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TO THE EDITOR:

Incidence of preexisting B-cell aplasia in B-ALL: implications for post–CAR T-cell monitoring

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B-cell aplasia (BCA) is a recognized toxicity of chimeric antigen receptor (CAR) T cells targeting relapsed/refractory B-cell hematologic malignancies.[1,2] Although prolonged and/or profound BCA may have implications on infection and immune function,[2] achieving and maintaining BCA is generally considered a desired outcome, theoretically aligning with ongoing CAR T-cell functionality. Accordingly, routine monitoring for BCA is increasingly used as a surrogate marker for functional CAR T-cell persistence.[3] Although BCA monitoring alone is insufficient in informing the risk of antigen-negative escape,[4] its effectiveness as a biomarker for CAR T-cell outcomes in the short term is being actively explored and/or implemented in real-world settings.[5]

Although interest in monitoring BCA is growing, particularly in B-cell acute lymphoblastic leukemia (B-ALL) in which CAR T-cell persistence is critical in maintaining durable remissions,[6] CAR T cells are not unique in causing BCA. Both blinatumomab (a bispecific T-cell engager targeting CD19 and CD3) and inotuzumab ozogamicin (a CD22-targeted conjugated antibody) cause BCA[7] and are increasingly incorporated earlier in treatment algorithms for B-ALL. Moreover, even if not antigen-targeted, systemic lymphoid-directed B-ALL chemotherapy induces BCA. Although prolonged BCA after the cessation of ALL therapy is rare, delayed recovery is common[8] and further impaired if additional treatment is needed (eg, with relapse). Lastly, patients with prior hematopoietic stem cell transplantation (HSCT) can have depletion of B cells for years after HSCT.[9,10] Considering that patients receiving CAR T cells are frequently those in whom standard chemotherapeutic and immunotherapeutic agents and/or HSCT have recently failed, preexisting BCA before CAR T-cell therapy is likely, as is the risk of prolonged time to recovery.

Because multiple therapeutics can induce BCA, its use as a surrogate for CAR T-cell functionality may present problems if it was preexisting. The incidence of pre–CAR T-cell BCA, however, is unknown. Here, we report on the incidence of preinfusion BCA in children, adolescents, and young adults with B-ALL across several phase 1 CAR T-cell trials.

Through a National Cancer Institute Institutional Review Board-approved protocol for patients who were enrolled on a CAR T-cell trial at our institution, we retrospectively reviewed pretreatment BCA in patients with relapsed/refractory B-ALL enrolled for treatment with CAR T cells from 2012 to 2023 (NCT03827343). Patients were treated across 4 unique phase 1 CAR T-cell trials conducted at the National Cancer Institute, including CD19.28z (NCT01593696), CD22.41BBz (NCT02315612), bivalent CD19/22.41BBz (NCT03448393), and bicistronic CD19.28z/22.41BBz (NCT05442515) CAR T-cell constructs.

The primary objective was to determine the incidence of peripheral blood BCA before initiation of lymphodepleting (LD) chemotherapy. The term "pretreatment" refers to the most proximal pre-LD/pre–CAR T-cell time point. BCA was defined as an absolute CD19 count of <50 cells per μL in peripheral blood, which is lower than all age-appropriate normal values[11] and used across other CAR T-cell trials,[6,12] and was assessed by lymphocyte phenotyping using flow cytometry. Secondary objectives were

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Data are available on request from the corresponding author, Nirali N. Shah (nirali.shah@nih.gov).

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to describe the relationship of prior therapy and/or other disease-specific characteristics with pretreatment BCA. Pretreatment characteristics, including complete blood counts, lymphocyte phenotyping, disease burden at presentation, and prior treatments, were collected by investigators from the electronic medical record for further analyses. Disease burden classification followed conventional morphologic criteria: M1, less than 5% blasts; M2, 5% to 25% blasts; and M3, greater than or equal to 25% blasts.

Patient demographics, prior treatments, incidence of BCA, and disease characteristics were analyzed with standard descriptive statistics. Associations between pretreatment characteristics and incidence of BCA were analyzed using Fischer exact test ($P = .05$). Marrow disease burden was dichotomized into M2 or higher marrow (high disease burden) and less than M2 marrow (ie, M1 marrow, low disease burden).

Collectively, 162 patients with a median age of 15.8 years (range, 4.3-38.7) were analyzed. (Table 1) Most were male (n = 112 [69.1%]) and had high disease burden (n = 106 [65.4%]). Prior, nonmutually exclusive therapies included CAR T cells (n = 64 [39.5%]), HSCT (n = 89 [54.9%]), blinatumomab (n = 56 [34.6%]), and inotuzumab (n = 30 [18.5%]; Figure 1A). Maintenance-type chemotherapy (n = 80 [49.4%]) was the most frequent regimen used as the most proximal intervention before initiation of LD (Table 1).

A total of 114 (70.4%) had pretreatment BCA, assessed at a median of 10 days before LD (range, 0-47; Figure 1B), 50% of whom had absolute CD19 values <5 cells per μL (n = 81 [50.0%]; Figure 1C). As a referral institution for alternative targeting (eg, CD22), among 45 patients with $CD19^{-}$ relapses (44 after CD19 targeting), 38 (84.4%) had pretreatment BCA.

Between those with pretreatment BCA (n = 114 [70.4%]) and without pretreatment BCA (n = 48 [29.6%]), there was no obvious difference in the prior number of lines of therapy or exposure to blinatumomab, inotuzumab, and/or CAR T cells. Overall immunotherapy exposure between those with and without BCA was 61.4% vs 66.7% ($P = .53$). However, a higher proportion of patients without preexisting BCA had prior HSCT (75.0% v 46.5%; $P = .001$; Table 1).

The most proximal pre-CAR therapy, generally based on agents used to bridge to CAR T cells, in the BCA cohort was maintenance-type chemotherapy (n = 59 [51.8%]). In the non-BCA cohort, there was an even split between maintenance-type chemotherapy (n = 21 [43.8%]) or no therapy (n = 21 [43.8%]).

Lastly, a higher proportion of patients with BCA (80/114 [70.2%]) had high disease burden than those without BCA (26/48 [54.2%]; $P = .07$). Collectively, 80 of 106 patients (75.5%) with M2 or higher marrow had BCA. (Figure 1D)

Table 1. Patient characteristics

Demographics	Total cohort (N = 162)	BCA (n = 114)	No BCA (n = 48)	*P* value
Median age (range)	15.8 (4.3-38.7)	16.9 (4.3-38.7)	14.9 (4.8-34.6)	.70
Sex				
Male	112 (69.1%)	78 (68.4%)	34 (70.8%)	.85
Female	50 (30.9%)	36 (31.6%)	14 (29.2%)	
Prior therapy				
Median number of prior treatments (range)	4 (1-14)	4.5 (1-13)	4 (2-14)	.56
Prior transplant (HSCT)	89 (54.9%)	53 (46.5%)	36 (75.0%)	.001
Median time from prior HSCT* (range), mo	22.2 (3.8-178.1)	20.9 (3.8-178.1)	24.0 (7.3-174.3)	.99
Prior CAR T cells	64 (39.5%)	48 (42.1%)	16 (33.3%)	.38
Prior blinatumomab	56 (34.6%)	38 (33.3%)	18 (37.5%)	.72
Prior inotuzumab	30 (18.5%)	20 (17.5%)	10 (20.8%)	.66
Any prior immunotherapy (including CAR T cells, blinatumomab, or inotuzumab)	102 (63.0%)	70 (61.4%)	32 (66.7%)	.53
Marrow disease burden before LD chemotherapy				
M1 marrow	56 (34.6%)	34 (29.8%)	22 (45.8%)	.07
M2 or greater	106 (65.4%)	80 (70.2%)	26 (54.2%)	
Most proximal prior therapy				
None	49 (30.2%)	28 (24.6%)	21 (43.8%)	N/A
Maintenance	80 (49.4%)	59 (51.8%)	21 (43.8%)	
Intensive chemotherapy	28 (17.3%)	22 (19.3%)	6 (12.5%)	
Immunotherapy	1 (0.6%)	1 (0.9%)	0 (0.0%)	
Other	4 (2.5%)	4 (3.5%)	0 (0.0%)	

BCA as defined by BCA <50 cells per μL. High disease burden defined as ≥M2 marrow and low disease burden defined as <M2 marrow.
N/A, not applicable.
*Date of prior HSCT available on 85 (of 89) patients: 51 of 53 patients with BCA and 34 of 36 patients without BCA.

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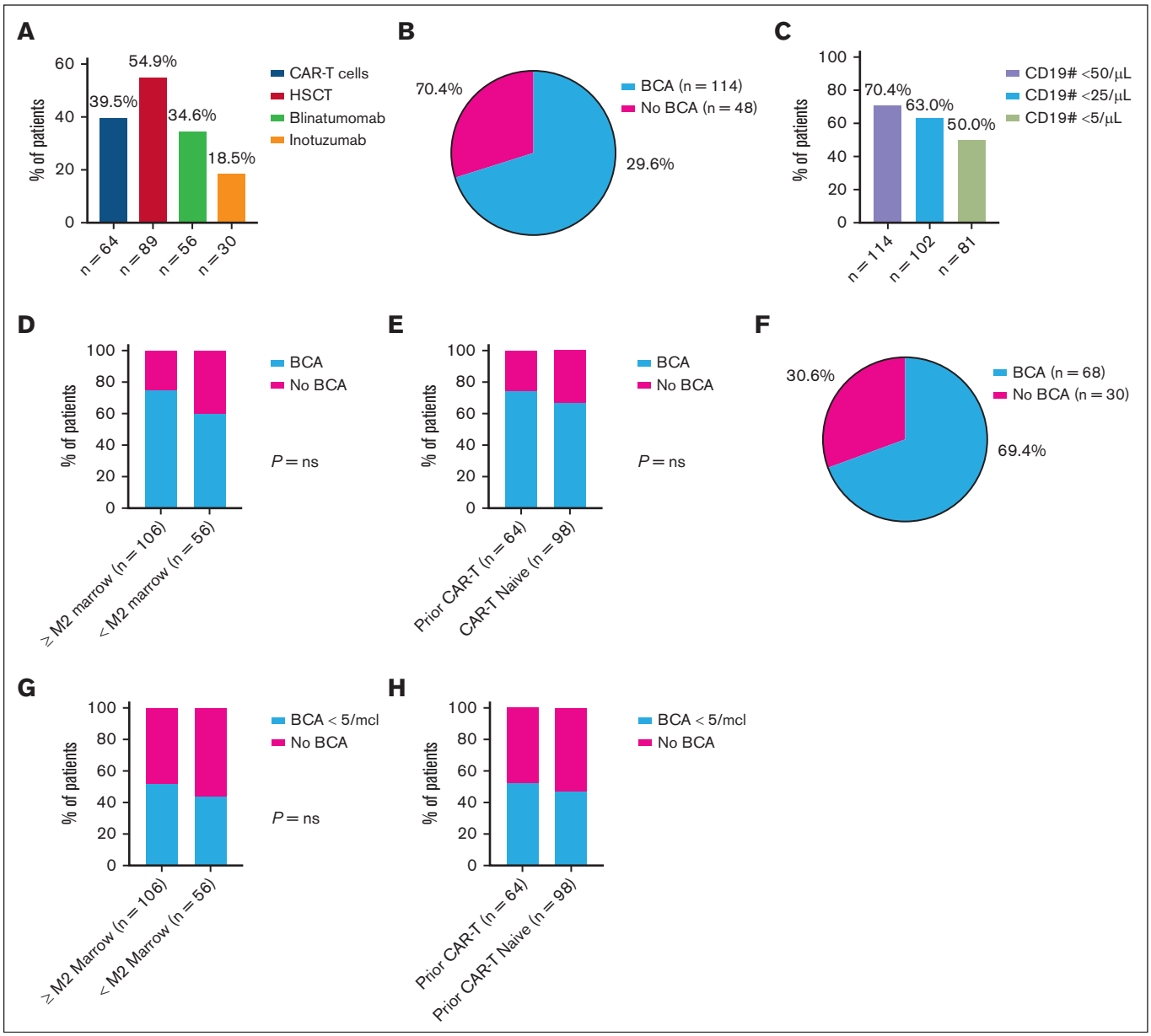

Figure 1. Key demographics across patients with and without BCA. (A) Prior nonmutually exclusive therapies, most patients had prior HSCT (54.9%), followed by CAR T-cell therapy (39.5%). (B) Percent of patients with BCA before the initiation of LD chemotherapy; most patients had BCA before LD chemotherapy in our cohort (70.4%). (C) Pre–CAR T-cell BCA CD19 cell count; various studies indicate different CD19 thresholds as indicator for BCA. Most patients achieve CD19 values of <50 cells per μL (70.4%). (D) High and low disease burden for patients with and without BCA; relatively similar proportion of patients with and without BCA in each cohort. (E) Prior CAR T-cell vs prior CAR T-cell naïve BCA; relatively similar proportion of patients with and without BCA in each cohort. (F) Percent of MRD-negative CR patients with pre–CAR T-cell BCA; greater percentage of MRD-negative CR patients had BCA (69.4%). Panels A-E describe the full cohort (N = 162). Panel F describes the MRD-negative CR cohort (n = 98). (G) High and low disease burden for patients with and without BCA, using BCA cutoff of <5 cells per μL. (H) Prior CAR T-cell vs prior CAR T-cell naïve BCA, using BCA cutoff of less than 5 cells per μL. BCA as defined by BCA less than 50 cells per μL; high disease burden defined as ≥M2 marrow and low disease burden defined as <M2 marrow. MRD-negative CR, minimal residual disease negative clinical response; ns, not significant.

Because prior CAR T cells may render subsequent BCA, we separately compared patients who had and had not received prior CAR T cells. Although those receiving prior CAR T cells were more likely to have BCA, this was not significantly different from those who were CAR T-cell naïve (75.0% v 67.3%, respectively; $P = .38$; Figure 1E).

After CAR T cells, a total of 98 patients (60.5%) achieved a minimal residual disease (MRD)–negative complete remission at day 28. Correspondingly, across 94 patients who had assessments for B-cell counts (at day 28 or beyond), all 94 (100%) had confirmed post–CAR T-cell BCA. Across those achieving MRD negativity, 68 (69.4%) had pretreatment BCA, with a median

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B-cell value of 0 cells per μL (range from 0 cells per μL to 4 cells per μL; Figure 1F).

Anticipating that more stringent values for BCA may be applied as the field advances, the above analysis was also performed by dichotomizing patients with BCA values of <5 cells per μL and those without. Across disease burden (Figure 1G) and prior CAR T-cell exposure (Figure 1H), no substantial differences were seen.

Because of the limited ability to track CAR T cells in real time and/or assess ongoing effectiveness, BCA is increasingly being used as a proxy for CAR T-cell functionality. Therefore, understanding the limitations of its use as a surrogate marker is essential.[13] Established concerns include the inability to use BCA to predict impending antigen-negative relapse[4] and its limited utility beyond 6 months after infusion.[3] Additionally, because all B-ALL therapy is lymphoid targeted (including pre–CAR T-cell LD) and may induce BCA, pre–CAR T-cell BCA may be more common than previously appreciated. This is particularly challenging because it precludes our ability to ascertain whether ongoing BCA is due to functional CAR T-cell persistence (as we would hope) or delayed recovery from prior therapy, including LD.

We report a high incidence of preexisting BCA in a cohort of CAR T-cell patients (70.4%). Given the prior therapy these patients receive, including other B-cell antigen–targeted therapy, this finding was expected but nonetheless striking. Although this incidence may be skewed in our heavily pretreated population, as antigen-targeted therapies move into frontline, monitoring for BCA will be essential. Moreover, although we defined BCA as <50 cells per μL, even when using more stringent definitions, the majority still had BCA (Figure 1C), and this generally did not differ across therapeutic exposures, except for prior HSCT. We hypothesize that post-HSCT patients may have improved immune reconstitution before proceeding with CAR T-cell infusion, but systematic evaluation is warranted.

In light of an evolving field in which antigen-burden, including the presence of normal B cells, affects CAR T-cell engraftment[14] and in which early loss of BCA after infusion may inform initiation of preemptive relapse prevention strategies,[12] understanding how to optimally use BCA is imperative. Accordingly, the high incidence of pretreatment BCA provocatively questions its use as a surrogate for CAR T-cell functionality, especially in the shorter term. Future efforts should focus on establishing systematic parameters for defining and monitoring BCA, particularly as patients are increasingly receiving antigen-targeted therapies, to better understand how preinfusion BCA affects post–CAR T-cell outcomes.

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