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Full Length Article
Cord Blood

Immune Reconstitution Profiling Suggests Antiviral Protection after Transplantation with Omidubicel: A Phase 3 Substudy

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A B S T R A C T

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for hematologic malignancies and nonmalignant disorders. Rapid immune reconstitution (IR) following allogeneic HCT has been shown to be associated with improved clinical outcomes and lower infection rates. A global phase 3 trial (ClinicalTrials.gov NCT02730299) of omidubicel, an advanced cell therapy manufactured from an appropriately HLA-matched single umbilical cord blood (UCB) unit, showed faster hematopoietic recovery, reduced rates of infection, and shorter hospitalizations in patients randomized to omidubicel compared with those randomized to standard UCB. This optional, prospective substudy of the global phase 3 trial characterized the IR kinetics following HCT with omidubicel compared with UCB in a systematic and detailed manner. This substudy included 37 patients from 14 global sites (omidubicel, n = 17; UCB, n = 20). Peripheral blood samples were collected at 10 predefined time points from 7 to 365 days post-HCT. Flow cytometry immunophenotyping, T cell receptor excision circle quantification, and T cell receptor sequencing were used to evaluate the longitudinal IR kinetics post-transplantation and their association with clinical outcomes. Patient characteristics in the 2 comparator cohorts were overall statistically similar except for age and total body irradiation (TBI)-based conditioning regimens. The median patient age was 30 years (range, 13 to 62 years) for recipients of omidubicel and 43 years (range, 19 to 55 years) for UCB recipients. A TBI-based conditioning regimen was used in 47% of omidubicel recipients and in 70% of UCB recipients. Graft characteristics differed in their cellular composition. Omidubicel recipients received a 33-fold higher median dose of $CD34^+$ stem cells and one-third of the median $CD3^+$ lymphocyte dose infused to UCB recipients. Compared with UCB recipients, omidubicel recipients exhibited faster IR of all measured lymphoid and myelomonocytic subpopulations, predominantly in the first 14 days post-transplantation. This effect involved circulating natural killer (NK) cells, helper T (Th) cells, monocytes, and dendritic cells, with superior long-term B cell recovery from day +28. At 1 week post-HCT, omidubicel recipients exhibited 4.1- and 7.7 -fold increases in the median Th cell and NK cell counts, respectively, compared to UCB recipients. By 3 weeks post-HCT, omidubicel recipients were 3-fold more likely to achieve clinically relevant Th cell and NK cell counts ≥100

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cells/μL. Similar to UCB, omidubicel yielded a balanced cellular subpopulation composition and diverse T cell receptor repertoire in both the short term and the long term. Omidubicel's $CD34^+$ cell content correlated with faster IR by day +7 post-HCT, which in turn coincided with earlier hematopoietic recovery. Finally, early NK and Th cell reconstitution correlated with a decreased rate of post-HCT viral infections, suggesting a plausible explanation for this phenomenon among omidubicel recipients in the phase 3 study. Our findings suggest that omidubicel efficiently promotes IR across multiple immune cells, including $CD4^+$ T cells, B cells, NK cells, and dendritic cell subtypes as early as 7 days post-transplantation, potentially endowing recipients of omidubicel with early protective immunity.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for hematologic malignancies and nonmalignant disorders [1–3]. Durable and early hematopoietic recovery after HCT is essential to guarantee a functioning bone marrow and circulating blood system [4]. Fast reconstitution of the adaptive immune system is indispensable For full patient recovery. Immune reconstitution (IR) is influenced by multiple factors [5,6], including patient characteristics (eg, age, disease), HLA compatibility, preparative chemotherapy regimen, stem cell graft source, and post-transplantation supportive care [7,8].

Umbilical cord blood (UCB) has been an important source of hematopoietic stem cells for use in allogeneic HCT for more than 3 decades [9–12]. Following UCB transplantation, cellular recovery of the immune system begins with the appearance of innate immune cell subsets—neutrophils, monocytes, macrophages, myeloid-derived dendritic cells (DCs), and natural killer (NK) cells—in the peripheral blood (PB), followed by adaptive immune subsets (T cells and B cells). Earlier immune cell recovery is associated with improved outcomes. Faster NK cell reconstitution has a favorable impact on the incidence of chronic graft-versus-host disease (GVHD) and cytomegalovirus reactivation events [13], and a low PB circulating NK cell count (<50 cells/μL) at 1 month post-transplantation is associated with a lower rate of disease-free survival and a higher rate of nonrelapse mortality [14]. In contrast to adaptive immunity [15], NK cells mature and become functionally competent quickly (2 to 4 weeks after transplantation) and are relatively unaffected by GVHD prophylaxis medications other than glucocorticoids [16]. In addition, above-normal levels of NK cells in the first months after UCB transplantation seem to act as a compensatory mechanism during the post-transplantation T and B lymphopenia period [17–20].

With adaptive immunity, a higher amount of transfused UCB $CD3^+$ cells could predict early lymphocyte recovery [21] and faster thymic regeneration. Moreover, a relative abundance of T cell receptor (TCR) excision circle (TREC) transcripts in PB mononuclear cell (PBMC)-derived DNA has been associated with a reduced rate of viremia reactivation and increased overall survival (OS) [22]. Sufficient thymopoiesis kinetics and subsequent development of de novo derived naive T lymphocytes significantly improve disease-free survival, reduce the relapse rate, and are beneficial for long-term clinical outcomes in adult recipients [22,23].

Omidubicel, an investigational advanced cell therapy manufactured from an appropriately HLA-matched single UCB unit, consists of an ex vivo expanded $CD133^+$ fraction and a nonexpanded $CD133^-$ fraction (Figure 1A). The proprietary nicotinamide technology used to manufacture omidubicel inhibits the differentiation and enhances the functionality of hematopoietic stem and progenitor cells [24].

In the previously published phase 3 randomized trial (NCT02730299), transplantation with omidubicel demonstrated faster hematopoietic recovery and shorter post-transplantation hospitalizations compared with UCB transplantation [25]. Lower rates of bacterial and invasive fungal infections were observed in concert with rapid and robust neutrophil engraftment. The study also demonstrated a nearly 3-fold decrease in the number of grade 3 viral infections following transplantation with omidubicel, thus suggesting early lymphocyte-centric IR.

In this substudy, we hypothesized that omidubicel transplant recipients experience faster immune system reconstitution compared to UCB transplant recipients, and that rapid reconstitution of lymphocyte subsets endowed with the ability to mediate antiviral immune responses correlates with fewer viral infections post-HCT. To test this hypothesis, we compared the temporal IR kinetics following myeloablative conditioning and transplantation with omidubicel versus UCB using longitudinal immunophenotyping of the lymphoid and myelomonocytic lineages and assessment of T cell repertoire diversity, and then correlating these parameters with clinical outcomes.

METHODS

Trial Design

This prospectively planned optional IR substudy using standardized specimen collection and analysis at a central laboratory was instituted as part of the phase 3 study [25]. The phase 3 trial design has been described previously [25]. In brief, 125 patients age 13 to 65 years with hematologic malignancies were randomized to omidubicel or UCB transplantation. All patients received myeloablative conditioning and GVHD prophylaxis. The trial was approved by the Institutional Review Boards of all participating institutions and the appropriate national regulatory authorities. All patients provided written informed consent. The study was performed in accordance with the International Conference on Harmonization, local regulations, and the principles of the Declaration of Helsinki.

Blood samples were collected according to the schedule summarized in Figure 1B. Following completion of the phase 3 study, the frozen samples were analyzed at a central laboratory. Flow cytometry, TREC, and TCR sequencing methodologies were qualified, validated, and managed by Labcorp Central Laboratory Services Sàrl (Meyrin, Switzerland).

Immunophenotyping

A 16-color, 2-tube, stain/wash/fix flow cytometric assay was used to evaluate the T cell and NK cell subset composition, with corresponding expression of memory/naive and helper T (Th) 1/2 markers of T cells (Supplementary Figures S1 and S2) [26–30]. A 14-color, 2-tube, stain/wash/fix flow cytometric assay was used to evaluate the B cell, monocyte, and DC subset composition (Supplementary Figure S3) [26–30]. Normal age reference ranges were derived from the American Board of Internal Medicine [31] and Shearer et al. [32].

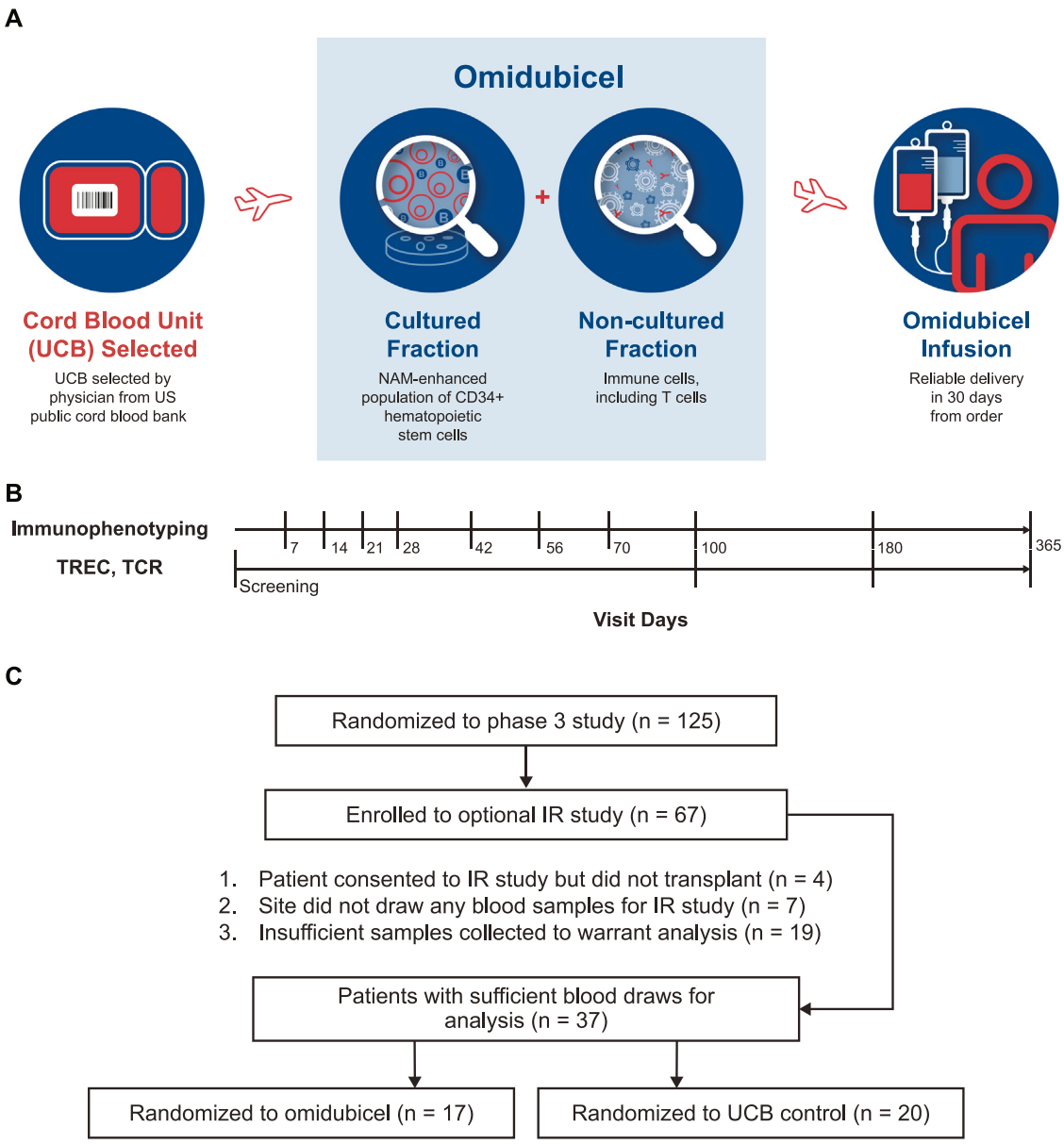

Figure 1. Study conduct. (A) Schematics of omidubicel composition and production. (B) Blood sampling schedule. Blood samples were collected for immunophenotyping analysis on days +7, +14, +21, +28, +42, +56, +70, +100, +180, and +365 post-transplantation. Blood samples were collected for TREC and TCR analysis at screening and on days +100, +180, and +365 post-transplantation. (C) Patient disposition.

TREC

The QIAamp DNA Blood Mini Kit (QIAGEN, Germantown, MD) and quantification by RT-PCR were used to determine TREC levels in DNA samples derived from patient PBMCs. TREC quantification was normalized using beta-actin as a housekeeping gene with respect to genomic DNA input (200 ng per reaction). Samples were tested in triplicate along with a standard curve using a calibrator ranging from 10 to 10^6 copies per reaction to confirm efficiency equivalency between targets and linearity.

TCR Repertoire Analysis

A TCRβ repertoire short-read (SR), next-generation sequencing-based assay was used to evaluate T cell diversity and clonal expansion by sequencing TCRβ chain rearrangements. The TCRβ SR test used a highly multiplexed targeted resequencing panel to generate libraries from 200 ng of DNA extracted from PBMCs. DNA evaluation of TCRβ chain rearrangements targeting CDR3 (with up to 150-bp read-length amplicons) was performed using NovaSeq 6000 (Illumina, San Diego, CA) and Ion Torrent S5XL (Thermo Fisher Scientific, Waltham, MA) sequencing systems. Overall, 60 TCRβ variable genes, 14 TCRβ joining genes, and 2 TCRβ constant genes were analyzed for diversity from FR3 to J genes. The libraries were sequenced using the NovaSeq 6000 platform. Sequence data were then processed using an analysis pipeline designed to detect the TCRβ VDJ rearrangements (clonotypes) and secondary analysis of repertoire features.

Statistical Analyses

For immunophenotyping (including recent thymic emigrants [RTEs]) and TREC analysis, data normality was assessed using the D'Agostino-Pearson and Shapiro-Wilk normality tests. Non-normally distributed datasets were predominantly

of lognormal distribution. These datasets were evaluated using nonparametric tests, including the Mann-Whitney *U* test. Correction for multiple comparisons was performed by adjusting *P* values using the Holm-Sidak method. In various analyses, Kaplan-Meier plots were constructed, and their difference was evaluated using the log-rank (Mantel-Cox) test. Correlative studies were performed to assess the degree of correlation between different datasets. These included graft characteristics (ie, total number of viable cells, $CD34^+$ and $CD3^+$ cell counts), IR parameters (ie, quantitative reconstitution of Th, cytotoxic T, NK, and B cells) and clinical outcomes (ie, time to neutrophil and platelet engraftment). Given the continuous nature of the variables composing these datasets, correlations were evaluated using the Pearson correlation coefficient (r) followed by fitting of linear regression models, including determination of threshold significance ($\alpha = .05$) and goodness of fit (R^2).

For the TCR repertoire analysis, total clones, V-J pairs, Shannon diversity, evenness, and Gini Clone Index were evaluated (see Supplementary Data, Methods for more information), and *P* values were determined using the Wilcoxon test.

Data Sharing Statement

Queries regarding the data can be addressed to the corresponding author at roeim@gamida-cell.com.

RESULTS

Patient Disposition

Of the 125 patients randomized in the phase 3 study, 67 patients consented to participate, and 37 patients from 14 global sites were included (omidubicel group, n = 17; UCB group, n = 20) (Figure 1C).

Baseline Patient and Graft Characteristics

The demographics and baseline characteristics of patients included in the IR substudy (Table 1) were generally similar to those observed in the phase 3 study population. Patients in the omidubicel group received a median $CD3^+$ cell dose of 1.66×10^6 cells/kg (range, 1.1 to 7.53×10^6 cells/kg) compared with patients in the UCB group, who received a 3-fold higher median $CD3^+$ dose of 4.93×10^6 cells/kg (range, 1.71 to 9.89×10^6 cells/kg). Overall, the T cell dose was 66% lower in the omidubicel recipients compared with the UCB recipients. Neither cohort received serotherapy, so in vivo lymphocyte depletion was not a confounder.

Clinical Outcomes

Clinical outcomes in the IR substudy were consistent with those from the phase 3 study (Table 2). The median time to neutrophil engraftment was significantly lower in the omidubicel group compared with the UCB group (10.0 days versus 18.5 days; $P < .0001$). The rate of grade 2/3 infections in the first year was significantly lower with omidubicel than with UCB (29% versus 70% [$P = .032$] for bacterial infections and 6% versus 45% [$P = .042$] for viral infections). There were no differences in the incidence of acute GVHD and chronic GVHD between the 2 groups.

Omidubicel recipients exhibit early and robust IR across multiple cell populations

The omidubicel arm demonstrated an up to 70-fold advantage over the UCB arm in median cell counts across most cell populations (Figure 2A), occurring predominantly in the short-term post-transplantation setting. Omidubicel recipients exhibited superior reconstitution of T and NK cells, monocytes, and DCs compared with UCB recipients in the first 2 weeks post-transplantation (Figure 2A-C). Further longitudinal kinetics analysis (Figure 2B,C) demonstrated a significant post-transplantation increase in the omidubicel arm versus the UCB arm in Th ($CD4+$) cell, DC, and monocyte subpopulations at days +7 and +14; in NK cells at days +7, +14, and +21; and in B cells at days +28, +42, and +56 post-transplantation. The increase in cytotoxic T ($CD8^+$) cells during the first 2 weeks post-transplantation was greater in the omidubicel arm versus the UCB arm, but the difference did not reach significance. In the B cell milieu, substantial differences were found as early as day +28 and remained superior in the omidubicel arm until the end of the study.

Omidubicel showed a significant advantage over UCB in the proportion of patients who achieved IR of Th, NK, and B cell counts of 50 cells/μL and 100 cells/μL in PB (Figure 2D). At 3 weeks post-transplantation, 76.5% and 70.6% of omidubicel recipients reached 50 cells/μL and 100 cells/μL of Th cells in PB, respectively, compared with 40% and 25%, respectively, in the UCB arm. Similar trends were seen for NK cells; 70.6% and 62.5% of omidubicel recipients achieved 50 cells/μL and 100 cells/μL, respectively, by day +21, compared with 35% and 20% of UCB recipients. Finally, 82.3% and 64.7% of omidubicel recipients achieved a B cell count of 50 cells/μL and 100 cells/μL, respectively, by day 42 post-transplantation, compared with 20% and 15% of UCB recipients.

These data suggest that omidubicel transplantation facilitates faster lymphocyte, monocyte, and DC reconstitution compared with UCB transplantation, with significant quantitative advantages before day +28 post-transplantation, the earliest historically reported milestone. For B cells, the quantitative advantage noted in omidubicel recipients persists for up to 1 year.

Immunologic recovery following omidubicel retains mononuclear cellular proportions and TCR repertoire diversity

To regain its functionality, the immune system must achieve the adequate cellularity required for proper immune function and retain a balanced subpopulation composition. We evaluated the relative fractions of different subpopulations that compose 5 key leukocyte subsets: Th and cytotoxic T cells, B cells, monocytes, and DCs (Figure 3A). In all subsets examined, omidubicel recipients exhibited short-, medium-, and long-term immune subset compositions comparable to those of UCB recipients.

As the infused preformed lymphocytes adapted to the host environment, the composition of different Th cell subsets changed substantially over time, without significant differences between the treatment arms. The 2 major subsets, $CD4^+$ effector memory (EM) and central memory (CM) cells, compose 50% to 70% of the total $CD4^+$ cell population. During the study period, the EM-to-CM ratio gradually and significantly increased from .57 to 2.26 for the omidubicel arm and from .66 to 2.08 for the UCB arm. The proportion of naive $CD4^+$ cells was higher at day +14 (13% to 23%) compared with days +100 (5%) and +365 (4%). As minor $CD4^+$ contributors, Th1, Th2, RTE, and regulatory $CD4^+$ cellular subsets did not change significantly over time (Figure 3A).

For the cytotoxic T cell compartment, the composition of subsets also changed substantially over time and did not differ significantly between the 2 treatment arms (Figure 3A). Cytotoxic EM cells made the greatest contribution at day +14 (48% to 63%), which gradually decreased to day +100 (22% to 45%). A similar, but lesser, trend was observed for the cytotoxic CM subset. In contrast, naive and RTE $CD8^+$ subsets increased over

Table 1
Baseline Patient and Graft Characteristics

Characteristic	Omidubicel Group	UCB Group
Total no. of patients	17	20
Sex, n (%)		
Male	10 (59)	12 (60)
Female	7 (41)	8 (40)
Age, yr, median (range)	30 (13-62)	43 (19-55)
Age group, n (%)		
12-17 yr	4 (23.5)	0 (0)
18-39 yr	7 (41.2)	8 (40)
40-59 yr	5 (29.4)	12 (60)
60-65 yr	1 (5.9)	0 (0)
Weight, kg, median (range)	91 (71-133)	90 (49-134)
Race, n (%)		
White	9 (53)	13 (65)
African American	5 (29.4)	3 (15)
Asian	0 (0)	2 (10)
Other/unknown	3 (17.6)	2 (10)
Primary diagnosis, n (%)		
Acute lymphoblastic leukemia	5 (29.4)	4 (20)
Acute myeloid leukemia	9 (53)	14 (70)
Chronic myeloid leukemia	2 (11.7)	0
Myelodysplastic syndrome	0	1 (5)
T cell non-Hodgkin lymphoma	1 (5.9)	0
Dendritic cell leukemia	0	1 (5)
Disease Risk Index, n (%)		
Low	5 (29.4)	5 (25)
Moderate	6 (35.3)	11 (55)
High/very high	6 (35.3)	4 (20)
HCT-Specific Comorbidity Index, n (%)		
0	1 (5.9)	1 (5)
1-2	5 (29.4)	6 (30)
3+	11 (64.7)	13 (65)
CMV seropositive, n (%)	10 (59)	13 (65)
Intended CBU transplant, n (%)		
Single	5 (29.4)	5 (25)
Double	12 (70.6)	15 (75)
Antigen-level HLA match score (intended treatment CBU 1), n (%)		
4/6	12 (70.6)	15 (75)
5/6	4 (23.5)	5 (25)
6/6	1 (5.9)	0 (0)
Antigen-level HLA match score (intended treatment CBU 2), n (%)*		
4/6		9 (60)
5/6		6 (40)
6/6		
ABO blood type mismatch, n (%)†	10 (62.5)	7 (36.8)
Graft parameters, median (range)		
TNC count, $\times 10^9$ cells		
Omidubicel CF	2.7 (1.3-4.8)	
Omidubicel NF	.83 (.53-1.4)	
CBU 1		2.04 (1.29-3.35)
CBU 2 (n = 15)		1.8 (1.44-4.03)
Total TNC count	3.45 (2.05-5.8)	3.39 (2.3-7.02)
TNC dose, $\times 10^7$ cells/kg	4.3 (1.75-9.81)	3.84 (2.55-7.34)
$CD34^+$ cell count, $\times 10^8$ cells		
CBU 1 (n = 16)		.15 (.06-1.43)
CBU 2 (n = 12)		.11 (.03-.29)
Total $CD34^+$ cell count	5.9 (2.8-15)	.29 (.1-1.5)
$CD34^+$ cell dose, $\times 10^6$ cells/kg	10.69 (2.15-23.21)	.32 (.11-1.81)

(*continued*)

Table 1 (*Continued*)

Characteristic	Omidubicel Group	UCB Group
$CD3^+$ cell count, $\times 10^8$ cells		
CBU 1 (n = 9)		2.94 (.33-6.76)
CBU 2 (n = 6)		2.38 (.89-3.76)
Total $CD3^+$ cell count	1.7 (.71-5.8)	4.99 (1.83-9.9)
$CD3^+$ cell dose, $\times 10^6$ cells/kg	1.66 (1.1-7.53)	4.93 (1.71-9.89)
Myeloablative conditioning, n (%)		
TBI/Flu/Thio	3 (17.6)	4 (20)
TBI/Flu/Cy	5 (29.4)	10 (50)
Thio/Bu/Flu	9 (53)	6 (30)
TBI-based	8 (47)	14 (70)
Non-TBI-based	9 (53)	6 (30)

CMV indicates cytomegalovirus; Flu, fludarabine; Thio, thiotepa; Bu, busulfan; CBU, cord blood unit.

* n = 15; percentages calculated out of the total number of patients receiving 2 CBUs.

† UCB group, n = 19; omidubicel group, n = 16. Percentages calculated of the number of patients for whom data were available.

time. The greatest increase across subpopulations was observed in the naive $CD8^+$ subset, the proportion of which rose from 6% to 10% at day +14 to 20% to 33% at days +100 and +365. Remarkable enrichment was observed for $CD8^+$ RTEs, which increased from 3% to 5% at day +14 to 15% to 19% at 1 year. Nonetheless, comparable levels of TREC transcripts were identified at days +100, +180, and +365 post-transplantation in both cohorts.

The major B cell subset was naive B cells, which increased from 50% at day +14 to >85% at days +100 and +365. Minor differences in monocyte and DC subset composition were found between the omidubicel and UCB arms, without any significant changes observed between the treatment arms over time. Classical and nonclassical monocytes comprised approximately 94% to 98% of the entire monocyte population. Most of the DC population was of myeloid origin (>92%), with a small fraction comprising the plasmacytoid compartment (Figure 3A).

An important aspect of T cell functional reconstitution is achieving a diversified TCR repertoire. Next-generation sequencing of TCR-βV and TCR-βJ genes was performed to evaluate the distribution of gene groups (Figure 3B,C). The distributions of TCR-βV and TCR-βJ gene transcripts were similar in the 2 treatment arms, without any significant differences over time. The number of V-J pairs and their diversity across pretransplantation and post-transplantation samplings were not significantly different between the treatment arms (Figure 3D). During the post-transplantation period, there was an increase over time in the number of V-J pairs and improvement of the TCR diversity indices, with no significant differences between the 2 treatment arms. These data demonstrate that omidubicel recipients can recover their mononuclear cellular diversity and TCR repertoire diversity in a similar fashion as UCB recipients.

Omidubicel $CD34^+$ progenitor cell mass correlates with faster IR at 1 week post-transplantation, which in turn coincides with superior hematopoietic recovery

To determine whether different cellular components measured in the omidubicel production process correlate with early post-transplantation lymphocyte reconstitution, 4 omidubicel intrinsic cellular parameters were evaluated: the total number of viable cells in both the cultured fraction (CF) and the noncultured fraction (NF), specific $CD34^+$ progenitor cells in the CF, and $CD3^+$ lymphocytes in the NF. Because early IR is relevant in the clinical context, these parameters were assessed versus 5 common lymphocyte subsets measured in the PB of transplantation recipients at 7 days post-transplantation: $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+$ cells.

To screen for correlations, the significance levels of linear regression models calculated for all 20 paired datasets in the correlation matrix were plotted. Robust positive correlations were identified between cellular elements from the CF—specifically, $CD34^+$ cell content—and reconstitution of T cells and NK cells (Figure 4A). No correlations were found for the NF parameters. For the correlation of T cells, highly significant linear

Table 2
Clinical Outcomes

Outcome	Omidubicel Group (N = 17)	UCB Group (N = 20)	*P* Value*
Time to neutrophil engraftment, d, median (range)	10 (6-28)	18.5 (14-40)	<**.0001**
Patients with >95% chimerism on day +21/28, n (%)	17 (100)	20 (100)	NS
Grade 2/3 infections during first 365 days, n (%)			
Bacterial infections	5 (29)	14 (70)	**.032**
Viral infections	1 (6)	9 (45)	**.042**
GVHD, n (%)			
Acute grade II-IV†	11 (65)	12 (60)	.77
Acute grade III-IV†	1 (6)	7 (35)	.08
Chronic (moderate/severe)	3 (18)	7 (35)	.26

NS indicates not significant.

* *P* values for the median time to neutrophil engraftment were calculated using the Mann-Whitney U test. All other *P* values were calculated using the relative risk test. Significant *P* values are in bold type.

† Maximum grade to day +180.

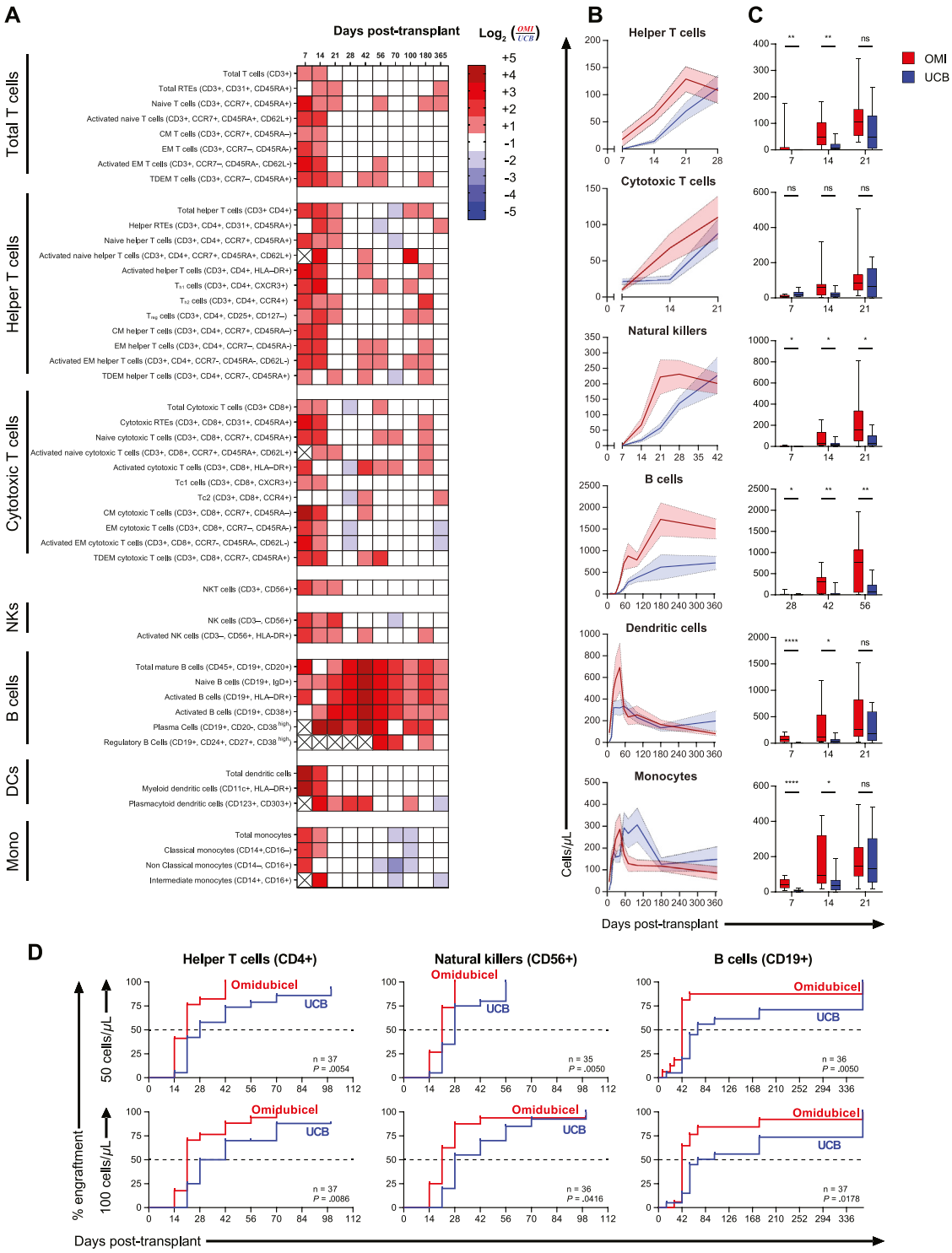

Figure 2. Transplantation with omidubicel facilitated rapid reconstitution of the lymphoid and myelomonocytic lineages. (A) Heatmap showing the log2 ratio between the median cell counts of omidubicel transplant recipients and UCB transplant recipients. (B) Longitudinal kinetics of the absolute quantities of various cell populations in omidubicel transplant recipients and UCB transplant recipients. Plots represent the mean values, and dashed lines represent the standard error of the mean. (C) Quantitative differences in the absolute cell counts between the 2 treatment groups. Central lines depict median value, the box represents interquartile range, and whiskers represent the entire range. *P* values were determined using the Mann-Whitney U test and were adjusted to multiple comparisons using the Holm-Sidak method. $^{*}P \leq .05$; $^{**}P \leq .01$; $^{***}P \leq .001$; $^{****}P \leq .0001$. (D) Kaplan-Meier curves of the percentage of patients achieving Th, NK, and B cell engraftment in omidubicel and UCB recipients. Engraftment was determined as achieving 50 cells/μL (upper panel) and 100 cells/μL (lower panel) at a given time point. Statistical significance between curves was determined using the log-rank (Mantel-Cox) test. CCR7, chemokine (C-C motif) receptor 7; Mono, monocyte; OMI, omidubicel; ns, not significant ($P > .05$); TDEM, terminally differentiated effector memory.

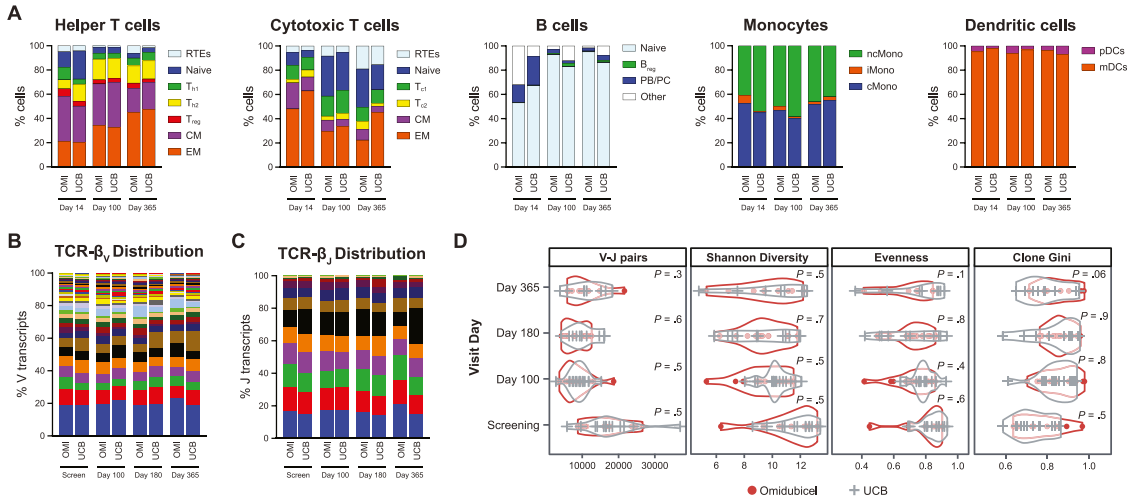

Figure 3. Transplantation with omidubicel culminated in a post-transplantation mononuclear subpopulation and TCR repertoire diversity similar to that observed with UCB transplantation. (A) Bar plots depicting the longitudinal relative abundance of T cells, B cells, monocytes, and DC subpopulations among omidubicel recipients compared with UCB recipient. For each cell population, early (day +14), mid-term (day +100), and long-term (day +365) values are presented. Plotted values represent the median relative value for each cell type. (B and C) Bar plots depicting the relative abundance of TCRβ V (B) and J (C) genes obtained via TCRβ sequencing of PB T cells sampled before myeloablation (at screening) and at days +100, +180, and +365 post-transplantation with omidubicel or UCB. Each color represents a distinct TCRβ V/J gene. (D) Violin plots of the number of distinct TCRβ V-J pairs and the Shannon diversity, evenness, and Gini indices describing TCRβ diversity. *P* values were determined using the Wilcoxon test. Breg, regulatory B cell; cMono, classical monocyte; iMono, intermediate monocyte; mDC, myeloid dendritic cell; ncMono, nonclassical monocyte; PB/PC, plasma blasts/plasma cell; pDC, plasmacytoid dendritic cell; Tc, cytotoxic T cell; Treg, regulatory T cell.

regression models were calculated (P = .0008 to .006), and strong positive correlations were identified (r = .74 to .83) (Figure 4B).

Correlations between the main lymphocyte subpopulations at 7 days post-transplantation and neutrophil/platelet engraftment times as reported in the original phase 3 study [25] were evaluated to determine whether faster lymphocyte IR coincided with rapid hematopoietic recovery. Calculated linear regression models identified significant correlations in the omidubicel arm between reconstituted $CD3^+$ and $CD4^+$ T cells and neutrophil engraftment (Figure 4C, upper panel) and between $CD3^+/CD8^+/CD19^+$-expressing cells and platelet engraftment (Figure 4C, lower panel).

These data suggest that in omidubicel recipients, donor sources harboring a higher CD34+ cell content in their CF may promote an immediate IR and prompt hematopoietic recovery. Conversely, the IR kinetics were not influenced by the graft $CD3^+$ cell content.

Early NK and Th cell reconstitution correlated with superior antiviral immunity post-transplantation

In the phase 3 study, omidubicel was shown to confer a nearly 3-fold decrease in the rate of post-transplantation grade 3 viral infections compared with UCB [25]. Consequently, it was hypothesized that faster IR led to this phenomenon. Kaplan-Meier estimation analyses were performed to examine the rate of grade III viral infections after a predetermined IR threshold. Data from a combined omidubicel/UCB-treated patient cohort were used, and patients were evaluated according to their ability to achieve cell counts of 50 cells/μL and 100 cells/μL of blood by day +21 for NK and Th cells and by day +42 for B cells. Early reconstitution of various lymphocyte subsets achieving values within this range was previously reported to correlate with multiple favorable clinical outcomes [33–37]. Reconstitution of both NK and Th cells correlated with a decreased rate of post-transplantation post-IR grade 3 viral infections (NK, P = .017 to .023; Th cells, P = .0075 to .0368), whereas B cell reconstitution had no such effect (Figure 5A).

NK and Th cells may have an additive protective effect, as the rate of post-transplantation, post-IR grade 3 viral infections was even lower in those with both Th and NK cell counts >100 cells/μL (Figure 5B). These data suggest that early IR of both NK and Th cells contributes to superior antiviral control in the post-transplantation, post-IR period and may account for the lower rate of grade 3 viral infections observed among omidubicel recipients.

DISCUSSION

Omidubicel is the first hematopoietic cell donor source composed entirely of ex vivo expanded UCB-derived HSCs [24]. Enhanced hematopoietic recovery following omidubicel has been well documented in several multicenter clinical trials [25,38,39]. Yet, although prompt post-HCT IR is known to correlate with superior clinical outcomes [13,14,17-20,22,23,40-44], the IR kinetics following omidubicel transplantation have not been investigated previously. As such, the focus of this substudy was on evaluating the IR kinetics of omidubicel versus UCB in a systematic manner, and on investigating whether these could account for the decrease in post-HCT viral infections as observed in the phase 3 parent study.

Evidently, omidubicel provided significantly earlier reconstitution of all major lymphoid lineages and achieved a 4- to 6-fold short-term advantage in T cell counts in recipients, even though they received substantially fewer $CD3^+$ cells compared with UCB recipients. The equal thymic functionality in the 2 arms, evaluated by RTE and TREC quantification, points to rapid homeostatic proliferation and/or less lymphocyte attrition in the immediate post-transplantation period as possible reasons for the faster lymphocyte reconstitution with omidubicel. Other possible causes for this phenomenon could be increased PB levels of lymphoid supportive cytokines and bone marrow niche invigoration achieved by early engraftment. Indeed, these postulations are indirectly supported by the significant correlations observed between the

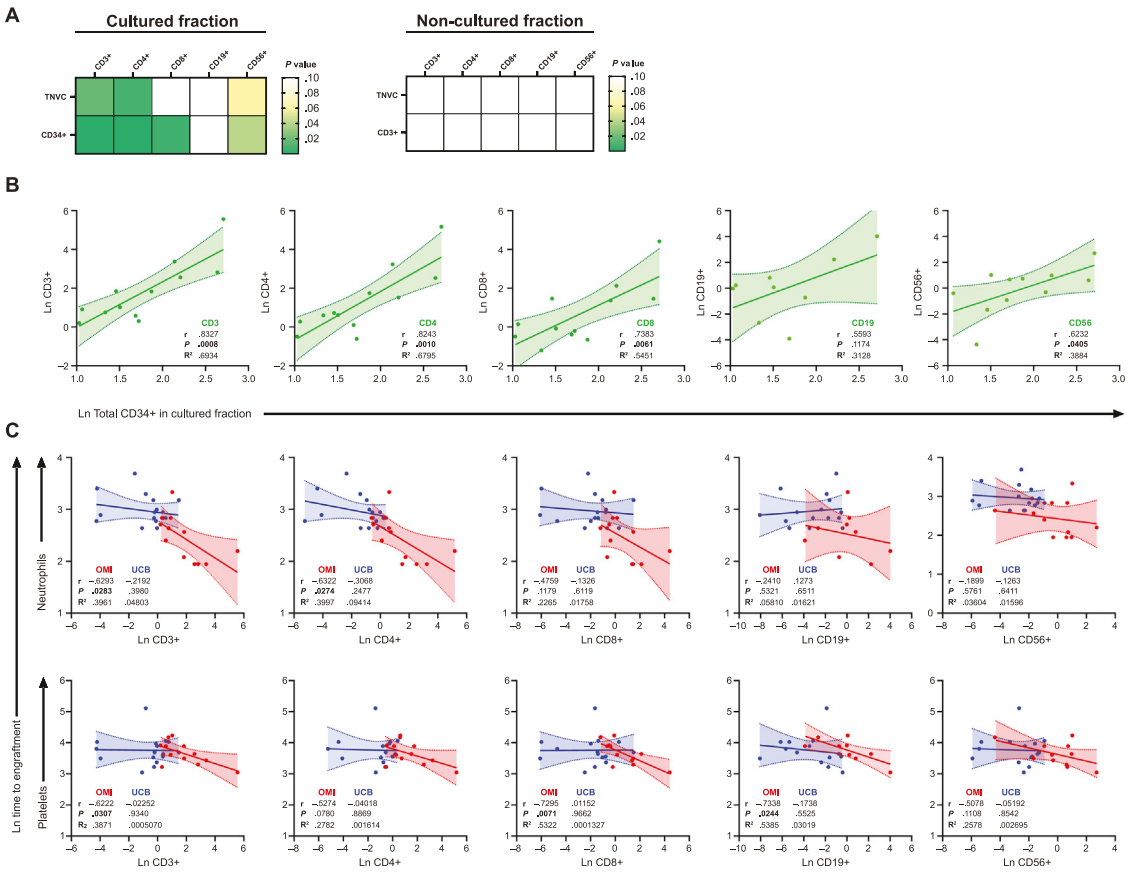

Figure 4. Correlation studies suggest a link between high CD34$^+$ progenitor cell mass in the omidubicel cultured fraction and rapid lymphocyte reconstitution at 1 week after transplantation. (A) Heatmaps depicting *P* values originating from Pearson correlation studies between omidubicel-related cellular parameters and reconstitution of lymphocyte subsets on day +7 post-transplantation. White indicates nonsignificant ($P \geq .1$); yellow gradient, marginally significant ($P = .05$ to $.1$); green gradient, significant ($P < .05$). (B) Pearson correlation analyses of the correlations between the absolute CD34$^+$ progenitor cell count in the omidubicel cultured fraction and the reconstitution of T cells, B cells, and NK cells at 7 days post-transplantation. Dots represent actual measurements; lines represent the linear regression models; and dashed lines represent the 95% confidence interval (CI) of the linear regression models. Bolded *P* values traverse the significance threshold. (C) Pearson correlation analyses of the correlations between neutrophil engraftment times (upper panel) or platelet engraftment times (lower panel), and the proportions of various lymphocyte subpopulations as measured by flow cytometry at 7 days post-transplantation with omidubicel (red) or UCB (blue). Dots represent actual measurements, lines represent the linear regression models, and dashed lines represent the 95% CIs of the linear regression models. Bolded *P* values traverse the significance threshold. Pearson correlation studies and linear regression models were used to determine the *P*, *r*, and R^2 values. TNVC, total number of viable cells.

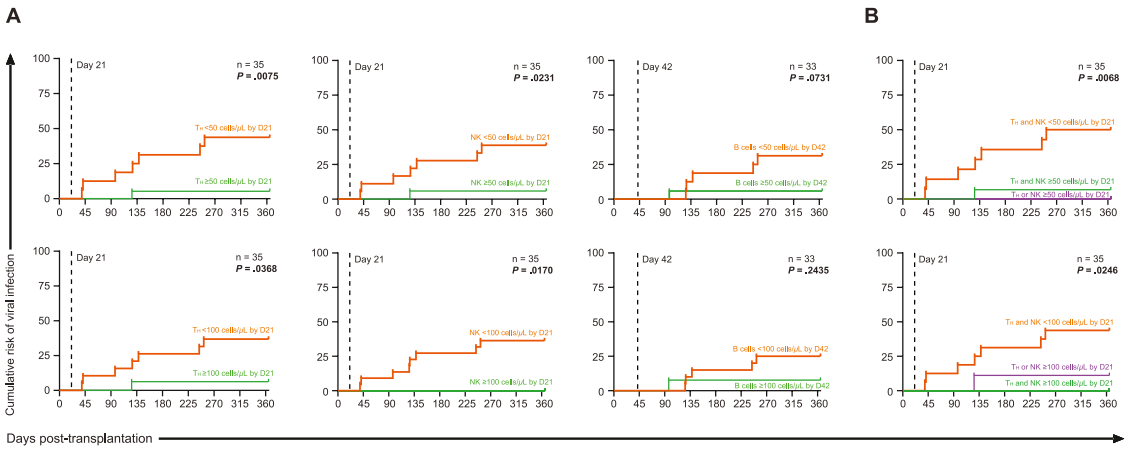

Figure 5. Short-term NK cell and Th cell reconstitution coincided with a decreased rate of severe post-transplantation viral infections. (A) Kaplan-Meier curves depict the percentage of HCT recipients who developed grade 3 viral infections from a predefined immune reconstitution time point post-transplantation (dashed vertical line) and beyond. Successful immune reconstitution of Th cells, NK cells, and B cells was predefined as achieving 50 cells/μL (upper panel) or 100 cells/μL (lower panel) of blood by 21 days post-transplantation for Th and NK cells and by 42 days post-transplantation for B cells. (B) A similar compound analysis accounted for both NK and Th cell reconstitution and their effect on the rate of viral infections. The statistical significance between curves was determined using the log-rank (Mantel-Cox) test. Statistically significant *P* values are in bold type.

$CD34^+$ cell mass infused to omidubicel recipients and faster lymphocyte recovery times at 1 week post-HCT. Of note, a significant correlation between $CD34^+$ cell content in the omidubicel CF and faster neutrophil engraftment time was reported in the phase 3 study as well [25]. These results emphasize the significant effect of omidubicel on the 2 most critical clinical processes defining patient recovery following HCT: neutrophil engraftment and IR. The phase 3 study also disclosed a significant, nearly 3-fold decrease in the risk of severe viral infections with omidubicel compared to UCB [25]. Both NK and Th cells mediate antiviral immune responses. Consistent with the published literature [35], we found that early and specific NK and Th cell reconstitution coincided with superior protection against viral infections, further underscoring the significance of short-term IR and its implications on antiviral immunity.

Although this study was not powered to detect a difference in OS, a trend favoring omidubicel was apparent in the phase 3 trial. The lack of a statistically significant difference might have stemmed from the limited sample size and the heterogenous causes of mortality, many of which occur independent of an intact immune system. Moreover, our substudy also harbors an intrinsic selection bias, as patients who were able to provide longitudinal samples, according to definition survive longer than those who perish soon after transplantation. It is tempting to assume that patients who rapidly reconstitute their immune system post-HCT benefit from better OS. Future dedicated studies should elucidate that hypothesis.

When planning for HCT, one key clinical consideration is graft availability. In the phase 3 study, efficacy outcomes, including relapse and disease-free OS, were measured from the time of randomization, incorporating the time required for omidubicel production. Although patients treated with omidubicel underwent HCT at a median of 41 days postrandomization, compared with 26 days in the UCB arm, there was no determinant in these outcomes associated with omidubicel production time [25]. In addition, in current clinical practice, most patients undergoing allogeneic HSCT receive a graft from an unrelated donor; it can take 2 to 3 months to identify and confirm an appropriate donor on the registry for a matched or mismatched unrelated donor. In contrast, omidubicel as a graft source can be reliably delivered within 30 days. In addition to the clinical benefit associated with omidubicel transplantation, the value of omidubicel to the health care system has been demonstrated by the reductions in hospitalization and overall health care resource utilization [45].

It is important to note the limitations of this study, especially when considering the paucity of literature describing early IR comparison between donor sources. Our findings are based on a limited sample size of 37 patients. Moreover, although a complete longitudinal set of samples was retrieved from most patients, there was a natural dropout during the study due to death, relapse, and other causes. Nonetheless, analyses of long-term IR kinetics at 1 year disclosed similar baseline normalization for nearly all immune cell subsets. Although the baseline patient characteristics generally were well balanced between the treatment arms, there were differences in the median age and the proportion of patients who received TBI-based regimens. Age variation may influence IR outcomes, as younger patients potentially could recover lymphocyte counts faster than older patients. However, our main findings were observed within 2 weeks post-HCT, well before thymic output is expected, and an age-adjusted analysis found no age-specific effect. Although TBI could slow down immune recovery compared to chemotherapy conditioning only, a TBI-adjusted analysis found that the IR effects observed were not attributable to TBI exposure.

In conclusion, the data presented in this study support our hypothesis by demonstrating a quantitative advantage in lymphocyte reconstitution for omidubicel recipients compared to UCB recipients. Moreover, these data provide supportive evidence for the decrease in post-HCT severe viral infections in patients who present with robust and early IR. Indeed, HCT recipients are most vulnerable to infections when lacking a functional immune system in the immediate post-HCT period. Our findings are important, as they shed light on the biology of IR in that critical period. Therefore, the immunologic advantage of omidubicel is limited not only to recovery of the myeloid compartment, as evident in the phase 3 study, but also to the lymphoid compartment. Collectively, our findings comprehensively demonstrate the advantageous qualities of omidubicel as a donor source capable of generating a rapid, balanced, and functional recuperation of the immune system across all cellular compartments.

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Authorship statement: P. Szabolcs, R.D.M., D.Y., S.L., and M.E.H. conceptualized the trial and developed the methodology. P. Stiff, A.R., R.H., A.K., C.L., J.W., N.K., J.M., N.H., I.L.T., G.S., D.V. and M.E.H. provided study resources. R.D.M., D.Y., S.L., P. Stiff, A.R., R.H., A.K., C.L., J.W., N.K., J.M., N.H., I.L.T., G.S., D.V., and M.E.H conducted the investigation. R.D.M., D.Y., and S.L. validated the results/experiments. G.S. analyzed the results. R.D.M., D.Y., and S.L. curated the data and wrote the initial draft of the manuscript. P. Szabolcs, R.D.M., D.Y., and S.L. performed a formal analysis of the study data. P. Szabolcs, R.D.M., D.Y., S.L., P. Stiff, R.H., A.K., C.L., J.W., N.K., J.M., N.H., D.V., and M.E.H. critically reviewed the manuscript. R.D.M. and D.Y. created the visualization of data for the manuscript. P. Szabolcs, R.D.M., and M.E.H. provided project supervision. P. Szabolcs and R.D.M. contributed equally to this work as co-first authors.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2023.04.018.

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