# Tumor burden-guided dosing contributes to mitigation of immunotoxicities following treatment with obecabtagene autoleucel in adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia

Elias Jabbour,[1] Karamjeet S. Sandhu,[2] Paul Shaughnessy,[3] Aaron C. Logan,[4] Mehrdad Abedi,[5] Bijal D. Shah,[6] Michael R. Bishop,[7] Jae H. Park,[8] Daniel J. DeAngelo,[9] Eleni Tholouli,[10] Deborah Yallop,[11] Sridhar Chaganti,[12] Katharine Hodby,[13] Pere Barba,[14] Manuel Guerreiro,[15] Tobias Menne,[16] Justin Shang,[17] Pierre Lao-Sirieix,[18] Wolfram Brugger[19] and Claire Roddie[20]

[1]University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]City of Hope National Medical Center, Duarte, CA, USA; [3]Sarah Cannon Transplant and Cellular Therapy Program, Methodist Hospital, San Antonio, TX, USA; [4]Hematology, Blood and Marrow Transplantation, and Cellular Therapy Program, University of California at San Francisco, San Francisco, CA, USA; [5]University of California Davis, Davis, CA, USA; [6]Moffitt Cancer Center, Tampa, FL, USA; [7]The David and Etta Jonas Center for Cellular Therapy, University of Chicago, Chicago, IL, USA; [8]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [9]Dana-Farber Cancer Institute, Boston, MA, USA; [10]Manchester Royal Infirmary, Manchester, UK; [11]King's College Hospital NHS Foundation Trust, London, UK; [12]University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK; [13]University Hospitals Bristol NHS Foundation Trust, Bristol, UK; [14]Hospital Universitari Vall d'Hebron-Universitat Autónoma de Barcelona, Barcelona, Spain; [15]Hospital Universitari I Politècnic La Fe, Valencia, Spain; [16]Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, UK; [17]Autolus Therapeutics, Rockville, MD, USA; [18]Autolus Therapeutics, London, UK; [19]Autolus Therapeutics, Munich, Germany and [20]University College London Cancer Institute, London, UK

**Correspondence:** E. Jabbour
ejabbour@mdanderson.org

**Received:** July 2, 2025.
**Accepted:** December 24, 2025.
**Early view:** January 8, 2026.

**https://doi.org/10.3324/haematol.2025.288587**



## Abstract

Obecabtagene autoleucel (obe-cel) is a CD19-targeted autologous chimeric antigen receptor T-cell therapy (CAR T) with a fast off-rate binding domain, administered as split-dose infusions guided by pre-lymphodepletion tumor burden (low-tumor-burden [TB] group: ≤20%; high-TB group: >20% bone marrow [BM] blasts). Obe-cel treatment in adult relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) was investigated in the phase Ib/II FELIX trial. Here, we report pharmacokinetics, safety, and efficacy outcomes in patients with low or high tumor burden and discuss the evidence/rationale justifying the spilt-dose strategy and threshold used to classify the groups. Tumor burden at lymphodepletion was a critical driver of CAR T-cell expansion; a 50% increase, e.g., 70% *versus* 20% BM blasts, was associated with a 1.9-fold increase (95% confidence interval: 1.4-2.6) in maximal expansion of CAR T cells. Robust CAR T-cell expansion was observed in both tumor burden groups. The incidence of grade ≥3 cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome was minimal in both the low- and high-TB groups (2% *vs*. 3% and 4% *vs*. 9%, respectively). Although the overall remission rate was higher in the low-TB group (85%), it also remained high in the high-TB group (73%). Evidence from FELIX suggests that use of tumor burden--guided dosing may mitigate the typical effects of immunotoxicity while maintaining substantial efficacy. Although further study is needed to better characterize the effects of the split-dosing strategy, the clinical evidence supports its use when administering obe-cel for the treatment of R/R B-ALL. Trial registered at www.clinicaltrial.gov (*clinicaltrials gov. Identifier: NCT04404660*).

## Introduction

Chimeric antigen receptor T-cell therapy (CAR T) has transformed the management of certain hematologic malignancies, including relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL), producing durable

remissions in patients otherwise not responsive to standard therapies.[1-3] However, for many patients the promise of CAR T can be limited by the potential of severe immunotoxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS),[4,5] with severity of CRS and ICANS correlating with pre-treatment tumor burden (assessed as bone marrow [BM] blast percentage).[6-9]

Obecabtagene autoleucel (obe-cel) is an autologous 4-1BB-ζ CD19-directed CAR T that uses a fast off-rate target binding domain, engineered to mimic natural T-cell engagement, a design intended to improve persistence and reduce immunotoxicity.[2,10] The scFv binding domain of obe-cel has >40-fold lower binding affinity compared with FMC63,[11] which is used in other available CAR T-cell therapies.[12,13] The lower binding affinity of obe-cel more closely approximates physiologic binding,[10,11] facilitating reduced CAR T-cell exhaustion, reduced cytokine release and toxicity, and enhanced CAR T-cell persistence.[10]

To further mitigate the potential for immunotoxicity related to high tumor burden, specifically in adult patients with B-ALL who are vulnerable to immunotoxicities,[10] a dosing strategy guided by tumor burden at lymphodepletion (threshold of 20% BM blasts) was developed.[2,10] Use of the pre-specified BM blast percentage threshold was chosen to simplify clinical decision making and dosing selection. The choice of the 20% BM blasts threshold was based on a preliminary study by Turtle *et al.*, where a risk-adapted dosing strategy was adopted due to a notable occurrence of severe CRS and neurotoxicity in patients treated with a high CAR T-cell dose.[14] Ten adult patients with >20% BM blasts (high tumor burden) were treated with a low dose of CAR T cells compared with patients with ≤20% BM blast percentage. Prior to the adoption of the risk-adapted dosing strategy, all six patients with high tumor burden treated with the high dose required intensive care unit admission, while only one of ten patients with high tumor burden treated using the risk-adapted dosing strategy required intensive care unit admission.[14]

In the FELIX study, patients with both low and high tumor burden were administered the same target total dose of obe-cel; however, split-dose infusions were used on days 1 and 10 (±2 days), with patients with high tumor burden receiving a low first dose compared with patients with low tumor burden.[2,10]

Treatment of adults with R/R B-ALL with obe-cel demonstrated promising results in the phase I ALLCAR19 study (clinicaltrials gov. *Identifier: NCT02935257*)[10] and the pivotal phase Ib/II FELIX study (clinicaltrials gov. *Identifier: NCT04404660*),[2] with high rates of durable responses and a low incidence of immunotoxicity overall.[2] In the analyses presented here, we describe evidence supporting the use of tumor burden-guided dosing as a strategy to reduce the severe adverse events commonly associated with CAR T.

# Methods

The benefit of the tumor burden-guided dosing schedule was investigated in the FELIX study by assessing CAR T-cell expansion and incidence of immunotoxicity, as well as efficacy based on a 20% BM blast percentage threshold (tumor burden-guided dosing strategy).

## FELIX study design

FELIX (clinicaltrials gov. *Identifier: NCT04404660*) is an open-label, multicenter, global, single-arm, phase Ib/II study evaluating the safety and efficacy of obe-cel in patients aged ≥18 years with CD19-positive R/R B-ALL. Details of the FELIX study design have been published previously.[2] Following institutional review board approval of the trial protocol, this study was carried out in accordance with the principles founded in the Declaration of Helsinki and the International Council on Harmonization Guideline for Good Clinical Practice. All patients provided written informed consent.

## Treatment/dosing schedule

Obe-cel was administered according to a tumor burden-guided dosing schedule, as described previously.[2] Patients with >20% BM blasts (high-tumor burden [high-TB] group) at lymphodepletion received a smaller starting dose of obe-cel to mitigate immunotoxicities,[14] while patients with ≤20% BM blasts (low-TB group) received a higher starting dose. On day 10 (±2), patients received a second infusion for a total target dose of $410\times10^6$ CAR T cells. Detailed treatment/dosing are outlined in the *Online Supplementary Appendix*.

## Assessments and endpoints

Obe-cel pharmacokinetics (PK) were assessed using droplet digital polymerase chain reaction on peripheral blood samples collected pre-lymphodepletion, on days 1, 3, 6, 9, 12, 15, 22, and 28, and months 2, 3, 4, 6, and every 3 months thereafter until end of study. The obe-cel PK endpoints included maximal expansion of transgene post-infusion ($C_{max}$; copies/μg DNA), time to maximal expansion (days), and exposure up to 28 days (area under the curve $[AUC]_{0-28days}$; day×copies/μg DNA). Serum cytokine levels were measured using the MSD V-PLEX Proinflammatory Panel 1 Human Kit assay system and the V-PLEX Cytokine Panel 1 Human Kit (Meso Scale Diagnostics, Rockville MD, USA).

Response evaluations were performed by a central independent response review committee as previously described.[2] Response evaluations included the overall remission rate (ORR; defined as complete remission [CR] or CR with incomplete hematologic recovery [CRi]), duration of remission (DoR), event-free survival (EFS), and overall survival (OS). Detailed definitions of response evaluations were previously reported.[2] Assessment de-

tails are outlined in the *Online Supplementary Appendix*.

### Statistical analysis

The infused set comprised all patients who received at least one dose of obe-cel (N=127). All safety and efficacy analyses were conducted in the infused set, stratified by pre-lymphodepletion tumor burden (low-TB group: ≤20%; high-TB group: >20% BM blasts). Obe-cel PK data analyses were conducted in the infused set, and in patients who received both doses of obe-cel (N=120).

Descriptive statistics were summarized for baseline demographics and disease characteristics. Linear regression analyses were performed to investigate the impact of tumor burden at lymphodepletion on CAR T-cell expansion. Geometric least squares means ratios with 90% confidence intervals (CI) were generated for comparison of PK parameters between subgroups based on patient and disease characteristics.[15] Time-to-event outcomes were summarized using the Kaplan-Meier method.

## Results

### Patients and baseline characteristics

A total of 127 patients were infused with obe-cel, comprising the infused set. Overall, 52 of 127 (41%) patients had ≤20% BM blasts at lymphodepletion (low-TB group), while 75 of 127 (59%) had >20% BM blasts at lymphodepletion (high-TB group). Baseline characteristics are summarized in *Online Supplementary Table S1* and were overall similar between the two tumor burden groups. As a result of the dosing strategy, the median BM blast percentage was 2% (range, 0-20) in the low-TB group while it was 80% (range, 26-100) in the high-TB group. Patients in the low-TB group were slightly younger (median 44 years; range, 20-81) compared with patients in the high-TB group (median 49 years; range, 20-79). A numerically higher incidence of prior treatment with allogeneic hematopoietic stem cell transplant was observed in the low-TB group compared with the high-TB group (58% *vs*. 35%, respectively), while more patients in the high-TB group were Hispanic or Latino (36% *vs*. 21%). Eighteen (14%) patients infused with obe-cel received bridging therapy with inotuzumab ozogamicin, 14 of whom had low tumor burden and four of whom had high tumor burden at lymphodepletion.

Of the 127 infused patients, 120 (94%) received two doses; seven (6%) patients received only dose 1, five of whom were in the high-TB group. The reasons for patients only receiving one dose were adverse events (N=3), progressive disease (N=2), death (N=1), and product manufacturing failure (N=1). Characteristics, including data on CAR T-cell expansion, and outcomes for the seven patients who received only dose 1, are summarized in *Online Supplementary Table 2.* Additionally, four of the 120 patients who received both doses did not receive the full target dose, but are stratified in reported analyses by their BM blasts at lymphodepletion and planned first dose.

### Impact of pre-lymphodepletion tumor burden and patient characteristics on CAR T-cell expansion

Overall, CAR T-cell expansion and persistence were observed in all patients who received two doses (N=120), regardless of tumor burden (Figure 1A); however, a slower initial rate of expansion was observed in patients with high tumor burden (Figure 1B). Time to peak expansion (time to maximal expansion) was numerically similar for patients with low and those with high tumor burden, day 11 (range, 2-28) and day 15 (range, 6-55), respectively (Figure 1B). CAR T-cell expansion was also observed in patients who received only one dose of obe-cel (*Online Supplementary Table S2*). Peak CAR T-cell expansion increased with tumor burden, with a $C_{max}$ geometric mean (coefficient of variation [CV]%) of 72,440 (171.6) copies/μg DNA *versus* 150,764 (264.6) copies/μg DNA, and an AUC geometric mean from day 0 to 28 (CV%) of 647,355 (201.4) day×copies/μg DNA *versus* 1,631,748 (176.5) day×copies/μg DNA for the low-TB and high-TB groups, respectively. Linear regression analysis of blast level in the BM at lymphodepletion showed that a 50% increase in tumor burden (e.g., patients with 70% *vs*. 20% BM blasts) was associated with a 1.9-fold increase in $C_{max}$ (95% CI: 1.4-2.6; Figure 1C) and a 2.0-fold increase in $AUC_{0-28days}$ (95% CI: 1.5-2.7; Figure 1D).

CAR T-cell expansion did not appear to be influenced by the age of patients at screening, with older patients (≥55 years old) having comparable $C_{max}$/$AUC_{0-28days}$ to younger patients (<55 years old) (Table 1). Although no formal statistical analyses can be performed, as stated previously, a ≤1.6-fold difference in $C_{max}$ and $AUC_{0-28days}$ was observed when comparing patient subgroups based on demographic or clinical characteristics (geometric least squares means ratio range, 93-162%); therefore, these characteristics are unlikely to influence CAR T-cell expansion (Table 1).

### Cytokine levels

The geometric mean of peak cytokine levels were numerically higher in the high-TB group compared with the low-TB group (*Online Supplementary Figure S1A*). Cytokine levels peaked at approximately day 12 following infusion of obe-cel and returned to baseline levels within 28 days (*Online Supplementary Figure S1B*).

### Immunotoxicity

The incidence of any-grade CRS and ICANS was higher in the high compared with the low-TB group (Table 2). Overall, 51% of patients in the high-TB group developed any-grade CRS and 11% developed any-grade ICANS following the first infusion; 29% and 19% developed any-grade CRS and ICANS following the second infusion, respectively. Of the patients in the low-TB group, 35% developed any grade CRS and 6% any-grade ICANS following the first infusion,

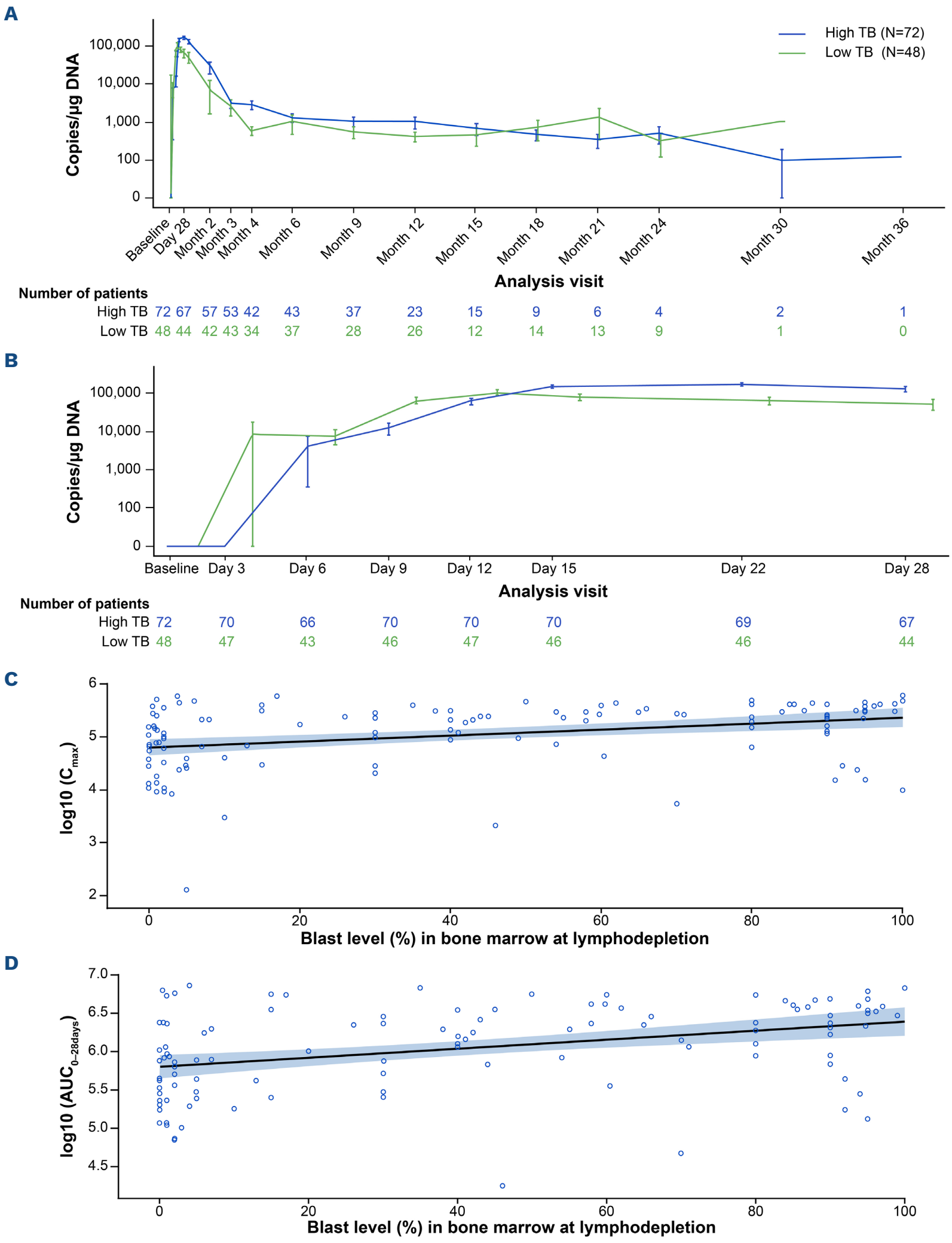


**Figure 1. Pharmacokinetic parameters in patients who received both doses (N=120).** (A) Mean (standard error [SE]) concentration of obe-cel transgene by droplet digital polymerase chain reaction (ddPCR) in peripheral blood by tumor burden-guided dosing regimen up to 36 months post-infusion. (B) Mean (SE) concentration of obecabtagene autoleucel (obe-cel) transgene by ddPCR in peripheral blood by tumor burden-guided dosing regimen up to 1-month post infusion. (C) Linear regression of log10 of maximal expansion of transgene ($C_{max}$) by bone marrow (BM) blast level (%) at lymphodepletion. (D) Linear regression of log10 of exposure up to 28 days ($AUC_{0–28days}$) by BM blast level (%) at lymphodepletion. Low tumor burden (Low TB) refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion.

**Table 1.** Pharmacokinetic parameters by patient and disease characteristics and best overall response.

| Characteristics | $C_{max}$ | | $AUC_{0-28days}$ | | $T_{max}$ |
|---|---|---|---|---|---|
| | Geometric mean (CV%), copies/µg DNA | GLSM ratio, % (90% CI) | Geometric mean (CV%), day×copies/µg DNA | GLSM ratio, % (90% CI) | Median days (range) |
| Sex | | | | | |
| Female, N=61 | 136,789 (234.0) | 149.3 | 1,424,726 (154.9) | 160.7 | 14 (7-28) |
| Male, N=66 | 91,636 (265.6) | (98.5-226.3) | 886,825 (258.6) | (107.1-240.9) | 14 (2-55) |
| Age, years | | | | | |
| ≥55, N=48 | 143,923 (169.9) | 152.4 | 1,267,322 (209.6) | 124.9 | 14 (8-28) |
| <55, N=79 | 94,460 (309.8) | (99.3-233.7) | 1,014,797 (214.0) | (82.0-190.3) | 14 (2-55) |
| Body weight (BMI) | | | | | |
| ≥25, N=78 | 121,563 (204.2) | 111.7 | 1,150,650 (204.0) | 112.7 | 14 (2-55) |
| <25, N=47 | 108,817 (214.8) | (75.1-166.1) | 1,021,336 (234.4) | (73.5-172.6) | 14 (7-28) |
| Race | | | | | |
| Non-White N=33 | 158,518 (146.9) | 162.3 | 1,565,767 (145.4) | 160.0 | 14 (2-28) |
| White N=94 | 97,692 (293.7) | (101.2-260.2) | 978,518 (232.7) | (100.7-254.3) | 14 (7-55) |
| Ethnicity | | | | | |
| Hispanic/Latino, N=38 | 141,771 (206.4) | 132.1 | 1,468,134 (157.8) | 138.2 | 14 (6-28) |
| Not Hispanic/Latino, N=80 | 107,359 (273.8) | (83.7-208.3) | 1,062,705 (233.9) | (88.8-214.9) | 14 (2-55) |
| Philadelphia chromosome | | | | | |
| Positive, N=36 | 129,672 (134.3) | 124.5 | 1,265,028 (159.5) | 122.0 | 13 (8-28) |
| Negative, N=91 | 104,171 (316.5) | (78.3-197.9) | 1,036,671 (240.7) | (78.7-189.2) | 14 (2-55) |
| Prior allo-SCT | | | | | |
| No, N=71 | 129,794 (317.6) | 142.5 | 1,375,872 (219.0) | 161.8 | 14 (2-28) |
| Yes, N=56 | 91,096 (183.3) | (93.7-216.7) | 850,410 (191.7) | (107.9-242.7) | 13 (8-55) |
| Prior blina | | | | | |
| Yes, N=53 | 114,604 (222.4) | 105.8 | 1,116,857 (197.5) | 101.8 | 14 (8-55) |
| No, N=74 | 108,363 (281.6) | (69.0-162.0) | 1,096,806 (226.0) | (67.2-154.4) | 14 (2-28) |
| Prior inotuzumab | | | | | |
| No, N=87 | 124,060 (175.3) | 143.7 | 1,135,031 (200.7) | 109.5 | 14 (2-55) |
| Yes, N=40 | 86,346 (518.9) | (91.5-225.6) | 1,036,855 (247.3) | (69.8-171.8) | 14 (6-28) |
| Prior blina and inotuzumab | | | | | |
| No, N=106 | 116,248 (245.8) | 134.6 | 1,125,479 (210.0) | 112.0 | 14 (2-55) |
| Yes, N=21 | 86,383 (306.2) | (75.9-238.7) | 1,004,947 (233.9) | (64.0-195.9) | 14 (8-28) |
| Refractory to all prior lines of therapy | | | | | |
| Yes, N=13 | 123,989 (204.7) | 113.3 | 1,392,359 (205.6) | 129.5 | 14 (6-28) |
| No, N=114 | 109,482 (261.9) | (56.8-225.8) | 1,074,962 (213.8) | (66.7-251.3) | 14 (2-55) |
| Refractory to first-line therapy within 12 months | | | | | |
| No, N=67 | 117,611 (180.6) | 113.1 | 1,022,106 (188.7) | 84.5 | 14 (2-28) |
| Yes, N=60 | 103,952 (362.1) | (74.3-172.2) | 1,209,449 (243.3) | (56.0-127.5) | 14 (8-55) |
| Refractory to last prior line of therapy | | | | | |
| Yes, N=66 | 115,767 (255.1) | 108.1 | 1,079,038 (255.2) | 93.2 | 14 (2-28) |
| No, N=60 | 107,092 (260.6) | (70.8-165.0) | 1,157,222 (176.7) | (61.7-140.9) | 14 (7-55) |
| BOR | | | | | |
| CR/CRi, N=99 | 114,409 (193.1) | 115.7 | 1,058,244 (218.3) | 75.1 | 14 (2-55) |
| Not CR/CRi, N=28 | 98,915 (652.8) | (69.4-192.9) | 1,408,464 (180.2) | (42.5-133.0) | 15 (6-28) |

ANOVA model, including group as fixed effects, was used for comparison. allo-SCT: allogeneic stem cell transplant; $AUC_{0-28days}$: exposure up to 28 days; blina: blinatumomab; BMI: body mass index; BOR: best overall response; CI: confidence interval; $C_{max}$: maximal expansion of transgene post-infusion; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; CV%: percentage coefficient of variation; GLSM: geometric least-square mean; inotuzumab: inotuzumab ozogamicin; PK: pharmacokinetics; $T_{max}$: time to maximal expansion.

and 17% and 8% developed any-grade CRS and ICANS following the second infusion, respectively. The median time to onset of CRS and ICANS was similar in the high- and low-TB groups; CRS: 9.0 days (range, 1-13) *versus* 7.0 (range, 1-23); ICANS: 12.0 days (range, 1-31) *versus* 12.0 days (range, 2-18), respectively. Incidence of grade ≥3 CRS was 2% *versus* 3% and incidence of grade ≥3 ICANS was 4% *versus* 9% in the low- and high-TB groups, respectively (Table 2). A general trend of increased incidence of any-grade CRS and ICANS with higher tumor burden at lymphodepletion was observed when tumor burden was separated into more discrete groups (<5% [N=36], ≥5-≤20% [N=16], >20-≤75% [N=35], and >75% [N=40] BM blasts; Figure 2). The use of tocilizumab (Figure 3A) and/or corticosteroids (Figure 3B) to treat CRS and ICANS did not appear to influence CAR T-cell expansion over time.

### Efficacy by tumor burden group

At data cut-off, median follow-up was 21.5 months (range, 8.6-41.4). In the infused set of patients, the ORR (CR/CRi) by independent response review committee was 85% (95% CI: 72-93) and 73% (95% CI: 62-83) in the low-TB (44/52) and high-TB (55/75) groups, respectively (Table 2). Among responders with evaluable measurable residual disease (MRD) samples post infusion, MRD-negative CR/CRi ($<10^{-4}$ leukemic cells) was achieved by 98% of patients with low tumor burden compared with 92% of patients with high tumor burden (Table 2). In patients who received two doses of obe-cel, the ORR was 81% (95% CI: 73-87). Two of the seven patients who received only one dose of obe-cel achieved CR/CRi (*Online Supplementary Table S2*).

CAR T-cell expansion was similar between patients who responded (N=99) and those who did not (N=28), with a $C_{max}$ geometric mean (CV%) of 114,409 (193.1) copies/μg DNA *versus* 98,915 (652.8) copies/μg DNA, and an AUC geometric mean from day 0 to 28 (CV%) of 1,058,244 (218.3) day×copies/μg DNA *versus* 1,408,464 (180.2) day×copies/μg DNA, respectively (Table 1). Despite similar expansion between the high- and low-TB groups, patients in the high-TB group had shorter DoR (Figure 4A), EFS (Figure 4B), and OS (Figure 4C) compared with patients in the low-TB group.

## Discussion

Findings from the FELIX study suggest that the application of a tumor burden-guided dosing strategy for obe-cel infusions, using a 20% BM blast threshold, resulted in substantial efficacy while mitigating the increased immunotoxicity often associated with high tumor burden.[6-9] Robust CAR T-cell expansion and durable persistence was observed in all patients who received the total target dose of obe-cel, regardless of pre-lymphodepletion tumor burden. Through detailed PK analyses, pre-lymphodepletion tumor burden was identified as a key driver for obe-cel CAR T-cell expansion *in vivo*, with no overt observed impact of any other patient characteristics. Patients with a high tumor burden showed greater maximum obe-cel expansion than those with a low tumor burden but a slower initial rate of CAR T-cell expansion, as assessed in the peripheral blood. While the mechanism for the slower initial rate is not fully clear, this is possibly because these patients received a lower first dose of obe-cel; however, it is also possible that, in patients with high tumor burden in the BM, CAR T-cell trafficking to and occupancy in the BM leads to lower CAR T-cell levels in peripheral blood.[16,17] The use of tocilizumab or corticosteroids to treat CRS and ICANS following obe-cel treatment did not appear to influence CAR T-cell expansion or persistence.

Of note, an association between tumor burden and CAR T-cell expansion in adult R/R B-ALL was previously identified in the phase I ALLCAR19 study of obe-cel,[10] and also in the phase II ZUMA-3 study of brexucabtagene autoleucel,[3]

**Table 2.** Treatment-emergent adverse events and overall response* by independent response review committee and post obe-cel infusion by tumor burden group.

| **Safety** | **Low TB N=52** | **High TB N=75** |
|---|---|---|
| Grade ≥3 TEAE, N (%) | 41 (79) | 63 (84) |
| Obe-cel related | 34 (65) | 43 (57) |
| TEAE of interest, N (%) | | |
| Any grade CRS | 27 (52) | 60 (80) |
| Grade ≥3 CRS | 1 (2) | 2 (3) |
| Any grade ICANS | 7 (13) | 22 (29) |
| Grade ≥3 ICANS | 2 (4) | 7 (9) |
| **Efficacy** | **Low TB N=52** | **High TB N=75** |
| Best overall response, N (%) | | |
| CR | 31 (60) | 42 (56) |
| CRi | 13 (25) | 13 (17) |
| No response | 5 (10) | 16 (21) |
| Unknown | 3 (6) | 4 (5) |
| Overall remission rate, N (%) | 44 (85) | 55 (73) |
| 95% CI, % | 72-93 | 62-83 |
| Among responders (CR/CRi) with evaluable MRD samples post infusion, N (%) | 42 | 49 |
| MRD-negative CR/CRi | 41 (98) | 45 (92) |
| MRD-positive CR/CRi | 1 (2) | 4 (8) |

*Patients in complete remission/complete remission with incomplete hematologic recovery (CR/CRi) at lymphodepletion are included. Low tumor burden (TB) refers to patients with ≤20% bone marrow (BM) blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. Adverse events (AE) were coded using MedDRA 26.0. A treatment-emergent AE (TEAE) was defined as any AE with post-obe-cel infusion onset. Measurable residual disease (MRD)-negative refers to patients with <1 in $10^{-4}$ leukemic cells, measured by next-generation sequencing (NGS), polymerase chain reaction (PCR), or flow cytometry. CI: confidence interval; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; MedDRA: Medical Dictionary for Regulatory Activities; obe-cel: obecabtagene autoleucel.

the only other licensed CD19-directed CAR T-cell therapies product for adults with R/R B-ALL.[18] While expansion for obe-cel appears to be driven by tumor burden, expansion for brexucabtagene autoleucel, which is administered as a single infusion, was reported to be inversely correlated with tumor burden.[3] Whether this is due to differences in the design of the two CAR T therapies, or to their dosing strategy, remains to be elucidated.

Multiple analyses have reported an association between higher tumor burden and increased severity of CRS and

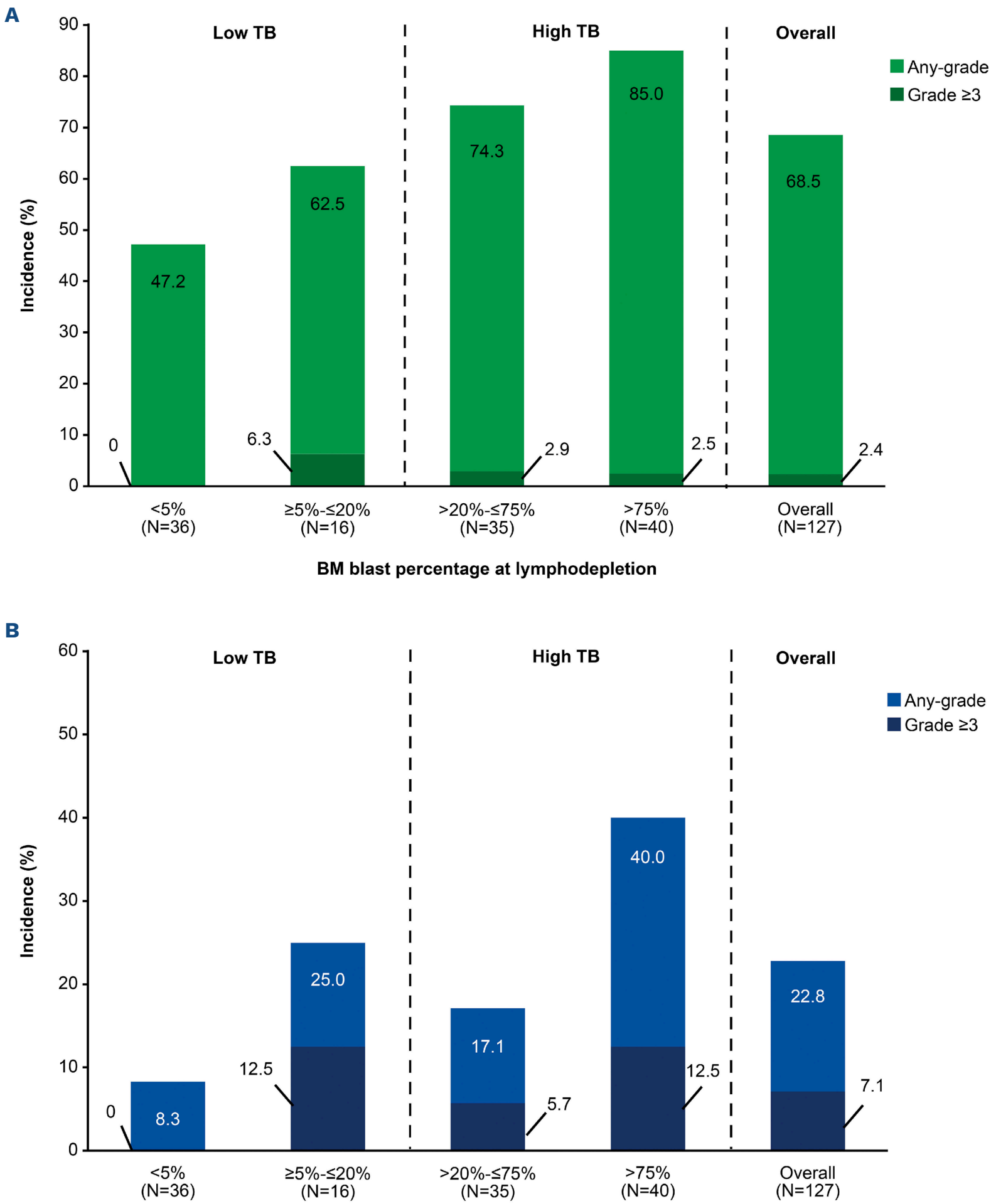


**Figure 2. Incidence of any-grade and grade ≥3 treatment-emergent adverse events of special interest.** (A) Cytokine release syndrome (CRS). (B) Immune effector cell-associated neurotoxicity syndrome (ICANS). Low tumor burden (TB) refers to patients with ≤20% bone marrow (BM) blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion.

neurologic toxicities/ICANS post CAR T.[2,5-7,9,14] Additionally, an association between CAR T-cell expansion and any-grade CRS and ICANS was previously reported in FELIX.[2] In this analysis, low incidence of immunotoxicity was observed after obe-cel treatment in FELIX, even when comparing safety outcomes in the high-TB group with safety outcomes for all patients receiving brexucabtagene autoleucel in the ZUMA-3 study.[3] Incidence of grade ≥3 CRS and ICANS were 3% and 9% for obe-cel in the high-TB group, compared with 24% and 25% for brexucabtagene autoleucel, respectively.[3] Results from the Real-World Outcomes Collaborative of CAR T in Adult ALL showed that 11% of patients treated with brexucabtagene autoleucel across 31 US centers developed grade 3-4 CRS, and 31% developed grade 3-4 ICANS, which

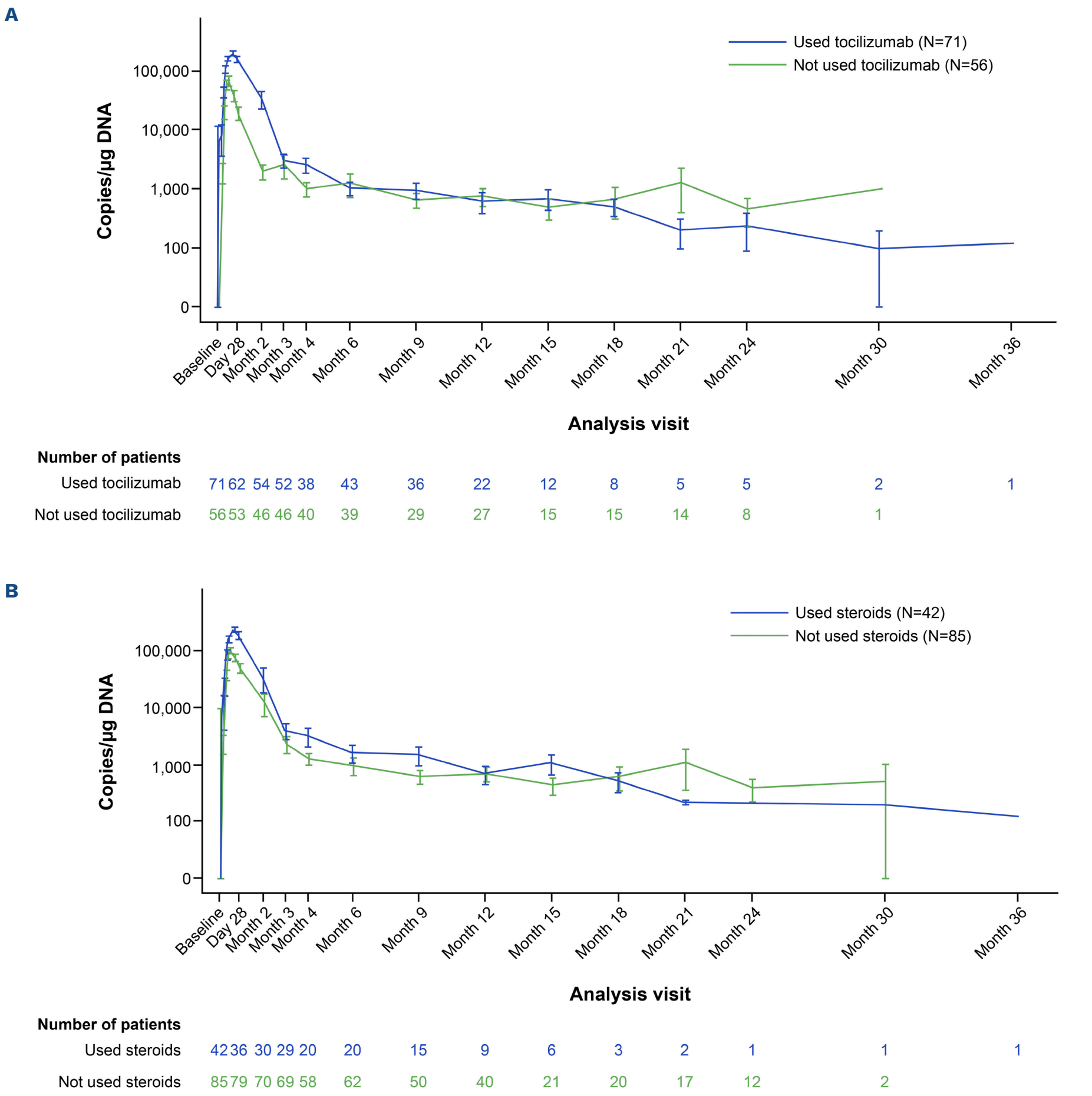


**Figure 3. Mean (standard error) concentration of obe-cel transgene level by droplet digital polymerase chain reaction in peripheral blood by type of treatment used within 28 days post obe-cel infusion.** (A) Tocilizumab. (B) Corticosteroids. obe-cel: obecabtagene autoleucel.

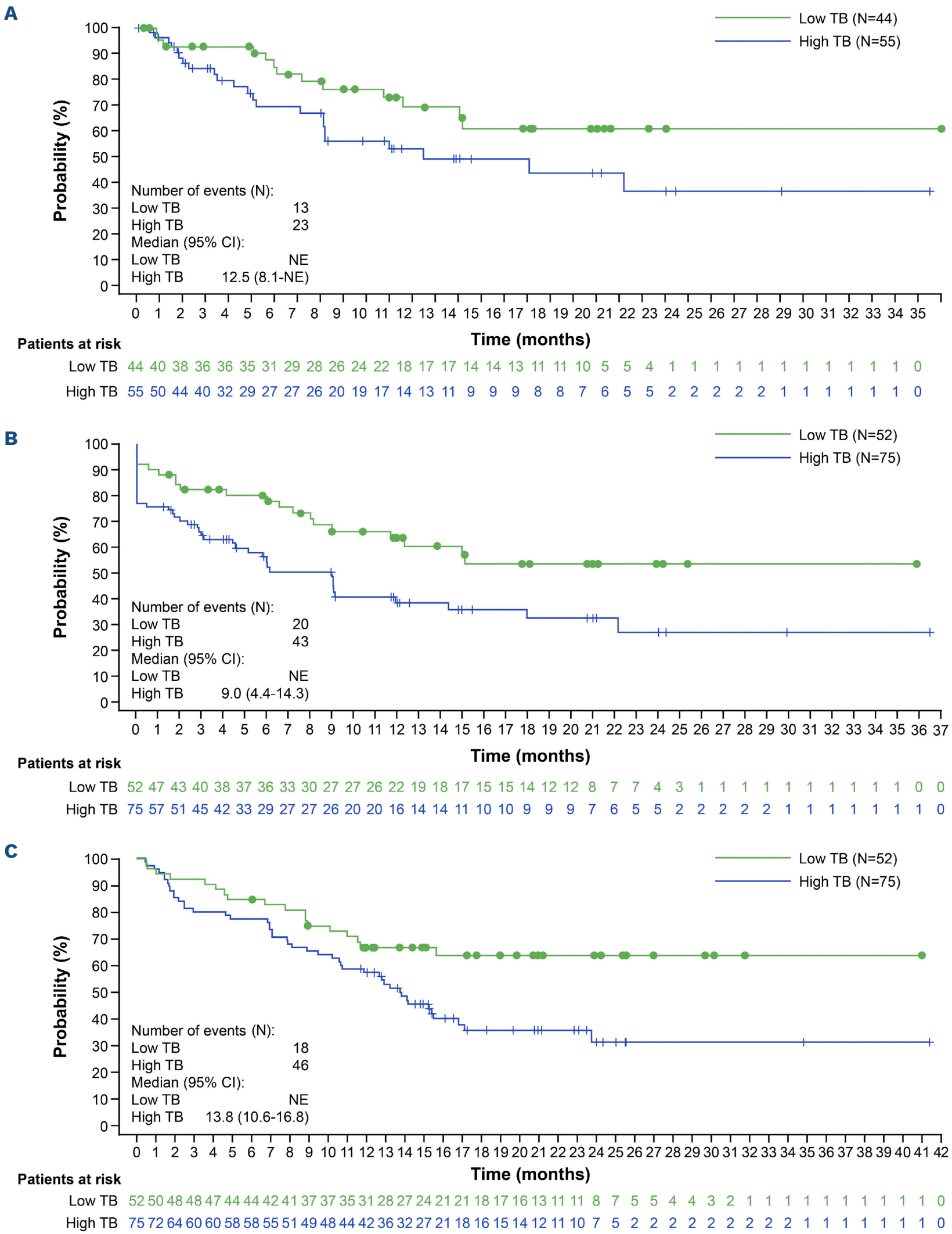


**Figure 4. Kaplan-Meier plots of efficacy outcomes for all infused patients in low- and high-tumor burden groups.** (A) Duration of remission (responders: N=99). (B) Event-free survival. (C) Overall survival. Duration of remission and event-free survival were censored for non-protocol anti-cancer therapies, including consolidative stem cell transplant, with disease assessment by independent response review committee. Overall survival was not censored for consolidative stem cell transplant. Low tumor burden (Low TB) refers to patients with ≤20% bone marrow (BM) blasts at lymphodepletion and High TB refers to patients with >20% BM blasts at lymphodepletion. CI: confidence interval; NE: not estimable.

seem comparable with rates reported in ZUMA-3.[19] In the ELIANA phase II study, tisagenlecleucel, which like obe-cel uses a 4-1BB co-stimulatory domain, was administered to 75 children and young adults with B-ALL (age range, 3-23). The median BM blast percentage was 74%, which is similar to that observed in the high-TB group reported in the FELIX study. The incidence of grade 3 and grade 4 CRS was 21% and 25%, respectively and the incidence of grade 3 neurotoxicity was 13% in patients treated with tisagenlecleucel.[20] The mitigation of severe immunotoxicities with a fractionated dosing strategy *versus* a single dose was previously reported for ARI001, a CD-19 directed CAR T-cell therapy in the CART19-BE-01 trial.[21] Ortiz-Maldonado *et al*. reported a grade ≥3 CRS incidence of 27% in patients who received a single dose while it was only 5% in patients who received three fractionated doses, similar to results in FELIX. The low first dose of obe-cel for patients in the high-TB group, and subsequent slower rate of expansion, may explain the offset of the expected severe immunotoxicities in the FELIX study; however, cross-trial comparisons must be interpreted with caution due to differences in patient populations and study designs. The exact contributions of obe-cel's design, such as its fast off-rate, *versus* the tumor burden-guided dosing strategy cannot be elucidated with existing data and requires further study; however, the low incidence of severe immunotoxicities following obe-cel treatment supports the selection of the 20% BM blast threshold.

In FELIX, increased peak cytokine levels were previously reported in patients with higher grade CRS and ICANS.[2] Here, as expected, higher peak cytokine levels were also observed following treatment with obe-cel in patients with high tumor burden; however, peak cytokine levels in either tumor burden group were lower or comparable with those observed following brexucabtagene autoleucel treatment.[3] The relative low peak cytokine levels post obe-cel infusion may explain the low incidence of grade ≥3 CRS and ICANS. Similar to the results with brexucabtagene autoleucel, cytokine levels for patients treated with obe-cel peaked early, and then returned to baseline within 28 days.[18]

Although high CAR T-cell expansion was observed in both tumor burden groups following obe-cel infusion, the low-TB group had better efficacy outcomes, including ORR, DoR, EFS, and OS, compared with the high-TB group. An association between low tumor burden and improved outcomes was previously reported in adult R/R B-ALL.[9] Additionally, the efficacy outcomes in the high TB-group are comparable with those reported for adult patients with R/R B-ALL treated with other CAR T. The overall remission rate was 69% and 71% in all adult patients treated with brexucabtagene autoleucel in the ZUMA-3 phase I and II trials, respectively,[3,22] and 81% in children and young adults treated with tisagenlecleucel in the ELIANA phase II trial;[20] these results are similar to the 73% observed in the high-TB group following obe-cel treatment. DoR and OS were also consistent with results reported in the ZUMA-3 phase I and II trials.[3,22]

The better efficacy outcomes observed for obe-cel in the low-TB group compared with the high-TB group are most likely driven by the lower tumor burden, or other patient or disease characteristics and not CAR T-cell expansion. Expansion was similar in patients who responded to obe-cel and in patients who had no response. In contrast, in the ZUMA-3 study median peak CAR T-cell expansion was highest in patients with R/R B-ALL who responded to brexucabtagene autoleucel,[23] and was significantly associated with MRD-negative remission in the phase I clinical trial of 19–28z CAR T cells.[9] It is important to note that although longer median DoR following obe-cel treatment was observed in patients with low tumor burden compared with patients with high tumor burden, a potential long-term plateau was observed in both tumor burden groups, potentially associated with ongoing CAR T-cell persistence rather than expansion.[24] Further investigation of long-term outcomes and correlation with obe-cel persistence in patients with high tumor burden is needed.

The strengths of the current study include detailed PK analyses in a large population of patients with R/R B-ALL, including the use of droplet digital polymerase chain reaction as a sensitive technology for detection and tracking of CAR T-cell kinetics compared with conventional flow cytometry.[25] Limitations include the smaller sample sizes for subgroup analyses, which limits investigation into the potential impact of patient and disease characteristics on CAR T-cell expansion. Additionally, more detailed subgroup analyses are limited by reduced sample sizes. We have limited data on patients who received only a single dose of obe-cel, which makes it difficult to determine whether one dose is sufficient; however, two of the seven patients who received only one infusion achieved CRi, one of them in the high-TB group. Substantial expansion was still observed in the five patients who received the low first dose, potentially due to the high tumor burden. The heterogeneity observed in the baseline patient and disease characteristics also adds a layer of complexity to any analysis investigating safety and efficacy outcomes following CAR T-cell therapy. In addition to high BM blasts, CAR T-cell therapy doses, and patient age,[5-7,9,10,14] specific treatment-related factors, such as lymphodepletion, and patient-specific factors, such as baseline thrombocytopenia and elevated levels of endothelial activation, have been linked to the development of severe CRS and neurotoxicity.[26] Extended follow-up of patients treated with obe-cel and real-world studies will be conducted to further characterize optimal administration and identify additional factors that may impact post-infusion immunotoxicity.

Overall, high CAR T-cell expansion was observed irrespective of tumor burden at lymphodepletion. Our data support the use of the tumor burden-guided dosing strategy when administering obe-cel in the treatment of adult R/R B-ALL. Overall, treatment with obe-cel using the tumor burden-guided dosing strategy, led to a reduction in expected incidence

of grade ≥3 CRS and ICANS while maintaining a clinically meaningful response, regardless of tumor burden group at lymphodepletion.

**Disclosures**

*The following authors have received payments for grants, consulting fees, honoraria, and meetings: EJ from AbbVie, Adaptive Biotechnologies, Amgen, Ascentage, Astellas Pharma, Astex, Autolus Therapeutics, Bristol Myers Squibb, Daiichi-Sankyo, Genentech Inc., Hikma, Kite, Novartis, Pfizer, Rigel Pharmaceuticals Inc., Takeda, TargetRx, and TERN Pharmaceuticals; KSS from Autolus Therapeutics; PS from Autolus Therapeutics, Bristol Myers Squibb and Sanofi; ACL from Actinium, Amgen, Astellas Pharma, Autolus Therapeutics, Gilead Sciences, Kite, Pharmacyclics, Pfizer, Sanofi, Takeda and Talaris; MA from AbbVie, Autolus Therapeutics, Bristol Myers Squibb and Gilead Sciences, and personal fees from CytoDyn; BDS from Adaptive Biotechnologies, Amgen, AstraZeneca, Autolus Therapeutics, BeiGene, Bristol Myers Squibb, Century Therapeutics, Deciphera, Gilead Sciences, Jazz Pharmaceuticals, Kite, Lilly, Pepromene Bio, Pfizer, Precision Biosciences, Servier, Novartis, and Takeda; MRB from AbbVie, Achieve Clinics, ADC Therapeutics, Arcellx, Autolus Therapeutics, Bristol Myers Squibb, Chimeric Therapeutics, CRISPR Therapeutics, Gilead Sciences, In8Bio, InCyte, Iovance Biotherapeutics, Kite, Lyell, Optum Health, Novartis, Sana Biotechnology, Sanofi and Servier, and personal fees from Achieve Clinics and In8Bio; JHP from Adaptive Biotechnologies, Affyimmune, Allogene, Amgen, Artiva Biotherapeutics, Autolus Therapeutics, Bright Pharmaceutical Services, Bristol Myers Squibb, Caribou Biosciences, Curocell, Fate Therapeutics, Galapagos, Genentech Inc., Gilead Sciences, InCyte, Intellia, Kite, Novartis, Pfizer, Servier, Sobi, Syntheklne, and Takeda, and personal fees from Curocell; DJD from AbbVie, Amgen, Autolus Therapeutics, Blueprint Medicines, Daiichi-Sankyo, FibroGen, Gilead Sciences, GlycoMimetics, InCyte, Jazz Pharmaceuticals, Novartis, Mt Sinai MPN Consortium, Pfizer, Servier, and Takeda; ET from Autolus Therapeutics, Bristol Myers Squibb/Celgene, Gilead Sciences, Janssen, Jazz Pharmaceuticals, Kite, Pfizer, Sobi, Takeda and Vertex; DY from Amgen, Autolus Therapeutics, Clinigen, Gilead Sciences, and Pfizer; SC from AbbVie, Adicet Bio, Atara Bio, Gilead Sciences, InCyte, Janssen, Miltenyi Biomedicine, Novartis, Orion Pharma, Pierre Fabre, Roche, and Takeda; KH from Autolus Therapeutics; PB from Allogene, Amgen, Bristol Myers Squibb, InCyte, Gilead Sciences, Jazz Pharmaceuticals, Miltenyi Biomedicine, Nektar, Novartis, Pfizer, and Pierre Fabre; MG from Bristol Myers Squibb, Gilead Sciences, MSD, Novartis, and Pierre Fabre; TM from Amgen, Atara Biotherapeutics, Bristol Myers Squibb, Daiichi-Sankyo, Gilead Sciences, InCyte, Janssen, Novartis, Pfizer, Roche, Servier, and Takeda; CR from Autolus Therapeutics, Bristol Myers Squibb, Gilead Sciences, and Janssen. JS, PL-S and WB are employees of and report personal fees from Autolus Therapeutics. PL-S also reports personal fees from the Medical Research Council, UK. All authors received non-financial support (assistance with manuscript preparation) from Ashfield MedComms, who received funding from Autolus Therapeutics.*

**Contributions**

*EJ, JS, PL-S, and WB designed the research and analyzed and interpreted the data. EJ, KSS, PS, ACL, MA, BDS, MRB, JHP, DJD, ET, DY, SC, KH, PB, MG, TM, and CR contributed vital new reagents or analytical tools and collected the data. JS and PL-S performed the statistical analysis. All authors performed the research and wrote the manuscript.*

**Acknowledgments**

*The authors thank all the patients and their families, and the participating sites and their staff for their support of the FELIX study. Third-party medical writing assistance, under the direction of the authors, was provided by Michaella Hulley, PhD, of Ashfield MedComms (Johannesburg, South Africa), an Inizio Company, and was funded by Autolus Therapeutics PLC.*

**Funding**

*This study was sponsored by Autolus Therapeutics PLC. CR is supported by the University College London NIHR Biomedical Research Center and by core funding through the CRUK London Major Center.*

**Data-sharing statement**

*The data that support the findings of this study will be made available to qualified researchers for agreed pre-specified purposes upon written request after the approval of the investigational therapy in the United States and Europe. All data access requests should be sent to: clinicaltrials@autolus.com.*

# References

1. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321-3330.
2. Roddie C, Sandhu KS, Tholouli E, et al. Obecabtagene autoleucel in adults with B-cell acute lymphoblastic leukemia. N Engl J Med. 2024;391(23):2219-2230.
3. Shah BD, Ghobadi A, Oluwole OO, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet. 2021;398(10299):491-502.
4. Hughes AD, Teachey DT, Diorio C. Riding the storm: managing cytokine-related toxicities in CAR T-cell therapy. Semin Immunopathol. 2024;46:5. https://doi.org/10.1007/s00281-024-01013-w

5. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant. 2019;25(4):625-638.
6. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014;6(224):224ra225.
7. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385(9967):517-528.
8. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. Nat Rev Immunol. 2022;22(2):85-96.
9. Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378(5):449-459.
10. Roddie C, Dias J, O'Reilly MA, et al. Durable responses and low toxicity after fast off-rate CD19 chimeric antigen receptor-T therapy in adults with relapsed or refractory B-cell acute lymphoblastic leukemia. J Clin Oncol. 2021;39(30):3352-3363.
11. 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(9):1408-1414.
12. Mao R, Hussein MS, He Y. Chimeric antigen receptor engineered T cells and their application in the immunotherapy of solid tumours. Expert Rev Mol Med. 2022;24:e7.
13. Seigner J, Zajc CU, Dötsch S, et al. Solving the mystery of the FMC63-CD19 affinity. Sci Rep. 2023;13(1):23024.
14. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126(6):2123-2138.
15. Vaillant M, Olliaro P. Geometric least squares means ratios for the analysis of Plasmodium falciparum in vitro susceptibility to antimalarial drugs. Malar J. 2007;6:156.
16. Martínez-Rubio Á, Chulián S, Blázquez Goñi C, et al. A mathematical description of the bone marrow dynamics during CAR T-Cell therapy in B-Cell childhood acute lymphoblastic leukemia. Int J Mol Sci. 2021;22(12):6371.
17. Mueller KT, Maude SL, Porter DL, et al. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. Blood. 2017;130(21):2317-2325.
18. Bouchkouj N, Lin X, Wang X, et al. FDA approval summary: brexucabtagene autoleucel for treatment of adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia. Oncologist. 2022;27(10):892-899.
19. Roloff GW, Aldoss I, Kopmar NE, et al. Outcomes after brexucabtagene autoleucel administered as a standard therapy for adults with relapsed/refractory B-cell ALL. J Clin Oncol. 2025;43(5):558-566.
20. 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(5):439-448.
21. Ortiz-Maldonado V, Rives S, Español-Rego M, et al. Factors associated with the clinical outcome of patients with relapsed/refractory CD19(+) acute lymphoblastic leukemia treated with ARI-0001 CART19-cell therapy. J Immunother Cancer. 2021;9(12):e003644.
22. 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.
23. Shah BD, Ghobadi A, Oluwole OO, et al. Two-year follow-up of KTE-X19 in patients with relapsed or refractory adult B-cell acute lymphoblastic leukemia in ZUMA-3 and its contextualization with SCHOLAR-3, an external historical control study. J Hematol Oncol. 2022;15(1):170.
24. Jabbour E, Tholouli E, Sandhu KS, et al. Obecabtagene autoleucel (obe-cel, AUTO1) in adults with relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL): overall survival (OS), event-free survival (EFS) and the potential impact of chimeric antigen receptor (CAR)-T cell persistency and consolidative stem cell transplantation (SCT) in the open-label, single-arm FELIX phase Ib/II study. J Clin Oncol. 2024;42(16 Suppl.):6504.
25. Day W, Raymond M, Roddie C, et al. Droplet digital PCR and flow cytometry sensitivity for measuring CAR T-cell kinetics in adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) treated with obecabtagene autoleucel. Hemasphere. 2024;8(S1):2713.
26. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. Blood Rev. 2019;34:45-55.