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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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Data sharing: 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 it 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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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, a paucity of detailed HCT safety and outcome data exists 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% in the preemptive cohort, respectively, and 67%, 20%, and 15% in the relapse cohort, respectively. The cumulative incidence of grade III-IV acute graft-versus-host disease (GVHD) was 14% in the preemptive 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 and 31% in the relapse cohort. In appropriate patients, HCT can be an effective strategy for attaining durable B-ALL remission when used preemptively post-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 to HCT for remission consolidation in children with limited CAR T cell persistence after CD19/4-1BB-based CARs (6) or in children treated with CD19/CD28-based CARs, (7) which are inherently shorter-persisting. (8) The benefit of preemptive HCT for other patient sub-groups is unclear. The overall prognosis of children with post-CAR19 relapse is dismal, with a median survival of approximately one year. (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, a paucity of HCT-related toxicity and outcome data exists 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 CR1 or CR2. Limited historic data of children transplanted in CR3 found long-term disease-free survival (DFS) rates of 30-32%, (11, 12) but it is not known if outcomes for these patients are improved in the CAR19 era. It is also unknown if 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 morphologic 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 by the Institutional Review Board at CHOP.

Transplant approach

Bone marrow grafts from HLA-matched related donors (MRDs) were used when available. Alternative donor sources included matched unrelated donor (URD) bone marrow or peripheral stem cells (PSC), or mismatched related donor (MMRD) PSCs. All PSCs 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 multiparameter flow cytometry (MFC), central nervous system (CNS)-1] prior to receipt of myeloablative conditioning with total body irradiation (TBI; 1200 cGy), cyclophosphamide and thiotepa. Children <3 years of age received clofarabine, thiotepa and melphalan. (16, 17) GVHD prophylaxis was based on donor and graft source. Additional details about transplant approach are included in the Data Supplement.

Study endpoints

The primary endpoint was disease-free survival (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 graft-versus-host disease (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 patient 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 TRM were considered competing risks for GVHD and VOD/SOS. Analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC), R v4.4.0., and Stata, version 14.0 (StataCorp, College Station, TX).

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 (pre-emptive cohort, n=21) or relapse therapy (relapse cohort, n=26) during the study period (Fig. 1). Baseline characteristics appear in Table 1. 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 5 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 3 (12%) were combined bone marrow and CNS3 (12%) and 1 (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 3 who did not meet Ponte di Legno Consortium criteria but were treated as 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 for 77% (n=20) of the relapse cohort. One patient in the relapse cohort had detectable MFC MRD pre-HCT of 0.027% of mononuclear cells on pre-HCT evaluation.

Interval therapy between CAR19 and HCT

In the preemptive cohort, bridging therapies between CAR19 administration and HCT varied by transplant indication. Among the 5 patients who received a pre-planned consolidative HCT, none received bridging therapy. Of 15 with early loss of B cell aplasia, 6 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 3-5 (n=8), 6 (n=9), and 12 (n=1) total doses administered. The therapies that ultimately induced HCT-acceptable remissions included: inotuzumab (n=15), cytotoxic chemotherapy (n=5), blinatumomab (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, median follow-up was 50 months from transplant. Three-year DFS was 90% (95% CI, 78-100) and 3-year OS was 95% (95% CI, 87-100) (Fig. 2A). The 3-year CIR rate was 5% (95%, 0-22) and the cumulative incidence of NRM by 6 months was 5% (95% CI, 0-20) (Fig. 2B). The one NRM event was due to disseminated adenovirus.

In the relapse cohort, median follow-up was 48 months from transplant. Three-year DFS was 64% (95% CI, 48-86) overall (Fig. 2C). For CD19-positive disease, DFS at 3 years was 73% (95% CI, 53-100), and for CD19-negative disease, DFS was 55% (95% CI, 32-94) (Data Supplement). Three-year OS was 67% (95% CI, 50-89) (Fig. 2C). The 3-year CIR rate was 20% (95% CI, 7-38) and cumulative incidence of NRM by 6 months was 15% (95% CI, 5-32) (Fig. 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 IV GVHD (n=1) and disseminated adenovirus (n=1).

Pre-transplant NGS-MRD

Twenty patients (preemptive, n=8; relapse, n=12) had NGS-MRD assessed pre-HCT using the clonoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA) (19) (Table 1). In the preemptive cohort, 4/4 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 (LOD) remained in remission, and the other died of NRM. No patients had quantifiable NGS-MRD. In the relapse cohort, 7/7 patients with negative NGS-MRD remained in remission. Two of three with NGS-MRD below the LOD remained in remission, while the other died of NRM. Both patients with quantifiable NGS-MRD relapsed post-HCT (Fig. S2).

Engraftment and graft-versus-host disease

Median time to neutrophil engraftment was 16 days (range, 11-22) in the preemptive 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 II-IV) and severe (grade III-IV) 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 post-HCT, 5/21 (24%) patients in the preemptive and 3/21 (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) post-HCT, 2 in the preemptive, and 8 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 (Fig. 3C). VOD/SOS occurred in 7/20 (35%) of patients treated with inotuzumab between CAR19 and HCT as compared to 3/27 (11%) inotuzumab-unexposed patients. The two VOD/SOS cases in the preemptive cohort were not associated with other organ failure whereas 3/8 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 4 patients (preemptive, n=3; relapse, n=1), pulmonary toxicity in 9 (preemptive, n=2; relapse, n=7), bleeding in 3 (preemptive, n=2; relapse, n=1), and neurologic toxicity in 2 [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 treatment, 10/21 (47.6%) in the preemptive and 12/26 (46.2%) in the relapse cohort (Table 2). CMV (n = 15), adenovirus (n = 6), and BK virus (n = 6) were 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 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 six months, one year, and two years post-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 one year post-transplant. Despite quantitatively normal B cell numbers, long-term immunoglobulin replacement dependence was common to maintain serum Immunoglobulin G (IgG) levels ≥400mg/dL; 9/20 (45%) still required replacement at 4 years post-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 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%). 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 higher.

Survival outcomes after first HCT for CAR19 remission consolidation were excellent. The 3-year CIR of 5% and NRM of 5% compare favorably to large, contemporary cohorts of CAR19-unexposed children undergoing HCT for relapsed B-ALL. (20-22) Comparable HCT outcome data in CAR19-exposed children remains limited, however. Seattle Children's reported similarly impressive outcomes for first HCT for CAR19 remission consolidation, with 12/13 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 with pre-transplant NGS-MRD testing were negative or below the LOD. This likely contributed to the very low relapse rate. Notwithstanding the outstanding survival and relapse rates, the morbidity associated with HCT remains significant, though

similar to 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 versus which 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 in CR3 and another 27% in CR4 or beyond, DFS, CIR and NRM rates were comparable to contemporary cohorts of children transplanted in CR2. (20-22) Three-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 accompanied by a significant toxicity profile, however. 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 that 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 18/26 (70%) patients, likely contributed to the high incidence of VOD/SOS. (23, 24) Notably, all patients with NRM were adolescents or young adults (AYA), corroborating prior HCT studies showing inferior survival for AYAs 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. This included 3 patients with low-level, flow-based MRD that did not meet the PDL-defined threshold, but were determined to represent 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 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 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 >400mg/dL, even 4 years post-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.[33] Two-thirds of patients in this study received prophylactic rituximab on day +1 to prevent EBV 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 non-B cell histologies, >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 contributes 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. (28, 29) 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 considerable, though, so prospective efforts to identify patients at highest risk for 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 transplant is performed in ≥CR3. 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.

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Table 1. Demographic and clinical characteristics of patients undergoing HCT after CAR19

Characteristic	Preemptive cohort (n = 21)	Relapse cohort (n = 26)	Total (n = 47)
Median age at HCT (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 bone marrow 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 bone marrow only	--	5 (19.2)[a]	--
Isolated morphologic bone marrow	--	18 (69.2)	--
Isolated extramedullary[b]	--	1 (3.8)	--
Combined bone marrow and CNS3	--	3 (11.5)	--
Median months from CAR19 to post-CAR19 relapse (range)	--	7.9 (1.9-37.3)	--
Median months from CAR19 to HCT (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)
Transplant type, n (%)			
Matched related marrow	7 (33.3)	5 (19.2)	12 (25.5)
Mismatched related PSCT	3 (14.3)	5 (19.2)	8 (17.0)
Unrelated marrow	3 (14.3)	2 (7.7)	5 (10.6)
Unrelated PSCT	8 (38.1)	14 (53.8)	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]2/5 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

Abbreviations: CAR19, CD19-directed chimeric antigen receptor T-cell therapy; Clo/Mel/Thio, clofarabine, melphalan, and thiotepa; CNS, central nervous system; CR, complete remission; EM, extramedullary; HCT, hematopoietic cell transplant; huCAR19, humanized CAR19; MFC MRD, minimal residual disease as measured by multiparameter flow cytometry; NGS MRD; minimal residual disease as measured by next generation sequencing; PSCT, peripheral stem cell transplant; TBI/Cy/Thio, total body irradiation, cyclophosphamide, and thiotepa

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

[a]Pseudotumor cerebrii
[b]Posterior reversible encephalopathy syndrome
[c]Duodenal hematoma (n=1), diffuse gastrointestinal bleed (n=1), retinal hemorrhage (n=1)
[d]Stevens-Johnson syndrome
Abbreviations: VOD/SOS, Veno-occlusive disease/sinuosoidal obstruction syndrome

Figure Legends

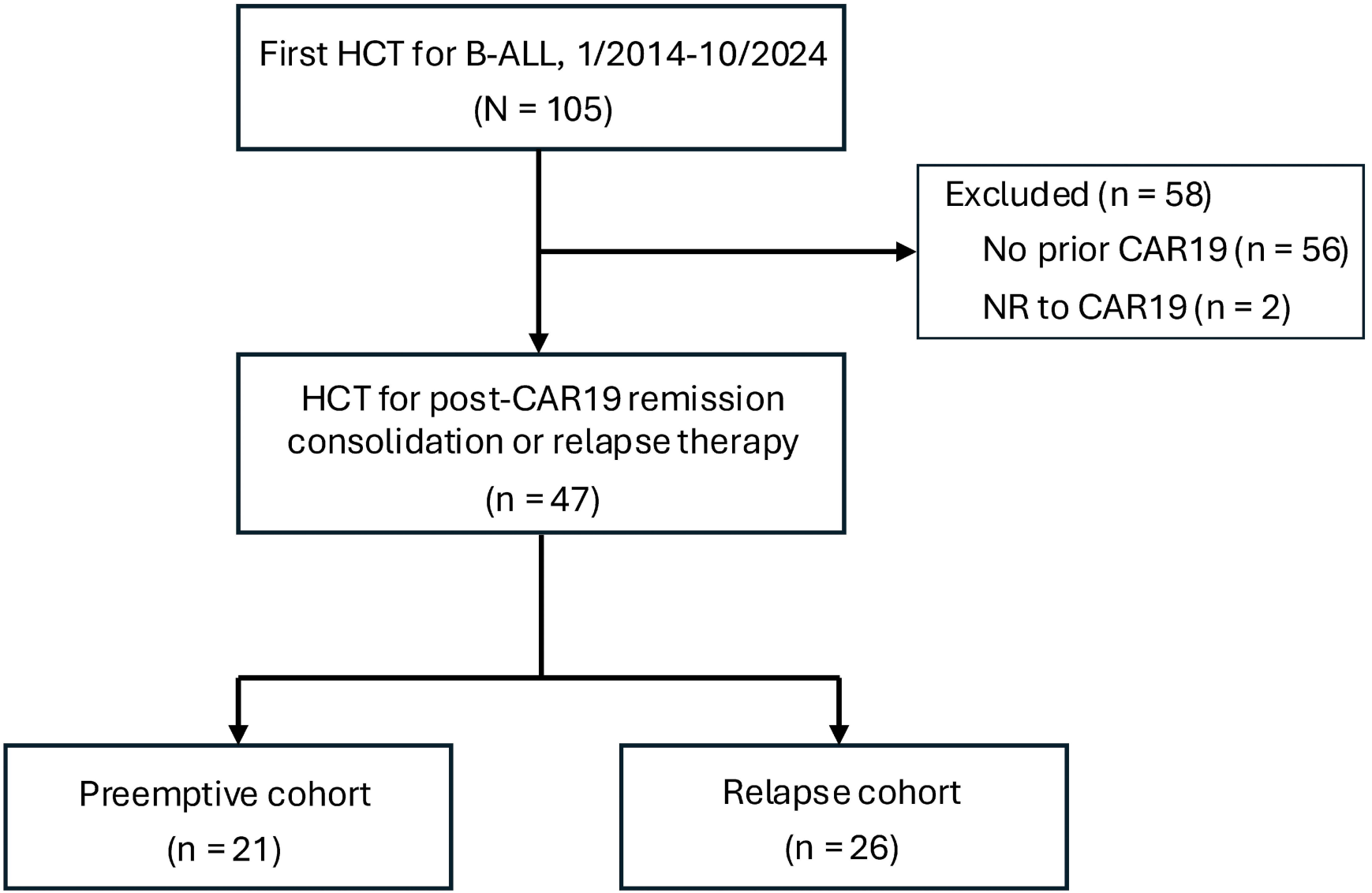
Figure 1. Flow diagram for patient inclusion in the analysis. Of 105 patients who underwent first HCT for B-ALL at Children's Hospital of Philadelphia between 1/2014-10/2024, 56 did not receive prior CAR19 and 2 did not achieve a complete response to prior CAR19 (eventually achieved a transplantable remission with other antileukemia therapies). Of 47 patients who met 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; CAR19, CD19-directed chimeric antigen receptor T-cell therapy; NR, no response

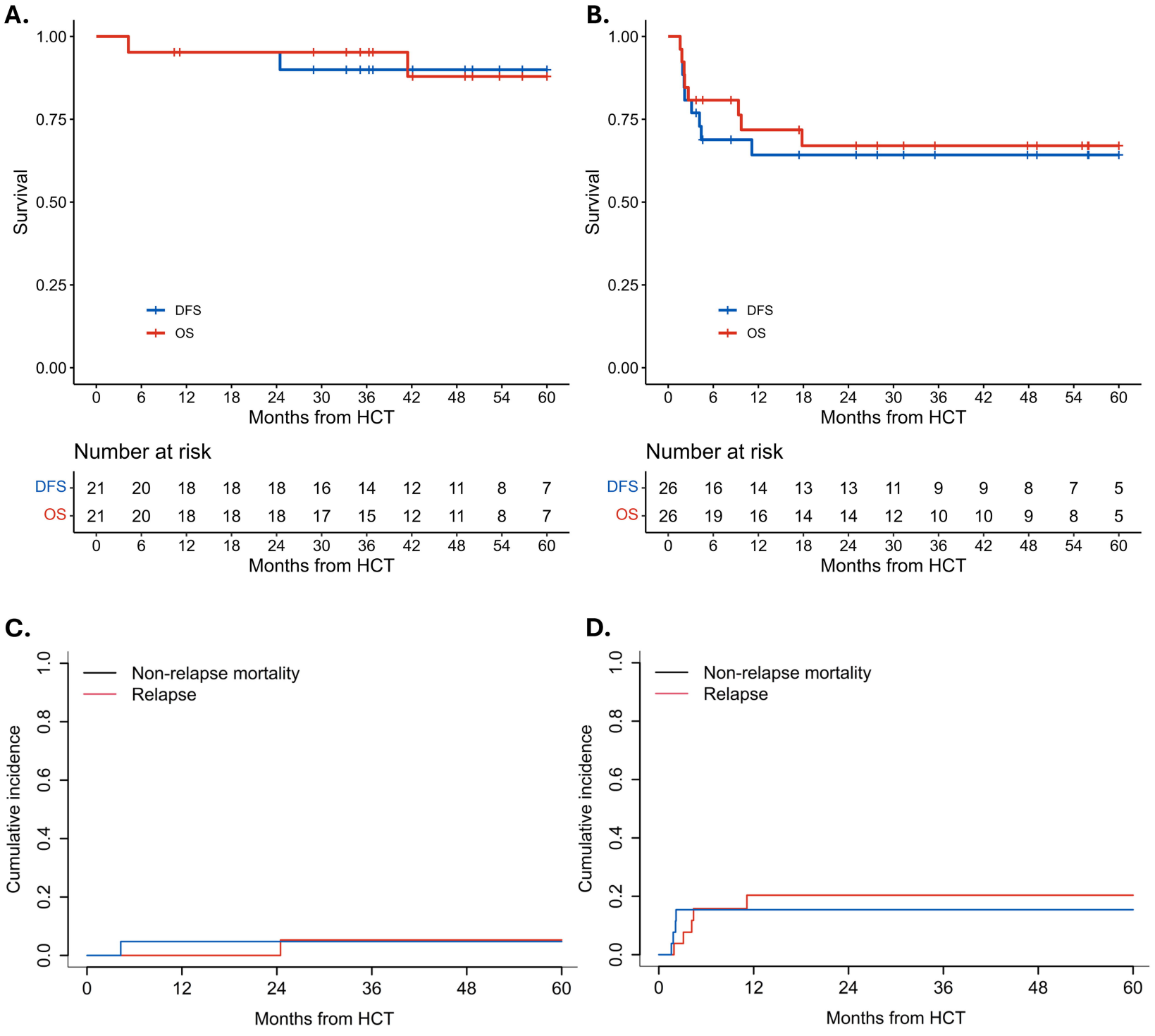
Figure 2. Survival outcomes among patients who underwent HCT for post-CAR19 remission consolidation or relapse therapy. (A) Disease-free survival (DFS) and overall survival (OS) for the preemptive cohort (n=21). DFS was defined as time from transplant to relapse or death from any cause. OS was defined 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.
CAR19, CD19-directed chimeric antigen receptor T-cell therapy; HCT, hematopoietic cell transplant.

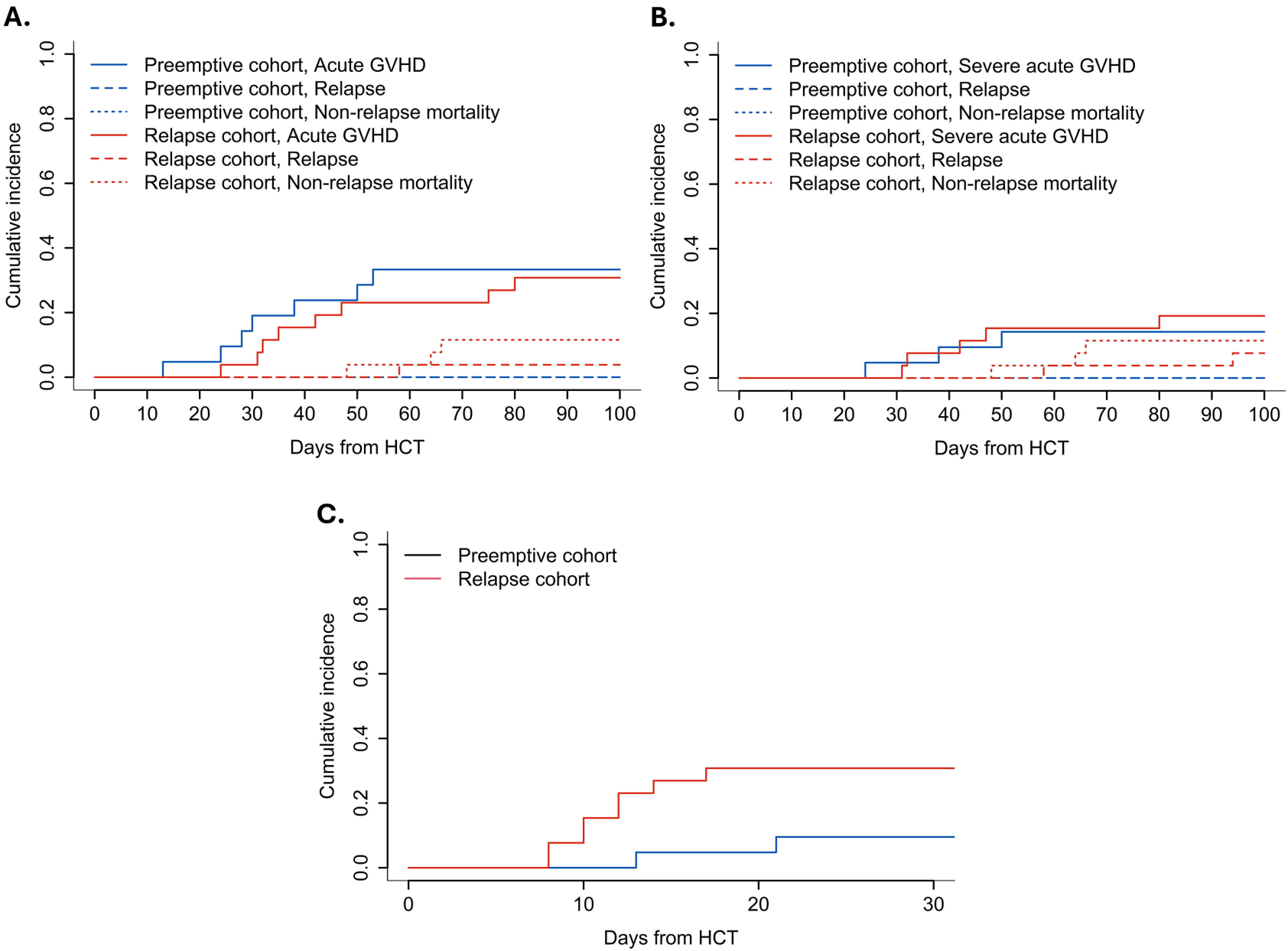
Figure 3. Cumulative incidence of GVHD and VOD/SOS among patients who underwent HCT for post-CAR19 remission consolidation or relapse therapy. (A) Cumulative incidence of grade ≥2 acute GVHD from HCT to day +100. (B) Cumulative incidence of severe (grade ≥3) acute GVHD from HCT to day +100. (C) Cumulative incidence of VOD/SOS from HCT to day +30. No VOD/SOS events were observed after day +30. NRM was considered a competing event, but no NRM occurred prior to day +30.
CAR19, CD19-directed chimeric antigen receptor T-cell therapy; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplant; NRM, non-relapse mortality; VOD/SOS, veno-occlusive disease/sinusoidal obstruction syndrome.

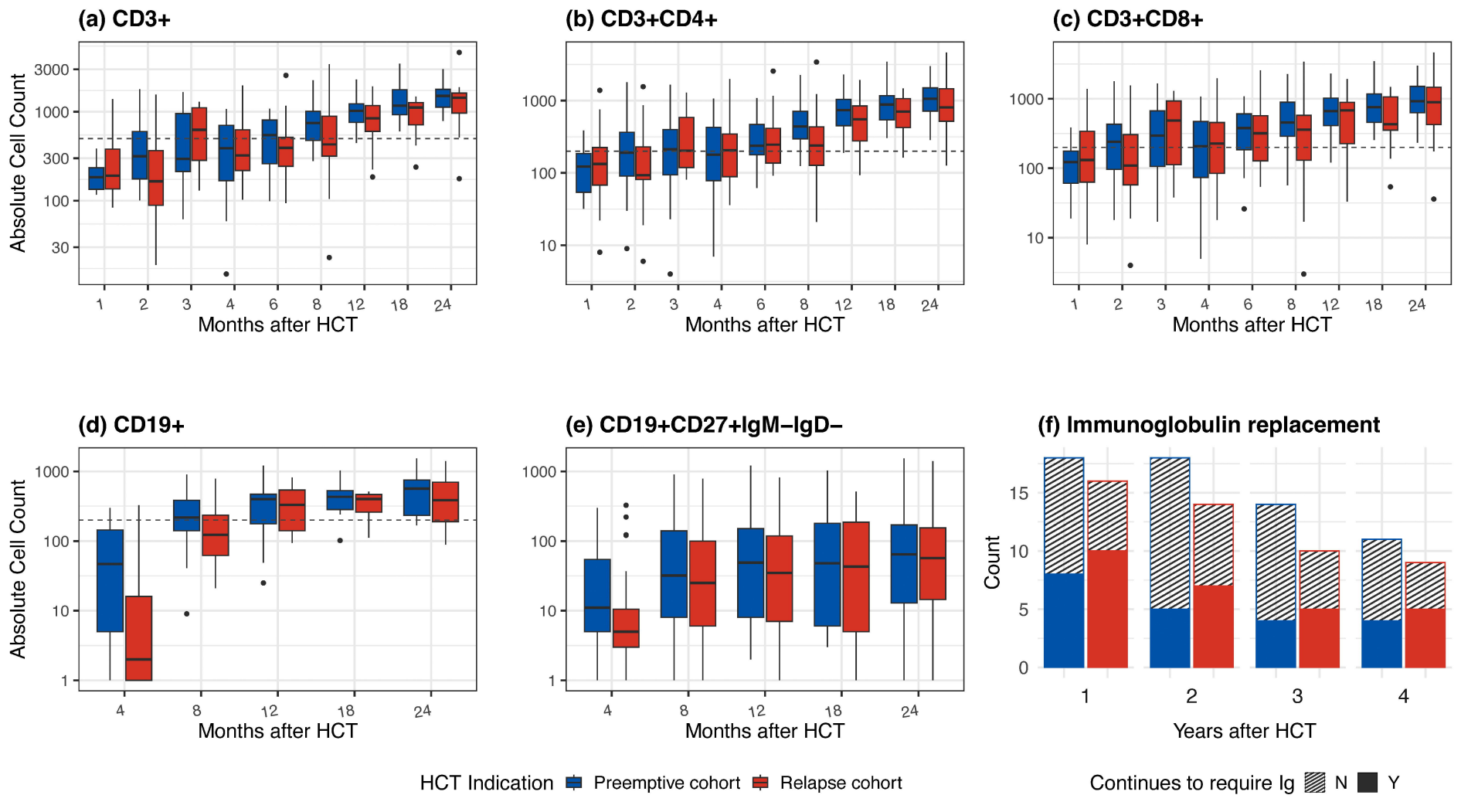
Figure 4. Immune reconstitution after HCT. (A – E) Box and whisker plots displaying cellular immune reconstitution from 1 month to 24 months post-transplant. Boxes show median, first quartile and third quartile absolute cell counts. Whiskers represent the data ranges and dots represent outliers. Reference 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 post-transplant. Proportion of patients requiring replacement is shown in solid colors and 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 time of relapse.

HCT, hematopoietic cell transplant.


First HCT for B-ALL, 1/2014-10/2024
(N = 105)
Excluded (n = 58)
No prior CAR19 (n = 56)
NR to CAR19 (n = 2)
HCT for post-CAR19 remission consolidation or relapse therapy
(n = 47)
Preemptive cohort
(n = 21)
Relapse cohort
(n = 26)


A.
1.00
0.75
0.50
0.25
0.00
Survival
DFS
OS
0 6 12 18 24 30 36 42 48 54 60
Months from HCT
Number at risk
DFS 21 20 18 18 18 16 14 12 11 8 7
OS 21 20 18 18 18 17 15 12 11 8 7
0 6 12 18 24 30 36 42 48 54 60
Months from HCT
B.
1.00
0.75
0.50
0.25
0.00
Survival
DFS
OS
0 6 12 18 24 30 36 42 48 54 60
Months from HCT
Number at risk
DFS 26 16 14 13 13 11 9 9 8 7 5
OS 26 19 16 14 14 12 10 10 9 8 5
0 6 12 18 24 30 36 42 48 54 60
Months from HCT
C.
Non-relapse mortality
Relapse
Cumulative incidence
0.0 0.2 0.4 0.6 0.8 1.0
0 12 24 36 48 60
Months from HCT
D.
Non-relapse mortality
Relapse
Cumulative incidence
0.0 0.2 0.4 0.6 0.8 1.0
0 12 24 36 48 60
Months from HCT


A.
Preemptive cohort, Acute GVHD
Preemptive cohort, Relapse
Preemptive cohort, Non-relapse mortality
Relapse cohort, Acute GVHD
Relapse cohort, Relapse
Relapse cohort, Non-relapse mortality
Cumulative incidence
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
70
80
90
100
Days from HCT
B.
Preemptive cohort, Severe acute GVHD
Preemptive cohort, Relapse
Preemptive cohort, Non-relapse mortality
Relapse cohort, Severe acute GVHD
Relapse cohort, Relapse
Relapse cohort, Non-relapse mortality
Cumulative incidence
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
70
80
90
100
Days from HCT
C.
Preemptive cohort
Relapse cohort
Cumulative incidence
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
Days from HCT


(a) CD3+
(b) CD3+CD4+
(c) CD3+CD8+
(d) CD19+
(e) CD19+CD27+IgM−IgD−
(f) Immunoglobulin replacement
Absolute Cell Count
Months after HCT
Count
Years after HCT
HCT Indication
Preemptive cohort
Relapse cohort
Continues to require Ig
N
Y

Hematopoietic cell transplantation for post-CD19 chimeric antigen receptor T-cell therapy remission consolidation or relapse treatment in pediatric acute lymphoblastic leukemia

DATA SUPPLEMENT

Supplemental Methods

Transplant Approach

Bone marrow grafts from HLA-matched related donors (MRDs) were used when available. Alternative donors were otherwise selected based on donor availability and recipient clinical status: 9- or 10-allele matched unrelated donor (URD) unmanipulated bone marrow (BM) or peripheral stem cells (PSC), or mismatched related donor (MMRD) PSCs. All PSCs underwent *ex vivo* partial T cell depletion using the CliniMACS Plus device (NCT02356653, NCT02323867, NCT03810196).[1,2]

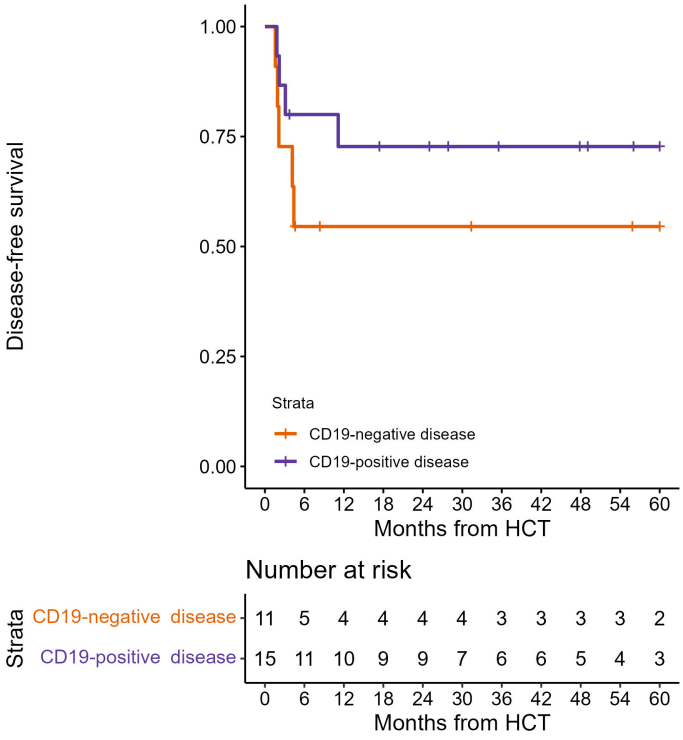
All patients had pre-HCT disease restaging to confirm adequate remission [<0.1% marrow blasts by multiparameter flow cytometry (MFC), central nervous system (CNS)-1]. All received myeloablative conditioning with total body irradiation (TBI; 1200 cGy), cyclophosphamide and thiotepa with the exception of children <3 years of age, who received clofarabine, thiotepa and melphalan.[3,4] Thymoglobulin (9mg/kg) was added for URD BMTs or MMRD PSCTs. GVHD prophylaxis included a calcineurin inhibitor +/- methotrexate for BMTs, [5] or a calcineurin inhibitor (in cases of CD3 or CD45RA-depleted addback) or no prophylaxis for partially T cell-depleted PSCTs.

All patients received bacterial, fungal and *Pneumocystis jirovecii* prophylaxis. Prophylactic acyclovir was used for patients with herpes simplex or varicella virus seropositivity, foscarnet or letermovir for patients with positive cytomegalovirus (CMV) serology, and rituximab for Epstein-Barr virus (EBV) seropositivity after serotherapy or *ex vivo* T cell depletion. CMV, adenovirus, and EBV were monitored weekly by polymerase chain reaction. Cellular immune reconstitution was assessed at 4, 8, 12 and 24 months. Immunoglobulin G (IgG) was followed monthly with replacement for serum levels <400mg/dL.

Definitions of Toxicity Endpoints

1. Neutrophil engraftment was defined as the first of 3 successive days with an absolute neutrophil count ≥500/μL.[6]
2. Acute and chronic GVHD were defined by the modified Glucksberg scale or the National Institute of Health consensus criteria, respectively.[7,8]
3. VOD/SOS was defined and graded by the European Society for Blood and Marrow Transplantation criteria for children[9] and further classified as VOD/SOS with or without other organ failure.

Figure S1. Disease-free survival among patients who underwent HCT for post-CAR19 relapse, stratified by relapse immunophenotype.



Disease-free survival, defined as time from transplant to relapse or death from any cause.

CAR19, CD19-directed chimeric antigen receptor T-cell therapy; HCT, hematopoietic cell transplant.

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