Received: 22 February 2021 | Revised: 27 July 2021 | Accepted: 13 August 2021

DOI: 10.1002/pbc.29323

Pediatric Blood & Cancer

ONCOLOGY: BRIEF REPORT

CD19 expression in pediatric patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia pre- and post-treatment with blinatumomab

Ester Mejstrikova[1] | Matthias Klinger[2] | Ana Markovic[3] | Gerhard Zugmaier[2] | Franco Locatelli[4]

[1] Department of Pediatric Hematology/Oncology, CLIP, Charles University, University Hospital Motol, Prague, Czech Republic

[2] Amgen Research (Munich) GmbH, Munich, Germany

[3] Global Clinical Development, Amgen, Thousand Oaks, California, USA

[4] Department of Pediatric Hematology-Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Sapienza University of Rome, Rome, Italy

Correspondence
Gerhard Zugmaier, Amgen Research (Munich) GmbH, Staffelseestrasse 2, 81477 Munich, Germany.
Email: gerhardz@amgen.com

Funding information
Amgen, Inc.

Abstract

Blinatumomab is a BiTE® (bispecific T-cell engager) immuno-oncology therapy, which has demonstrated significant activity in patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (R/R B-ALL); however, a subset of patients relapse. Monitoring expression of cluster of differentiation (CD)19 in relapsed patients is critical to inform sequencing of subsequent therapies. The expression of CD19 in 59 pediatric patients with R/R B-ALL was analyzed on the day of diagnosis of R/R B-ALL and on days 15 and 29 of cycle 1 of blinatumomab. Most patients treated with one cycle of blinatumomab retained expression of CD19, and would therefore be eligible for subsequent anti-CD19 CAR T-cell therapy.

1 | INTRODUCTION

Cluster of differentiation (CD)19-directed immunotherapies have shown efficacy in patients with B-cell precursor acute lymphoblastic leukemia (B-ALL).[1] Blinatumomab, a novel BiTE® (bispecific T-cell engager) immuno-oncology therapy, which redirects CD3-positive T cells to engage and lyse CD19-positive target cells, has demonstrated a significant improvement in the survival of patients with B-ALL who are refractory to or relapse following chemotherapy (R/R B-ALL).[2] However, a subset of patients relapse following treatment with blinatumomab. Previously published results from this phase 1/2 open-label study in pediatric patients with R/R B-ALL demonstrated that of the 70 patients who received the recommended phase 2 dose of blinatumomab, 27 patients (39%) achieved complete remission (CR) within the first two cycles of treatment.[3] At the end of a 2-year follow-up, 19 of those 27 patients (70%) relapsed, of which two were alive at last assessment, 15 died, and two withdrew consent.[3]

Patients who relapse with CD19-negative disease generally have poor prognosis with limited treatment options.[4] Monitoring the expression of CD19 in patients with R/R B-ALL who relapse upon treatment with blinatumomab could help gain critical insights into the mechanism of relapse and thus aid in the sequencing of subsequent anti-CD19 therapies. The aim of this report was to evaluate the expression of CD19 in pediatric patients with R/R B-ALL at the end of one cycle of treatment with blinatumomab.

Abbreviations: B-ALL, B-cell precursor acute lymphoblastic leukemia; BiTE, bispecific T-cell engager; CART-19, anti-CD19 chimeric antigen receptor T-cell therapy; CD, cluster of differentiation; CR, complete remission; FC, flow cytometry; MRD, minimal residual disease; R/R B-ALL, relapsed or refractory B-cell precursor acute lymphoblastic leukemia

The expression of CD19 was assessed by flow cytometry (FC) on the day of confirmation of diagnosis of R/R B-ALL, and on day 15 and day 29 of cycle 1 of blinatumomab. Detailed description of the study design, patient eligibility, dose modifications, interruptions, discontinuations, and the methodology used for FC and minimal residual disease (MRD) analysis (by FC or polymerase chain reaction) were previously reported and are summarized in Supplementary Methods.[3] Hematologic remission was defined per von Stackelberg et al.[3] and described in Supplementary Methods. CR was subclassified on the basis of recovery of peripheral blood counts.

2 | RESULTS

Of the 70 patients who received the recommended phase 2 dose in the phase 1/2 study, the expression of CD19 was analyzed retrospectively in 59 patients for whom CD19 expression data were available. Baseline characteristics for patients included in this analysis are shown in Supplementary Table S1. Of the 59 patients included in this analysis, 29 patients were treated with 5, 15, 30 μg/m^2/day, or a stepwise regimen of 15 μg/m^2/day for 7 days, followed by 30 μg/m^2/day for 21 days of blinatumomab in the phase 1 portion of the primary study,[3] and 29 patients were treated with a stepwise dose of 5 μg/m^2/day for 7 days, followed by 15 μg/m^2/day for 21 days (5/15 μg/m^2/day) of blinatumomab in the phase 2 portion of the primary study.[3] Overall, 44 of 59 patients received blinatumomab at the recommended stepwise dose of 5/15 μg/m^2/day. Hematologic and MRD response and the status of CD19 expression on the day of diagnosis of R/R B-ALL and on days 15 and/or 29 of cycle 1 are listed in Table 1 and summarized in Supplementary Table S2. Analysis of CD19 expression on the day of diagnosis versus day 15 or 29 in patients stratified per response to blinatumomab showed that the majority of patients across response groups retained the expression of CD19 upon completion of one cycle of blinatumomab (Supplementary Figure S1). The data on expression of CD19 in patients who were MRD-negative ($<1 \times 10^{-4}$ detectable blasts) on days 15 and/or 29 of cycle 1 of treatment with blinatumomab were assessed for the expression of CD19; however, these patients did not have detectable leukemic blasts.

Seven of the 59 patients analyzed in the current analysis experienced a disease relapse due to the emergence of CD19-negative blasts (patients #4, #10, #27, #29, #32, #33, and #47; Table 1). Of the 19 patients who were reported to have relapsed in the primary study,[3] the proportion of patients who relapsed with CD19-negative disease was 37% (7/19) per data presented in the current analysis. A prior publication[5] that analyzed 18 patients with evaluable data from the same primary study[3] had reported that 22% (4/18) of patients had CD19-negative relapse. The current analysis assessed evaluable data from an additional 41 patients; therefore, of a total of 59 patients analyzed in the current analysis, 11.9% (7/59) of patients had CD19-negative relapse. Patients #4, #10, #27, and #33 showed a decrease/loss of CD19 expression at the end of cycle 1 of blinatumomab, and patients #29, #32, and #47 showed a decrease/loss of CD19 expression at the end of cycle 3. Analysis of CD19 expression on B-ALL leukemic blasts from patients #4 and #10 on the day of diagnosis showed a heterogeneous population of blasts that expressed CD19 (Supplementary Figure S2).

3 | DISCUSSION

Overall, the majority of pediatric patients with R/R B-ALL continued to express CD19 at the end of treatment with one cycle of blinatumomab. Of the total number of patients who relapsed in the primary study, 37% of patients relapsed with CD19-negative disease. Among pediatric/adolescent patients with R/R B-ALL who relapsed following treatment with anti-CD19 chimeric antigen receptor T-cell therapy (CART-19), the proportion of patients with CD19-negative relapse has been reported to be higher (39%–100%) compared with that reported in the current analysis (37%), possibly due to the high therapeutic pressure applied by CART-19.[6–9] Patients who relapse upon treatment with blinatumomab have been shown to respond to re-treatment with blinatumomab or subsequent treatment with CART-19.[10] In adult patients, recent data from phase 1 of the ZUMA 3 trial showed that of the 21 patients with R/R B-ALL who received prior treatment with blinatumomab, 12 (57%) patients responded to subsequent treatment with CART-19.[11] Notably, in another analysis of 38 adult patients with R/R B-ALL who progressed after CART-19, the overall prognosis of patients was poor, though a subset of patients achieved sustained remission to salvage treatments including blinatumomab and/or inotuzumab ozogamicin (seven of 12 patients, 58.3%) or re-infusion of CAR T cells (four of 10 patients, 40%).[12] On the contrary, an analysis of 166 patients with R/R B-ALL who received CART-19 showed that patients with prior treatment with blinatumomab had a higher rate of failure to achieve MRD-negative remission.[13] Another report based on an analysis of 420 patients showed that previous blinatumomab treatment increased the risk of nonresponse to CART-19 and was associated with a worse 6-month probability of relapse-free and event-free survival in 13 of 71 patients (18.3%).[14] However, patients treated with blinatumomab had a greater incidence of *MLL* rearrangement compared with patients who had not been previously exposed to blinatumomab, which could explain the increased risk of nonresponse to CART-19, as it is known that patients with *MLL* rearrangement are more prone to a switch in the phenotype/lineage of the leukemic blasts, thus rendering the escape from immunotherapy more likely.[15,16]

The mechanisms underlying CD19-negative relapse are not fully elucidated; however, their characterization is critical for the optimization of the sequencing of CD19-directed immunotherapies. Possible mechanisms of relapse include loss of surface CD19 antigen, expression of a genetically altered variant of *CD19*, low expression of *CD19* RNA, mutations in the CD19 signaling complex member *CD81*, aneuploidy-mediated loss of the nonmutated *CD19* allele, and a potential switch from lymphoid to myeloid phenotype of leukemic blasts.[17–20] Although none of the patients in the current analysis showed a clear lineage switch, patient #27 showed a gain of monocytic phenotype upon disease progression.[5]

TABLE 1 Hematologic response and status of CD19 expression at the end of cycle 1 of blinatumomab in pediatric patients with R/R B-ALL

	Treatment response			MRD by flow [neg. = 1.00E-05]		%CD19 expression on blasts		
Patient no.	Cycle 1 Day 15	Cycle 1 Day 29	MRD negativity by flow	Cycle 1 Day 15	Cycle 1 Day 29	Confirmation of diagnosis	Cycle 1 Day 15	Cycle 1 Day 29
1	NR	NR	No	3.57E-01	0.73	50.6	65.7	60.8
2	PR	PR	Cycle 1 Day 29	2.10E-03	1.00E-05	100.0	N/A	NLD
3	NR	NR	No	8.80E-01	8.00E-01	91.6	99.0	78.3
4	NR	CRn	No	9.00E-02	2.90E-02	58.5	38.1	28.4
5	PD	N/A	No	8.80E-01	N/A	99.0	96.5	N/A
6	NR	N/A	No	6.10E-01	N/A	98.0	77.8	N/A
7	NR	N/A	No	3.20E-01	N/A	56.0	93.0	N/A
8	PR	PR	No	2.69E-02	7.20E-02	96.3	97.3	91.0
9	NR	NR	No	5.90E-01	7.80E-01	96.7	93.3	89.8
10	NR	N/A	No	9.00E-01	N/A	59.8	0.6	N/A
11	NR	N/A	No	4.80E-01	N/A	97.0	99.4	N/A
12	CRi	CRc	Cycle 1 Day 15	1.00E-05	1.00E-05	98.4	NLD	NLD
13	NR	NR	No	6.40E-01	4.21E-01	95.2	84.5	93.4
14	N/A	PR	No	N/A	5.30E-02	94.4	77.9	79.4
15	NR	N/A	No	7.50E-01	N/A	98.9	99.4	N/A
16	NR	NR	Cycle 2 Day 29	4.70E-01	7.60E-01	95.7	19.5	75.2
17	NR	PD	No	9.18E-02	5.17E-01	93.9	95.6	95.2
18	CRc	CRc	Cycle 1 Day 15	1.00E-05	1.00E-05	100.0	NLD	NLD
19	CRi	CRc	Cycle 1 Day 15	1.00E-05	1.00E-05	99.9	NLD	NLD
20	NR	N/A	No	9.80E-01	N/A	99.9	98.5	N/A
21	PR	PR	Cycle 1 Day 15	1.00E-05	1.00E-05	71.0	NLD	NLD
22	NR	NR	No	4.20E-01	7.90E-01	94.7	99.2	97.4
23	CRc	PR	No	1.03E-02	1.97E-01	91.7	91.9	94.0
24	PD	PD	No	1.30E-01	2.23E-01	88.2	83.6	94.6
25	CRi	CRc	Cycle 2 Day 15	4.06E-03	2.52E-03	87.0	75.4	89.4
26	CRc	CRc	No	4.70E-03	N/A	99.9	97.3	98.3
27[a]	CRn	CRn	Cycle 1 Day 15	1.00E-05	1.00E-05	97.7	NLD	NLD
28	CRn	CRi	No	N/A	3.60E-03	98.0	N/A	99.5
29[b]	CRn	PR	Cycle 1 Day 15	1.00E-05	4.30E-02	98.9	NLD	91.4
30	N/A	PD	No	N/A	8.50E-01	98.6	N/A	99.2
31	NR	NR	No	N/A	8.70E-01	98.3	95.8	91.9
32[c]	PR	PR	No	N/A	1.64E-03	97.6	N/A	93.4
33[d]	PR	PR	Cycle 1 Day 15	1.00E-05	1.00E-05	99.3	NLD	NLD
34	CRc	PR	Cycle 2 Day 29	1.60E-02	3.39E-04	92.4	48.3	99.6
35	NR	PR	Cycle 3 Day 29	3.30E-01	1.41E-02	93.5	93.0	96.7
36	NR	NR	No	4.10E-01	4.20E-02	97.4	95.3	95.9
37	PR	CRn	No	2.24E-02	N/A	99.0	100.0	100.0
38	PD	PD	No	2.40E-01	8.00E-01	97.4	99.6	99.8
39	NR	NR	No	6.70E-01	7.08E-01	99.3	98.5	98.5
40	PR	PD	No	5.30E-02	2.28E-01	96.7	91.5	100.0

(Continues)

TABLE 1 (Continued)

Patient no.	Treatment response		MRD negativity by flow	MRD by flow [neg. = 1.00E-05]		%CD19 expression on blasts		
	Cycle 1 Day 15	Cycle 1 Day 29		Cycle 1 Day 15	Cycle 1 Day 29	Confirmation of diagnosis	Cycle 1 Day 15	Cycle 1 Day 29
41	CRn	CRc	Cycle 1 Day 15	1.00E-05	1.00E-05	98.7	NLD	NLD
42	CRn	CRi	Cycle 1 Day 15	1.00E-05	7.56E-04	99.6	NLD	100.0
43	NR	NR	No	4.70E-01	7.70E-01	86.3	98.7	98.7
44	NR	NR	No	8.60E-01	7.40E-01	99.8	99.8	95.0
45	NR	PR	No	2.40E-01	5.67E-02	98.6	98.0	93.0
46	CRn	CRi	Cycle 1 Day 15	1.00E-05	1.00E-05	99.8	NLD	NLD
47[e]	CRn	CRn	Cycle 1 Day 15	1.00E-05	1.00E-04	99.6	NLD	100.0
48	PD	N/A	No	9.67E-01	N/A	94.4	83.5	N/A
49	N/A	NR	No	N/A	7.90E-01	93.4	N/A	88.9
50	PD	PD	No	7.70E-01	9.00E-01	97.4	97.9	99.0
51	NR	NR	No	N/A	9.70E-01	98.6	100.0	98.2
52	PR	PD	No	6.60E-01	8.60E-01	98.8	99.9	99.6
53	PR	CRi	Cycle 1 Day 15	1.00E-05	1.00E-05	97.7	NLD	NLD
54	CRn	CRn	Cycle 1 Day 15	1.00E-05	1.00E-05	99.0	NLD	NLD
55	PD	PD	No	7.40E-01	6.80E-01	99.3	100.0	100.0
56	PD	N/A	No	9.10E-01	N/A	98.0	99.5	N/A
57	N/A	CRn	No	N/A	4.10E-02	86.1	N/A	79.9
58	N/A	N/A	No	N/A	N/A	75.6	98.7	N/A
59	CRi	PD	No	2.43E-02	1.20E-03	94.4	86.6	N/A

Note: Expression of CD19 in patients who were MRD-negative and thus had no detectable blasts ($<1 \times 10^{-4}$ blasts) on days 15 and/or 29 of cycle 1 of treatment with blinatumomab has been described as NLD. Data on response to treatment with blinatumomab for Patient #58 were not available. This patient was excluded from the analysis. CR defined as <5% blasts in bone marrow and no evidence of disease and was subclassified on the basis of recovery of peripheral blood counts; CRc defined as <5% blasts in bone marrow and no evidence of disease with complete hematologic recovery; CRi defined as <5% blasts in bone marrow with incomplete hematologic recovery; CRn defined as <5% blasts in bone marrow with responses that did not qualify as CRc or CRi, OR (blast-free) hypoplastic or aplastic bone marrow. PR was defined as complete disappearance of circulating blasts, ≥5% or <25% blasts in bone marrow and appearance of normal progenitor cells; PD was defined as an increase of at least 25% or an increase of at least 5000 cells/μl (whichever was greater) in the number of circulating leukemia cells, development of extramedullary disease, or other clinical evidence of PD. NR or stable disease was defined as failure of the patient to qualify for CR, PR, or PD.

Abbreviations: CR, complete remission; CRc, complete remission with complete hematologic recovery; CRi, complete remission with incomplete hematologic recovery; CRn, complete remission that did not qualify for CRc or CRi OR (blast-free) hypoplastic or aplastic bone marrow; E, exponent to the power 10; MRD, minimal residual disease; N/A, data not available; neg., negative; NLD, no leukemia detected; NR, no response; PD, progressive disease; PR, partial remission; R/R ALL, relapsed/refractory acute lymphoblastic leukemia.

[a]Patient #5 from Mejstríková et al.[5]

[b]Patient #2 from Mejstríková et al.[5]

[c]Patient #3 from Mejstríková et al.[5]

[d]Patient #4 from Mejstríková et al.[5]

[e]Patient #1 from Mejstríková et al.[5]

Analysis of the expression of CD19 on blasts from patients who experienced CD19-negative relapse showed a heterogeneous population of CD19-expressing blasts on the day of diagnosis of R/R B-ALL. These data indicate a possibility for the existence of a small population of immunophenotypically invisible CD19-negative blasts prior to the initiation of treatment with blinatumomab, which could eventually emerge to become the dominant population once the CD19-positive blasts are depleted by blinatumomab. This possibility is supported by a recent report that demonstrated the use of a single-cell RNA sequencing approach to detect the presence of CD19-negative leukemic cells in a patient with R/R B-ALL prior to the start of CART-19.[21]

Patients with CD19-negative relapse have limited treatment options. A subset of pediatric patients with CD19-negative relapse following treatment with blinatumomab has been known to respond to chemotherapy.[5] In addition, patients experiencing CD19-negative relapse could be approached with immunotherapies that target other antigens, such as inotuzumab ozogamicin or daratumumab, which target CD22 and CD38, respectively. A recent report based on a

retrospective analysis of 56 patients (55 with R/R B-ALL), majority of whom had relapsed or were refractory to CD19-targeted therapies, demonstrated that 29% of patients who were CD19-negative or had partial expression of CD19 regained the expression of CD19 over the follow-up period.[22] Thus, in addition to detection of the presence of CD19-negative leukemic blasts prior to the start of treatment with blinatumomab, tracking disease progression by analyzing expression of CD19 and other B-cell markers on leukemic blasts would be critical to inform sequencing of subsequent therapies in patients with CD19-negative relapse.

The current analysis has limitations. Lack of data points due to the small patient size and inadequate follow-up data prevented further assessment of the effect of subsequent cycles of blinatumomab on the expression of CD19. Furthermore, expression of CD19 in patient samples was categorized as dim/bright at the stage of diagnosis of R/R B-ALL based on the presence of the predominant population of leukemic blasts; however, modulation in the expression of CD19 was not assessed during the course of treatment with blinatumomab. Assessment of the modulation of CD19 during and after treatment with blinatumomab would be critical prior to subsequent treatment with CART-19. In conclusion, data from the current retrospective analysis suggest that leukemic blasts from the majority of patients retain the expression of CD19 upon treatment with one cycle of blinatumomab. A subset of patients experienced CD19-negative relapse with no incidence of ALL-to-AML lineage switch. Prior exposure to blinatumomab would not exclude most pediatric patients with R/R B-ALL from the potential benefits of subsequent immunotherapy such as CART-19 or re-treatment with blinatumomab.

ACKNOWLEDGMENTS

The authors thank all the site investigators and patients who participated in the study. Swapnil Kher, PhD, of Cactus Life Sciences (part of Cactus Communications) provided medical writing assistance funded by Amgen, Inc.

AUTHOR CONTRIBUTIONS

All authors contributed toward analysis, interpretation of the data, and review of the manuscript.

DATA SHARING STATEMENT

Qualified researchers may request data from Amgen clinical studies. Complete details are available at: http://www.amgen.com/datasharing/.

CONFLICT OF INTEREST

Ester Mejstrikova performed data assessments as a service to Amgen. Matthias Klinger and Gerhard Zugmaier are employed by Amgen Research (Munich) GmbH, own stock in Amgen, Inc., and hold patents relating to blinatumomab. Ana Markovic is an employee of and owns stock in Amgen, Inc. Franco Locatelli has no competing financial interests.

ORCID

Ester Mejstrikova https://orcid.org/0000-0001-5169-4653
Matthias Klinger https://orcid.org/0000-0001-7468-1951
Gerhard Zugmaier https://orcid.org/0000-0001-6879-2671
Franco Locatelli https://orcid.org/0000-0002-7976-3654

REFERENCES

1. Aldoss I, Forman SJ. How I treat adults with advanced acute lymphoblastic leukemia eligible for CD19-targeted immunotherapy. *Blood*. 2020;135:804-813.
2. Kantarjian H, Stein A, Gökbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med*. 2017;376:836-847.
3. von Stackelberg A, Locatelli F, Zugmaier G, et al. Phase I/phase II study of blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. *J Clin Oncol*. 2016;34:4381-4389.
4. Ruella M, Maus MV. Catch me if you can: leukemia escape after CD19-directed T cell immunotherapies. *Comput Struct Biotechnol J*. 2016;14:357-362.
5. Mejstríková E, Hrusak O, Borowitz MJ, et al. CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. *Blood Cancer J*. 2017;7:659.
6. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-448.
7. Lee D, Stetler-Stevenson M, Yuan C, et al. Long-term outcomes following CD19 CAR T cell therapy for B-ALL are superior in patients receiving a fludarabine/cyclophosphamide preparative regimen and post-CAR hematopoietic stem cell transplantation. *Blood*. 2016; 128:218.
8. Gardner RA, Finney O, Annesley C, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood*. 2017;129:3322-3331.
9. Ghorashian S, Kramer AM, Onuoha S, et al. Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. *Nat Med*. 2019;25:1408-1414.
10. Topp MS, Stelljes M, Zugmaier G, et al. Blinatumomab retreatment after relapse in patients with relapsed/refractory B-precursor acute lymphoblastic leukemia. *Leukemia*. 2018;32:562-565.
11. Shah BD, Bishop MR, Oluwole OO, et al. KTE-X19 anti-CD19 CAR T-cell therapy in adult relapsed/refractory acute lymphoblastic leukemia: ZUMA-3 phase 1 results. *Blood*. 2021;138(1):11-22.
12. Wudhikarn K, Flynn JR, Rivière I, et al. Interventions and outcomes of adult patients with B-ALL progressing after CD19 chimeric antigen receptor T cell therapy. *Blood*. 2021;138(7):531–543.
13. Pillai V, Muralidharan K, Meng W, et al. CAR T-cell therapy is effective for CD19-dim B-lymphoblastic leukemia but is impacted by prior blinatumomab therapy. *Blood Adv*. 2019;3:3539-3549.
14. Taraseviciute A, Steinberg S, Myers R, et al. Pre-CAR blinatumomab is associated with increased post-CD19 CAR relapse and decreased event free survival. *Blood*. 2020;136:13-14.
15. Wölfl M, Rasche M, Eyrich M, Schmid R, Reinhardt D, Schlegel PG. Spontaneous reversion of a lineage switch following an initial blinatumomab-induced ALL-to-AML switch in MLL-rearranged infant ALL. *Blood Adv*. 2018;2:1382-1385.
16. Gardner R, Wu D, Cherian S, et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. *Blood*. 2016;127:2406-2410.
17. Braig F, Brandt A, Goebeler M, et al. Resistance to anti-CD19/CD3 BiTE in acute lymphoblastic leukemia may be mediated by disrupted CD19 membrane trafficking. *Blood*. 2017;129:100-104.

18. Sotillo E, Barrett DM, Black KL, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov*. 2015;5:1282-1295.
19. Rayes A, McMasters RL, O'Brien MM. Lineage switch in MLL-rearranged infant leukemia following CD19-directed therapy. *Pediatr Blood Cancer*. 2016;63:1113-1115.
20. Zhao Y, Aldoss I, Qu C, et al. Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL. *Blood*. 2021;137:471-484.
21. Rabilloud T, Potier D, Pankaew S, Nozais M, Loosveld M, Payet-Bornet D. Single-cell profiling identifies pre-existing CD19-negative subclones in a B-ALL patient with CD19-negative relapse after CAR-T therapy. *Nat Commun*. 2021;12(1):865.
22. Libert D, Yuan CM, Masih KE, et al. Serial evaluation of CD19 surface expression in pediatric B-cell malignancies following CD19-targeted therapy. *Leukemia*. 2020;34:3064-3069.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mejstrikova E, Klinger M, Markovic A, Zugmaier G, Locatelli F. CD19 expression in pediatric patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia pre- and post-treatment with blinatumomab. *Pediatr Blood Cancer*. 2021;e29323
https://doi.org/10.1002/pbc.29323.