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GUIDELINES - CONSENSUS-BASED

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Harmonization on defining B-cell recovery post CD19-CAR T-cell therapy in B-cell acute lymphoblastic leukemia: An international consensus statement

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Abstract

Relapse following CD19-targeting chimeric antigen receptor T-cell therapy (CD19-CAR) remains a major barrier to long-term cure in relapsed/refractory B-cell acute lymphoblastic leukemia, with nearly 50% of patients relapsing within 6 months. Early B-cell recovery (BCR), as detected by the re-emergence of CD19-positive cells, has been strongly associated with relapse risk and serves as a surrogate marker for loss of CAR T-cell persistence. However, clinical use of BCR is hindered by variability in monitoring practices, including inconsistent definitions, timing, and measurement across institutions. To address this gap, we convened an international working group of pediatric cellular therapy experts to establish a consensus definition for BCR. Our collaborative effort outlines standardized criteria for BCR assessment aimed at improving comparability across studies and guiding post-CAR T-cell surveillance strategies.

INTRODUCTION

While CD19-targeted chimeric antigen receptor (CAR) T-cell therapy (CD19-CAR) has a remarkable remission induction rate in patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL), relapse remains an ongoing challenge. With approximately 50% of patients experiencing relapse,[1] and dismal outcomes for those with post-CAR T-cell relapse,[2] it remains critical to identify biomarkers prognostic for relapse to inform relapse prevention strategies.

B-cell aplasia (BCA) is an on-target/off-tumor permissive toxicity that develops following treatment with B-cell targeting CAR T cells. Therefore, ongoing BCA is a surrogate biomarker for functional CD19-CAR persistence.[3] As CD19-positive cells can be easily enumerated in peripheral blood (PB) using multiparameter flow cytometry (MFC), routine monitoring is generally feasible with a clinical assay available in all major medical centers. In contrast, the ability to directly measure CAR T cells is limited, and while commercial assays are available, none have been validated against clinical outomes.[4]

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Moreover, detection of the presence of CAR T cells does not confirm function, which is particularly important in B-ALL, where functional persistence is critical for the immune surveillance necessary to maintain remission.[5,6]

Early B-cell recovery (BCR), typically defined as within 6 months of CAR infusion, can be considered as a surrogate for loss of functional CAR T cells and is highly associated with risk of B-ALL relapse.[3,7–10] While the optimal duration of BCA has yet to be established, pooled data from the ELIANA/ENSIGN trials suggest risk of relapse is a spectrum, with earlier BCR being associated with greater risk of CD19-positive relapse.[3] Patients with BCR at 3- and 6-month post-CAR had a predicted 2-year event-free survival (EFS) of 9% and 14%, respectively. Thus, most treatment centers employ serial post-CAR BCR monitoring with surveillance intervals varying from every 2 weeks in the immediate post-infusion period to every 3 months the further patients get from infusion.[11] Due to this increased relapse risk, some clinicians advocate for additional therapies for those patients with early BCR, none of which have been studied systematically. These therapeutic approaches for relapse prevention may include consolidative allogeneic hematopoietic stem cell transplant (HSCT), reinfusion of previously manufactured CAR T cells, and/or maintenance-style chemotherapy.[12–14]

Although MFC is well validated for monitoring lymphocyte subsets in the PB, there is a lack of standardization in both B-cell quantification in the bone marrow (BM) and the frequency of post-infusion monitoring (Supporting Information S1: Figure 1). This lack of standardization is related to the technical limitations of MFC and variations in clinical monitoring embedded in prior clinical trials, which impacts clinical approaches to utilize this modality and/or interpreting results. Moreover, the threshold of detection of B cells that defines ongoing aplasia or recovery has also been variable (Supporting Information S2: Table 1). Understanding the potential pitfalls in BCA monitoring is important, as it can impact optimal utilization of this biomarker.

Given the known utility of BCA as a prognostic biomarker and the current lack of standardization, we established an international pediatric CAR T-cell working group to (1) explore the current landscape of B-cell monitoring to identify diagnostic pitfalls and (2) develop a consensus definition for BCR. In this report, we will review normal B-cell development, outline current approaches to BCA monitoring, and provide a thorough analysis of the limitations of this biomarker. We then propose a consensus definition of BCR and guidance on its optimal incorporation in monitoring post-CD19-CAR infusion.

NORMAL B-CELL DEVELOPMENT AND KINETICS

Understanding normal B-cell developmental physiology is essential to the sensitivity of BCA monitoring, particularly identifying the points at which B-cell progenitors would be targeted by CD19-targeting therapies. B-cell development is characterized by the coordinated and sequential expression of cell surface proteins along with rearrangement of immunoglobulin genes, leading to mature B cells capable of producing antibodies (Figure 1).[15,16]

The early stages of B-cell development (encompassing CD34+, CD10+, TdT+, CD19dim pro-B cells to CD34^{neg}, CD10+, CD20+, and sIg+ immature B cells) occur within the BM and are termed "hematogones" by hematopathologists.[17,18] Expression of CD19 becomes more prominent as cells transition from the earliest pro-B cells to pre-B cells. This transition includes the initiation of gene rearrangement of the immunoglobulin heavy chain and co-expression of enzymes related to this process, such as TdT. CD22 expression becomes more prominent during the pre-B-cell stage, and rearrangement of immunoglobulin light chain genes begins. As B cells mature, CD20 and newly assembled immunoglobulin molecules are expressed on the surface.[19] Importantly, CD19 expression decreases as B cells terminally differentiate into CD138+CD38+ plasma cells, with CD19 expression retained only on a subset.[20] Given the evolution of CD19 expression during normal B-cell development, few cells past the common lymphoid-progenitor stage should be seen in the setting of functional CD19-CAR persistence. Conversely, following loss of CD19-CAR, emergence of hematogones in the BM would be expected, followed by B-cell maturation and trafficking to the PB and secondary immune organs.

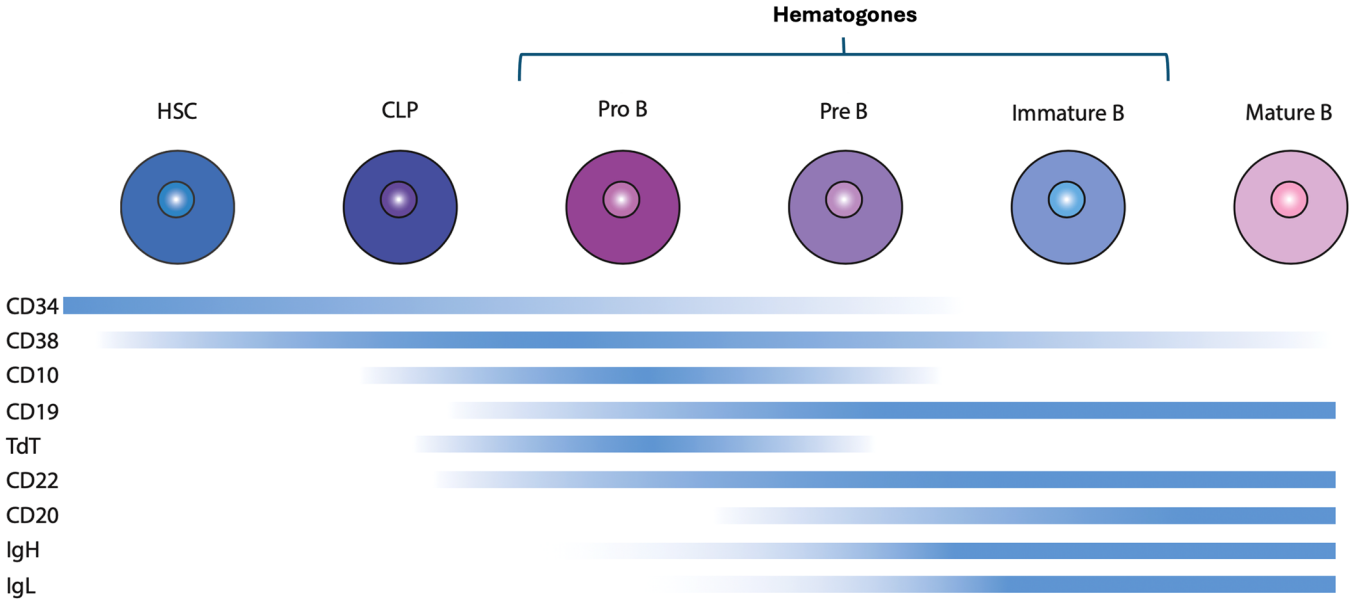

FIGURE 1 **Normal B-cell development based on surface antigen expression.** CLP, common lymphoid progenitor; HSC, hematopoietic stem cell.

A phenomenon of transient BCR following treatment with CD19-CAR has been described, where low levels of immature B cells will temporarily recover, but then the patient will regain and maintain BCA on subsequent measures.[21] Since B cells originate from a self-renewing cell pool, it is anticipated that the BM continuously attempts to produce normal B cells, even in the presence of ongoing CD19-CAR persistence. Additionally, there is a delay between B-cell development in the BM and migration into the PB, illustrating how patients may initially have evidence of BCR in the BM and not the PB.[15] These concepts highlight the importance of systematic B-cell measurement and not relying on a single compartment or timepoint to determine a patient's BCR status.

As B cells/plasma cells are responsible for antibody production, there is potential clinical utility in immunoglobulin monitoring as an additional form of surveillance. Patients are frequently rendered hypogammaglobulinemic following CD19-CAR but typically receive immunoglobulin replacement, prohibiting the use of IgG levels as a functional surrogate.[22] Less patients will become IgA-deplete following CD19-CARs, potentially due to the long lifespan of IgA-producing plasma cells, which may similarly restrict the clinical utility of IgA monitoring.[23] In contrast, IgM-producing plasma cells have a shorter lifespan, rendering a majority of patients IgM depleted following CD19-CARs. The utility of monitoring IgM levels post-CAR as an additional biomarker of functional persistence is an area of active investigation.

BARRIERS TO MONITORING FOR BCR

Despite its prognostic significance, there are multiple technical and practical barriers to the successful incorporation of BCA as a surrogate for functional CD19-CAR persistence (Table 1). These challenges have, in some instances, prevented the widespread adoption of BCR surveillance, as well as limited our ability to systematically study data from separate trials.

TABLE 1 Barriers and limitations of B-cell aplasia (BCA) utilization post-infusion.

Technical barriers
• Bone marrow B cells not universally reported
• False positives (e.g., compensation artifact, debris, and nonspecific staining of cells)
• False negatives (e.g., high background fluorescence)
• Inconsistent PB (e.g., absolute B-cell number vs. percentage of lymphocytes) and BM (e.g., B-cell percentage of total WBCs vs. MNCs) reporting
• Inter- and intra-user gating variability
Practical barriers
• Lack of universal definition of BCR
• Optimal surveillance schedule not defined
• Invasiveness of BM sampling
• Optimal management of patients experiencing BCR
Biologic limitations
• Antigen escape
• Preexisting BCA
• Transient BCR
• Different BCR kinetics based on compartment (e.g., BM vs. PB) or compared to B-ALL
• Compartment-specific loss of persistence (e.g., CNS)

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; BCR, B-cell recovery; BM, bone marrow; CNS, central nervous system; MNC, mononuclear cell; PB, peripheral blood; WBC, white blood cell.

Conventional MFC is used by most clinical laboratories to detect frequencies and phenotypic features of malignant and nonmalignant hematologic cells. General technical limitations related to MFC impacting assay sensitivity are well described and include high background fluorescence, compensation artifact, debris, nonspecific staining of cells, and varied processing methodologies.[24] These challenges can become even more pronounced when analyzing a low number of events (i.e., rare cells), hence are especially relevant to analyses of CD19-CAR recipients.[25] Additionally, data analysis is traditionally performed using manual gating, which varies based on user expertise and institutional preference. Supervised and unsupervised analyses are being explored but are not routinely used in a clinical setting.[26]

PB MFC is the most standardized modality for BCA surveillance. Monitoring lymphocyte subsets in the PB is a validated assay for several nonmalignant conditions (e.g., immunodeficiencies and inflammatory disorders), and repurposing this assay for CD19-CAR-related indications can be done with minimal (if any) modification.[27,28] However, unlike in the setting of immune disorders, the significance of detection of a low number versus complete absence of B-cell events is of great importance in patients treated with CD19-CAR, and therefore focused training may be required to ensure adequate stringency of interpretation. B cells in the PB are typically enumerated as a proportion/percentage of total lymphocytes (%) from which an absolute number (/mcL) is calculated. The B-cell percentage is contingent on the total lymphocytes, which may be influenced by other biologic factors (e.g., CAR expansion, viral infection, and immune recovery). The absolute number of B cells is a calculation based on the absolute lymphocyte count and the percentage of B cells enumerated by MFC.

The minimal invasiveness of PB sampling facilitates frequent monitoring, which is essential in the early post-CAR period. B-cell monitoring in the BM is less standardized and requires an invasive procedure but can be coupled to routine disease assessments and timepoints, such as minimal residual disease (MRD) assessment by MFC. MRD antibody panels and assays have been developed to detect abnormal populations and distinguish them from normal background cells; however, laboratories may not be accustomed to enumerating "minimal residual" normal CD19-positive B cells, such as is done for measurable residual disease analyses, particularly at levels that may be clinically relevant in the context of CD19-CAR. Since MFC sensitivity is also influenced by the number of events captured, there may be variability in the BM results based on the amount of PB contamination, paucicellularity in suboptimal aspirate samples, the presence/absence of nucleated red blood cells, or the time between sample collection and MFC testing.

Another factor limiting BM for BCA monitoring is the impact of pre-analytical processing of MFC samples on the enumeration of normal B cells. In processing PB and BM for MFC, samples are typically treated with a red blood cell lysing solution. Such reagents are efficient at lysing mature red blood cells; however, the nucleated erythroid precursors present in BM samples may be incompletely lysed. Therefore, the enumeration of B cells can be impacted by intact erythroids and whether these erythroid events are excluded from downstream analyses via gating strategies.

Furthermore, there is institutional variation in how cells are enumerated and/or the number of total captured events in MFC assays. Similar to known differences in MRD quantification, the percentage of B cells in the BM can be calculated as a proportion of a variety of different populations, most frequently either total white blood cells or mononuclear cells.[29] Depending on the denominator used, significant variations in B-cell reporting in the BM may result (Figure 2A,B). For instance, in the

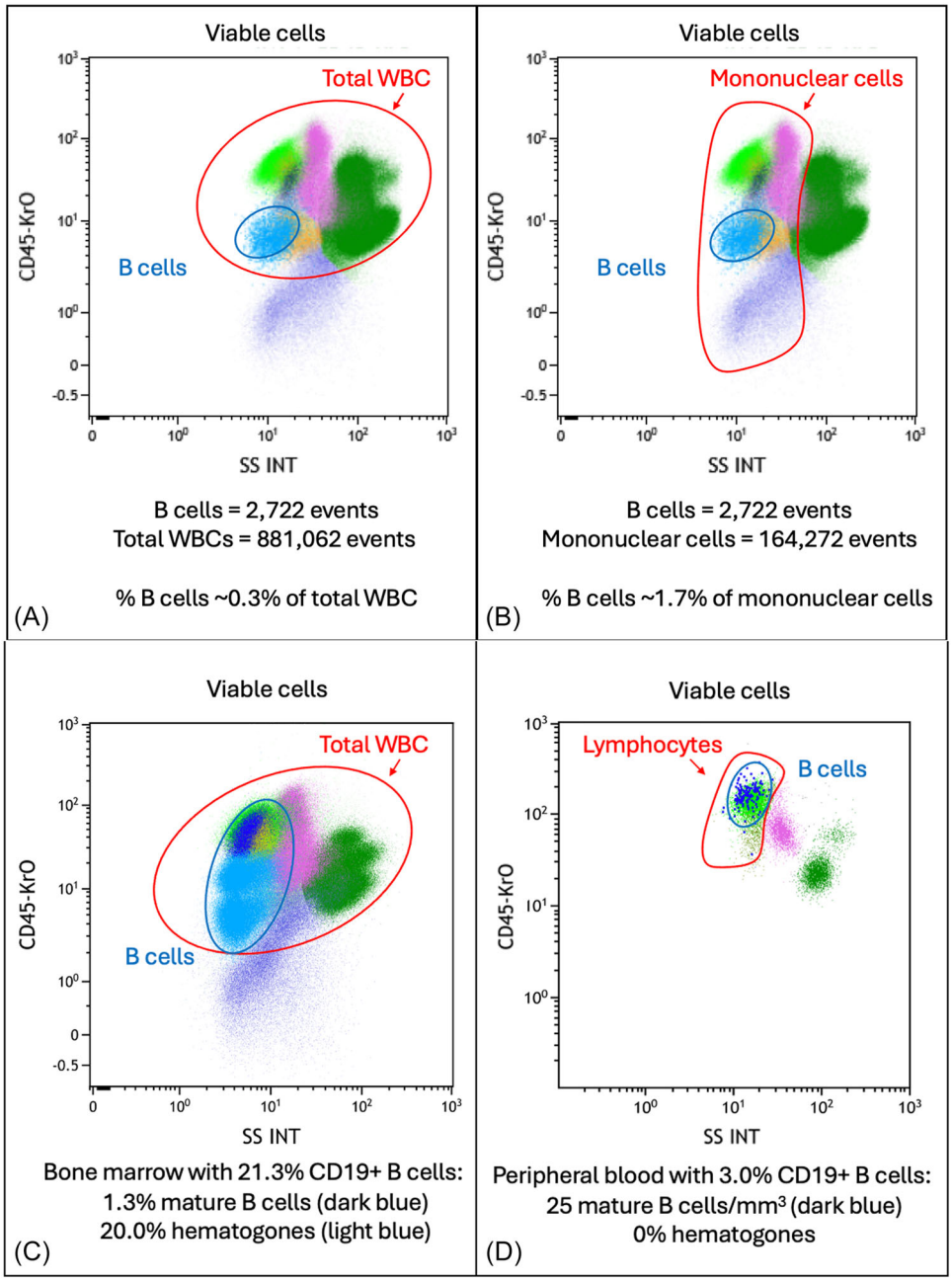

FIGURE 2 **Challenges to B-cell gating. (A, B)** Bone marrow evaluation from a patient on Day 28 following tisagenlecleucel. Flow cytometry dot plots depicting the same sample with two different gating strategies for enumeration (a, total white blood cell [WBC] vs. b, mononuclear cells). Cell populations colored as follows: light blue = B cells (hematogones), light green = mature T and NK cells (no mature B cells at this timepoint), pink = monocytes, dark green = maturing myeloid/granulocytic cells, orange = myeloid blasts, and purple = erythroid precursors (unlysed). **(C, D)** B-cell aplasia (BCA) monitoring in a patient following tisagenlecleucel. Cell populations colored as described above, with the addition of mature B cells appearing in dark blue. **(C)** Bone marrow evaluation on Day 60 with frank B-cell recovery (BCR). **(D)** Peripheral blood evaluation on Day 74 with evidence of low-level BCR.

setting of increased neutrophils, the latter technique may overestimate the percentage of B cells compared to the former technique, complicating comparability between patients treated with identical therapies.

Despite these hurdles, monitoring for ongoing BCA in the PB and BM is an essential part of management in patients treated with CD19-CAR. While many of the limitations discussed above may lead to quantitative differences, the prognostic significance of BCR is largely a qualitative question, and thus any number of detectable B cells, regardless of the level, may be clinically relevant.

CONSENSUS RECOMMENDATION

Given the heterogeneity in approaches to B-cell monitoring, it is critical to align around both a consensus definition of BCR and a robust monitoring plan. This will give treating teams the ability to act in a timely fashion in cases of early loss of functional CD19-CAR persistence. Furthermore, harmonization will allow for meaningful comparison of results across centers and future trials. In developing consensus recommendations, all aforementioned barriers need to be taken into consideration (Table 1).

We recommend using the absolute number of B cells (/mcL) for reporting, as this value provides a more detailed view of the B cells compared to percentage alone. Due to the implications of early BCR, PB should be checked at a minimum of monthly for the first 6 months following CAR infusion. As the risk of relapse in the setting of BCR after 6 months is less clear, monitoring frequency may be individualized to the patient and specific clinical situation (Table 2).

As BCR is expected to occur in the BM before the PB (Figure 2C,D), surveillance of B cells in the BM should be considered an essential part of early surveillance following CD19-CAR, when any additional lead time between BCR and overt leukemic relapse may be clinically relevant and permit intervention. Since MFC has not been validated for the detection of BCR in the BM, a BM aspirate should not be performed for the sole indication of B-cell enumeration. Instead, for BM obtained at times of standard disease evaluation, we recommend that MFC reports include a quantitative value of both mature and immature normal B cells that express CD19. Many high-volume CAR T-cell centers (e.g., >20 pediatric CAR infusions/year or participation in ≥2 multicenter CAR trials) perform BM disease evaluations at 1-, 2-, 3-, 6-, 9-, and 12-month post-CAR, and we recommend using these times to also assess for BCR in the BM (Table 2). We recognize that practice patterns may evolve as molecular and cellular assays become more accessible and validated in the post-CAR T setting. While our recommendations reflect prevailing practice among high-volume centers, future adaptations may increasingly rely on noninvasive monitoring tools to reduce patient burden.

Cell counts are not routinely performed in the BM and, as such, percentages are the only reporting option. As discussed previously, BM percentages can be reported as a proportion of a variety of different populations (e.g., total white blood cells, mononuclear cells), which can greatly impact values, particularly at low percentages. In terms of reporting CD19-positive B-cell percentages, there is no data to suggest one approach is superior to another. However, information should be recorded as to which denominator was used. Consistent reporting will promote comparability between patients and the establishment of prognostically relevant thresholds.

Upon detection of CD19-positive B cells in either the PB or BM, we consider it essential to obtain confirmation by repeat measures in most cases. This reduces the chance of falsely identifying a patient as having experienced BCR due to false positives or transient BCR, and therefore intervening in a way that could be deleterious to active CD19-CARs and/or harmful to the patient. There is no consensus on the timeframe between repeated measures, but we suggest 2–4 weeks between measures and anticipate that the confirmatory value will be higher than the initial value. Confirmatory samples can be obtained from alternative sources (e.g., PB or BM). However, as previously mentioned, the kinetics of BCR in the PB is often delayed compared to the BM; therefore, caution should be taken if the first sample is a BM and the confirmatory is PB. Although we do require two consecutive samples showing BCR to confirm loss of functional CD19-CAR persistence, the onset of BCR is defined by the date of the initial sample.

There are rare situations where normal B-cell numbers are high enough that a confirmatory sample may not be necessary. Given the potential implications of BCR and the minimal invasiveness of a PB draw, it is our recommendation to confirm all episodes of BCR, unless otherwise contraindicated. We have chosen ≥10 cells/mcL in the PB and ≥1% CD19-positive B cells in the BM as the threshold to define BCR, acknowledging that it is possible that a lower level of detection in either compartment may reflect loss of functional persistence of CD19-CAR. Since levels below these thresholds may be clinically significant, it would be reasonable to increase the frequency of surveillance for any detected CD19-positive B cells (Table 3). This threshold also reduces the risk of false positives, and there is a current lack of data to support a lower threshold. In contrast, while some historic protocols have utilized higher thresholds, inappropriately labeling a patient as having ongoing functional persistence may be clinically deleterious and cause patients to miss a therapeutic window for consolidative therapy.

Consensus definition (Table 4): Based on our expert panel, we provide the following definitions.

> *B-cell recovery is defined as peripheral blood ≥ 10 CD19-positive B cells/mcL OR ≥ 1% CD19-positive B cells in the bone marrow (as measured out of WBC or mononuclear cells). Results must be confirmed on a subsequent test ≥ 2 weeks apart with timing of B-cell recovery defined as the date of the initial sample met the aforementioned criteria.*

The proposed definition of BCR was developed through a structured consensus-building process involving international experts in CAR T-cell therapy. First, we conducted a comprehensive literature review to evaluate existing definitions of BCR and/or what constituted BCA and identified thresholds that previously correlated with clinical outcomes. Next, we distributed a survey to key opinion leaders and representatives from established cellular therapy centers to capture prevailing practice and perspectives on B-cell monitoring.

TABLE 2 Recommended timepoints for post-CD19-chimeric antigen receptor (CAR) T-cell monitoring.

			Month post-infusion											
Source	Measure	Technique	1	2	3	4	5	6	7	8	9	10	11	12
PB	BCA[a]	MFC	X	X	X	X	X	X			X			X
BM	MRD[b]/BCA[c]	MFC ± PCR/NGS	X	X	X			X			X			X
CSF	Disease	CSF assessment[d]	X											

Note: "Source" refers to sample location; "Measure" indicates the clinical or biologic readout; and "Technique" refers to the assay or platform used. Centers should tailor surveillance based on patient-specific risk, resource availability, and assay access.

Abbreviations: BCA, B-cell aplasia; BM, bone marrow; CSF, cerebrospinal fluid; MFC, multiparameter flow cytometry; MRD, minimal residual disease; NGS, next-generation sequencing; PB, peripheral blood; PCR, polymerase chain reaction; WBC, white blood cell.

[a]PB NGS-MRD and direct CAR enumeration are emerging adjuncts to BCA monitoring. These approaches may be considered in select clinical scenarios, but their role in the post-CAR T setting remains investigational and requires prospective validation.

[b]More than one MRD modality should be employed, typically PCR or NGS in combination with MFC to assess for antigen loss.

[c]Quantitative values of both mature and immature normal B cells should be included with any BM evaluation and reported as % of total white blood cell (WBC) or mononuclear cells, with gating strategy noted.

[d]CSF MFC can be helpful in certain circumstances, particularly to assess the immunophenotype of a post-immunotherapy relapse.

TABLE 3 Algorithm for assessing B-cell results.

B-cell status	Definition	Action
Ongoing BCA	• <0.01% $CD19^+$ B cells[a] in a bone marrow sample; AND • 0 $CD19^+$ cells/mcL in the peripheral blood	• Continue standard surveillance schedule
Potential BCR	• 0.01 to <1% $CD19^+$ cells[a] in a bone marrow sample; OR • 1–9 $CD19^+$ cells/mcL in the peripheral blood	• Consider intensifying surveillance schedule
Impending BCR	Single measure: • ≥1% $CD19^+$ cells[a] in a bone marrow sample; OR • ≥10 $CD19^+$ cells/mcL in the peripheral blood	• Repeat peripheral blood lymphocyte subsets and/or obtain bone marrow aspirate in 2 weeks • If repeat is stable or rising, then consistent with confirmed BCR
Confirmed BCR	Repeat measure: • ≥1% $CD19^+$ cells[a] in a bone marrow sample; OR • ≥10 $CD19^+$ cells/mcL in the peripheral blood	• Early BCR—consider consolidative strategies • Late BCR—disease surveillance or intervention per provider discretion based on patient history and timing

Abbreviations: BCA, B-cell aplasia; BCR, B-cell recovery.

[a]As measured out of total white blood cells or mononuclear cells.

TABLE 4 Consensus definition of B-cell recovery (BCR) and rationale.

"B-cell recovery is defined as peripheral blood ≥ 10 CD19-positive B cells/mcL OR ≥ 1% CD19-positive B cells in the bone marrow (as measured out of WBC or mononuclear cells). Results must be confirmed on a subsequent test ≥ 2 weeks apart with timing of B-cell recovery defined as the date of the initial sample."

Criteria	Rationale	Strength of agreement
BCR thresholds	• Any level of detectable B cells should raise concern for BCR with higher thresholds risking inappropriately labeling patients as having ongoing functional persistence. • A lower threshold risks inappropriately labeling a patient as losing functional persistence in the setting of compensation artifact, debris, etc.	Moderate agreement
Peripheral blood absolute number	• Considers absolute lymphocyte count and provides more detailed view of B-cell status compared to percentage.	Unanimous agreement
Bone marrow reporting	• B-cell reporting in the marrow may vary based on institution (e.g., as measured out of total WBC or mononuclear cells), and there is no superiority of one over the other. Information regarding gating strategy should be recorded to facilitate comparisons.	Strong majority
Confirmation	• Reduces the chance of inappropriately identifying a patient as having experienced BCR due to false positives, transient BCR, etc.	Unanimous agreement

Abbreviation: WBC, white blood cells.

Finally, we convened an international expert panel as part of the Insights in Pediatric CAR T-cell Immunotherapy: Recent Advances and Future Directions (INSPIRED) symposium, where the absence of a harmonized BCR definition was recognized as a major gap in the field.[30] The panel represented diverse institutions and geographic regions and included clinicians, translational researchers, and cellular therapy program leaders. Through real-time discussion and iterative refinement, a consensus definition was established based on shared clinical rationale and practical implementation considerations. Table 4 outlines each component of the final definition, the rationale behind it, and a qualitative assessment of agreement among the expert panel.

LIMITATIONS OF MONITORING BCA

Despite its potential prognostic utility, it is important to note that BCA is an imperfect proxy for ongoing remission (Figure 3). For instance, patients with ongoing BCA can still emerge with antigen-negative disease or even antigen-positive disease, particularly in an immune-privileged compartment (e.g., central nervous system [CNS]).[31] Furthermore, non-CAR T-cell induction of BCA (e.g., from lymphodepleting chemotherapy or prior therapies), different proliferative capacities of B cells compared to leukemia, and varying kinetics of BCR based on the compartment tested (e.g., PB compared to BM) all impact the potential utility of ongoing BCA as a surrogate for CD19-CAR functionality.[31]

Preexisting BCA

Patients with relapsed/refractory B-ALL often have preexisting BCA before CD19-CAR, which limits its reliability as a surrogate for CD19-CAR functionality. B-ALL treatment is inherently lymphotoxic, and prior therapies, including allogeneic HSCT, can cause prolonged lymphopenia. With the growing use of immunotherapies in frontline and relapse settings, many patients undergoing CD19-CAR therapy will have prior exposure to B-cell-targeting agents, such as blinatumomab, inotuzumab, or rituximab, each contributing to varying durations of BCA. For instance, rituximab, commonly used in CD20-positive B-ALL, leads to prolonged BCA lasting 6–9 months.[32,33] In contrast, blinatumomab and inotuzumab deplete B-cells rapidly, but the duration of their BCA effect is not well established. Patients

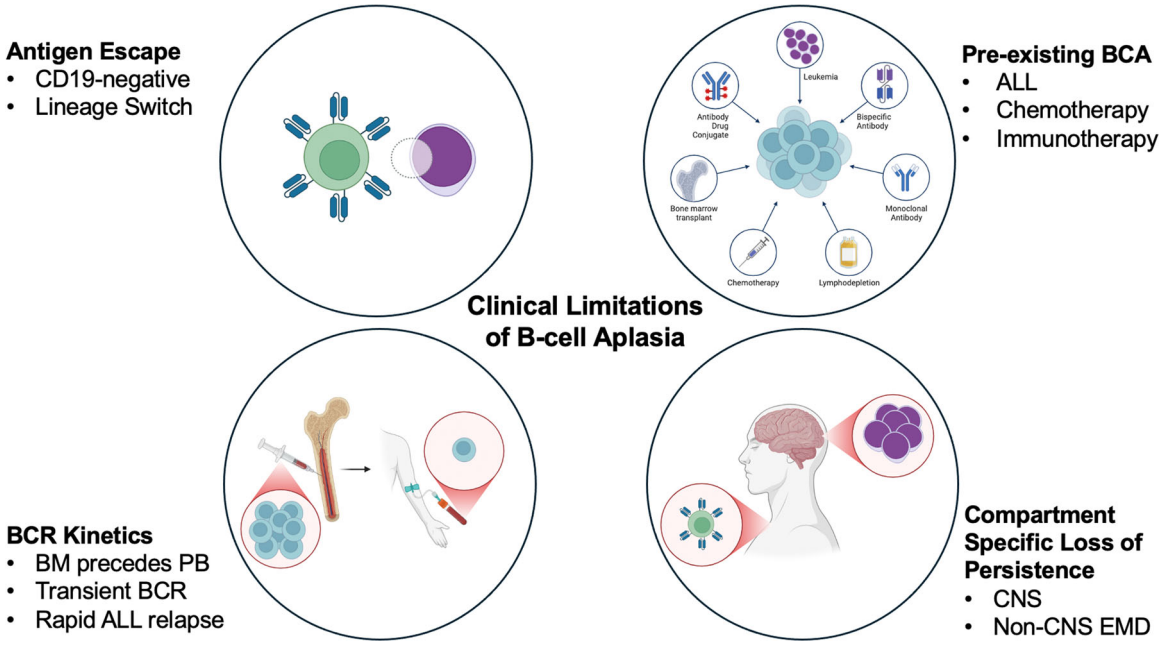

FIGURE 3 **Limitations of monitoring B-cell aplasia (BCA).** ALL, acute lymphoblastic leukemia; BCR, B-cell recovery; BM, bone marrow; CNS, central nervous system; EMD, extramedullary disease; PB, peripheral blood.

previously exposed to inotuzumab proceeding CAR, particularly those using this agent as bridging, have a high risk of BCA before lymphodepletion.[34,35] The clinical significance of this exposure is unclear, as studies have reported mixed outcomes, which may or may not be related to impacts on the B-cell compartment.[35,36]

HSCT also delays immune reconstitution, particularly for B cells and CD4-positive T cells, often requiring 6–12 months for recovery.[37,38] Many patients referred for CD19-CAR therapy after HSCT may still have incomplete B- and T-cell reconstitution. The timing of relapse post-HSCT may impact outcomes post-CD19-CAR, with at least one study showing those who relapsed within 6 months following HSCT exhibited inferior EFS following tisagenlecleucel.[39] It is hypothesized that this may be due to poor T-cell functionality early posttransplant, a more aggressive leukemia disease phenotype, and/or the lack of normal B cells to promote CD19-CAR proliferation.

Relapse despite ongoing BCA

Another limitation when using BCA as a measure of ongoing disease control after CD19-CAR is that leukemic relapse may occur despite ongoing BCA, typically in the setting of antigen escape, which would not be prevented despite ongoing CD19-CAR persistence. Loss of CD19 on leukemic blasts is reported to occur in 15%–20% of patients after treatment with CD19-CAR, with higher rates of CD19-negative relapse reported in cohorts treated with high disease burden cells.[30,31,40] Several mechanisms have been implicated in the negative expression of CD19 on ALL blasts, including alternative splicing, CD19 mutations, preexisting CD19-negative subclones, and lineage switch.[30,41–43] BCR is not associated with an increased incidence of CD19-negative relapses, and CD19-negative cells have been identified despite ongoing CD19-CAR persistence.[44] Thus, although an essential supplement to disease surveillance, the finding of ongoing BCA is insufficient evidence for leukemia control for cases in which the target antigen is no longer expressed. Therefore, alternative methods of direct-disease monitoring (e.g., immunoglobulin or T-cell receptor [Ig-TCR] rearrangements by allele-specific polymerase chain reaction [PCR] or next-generation sequencing [NGS]) are necessary in this high-risk patient population.[30,44]

In addition to antigen escape, extramedullary disease, including CNS involvement, may influence the trafficking and persistence of CAR T cells due to distinct microenvironmental factors. Barriers such as the blood-brain barrier in the CNS or unique immune landscapes in other extramedullary sites can impact the distribution and efficacy of CD19-CARs.[45,46] Variations in target antigen levels and inhospitable immune microenvironments may lead to differential CAR T-cell persistence and activity, potentially contributing to delayed or incomplete eradication of B-ALL in these compartments. While prolonged systemic CD19-CAR persistence is considered critical for durable remission, the optimal duration of persistence in extramedullary sites remains unclear. The absence of normal B cells in compartments such as the CNS complicates monitoring compartment-specific functional persistence, and direct quantification of CD19-CARs in cerebrospinal fluid or other extramedullary sites is largely confined to research settings. Further investigation is needed to better understand how microenvironmental factors influence CAR T-cell kinetics and therapeutic efficacy across extramedullary sites.

Product-specific limitations

The clinical utility of BCA as a surrogate for CD19-CAR T-cell functionality is largely dependent on the persistence profile of the CAR construct. In B-ALL, particularly following treatment with 4-1BB-based CARs such as tisagenlecleucel, longer term persistence is common and correlates with durable remissions. However, for CAR constructs that incorporate CD28 costimulatory domains, such as brexucabtagene autoleucel, persistence is generally limited.[47,48] As a result, BCA monitoring has more limited prognostic value, as most patients will require HSCT for durable disease control regardless of the timing or presence of BCR.[49]

COMPLEMENTARY POST-CAR BIOMARKERS

Incorporating the use of molecular MRD

Molecular MRD monitoring, via PCR or NGS, serves as a surveillance metric complementary to BCA in the post-CAR setting. Clone-specific Ig-TCR rearrangement PCR generally leads to sensitivity in the range of 10^{-4} to 10^{-5} cells. NGS of VDJ complementary determining regions (ClonoSEQ®) captures unique B-cell receptor and TCR sequences and can be used to identify expanded diagnostic clones and track sequences over time at a threshold of 10^{-6} cells. In contrast, MFC, the clinical standard for leukemia surveillance, detects disease at levels of 10^{-4} cells.[50] Analysis of clinical biomarkers predictive of post-CD19-CAR relapse in children and young adults with B-ALL identified the combination of both BCA and NGS-MRD to be predictive of relapse, with any nonzero detectable NGS-MRD measure post-CAR highly predictive of relapse.[3] Additionally, increased lead time before morphologic relapse was identified with NGS-MRD compared to MFC.[3] NGS-MRD is therefore becoming increasingly adopted as a clinically relevant post-CAR surveillance measure in the United States. PB-based NGS-MRD offers an appealing, noninvasive surveillance approach, but remains investigational and should be considered experimental at present.[51]

Re-emergence of disease post-CAR by molecular MRD can prompt early intervention, facilitating disease control at a time of lower disease burden. However, rising clone frequency detected via NGS or PCR does not inform surface antigen expression of CD19 and/or CD22, which can lead to therapeutic dilemmas. An additional challenge is that non-leukemia B and/or T cells can clonally expand (e.g., infection, autoimmunity), and sequences related to diagnostic clones can be stoichiometrically detected that are unrelated to active leukemic expansion. Detection of subthreshold or newly emergent clones can cause uninvited angst to patients, families, and providers, and molecular MRD management carries significant practice variability amongst pediatric CD19-CAR prescribers.[52,53] Nonetheless, molecular MRD is highly prognostic for post-CAR disease relapse, and ongoing studies are needed to standardize intervention based on results from these monitoring tools.

Efforts are underway to further understand the optimal collective clinical use of NGS-MRD and B-cell monitoring following treatment with CD19-CAR in the pediatric and young adult patient population. CAR-CURE (NCT05621291) is a multicenter clinical trial with the objective of evaluating the efficacy of monitoring NGS-MRD and BCA to guide management for HSCT-naïve patients in ongoing CD19-CAR mediated remissions. This prospective study will provide invaluable data on post-CAR management using a biomarker-guided approach and further inform our consensus monitoring recommendations. Additionally, with increased clinical use of NGS-MRD and B-cell tracking following commercial tisagenlecleucel, the real-world experience serves as a rich data source to study biomarker impact on patient outcomes. The Pediatric Real World CAR Consortium (PRWCC) multi-institutional Foresight trial (NCT05865391) aims to follow real-world outcomes of children and young adults receiving commercial tisagenlecleucel. Within this effort, a retrospective study of serial BM and PB CD19-positive B-cell values in the post-CAR follow-up window with parallel NGS-MRD values is ongoing. Merged analysis of B cell and NGS-MRD measures with post-CAR treatment course and relapse will inform the limit of detection of BCR and NGS that impact EFS. These ongoing efforts will add to existing knowledge on the prognostic significance of BCR and NGS-MRD detection to algorithmically guide management in this population and further refine our consensus BCR definition.

Direct CAR enumeration

Given that monitoring BCA is an indirect measure of CD19-CAR persistence, direct measures have been applied. The most frequently used approaches are the following: (i) MFC, which measures CAR protein expression on the cell surface and (ii) quantitative PCR (qPCR) or digital droplet (dd)PCR, which measures CAR transgene copies on a genomic level.[54] PCR assays are highly sensitive and can measure low levels of circulating CD19-CAR found early after infusion or later in the treatment course. Unlike BCA, which is a functional readout, these techniques allow quantification of circulating CD19-CAR to permit correlation with response and toxicity. MFC can characterize CD19-CAR subsets to inform evolution and enrichment of distinct dominant CD19-CAR subsets over time. Further, in patients with relapse, utilization of both MFC and PCR assays theoretically could allow for the distinction between CAR loss and CAR exhaustion. Thus far, these approaches have been predominantly used in the clinical trial setting and, as a result, extensive heterogeneity exists in methodologies used and reporting across trials. There are now commercially available CAR T-cell transgene assays, although limited data are available on their clinical correlation.[4] These assays have the potential to identify CAR loss preceding BCR or NGS-MRD positivity, but ongoing studies are needed. Ultimately, the goal is to standardize reporting and evaluate the utility of these measurements to guide prognostication and clinical decision-making.

While each assay described above has unique strengths and limitations, NGS-MRD and BCA monitoring should be viewed as complementary rather than competing tools in the post-infusion setting. NGS-MRD provides highly sensitive disease detection, whereas BCA captures functional bioactivity in real time. Direct CAR enumeration assays have the potential to provide mechanistic insight into cell persistence, but until prospective studies define an optimal integrated surveillance strategy, BCA monitoring, used alongside MRD assessment, remains the most practical approach for pediatric and young adult B-ALL patients.

CONCLUSION

In conclusion, BCA serves as an important, though imperfect, surrogate for functional persistence of CD19-CAR. While BCA monitoring can provide valuable insights into CD19-CAR disease control, multiple factors—including biologic variability, preexisting conditions, antigen escape, and technical limitations—complicate the use of BCA as a predictor of long-term remission. Establishing consensus definitions and standardized protocols for BCR in both PB and BM is critical to ensuring uniformity across clinical settings and research trials. By defining specific thresholds and promoting frequent monitoring, clinicians can better predict outcomes and manage potential relapses, particularly in the early months post-CAR. Moving forward, a more comprehensive understanding of CD19-CAR persistence, combined with harmonized B-cell monitoring strategies, will be essential to optimizing treatment for patients with B-ALL.

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AUTHOR CONTRIBUTIONS

Adam Lamble: Conceptualization; writing—review and editing; visualization; writing—original draft. **Sandra D. Bohling**: Writing—review and editing; visualization; writing—original draft. **Kara L. Davis**: Writing—original draft; writing—review and editing; visualization. **Aimee C. Talleur**: Writing—original draft; writing—review and editing. **Kevin O. McNerney**: Writing—original draft; writing—review and editing. **Swati Naik**: Writing—original draft; writing—review and editing. **Priya Kumar**: Writing—original draft; writing—review and editing. **Rebecca Thomas**: Writing—original draft; writing—review and editing. **Hao-Wei Wang**: Writing—original draft; writing—review and editing. **Constance M. Yuan**: Writing—original draft; writing—review and editing. **Elad Jacoby**: Writing—original draft; writing—review and editing. **Andre Baruchel**: Writing—original draft; writing—review and editing. **Sara Ghorashian**: Writing—original draft; writing—review and editing. **Michael A. Pulsipher**: Writing—original draft; writing—review and editing. **Liora Schultz**: Writing—original draft; writing—review and editing. **Rebecca A. Gardner**: Writing—original draft; writing—review and editing. **Nirali N. Shah**: Writing—original draft; writing—review and editing; visualization; conceptualization.

CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

DISCLAIMER

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

ETHICS STATEMENT

Not applicable. This article does not include original research involving human participants or animals.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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