* T-cell depletion with alemtuzumab, as outlined earlier, for patients receiving myeloablative conditioning. GVHD prophylaxis was with cyclosporine alone, which was withdrawn over 4 weeks from day 50, where possible, in both arms. Acute and chronic GVHD was graded according to Seattle and National Institutes of Health criteria, respectively.

Generation of ADTs

Generation of ADTs was performed under current Good Manufacturing Practice conditions at Great Ormond Street Hospital for Children, London, UK. Patient-derived mature DCs were generated from CD14-selected PBMCs from autologous apheresis performed 2 weeks prior to transplant as previously described [27,30]. Donor PBMCs were obtained a week prior to transplant via unstimulated leukapheresis or 500-mL blood draw. Host DCs were irradiated at 30 Gy and co-cultured 1:10 with donor PBMCs in serum-free medium for 4 days. Activated allogeneic donor T cells were then incubated with an anti-CD71-biotin antibody followed by CliniMACS anti-biotin and anti-CD25 beads (Miltenyi Biotec Ltd, Woking, UK). CD25/CD71 cells were then removed through immunomagnetic depletion on the CliniMACS Plus instrument (Miltenyi Biotec Ltd). The resulting ADTs were tested for sterility and residual alloreactivity and cryopreserved. Release criteria for the final product included <0.2% CD3+CD25+ and <0.2% CD3+CD71+, as assessed by flow cytometry, and <10% residual proliferation against host cells, as seen in secondary mixed leukocyte reaction.

Monitoring immune reconstitution

Immune reconstitution was studied monthly for the first 6 months and then every 2 months until 1 year post-SCT by analyzing PBMC flow cytometrically for lymphocyte subpopulations [33]. At 4 months, 6 months and 12 months post-SCT, T-cell function was analyzed via measurement of proliferative responses to mitogenic stimulation with phytohemagglutinin (PHA) using ^{3}H-thymidine uptake. Immunoglobulin levels and T-cell Vβ repertoire were assayed by

polymerase chain reaction spectratyping. A normal spectratype consisted of five to eight bands per family with a Gaussian size distribution. Anti-viral responses to CMV and EBV peptides were evaluated in batched cryopreserved PBMCs using IFN-γ ELISpot assays. Anti-CMV response was further analyzed with pentamer analysis in patients who experienced CMV reactivation.

Data analysis

The study was designed with 80% power and one-sided test of statistical significance of 15%, requiring 16 patients in the experimental arm and eight in the control arm to detect a standardized mean difference $\geq$0.85 in circulating CD3 count at 4 months post-SCT. A one-sided significance level of 5–20% is common in early phase 2 trials [36]. CD3 count at 4 months was considered to be a meaningful time point with regard to immune reconstitution. A large effect size (0.85) was used because CD3 count is a surrogate endpoint, and it has generally been observed in cancer trials that large effects on a surrogate are associated with smaller/more moderate effects on clinical endpoints such as relapse and survival.

Statistical analysis in this study was predominantly descriptive. Wilcoxon rank-sum test was used to compare median values for lymphocyte recovery at 4 months, as data were not normally distributed. Similar analysis was done to evaluate differences in median CD3, CD4 and CD8 counts between the control and ADT groups at different time points (4 months, 6 months and 12 months post-SCT). The area under the curve for CD3 counts from 0 months to 4 months was calculated using the trapezoid rule. GVHD and toxicity were reported in terms of frequencies and percentages by treatment group based on the worst grade reported during the study. Mortality and DFS were analyzed using Kaplan–Meier curves and Cox regression.

Results

Recruitment and patient characteristics

Between August 2014 and February 2019, a total of 37 patients were recruited and randomized (Figure 1). A total of 16 patients were taken off the trial because of donor refusal (seven), acute GVHD grade >1 (three), death (one), withdrawal of consent (one), leukapheresis labeling error (one), oxygen requirement (one), cytopenia (one) and intercurrent illness preventing continuation in the study (one). Although the study was designed for a total of 24 evaluable patients (16 in the ADT arm and eight in the control arm), the sample size achieved (21 evaluable patients) was slightly less as a result of these issues. Of the 21 evaluable patients, 13 were treated in the ADT arm and eight were treated in the control arm. All 21 patients were evaluable for the primary endpoint (T-cell recovery at 4 months post-transplant), and 16 patients reached the 12-month follow-up. Patients who did not reach this endpoint included three patients who died prior to 12 months post-SCT (two because of disease progression in the treatment arm and one because of TRM in the control arm) and two patients in the ADT arm who withdrew from the trial.

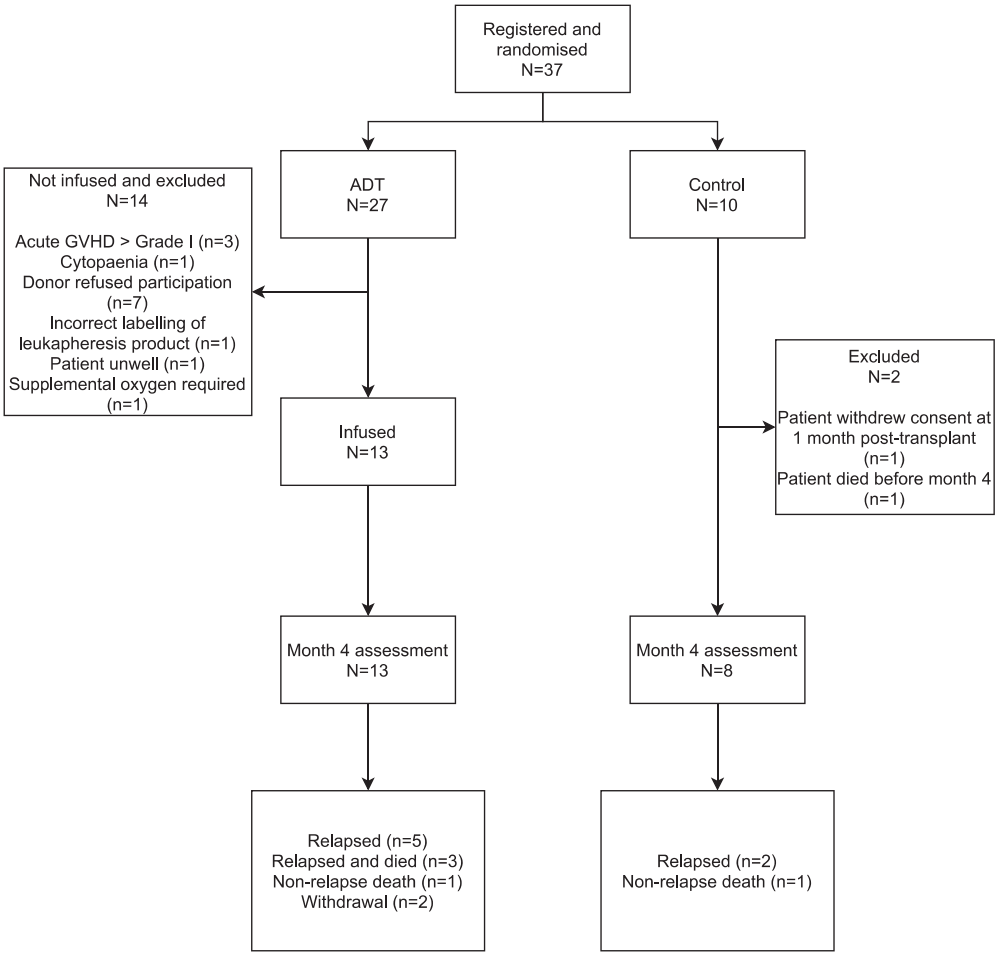

Fig. 1. CONSORT diagram of ICAT recruitment and outcome summary. CONSORT, Consolidated Standards of Reporting Trials; ICAT, Individualized Cancer Therapy.

Table 1
Baseline patient characteristics.

		ADT (n = 13)		Control (n = 8)	
		n		n	
Age in years	median (range)	13	54 (36–68)	8	48 (34–62)
Sex					
	Female	3	23%	4	50%
	Male	10	77%	4	50%
Diagnosis					
	AML	9	69%	2	25%
	DLBCL	2	16%	1	13%
	FL	0	0%	1	13%
	HL	0	0%	1	13%
	MCL	0	0%	1	13%
	CLL	1	8%	0	0%
	ALL	0	0%	0	0%
	CML	0	0%	2	25%
	Myelodysplasia	1	8%	0	0%
Disease status					
	Morphological CR	8	62%	3	38%
	Cytogenetic CR	1	8%	0	0%
	Partial metabolic response	0	0%	1	13%
	Partial response	1	8%	1	13%
	Stable disease	1	8%	1	13%
	Chronic phase	0	0%	1	13%
	Progressive disease	1	8%	1	13%
	Missing	1	8%	0	0%
Conditioning					
	Myeloablative	2	15%	2	25%
	Non-myeloablative	11	85%	6	75%

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma.

Baseline patient characteristics are shown in Table 1. Given the size of the study, baseline characteristics were not expected to be equally distributed between the two cohorts. More patients in the ADT group had acute myeloid leukemia (nine of 13 [70%] versus two of eight [25%] in the control group) and were in complete remission (nine of 13 [70%] versus three of eight [38%] in the control group). Only one patient in each cohort had progressive disease prior to transplant. Seventeen patients received reduced-intensity conditioning (11 of 13 in the ADT arm and six of eight in the control arm) and two patients in each group underwent myeloablative conditioning, the details of which are provided in supplementary Table 1. All but one patient received conditioning regimens comprising 50–100 mg of intravenous alemtuzumab in fractions; the remaining patient had the graft treated with 20 mg of alemtuzumab in the bag.

ADT and treatment compliance

Characteristics of the ADT products released for the 13 infused patients are summarized in supplementary Table 2. Of the 13 patients treated in the ADT arm, five received one dose, four received two doses and four received three doses. With regard to the nine of 13 patients who did not receive the three planned escalating doses, the reasons were as follows: three already had circulating T cells >700/μL, five had acute GVHD grade >1 at the infusion time point and the dose was not available for one. Table 2 shows a summary of treatment compliance, complications and outcomes for each patient.

Immune reconstitution

The primary endpoint of the study was T-cell reconstitution at month 4 post-transplant (Figure 2A). Data were not normally distributed (Figure 2B), and non-parametric tests did not show a significant difference in median T-cell count between both cohorts, with 230/μL (range, 10–4080) in the treatment cohort and 145/μL (range, 10–500) in the control cohort (one-sided P = 0.18) (Figure 2C). Using log-transformed data, the standardized mean difference was 0.43 (one-sided P = 0.17). Median area under the curve for CD3 for months 0–4 was also not significantly different between the two groups (590 μL/4-month ADT group versus 367.5 μL/4-month control group, two-sided P = 0.38). Three patients (all in the ADT arm) had a normal CD3+ T-cell count of more than 700/μL at 4 months post-SCT, skewing the mean for that population. These patients were C (4080/μL), D (1470/μL) and G (1720/μL) (Table 2). In patients C and G, the increase in CD3+ count was predominantly seen in the CD8+ compartment, whereas in patient D, it was observed in CD4+ T cells. All three of these patients experienced GVHD, but none required treatment with systemic steroids. Patients C and G had early low-level CMV reactivation (Table 2).

As shown in Figure 2D, time to normal CD3+ T-cell count was somewhat more rapid in the ADT cohort, though this was not statistically significant (P = 0.35). At 4 months post-SCT, three of 13 (23%) ADT patients had a normal T-cell count versus zero of eight (0%) patients in the control arm. At 6 months, four of 11 (36%) evaluable patients in the ADT cohort had a normal T-cell count versus one of eight (13%) patients in the control cohort. Median CD4 and CD8 counts were comparable between the control and ADT groups at all time points (see supplementary Figure 1).

To assess T-cell functionality, the authors determined the ability of PBMCs derived from patients post-SCT to proliferate in response to the mitogen PHA. Although a minority of patients in the ADT arm appeared to show improved responses, the median PHA stimulation index was comparable between the two cohorts at 4 months, at 3.3 (range, 0.67–73.1) for the ADT group and 2.7 (range, 1.1–8.2) for the control group (Figure 3A). Figure 3B illustrates the diversity of the TCR repertoire as assessed by Vβ spectratyping at 4 months and 6 months post-SCT. Neither the median number of peaks nor the number of Gaussian families for CD4 and CD8 subsets was significantly different between patients in the ADT and control cohorts. In the ADT

Table 2
Summary of treatment compliance, complications and outcomes.

Patient	Cohort	ADT doses	Reason for stopping ADT treatment	AUC for CD3 from 0 months to 4 months, count/μL	Acute GVHD $\geq$2	Chronic GVHD	Systemic steroids before month 4	CMV serostatus, Donor/ Recipient	EBV serostatus, Donor/ Recipient	Viral reactivation within first 6 months (maximum copies/mL - month post-SCT)	Other significant complications	Relapse/ progressive disease	Status at 12 months post-SCT	Months to death post-transplant (cause)
A	ADT	3	Not applicable	890	Yes, grade 3	No	No	+/+	+/−	CMV (18 600 -M6) EBV (1835 - M6) ADV (37 939 - M5)	None	No	CR	
B	ADT	2	Unable to	manufacture	15	Yes, grade 2	No	Yes	−/−	−/+	No	None	No	CR
C	ADT	1	T cells >700 prior to day 60	5420	No	No	No	+/+	+/+	CMV (320 -M1)	None	Yes, at 10.2 months post-SCT	PD	12.1 (relapse/ PD)
D	ADT	1	GVHD grade >1 prior to day 60	1725	Yes, grade 3	No	No	−/−	+/+	EBV (106 772 - M5)	Device-related infection	Yes, at 6.5 months post-SCT	PD	
E	ADT	2	GVHD grade >1 prior to day 90	590	No	Yes,		moderate	No	+/+	−/+	CMV (2244 - M2)	None	Yes, at 11 months post-SCT
PD														
F	ADT	3	NA	495	No	No	No	−/−	+/−	EBV (1522 -M6)	None	Yes, at 20.2 months post-SCT	CR	20.9 (relapse/ PD)
G	ADT	2	T cells >700 prior to day 90	2080	No	No	No	+/NA	+/NA	CMV (948 - M1)	Parainfluenza, device-related infection	No	CR	
H	ADT	2	GVHD grade >1 prior to day 90	365	Yes, grade 2	No	No	−/−	+/+	EBV (22 052 -M6)	Death due to pneumonia 14.5 months post-SCT	No	CR	14.5 (NRM)
I	ADT	3	Not applicable	90	No	No	No	−/−	+/−	No	None	No	Not assessed, no clinical evidence of disease	
J	ADT	1	GVHD grade >1 prior to day 60	615	Yes, grade 2	No	No	−/−	+/+	No	None	NA	NA, last follow- before 12 months	
K	ADT	1	T cells >700 prior to day 60, unwell, with oxygen requirement and respiratory infection	1450	Yes, grade 2	Yes, mild	Yes	−/−	+/+	No	Intracranial hemorrhage, chest infection	Yes, at 3.4 months post-SCT	NA, dead before 12 months	5.6 (relapse/PD)

(*continued on next page*)

Table 2 (*Continued*)

Patient	Cohort	ADT doses	Reason for stopping ADT treatment	AUC for CD3 from 0 months to 4 months, count/μL	Acute GVHD ≥2	Chronic GVHD	Systemic steroids before month 4	CMV serostatus, Donor/ Recipient	EBV serostatus, Donor/ Recipient	Viral reactivation within first 6 months (maximum copies/mL - month post-SCT)	Other significant complications	Relapse/ progressive disease	Status at 12 months post-SCT	Months to death post-transplant (cause)
L	ADT	1	GVHD grade >1 prior to day 60	305	Yes, grade 3	No	Yes	−/−	−/−	No	None	No	Not assessed, no clinical evidence of disease	
M	ADT	3	Not applicable	255	No	Yes, severe	Yes	−/−	−/+	No	None	No	CR	
N	Control	–	–	920	No	Yes, mild	No	−/−	+/+	EBV (2680 -M2)	None	Yes, at 6.3 months post-SCT	PD	
O	Control	–	–	90	Yes, grade 3	No	Yes	−/−	+/−	No	Heart failure (resolved)	No	Not assessed, no clinical evidence of disease	
P	Control	–	–	390	No	Yes, mild	No	−/−	+/+	No	None	No	Not assessed, no clinical evidence of disease	
Q	Control	–	–	345	No	No	No	−/−	+/−	No	None	Yes, at 7.1 months post-SCT	PD	
R	Control	–	–	630	No	No	No	−/−	+/+	No	Hypertension	No	Stable disease	
S	Control	–	–	105	Yes, grade 3	No	Yes	−/−	+/−	No	None	No	CR	
T	Control	–	–	30	Yes, grade 2	Yes, mild	Yes	NA/−	NA/+	No	NRM, death due to viral infection at 8 months	No	NA, dead before 12 months	8.2 (NRM)
U	Control	–	–	1030	Yes, grade 2	No	Yes	+/+	+/+	CMV (2800-M5)	None	No	CR	

ADT: allodepleted T-cell cohort; ADV: adenovirus; AUC, area under the curve; CMV: citomegalovirus; CR, complete remission; EBV: Ebstein-Barr virus; GVHD: graft vs host disease; NA, not applicable/not available; NRM, non-relapse mortality; PD, progressive disease; SCT: stem-cell transplant

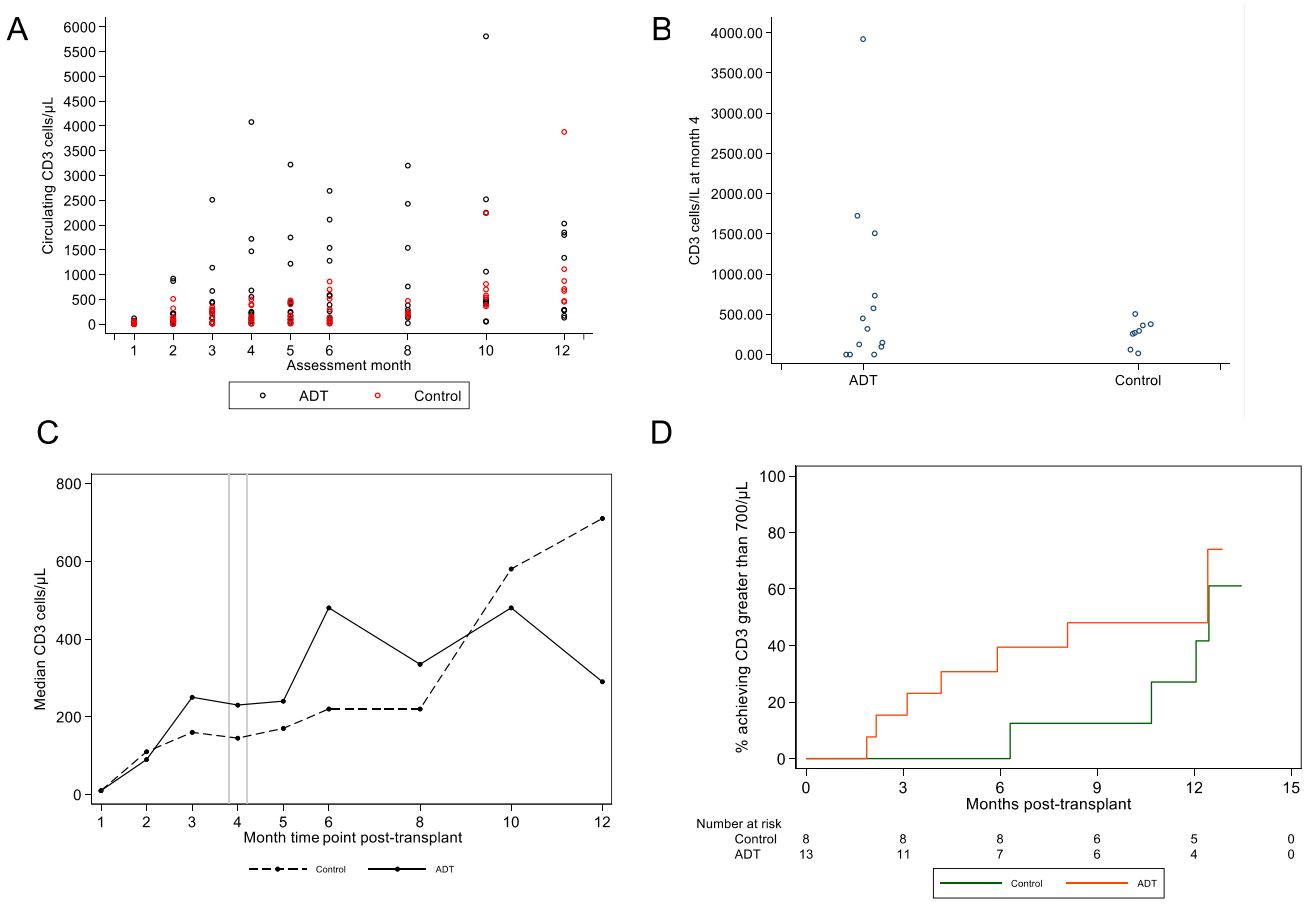

Fig. 2. Median CD3 T-cell recovery between ADT and control cohorts at 4 months. (A) Circulating CD3/μL for all 21 patients post-HSCT. (B) Circulating CD3/μL at 4 months post-SCT. (C) Median circulating CD3 for control and ADT groups at different time points after HSCT. Wilcoxon two-sided $P > 0.05$ at all time points. (D) Time to normal CD3/μL count after HSCT in both cohorts.

and control groups, respectively, the median number of peaks for CD4 at 4 months was six (range, 4–7) and five (range, 4–7) and the median number of peaks for CD8 at 6 months was four (range, 2.5–6) and four (range, 1.5–7). With regard to Gaussian families, in the ADT and control arms, respectively, the median number for CD4 at 4 months was 2.5 (range, 0–10) and three (range, 0–13) and the median number for CD8 at 6 months was zero (range, 0–2) and zero (range, 0–4). However, 10 of 11 (91%) evaluable patients in the ADT group achieved near-normal TCR diversity by month 4, with a median number of peaks between six and seven, compared with two of six (33%) evaluable patients in the control group (Fisher exact test P=0.028). As expected, both B-cell and natural killer cell recovery and IgG and IgM immunoglobulin levels were similar between the two cohorts at 4 months, 6 months and 12 months post-transplant (see supplementary Figure 2).

Virus-specific immunity

To assess virus-specific immune reconstitution, *in vitro* T-cell responses to EBV and CMV peptides were assayed by IFN-γ ELISpot. Four of 12 evaluable patients in the ADT arm and one of eight evaluable patients in the control arm were at risk of CMV reactivation by virtue of patient/donor seropositivity (Table 2), and they all experienced CMV reactivation. As shown in Figure 4A, four of 12 patients in the ADT arm had a significant response (>20 spot-forming units/10^5 cells) to CMV compared with one of eight control patients. In each case, this was associated with CMV reactivation in patients with CMV-seropositive donors. Five patients experienced CMV reactivation, and the relationship between reactivation and CMV-specific IFN-γ response is illustrated in supplementary Figure 3A. In the four patients treated with ADTs, IFN-γ responses were detectable following ADT infusion (at 2–3 months post-SCT) but not before, suggesting that ADT infusion enhanced the T-cell response to virus reactivation.

Of the five patients who experienced CMV reactivation, four were evaluable by CMV-specific pentamer analysis (three patients in the ADT group and one patient in the control group). CMV-specific CD8 responses were detectable in two of these patients by 4 months post-SCT (see supplementary Figure 3), in each case correlating with prior CMV viremia. The remaining eight of 12 ADT patients and seven of eight control patients did not experience CMV reactivation and did not show significant IFN-γ responses to CMV.

All 21 patients were at risk of EBV reactivation through either patient or donor seropositivity (Table 2). Four patients in the ADT arm and one patient in the control arm experienced EBV reactivation. Seven of 12 evaluable patients in the ADT group had significant (>20 spot-forming units/10^5 cells) IFN-γ responses to EBV peptides compared with four of eight controls. Once again, these EBV- specific responses were seen mostly in patients with EBV reactivation and tended to occur somewhat later than recovery of CMV-specific responses (Figure 4B).

GVHD and toxicity

The incidence of acute and chronic GVHD was comparable in the ADT and control cohorts (Table 3). Overall, acute GVHD 1–4 occurred in 17 of 21 patients. Acute GVHD grade ≥2 occurred in seven of 13 (54%) patients in the ADT group versus four of eight (50%) patients in

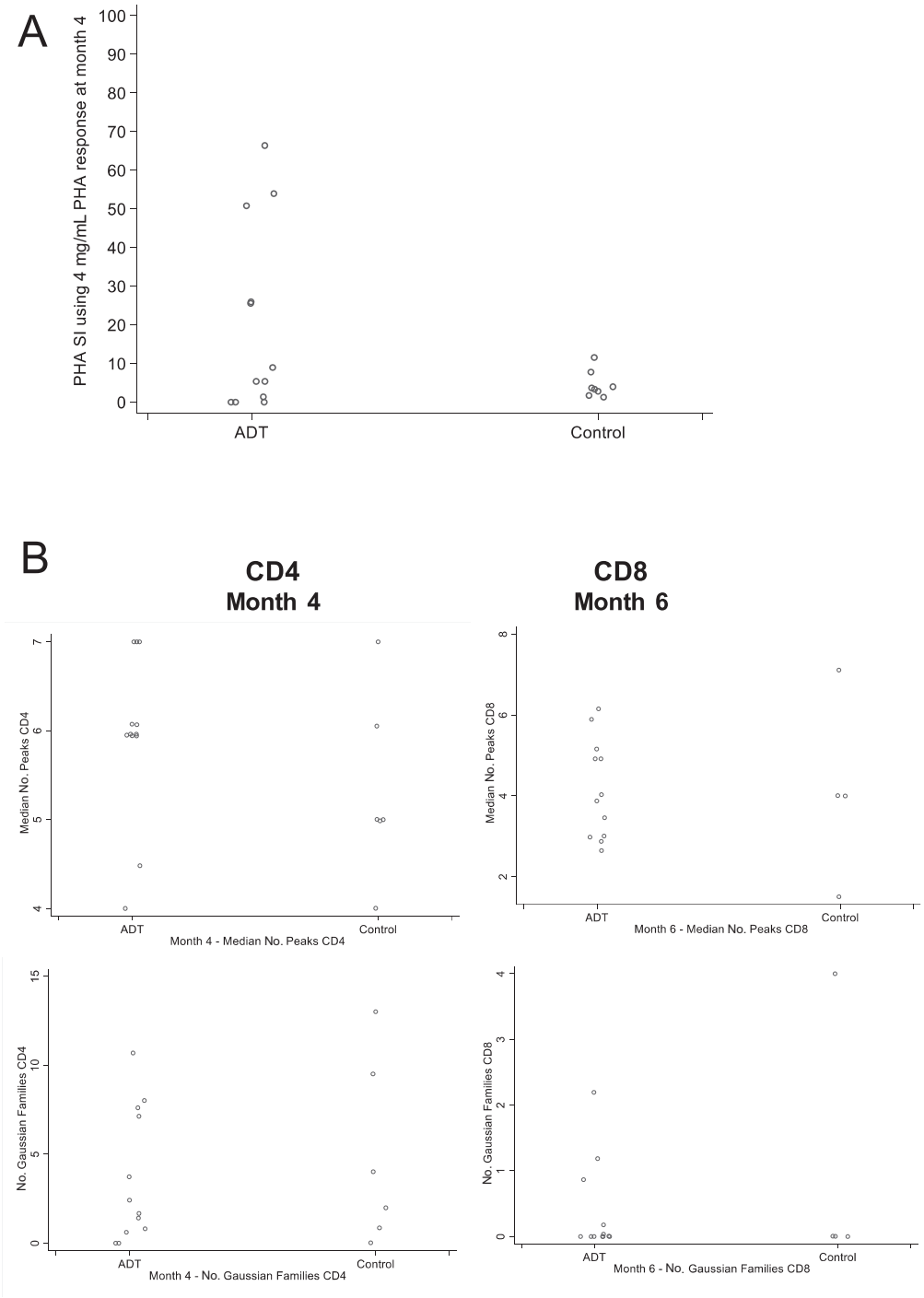

Fig. 3. Functional immune reconstitution tests were not different between ADT and control cohorts. (A) PHA SI at month 4 and month 12 for patients in both cohorts. (B) Vβ repertoire at 4 months and 6 months post-SCT represented by median number of peaks (upper panels) and median number of Gaussian families (lower panels) in CD4 and CD8 subsets. SI, stimulation index.

the control group ($P > 0.99$). Severe acute GVHD (grade 3) occurred in three of 13 (23%) ADT patients and two of eight (25%) control patients ($P > 0.99$). No cases of grade 4 acute GVHD were observed. Chronic GVHD affected three patients in each cohort (one mild, one moderate and one severe in the ADT group and three mild in the control group).

Overall, eight of 21 patients received systemic steroid treatment for acute or chronic GVHD within the first 4 months post- SCT: four of 13 in the ADT arm and four of eight in the control arm. Overall, toxicity was mostly transplant-related; grade 3–4 toxicities are summarized in supplementary Table 3. A total of 11 of 13 (85%) patients in the ADT group experienced at least one grade 3–4 toxicity versus four of eight (50%) patients in the control arm. Cytopenias accounted for most of these events in both arms.

Chimerism

Four patients had mixed chimerism at month 1 post-SCT, of whom two were in the ADT group and two were in the control group. Two of these patients converted to full chimerism by month 12. Three

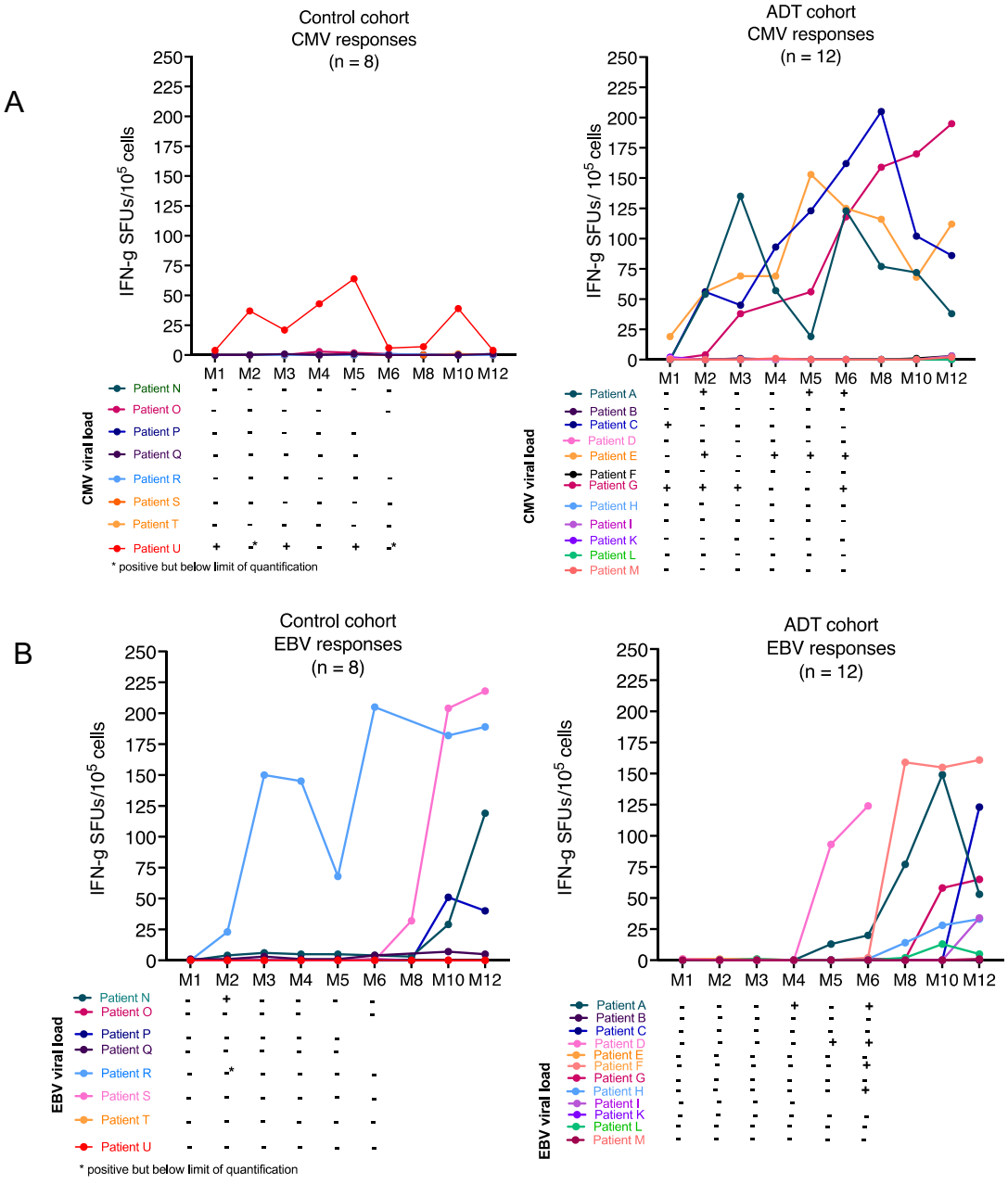

Fig. 4. (A) CMV and (B) EBV responses of ADT and control cohort patients post-SCT. At each time point post-SCT, T-cell responses to EBV and CMV peptides were assayed by IFN-γ ELISpot and viral load was measured by peripheral blood PCR. PCR, polymerase chain reaction; SFUs, spot-forming units.

Table 3
Incidence of acute and chronic GVHD.

Type of GVHD	Reported grade	ADT arm, n (%) (N = 13)	Control arm, n (%) (N = 8)
Acute GVHD	1	4 (31)	2 (25)
	2	4 (31)	2 (25)
	3–4[a]	3 (23)	2 (25)
Chronic GVHD	Mild	1 (8)	3 (38)
	Moderate	1 (8)	0 (0)
	Severe	1 (8)	0 (0)

[a]No cases of grade 4 acute GVHD occurred.

additional patients in the ADT arm went on to develop mixed chimerism between 2 months and 3 months post-transplant; all had converted to 100% chimerism by month 12.

Patient outcomes

The median follow-up time was 14 months. Seven patients relapsed or progressed: five of 13 in the study arm and two of eight in the control arm. The 1-year overall survival rate was 92% for patients treated with ADTs compared with 88% in the controls, and 1-year DFS rate was 68% in the ADT group versus 63% in the control group. Three patients died during the 12-month follow-up: two of 13 (C and K) in the ADT group as a result of disease progression/relapse and one of eight (T) in the control group as a result of TRM (viral

infection). Two further patients, both in the ADT arm, died after 12-month follow-up: patient F as a result of relapse at 20.9 months post-transplant and patient H as a result of pneumonia at 14.5 months post-transplant.

Discussion

The authors report the outcome of the first prospective randomized clinical trial assessing the safety and biological efficacy of ADTs in patients undergoing alemtuzumab-conditioned MUD SCT. This approach proved to be feasible and safe, with comparable 1-year overall survival and DFS for the treatment and control groups.

Importantly, the authors noted no significant increase in GVHD associated with ADT infusion in the study. The overall incidence of significant (grade 2–4) acute GVHD (seven of 13 patients in the ADT group versus four of eight patients in the control group) and chronic GVHD (three of 13 patients in the ADT arm versus three of eight patients in the control arm) did not differ significantly between the two cohorts. It should be noted, however, that the incidence of significant acute GVHD (11 of 21 [52%] patients) in the authors' cohort is higher than that reported in other studies on unrelated donor HSCT using alemtuzumab [2,4,37–42]. This may reflect the use of peripheral blood stem cells as the stem cell source together with single-agent cyclosporine as GVHD prophylaxis and early withdrawal of immunosuppression. André-Schmutz *et al.* [26] found that GVHD was more common if residual alloreactivity exceeded 1%. In the authors' study, residual alloreactivy measured as the median residual T cell proliferation in an ADT/host cell co-culture in patients receiving ADTs was 0% in both patients who developed significant acute GVHD (n = 7) and those who did not (n = 6). Relapse rates were comparable between the ADT arm and control arm. It should be noted that ADTs are depleted of alloreactive T cells that mediate graft-versus-leukemia responses and would therefore not be predicted to prevent relapse or shift chimerism.

The primary endpoint of the study was T-cell immune reconstitution at 4 months post-transplant, as this is the period at which patients are most likely to succumb to infection due to insufficient T-cell recovery, particularly in the setting of alemtuzumab, where T-cell reconstitution is typically not seen until 6–8 months post-transplant [1,5,43]. In this study, patients treated with ADTs did not experience significant improvement in T-cell reconstitution; however, there were improved T-cell counts and function in a minority of patients in the ADT arm, although this was associated with CMV reactivation in patients C and G and acute GVHD in patient D. The authors and others have previously reported virus reactivation and GVHD as drivers of accelerated T-cell reconstitution after infusion of ADTs [26,33]. Moreover, although the T-cell repertoire was not significantly more diverse in the ADT group at 4 months post-SCT, almost all patients in this group achieved near-normal TCR diversity compared with only a minority in the control group. These data suggest that ADTs may have an effect on T-cell recovery, at least in some patients. The overall small study size and the fact that slightly fewer patients than planned were recruited on the treatment arm (13 versus 16)—with donor refusal to participate in the study being the main limiting factor—may have contributed to this failure to reach statistical significance for the primary endpoint. The observed effect (standardized mean difference, 0.43) was smaller than the (large) target of 0.85, so the authors' study may have missed a modest treatment effect.

Viral infections/reactivations are a leading cause of morbidity and non-relapse mortality after unrelated donor SCT [44–46]. Although the authors did not demonstrate significant differences in virus-specific T-cell responses in this study, it is noteworthy that four patients in the ADT arm displayed early CMV-specific responses, suggesting that patients treated with ADTs can respond to CMV at early time points in the context of reactivation and when the graft received is from a CMV-positive donor. The low frequency of CMV reactivation in the control group precluded assessment of whether they could mount CMV-specific T-cell responses at this stage, but previous data suggest that this is unlikely [47]. This suggests that virus reactivation post-HSCT may trigger an expansion of ADTs.

There are a number of potential allodepletion methodologies using different host antigen-presenting cells and depletion strategies [31]. At present, it is not possible to define which methodology is superior both because of a lack of standardized assays of residual alloreactivity/anti-viral responses and because clinical studies are not easily comparable. The authors utilized stimulation of donor T cells with host DCs followed by immunomagnetic CD25/CD71 depletion. Although the authors' production methodology is relatively straightforward, it proved logistically challenging to obtain unstimulated donor leukapheresis prior to stem cell harvest. This is a hurdle that is more easily overcome in the haploidentical SCT setting.

Roy *et al.* [34,35] recently reported promising results in CD34-selected haploidentical SCT patients receiving ATIR101, an ADT product obtained through photodepletion. Photodepletion seems to spare some regulatory T cells [29] that would be depleted with an anti-CD25 approach. In phase 1 and phase 2 clinical trials [34,35], ATIR101 proved to be safe with a low rate of significant acute GVHD (four of 23 [17%] patients), no severe acute GVHD and reduced non-relapse mortality compared with T-cell-depleted haploidentical transplant, resulting in rates of GVHD- and relapse-free survival similar to those of MUD SCT. Unfortunately, however, a phase 3 registration study of this product is no longer being taken forward.

Conclusions

To date, adoptive transfer of ADTs has shown great promise in improving immune reconstitution after *ex vivo* T-cell-depleted haploidentical SCT [26,33,35]. By contrast, in the present randomized study in the unrelated donor setting, the authors were not able to demonstrate accelerated T-cell recovery with ADTs. A number of potential factors may contribute to this difference. In haploidentical SCT, anti-thymocyte globulin is given as serotherapy, whereas in the authors' unrelated donor cohort, alemtuzumab was used. Alemtuzumab is known to have a longer half-life *in vivo* than anti-thymocyte globulin and—although pharmacokinetics are highly variable between patients—can remain in the circulation at potentially lympholytic levels at day 30 and up to 2 months post *in vivo* administration at doses similar to those utilized in the authors' study [48,49]. Unfortunately, the authors did not measure circulating alemtuzumab levels in the present study, and it is possible that this may have abrogated the beneficial effect of ADTs. It should be noted, however, that the level of alemtuzumab that is truly lytic to T cells *in vivo* is not clear, and the authors have previously demonstrated expansion of CMV-specific cytotoxic T lymphocytes adoptively transferred as early as day 32 after alemtuzumab-based reduced-intensity conditioning [48]. Further *ex vivo* T-cell depletion in the haploidentical setting results in more profound T-cell depletion than that achieved through alemtuzumab [49]. T-cell-depleted haploidentical SCT patients therefore have *a priori* a lower risk of GVHD. However, in MUD transplants, where the graft is less rigorously T-cell-depleted, ADTs are suppressed by cyclosporine and GVHD is more frequent, resulting in the use of steroids, which further suppress ADT expansion. In this study, 11 of 13 patients in the ADT arm were on cyclosporine at the time of their last ADT dose. Moreover, eight of 21 patients (including four of 13 patients in the ADT arm) received early systemic steroids post-transplant compared with two of 16 patients in the authors' previous study conducted in the haploidentical setting [33]. Thus, the authors' data indicate that immunotherapy with ADTs may be best applied in transplant platforms where the graft is rigorously T-cell-depleted (e.

g., with CD34 selection). Whether this approach can be effective in improving immune reconstitution after haploidentical HSCT following TCR-$\alpha\beta$/CD19 depletion or post-transplant cyclophosphamide will require further studies.

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Author Contributions

Conception and design of the study: KSP, SJA, PJA, BP, FC, KC, OS, LC, AL, TT, TP, MF, SM and ET. Acquisition of data: KSP, SJA, CI, RR, JC, RW, AG, SG, AC, MS, BF, TT, TP, MF, SM and ET. Analysis and interpretation of data: KSP, PJA, MO, CI, RR, JC, RW, AG, SG, AC, MS, BF, AL, SA and KCG. Drafting or revising the manuscript: MO, PJA, KSP, SJA, BP, FC, KC, OS and LC. All authors have approved the final article.

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2022.08.010.

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