

Biomarker discrepancies following chimeric antigen receptor T cells: implications in B-cell acute lymphoblastic leukemia

by Chelsea Lamplugh, Julie Ma, Bonnie Yates, Hao-Wei Wang, Constance M. Yuan, Chloe Hoang, Sara K. Silbert, Tanvi Verma, David Peske, Benjamin L. Green, Ilan Kirsch, Challice Bonifant and Nirali N. Shah

Received: July 23, 2026.

Accepted: September 29, 2026.

Citation: Chelsea Lamplugh, Julie Ma, Bonnie Yates, Hao-Wei Wang, Constance M. Yuan, Chloe Hoang, Sara K. Silbert, Tanvi Verma, David Peske, Benjamin L. Green, Ilan Kirsch, Challice Bonifant and Nirali N. Shah. Biomarker discrepancies following chimeric antigen receptor T cells: implications in B-cell acute lymphoblastic leukemia.

Haematologica. 2026 Oct 8. doi: 10.3324/haematol.2026.301688 [Epub ahead of print]

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science.

Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.

E-publishing of this PDF file has been approved by the authors.

After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval, the final version of the manuscript will then appear in a regular issue of the journal.

All legal disclaimers that apply to the journal also pertain to this production process.

**Biomarker discrepancies following chimeric antigen receptor T cells:
implications in B-cell acute lymphoblastic leukemia**

Chelsea Lamplugh^{1,2}, Julie Ma^{1*}, Bonnie Yates¹, Hao-Wei Wang³, Constance M. Yuan³, Chloe Hoang¹, Sara K. Silbert¹, Tanvi Verma⁴, David Peske⁴, Benjamin L. Green⁵, Ilan Kirsch⁵, Challice Bonifant², Nirali N. Shah^{1*}

¹Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD; ²Department of Pediatric Oncology, Johns Hopkins Children's Center, Baltimore, MD; ³Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, MD; ⁴Johns Hopkins School of Medicine, Department of Pathology, Baltimore, MD; ⁵Adaptive Biotechnologies, Seattle, WA

*Current affiliation: Julie Ma, Division of Pediatric Hematology, Oncology, Stem Cell Transplantation & Regenerative Medicine, Stanford University School of Medicine, Palo Alto, CA; Nirali N. Shah, University of Maryland, School of Medicine, Baltimore, MD

Correspondence:

Nirali N. Shah, MD, MHSc (*Current location)
University of Maryland School of Medicine
22 S. Greene Street
Baltimore, MD 21201 USA.
E-mail: Nirali.Shah@som.umaryland.edu
Phone: 410-328-2955

Running title: MRD and BCA post CAR

Disclaimer: This research was supported in part by the Intramural Research Program of the National Institutes of Health (NIH). The contributions of the NIH author(s) were made as part of their official duties as NIH federal employees, are in compliance with agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the author(s) and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

Keywords: chimeric antigen receptor T-cell, immunotherapy, B-cell acute lymphoblastic leukemia, minimal residual disease, B-cell aplasia, next generation sequencing

Author contribution

C.L. and N.N.S designed the study concept, conducted the primary analysis and wrote the first draft of the manuscript. All authors contributed with data and critical input into the data analysis and writing. C.M.Y., H.W.W. performed and analyzed flow cytometry. J.M., B.Y., S.K.S., C.B., and N.N.S., provided patient care. I.K. and B.G. provided critical insights with NGS data. All authors contributed significantly to the final version of the manuscript and agree to its submission.

Disclosures

N.N.S. receives research funding from Lentigen, VOR Bio, and CARGO Therapeutics. N.N.S. has attended advisory board meetings (no honoraria) for VOR, ImmunoACT, and Sobi. N.N.S. received royalties from NIH for development of CD22 CAR. . C.L.B. has been awarded and has pending patents in the field of engineered cellular therapy. BLG are LK are employees at Adaptive Biotechnologies Corporation.

Data sharing

De-identified participant data that support the findings of this article will be shared upon approved written request to researchers who provide a methodologically sound proposal for the purposes of achieving specific aims outlined in that proposal. To gain access, data requesters will need to sign a data access agreement and to confirm that data will only be used for the agreed purpose for which access was granted. Requests can be directed to Nirali N. Shah, MD: nirali.shah@nih.gov

Acknowledgements

This work was supported in part by the Intramural Research Program, Center of Cancer Research, National Cancer Institute and NIH Clinical Center, National Institutes of Health (ZIA BC 011823, N.N.S). C.L.B was partially supported by an Alex's Lemonade Stand Million Mile Award. We would like to acknowledge Kris Mahadeo and Roland Matsouaka for being part of the NIH-Duke Clinical Research Training Program (CRTP) master's thesis committee for C.L.

To the Editor,

While chimeric antigen receptor T-cell therapy (CAR-T) has been widely utilized for treatment of B-cell acute lymphoblastic leukemia (B-ALL) in children, adolescent, and young adult (CAYA), relapse remains a concern. Measurable residual disease (MRD) and B-cell aplasia (BCA) are key biomarkers utilized in the post-CAR setting to monitor for response, yet data on how post-infusion biomarkers cross compare with one another remains limited (1-5). One particular area of concern with MRD and BCA monitoring is its applicability in the setting of extramedullary disease (EMD), as these modalities rely on peripheral blood (PB) and bone marrow (BM) assessment (6-8). Here, we report on a cross-comparison of key biomarkers in a cohort of CAYA patients with B-ALL who achieved a FC-MRD^{neg} complete remission (CR) following CD19 and/or CD22-targeted CAR T-cell therapy, incorporating a sub analysis of those with EMD.

This National Institutes of Health IRB approved retrospective study evaluated CAYA patients with r/r B-ALL who achieved a FC MRD^{neg} CR at approximately day +28 (D+28) following CAR T-cell therapy administered between February 15, 2013 and April 29, 2025 at the National Cancer Institute (NCI) across phase I clinical trials for investigational CD19 (NCT01593696), CD22 (NCT02315612), bivalent CD19/CD22 (NCT03448393), and bicistronic CD19/CD22 (NCT05442515) or Johns Hopkins Children's Center (JHCC) who received commercial or investigational (tisagenlecleucel) CD19 CAR T-cell therapy (**Supplemental Appendix Table 1**). The primary aim was to assess for discrepancies in post-CAR T-cell infusion biomarkers. We additionally described disease detection metrics and BCA trends pre and post-infusion. For patients with available clonoSEQ (Adaptive Biotechnologies Corporation, Seattle WA) reports, MRD was assessed based on B-cell rearrangement. Patients with CNS disease and EMD were evaluated with CSF studies and fluorodeoxyglucose-positron emission tomography (PET) scan, respectively. For EMD, those with partial responses (PR) or CR were included. BCA

was evaluated in both the BM (<1% B-cells) by FC and NGS and PB (absolute CD19+ count <10 cells/ μ L (4)) using FC. To calculate B-cells by NGS, the total number of immunoglobulin heavy chain sequences was divided by the total number of nucleated cells and multiplied by 100 (**Supplemental Appendix Figure 1**). Primary statistical analysis was descriptive. Non-parametric t-tests, Chi squared tests, Fisher's exact test, and log-rank tests were used as appropriate using GraphPad Prism version 10.1. Tests were performed using non-parametric analyses with a significance threshold of $p < 0.05$.

Collectively 196 patients with B-ALL received CAR T-cells, of which 129 (65.8%) achieved BM MRD^{neg} remission by FC (**Figure 1A**). Patient characteristics are in **Table 1**. Across 129 patients, 46 (35.7%) had BM NGS at restaging (**Table 1**). In 17 (37%), NGS-MRD was positive despite FC-MRD negativity (**Figure 1B**). The median number of clones detected by NGS-MRD was 11 per million nucleated cells (range 1-264), using the most highly detected sequence. Twenty-one (45.7%) patients relapsed, with higher rates of relapse in NGS-MRD positive patients (9/17, 52.9%) versus those who were NGS-MRD^{neg} (12/29, 41.4%) ($p=0.54$). Similarly, median time to relapse was shorter for those with NGS-MRD positivity (9 months, $n=17$ versus not defined, $n=29$; $p=0.09$) (**Figure 1C**).

_____ Across 129 patients, 122 (94.6%) had post-CAR PB BCA assessment. All 122 (100%) had PB FC-BCA (**Figure 1D**). Interestingly, 54.8% of patients had pre-existing PB FC-BCA.

Across 125 patients with post-CAR BM FC-BCA assessments, 106 (84.8%) had BM FC-BCA (**Figure 1E**). Interestingly, 18 subjects with PB FC-BCA did not have BM FC-BCA. In comparing outcomes between those with and without FC BM BCA, 58/106 (54.7%) with BCA versus 8/19 (42.1%) without FC-BCA ($p=0.33$) experienced relapse with no difference in time to relapse ($p=0.32$) (**Figure 1F**).

Of 46 subjects with NGS analysis available, 40 (87.0%) had BM NGS-BCA (**Figure 1G**). While more patients without NGS-BCA experienced relapse (5 of 6, 83.3% without NGS-BCA to 15 of 40 (40%) with BCA) $p=0.08$, the smaller numbers limit the analysis. Those without BM NGS-BCA also relapsed earlier (median time, 7.5 months for those without BM NGS-BCA versus undefined ($p=0.05$) (**Figure 1H**).

Comparing FC to NGS results for BCA, while all had post-CAR PB FC-BCA, 39 (86.7%) had post-CAR BM FC-BCA (one did not have BM FC-BCA assessed) and 40 (87.0%) had post-CAR BM NGS-BCA. Importantly, discrepancies between BM FC-BCA and NGS-BCA were not seen, and all subjects who had BM NGS-BCA also had concurrent FC-BCA (**Figure 2A**).

_____Of the 17 subjects with BM NGS-MRD positivity at restaging, all demonstrated PB FC-BCA (one did not have PB FC-BCA assessed) and 16 (94.1%) had BM FC-BCA and BM NGS-BCA. The other subject demonstrated BCR (**Figure 2B**). Of the 29 subjects who were BM NGS-MRD^{neg}, all demonstrated PB FC-BCA, with 23/28 (82.1%) having BM FC-BCA (one did not have BM FC-BCA measured) and 24/29 (82.8%) having BM NGS-BCA (**Figure 2C**).

Combining the various BM biomarkers with outcomes, of the 25 patients who were NGS-MRD^{neg} and had NGS-BCA, 8 (32%) relapsed. Of the 4 patients who were NGS-MRD^{neg} and did not have NGS-BCA, all 4 (100%) relapsed. In contrast, in the 15 patients who were NGS-MRD positive and had NGS-BCA, 8 (53.3%) relapsed compared to the 2 patients who were NGS-MRD positive and did not have NGS-BCA, of whom 1 (50%) relapsed (**Supplemental Table 2**).

Across 19 patients with active CNS EMD (CNS1 with flow detectable or CNS2 status), 15 (78.9%) had concurrent BM disease. All 19 achieved FC MRD^{neg} CR; all had PB FC-BCA and 14 (73.7%) had BM FC-BCA post-CAR. Twelve (63.2%) relapsed, at a median of 5.2 months.

Relapse was seen in 8 of 14 (57.1%) patients with BM FC-BCA compared to 4/5 (80%) without BM FC-BCA, warranting further study of FC-BCA as a risk factor in those with CNS disease.

In 23 patients (17.8%) with non-CNS EMD, 16 had concurrent BM disease (all achieved FC MRD^{neg} CR). In those with post-CAR PB evaluation for BCA (n=21), all had PB BCA and 17 (73.9%) had post-CAR BM FC-BCA. Twelve (52.5%) had NGS results available and 2 (16.7%) were NGS-MRD positive and 8 (66.7%) had NGS-BCA. Despite small numbers, in those with non-CNS EMD, the percentage of those who were NGS-MRD positive at restaging was lower compared to the overall cohort (16.7% vs 37%), even though many patients also had medullary disease. Additionally, the percentage of those with post-infusion BM FC and NGS-BCA was lower in this group (73.9% and 66.7%, respectively) compared to the overall cohort (84.8% and 87%, respectively). This suggests that the typical biomarkers used post CAR T-cell infusion may not be as applicable in this unique group of patients.

Collectively, in patients with either CNS or non-CNS EMD, BM BCA was less commonly achieved compared to those with BM involvement alone. Specifically, the percentage of patients having BM FC-BCA was 73.7% (14/19 patients) in those with CNS disease; 73.9% (17/23 patients) in those with non-CNS EMD), and 88.4% (76/86 patients) in those without any EMD

This exploratory analysis of post-CAR biomarkers, incorporating the novel use of NGS in assessing BCA, identified relatively high rates of discrepancy across disease detection parameters with general concordance across BCA assessment. As expected, a substantial proportion of patients who were BM FC-MRD^{neg} at initial restaging timepoint were NGS-MRD positive, highlighting the importance of utilizing NGS. In contrast, FC-BCA and NGS-BCA in the BM were fully concordant, with most patients having NGS-BCA, suggesting FC-BCA may be sufficient for monitoring. Interestingly, that same concordance was not seen across compartments. Specifically,

15.2% did not have BM FC-BCA while all had PB FC-BCA, highlighting the importance of assessing BM BCA using any modality. Importantly, for NGS-BCA, additional samples are not needed as the data used for the computation is already included in the clinical NGS-MRD report, supporting prospective evaluation of NGS-BCA.

In addition to our comprehensive analysis, we assessed both CNS EMD and non-CNS EMD separately. In both cohorts, rates of post-infusion BCA were lower compared to patients without EMD where high rates of relapse (82.6%) were seen, raising concern that these biomarkers may not be as reliable in EMD (7).

While trial and construct heterogeneity, along with limited serial monitoring given substantial use of consolidative HSCT, impacts our results, further study of the relative predictive value of relapse with NGS-MRD positivity compared to early loss of BCA in the BM warrants prospective study. Additionally, the lack of a standardized definition for BM BCA could change how these results are interpreted (4). Lastly, the generalizability of our data based on patients with extensively relapsed disease, may limit applicability

In conclusion, our comprehensive analysis of post-CAR T-cell biomarkers provides insights into the utilization of multiple modalities for monitoring both disease detection and relapse risk in CAYA patients with B-ALL. Discrepancies between FC-MRD and NGS-MRD at identical timepoints highlights the importance of NGS for disease reassessment. Additionally, we found high levels of concordance between BM FC-BCA and BM NGS-BCA, suggesting either of these methods are likely sufficient. Further exploration is warranted to evaluate these metrics in the upfront setting in treatment of B-ALL.

References

1. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood*. 2008;111(12):5477-5485.
2. Short NJ, Aldoss I, DeAngelo DJ, et al. Clinical use of measurable residual disease in adult ALL: recommendations from a panel of US experts. *Blood Adv*. 2025;9(6):1442-1451.
3. Pulsipher MA, Han X, Maude SL, et al. Next-generation sequencing of minimal residual disease for predicting relapse after tisagenlecleucel in children and young adults with acute lymphoblastic leukemia. *Blood Cancer Discov*. 2022;3(1):66-81.
4. Lamble A, Bohling SD, Davis KL, et al. Harmonization on defining B-cell recovery post CD19-CAR T-cell therapy in B-cell acute lymphoblastic leukemia: an international consensus statement. *Hemasphere*. 2025;9(11):e70247.
5. Hamidi R, Bhatt D, Zhang A, et al. Establishing Concordance of next generation sequencing and flow cytometry measures of B-cell recovery post-CAR T Cell therapy in B-ALL. *Transplant Cell Ther*. 2025;31(2):S205-S206.
6. Johns C, Erickson C, Jacobs A, et al. Case report: sub-clinical extramedullary B-ALL in the setting of relapse following targeted therapy. *Front Immunol*. 2024;15:1423487.
7. Holland EM, Yates B, Ling A, et al. Characterization of extramedullary disease in B-ALL and response to CAR T-cell therapy. *Blood Adv*. 2022;6(7):2167-2182.
8. Friedes BD, DiNofia AM, Iannone E, et al. Therapies and outcomes following extramedullary relapse of pediatric and young adult ALL after CD19 CAR T-cell therapy. *Blood Adv*. 2025;9(2):354-359.

Table 1

Table 1: Patient Characteristics		
	All Patients (n=129)	Patients with available NGS data (n=46)
Median age	15.3 (range 0.8-38.7)	16.0 (range 0.8-38.1)
Sex		
Male	81 (62.8%)	25 (54.3%)
Female	48 (37.2%)	21 (45.7%)
Race		
Caucasian	88 (68.2%)	29 (63.0%)
Black or African American	6 (4.7%)	4 (8.7%)
Asian	13 (10.1%)	6 (13.0%)
Hawaiian or Pacific Islander	2 (1.6%)	0 (0.0%)
American Indian or Alaskan Native	0 (0%)	0 (0.0%)
Multiracial	4 (3.1%)	2 (4.3%)
Other	3 (2.3%)	2 (4.3%)
Unknown	13 (10.1%)	3 (6.5%)
Ethnicity		
Hispanic	46 (35.7%)	18 (39.1%)
Non-Hispanic	80 (62.0%)	26 (56.5%)
Unknown	3 (2.3%)	2 (4.3%)
Type of CAR		
Investigational	106 (82.2%)	35 (76.1%)
Commercial	23 (17.8%)	11 (23.9%)
Prior therapy		
Prior CAR	33 (25.6%)	10 (21.7%)
Prior blinatumomab	44 (34.1%)	17 (37.0%)
Prior inotuzumab	16 (12.4%)	7 (15.2%)
Prior HSCT	55 (42.6%)	20 (43.5%)
CAR Construct		
CD19 (NCI)	27 (20.9%)	0 (0.0%)
CD22 (NCI)	41 (31.8%)	8 (17.4%)
Bivalent CD19/22 (NCI)	27 (20.9%)	18 (39.1%)
Bicistronic CD19/22 (NCI)	10 (7.8%)	9 (19.6%)
CD19 (JHH)	1 (0.8%)	0 (0.0%)
Tisagenlecleucel	23 (17.8%)	11 (23.9%)
BM disease status pre-CAR		
Active disease pre CAR	127 (98.4%)	46 (100%)
No BM disease	13 (10.1%)	5 (10.9%)
M1	53 (41.1%)	21 (45.7%)
M2	16 (12.4%)	6 (13.0%)

M3	47 (36.4%)	14 (30.4%)
Other sites of disease		
CNS disease pre-treatment	19 (14.7%)	3 (6.5%)
Non-CNS EMD	23 (17.8%)	12 (26.1%)
Abbreviations: CAR: Chimeric Antigen Receptor HSCT: Hematopoietic Stem Cell Transplant BM: Bone marrow M1: <5% blasts in bone marrow M2: 5-25% blasts in bone marrow M3: >25% blasts in bone marrow CNS: Central nervous system EMD: Extramedullary disease		

Figure legends:

Figure 1: Disease detection metrics and biomarkers and their impact on patient outcomes. (A) Consort diagram for the analyses performed, including those of disease detection as well as BCA assessment. (B) Disease detection discrepancies as outlined by percentage of patients who were BM NGS-MRD+ and BM NGS-MRD- despite BM FC-MRD negativity and (C) time to relapse in those who were BM NGS-MRD+ vs BM NGS-MRD-. BCA discrepancies post CAR T-cell infusion, including (D) PB FC-BCA, (E) BM FC-BCA, (F) time to relapse based on BM FC-BCA status, (G) BM NGS-BCA and (H) time to relapse based on BM NGS-BCA status.

Abbreviations:

BCA: B-cell aplasia

BM: Bone marrow

NGS: Next generation sequencing

MRD: Measurable residual disease

FC: Flow cytometry

CAR: Chimeric antigen receptor

PB: Peripheral blood

Figure 2: Cross comparison of biomarkers post CAR T-cell infusion. (A) Heat map showing a cross comparison of post-CAR T-cell therapy biomarkers (PB FC-BCA, BM FC-BCA, BM NGS-BCA, BM NGS-MRD) and frequency of relapse (B) Cross comparison of post-CAR biomarkers in patients who were NGS-MRD positive (C) Cross comparison of post-CAR biomarkers in patients who were NGS-MRD^{neg}.

Abbreviations:

CAR: Chimeric antigen receptor

PB: Peripheral blood

FC: Flow cytometry

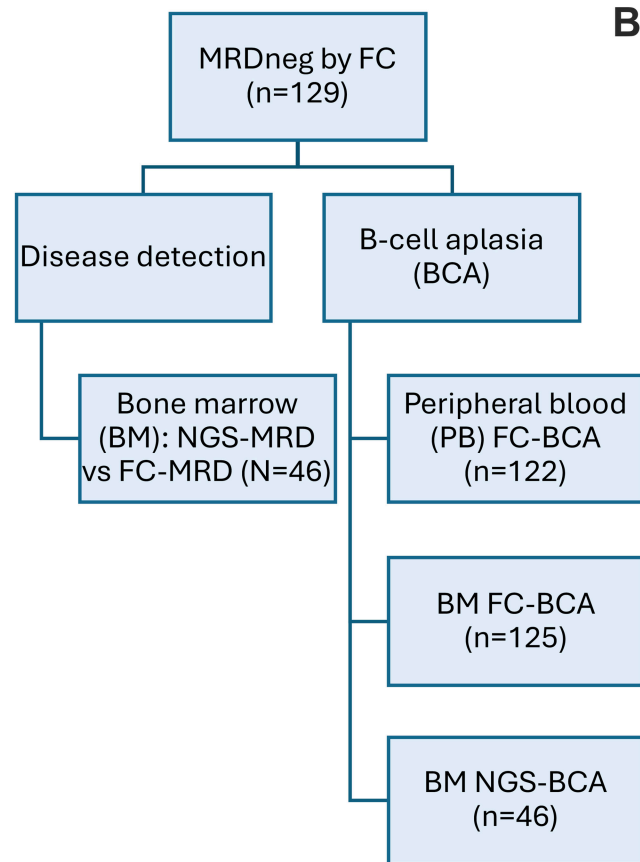
BCA: B-cell aplasia

BM: Bone marrow

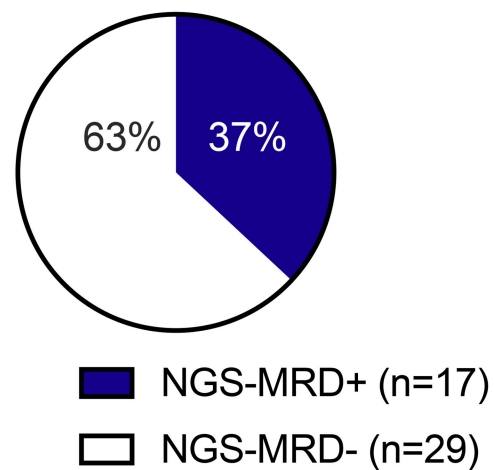
NGS: Next generation sequencing

MRD: Measurable residual disease

A

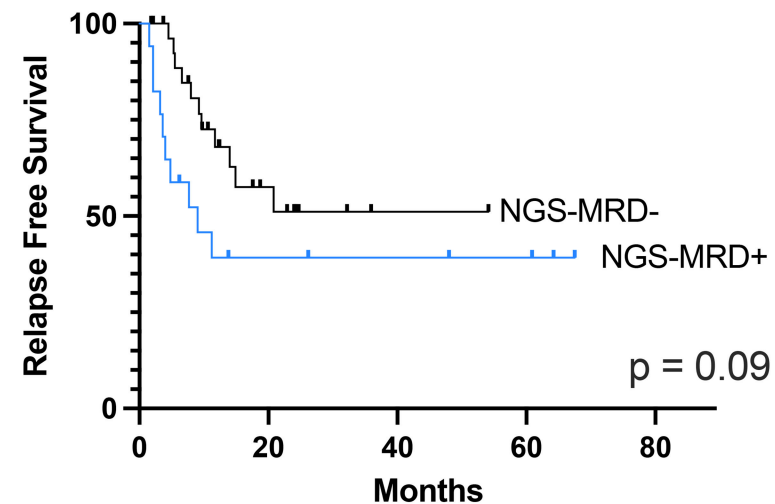


B



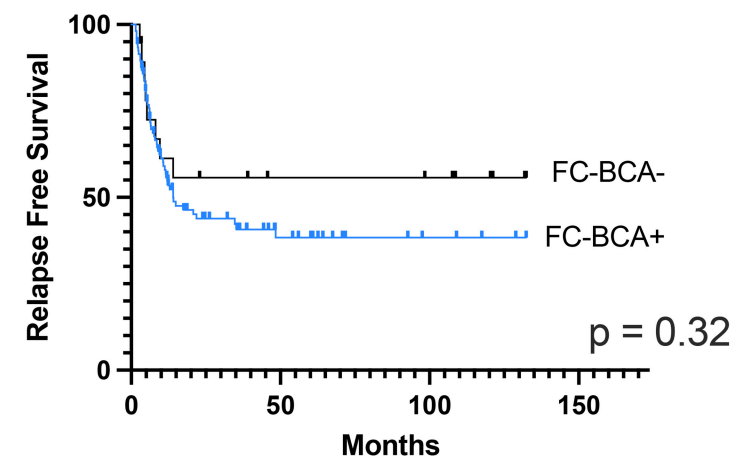
C

Time to Relapse Based on NGS-MRD Status



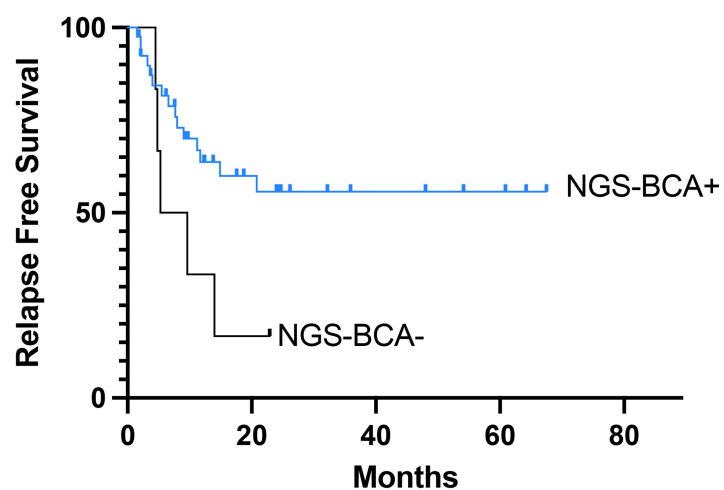
F

Time to Relapse Based on FC-BCA Status

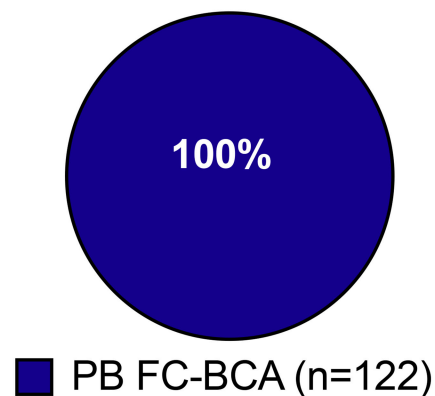


H

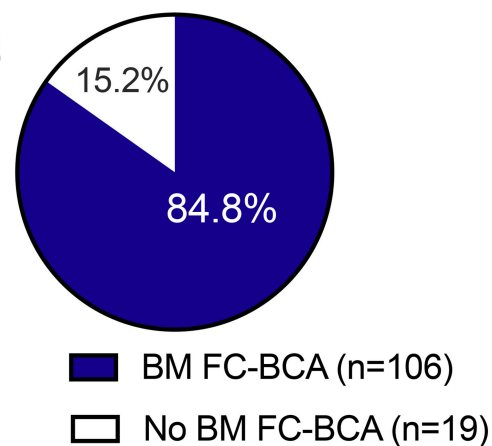
Time to Relapse Based on NGS-BCA Status



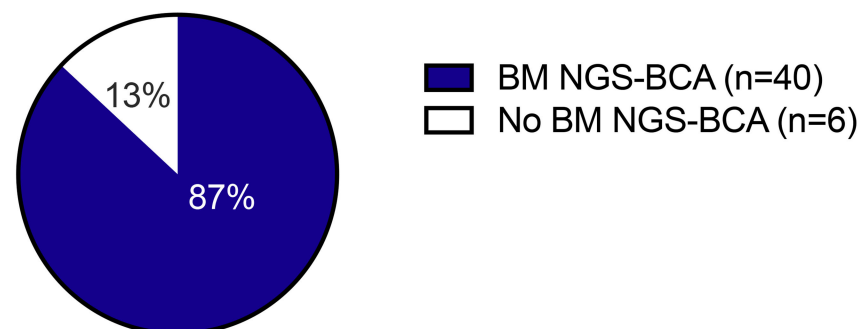
D

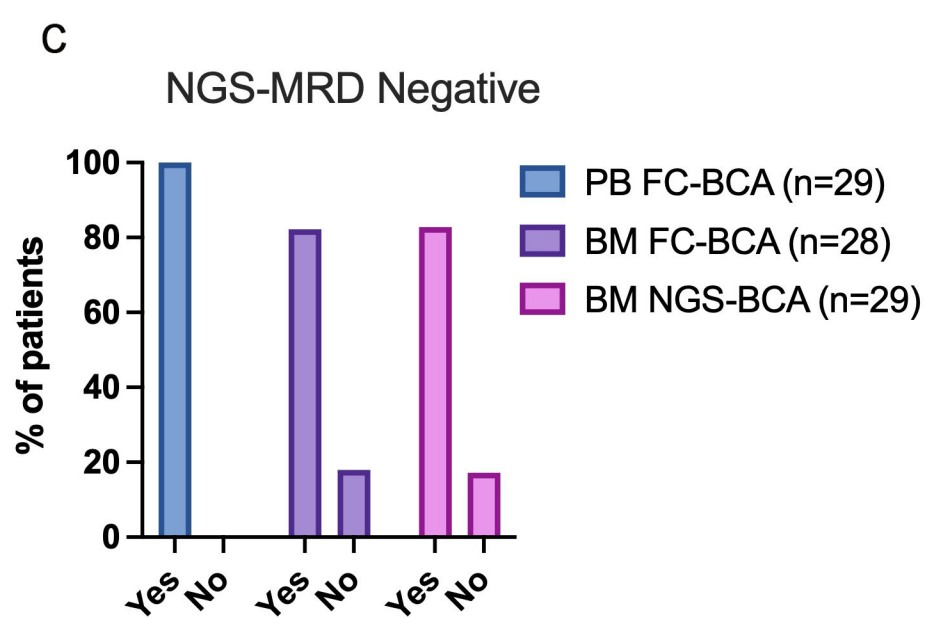
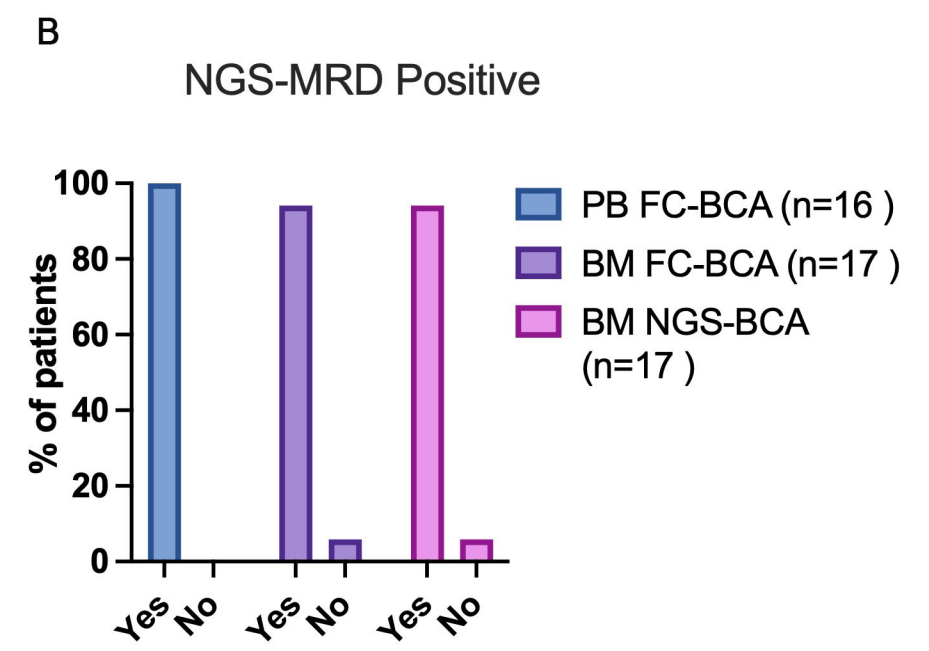
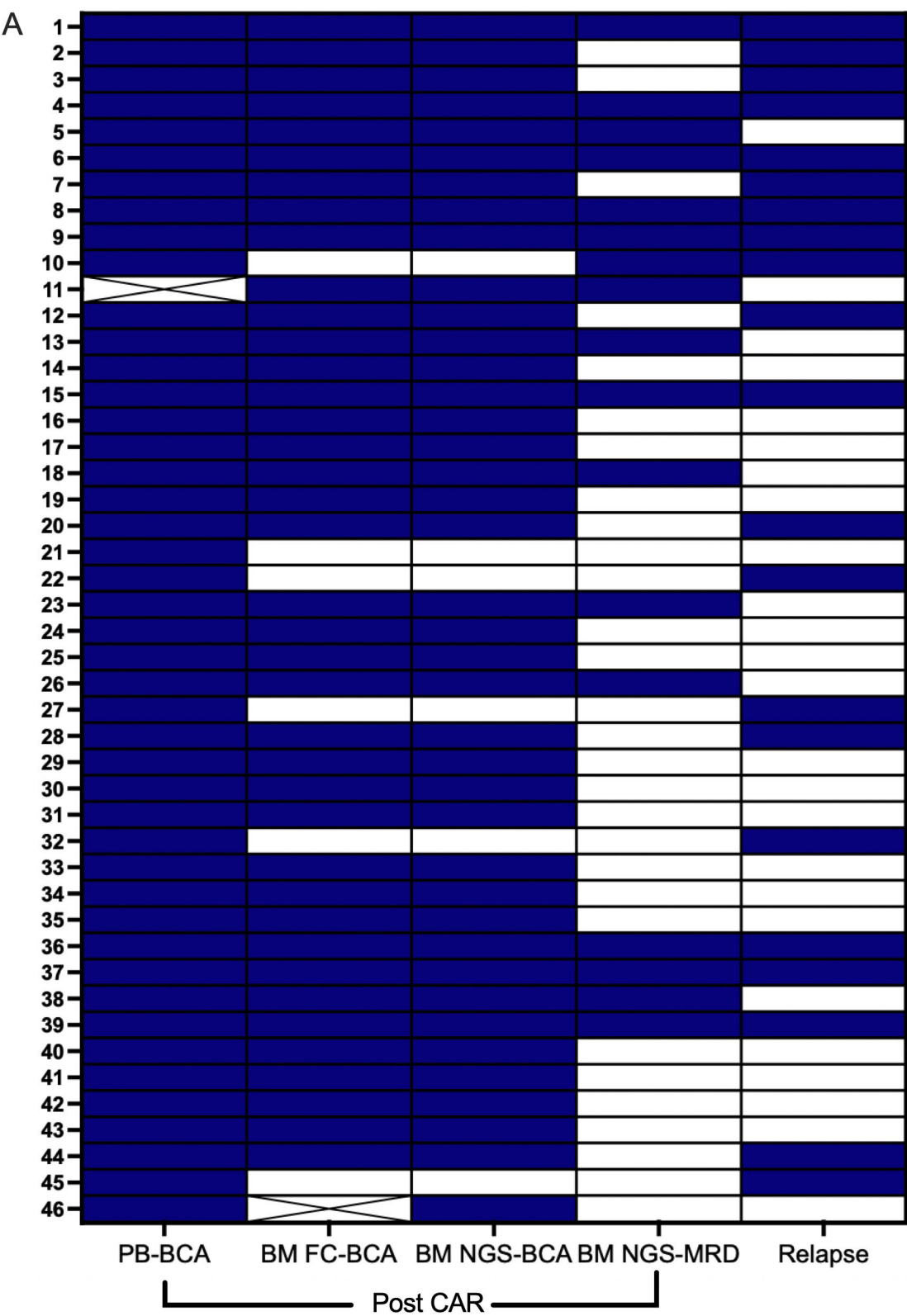


E



G





Yes/positive
 No/negative

X

 Not available

Supplemental Appendix:

1.0 Supplemental Figures & Tables

1.1 Supplemental Table 1

1.2 Supplemental Figure 1

1.3 Supplemental Table 2

1.1 Supplemental Table 1: Description of disease detection and BCA analysis metrics used in the analysis.

Disease detection modalities				
Assay	Methodology	Sensitivity	Limitations	Comments
Flow cytometry	Antibody based	1×10^{-4}	Lower limit of detection compared to NGS	Standard of care for MRD detection
Next generation sequencing (NGS), clonoSEQ®	DNA/RNA sequencing	1×10^{-6}	Cost, availability, not run in house	
PET-CT	Nuclear medicine, tracer injected and uptake shows sites of disease	Approximately 90%	Cost if not experimental due to insurance, requires sedation in younger patients, can be difficult to interpret in diabetic patients, etc.	Experimental in leukemia, standard of care in other malignancies
B-cell monitoring assays				
Assay	Methodology	Sensitivity	Limitations	Comments
Flow cytometry	Antibody based			
Next generation sequencing (NGS), clonoSEQ®	DNA/RNA sequencing		Cost, availability, not run in house	Experimental, not validated for use for this purpose

1.2 Supplemental Figure 1: Analysis of BCA utilizing adaptive ClonoSEQ platform (NGS). Total IgH sequences are divided by total nucleated cells and multiplied by 100 to obtain a percentage of B-cells in the BM.

APPENDIX

SUPPLEMENTAL SAMPLE INFORMATION

SAMPLE CLONALITY ¹	TOTAL NUCLEATED CELLS ²	LOCI	TOTAL SEQUENCES ³	TOTAL UNIQUE SEQUENCES ⁴
0.02	3,752,824	IGH	53,746	52,703
		IGK	≥3,945	3,945
		IGL	≥349	349

$$\frac{\text{Total sequences (represents total \# of B-cells)}}{\text{Total nucleated cells}} \times 100 = \% \text{ B-cells in marrow}$$

1.3 Supplemental Table 2: Crossover of BM post CAR-T cell infusion biomarkers and association with patient outcomes

Biomarker	Number of patients with biomarker	Number of patients who relapsed
NGS-MRD ^{neg} with NGS-BCA	25	8
NGS-MRD ^{neg} without NGS-BCA	4	4
NGS-MRD ^{pos} with NGS-BCA	15	8
NGS-MRD ^{pos} without NGS-BCA	2	1