Hematopoietic cell transplantation after CD19-directed CAR T-cell therapy for remission consolidation or relapse treatment in pediatric acute lymphoblastic leukemia

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Abstract

Relapse of B-cell acute lymphoblastic leukemia (B-ALL) after CD19-targeted chimeric antigen receptor T-cell therapy (CAR19) remains a substantial challenge. Allogeneic hematopoietic cell transplant (HCT) represents an approach for both post-CAR19 relapse prevention and relapse therapy. However, there is a paucity of detailed HCT safety and outcome data in this population. We conducted a retrospective review of 47 children and young adults with B-ALL who underwent first HCT for post-CAR19 remission consolidation (preemptive cohort, N=26) or relapse therapy (relapse cohort, N=21). With a median follow-up of 4.1 years, 3-year disease-free survival was 90% in the preemptive cohort and 64% in the relapse cohort. Overall survival, cumulative incidence of relapse, and non-relapse mortality at 3 years were 95%, 5%, and 5%, respectively, in the preemptive cohort and 67%, 20%, and 15%, repectively, in the relapse cohort. The cumulative incidence of grade 3-4 acute graft-*versus*-host disease (GvHD) was 14% in the preemptive cohort and 19% in the relapse cohort. Chronic GvHD developed in 24% and 14% of patients alive at 100 days in the preemptive and relapse cohorts, respectively. Veno-occlusive disease/sinusoidal obstruction syndrome was the most common non-GvHD severe organ toxicity, with a cumulative incidence of 10% in the preemptive cohort and 31% in the relapse cohort. In appropriate patients, HCT can be an effective strategy for attaining durable B-ALL remission when used preemptively after CAR19 or as part of post-CAR19 relapse salvage therapy.

Introduction

CD19-directed chimeric antigen receptor T-cell therapy (CAR19) has revolutionized the treatment paradigm for relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL). Initial complete remission (CR) rates exceed 90% in some studies.[1,2] However, disease recurrence remains a persistent obstacle to cure with half of children and young adults experiencing subsequent relapse.[3-5] Therefore, identifying effective strategies to prevent and treat post-CAR19 relapse is vital.

Allogeneic hematopoietic cell transplant (HCT) represents an approach for both post-CAR19 relapse prevention and relapse therapy. Previous studies demonstrate a clear

benefit of HCT for remission consolidation in children with limited CAR T-cell persistence after treatment with CD19/4-1BB-based CAR constructs[6] or in children treated with CD19/CD28-based CAR constructs,[7] which are inherently shorter-persisting.[8] The benefit of preemptive HCT for other subgroups of patients is unclear. For children who do experience relapse, the overall prognosis is dismal, with a median survival of approximately 1 year after post-CAR19 relapse.[9,10] For those who achieve remission following a post-CAR19 relapse, HCT is the only known curative therapy. Although HCT after CAR19 is ultimately utilized for many patients, there is a paucity of HCT-related toxicity and outcome data in this population. This knowledge gap is particularly relevant as children undergoing HCT after CAR19 are often in third complete remission (CR3) or beyond. In contrast, most published pediatric HCT outcome data are focused on children transplanted in first or second CR (CR1 or CR2, respectively). Limited historic data regarding children transplanted in CR3 documented long-term disease-free survival (DFS) rates of 30-32%,[11,12] but it is not known whether outcomes for these patients are improved in the CAR19 era. It is also unknown whether the transplant experience is different for children treated with CAR19 compared to children who previously would have required intensive chemotherapy to achieve CR3. Specifically, the impact of prior extensive immunotherapy on transplant complications, immune recovery, and long-term outcomes remains undefined.

To address these gaps, we conducted a retrospective review to assess outcomes of HCT for post-CAR19 remission consolidation or treatment of post-CAR19 relapse in children and young adults with B-ALL. The primary objective was to determine 3-year DFS by cohort. Secondary objectives were to describe additional survival and relapse outcomes, the frequency and severity of post-transplant toxicities, and patterns of immune reconstitution.

Methods

Patients and study design

We assembled a retrospective cohort of children and young adults with relapsed/refractory B-ALL who underwent first HCT at Children's Hospital of Philadelphia (CHOP) between 2014-2024 either to consolidate a CAR19-induced remission (preemptive cohort) or treat post-CAR19 B-ALL recurrence (relapse cohort). Preemptive HCT was performed for loss of B-cell aplasia within 6 months of CAR19, detectable measurable residual disease by next-generation sequencing (NGS-MRD) after CAR19 without flow-detectable disease, or as a pre-planned procedure based on patient, family or physician preference. Relapse therapy HCT was performed for morphological relapse or emergence of multiparameter flow cytometry (MFC)-based MRD of >0.01%. Prior CAR19 therapy was administered on one of six clinical trials (CTL019: NCT01626495,[13] NCT02906371,[1] NCT04276870; humanized CAR19: NCT02374333;[2] NCT03792633; brexucabtagene autoleucel: NCT02625480) or with commercial tisagenlecleucel. Data were abstracted from electronic medical records. This study was reviewed and considered exempt from ongoing Institutional Review Board oversight.

Transplant approach

Bone marrow grafts from HLA-matched related donors were used when available. Alternative donor sources included matched unrelated donor bone marrow or peripheral stem cells, or mismatched related donor peripheral stem cells. All peripheral stem cells underwent *ex vivo* partial T-cell depletion using the CliniMACS Plus device (NCT02356653, NCT02323867, NCT03810196).[14,15]

Disease restaging was performed to confirm adequate remission (<0.1% marrow blasts by MFC, central nervous system-1) prior to receipt of myeloablative conditioning with total body irradiation (1,200 cGy), cyclophosphamide and thiotepa. Children <3 years of age were conditioned with clofarabine, thiotepa and melphalan.[16,17] Graft-*versus*-host disease (GvHD) prophylaxis was based on donor and graft source. Additional details about the transplant approach are included in the *Online Data Supplement*.

Study endpoints

The primary endpoint was DFS, defined as time from transplant to relapse or death from any cause. Ponte di Legno Consortium consensus recommendations were used to define relapse.[18] Secondary survival endpoints included overall survival (OS; defined as time from transplant to death from any cause), cumulative incidence of relapse (CIR), and non-relapse mortality (NRM; defined as time to death without relapse). NRM was considered a competing risk for CIR, and relapse was considered a competing event for NRM. Toxicity endpoints included time to neutrophil engraftment, cumulative incidence of GvHD and veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS), frequency of clinically significant infections or non-GvHD severe organ toxicity, and immune reconstitution metrics.

Statistical analysis

Analyses of the preemptive and relapse cohorts were performed separately. Standard descriptive statistics were calculated to summarize patients' characteristics, neutrophil engraftment, toxicities, and immune reconstitution (until relapse). For time-to-event outcomes, patients were followed from day 0 of HCT to the event of interest or last follow-up except where noted, with a data cutoff of January 1, 2025. DFS and OS were evaluated using Kaplan-Meier methods. Relapse, NRM, GvHD, and OS were estimated using the cumulative incidence function. Relapse and transplant-related mortality were considered competing risks for GvHD and VOD/SOS. Analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC,

USA), R v4.4.0., and Stata, version 14.0 (StataCorp, College Station, TX, USA).

Results

Patient, disease and treatment characteristics

Forty-seven patients (median age 13.1 years; range, 2.8-23.5) underwent a first HCT for post-CAR19 remission consolidation (preemptive cohort, N=21) or relapse therapy (relapse cohort, N=26) during the study period (Figure 1). Baseline characteristics are shown in Table 1. The median time from CAR19 infusion to HCT was 5.2 months (range, 2.6-10.4) in the preemptive cohort and 14.2 months (range, 4.7-39.9) in the relapse cohort. Indications for HCT in the preemptive cohort included early loss of B-cell aplasia (N=15, 71%), detectable NGS-MRD (N=1, 5%), or pre-planned consolidative HCT (N=5, 24%). Of the five pre-planned transplants, one was recommended due to treatment with a CD28-containing CAR; the other four were performed electively. In the relapse cohort, 14 (56%) post-CAR19 relapses were CD19-positive and 11 (44%) were CD19-negative. Most relapses occurred only in the bone marrow (N=23, 88%), but three (12%) were combined bone marrow and central nervous system-3 (12%) and one (4%) was isolated to the bones in a patient with a history of B-lymphoblastic lymphoma (who had prior leukemic disease). Five patients had relapse detected by MFC-MRD only, including three who did not meet Ponte di Legno Consortium criteria but were treated as having a relapse by the clinical team. Disease status at HCT was CR1/CR2 for 81% (N=17) of the preemptive cohort; in contrast, disease status was CR3 or beyond for 77% (N=20) of the relapse cohort. One patient in the relapse cohort had detectable MFC-MRD before HCT of 0.027% of mononuclear cells at the pre-HCT evaluation.

Interval between CD19-targeted chimeric antigen receptor T-cell therapy and hematopoietic cell transplantation

In the preemptive cohort, bridging therapies between CAR19 administration and HCT varied by transplant indication. Among the five patients who received a pre-planned consolidative HCT, none received bridging therapy. Of 15 with early loss of B-cell aplasia, six received bridging therapy (low- or medium-intensity cytotoxic chemotherapy, N=4; blinatumomab, N=1; inotuzumab [3 doses], N=1). Eight patients also received at least one CAR19 reinfusion with a goal of prolonging CAR T-cell persistence. The aforementioned patient with emergence of NGS-MRD was bridged with inotuzumab (3 doses).

Various salvage therapies were utilized in the relapse cohort. Eighteen patients in the relapse cohort received inotuzumab between CAR19 and HCT, with three to five doses (N=8), six doses (N=9), and 12 (N=1) doses administered. The therapies that ultimately induced HCT-acceptable remissions included inotuzumab (N=15), cytotoxic chemotherapy (N=5), blinatu-

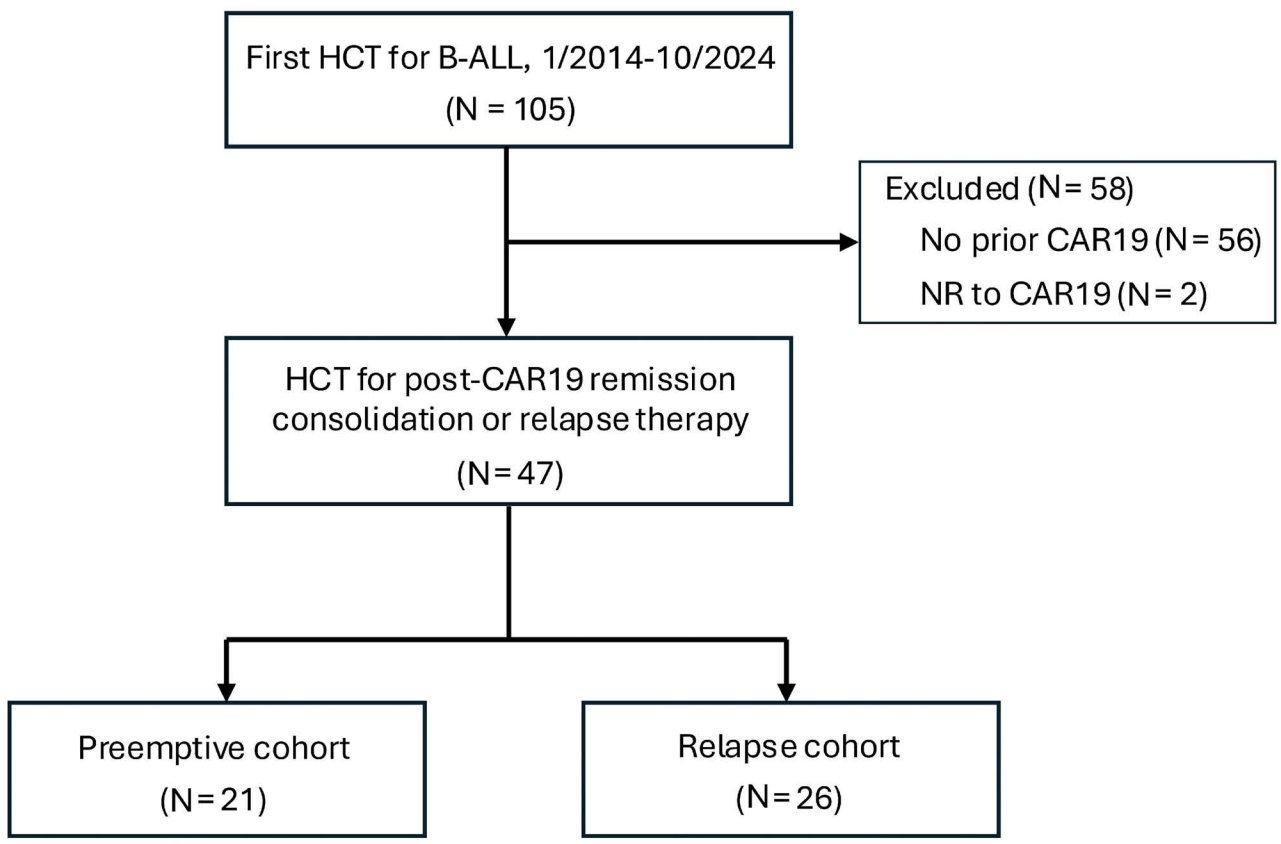


Figure 1. Flow diagram for patient inclusion in the analysis. Of 105 patients who underwent first hematopoietic cell transplantation (HCT) for B-cell acute lymphoblastic leukemia at the Children's Hospital of Philadelphia between January 2014 and October 2024, 56 did not receive prior CD19-directed chimeric antigen receptor T-cell therapy (CAR19) and two did not achieve a complete response to prior CAR19 (eventually achieved a transplantable remission with other antileukemia therapies). Of 47 patients who met the inclusion criteria, 21 underwent HCT for post-CAR19 remission consolidation (preemptive cohort) and 26 for post-CAR19 relapse therapy (relapse cohort). B-ALL: B-cell acute lymphoblastic leukemia; NR: no response.

Table 1. Demographic and clinical characteristics of patients undergoing hematopoietic cell transplantation CD19-targeted chimeric antigen receptor T-cell therapy.

Characteristic	Preemptive cohort N=21	Relapse cohort N=26	Total N=47
Age at HCT, years, median (range)	13.1 (2.8-21.4)	12.8 (4.4-23.5)	13.0 (2.8-23.5)
Female sex, N (%)	4 (19.1)	14 (53.9)	18 (38.3)
Race, N (%)			
White	14 (66.7)	15 (57.7)	29 (61.7)
Black or African American	2 (9.5)	5 (19.2)	7 (14.9)
Asian	0 (0.0)	1 (3.9)	1 (2.3)
Other	5 (23.8)	5 (19.2)	10 (21.8)
Hispanic ethnicity, N (%)	3 (14.3)	7 (26.9)	9 (22.0)
Down syndrome, N (%)	0 (0.0)	3 (11.5)	3 (6.4)
CAR19 product, N (%)			
Investigational CTL019	7 (33.3)	5 (19.2)	12 (25.5)
Humanized CART19	8 (38.1)	11 (4.2)	19 (40.4)
Commercial tisagenlecleucel	5 (2.4)	10 (38.5)	15 (31.2)
Brexucabtagene autoleucel	1 (4.8)	0 (0.0)	1 (2.1)
Prior inotuzumab, N (%)	3 (14.3)	19 (73.0)	22 (46.8)
Pre-CAR19 BM disease burden, N (%)			
MRD-negative (<0.01% blasts)	8 (38.1)	8 (30.8)	16 (34.0)
0.01-5% blasts	6 (28.6)	7 (26.9)	13 (27.7)
≥5% blasts	7 (33.3)	11 (44.0)	18 (38.3)
Indication for preemptive HCT, N (%)			
Pre-planned consolidative HCT	5 (23.8)	-	-
Early loss of B-cell aplasia	15 (71.4)	-	-
Detectable NGS-MRD	1 (4.8)	-	-
CD19 immunophenotype at post-CAR19 relapse, N (%)			
CD19-positive	-	14 (56.0)	-
CD19-negative	-	11 (44.0)	-
Post-CAR19 relapse sites, N (%)			
MFC-MRD level BM only	-	5 (19.2)[a]	-
Isolated morphological BM	-	18 (69.2)	-
Isolated extramedullary[b]	-	1 (3.8)	-
Combined BM and CNS3	-	3 (11.5)	-
Interval from CAR19 to post-CAR19 relapse, months, median (range)	-	7.9 (1.9-37.3)	-
Interval from CAR19 to HCT, months, median (range)	5.2 (2.6-10.4)	14.2 (4.7-39.9)	8.1 (2.6-39.9)
Clinical stage at HCT, N (%)			
CR1	9 (42.9)	0 (0.0)	9 (19.2)
CR2	8 (38.1)	6 (23.1)	14 (29.8)
CR3	4 (19.1)	13 (50.0)	17 (36.1)
CR4	0 (0.0)	6 (23.1)	6 (12.3)
≥CR5	0 (0.0)	1 (3.9)	1 (2.1)
Positive MFC-MRD (>0.01%) pre-HCT, N (%)	0 (0.0)	1 (3.8)	1 (2.1)
NGS-MRD pre-HCT, N (%)	8 patients	12 patients	20 patients
NGS-MRD negative	4 (50.0)	7 (58.3)	11 (55.0)
NGS-MRD positive below limit of detection	4 (50.0)	3 (25.0)	7 (35.0)
NGS-MRD quantifiable	0 (0.0)	2 (16.7)	2 (10.0)
Conditioning regimen, N (%)			
TBI/Cy/Thio	19 (90.5)	26 (100.0)	35 (95.7)
Clo/Mel/Thio	2 (9.5)	0 (0.0)	2 (4.3)
Receipt of thymoglobulin, N (%)	6 (28.6)	7 (26.9)	13 (27.7)

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Transplant type, N (%) Matched related BM Mismatched related PSCT Unrelated BM Unrelated PSCT	 7 (33.3) 3 (14.3) 3 (14.3) 8 (38.1)	 5 (19.2) 5 (19.2) 2 (7.7) 14 (53.8)	 12 (25.5) 8 (17.0) 5 (10.6) 22 (46.8)
Ex-vivo T-cell depletion, N (%)	11 (52.4)	19 (73.1)	30 (63.8)
Receipt of rituximab on day +1, N (%)	10 (47.6)	20 (76.9)	30 (63.8)

[a]Two of five patients met the Ponte-di-Legno consortium relapse definition; 3/5 started relapse therapy prior to meeting the consortium definition. [b]Disease sites included multiple bones. HCT: hematopoietic cell transplant; CAR19: CD19-directed chimeric antigen receptor T-cell therapy; BM: bone marrow; MRD: minimal residual disease; NGS-MRD; minimal residual disease as measured by next-generation sequencing; MFC-MRD: minimal residual disease as measured by multiparameter flow cytometry; CNS: central nervous system; CR: complete remission; TBI/Cy/Thio: total body irradiation, cyclophosphamide, and thiotepa; Clo/Mel/Thio: clofarabine, melphalan, and thiotepa; PSCT: peripheral stem cell transplant.

momab (N=2), CD22-targeted CAR (N=2), CAR19 reinfusion (N=1), and pembrolizumab (N=1, patient with lymphomatous relapse).

Relapse and survival outcomes

In the preemptive cohort, the median follow-up was 50 months from transplant. Three-year DFS was 90% (95% confidence interval [95% CI]: 78-100) and 3-year OS was 95% (95% CI: 87-100) (Figure 2A). The 3-year CIR rate was 5% (95% CI: 0-22) and the cumulative incidence of NRM by 6 months was 5% (95% CI: 0-20) (Figure 2B). The one NRM event was due to disseminated adenovirus.

In the relapse cohort, the median follow-up was 48 months from transplant. The 3-year DFS was 64% (95% CI: 48-86) overall (Figure 2C), 73% (95% CI: 53-100) for CD19-positive disease and 55% (95% CI: 32-94) for CD19-negative disease (*Online Data Supplement*). The 3-year OS was 67% (95% CI: 50-89) (Figure 2C). The 3-year CIR rate was 20% (95% CI: 7-38) and the cumulative incidence of NRM by 6 months was 15% (95% CI: 5-32) (Figure 2D). No NRM occurred after 6 months. Causes of NRM included multisystem organ failure due to VOD/SOS (N=2), multisystem organ failure in the setting of grade 4 GvHD (N=1) and disseminated adenovirus (N=1).

Pre-transplant minimal residual disease detected by next-generation sequencing

Twenty patients (preemptive, N=8; relapse, N=12) had NGS-MRD assessed before HCT using the clonoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA, USA)[19] (Table 1). In the preemptive cohort, four of four patients with negative NGS-MRD (0 clones) remained in remission during the follow-up period. Three of four with positive NGS-MRD below the limit of detection remained in remission, and the other died of NRM. No patients had quantifiable NGS-MRD. In the relapse cohort, seven of seven patients with negative NGS-MRD remained in remission. Two of three with NGS-MRD below the limit of detection remained in remission, while the other died of NRM. Both patients with quantifiable NGS-MRD relapsed after HCT (*Online Supplementary Figure S2*).

Engraftment and graft-*versus*-host disease

The median time to neutrophil engraftment was 16 days (range, 11-22) in the preemptive cohort and 13 days (range, 9-19) in the relapse cohort. No patients experienced primary or secondary graft failure.

The cumulative incidences of clinically significant (grade 2-4) and severe (grade 3-4) acute GvHD were 33% (95% CI: 14-54) and 14% (95% CI: 3-33), respectively, in the preemptive cohort; and 31% (95% CI: 14-49) and 19% (95% CI: 7-36), respectively, in the relapse cohort. Among patients who were alive at 100 days after HCT, five of 21 (24%) patients in the preemptive cohort and three of 21 (14%) patients in the relapse cohort developed chronic GvHD requiring systemic immunosuppression.

Veno-occlusive disease/sinusoidal obstruction syndrome and organ toxicity

Ten patients developed VOD/SOS at a median of 11 days (range, 7-20) after HCT, two in the preemptive cohort and eight in the relapse cohort (Table 2). The cumulative incidences of VOD/SOS by day +30 were 10% (95% CI: 0-21) and 31% (95% CI: 11-46) in the preemptive and relapse cohorts, respectively (Figure 3C). VOD/SOS occurred in seven of 20 (35%) patients treated with inotuzumab between CAR19 and HCT as compared to three of 27 (11%) inotuzumab-unexposed patients. The two VOD/SOS cases in the preemptive cohort were not associated with other organ failure whereas three of eight cases in the relapse cohort were complicated by respiratory failure requiring invasive mechanical ventilation and renal failure requiring renal replacement therapy.

Non-GvHD severe organ toxicities included transplant-associated microangiopathy in four patients (preemptive, N=3; relapse, N=1), pulmonary toxicity in nine (preemptive, N=2; relapse, N=7), bleeding in three (preemptive, N=2; relapse, N=1), and neurological toxicity in two (preemptive, N=1 [pseudotumor cerebri]; relapse, N=1 [posterior reversible encephalopathy syndrome]).

Infections and immune reconstitution

Viral infections occurred commonly; 22 (46.8%) patients developed at least one viral infection that required treat-

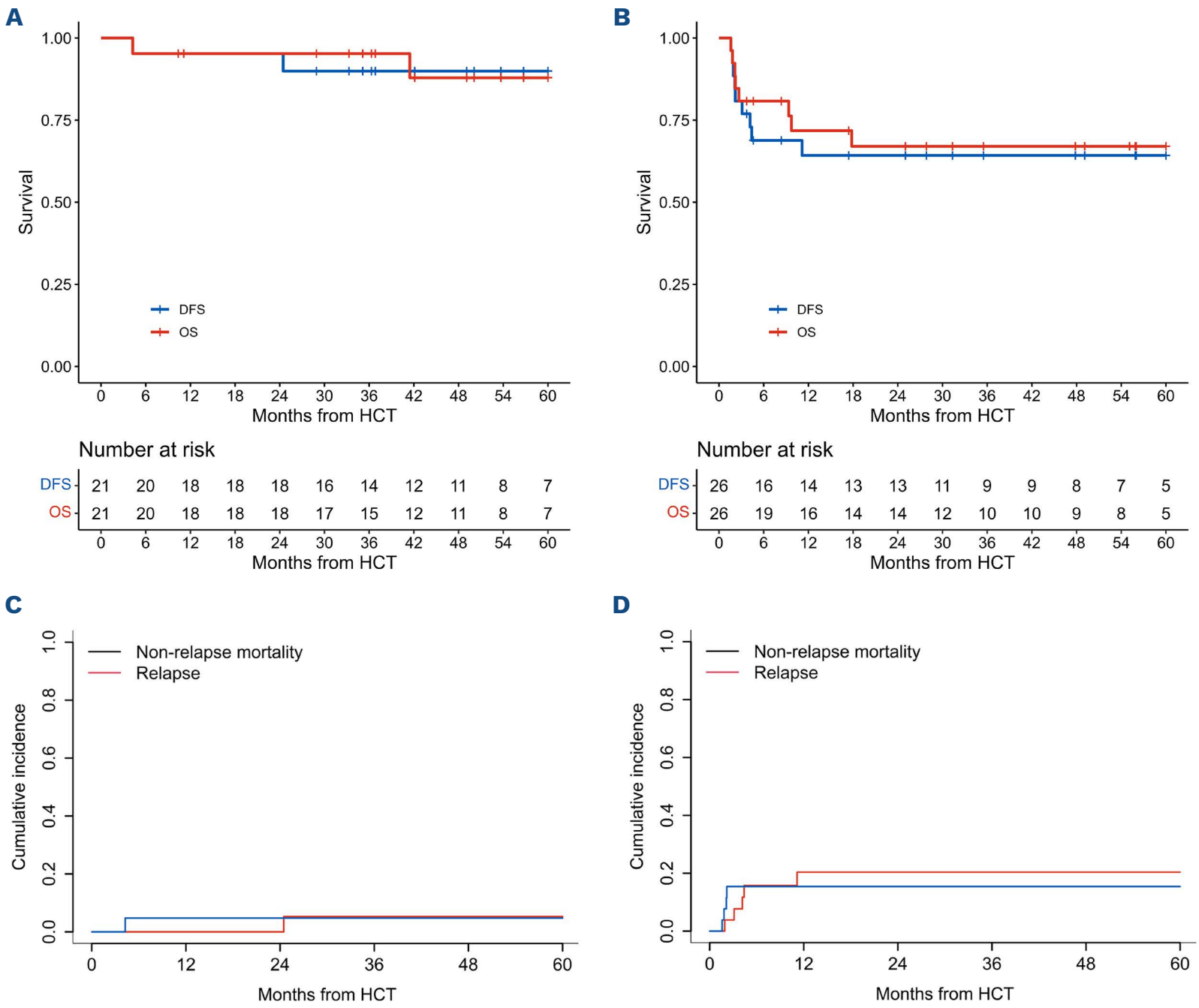


Figure 2. Survival outcomes among patients who underwent hematopoietic cell transplantation for post-CD19-targeted chimeric antigen receptor T-cell remission consolidation or relapse therapy. (A) Disease-free survival (DFS) and overall survival (OS) for the preemptive cohort (N=21). DFS was defined as time from transplant to relapse or death from any cause. OS was defined as time from transplant to death from any cause. (B) DFS and OS for the relapse cohort (N=26). (C) Cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) for the preemptive cohort. For CIR, NRM was considered as a competing risk. NRM was defined as time from transplant to death without relapse, with relapse considered as a competing risk. (D) CIR and NRM for the relapse cohort. Data were censored at the data cutoff of January 1, 2025. HCT: hematopoietic cell transplantation.

ment, ten of 21 (47.6%) in the preemptive cohort and 12 of 26 (46.2%) in the relapse cohort (Table 2). Cytomegalovirus (N=15), adenovirus (N=6), and BK virus (N=6) were the most frequent. In addition, one patient in each cohort developed a possible pulmonary fungal infection. The patient in the preemptive cohort had progression of lung nodules that pre-dated the transplant and improved with antifungal medication only. The patient in the relapse cohort developed cavitary lung nodules and died of multisystem organ failure before additional diagnostics were obtained.

Cellular immune reconstitution was assessed at 4, 8, 12 and 24 months (Figure 4). T-cell immune reconstitution was qualitatively similar across cohorts; 61%, 91% and 96% of patients achieved absolute $CD3^+/CD4^+$ counts >200 cells/μL by 6 months, 1 year and 2 years after transplant, respectively. B-cell immune reconstitution was slower, with most patients achieving normal $CD19^+$ counts (>200 cells/μL) and detectable switched memory B cells by 1 year after transplant. Despite quantitatively normal B-cell numbers, long-term immunoglobulin replacement dependence was common to maintain serum immunoglobulin G (IgG) levels ≥400 mg/dL; nine of 20 (45%) patients still required replacement at 4 years after HCT (preemptive, 4/11; relapse, 5/9).

Discussion

Identifying strategies to prevent and treat post-CAR19 B-ALL relapse is critical for optimizing this transformative therapy in children and young adults. Allogeneic HCT is a

Table 2. Infections and severe organ toxicities of special interest.

Outcomes	Preemptive cohort N=21	Relapse cohort N=26	Total N=47
Non-GvHD severe organ toxicity, N (%)			
VOD/SOS, any grade Grade ≥4 VOD/SOS VOD/SOS with other organ failure	2 (9.5) 1 (4.8) 0 (0.0)	8 (30.8) 7 (26.9) 3 (11.5)	10 (21.3) 8 (17.0) 4 (8.5)
Transplant-associated microangiopathy	3 (14.3)	1 (3.8)	4 (8.5)
Pulmonary toxicity	2 (9.5)	7 (26.9)	9 (19.1)
Cardiac toxicity	1 (4.8)	0 (0.0)	1 (2.1)
Neurological toxicity	1 (4.8)[a]	1 (3.8)[b]	2 (4.3)
Bleeding	2 (10.5)[c]	1 (3.8)	3 (6.1)
Other	0 (0.0)	1 (3.8)[d]	1 (2.1)
Infection, N (%)			
Clinically significant viral infection Cytomegalovirus Epstein-Barr virus Adenovirus BK virus Varicella-zoster virus Human herpesvirus 6	10 (47.6) 7 (33.3) 2 (9.5) 1 (4.8) 2 (9.5) 1 (4.8) 0 (0.0)	12 (46.2) 8 (30.8) 2 (7.7) 5 (19.2) 4 (15.4) 1 (3.8) 1 (3.8)	22 (46.8) 15 (31.2) 4 (8.5) 6 (12.3) 6 (12.3) 2 (4.3) 1 (2.1)
Invasive fungal disease (possible or probable)	1 (4.8)	1 (3.8)	2 (4.3)

[a]*Pseudotumor cerebrii*. [b]Posterior reversible encephalopathy syndrome. [c]Duodenal hematoma (N=1), retinal hemorrhage (N=1). [d]Stevens-Johnson syndrome. GvHD: graft-*versus*-host disease; VOD/SOS: veno-occlusive disease/sinuosoidal obstruction syndrome.

key tool in the armamentarium for both relapse prevention and relapse therapy. We report that children who proceeded to their first HCT for CAR19 remission consolidation for early loss of B-cell aplasia, pre-planned consolidation, or emergent NGS-MRD, had remarkably high 3-year DFS (90%) and OS (95%) rates. For children who underwent first HCT as part of post-CAR19 relapse therapy, 3-year DFS (64%) and OS (67%) were higher than expected given that most underwent transplant in CR3 or beyond.

Survival outcomes after first HCT for CAR19 remission consolidation were excellent. The 3-year CIR of 5% and NRM of 5% compare favorably to results in large, contemporary cohorts of CAR19-unexposed children undergoing HCT for relapsed B-ALL.[20-22] Comparable HCT outcome data in CAR19-exposed children do, however, remain limited. Seattle Children's Hospital reported similarly impressive outcomes for first HCT for CAR19 remission consolidation, with 12 of 13 patients achieving long-term DFS.[6] The National Cancer Institute and the Pediatric Real World CAR Consortium also reported promising survival outcomes, albeit at slightly lower rates than in our study. Of note, these analyses did not stratify outcomes by first or second HCT, potentially contributing to the observed survival differences.[4,7] Importantly, all patients in our study were HCT-naïve and in deep MFC-MRD-negative remissions at the time of transplant. All eight patients who underwent pre-transplant NGS-MRD testing were negative or below the limit of detection. This likely contributed to the very low relapse rate. Notwithstanding the outstanding survival and relapse rates, the morbidity associated with HCT remains significant, although similar to that in CAR-naïve patients. Three of 21 patients developed severe acute GvHD, two had VOD/SOS, several more had other severe organ toxicities, and one died of transplant-related complications. Differentiating which patients need consolidative HCT from those who can be cured with CAR19 alone is a major imperative.

We also report encouraging survival outcomes after first HCT for post-CAR19 B-ALL relapse in a very high-risk population. Despite 50% of the cohort being in CR3 and another 27% in CR4 or beyond, DFS, CIR and NRM rates were comparable to those in contemporary cohorts of children transplanted in CR2.[20-22] The 3-year DFS of 64% is a substantial improvement over historical DFS rates of 30-32% for children transplanted in CR3.[11,12] The promising DFS was, however, accompanied by a significant toxicity profile. Four of 26 patients experienced early NRM by day +65. Grade 4 VOD/SOS occurred in seven patients (27%), severe pulmonary toxicity in seven (27%), and severe acute GvHD in five (19%). The toxicity burden is likely reflective of the significant treatment history of this cohort of patients who came to transplant late in the disease course and required additional rounds of treatment to attain remission after the post-CAR19 relapse. Inotuzumab, which was used in 18 of 26 (70%) patients, likely contributed to

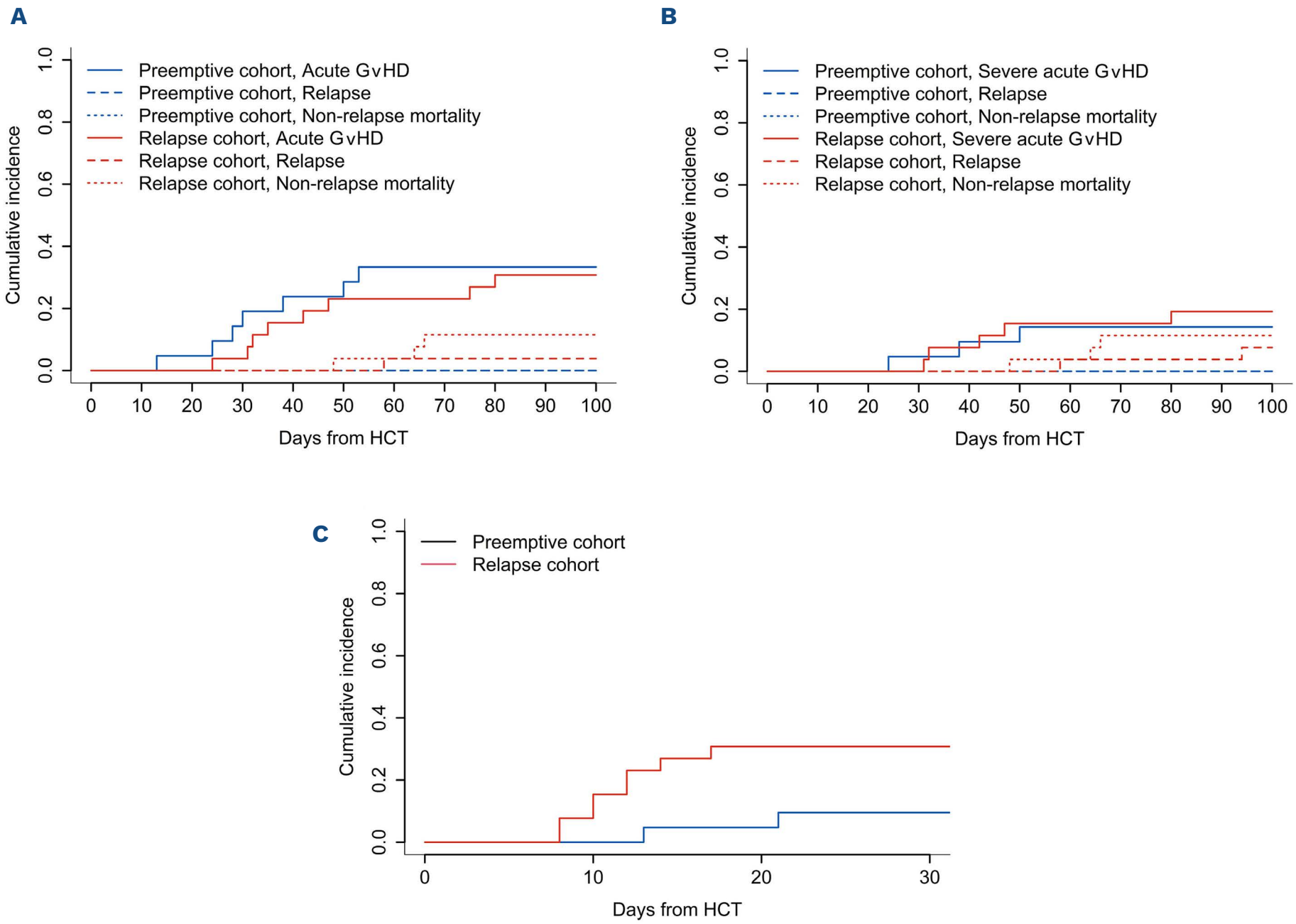


Figure 3. Cumulative incidence of graft-*versus*-host disease and veno-occlusive disease/sinusoidal obstruction syndrome among patients who underwent hematopoietic cell transplantation for post-CD19-targeted chimeric antigen receptor T-cell remission consolidation or relapse therapy. (A) Cumulative incidence of grade ≥2 acute graft-*versus*-host disease (GvHD) from hematopoietic cell transplantation (HCT) to day +100. (B) Cumulative incidence of severe (grade ≥3) acute GvHD from HCT to day +100. (C) Cumulative incidence of veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) from HCT to day +30. No VOD/SOS events were observed after day +30. Non-relapse mortality (NRM) was considered a competing event, but no NRM occurred prior to day +30.

the high incidence of VOD/SOS.[23,24] Notably, all patients with NRM were adolescents or young adults, corroborating prior HCT studies showing inferior survival for adolescents or young adults compared to younger children.[25] We also note that due to post-CAR19 disease surveillance protocols, some patients in this cohort had very early identification of relapse. These included three patients with low-level, MFC-MRD who did not meet the Ponte-di-Legno consortium threshold, but were determined to have relapse by the clinical team; therefore, salvage therapy was initiated prior to progression to overt relapse. In interpreting these results, it is important to recognize that our study only included children who achieved a transplantable remission after post-CAR19 relapse. It is unknown how many could not be successfully bridged to HCT. As such, these survival estimates cannot be applied to the overall post-CAR19 relapse population. Nevertheless, for patients who do enter another remission, these results demonstrate that HCT can be an effective and definitive component of salvage therapy.

Although toxicity and survival appeared to be more favorable in the preemptive cohort than the relapse cohort, the cohorts are not directly comparable given marked differences in patient populations. Patients in the preemptive cohort maintained CAR19-induced remissions to transplant; it is unknown how many would have remained in durable remissions without HCT. Though early loss of B-cell aplasia, which was the HCT indication for 71%, has been associated with a higher relapse risk, relapse is not universal.[26,27] For 24% of the cohort, consolidative HCT was pre-planned based on either CAR construct (N=1) or on patient, family, or physician preference, which was not

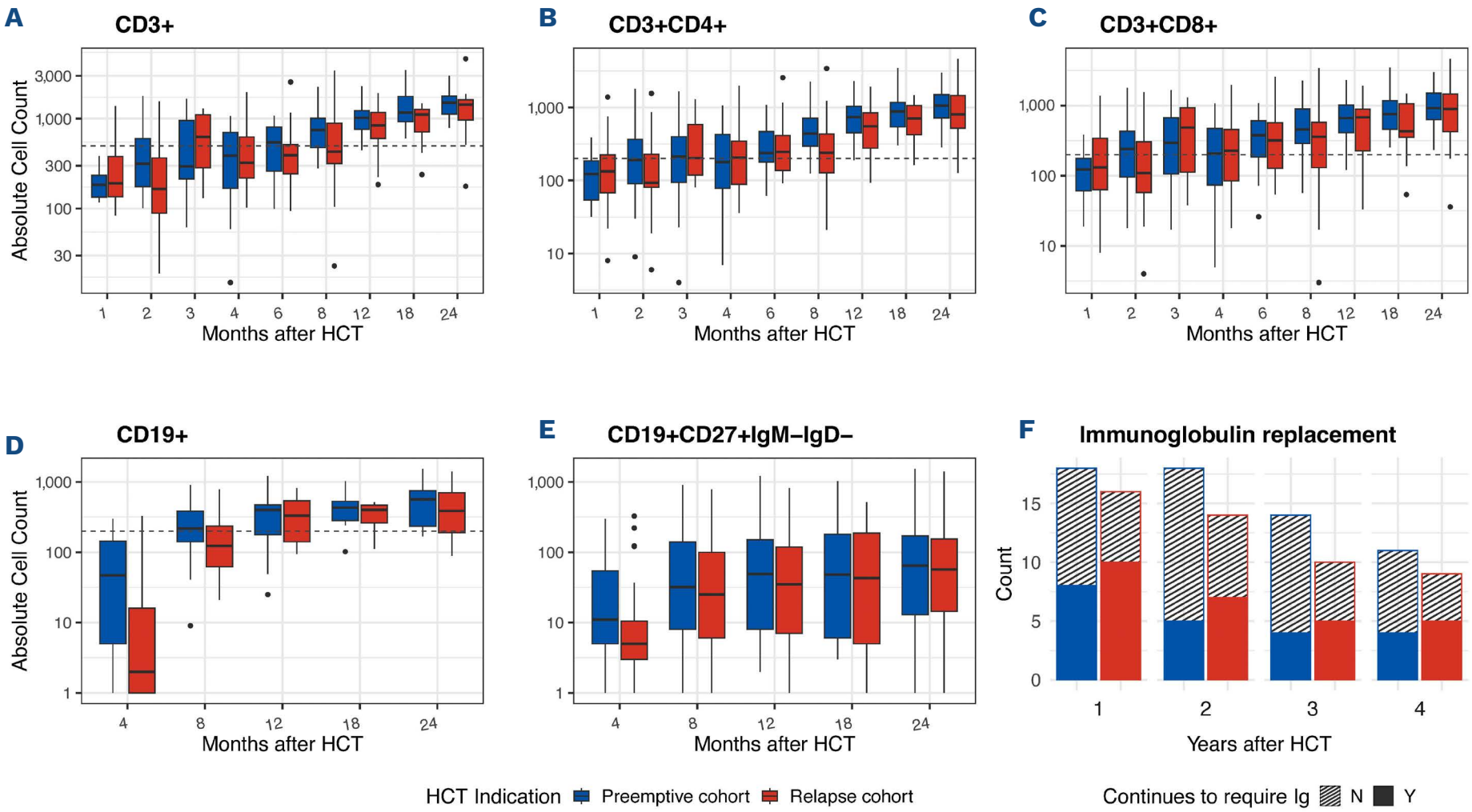


Figure 4. Immune reconstitution after hematopoietic cell transplantation. (A-E) Box and whisker plots displaying cellular immune reconstitution from 1 month to 24 months after transplant. Boxes show the median, first quartile and third quartile absolute cell counts. Whiskers represent the data ranges and dots represent outliers. Reference lines depict clinically relevant values: 500 cells (A), 200 cells (B-D). (F) Frequency of patients requiring routine immunoglobulin replacement at 1, 2, 3, and 4 years after transplant. The proportion of patients requiring replacement is shown in solid colors and the proportion not requiring replacement is shown with diagonal lines. For each panel, the preemptive cohort is shown in blue and the relapse cohort in red. Data collection ended at the time of relapse. HCT: hematopoietic cell transplant; Ig: immunoglobulins.

necessarily reflective of relapse risk. In contrast, patients in the relapse cohort proved to have CAR19-refractory disease and, thus, would be expected to be at high risk for relapse after HCT. Additionally, children in the relapse cohort came to transplant later in the disease course, so were more heavily pretreated than those in the preemptive cohort. Without the ability to make direct comparisons between cohorts, this study cannot be interpreted to indicate that patients should undergo HCT preemptively instead of after relapse; rather, our data show promising outcomes after first HCT even for patients who suffer relapse again after CAR19 therapy.

The patterns of immune reconstitution in this group of patients transplanted after CAR19 were unusual, regardless of cohort. Despite relatively rapid recovery of T- and B-cell counts, including evidence of class switching as early as 8 months after HCT, almost half of patients still required immunoglobulin replacement to maintain IgG >400 mg/dL, even 4 years after HCT. This pattern is distinct from the immune reconstitution observed after transplant for hematologic malignancies more broadly. Prolonged immunoglobulin dependence is described with early administration of CD20-directed antibodies after HCT, potentially due to impaired non-intrinsic effects on differentiation and isotype switching.[28] Two-thirds of patients in this study received prophylactic rituximab on day +1 to prevent Epstein-Barr virus infection, which may have contributed to immunoglobulin dependence. However, in a prior report from our center describing immune reconstitution after *ex vivo* T-cell depletion in hematologic malignancies inclusive of those with non-B-cell histology, >80% of patients received rituximab and were able to discontinue immunoglobulin replacement at a median of 8 months. Thus, the proportion of patients in this study with ongoing immunoglobulin dependence at 4 years is notable. Further research is needed to understand whether the pre-HCT CAR19 exposure contributed to this finding.

This study is limited by its retrospective design. The relatively small sample size did not allow for in-depth analyses of factors potentially associated with improved outcomes, including variations in pre-transplant therapy, donor and graft sources, and GvHD prophylaxis. The number of patients with pre-transplant NGS-MRD testing was limited as this testing was not standard practice at our center until 2020, which precluded more specific analysis of the potential impact of NGS-MRD results.[29,30] Nonetheless, this

analysis is one of the first to describe detailed safety and HCT outcomes in this population and provides important data to guide clinical decision making.

In conclusion, first HCT for post-CAR19 remission consolidation is associated with outstanding DFS and low NRM. HCT-related morbidity was, however, considerable, so prospective efforts to identify patients at highest risk of relapse with CAR19 as standalone therapy are critical. First HCT for post-CAR19 relapse therapy was also associated with encouraging DFS and NRM rates that are comparable to the broader HCT for B-ALL experience, even when the transplant is performed in CR3 or beyond. Although the overall toxicity profile mirrored the toxicity profile of HCT for children without prior CAR19 exposure, patterns of immune reconstitution were unique and warrant further study. HCT is a viable and effective strategy for attaining durable remissions when used preemptively after CAR19 or for salvage for post-CAR19 relapse.

Disclosures

CJD has received consulting fees from Merck. CWE has received honoraria from Miltenyi Biotec and Pierre Fabre Group and research funding from Jazz Pharmaceuticals, all for unrelated studies. SAG has received clinical trial support from Novartis, Servier, Cellectis, Vertex and Kite Pharma; has served on study steering committees, scientific advisory boards and/or consulted for Novartis, Allogene, Adaptive, Cabaletta, CRISPR/Vertex, Estrella, Eureka, BiolineRx, Gamida Cell, Beam and Verismo and has CAR T toxicity management patents managed by U Penn policies. SPH owns common stock in Amgen and has received honoraria from Jazz and Servier. SLM has received clinical trial support from Novartis and Wugen, served on advisory boards or study steering committees for Novartis, Wugen and Syndax and has a patent pending and licensed to Novartis Pharmaceuticals without royalty. SRR has consulted for AbbVie and Pfizer and her spouse works for OptiNose. SKT has received research funding from Incyte Corporation and Kura Oncology; has served on advisory boards for Aleta Biotherapeutics, AstraZeneca, C-Further/LifeArc, Jazz Pharmaceuticals, Kestrel Therapeutics, Syndax Pharmaceuticals and Wugen, Inc and received travel support from Amgen and Jazz Pharmaceuticals.

Contributions

RMM, AES and CWE conceptualized, designed and planned the study. RMM, SM, AES and CWE collected the data. RMM, YL, HL and LW performed the statistical analysis. All authors reviewed the analyses, contributed to interpretation of results and writing of the manuscript, and approved the final version of the submitted report.

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Data-sharing statement

De-identified, individual participant data may be shared with investigators. To access data, investigators will be required to provide a methodologically sound proposal with approved aims. Data will only be shared if this does not compromise an ongoing trial or study, if there is strong scientific rationale for the data to be used for the requested purpose and if the investigators who have invested time and efforts into development these trials have a period of exclusivity to pursue their own aims with the data. Proposals should be directed to the corresponding authors. To gain access, data requestors will need to sign a data use agreement.

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