In Vivo Cellular Expansion of Lisocabtagene Maraleucel and Association With Efficacy and Safety in Relapsed/Refractory Large B-Cell Lymphoma

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Lisocabtagene maraleucel (liso-cel) is an autologous, CD19-directed, chimeric antigen receptor T-cell product for the treatment of adult patients with relapsed or refractory large B-cell lymphoma (LBCL) after 2 or more lines of systemic therapy. *In vivo* cellular expansion after single-dose administration of liso-cel has been characterized. In this article, *in vivo* liso-cel expansion in the pivotal study TRANSCEND NHL 001 (ClinicalTrials.gov identifier, NCT02631044) was further characterized to assess the relationship between *in vivo* cellular expansion after single-dose administration of liso-cel and efficacy or safety after adjusting for key baseline characteristics. Two bioanalytical methods, quantitative polymerase chain reaction and flow cytometry, were used for the assessment of cellular kinetics of liso-cel, which showed high concordance for *in vivo* cellular expansion. Multivariable logistic regression analyses demonstrated that higher *in vivo* cellular expansion of liso-cel was associated with a higher overall response and complete response rate, and a higher incidence of cytokine release syndrome and neurological events in patients with relapsed or refractory LBCL. Age and tumor burden (by sum of the product of perpendicular diameters) were likely to confound the relationship between *in vivo* cellular expansion and efficacy, where the association became stronger after controlling for these factors. Repeat dosing of liso-cel was tested in the study; however, *in vivo* cellular expansion of liso-cel was lower after repeat dosing than after the initial dose. These findings should enable a comprehensive understanding of the *in vivo* cellular kinetics of liso-cel and the association with outcomes in relapsed/refractory LBCL.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Characterization of *in vivo* cellular expansion after single-dose lisocabtagene maraleucel (liso-cel) administration in large B-cell lymphoma (LBCL).

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What relationship exists between *in vivo* cellular expansion after single-dose liso-cel administration and efficacy/safety in patients with relapsed/refractory LBCL, after adjusting for key baseline characteristics? What was *in vivo* cellular expansion after repeat dosing of liso-cel?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Multivariable analysis demonstrated higher *in vivo* cellular expansion of liso-cel was associated with higher overall response and complete response rate, and higher incidence of cytokine release syndrome and neurological events. Age and high tumor burden are likely to confound the relationship between *in vivo* cellular expansion and efficacy, which became stronger after controlling for these factors. In addition, *in vivo* cellular expansion of liso-cel was lower after repeat dosing as a second dose or after relapse compared with that after the first dose.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These findings should enable a comprehensive understanding of the *in vivo* cellular kinetics of liso-cel and its association with outcomes in relapsed/refractory LBCL.

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Autologous chimeric antigen receptor (CAR) T-cell therapy is a novel treatment using T cells that are genetically modified to recognize and kill cells expressing a target antigen.[1] Exogenous DNA encoding an extracellular tumor recognition domain (e.g., single-chain variable fragment), a linking transmembrane domain, and intracellular T-cell activation domains (CD3ζ and costimulatory domain(s)) is introduced into the T cells.[2–4] CAR engagement of the specific antigen on target cells mediates signaling that results in T-cell activation and expansion, cytokine production, and cytolytic activity.[4,5] Decades of CAR T-cell clinical research resulted in the introduction of these novel therapies for use in hematologic malignancies.[3,4,6] Lisocabtagene maraleucel (liso-cel) is a CD19-directed, genetically modified, autologous T-cell immunotherapy administered at a defined composition of CAR^+-viable T cells consisting of separate $CD8^+$ and $CD4^+$ components at equal target doses.[7] The liso-cel CAR comprises an FMC63 monoclonal antibody–derived anti-CD19 single-chain variable fragment, immunoglobulin G4 hinge region, CD28 transmembrane domain, 4-1BB (CD137) costimulatory domain, and CD3ζ activation domain. In addition, liso-cel includes a nonfunctional truncated epidermal growth factor receptor (EGFRt) that is co-expressed on the cell surface with the CD19-specific CAR and can serve as a surrogate for CAR expression. Patient T cells are obtained from a standard leukapheresis procedure and liso-cel is prepared from the purified $CD8^+$ and $CD4^+$ T cells, which are separately activated and transduced with the replication-incompetent lentiviral vector containing the anti-CD19 CAR transgene. The transduced T cells are expanded in cell culture, washed, formulated into a suspension, and cryopreserved as separate $CD8^+$ and $CD4^+$ component vials that together constitute a single dose of liso-cel.

TRANSCEND NHL 001 (TRANSCEND; NCT02631044) is a phase I, multicenter, multicohort, seamless design study to determine the safety, antitumor activity, and cellular kinetics of liso-cel in patients with relapsed or refractory (R/R) aggressive B-cell non-Hodgkin lymphoma (NHL). Treatment with liso-cel resulted in a high rate of durable complete response (CR) and low incidence of severe cytokine release syndrome (CRS) and neurological events (NE) among patients with R/R, high-risk, aggressive large B-cell lymphoma (LBCL) in TRANSCEND.[7] Eligible patients underwent leukapheresis for collection of autologous peripheral blood mononuclear cells for manufacture of liso-cel. Once the liso-cel product was available and the patient was confirmed to be eligible for infusion, the patient received lymphodepleting chemotherapy (LDC; fludarabine 30 mg/m^2 and cyclophosphamide 300 mg/m^2 for 3 days). Liso-cel was administered as two sequential infusions of $CD8^+$ and $CD4^+$ CAR^+ T cells 2–7 days after LDC. A subset of patients received additional doses, either as a two-dose schedule or as re-treatment (see **METHODS**).

In vivo cellular expansion after single-dose administration of liso-cel in TRANSCEND was previously characterized by quantitative polymerase chain reaction (qPCR).[7,8] Association between *in vivo* cellular expansion and efficacy or safety end points was also assessed; however, any potential confounding factors (e.g., age and tumor burden) were not considered.[7] Because several baseline characteristics were associated with efficacy, safety,[7] and/or *in vivo* cellular expansion,[8] it was crucial to assess the relationship between *in vivo* cellular expansion and efficacy or safety controlling for potential confounding variables. Therefore, multivariable logistic regression analyses were conducted to develop a model describing the relationship between *in vivo* cellular expansion and efficacy or safety after adjusting for effects of significant covariates. In addition, *in vivo* cellular expansion was assessed after repeat dosing of liso-cel and after single-dose administration of the nonconforming product. Last, *in vivo* cellular expansion in peripheral blood was also evaluated by flow cytometry and compared with qPCR-based assessment.

METHODS

Clinical study data

Data from the liso-cel–treated LBCL cohort of TRANSCEND[7] were used for this analysis, including data from patients who received nonconforming product (i.e., one of the $CD8^+$ or $CD4^+$ cell components did not meet one of the requirements to be considered liso-cel but was considered safe for infusion). Liso-cel was administered as two sequential infusions of $CD8^+$ and $CD4^+$ CAR^+ T cells 2–7 days after LDC. Three of the following target dose levels were explored: 50×10^6 CAR^+ T cells (dose level 1), 100×10^6 CAR^+ T cells (dose level 2), and 150×10^6 CAR^+ T cells (dose level 3). Dose level 1 was also evaluated as a two-dose schedule 14 days apart without additional LDC. Patients who achieved a CR after liso-cel infusion and subsequently relapsed could receive re-treatment.[7] Full eligibility criteria as well as study design and procedures have been described previously.[7]

The study was ongoing as of August 12, 2019, which was the data cut-off date used for this analysis. The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation Good Clinical Practice guidelines, and applicable regulatory requirements. Institutional review boards approved the study protocol and amendments at participating institutions. All patients provided written informed consent.

Quantitative polymerase chain reaction

The qPCR assay was used to measure the liso-cel transgene in cells from peripheral blood. Blood sample collection and details of qPCR assays were described previously.[8]

Flow cytometry

$CD3^+$ $EGFRt^+$, $CD4^+$ $EGFRt^+$, and $CD8^+$ $EGFRt^+$ T cells in peripheral blood were enumerated at pre-infusion and 1, 3, 7, 10, 14, 21, and 28 days and 2, 3, 6, 9, and 12 months post-infusion of liso-cel. A flow cytometry method for the assessment of CAR T cells in human peripheral blood samples was developed using fluorescently labeled cetuximab to detect EGFRt on the surface of CAR T cells. Nonfunctional EGFRt is co-expressed with the CD19-specific CAR and the EGFRt expression is proportional to CAR expression; therefore, the detection of EGFRt serves as a surrogate for detection of CAR T cells in the peripheral blood.[9–11] Flow cytometry sample acquisition was carried out using a BD FACSCanto II Clinical Flow Cytometry System (BD Biosciences, Franklin Lakes, NJ) and data were analyzed using FlowJo Software (BD Biosciences). The lower limit of detection was determined to be 0.1 cells/μL with at least 25 events captured in the $EGFRt^+$ flow cytometry detection gate.

Cellular kinetic analysis

Cellular kinetic parameters were estimated using a noncompartmental analysis, including maximum expansion (C_{max}), time to C_{max} (t_{max}), area under the curve from 0 to 28 days post-infusion ($AUC_{0–28\ days}$), and area under the curve from 0 to 90 days post-infusion ($AUC_{0–90\ days}$).

Logistic regression analysis

Multivariable logistic regression analysis was conducted to develop models describing the relationship between *in vivo* cellular expansion

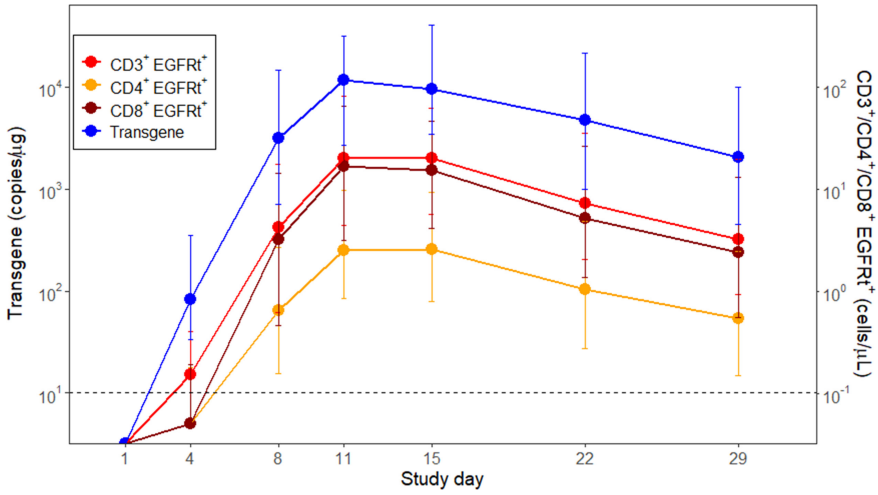

Figure 1 Median (Q1, Q3) transgene (qPCR) and EGFRt$^+$ T cell (flow cytometry) over time in patients with relapsed or refractory large B-cell lymphoma. EGFRt, truncated epidermal growth factor receptor; Q1, first quartile; Q3, third quartile; qPCR, quantitative polymerase chain reaction.

parameters and the probability of clinical outcomes after adjusting for effect of potential confounders. The probability that an event occurs as a function of *in vivo* cellular expansion parameters and/or covariates is described as follows:

$$\ln(\text{odds}) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n$$

where p is the probability of clinical outcome and $\exp(\beta_0)$ is the baseline odds of the clinical outcome. The exponentiated coefficients $\exp(\beta_1)$, ..., $\exp(\beta_n)$ represent the odds ratio corresponding to a 1-unit increase in the corresponding variables, $x_1, ..., x_n$.

The clinical end points included overall response (CR or partial response (PR); yes or no), CR (yes or no), any-grade CRS (yes or no), any-grade NE (yes or no), and grade 3 or higher NE (yes or no). Due to the low incidence of grade 3 or higher CRS ($n = 6$),[7] this end point was not included. Response was defined as a best overall response of CR or PR based on assessment by an independent review committee per Lugano criteria.[12] Covariates considered for the models were chosen based on clinical relevance and adequate subgroup size and included age (≥ 65 vs. < 65 years), sum of the product of perpendicular diameters (SPD) per independent review committee before LDC (≥ 50 vs. < 50 cm^2), lactate dehydrogenase before LDC (≥ 500 vs. < 500 U/L), C-reactive protein (CRP) at baseline (≥ 20 vs. < 20 mg/L), bridging therapy (yes vs. no), prior response status (relapsed vs. refractory), and prior hematopoietic stem cell transplantation (yes vs. no).[7,8]

Expansion parameters that were considered included C_{max} and $AUC_{0-28\ days}$, both of which were $\log_{10}$-transformed. For multivariable models, the expansion variable was held fixed in the model and other variables were selected from the covariates considered using a stepwise procedure with forward selection criteria $P < 0.10$ and backward deletion criteria $P > 0.15$. The stepwise procedure used only patients with complete data on all potential covariates and so the model was then refit using the selected variables. All statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patients

In vivo cellular expansion data were obtained from TRANSCEND. Baseline characteristics of patients who received liso-cel in TRANSCEND were previously reported.[7]

In vivo cellular expansion as assessed by qPCR and flow cytometry

Cellular kinetics of liso-cel were determined using two methods, qPCR and flow cytometry. The cellular kinetic analyses were based on qPCR and the cellular kinetic parameters (C_{max}, t_{max}, and $AUC_{0-28\ days}$) were previously reported.[7] Data from three single-dose levels were pooled because no apparent relationships were observed between the dose levels or dose and cellular kinetic parameters.[7,8] To confirm whether the first 28 days captured the overall exposure, $AUC_{0-90\ days}$ was additionally calculated. The median ratio of $AUC_{0-90\ days}$ to $AUC_{0-28\ days}$ was 1.24 (interquartile range, 1.09–1.57; $n = 192$). In addition, a high correlation between $AUC_{0-28\ days}$ and C_{max} or $AUC_{0-90\ days}$ was observed (**Figure S1**), which supports that the 28-day period (days 1–29) sufficiently captured the cell expansion phase and adequately reflected the overall exposure.

The assessments by flow cytometry (CD3$^+$ EGFRt$^+$, CD8$^+$ EGFRt$^+$, and CD4$^+$ EGFRt$^+$ T cells) were considered exploratory and supportive data that allow the evaluation of cellular kinetic parameters per drug product component. Flow cytometry–based assessment demonstrated the ability of both CD8$^+$ and CD4$^+$ drug product components to expand after liso-cel infusion. Higher expansion of CD8$^+$ EGFRt$^+$ T cells was observed compared with CD4$^+$ EGFRt$^+$ T cells (**Figure 1**, **Table S1**). High correlation between qPCR (transgene) and flow cytometry (CD3$^+$ EGFRt$^+$ T cells) cellular kinetic parameters was observed (**Figure 2**), with a correlation coefficient of 0.8775 for C_{max}, 0.9048 for $AUC_{0-28\ days}$, and 0.7449 for t_{max}, and *in vivo* cellular expansion as assessed by flow cytometry was generally consistent with the qPCR assessment. Accordingly, the following cellular kinetic analyses were reported only based on qPCR.

Relationship between *in vivo* cellular expansion and efficacy or safety: Univariable logistic regression analysis

We previously reported that higher median C_{max} and $AUC_{0-28\ days}$ were associated with response (CR or PR), higher baseline tumor burden, and higher incidence of any-grade CRS, any-grade NE, and grade 3 or higher NE, by Wilcoxon tests.[7] In this study,

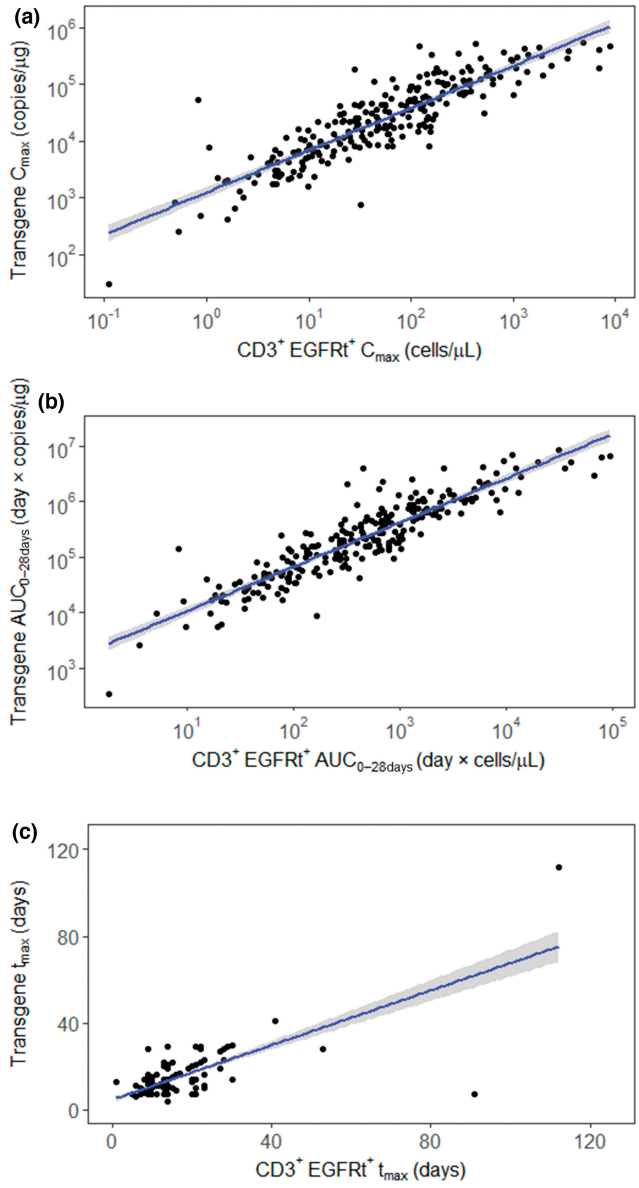

Figure 2 Correlation of *in vivo* cellular expansion parameters between transgene (qPCR) and $CD3^+$ $EGFRt^+$ T cells (flow cytometry): C_{max} (**a**), $AUC_{0-28\ days}$ (**b**), and t_{max} (**c**). $AUC_{0-28\ days}$, area under the curve from 0 to 28 days post-infusion; C_{max}, maximum expansion; EGFRt, truncated epidermal growth factor receptor; qPCR, quantitative polymerase chain reaction; t_{max}, time to maximum expansion. Black lines and gray areas denote regression lines and the 95% confidence intervals. [Colour figure can be viewed at wileyonlinelibrary.com]

multivariable logistic regression analysis was conducted to evaluate cellular kinetic parameters (C_{max} or $AUC_{0-28\ days}$), efficacy or safety variables, and baseline characteristics simultaneously to control for potentially confounding variables. First, univariable logistic regression analysis of clinical outcomes with *in vivo* cellular expansion was conducted and the odds ratios were reported for a 1-unit increase in log_{10}-transformed C_{max} (**Table 1**) or log_{10}-transformed $AUC_{0-28\ days}$ (**Table S2**). Owing to a high correlation between C_{max} and $AUC_{0-28\ days}$ (**Figure S1a**), association between *in vivo* cellular expansion and the efficacy or safety was presented mainly based on C_{max}. Results of the univariable logistic regression analysis for response (CR or PR), CRS, and NE were consistent with the previous Wilcoxon test.[7] Potential association between C_{max} and CR was also observed; however, the odds ratio for CR was smaller than that for response (CR or PR; **Table 1**). Similar results were observed for $AUC_{0-28\ days}$ (**Table S2**).

Table 1 Univariable and multivariable logistic regression analysis of clinical outcomes with C_{max} (qPCR)

Outcome variables Independent variables	Odds ratio estimate (95% CI)	
	Univariable analysis	Multivariable analysis
CR + PR		
Log_{10} C_{max} [a]	2.86 (1.76–4.65)	3.59 (2.09–6.16)
Pre-LDC SPD ≥ 50 cm^2 vs. < 50 cm^2		0.47 (0.22–1.01)
Age ≥ 65 years vs. < 65 years		2.15 (1.02–4.53)
CR		
Log_{10} C_{max} [a]	1.60 (1.09–2.35)	2.28 (1.46–3.55)
Age ≥ 65 years vs. < 65 years		2.29 (1.23–4.27)
Pre-LDC SPD ≥ 50 cm^2 vs. < 50 cm^2		0.38 (0.19–0.75)
Bridging therapy, received vs. not received		0.57 (0.32–1.04)
Any-grade CRS		
Log_{10} C_{max} [a]	2.17 (1.44–3.25)	2.29 (1.48–3.54)
Response to last therapy, relapsed vs. refractory		2.59 (1.31–5.11)
CRP ≥ 20 mg/L vs. < 20 mg/L		2.14 (1.19–3.87)
Bridging therapy, received vs. not received		1.92 (1.04–3.53)
Pre-LDC LDH ≥ 500 U/L vs. < 500 U/L		2.11 (1.02–4.36)
Any-grade NE		
Log_{10} C_{max} [a]	2.77 (1.74–4.41)	2.99 (1.85–4.86)
CRP ≥ 20 mg/L vs. < 20 mg/L		2.78 (1.51–5.14)
Grade ≥ 3 NE		
Log_{10} C_{max} [a]	4.84 (2.04–11.50)	5.11 (2.12–12.32)
CRP ≥ 20 mg/L vs. < 20 mg/L		3.27 (1.11–9.66)

CI, confidence interval; C_{max}, maximum expansion; CR, complete response; CRP, C-reactive protein; CRS, cytokine release syndrome; LDC, lymphodepleting chemotherapy; LDH, lactate dehydrogenase; NE, neurological event; PR, partial response; qPCR, quantitative polymerase chain reaction; SPD, sum of the product of perpendicular diameters.
[a]Expansion parameter C_{max} was log_{10}-transformed.

Relationship between *in vivo* cellular expansion and efficacy or safety: Multivariable logistic regression analysis

Next, a multivariable logistic regression analysis was conducted to evaluate the relationship between *in vivo* cellular expansion and efficacy or safety, controlling for potentially confounding baseline characteristics (**Table 1**, **Table S2**, **Figure 3**, and

Figure S2). The odds ratio for response (CR or PR) and CR associated with a 1-unit increase in $\log_{10}$-transformed C_{max} was numerically increased after controlling for age and SPD (odds ratio (95% confidence interval (CI)), 2.86 (1.76–4.65) to 3.59 (2.09–6.16), and 1.60 (1.09–2.35) to 2.28 (1.46–3.55), respectively; **Table 1**). The odds ratio for any-grade CRS associated with a 1-unit increase in $\log_{10}$-transformed C_{max} was similar after controlling for several baseline characteristics (odds ratio (95% CI), 2.17 (1.44–3.25) to 2.29 (1.48–3.54); **Table 1**). The odds ratio for any-grade NE and grade 3 or higher NE associated with a 1-unit increase in $\log_{10}$-transformed C_{max} was similar after controlling for baseline CRP (odds ratio (95% CI), 2.77 (1.74–4.41) to 2.99 (1.85–4.86), and 4.84 (2.04–11.50) to 5.11 (2.12–12.32), respectively; **Table 1**). Similar results were observed for $AUC_{0-28\ days}$ (**Table ,S2 Figure S2**).

In vivo cellular expansion after a second dose of the two-dose regimen or re-treatment after relapse

Patients could have received ≥ 1 dose of liso-cel in TRANSCEND as part of a two-dose schedule in dose level 1 (50×10^6 CAR^+ T cells), and at any dose level as re-treatment after relapse.[7] In TRANSCEND, dose level 1 was tested as both a single dose given at day 1 and as a two-dose schedule (dose level 1D), with a second dose of liso-cel given on day 15.[7] Patients in dose level 1D only received LDC before the first dose of liso-cel. In dose level 1D ($n = 6$), the second dose did not provide a distinguishable increase in C_{max} from the first dose (**Figure 4**).

Sixteen patients who achieved a CR after liso-cel treatment but later progressed received re-treatment with liso-cel.[7] All patients received LDC before re-treatment with liso-cel. C_{max} and $AUC_{0-28\ days}$ after re-treatment appeared to be lower compared with those after the first dose (**Figure 5**).

In vivo cellular expansion in patients who received the nonconforming product

Nonconforming product was defined as any product wherein one of the $CD8^+$ or $CD4^+$ cell components did not meet one of the requirements to be considered liso-cel. In TRANSCEND, 25 patients received a nonconforming CAR T-cell product.[7] Ranges of C_{max} and $AUC_{0-28\ days}$ were highly overlapping between patients

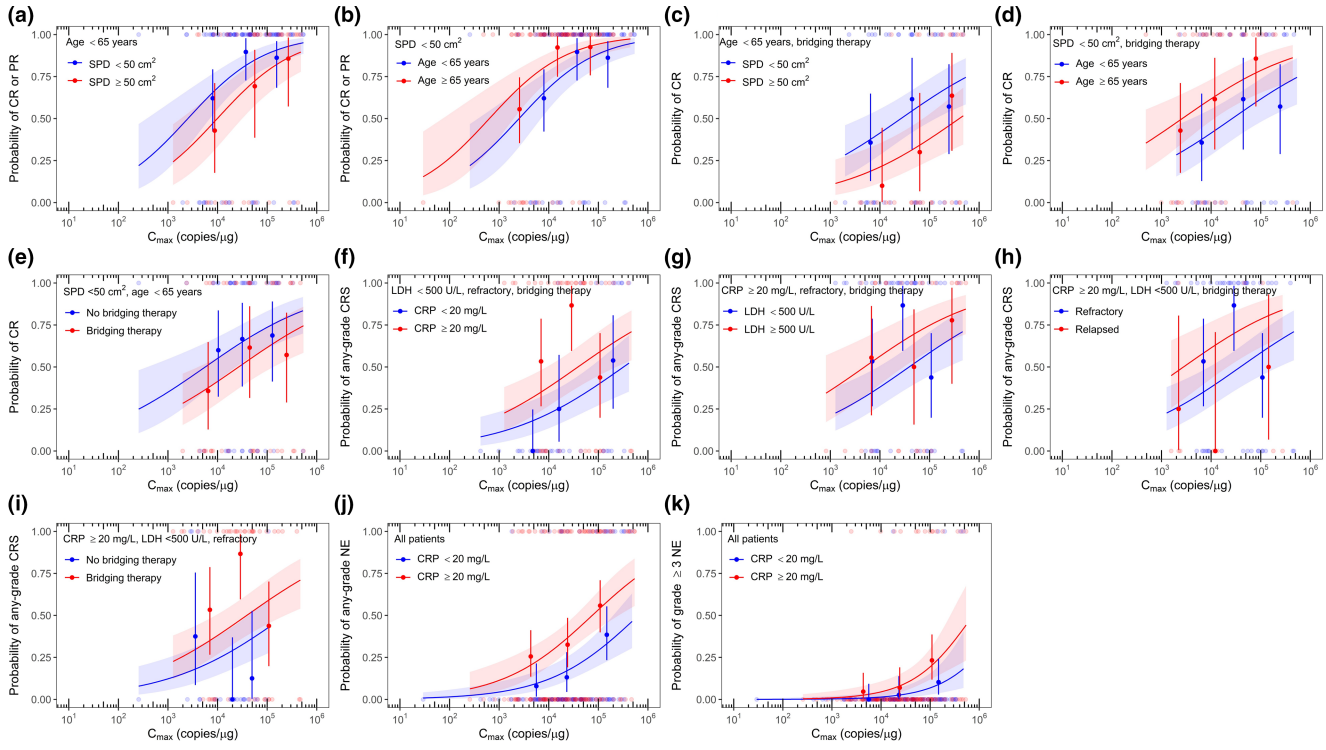

Figure 3 Relationship between C_{max} (qPCR) and probability of efficacy or safety outcomes by a certain covariate controlling for other covariates: overall response (CR or PR) by pre-LDC SPD ≥ 50 cm^2 vs. < 50 cm^2 in patients < 65 years (**a**) and by age ≥ 65 years vs. < 65 years in patients with pre-LDC SPD < 50 cm^2 (**b**); CR by pre-LDC SPD ≥ 50 cm^2 vs. < 50 cm^2 in patients < 65 years who received bridging therapy (**c**), by age ≥ 65 years vs. < 65 years in patients with pre-LDC SPD < 50 cm^2 who received bridging therapy (**d**), and by bridging therapy in patients < 65 years with pre-LDC SPD < 50 cm^2 (**e**); any-grade CRS by CRP ≥ 20 mg/L vs. < 20 mg/L in patients with refractory LBCL and pre-LDC LDH < 500 U/L who received bridging therapy (**f**), by pre-LDC LDH ≥ 500 U/L vs. < 500 U/L in patients with refractory LBCL and CRP ≥ 20 mg/L who received bridging therapy (**g**), by relapsed vs. refractory LBCL in patients with CRP ≥ 20 mg/L and pre-LDC LDH < 500 U/L who received bridging therapy (**h**), and by bridging therapy in patients with refractory LBCL, CRP ≥ 20 mg/L, and pre-LDC LDH < 500 U/L (**i**); any-grade NE by CRP ≥ 20 mg/L vs. < 20 mg/L in all evaluable patients (**j**); and grade ≥ 3 NE by CRP ≥ 20 mg/L vs. < 20 mg/L in all evaluable patients (**k**). Lines indicate logistic regression curve and 95% confidence bands. Closed circles and vertical error bars indicate observed proportion and the 95% confidence intervals in tertiles of C_{max} (qPCR) for each subgroup. On the y-axis, 1 and 0 indicate yes and no, respectively. C_{max}, maximum expansion; CR, complete response; CRP, C-reactive protein; CRS, cytokine release syndrome; LBCL, large B-cell lymphoma; LDC, lymphodepleting chemotherapy; LDH, lactate dehydrogenase; NE, neurological event; PR, partial response; qPCR, quantitative polymerase chain reaction; SPD, sum of the product of perpendicular diameters.

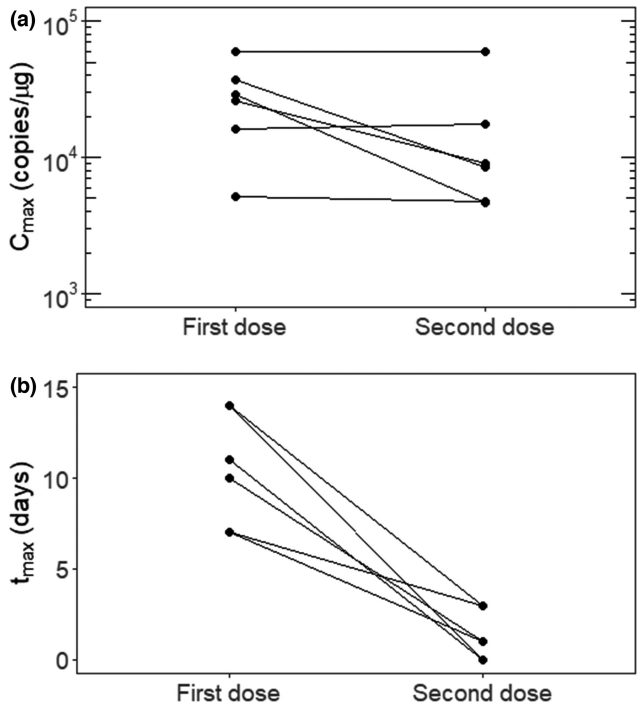

Figure 4 Comparison of *in vivo* cellular expansion parameters (qPCR) after the first dose with those after second dose at dose level 1 as a 2-dose schedule: C_{max} (**a**) and t_{max} (**b**). C_{max}, maximum expansion; qPCR, quantitative polymerase chain reaction; t_{max}, time to maximum expansion.

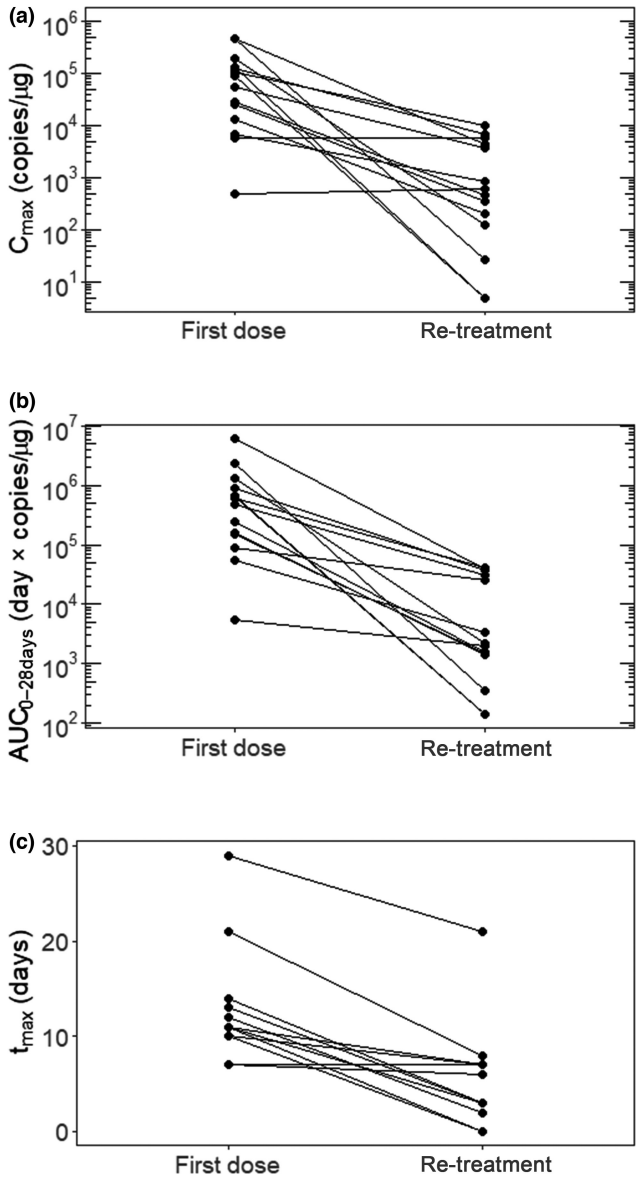

Figure 5 Comparison of *in vivo* cellular expansion parameters (qPCR) after the first dose with those after re-treatment[a]: C_{max} (**a**), $AUC_{0-28\ days}$ (**b**), and t_{max} (**c**). [a]Patients who experienced progressive disease after achieving a CR were eligible for re-treatment with liso-cel if additional doses were available. $AUC_{0-28\ days}$, area under the curve from 0 to 28 days post-infusion; C_{max}, maximum expansion; CR, complete response; liso-cel, lisocabtagene maraleucel; qPCR, quantitative polymerase chain reaction; t_{max}, time to maximum expansion.

who received a nonconforming product and patients who received liso-cel (**Figure 6**).

DISCUSSION

In vivo cellular expansion of liso-cel and the association with efficacy and safety in R/R LBCL from TRANSCEND were further characterized in this analysis. Association between *in vivo* cellular expansion and the efficacy or safety was confirmed after controlling for key baseline characteristics, including age and SPD, as potential confounding variables for the relationship between *in vivo* cellular expansion and efficacy. Data after the second dose in a two-dose schedule or re-treatment after relapse suggested that CAR T-cell expansion was lower after the second dose or re-treatment relative to the first liso-cel administration. Two methods, qPCR and flow cytometry, were used for the assessment of *in vivo* cellular kinetics of liso-cel in TRANSCEND and high concordance was observed for *in vivo* cellular expansion between the two analytical methods.

Multivariable logistic regression analysis was conducted to evaluate the relationship between *in vivo* cellular expansion and efficacy or safety, controlling for potentially confounding baseline characteristics. The odds ratios for response (CR or PR) and CR associated with *in vivo* cellular expansion was numerically increased after controlling for age and SPD, suggesting that age and/or SPD were confounding the relationship between *in vivo* cellular expansion and efficacy. Overall response rate (ORR) and CR rate in patients aged 65 years or older were comparable to but numerically higher than those in patients younger than 65 years,[7] whereas older age was associated with lower expansion.[8] Similarly, patients with a high tumor burden (i.e., SPD ≥ 50 cm^2) had numerically lower ORR and CR rate than patients with a low tumor burden (i.e., SPD < 50 cm^2),[7] whereas high tumor burden was associated with higher expansion.[8] Thus, consideration of age and SPD into the model increased the odds ratio for efficacy with *in vivo* cellular expansion. This result suggested that higher cellular expansion in younger patients and high tumor burden by SPD do not necessarily result in better responses.

The odds ratio for any-grade CRS, any-grade NE, or grade ≥ 3 NE associated with *in vivo* cellular expansion was similar even after controlling for baseline characteristics, which is

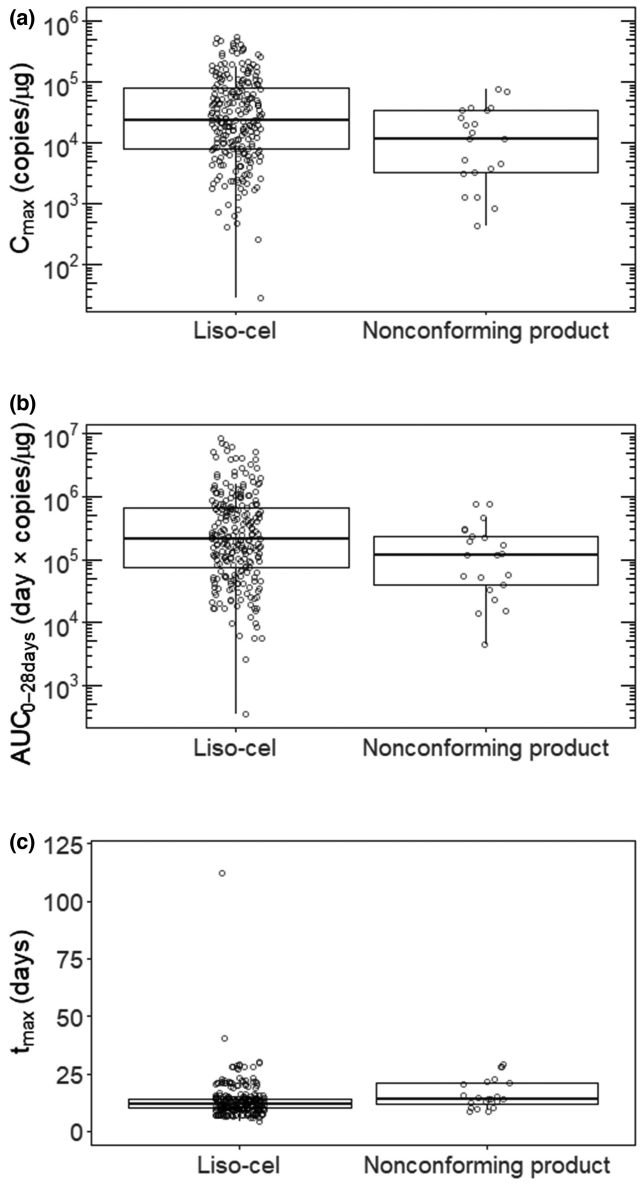

Figure 6 Comparison of *in vivo* cellular expansion parameters (qPCR) in patients who received liso-cel with patients who received nonconforming product[a]: C_{max} (**a**), $AUC_{0-28\ days}$ (**b**), and t_{max} (**c**). [a]Nonconforming product was defined as any product wherein one of the $CD8^+$ or $CD4^+$ cell components did not meet one of the requirements to be considered liso-cel. $AUC_{0-28\ days}$, area under the curve from 0 to 28 days post-infusion; C_{max}, maximum expansion; liso-cel, lisocabtagene maraleucel; qPCR, quantitative polymerase chain reaction; t_{max}, time to maximum expansion.

consistent with the finding that none of the characteristics that were adjusted in the model were associated with changes in C_{max} and $AUC_{0-28\ days}$.[8] Increased inflammatory marker (i.e., CRP ≥ 20 mg/L) was associated with higher incidence of CRS and NE, which was observed in the previous analysis.[7] Although patients with high tumor burden also had a higher incidence of CRS and NE in the previous (univariable) analysis,[7] high tumor burden by SPD did not meet the threshold to be included in the multivariable model for any safety end points, suggesting that association between high tumor burden and CRS or NE might be partially mediated through higher *in vivo* expansion by high tumor burden. Population cellular kinetic analysis of liso-cel in LBCL using a nonlinear mixed-effects modeling approach indicated that the use of tocilizumab and/or corticosteroids for the treatment of CRS and/or NE was associated with higher C_{max} and $AUC_{0-28\ days}$;[8] however, these factors were not considered as potential covariates in the multivariable models presented here because CRS and NE (clinical outcomes in the multivariable models) triggered the therapeutic intervention with tocilizumab and corticosteroids. The current analysis indicates that *in vivo* cellular expansion is associated with higher incidence of CRS and NE, regardless of baseline characteristics.

In R/R B-cell malignancies, other approved CD19-directed CAR T-cell therapies demonstrated generally similar findings to the univariable analysis for *in vivo* cellular expansion and efficacy or safety of liso-cel.[13–17] Higher *in vivo* CAR T-cell expansion was observed in responders than nonresponders for tisagenlecleucel in B-cell acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia,[14,15] axicabtagene ciloleucel in LBCL,[16] and brexucabtagene autoleucel in mantle cell lymphoma.[17] However, the cellular kinetics of tisagenlecleucel in LBCL were similar between responders and nonresponders.[13] Higher *in vivo* CAR T-cell expansion was also associated with higher incidence of grade ≥ 3 CRS or NE.[13–17] The reason for the differing results in the same indication (e.g., R/R LBCL) are not clear. Although the JULIET trial (tisagenlecleucel)[13] sample size was smaller and enrollment criteria were not identical to TRANSCEND, there were also sample size and enrollment criteria differences between the ZUMA-1 trial (axicabtagene ciloleucel)[16] and TRANSCEND, yet an association between higher expansion and response was observed in both of these LBCL studies. Higher *in vivo* CAR T-cell expansion in responders has also been observed for non–CD19-directed CAR T-cell therapy in other indications (multiple myeloma and non–small cell lung cancer).[18,19] TRANSCEND is the largest clinical study reported to date of CD19-directed CAR T-cell therapy in R/R LBCL.[7,10,16,20,21] This article, to the best of our knowledge, describes for the first time the relationship between *in vivo* cellular expansion and efficacy or safety of CAR T-cell therapy controlling for potentially confounding baseline factors.

Patients could have received more than 1 dose of liso-cel in TRANSCEND as a second dose at dose level 1 or as re-treatment after relapse.[7] The second dose at dose level 1 did not provide a distinguishable increase in C_{max} from the first dose, and therefore, testing of the two-dose schedule was not pursued further in the study. In addition, the C_{max} and $AUC_{0-28\ days}$ after re-treatment after relapse appeared lower compared with the C_{max} and $AUC_{0-28\ days}$ after the first dose, which is consistent with the low ORR by investigator's assessment (19%) after re-treatment.[7] Patients with response after re-treatment had higher C_{max} and $AUC_{0-28\ days}$ after re-treatment than patients without response after re-treatment (**Figure S3**), which is consistent with the observations in the entire TRANSCEND study patient population after the first dose. These analyses suggest that CAR T-cell expansion was lower after repeat dosing of liso-cel in LBCL. Lower *in vivo* expansion of other CD19-directed CAR T cells after re-treatment vs. first dose was observed in LBCL[22] and B-cell malignancies (pooled analysis of B-cell ALL, chronic lymphocytic leukemia, and NHL),[23] whereas the median C_{max} of CAR

T cells was similar at re-treatment compared with the first dose in patients with follicular lymphoma.[24] Potential mechanisms for lower CAR T-cell expansion after repeat dosing could include unfavorable alteration of the tumor microenvironment or downmodulation and/or loss of target antigen expression. Because of the small sample size of all reports with different hematologic malignancies, further investigation is warranted before drawing conclusions on re-treatment with CAR T cells, including liso-cel.

Liso-cel is a defined composition CAR T-cell product administered as separate $CD8^+$ and $CD4^+$ CAR^+ T-cell components at equal target doses. Each of the components is required to meet quality specifications. Nonconforming product is defined as any product wherein one of the $CD8^+$ or $CD4^+$ cell components did not meet one of the requirements to be considered liso-cel. Efficacy and safety among the 25 patients who received nonconforming product were similar to that of patients who received liso-cel,[7] and there was no apparent difference in C_{max} and $AUC_{0-28\ days}$ between the two groups. Efficacy and safety of CAR T-cell products that did not meet release specifications for tisagenlecleucel have been reported in B-cell ALL and NHL.[25–28] These out-of-specification (OOS) products showed similar efficacy and safety compared with tisagenlecleucel. Although *in vivo* cellular expansion data of the OOS products were not available, no clear relationship was suggested between *in vivo* cellular expansion of tisagenlecleucel and cell viability,[15] which was the main reason for OOS reported in the real-world setting from a cellular therapy registry of both B-cell ALL and NHL.[28]

Exploratory flow cytometry analysis indicates that both $CD8^+$ and $CD4^+$ components of liso-cel expanded *in vivo*, with higher expansion of $CD8^+$ $EGFRt^+$ T cells compared with $CD4^+$ $EGFRt^+$ T cells. $CD8^+$ and $CD4^+$ T cells are programmed to undergo extensive and limited proliferation, respectively.[29] $CD8^+$ T cells mediate direct cytotoxic activity toward targeted tumor cells, whereas $CD4^+$ T cells assist the immune response through both cytokine production, which supports $CD8^+$ T-cell proliferation and effector function, and direct cytotoxic activity.[30] These findings correspond to higher expansion of $CD8^+$ $EGFRt^+$ T cells than $CD4^+$ $EGFRt^+$ T cells after liso-cel administration.

In summary, multivariable logistic regression analysis demonstrated that higher *in vivo* cellular expansion of liso-cel was associated with higher overall response and CR rate, and higher incidence of CRS and NE in patients with R/R LBCL. Age and high tumor burden are likely to confound the relationship between *in vivo* cellular expansion and efficacy and the association became stronger after controlling for these factors. In addition, *in vivo* cellular expansion of liso-cel was lower after repeat dosing as a second dose or as re-treatment after relapse compared with expansion after the first dose. These findings should serve as the basis for a comprehensive understanding of *in vivo* cellular kinetics of liso-cel and the association with outcomes in R/R LBCL.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

K.O., J.L., T.M., J.D., C.H., J.S., L.P., and A.K. are employees of Bristol Myers Squibb and hold stock in Bristol Myers Squibb.

AUTHOR CONTRIBUTIONS

K.O. and J.L. wrote the manuscript. K.O., J.L., and A.K. designed the research. T.M., J.D., J.S., and C.H. performed the research. J.L. and K.O. analyzed the data. K.O., J.L., L.P., and A.K. interpreted the data.

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