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Preinfusion factors impacting relapse immunophenotype following CD19 CAR T cells

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Key Points

- The presence of a *KMT2A* rearrangement is highly associated with the development of a lineage switch after CD19-CAR.
- Outcomes following post–CD19-CAR relapse are poor and particularly dismal in patients with *KMT2A* rearrangements.

Relapse following chimeric antigen receptor (CAR) T-cell therapy directed against CD19 for relapsed/refractory B-acute lymphoblastic leukemia (r/r B-ALL) remains a significant challenge. Three main patterns of relapse predominate: CD19 positive ($CD19^{pos}$) relapse, CD19 negative ($CD19^{neg}$) relapse, and lineage switch (LS). Development and validation of risk factors that predict relapse phenotype could help define potential pre- or post-CAR T-cell infusion interventions aimed at decreasing relapse. Our group sought to extensively characterize preinfusion risk factors associated with the development of each relapse pattern via a multicenter, retrospective review of children and young adults with r/r B-ALL treated with a murine-based CD19-CAR construct. Of 420 patients treated with CAR, 166 (39.5%) relapsed, including 83 (50%) $CD19^{pos}$, 68 (41%) $CD19^{neg}$, and 12 (7.2%) LS relapses. A greater cumulative number of prior complete remissions was associated with $CD19^{pos}$ relapses, whereas high preinfusion disease burden, prior blinatumomab nonresponse, older age, and 4-1BB CAR construct were associated with $CD19^{neg}$ relapses. The presence of a *KMT2A* rearrangement was the only preinfusion risk factor associated with LS. The median overall survival following a post-CAR relapse was 11.9 months (95% CI, 9-17) and was particularly dismal in patients experiencing an LS, with no long-term survivors following this pattern of relapse. Given the poor outcomes for those with post-CAR relapse, study of relapse prevention strategies, such as consolidative hematopoietic stem cell transplantation, is critical and warrants further investigation on prospective clinical trials.

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The full-text version of this article contains a data supplement.

Introduction

Despite impressive remission induction rates for B-cell acute lymphoblastic leukemia (B-ALL) with CD19-directed chimeric antigen receptor (CD19-CAR) T cells,[1,2] relapse remains a significant problem.[3] Factors influencing a patient's risk of relapse following CD19-CAR are becoming better established and include disease burden, depth of remission attained, CD19-CAR construct used and functional CD19-CAR persistence.[4-6] Refining our ability to predict relapse following CD19-CAR is imperative to improve patient management and overall survival.

Complicating the landscape of post-CAR relapse is the immunophenotypic heterogeneity of relapse. Largely categorized into 3 predominant patterns, relapse can present with retention of CD19 (CD19-positive [$CD19^{pos}$]), loss of CD19 (CD19-negative [$CD19^{neg}$]), or lineage switch (LS) from a lymphoid to a myeloid phenotype (Figure 1A). Whereas $CD19^{pos}$ relapse generally represents loss of functional CD19-CARs, permitting disease recurrence, $CD19^{neg}$ relapses result from several potential mechanisms. Genomic changes, such as the acquisition of a splice variant or heterogeneous pre-CAR populations, are among the more common etiologies of $CD19^{neg}$ relapse.[7,8] The rarest but potentially most concerning pattern of relapse is LS. Whereas $CD19^{neg}$ relapses may be uniquely associated with immunotherapeutic pressure, B-ALL undergoing LS following conventional therapy is seen, particularly in patients with infant B-ALL with *KMT2A* rearrangements (*KMT2A*r).[9,10] Unfortunately, the incidence of LS has increased with the higher use of CD19 targeting.[11-15] Due to the rarity, literature surrounding LS has largely been limited to descriptions within study reports or single case reports.[11-23]

The type of relapse following CD19-CAR has therapeutic and likely prognostic implications. Therefore, identifying factors predisposing to a pattern of relapse could potentially inform post-CAR relapse prevention strategies, including hematopoietic stem cell transplantation (HSCT). While we previously identified risk factors associated with any relapse of B-ALL following CD19-CAR,[4] there is limited information about factors associated with each specific relapse phenotype.[24] Given the critical impact of relapse phenotype on salvage options and to facilitate potential consolidative measures to extend durable remissions in patients at high risk of relapse, we sought to compare the different patterns of relapse following CD19-CAR and identify preinfusion risk factors associated with each pattern.

Methods

Study design

With a primary focus on relapse immunophenotype following CD19-CAR in children and young adults with B-ALL, we retrospectively reviewed outcomes in those receiving a first CD19-CAR product, with one of 3 unique constructs across 7 different institutions. Inclusion criteria were age ≤25 years at B-ALL diagnosis, ≥1 disease assessment evaluation after CAR infusion, and 30 days of follow-up or an event (nonresponse, disease progression, second malignancy, or treatment-related mortality) prior to 30 days. The data reported here are a sub-aim of our initial analysis of overall outcomes in 420 patients previously analyzed and reported.[4] All patients received infusion between 1 January 2012, and 31 December 2019. Disease assessments were performed before and after CAR infusion at standard timepoints, as previously described.[4] High disease burden was classified as ≥5% bone marrow blasts. B-cell aplasia (BCA) definitions were institutional specific and are detailed in the appendix. Relapse phenotype was defined by CD19 status and LS only in those for whom relapse phenotype was available. LS was defined by retention of cytogenetics from initial diagnosis and/or rapid emergence of myeloid disease at the first disease restaging timepoint. The study was reviewed and approved or considered exempt by each center's Institutional Review Board. Additional methods are presented in the supplemental Appendix.

Statistical analysis

The primary objectives of this study were to evaluate the incidence and risk factors associated with relapse of $CD19^{pos}$, $CD19^{neg}$, and LS following CD19-CAR. Secondary objectives included evaluation of the cumulative incidence of relapse (CIR) as stratified by pattern of relapse alongside assessment of overall survival by relapse phenotype. Patient and disease demographics, along with outcomes (eg, response and relapse phenotype) were descriptively characterized.

Event-free survival (EFS) was defined as the time from CD19-CAR infusion to no response, relapse, or death from any cause. Patients who developed LS at the first restaging timepoint were categorized as relapse. Patients without any event were censored at last contact. Overall survival (OS) was defined as the time from CD19-CAR infusion to death from any cause or last contact. CIR, using D0 as the starting timepoint, was determined for each relapse outcome ($CD19^{pos}$, $CD19^{neg}$, and LS) with both death and an alternate relapse phenotype as competing risks, with Gray's test comparing CIR curves. Preinfusion baseline factors (age at diagnosis, cytogenetics, disease burden) were assessed to identify their association with relapse phenotype. Follow-up was estimated using the reverse Kaplan-Meier method. Preinfusion risk factors for each form of relapse based on the cumulative incidence results were evaluated for their joint effect using multivariable Cox proportional hazards analysis, censoring for either no relapse or an alternative relapse phenotype or death. Backward selection was used to identify the final model for each relapse phenotype. Factors of interest were initially identified by univariate analysis; factors associated with relapse with $P < .10$ were included in the multivariable model. Subgroup analysis was performed in patients with LS, *KMT2A*r, and infant ALL (defined as initial diagnosis prior to 12 months of age). Descriptive association of BCA with relapse was also performed.

Results

A total of 420 patients who received a first CD19-CAR were analyzed. Disease characteristics of the full cohort were recently reported[4] and listed in supplemental Table 1. Median follow-up was 30.1 months (interquartile range [IQR]: 21.5-48.4). Among 376 patients achieving a complete remission (CR) and 2 additional patients who had rapid emergence of LS at the first restaging, 166 (43.9%) experienced a relapse (Figure 1B). Clinical characteristics associated with worse EFS included high disease burden, active extramedullary disease, circulating peripheral blood blasts, CD19/28ζ CAR construct type, and poor response to blinatumomab, as recently reported.[4] The median OS following relapse was 11.9 months (95% CI, 9.0-17.0) (Figure 1C; supplemental Table 2). Patient, disease, and treatment characteristics of 163 patients for whom relapse immunophenotype was available are presented in Table 1 and constitute the analysis cohort.

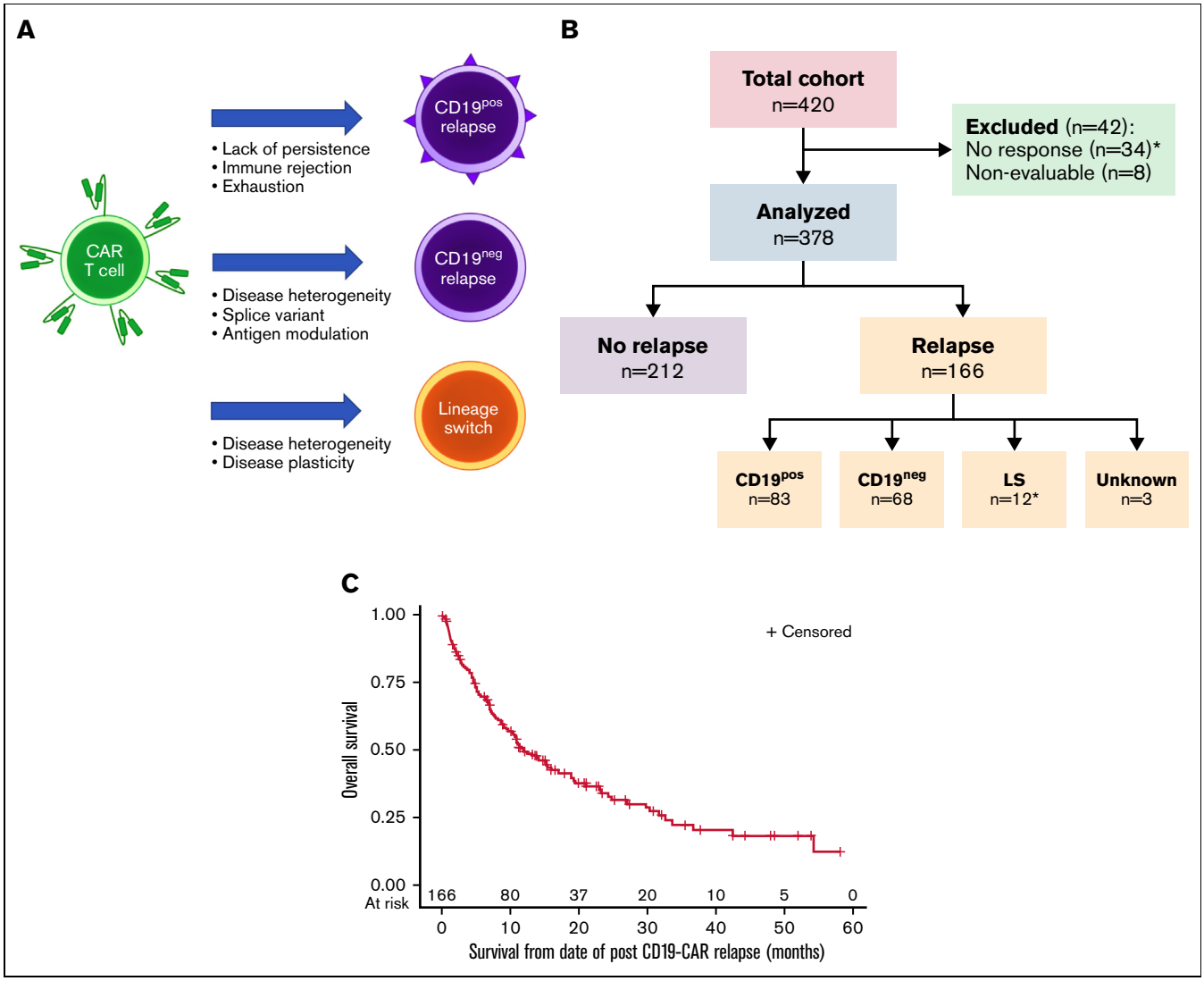

Figure 1. Categories of relapse phenotype and overall outcomes following relapse. (A) Categories of relapse phenotype. (B) CONSORT flow diagram. Figure made using BioRender. Unknown relapse category due to unavailable immunophenotype at time or relapse. *Includes 2 patients who had rapid emergence of LS at first restaging timepoint. (C) Overall survival following relapse (n = 166). Median OS was 11.9 months (95% CI, 9.0-17.0). The 6-, 12-, and 24-month OS rates were 69.8% (95% CI, 62.0% to 76.3%), 49.4% (95% CI, 41.1% to 57.2%), and 34.0% (95% CI, 25.7% to 42.5%), respectively.

CD19^{pos} relapse (n = 83)

Eighty-three of 163 (50.9%) relapses were CD19^{pos} (Figure 1B). The 24-month CIR for a CD19^{pos} relapse was 22% (95% CI, 17.7% to 26.5%) (Figure 2A). The median time from CAR infusion to CD19^{pos} relapse was 244 days (range, 36-1367) (Figure 2B). Median OS for CD19^{pos} patients following relapse was 18.9 months (95% CI, 11.2-27.0) (Figure 2C). Among the 78 patients with available data on BCA, 29 (37.2%) had ongoing BCA at a timepoint proximal to relapse (Figure 2D).

In a multivariable Cox regression model, 2 or more previous remissions was the only variable independently associated with an increased risk of CD19^{pos} relapse (Table 2). There were no significant associations with sex, age at CAR infusion, race, ethnicity, *KMT2A* status, prior HSCT, prior blinatumomab exposure, prior blinatumomab response, type of CAR costimulatory domain, or disease status before CAR.

CD19^{neg} relapse (n = 68)

Sixty-eight of 163 (41.7%) relapses were CD19^{neg} (Figure 1B). The 24-month CIR for a CD19^{neg} relapse was 16.3% (95% CI, 13.2% to 21.0%) (Figure 2A). The median time from CAR infusion to CD19^{neg} relapse was 148 days (range, 30-1159) (Figure 2B). Median OS following CD19^{neg} relapse was 9.7 months (95% CI, 6.9-15.9) (Figure 2C). Of 63 patients with available data, 43 (68.3%) had ongoing BCA at a timepoint proximal to relapse (Figure 2D). As previously reported, among patients with dim CD19 expression before CAR (n = 29), 4 were nonresponders and 13 experienced relapses, including 9 of 13 (69.2%) with CD19^{neg} disease.[4]

In a multivariable Cox regression model, age <7 years at CD19-CAR infusion, lack of *KMT2A*r, 4-1BB CAR type, prior blinatumomab nonresponse, and high disease burden (≥5% blasts) before CAR were each associated with an increased risk of CD19^{neg}

Table 1. Patient, disease, and treatment characteristics of all (n = 420) and relapsed (n = 163) patients

	All (n = 420)	Relapsed (n = 163*)
Demographics		
Female, n (%)	156 (37.1)	71 (37.1)
Median age at B-ALL diagnosis, y (IQR)	7.6 (3.4-13.8)	7.3 (0.02-24.3)
Median age at CAR infusion, y (IQR)	12.7 (7.1-17.5)	11.8 (0.8-30.4)
Race (%)		
White	275 (65.5)	108 (66.2)
Black	17 (4.0)	5 (3.1)
Asian	20 (4.8)	8 (4.9)
Other (mixed)/unknown	108 (25.7)	38 (23.3)
Ethnicity (%)		
Non-Hispanic	255 (60.7)	109 (66.9)
Hispanic	134 (31.9)	42 (25.8)
Unknown	31 (7.4)	12 (7.4)
Prior therapy (prior to CAR T cells)		
Primary refractory disease, n (%)	92 (21.9)	29 (17.8)
No. of prior CR, median (range)	2 (0-7)	2 (0-7)
Prior blinatumomab, n (%)	33 (7.9)	35 (21.5)
Prior HSCT, n (%)	159 (37.9)	76 (46.6)
Cytogenetics (%)		
Normal	41 (9.8)	18 (11)
ETV6-RUNX1	24 (5.7)	16 (9.8)
KMT2Ar	38 (9)	15 (9.2)
Ph+/Ph-like	61 (14.5)	17 (10.4)
Hypodiploid	12 (2.9)	7 (4.3)
Disease status pre-CAR (%)		
M1 or MRD-negative marrow	217 (51.7)	64 (39.3)
M2/M3 marrow	203 (48.3)	99 (60.7)
CNS3	4 (0.9)	1 (0.01)
Active EM disease	22 (5.2)	9 (5.5)
Active PB blasts	56 (13.3)	30 (18.4)
CAR T-cell construct infused (%)†		
CD19/4-1BB	277 (66.0)	115 (70.6)
Tisagenlecleucel (Kymriah)	88 (21.0)	34 (20.9)
CD19/28z	55 (13.1)	14 (8.6)
Relapse phenotype (%)		
$CD19^{pos}$	N/A	83 (50.9)
$CD19^{neg}$	N/A	68 (41.7)
Lineage switch	N/A	12 (7.4)

Blina, blinatumomab; EM, extramedullary; *KMT2Ar*, *KMT2A*-rearranged; MRD, minimal residual disease (defined as <.01% bone marrow blasts by multiparameter flow cytometry); N/A, not applicable; PB, peripheral blood.

*A total of 166 patients experienced relapse, but immunophenotype at relapse was only available in 163 patients, which constituted the analysis cohort.

†4-1BB CAR T-cell constructs were comprised of 1 of 2 available constructs, including the construct that eventually was FDA approved; tisagenlecleucel refers to the commercially available construct.

relapse (Table 2). There were no significant associations with sex, age at diagnosis, race, ethnicity, prior HSCT, or cumulative number of prior CRs.

LS (n = 12)

Twelve of 163 (7.4%) relapses were LS (Figure 1B). Eleven of 12 patients (91.7%) with LS converted to acute myeloid leukemia; 1 patient converted to mixed phenotype acute leukemia.

The 24-month CIR for LS was 3% (95% CI, 1.6% to 5.2%) (Figure 2A). All but 2 LSs occurred during the first year following CD19-CAR infusion, with a median time from CAR infusion to LS of 65.5 days (range, 21-1159) (Figure 2B). Median OS following LS was 3.7 months (95% CI, 1.2-7.0), substantially shorter than OS following either a $CD19^{pos}$ ($P < .0001$) or $CD19^{neg}$ ($P = .0018$) relapse (Figure 2C). Importantly, there were no long-term survivors following LS, with all patients dying from progressive disease. Subsequent therapy ranged from various myeloid-directed intensive chemotherapies to palliative care (supplemental Table 3). All 8 patients with available data had ongoing BCA at time of LS (Figure 2D).

KMT2Ar was the predominant cytogenetic abnormality seen in patients with LS, present in 9 of 12 (75%) patients with LS compared with 20 of 408 (7.1%) patients without LS patients ($P < .001$) (Table 3; Figure 3A). Given the association of *KMT2Ar* with infant ALL, patients with LS were expectedly younger at initial diagnosis compared with the remaining cohort (median age, 1.6 years vs 7.7 years; $P = .001$). In a multivariable Cox regression model, *KMT2Ar* was the only independent predictor of LS (hazard ratio, 32.35; $P < .0001$; Table 2). There were no significant associations with sex, age at CAR infusion, race, ethnicity, prior HSCT, cumulative number of prior CRs, prior blinatumomab exposure, prior blinatumomab response, type of CAR, or disease status before CAR.

KMT2Ar (n = 38)

Given the strong association between LS and *KMT2Ar*, as visualized in the intersection graph (Figure 3A), we further analyzed outcomes of all patients with *KMT2Ar*. Overall, 38 of 420 patients (9%) had a *KMT2Ar*, with 9 of 38 (23.7%) experiencing an LS. Outcomes for this cohort, stratified by disease burden prior to CAR infusion, are shown in Figure 3B. Patients with *KMT2Ar* were younger at diagnosis (median age, 0.6 years vs 8.5 years; $P < .0001$) and CAR infusion (median age, 3 years vs 13.3 years; $P < .0001$) compared with non-*KMT2Ar* patients. *KMT2Ar* patients were also more likely than non-*KMT2Ar* patients to have previously received blinatumomab (31.6% vs 17%%; $P = .04$) and had comparable CR rates to blinatumomab (75% vs 52.3%; $P = .21$). Otherwise, there were no substantial baseline differences between the 2 groups, including CD19-CAR response (Table 3). Relapse immunophenotype, however, was skewed toward LS in *KMT2Ar* patients, as discussed above (Figure 3C-D).

Individual outcomes for *KMT2Ar* patients are shown in Figure 3E. Thirty-one of 38 (81.6%) *KMT2Ar* patients achieved a CR after CAR. Seven patients received a consolidative HSCT in remission (representing 4 first and 3 second HSCTs) at a median of 100 days (range, 60-429) after CAR. Three (42.9%) patients receiving a consolidative HSCT remain alive in remission (median follow-up of 1164 days after CAR). Among the other 4 patients, there were 2 $CD19^{pos}$ relapses, 1 $CD19^{neg}$ relapse, and 1 transplant-related mortality after HSCT. No *KMT2Ar* patient experienced post-HSCT LS. Of the 24 *KMT2Ar* patients who did not receive HSCT after CAR, 3 (12.5%) experienced a $CD19^{pos}$ relapse, 7 (30.4%) developed LS, and 14 (60.9%) are alive with a median follow-up of 864 days after CAR. These 24

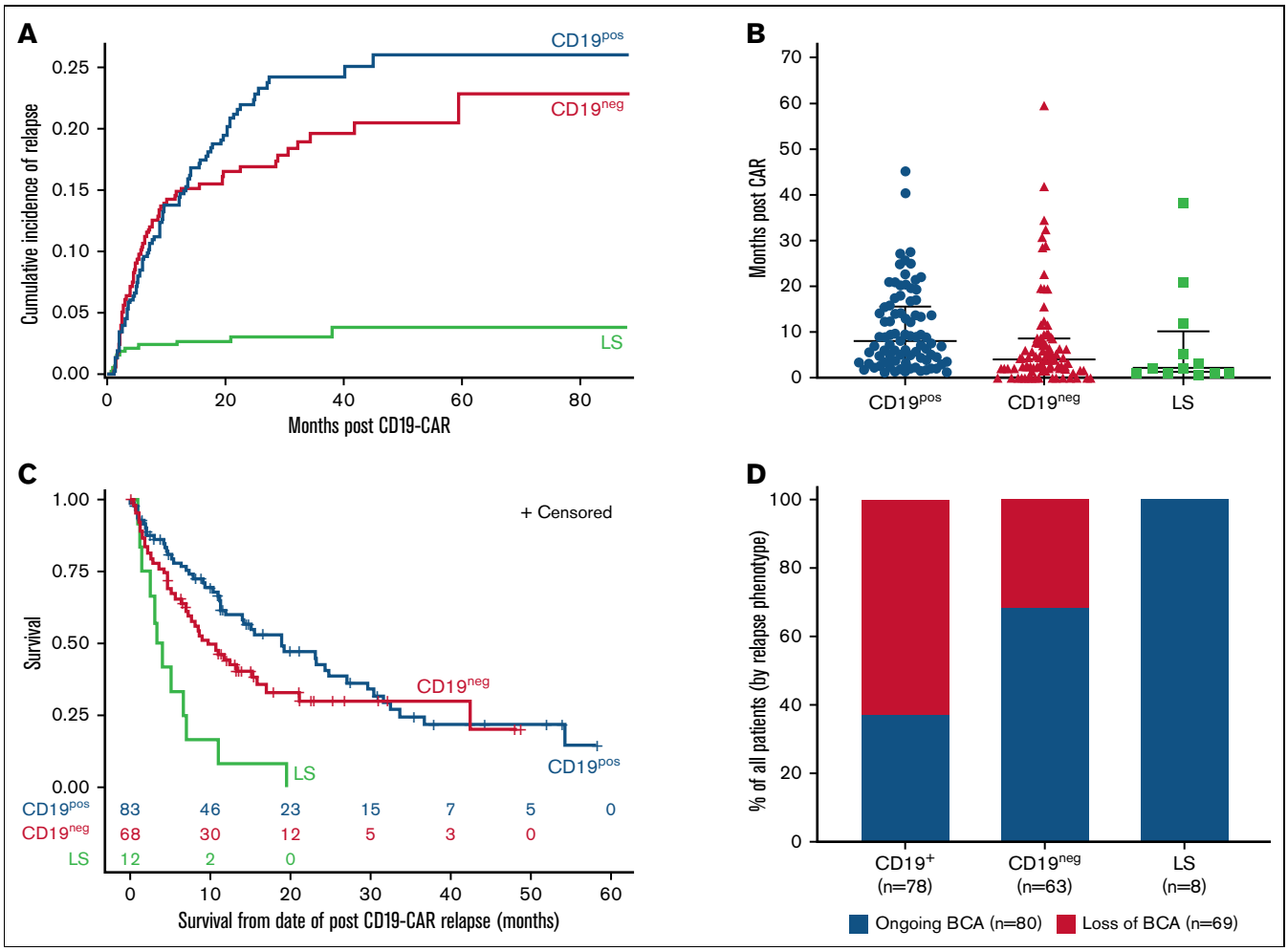

Figure 2. Outcomes stratified by relapse immunophenotype. (A) CIR stratified by relapse immunophenotype. Corresponding data in supplemental Table 2. (B) Median time to relapse, stratified by relapse immunophenotype. (C) OS following relapse, stratified by relapse immunophenotype. Median OS for $CD19^{pos}$ relapse was 18.9 months (95% CI, 11.2-27.0). Median OS for $CD19^{neg}$ relapse was 9.7 months (95% CI, 6.9-15.9). Median OS for LS was 3.7 months (95% CI, 1.2-7.0). (D) BCA at time of relapse, stratified by relapse immunophenotype. BCA, and loss thereof, was based on local assessment and was generally defined by 1 of the following parameters: (1) >1% bone marrow CD19+ hematogones; (2) >1% increase in $CD19^{+}$ cells in bone marrow or peripheral blood; or (3) >3% $CD19^{+}$ B cells of total peripheral blood lymphocytes or >50 $CD19^{+}$ cells/μL, verified by 2 consecutive timepoints.

Table 2. Multivariable analysis of factors that may be associated with each relapse immunophenotype

Parameter	Parameter estimate	Standard error	χ^2	*P*	Hazard ratio	95% CI
$CD19^{pos}$ relapse						
≥ 2 CRs	0.26	0.1	7.06	.008	1.3	1.07-1.58
$CD19^{neg}$ relapse						
Younger age (<7 y) at CAR	1.23	0.27	20.78	<.001	3.42	2.02-5.81
KMT2Ar presence	−2.51	1.02	6.03	.01	0.08	0.01-0.6
CAR type*	1.51	0.72	4.37	.04	0.22	0.05-0.91
High disease burden	1.64	0.29	32.28	<.001	5.17	2.93-9.11
Blina nonresponse	1.05	0.37	8	.005	2.85	1.38-5.89
Lineage switch						
KMT2Ar presence	3.48	0.67	27.11	<.0001	32.35	8.74-119.71

*CD28z CAR associated with lower risk of $CD19^{neg}$ relapse.

Table 3. Disease characteristics based on *KMT2A*r status

	KMT2A (n = 38)	Non-*KMT2A* (n = 382)	*P*
Demographics			
Median age at diagnosis, y (range)	0.6 (0.02-11.1)	8.5 (0.8-25.1)	**<.0001**
Median age at infusion, y (range)	3.0 (0.6-16.2)	13.3 (1.7-30.4)	**<.0001**
Female, n (%)	16 (42.1)	140 (36.6)	.6
Male, n (%)	22 (57.9)	242 (63.4)	
Prior therapy (%)			
Primary refractory	7 (18.4)	85 (22.2)	.68
Prior HSCT	18 (47.4)	141 (36.9)	.22
Prior blina	12 (31.6)	65 (17.0)	**.04**
Prior blina nonresponse	3 of 12 (25)	31 of 65 (47.7)	.21
Disease burden (%)			
M1	17 (44.7)	200 (52.4)	.4
≥M2	21 (55.3)	182 (47.6)	
CAR type (%)			
41BB	36 (94.7)	329 (86.1)	.20
CD28	2 (5.3)	53 (13.9)	
CAR response (%)*			
CR	31 (86.1)	345 (91.8)	.23
No CR (PR/SD/PD)	5 (13.9)	31 (8.2)	
Relapse phenotype (%)			
LS	9 (23.7)	3 (0.8)	**<.0001**
$CD19^{pos}$	5 (13.2)	78 (20.4)	
$CD19^{neg}$	1 (2.6)	67 (17.5)	

*Number of patients evaluable for response: *KMT2A* (n = 36); non-*KMT2A* (n = 376).

patients did not receive a consolidative HSCT in remission due to early LS (n = 5), pre-CAR HSCT (n = 11), or patient/provider preference (n = 8).

Of 7 (18.4%) CD19-CAR nonresponding patients with *KMT2A*r, 2 (28.6%) had rapid emergence of LS by the first restaging timepoint, and the remaining patients died of disease (n = 3) or CAR-related toxicity (n = 2).

The EFS for *KMT2A*r patients (median, 14.1 months; 95% CI, 12.2 not estimable [NE]) was similar to non-*KMT2A*r patients (20.2 months; 95% CI, 13.9-28.4; $P = .47$) (Figure 4A). However, the median OS for *KMT2A*r patients was inferior to non-*KMT2A*r patients (25.3 months [95% CI, 7.9 NE] vs 51.9 months [95% CI 42.0 NE]; $P = .02$) (Figure 4B). Specifically, no *KMT2A*r patient experiencing an event following CD19-CAR was a long-term survivor.

Other predominant cytogenetic alterations in our cohort included Ph^+ (n = 32), Ph-like (n = 29), *ETV6-RUNX1* (n = 24), and hypodiploidy (n = 12). There was no significant association between these alterations and a specific relapse immunophenotype (supplemental Table 4).

Infant ALL (n = 29)

Overall, 29 of 420 (6.9%) patients had infant ALL (defined as initial diagnosis within the first 12 months of life). The median age at CD19-CAR infusion was 2.1 years (range, 0.6-11.2), with only 3 patients receiving CD19-CAR during their first year of life. Twenty-seven of 29 (93.1%) patients with infant ALL had a *KMT2A*r, and 25 of 29 (86.2%) achieved a CR following CD19-CAR, 24 (96%) of which were minimal residual disease (MRD) negative.

Nine of 29 (31%) patients with infant ALL experienced relapse following CD19-CAR, including 2 $CD19^{pos}$ relapses, 1 $CD19^{neg}$ relapse, and 6 LSs. All patients with infant ALL experiencing an LS had a co-occurring *KMT2A*r. The median EFS (not reached for infants vs 19.5 months for noninfants; $P = .88$; Figure 4C) and median OS (35.8 months for infants vs 49.1 months for noninfants; $P = 015$; Figure 4D) for patients with infant ALL were similar to patients with noninfant ALL.

Discussion

While CD19-CAR has changed the landscape for treatment of relapsed/refractory B-ALL, relapse after CAR remains a major challenge. This risk of relapse has informed next-generation CAR strategies targeting relapse prevention, including bispecific CARs,[25] addition of checkpoint inhibitors,[26] and episodic antigen exposure.[27] However, the best approach to prevent post-CAR relapse remains unclear. To better understand post-CAR relapse and assist in the development of relapse prevention strategies, we evaluated relapse stratified by immunophenotype and identified pre-CAR risk factors specific to each pattern of relapse in a large, multicenter setting. These predictive factors are vital for treating clinicians and may inform optimal planning for peri-CAR strategies.

Among the 163 patients who relapsed after CD19-CAR, $CD19^{pos}$ relapses, $CD19^{neg}$ relapses, and LS accounted for 50.9%, 41.7%, and 7.4% of relapses, respectively. Focusing first on $CD19^{pos}$ and $CD19^{neg}$ relapse, Dourthe et al recently reported on risk factors associated with these events in a cohort of 51 patients following treatment with tisagenlecleucel.[24] They found that low disease burden and loss of BCA were associated with $CD19^{pos}$ relapse. In contrast, they observed that high disease burden and detectable MRD at day 28 after CD19-CAR were associated with an increased risk of $CD19^{neg}$ relapse. With a primary focus on preinfusion factors, we similarly identified high disease burden as the most important risk factor for $CD19^{neg}$ relapse, followed by prior blinatumomab nonresponse, age at CAR infusion, and CAR construct.

The association of $CD19^{neg}$ relapses with high disease burden may reflect a heterogenous pre-CAR disease population with a $CD19^{neg}$ clone obscured by bulk disease, a so-called "needle in the haystack" phenomenon. Following the eradication of bulk $CD19^{pos}$ disease, $CD19^{neg}$ disease could emerge unimpeded by ongoing CD19-CAR persistence. Notably, our prior efforts incorporated extensive evaluation of CD19 expression phenotype, and there was no association between pre-CAR CD19 expression and relapse immunophenotype, potentially highlighting the challenge of describing large populations of cells (supplemental Table 1).[4] This analysis is inherently limited because patients with decreased or partial expression of CD19 may have been ineligible for CD19-CAR treatment or allocated to alternative therapies.

Interestingly, several of the variables associated with $CD19^{neg}$ relapses have also been linked to prolonged CD19-CAR persistence. The 4-1BB CAR T-cell construct has longer persistence compared with its CD19/28ζ counterpart and is more associated

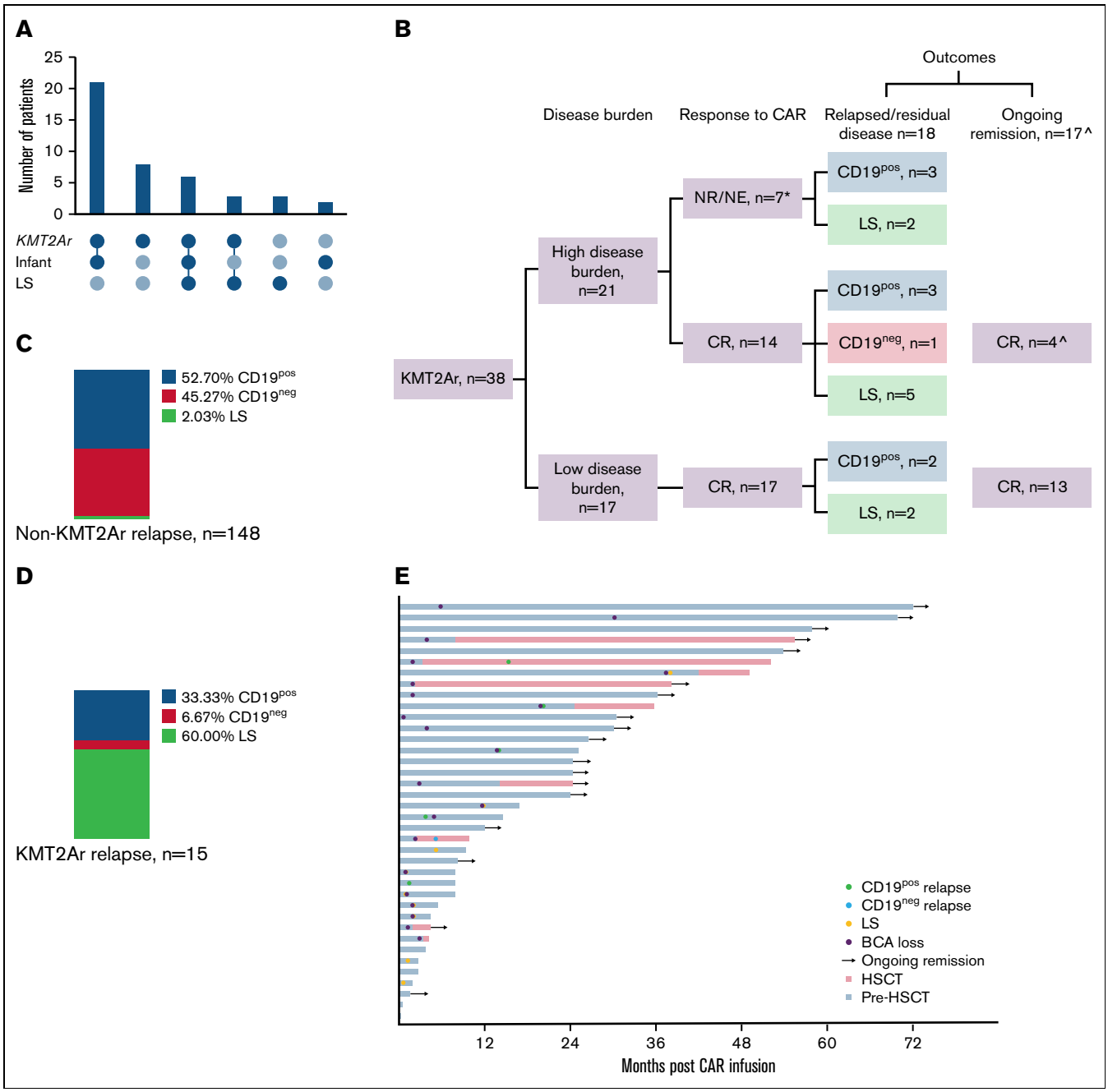

Figure 3. Outcomes for patients with *KMT2Ar* ALL. (A) Intersection graph showing association between *KMT2A*r, infant ALL diagnosis and lineage switch. (B) Flow diagram showing overall outcomes of patients with *KMT2A*r ALL following CD19 CAR. *2 patients died of CAR toxicity; ^1 died of post-HSCT TRM. CR, complete remission; NE, nonevaluable; NR, nonresponse (including partial response, stable disease, and progressive disease). (C) Relapse phenotype of non-*KMT2A*r patients. (D) Relapse phenotype of *KMT2A*r patients. (E) Outcomes for individual patients with *KMT2A*r ALL.

with CD19^{neg} relapse. Additionally, patients who receive a CD19/28ζ construct are typically allocated to a consolidative HSCT,[28] which may potentially prevent the development of LS. The observation that younger age at infusion is associated with CD19^{neg} relapses (and less likely to have CD19^{pos} relapse) warrants further study, specifically to evaluate if age at the time of collection influences T-cell function. Indeed, preclinical and clinical studies have shown that older patients have decreased persistence and effectiveness of their CAR product compared with younger patients, leading to an increased risk of CD19^{pos} relapses and, in turn, potentially decreasing the incidence of CD19^{neg} relapses.[29,30] These findings are consistent with the hypothesis that ongoing immunotherapeutic pressure associates with or potentially facilitates the emergence of CD19^{neg} disease. Further support for this hypothesis is the observation that most patients with

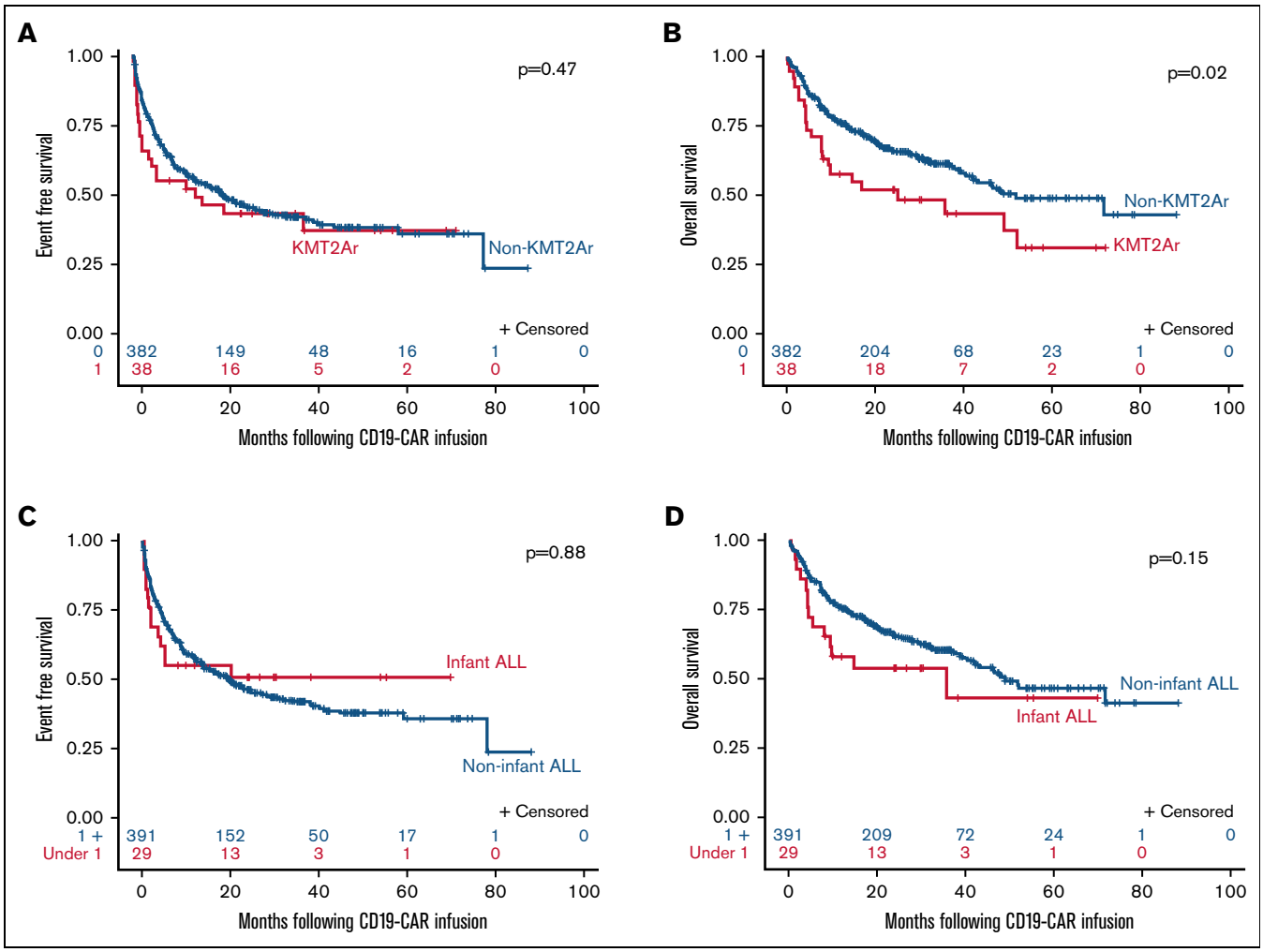

Figure 4. Overall and event-free survival in patients with *KMT2Ar* and infant ALL. (A) EFS *KMT2Ar* vs non-*KMT2Ar*. EFS for *KMT2Ar* patients at 6, 12, and 24 months was 55.3% (95% CI, 38.3% to 69.3%), 52.5% (95% CI, 35.6% to 66.9%), and 43.8% (95% CI, 27.6% to 58.8%), respectively, with a median EFS of 14.1 months (95% CI, 2.2 NE). EFS for non-*KMT2Ar* patients at 6, 12, and 24 months was 69.5% (95% CI, 64.6% to 73.9%), 58.0% (95% CI, 52.9% to 62.7%), and 47.0% (95% CI, 41.7% to 52.2%), respectively, with a median EFS of 20.2 months (95% CI, 13.9-28.4). $P = .47$. (B) OS *KMT2Ar* vs non-*KMT2Ar*. OS for *KMT2Ar* patients at 6, 12, and 24 months was 71.1% (95% CI, 53.9% to 82.8%), 57.7 (95% CI, 40.5% to 71.5%), and 51.9% (95% CI, 34.9% to 66.5%), respectively, with a median OS of 25.3 months (95% CI, 7.9 NE). OS for non-*KMT2Ar* patients at 6, 12, and 24 months was 85.6% (95% CI, 81.6% to 88.7%), 76.3 (95% CI, 71.7% to 80.3%), and 65.9% (95% CI, 60.6% to 70.7%), respectively, with a median OS of 51.9 months (95% CI, 42% NE). $P = .02$. (C) EFS infant ALL vs noninfant ALL. EFS for infant patients at 6, 12, and 24 months was 55.2% (95% CI, 35.6% to 71.0%), 55.2% (95% CI, 35.6% to 71.0%), and 50.9% (95% CI, 31.5% to 67.5%), respectively, with a median EFS not reached. EFS for noninfant patients at 6, 12, and 24 months was 69.2% (95% CI, 64.3% to 73.5%), 57.7% (95% CI, 52.6% to 62.5%), and 46.5% (95% CI, 41.2% to 51.6%), respectively, with a median EFS of 19.5 months (95% CI, 13.9-27.0). $P = .88$. (D) OS Infant ALL vs noninfant ALL. OS for infant patients at 6, 12, and 24 months was 69% (95% CI, 48.8% to 82.5%), 58.2% (95% CI, 38.3% to 83.8%), and 54.1% (95% CI, 34.2% to 70.3%), respectively, with a median OS of 35.8 months (95% CI, 5.6 NE). OS for noninfant patients at 6, 12, and 24 months was 85.4% (95% CI, 81.5% to 88.5%), 75.8 (95% CI, 71.2% to 79.8%), and 65.4% (95% CI, 60.2% to 74.2%), respectively, with a median OS of 49.1 months (95% CI, 42.0 NE). $P = .15$.

CD19^{neg} relapse in our cohort had ongoing BCA (a surrogate for functional CD19-CAR persistence) and could not be used to predict CD19^{neg} escape.

While blinatumomab exposure itself did not increase the risk of developing a CD19^{neg} relapse, a prior lack of response to blinatumomab increased the risk for eventual CD19^{neg} relapse, suggesting a highly refractory nature of these patients' disease. Although the reason for blinatumomab failure in this cohort was not the development of CD19^{neg} disease because these patients would not have been eligible for CD19-CAR therapy, a small proportion did have CD19 modulation after blinatumomab, which may have been the prelude to eventual antigen escape.[4] Given that blinatumomab nonresponders have worse long-term outcomes following CD19-CAR and that the predominant relapse type for this population is CD19^{neg}, for which there are limited salvage strategies, prioritizing these patients for next-generation CAR strategies or consolidative HSCT to decrease relapse risk could be considered.

In contrast to CD19^{neg} relapse, the only factor in our study associated with a CD19^{pos} relapse was the cumulative number of CRs, which serves as a proxy for additional lines of therapy needed and

overall treatment burden. Specifically, higher numbers of CRs were associated with worse EFS.[4] We have also previously shown that primary refractory patients have a more favorable EFS following CD19-CAR.[4] We hypothesize that these variables may reflect an element of T-cell fitness and surmise that heavily pretreated patients may generate CAR T cells with shortened functional persistence, predicating to CD19^{pos} relapse.

While LS events were rare overall, the presence of a *KMT2A*r was strongly identified as a risk factor. This profound enrichment of *KTM2A*r in patients developing LS following CD19-directed immunotherapy is consistent with the culmination of single case reports.[11-23] Notably, there were no LS events following treatment with a CD19/28ζ CAR. Similar to CD19^{neg} relapse, ongoing immunotherapeutic pressure likely facilitates LS, presumably via disease plasticity.

Patients with *KMT2A*r had a similar EFS compared with non-*KMT2A*r patients. However, the OS for patients with *KMT2A*r was inferior, with no long-term survivors following an event after CAR. This was largely driven by the increased proportion of LS events in the *KMT2A*r patients. Although there are some case reports of long-term survivors following LS,[15] this entity poses a challenging clinical situation for treating clinicians. Effective immunotherapeutic targets are often lacking, their clones are likely chemotherapy resistant given the heavy pretreatment, and many of these patients have already had an HSCT. Concerningly, the *KMT2A*r patients with non-LS events showed a similar lack of salvageability, which suggests that *KMT2A*r relapses are challenging to salvage regardless of the relapse immunophenotype.

Not answered by our data set is the question of our ability to prevent LS, specifically as it relates to post-CAR HSCT. Some have postulated that a consolidative HSCT may be important or required to prevent LS via high-dose conditioning or a graft-versus-leukemia effect. However, avoidance of HSCT, especially for younger patients, is often desired given the associated short- and long-term toxicity. While no patients who received a post-CAR HSCT experienced an LS, our numbers are too small to indicate a protective benefit of HSCT in these patients. Additionally, several patients were unable to proceed to HSCT because their event happened very early after CD19-CAR infusion, precluding HSCT. Given the association between *KMT2A*r and infant ALL, a population for whom bridging to CAR T-cell therapy and manufacturing a product is challenging and in whom there is equipoise regarding the role of HSCT,[31,32] it is abundantly clear that this population remains at very high risk. Importantly, we found that neither blinatumomab exposure nor blinatumomab failure put patients with *KMT2A*r at higher risk for LS. This is a key consideration given the upfront incorporation of blinatumomab in ongoing clinical trials and for treating clinicians seeking to prioritize salvage strategies.

While our study focused on pre-CAR risk factors, analysis of BCA revealed that most patients with CD19^{neg} and LS had ongoing BCA prior to their event, supporting the notion that BCA monitoring is suboptimal for prediction of approximately 50% of relapses occurring after CD19-CAR. Even more worrisome, however, are the data that 37.2% of patients with CD19^{pos} relapse had ongoing BCA proximal to their event, suggesting that its role as a predictive tool in monitoring for any relapse may be limited. Because of the variability in how BCA was captured in this retrospective analysis, our findings are descriptive at best. However, Pulsipher et al similarly describe in their cohort of 143 patients with more stringent definitions and well-defined monitoring of BCA that 22 of 25 (88%) patients with CD19^{neg} relapse had ongoing BCA, as did 3 of 14 (21.2%) patients with CD19^{pos} relapse, also raising concerns regarding the limited use of BCA monitoring in the post-CAR setting.[6]

An important, but unanticipated, finding was regarding the timing of relapse. Generally, most relapses have been reported within the first year after CD19-CAR.[1,6] In our dataset, which is both expansive and longitudinal, the median time to relapse varied by relapse immunophenotype, but the relapse risk remained present even at later timepoints, particularly for CD19^{pos} relapse. Indeed, the median time to CD19^{pos} relapse was 244 days (IQR, 107-480), suggesting that late relapses are occurring well beyond the first year. This presents a potential window of opportunity in which continued monitoring with methods such as next-generation sequencing as recently reported[6] may be highly informative in identifying those at high risk of relapse. Additionally, the mechanism of CD19^{pos} relapses may differ based on timing, with late relapses being more amicable to reinfusion of CD19-CAR, but further investigation is warranted. Unfortunately, but not unexpectedly, we demonstrate, as others in adult oncology have also reported, that survival following post–CD19-CAR relapse is dismal.[33-35] Relapse prevention strategies for patients in whom there is time to intervene prior to relapse will be essential.

In our analysis, we opted to use the first relapse as the primary event upon which to base our statistical analysis, particularly as it related to competing risk. We recognize, however, and particularly in the era of immunotherapy, that immunophenotypic expression is in evolution (eg, a patient whose first relapse phenotype is CD19^{pos} may evolve to CD19^{neg} in the future). Nevertheless, because the event of interest was first relapse, which would prompt additional therapy and represent failure following CD19-CAR, our approach of basing our analysis on the most predominant population seen at relapse remains highly informative with respect to post–CD19-CAR outcomes. We also opted to use only pre-CAR infusion characteristics to define risk but recognize the important role of post-CAR monitoring, particularly MRD and BCA in the context of a CD19-CAR response. Lastly, given the longer time to CD19^{pos} relapse, it is important to note that we were not able to determine if a consolidative HSCT triggered by loss of BCA changed the trajectory of CD19^{pos} relapse, a limitation of this retrospective analysis. In concert with our recent efforts that identified high disease burden and blinatumomab nonresponse as risk factors for relapse, our results identifying preinfusion risk factors stratified by relapse phenotype facilitate a more fine-tuned approach to individual patients and potential outcomes, allowing for more informed planning and decision-making for each child, adolescent, and young adult undergoing CD19-CAR.

In summary, in the context of the largest retrospective pediatric CD19-CAR dataset established to date, we provide a conclusive association of *KMT2A*r with LS, identify risk factors associated with CD19^{neg} and CD19^{pos} relapse, further characterize the timing of relapse by immunophenotype, and comprehensively describe outcomes of patients with *KMT2A*r and infant ALL who received CD19-CAR. Given the dismal outcomes for those with post-CAR relapse, relapse prevention strategies will be critical, and further development and validation of risk factors is a key next step. As next-generation CAR strategies become more widespread, the

incidence of LS and relapse overall will need to be established to see if these strategies show benefit. Similarly, larger studies will need to be performed to evaluate the role of post-CAR HSCT. Our efforts serve to further refine risk factors that may help to appropriately identify patients at highest risk following CD19-CAR.

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Authorship

Contribution: A.J.L. and N.N.S. wrote the first draft of the manuscript; A.T., R.M.M., L.G., P.A.B., M.A.P., D.B., S.M.S., and N.N.S designed the study and analysis plan; R.M.M., A.T., A.J.L., A.E.K., J.S., B.Y., T.F., P.C., L.C., C.A., S.J., D.B., P.A.B., T.W.L., L.G., R.A.G., S.A.G., S.R.R., M.A.P., and N.N.S. all contributed to data collection; S.M.S. and N.N.S. performed statistical analyses; A.E.K., M.J.B., B.W., M.S-S., C.M.Y., S.AG., and V.P. performed institution-specific flow cytometry review; and all authors substantially contributed to the final version of the manuscript and approved the submission.

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