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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

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Author 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.

Running header: TB-guided dosing of obe-cel in adult R/R B-ALL

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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.

Clinical trial registration

This trial was registered at www.clinicaltrials.gov as NCT04404660.

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Key Points:

- Tumor burden at lymphodepletion is a key driver of obe-cel CAR T-cell expansion in R/R B-ALL and is correlated with immunotoxicity
- Obe-cel's tumor burden-guided dosing and unique CAR construct result in low incidence of severe immunotoxicity, regardless of tumor burden

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 1b/2 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% *versus* 3% and 4% *versus* 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 registration number: NCT04404660 (www.clinicaltrials.gov).

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 pretreatment 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 Ts.[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 [TB]) 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 TB treated with the high dose required intensive care unit admission, while only one of 10 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 TB 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 TB receiving a low first dose compared with patients with low TB.[2,10]

Treatment of adults with R/R B-ALL with obe-cel demonstrated promising results in the phase 1 ALLCAR19 study (NCT02935257)[10] and the pivotal phase 1b/2 FELIX study (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 TB-guided dosing as a strategy to reduce the severe adverse events commonly associated with CAR T.

Methods

The benefit of the TB-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 (NCT04404660) is an open-label, multicenter, global, single-arm, phase 1b/2 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 Harmonisation 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-[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 410×10^6 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 $[AUC]_{0–28\,days}$; 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 details 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 (CIs) 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, 52/127 (41%) patients had ≤20% BM blasts at lymphodepletion (low-TB group), while 75/127 (59%) had >20% BM blasts at lymphodepletion (high-TB group). Baseline characteristics are summarized in Online Supplementary Table 1 and were overall similar between the two TB 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; 7 (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 7 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 [range]) was numerically similar for patients with low and those with high tumor burden, day 11 (2-28) and day 15 (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 2).

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 *vs.* 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 *vs.* 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 $AUC_{0–28\,days}$ (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}/$AUC_{0–28\,days}$ 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 $AUC_{0–28\,days}$ 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 means of peak cytokine levels were numerically higher in the high-TB group compared with the low-TB group (Online Supplementary Figure 1A). Cytokine levels peaked at approximately Day 12 following infusion of obe-cel and returned to baseline levels within 28 days (Online Supplementary Figure 1B).

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, 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- (44/52) and high- (55/75) tumor burden 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 7 patients who received only one dose of obe-cel achieved CR/CRi (Online Supplementary Table 2).

CAR T-cell expansion was similar between patients who responded (N=99) and those who did not (N=28) patients, 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 1 ALLCAR19 study of obe-cel,[10] and also in the phase 2 ZUMA-3 study of brexucabtagene autoleucel,[3] the only other licensed CD19-directed CAR T-cell 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 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 seem comparable with rates reported in ZUMA-3.[19] In the ELIANA phase 2 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.[3] 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.[2] 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 1 and 2 trials, respectively,[3,22] and 81% in children and young adults treated with tisagenlecleucel in the ELIANA phase 2 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 1 and 2 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 1 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 7 patients who received only one infusion achieved CRi, one of them in the high-TB group. Substantial expansion was still observed in the 5 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.

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Table 1. PK parameters by patient and disease characteristics and BOR.

	C_{max}		$AUC_{0-28days}$		T_{max}
	Geometric mean (CV%), copies/µg DNA	**GLSM ratio (%) (90% CIs)**	**Geometric mean (CV%), day×copies/µg DNA**	**GLSM ratio (%) (90% CIs)**	**Median (range), days**
Sex					
Female (N=61)	136,789 (234.0)	149.3 (98.5-226.3)	1,424,726 (154.9)	160.7 (107.1-240.9)	14 (7-28)
Male (N=66)	91,636 (265.6)		886,825 (258.6)		14 (2-55)
Age, years					
≥55 (N=48)	143,923 (169.9)	152.4 (99.3-233.7)	1,267,322 (209.6)	124.9 (82.0-190.3)	14 (8-28)
<55 (N=79)	94,460 (309.8)		1,014,797 (214.0)		14 (2-55)
Body weight (BMI)					
≥25 (N=78)	121,563 (204.2)	111.7 (75.1-166.1)	1,150,650 (204.0)	112.7 (73.5-172.6)	14 (2-55)
<25 (N=47)	108,817 (214.8)		1,021,336 (234.4)		14 (7-28)
Race					
Non-White (N=33)	158,518 (146.9)	162.3 (101.2-260.2)	1,565,767 (145.4)	160.0 (100.7-254.3)	14 (2-28)
White (N=94)	97,692 (293.7)		978,518 (232.7)		14 (7-55)
Ethnicity					
Hispanic/Latino (N=38)	141,771 (206.4)	132.1 (83.7-208.3)	1,468,134 (157.8)	138.2 (88.8-214.9)	14 (6-28)
Not Hispanic/Latino (N=80)	107,359 (273.8)		1,062,705 (233.9)		14 (2-55)
Philadelphia chromosome					
Positive (N=36)	129,672 (134.3)	124.5 (78.3-197.9)	1,265,028 (159.5)	122.0 (78.7-189.2)	13 (8-28)
Negative (N=91)	104,171 (316.5)		1,036,671 (240.7)		14 (2-55)
Prior allo-SCT					
No (N=71)	129,794 (317.6)	142.5 (93.7-216.7)	1,375,872 (219.0)	161.8 (107.9-242.7)	14 (2-28)
Yes (N=56)	91,096 (183.3)		850,410 (191.7)		13 (8-55)
Prior blina					
Yes (N=53)	114,604 (222.4)	105.8 (69.0-162.0)	1,116,857 (197.5)	101.8 (67.2-154.4)	14 (8-55)
No (N=74)	108,363 (281.6)		1,096,806 (226.0)		14 (2-28)
Prior inotuzumab					
No (N=87)	124,060 (175.3)	143.7 (91.5-225.6)	1,135,031 (200.7)	109.5 (69.8-171.8)	14 (2-55)
Yes (N=40)	86,346 (518.9)		1,036,855 (247.3)		14 (6-28)
Prior blina and inotuzumab					
No (N=106)	116,248 (245.8)	134.6 (75.9-238.7)	1,125,479 (210.0)	112.0 (64.0-195.9)	14 (2-55)
Yes (N=21)	86,383 (306.2)		1,004,947 (233.9)		14 (8-28)

Refractory to all prior lines of therapy					
Yes (N=13)	123,989 (204.7)	113.3 (56.8-225.8)	1,392,359 (205.6)	129.5 (66.7-251.3)	14 (6-28)
No (N=114)	109,482 (261.9)		1,074,962 (213.8)		14 (2-55)
Refractory to first-line therapy within 12 months					
No (N=67)	117,611 (180.6)	113.1 (74.3-172.2)	1,022,106 (188.7)	84.5 (56.0-127.5)	14 (2-28)
Yes (N=60)	103,952 (362.1)		1,209,449 (243.3)		14 (8-55)
Refractory to last prior line of therapy					
Yes (N=66)	115,767 (255.1)	108.1 (70.8-165.0)	1,079,038 (255.2)	93.2 (61.7-140.9)	14 (2-28)
No (N=60)	107,092 (260.6)		1,157,222 (176.7)		14 (7-55)
BOR					
CR/CRi (N=99)	114,409 (193.1)	115.7 (69.4-192.9)	1,058,244 (218.3)	75.1 (42.5-133.0)	14 (2-55)
Not CR/CRi (N=28)	98,915 (652.8)		1,408,464 (180.2)		15 (6-28)

ANOVA model, including group as fixed effects, was used for comparison.

allo-SCT: allogeneic stem cell transplant; $AUC_{0-28\,days}$: exposure up to 28 days; blina, blinatumomab; BMI: body mass index; BOR: best overall response; CI: confidence interval; C_{max}: maximal expansion of transgene/chimeric antigen receptor-positive T-cell levels 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.

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 CR/CRi at lymphodepletion are included.

Low TB refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. AEs were coded using MedDRA 26.0. A TEAE was defined as any AE with post-obe-cel infusion onset. MRD-negative refers to patients with <1 in 10^{-4} leukemic cells, measured by NGS, PCR, or flow cytometry.

AE: adverse event; BM: bone marrow; CI: confidence interval; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; MedDRA: Medical Dictionary for Regulatory Activities; MRD: measurable residual disease; obe-cel: obecabtagene autoleucel; PCR: polymerase chain reaction; NGS: next-generation sequencing; TB: tumor burden; TEAE: treatment-emergent adverse event.

Figure legends

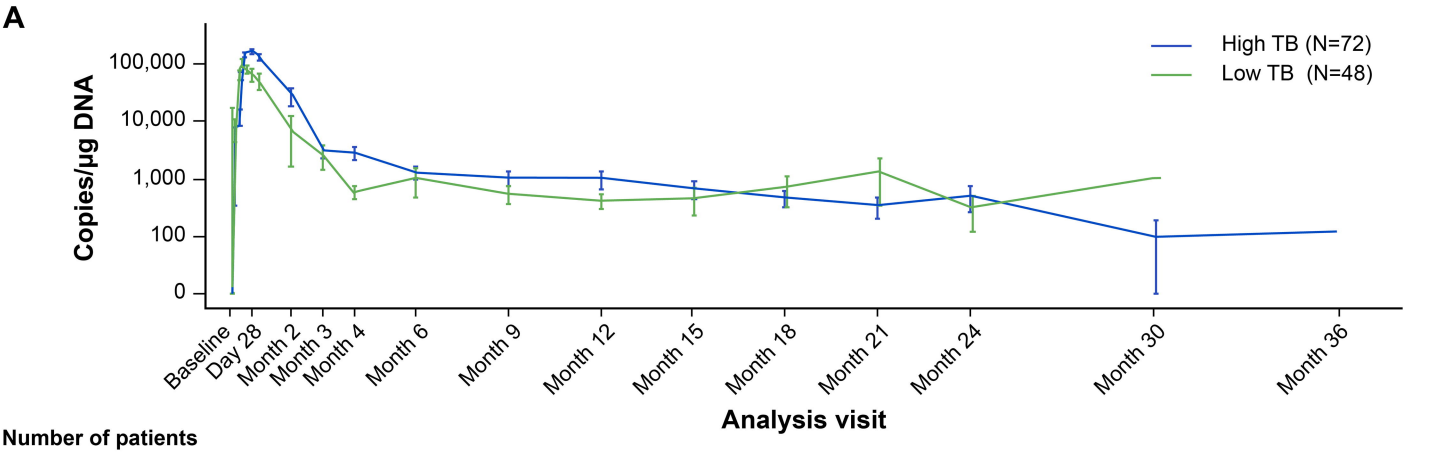
Figure 1. Pharmacokinetic parameters in patients who received both doses (N=120). (A) Mean (SE) concentration of obe-cel transgene by ddPCR in peripheral blood by tumor burden-guided dosing regimen up to 36 months post-infusion. (B) Mean (SE) concentration of obe-cel transgene by ddPCR in peripheral 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 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 TB refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. $AUC_{0–28\,days}$: area under the $curve_{0–28\,days}$; BM: bone marrow; C_{max}: maximal expansion of transgene post-infusion; ddPCR: droplet digital polymerase chain reaction; obe-cel: obecabtagene autoleucel; SE: standard error; TB: tumor burden.

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

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

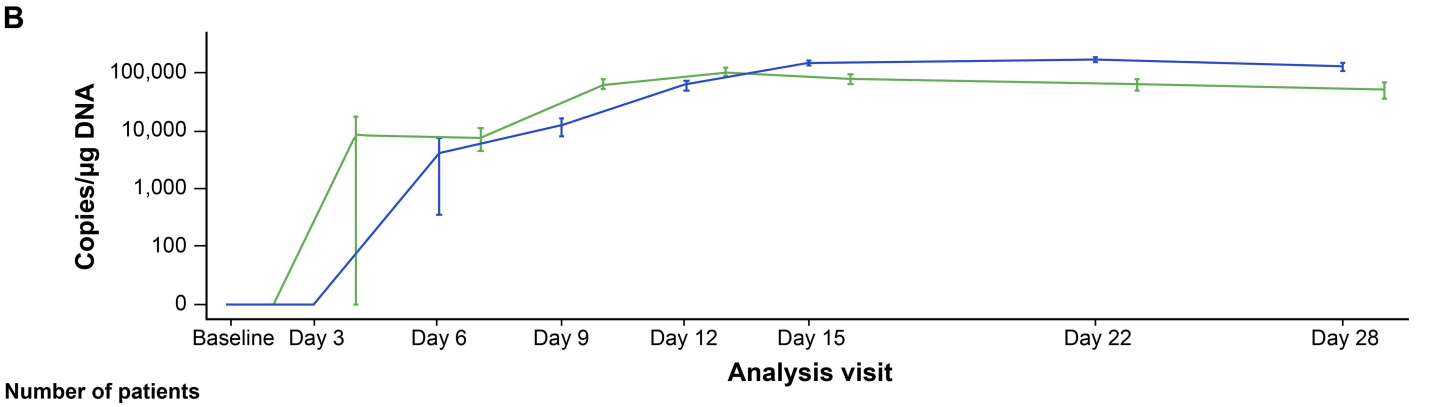
Figure 4. Kaplan–Meier plots of efficacy outcomes for all infused patients in low- and high-TB groups. (A) DoR (responders: N=99). (B) EFS. (C) OS. DoR and EFS were censored for non-protocol anti-cancer therapies, including consolidative stem cell transplant, with disease assessment by independent response review committee. OS was not censored for consolidative stem cell

transplant. Low TB refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. BM: bone marrow; CI: confidence interval; DoR: duration of remission; EFS: event-free survival; NE: not estimable; OS: overall survival; TB: tumor burden.



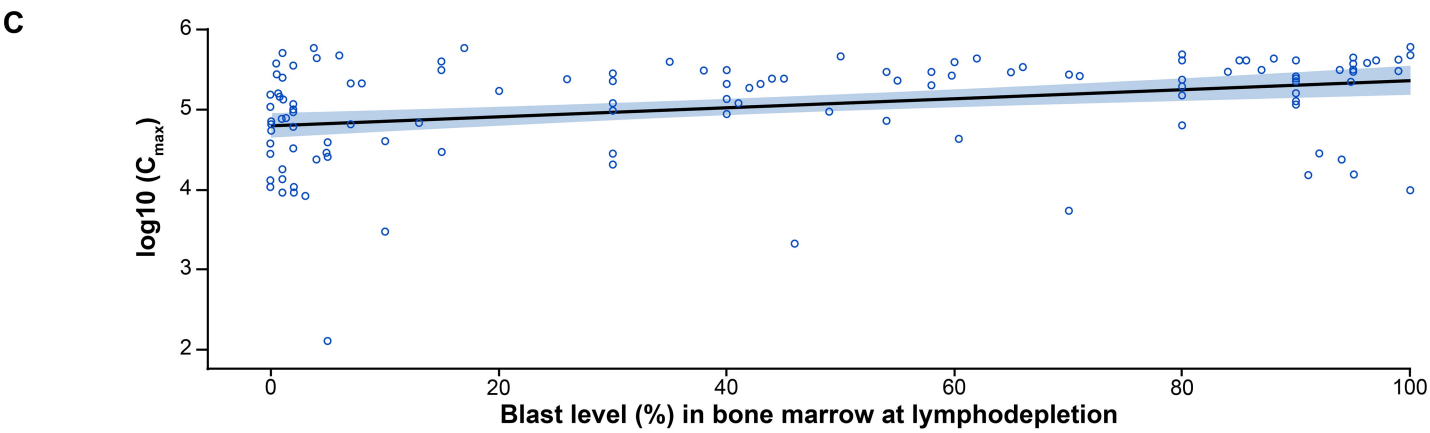
Number of patients

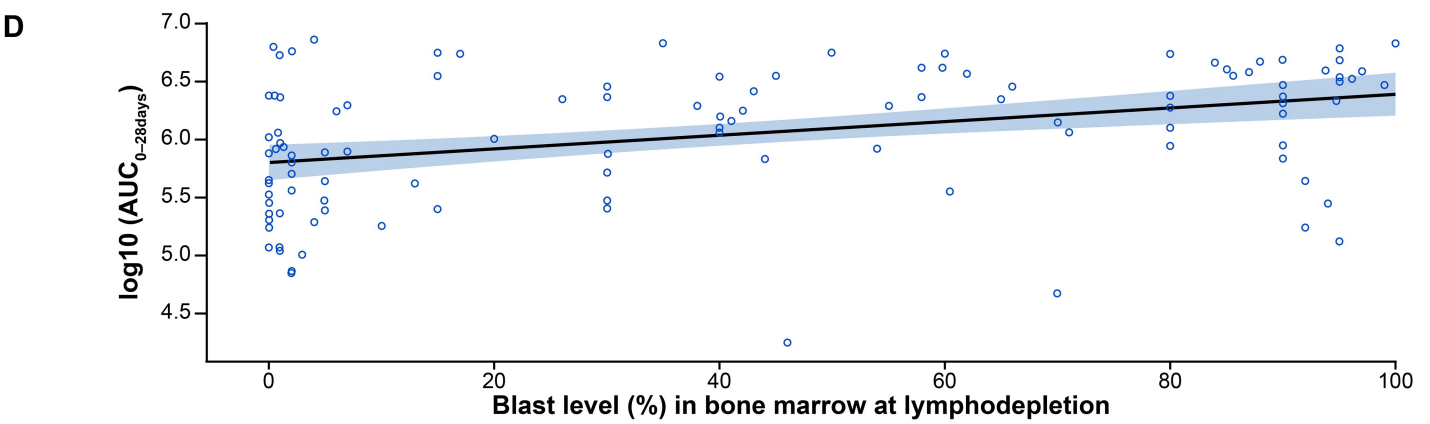
High TB	72	67	57	53	42	43	37	23	15	9	6	4	2	1
Low TB	48	44	42	43	34	37	28	26	12	14	13	9	1	0

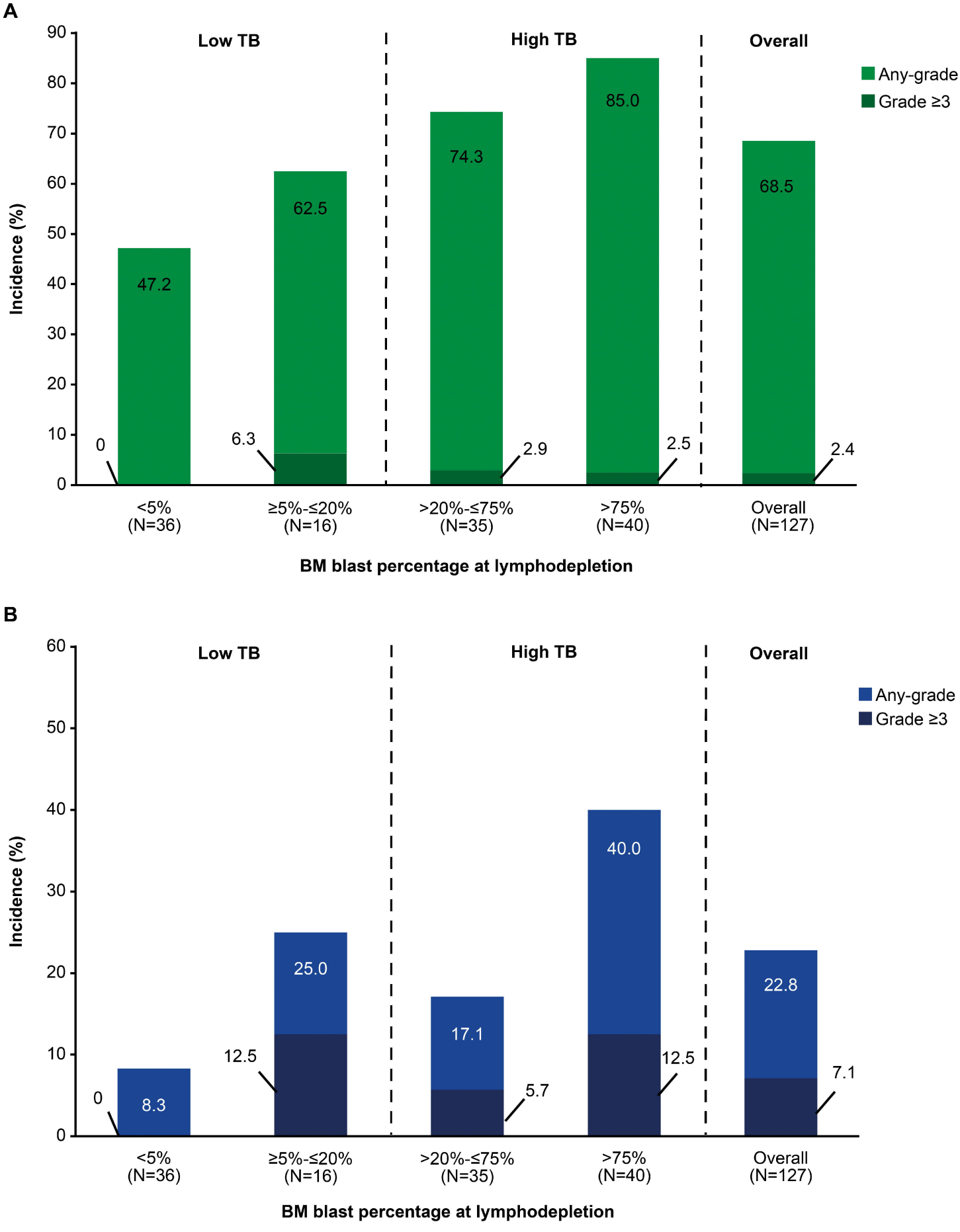


Number of patients

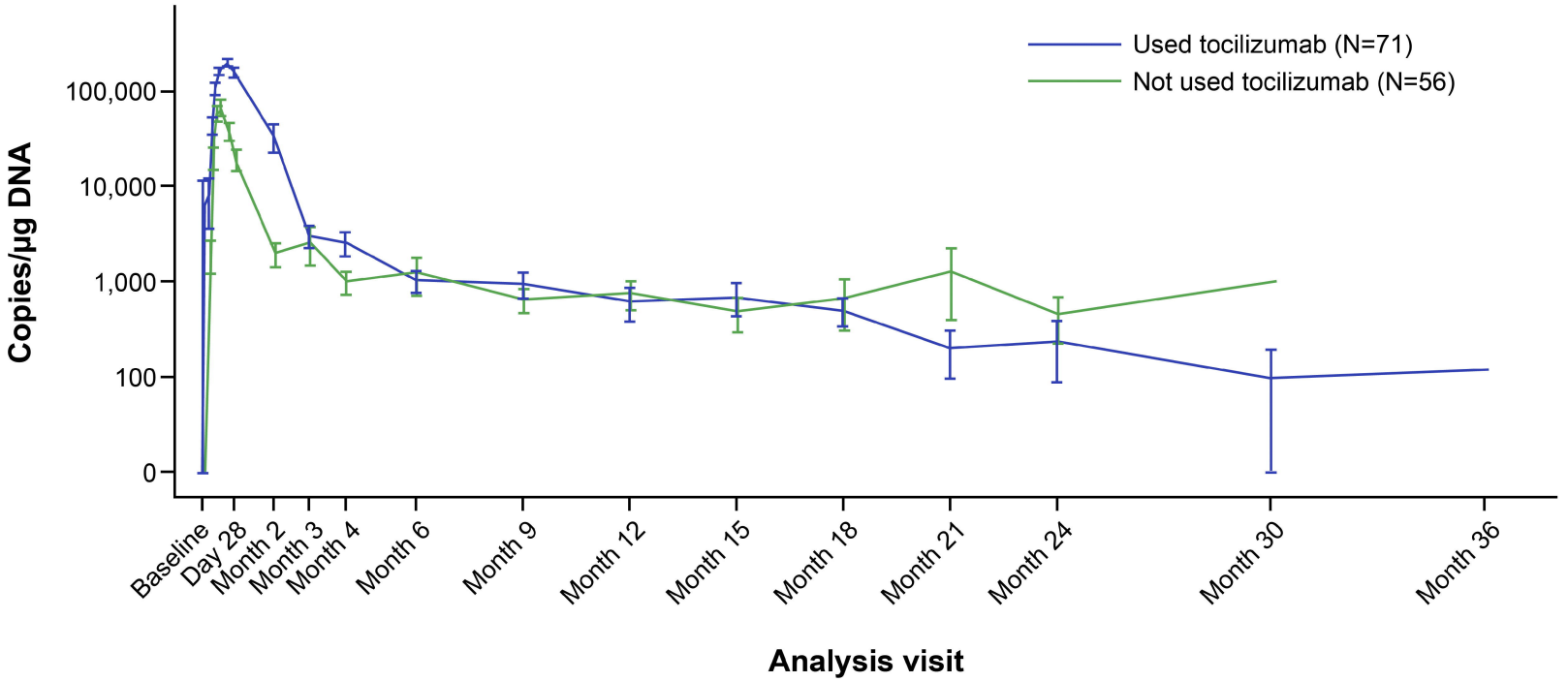
High TB	72	70	66	70	70	70	69	67
Low TB	48	47	43	46	47	46	46	44






A
Low TB
High TB
Overall
Any-grade
Grade ≥3
Incidence (%)
90
80
70
60
50
40
30
20
10
0
47.2
62.5
74.3
85.0
68.5
0
6.3
2.9
2.5
2.4
<5%
(N=36)
≥5%-≤20%
(N=16)
>20%-≤75%
(N=35)
>75%
(N=40)
Overall
(N=127)
BM blast percentage at lymphodepletion
B
Low TB
High TB
Overall
Any-grade
Grade ≥3
Incidence (%)
60
50
40
30
20
10
0
8.3
25.0
17.1
40.0
22.8
0
12.5
5.7
12.5
7.1
<5%
(N=36)
≥5%-≤20%
(N=16)
>20%-≤75%
(N=35)
>75%
(N=40)
Overall
(N=127)
BM blast percentage at lymphodepletion

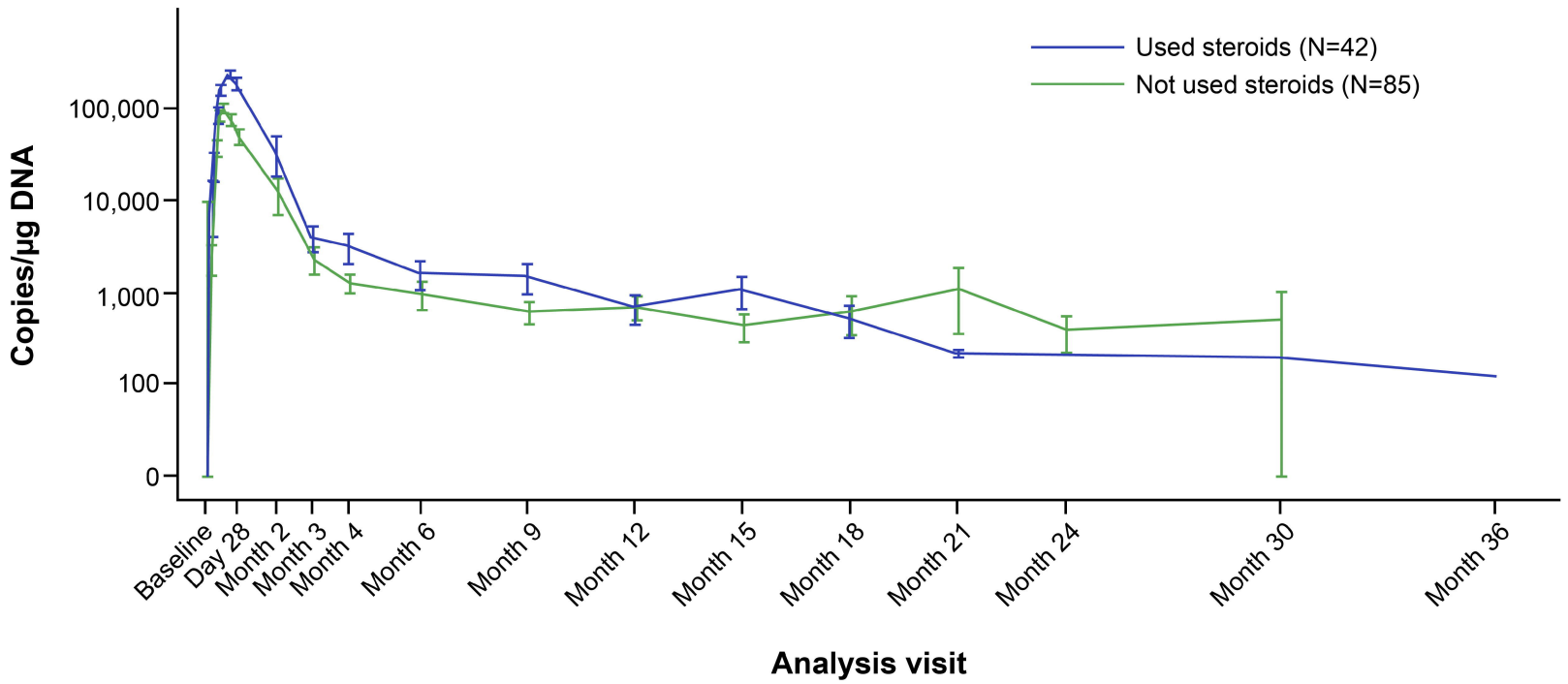
A



Number of patients

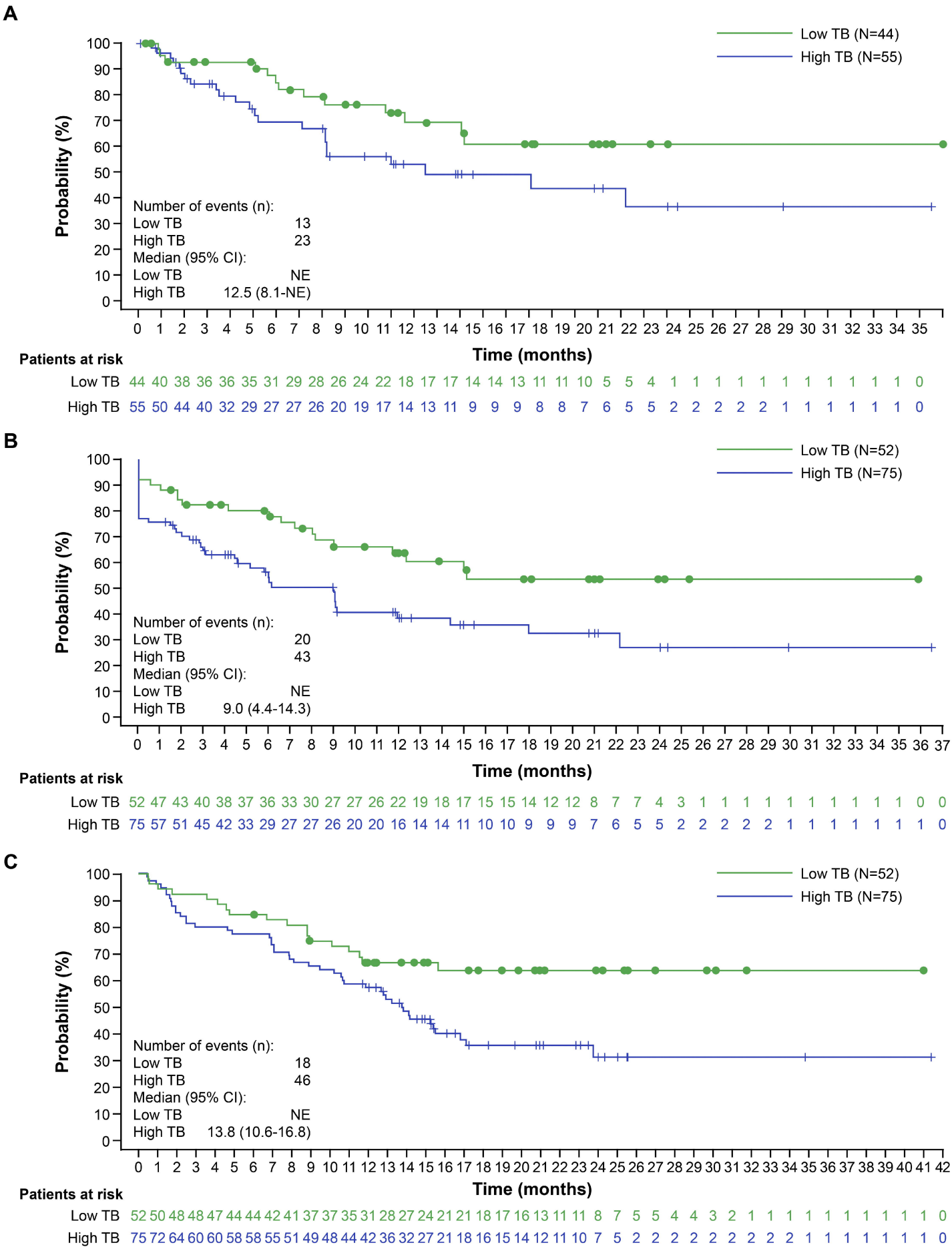
Used tocilizumab	71	62	54	52	38	43	36	22	12	8	5	5	2	1
Not used tocilizumab	56	53	46	46	40	39	29	27	15	15	14	8	1	

B



Number of patients

Used steroids	42	36	30	29	20	20	15	9	6	3	2	1	1	1
Not used steroids	85	79	70	69	58	62	50	40	21	20	17	12	2	


A
Low TB (N=44)
High TB (N=55)
Probability (%)
Number of events (n):
Low TB 13
High TB 23
Median (95% CI):
Low TB NE
High TB 12.5 (8.1-NE)
Time (months)
Patients at risk
Low TB 44 40 38 36 36 35 31 29 28 26 24 22 18 17 17 14 14 13 11 11 10 5 5 4 1 1 1 1 1 1 1 1 1 0
High TB 55 50 44 40 32 29 27 27 26 20 19 17 14 13 11 9 9 9 8 8 7 6 5 5 2 2 2 2 2 1 1 1 1 1 0
B
Low TB (N=52)
High TB (N=75)
Probability (%)
Number of events (n):
Low TB 20
High TB 43
Median (95% CI):
Low TB NE
High TB 9.0 (4.4-14.3)
Time (months)
Patients at risk
Low TB 52 47 43 40 38 37 36 33 30 27 27 26 22 19 18 17 15 15 14 12 12 8 7 7 4 3 1 1 1 1 1 1 1 1 1 0 0
High TB 75 57 51 45 42 33 29 27 27 26 20 20 16 14 14 11 10 10 9 9 9 7 6 5 5 2 2 2 2 2 1 1 1 1 1 1 0
C
Low TB (N=52)
High TB (N=75)
Probability (%)
Number of events (n):
Low TB 18
High TB 46
Median (95% CI):
Low TB NE
High TB 13.8 (10.6-16.8)
Time (months)
Patients at risk
Low TB 52 50 48 48 47 44 44 42 41 37 37 35 31 28 27 24 21 21 18 17 16 13 11 11 8 7 5 5 4 4 3 2 1 1 1 1 1 1 1 1 1 0
High TB 75 72 64 60 60 58 58 55 51 49 48 44 42 36 32 27 21 18 16 15 14 12 11 10 7 5 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1 1 0

Supplementary Appendix

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

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Administration in practice

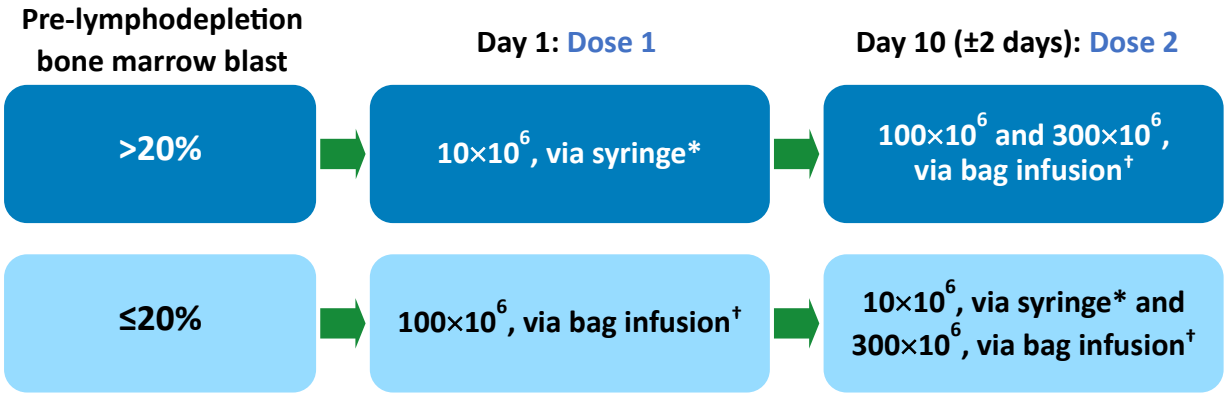
Administration in practice: AUCATZYL® (obecabtagene autoleucel [obe-cel]) suspension for intravenous infusion[1]

Obe-cel is a CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia.

Dosage and administration

- Obe-cel is a cell suspension for infusion and intended for autologous and intravenous use only.
- The total recommended dose of obe-cel is 410×10^6 CD19 chimeric antigen receptor (CAR)-positive viable T cells supplied in three to five infusion bags (10×10^6, 100×10^6, 300×10^6) for split dose administration.
- A bone marrow assessment must be available from a sample obtained within 7 days prior to the commencement of lymphodepleting chemotherapy treatment.
- The bone marrow assessment will be used to determine the dosage regimen based on a bone marrow blast of >20% or ≤20%.
- If the bone marrow assessment results are inconclusive, repeat the biopsy or aspirate (only if lymphodepleting chemotherapy treatment has not started).
- If results remain inconclusive, proceed with the bone marrow blast of >20% dosage.
- Administer the lymphodepleting chemotherapy regimen before infusion of obe-cel: fludarabine (FLU) 30 mg/m^2/day intravenously for 4 days and cyclophosphamide (CY) 500 mg/m^2/day intravenously for 2 days starting with the first dose of fludarabine (total dose: FLU 120 mg/m^2; CY 1,000 mg/m^2). Infuse obe-cel 3 days (± 1 days) after completion of lymphodepleting chemotherapy treatment (Day 1), allowing a minimum 48-hour washout.
- To minimize the risk of an infusion reaction, premedicate with acetaminophen approximately 30 minutes prior to obe-cel infusion.

- Confirm availability of tocilizumab prior to infusion for the potential treatment of cytokine release syndrome (CRS).
- Avoid prophylactic use of systemic corticosteroids as they may interfere with the activity of obe-cel.
- Proceed with obe-cel administration as outlined below:



*Volume specified per infusion bag, calculated based on the concentration of CD19 CAR-positive viable T cells, administer at a rate of 0.5mL/minute through a central venous line or large peripheral venous access line. †Administer the full content of the bag via a gravity or peristaltic pump assisted intravenous infusion through a central venous line or large peripheral venous access line at a rate of 0.1–27mL/minute.

- Monitor patients for signs and symptoms of CRS, neurologic toxicities/immune effector cell-associated neurotoxicity syndrome (ICANS) and other acute toxicities daily for at least 14 days at the healthcare facility following the first infusion.
- Continue to monitor patients for at least 4 weeks following each infusion.
- Discontinue treatment if, patients develop grade ≥3 CRS and/or grade ≥2 ICANS or grade ≥3 pulmonary or cardiac toxicities following the Dose 1. Dose 2 infusion for patients who develop maximum grade 2 CRS and/or grade 1 ICANS following infusion of Dose 1 may be delayed up to day 21, and administered only if CRS resolves to grade ≤1 and ICANS completely resolves.

Supplementary Materials and Methods

The design and conduct of the FELIX study was previously reported.[2]

Treatment/dosing schedule

Following leukapheresis, patients received bridging therapy at the discretion of the investigator to reduce the percentage of bone marrow blasts prior to lymphodepletion. Bone marrow blast percentage was assessed locally within 7 days prior to lymphodepletion and assessed by centralized morphologic review to determine tumor burden. Obe-cel was administered according to a tumor burden-guided dosing schedule following lymphodepletion with fludarabine and cyclophosphamide. The study design allowed for the treatment of adverse events prior to the administration of the second dose of obe-cel.

Tocilizumab was recommended for the treatment of grade ≥2 CRS as a 60-minute intravenous infusion (8 mg/kg in patients weighing ≥30 kg or 12 mg/kg in patients weighing <30 kg); a maximum of 800 mg per infusion was recommended. If no clinical improvement occurred following the first dose, up to three additional doses of tocilizumab were permitted with an interval of at least 8 hours between doses. Corticosteroids were recommended for the treatment of grade 3 CRS refractory to tocilizumab, grade 4 CRS, and grade ≥2 ICANS (recommended dose of methylprednisolone of 2 mg/kg intravenously every 12 hours over 5 days, and dexamethasone of 10 mg intravenously every 6 hours).

Assessments and endpoints

Serum cytokine levels were measured using peripheral blood samples collected pre-lymphodepletion and on days 1, 3, 6, 9, 12, 15, 22, and 28, and month 3 post obe-cel infusion. Post obe-cel infusion response/relapse was monitored at day 28, months 2, 3, 4, 6, and every 3 months thereafter until the end of study based on peripheral blood and/or BM assessments.

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) 26.0 and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0. CRS and ICANS were graded using the American Society for Transplantation and Cellular Therapy (ASTCT)/American Society for Blood and Marrow Transplantation (ASBMT) consensus guidelines.[3]

Statistical analyses

Statistical analyses for the comparison of efficacy outcomes and pharmacokinetic parameters between subgroups based on patient and disease characteristics were not performed, as these were not pre-specified endpoints in the trial.

Supplementary results

Supplementary Table 1. Baseline characteristics of the infused set of patients (N=127).

	Low TB (N=52)	**High TB (N=75)**
Age in years, median (range)	44 (20-81)	49 (20-79)
Male/female sex, N	28/24	38/37
Race, N (%)		
Asian	8 (15)	8 (11)
Black or African American	0 (0)	2 (3)
White	41 (79)	53 (71)
Unknown	3 (6)	12 (16)
Ethnicity, N (%)		
Hispanic or Latino	11 (21)	27 (36)
Not Hispanic or Latino	37 (71)	43 (57)
Unknown	4 (8)	5 (7)
Philadelphia chromosome-positive, N (%)	17 (33)	19 (25)
Philadelphia chromosome-like disease, N (%)	4 (8)	6 (8)
Prior lines of therapy, median (range)	2 (1-5)	2 (1-6)
≥3 prior lines, n (%)	22 (42)	23 (31)
Refractory to all prior lines of therapy, N (%)	3 (6)	10 (13)
Refractory to first-line therapy, N (%)	10 (19)	22 (29)
Refractory to last prior line of therapy, N (%)	27 (52)	39 (52)
Prior blinatumomab, N (%)*	20 (38)	33 (44)
Prior inotuzumab ozogamicin, N (%)*	16 (31)	24 (32)
Prior blinatumomab or inotuzumab ozogamicin, N (%)*	27 (52)	45 (60)
Prior blinatumomab and inotuzumab ozogamicin, N (%)*	9 (17)	12 (16)
Prior allo-SCT, N (%)	30 (58)	26 (35)

TB (BM blast %) at screening, median (range)	25 (0-100)	47 (0-100)
TB (BM blast %) at lymphodepletion, median (range)	2 (0-20)	80 (26-100)
Extramedullary disease at screening, N (%)	13 (25)	16 (21)

*Therapies administered prior to screening.

Low TB refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. allo-SCT: allogeneic stem cell transplant;

BM: bone marrow; TB: tumor burden.

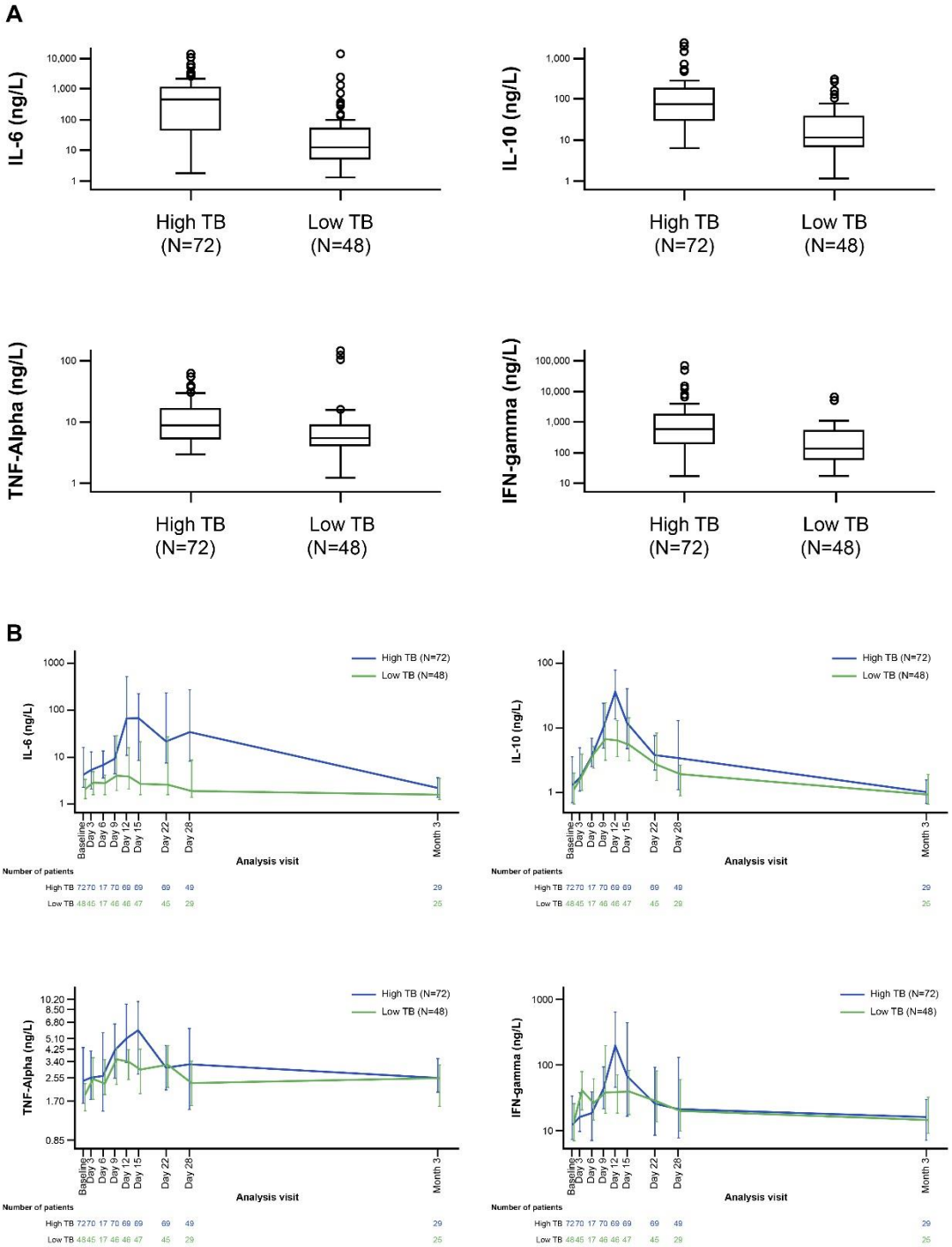
Supplementary Table 2. Characteristics and outcomes for patients who received only one dose of obe-cel.

Patient	% BM blasts at lymphodepletion	EMD at lymphodepletion	Planned Dose 1 regimen – CAR T-cells	Reason for not receiving Dose 2	Best overall response	C_{max}, copies/µg DNA
1	90	Yes	10×10^6	Grade 3 ICANS	No CR/CRi	245,000
2	98	No	10×10^6	Progressive disease	No CR/CRi	NE
3	99	No	10×10^6	Death due to cerebrovascular incident at day 14	No CR/CRi	308,000
4	30	No	10×10^6	Grade 3 CRS	CRi	288,000
5	80	Yes	10×10^6	Grade 3 ICANS	No CR/CRi	490,000
6	0	No	100×10^6	Low dose manufactured	CRi	10,900
7	10	No	100×10^6	Progressive disease	No CR/CRi	3,010

Table adapted from Roddie C et al. N Engl J Med. 2024;391(23):2219-2230 (Supplementary Appendix)[2] to include additional relevant data.

BM: bone marrow; CAR: chimeric antigen receptor; C_{max}: maximal expansion of transgene/chimeric antigen receptor-positive T-cell levels post-infusion; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; CRS: cytokine release syndrome; EMD: extramedullary disease; ICANS: immune effector cell-associated neurotoxicity syndrome; NE: not estimable; obe-cel: obecabtagene autoleucel.

Supplementary Figure 1. Interleukin-6, interleukin-10, tumor necrosis factor alpha, and interferon-gamma levels in patients who received both doses obe-cel (N=120). (A) Peak levels by tumor burden group. (B) Levels by visit. Low TB refers to patients with ≤20% BM blasts at lymphodepletion and high TB refers to patients with >20% BM blasts at lymphodepletion. BM: bone marrow; TB: tumor burden.



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1. Autolus Limited. AUCATZYL® (obecabtagene autoleucel) suspension for intravenous infusion, 11/2024 https://www.fda.gov/media/183463/download Accessed March 13, 2025.

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. 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.