

Clinical Pharmacology of Tisagenlecleucel in B-cell Acute Lymphoblastic Leukemia

Karen Thudium Mueller¹, Edward Waldron², Stephan A. Grupp^{3,4}, John E. Levine^{5,6}, Theodore W. Laetsch^{7,8}, Michael A. Pulsipher⁹, Michael W. Boyer¹⁰, Keith J. August¹¹, Jason Hamilton¹², Rakesh Awasthi¹, Andrew M. Stein¹³, Denise Sickert¹⁴, Abhijit Chakraborty², Bruce L. Levine¹⁵, Carl H. June¹⁵, Lori Tomassian², Sweta S. Shah², Mimi Leung², Tetiana Taran², Patricia A. Wood², and Shannon L. Maude^{3,4}



Abstract

Purpose: Tisagenlecleucel is an anti-CD19 chimeric antigen receptor (CAR19) T-cell therapy approved for the treatment of children and young adults with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL).

Patients and Methods: We evaluated the cellular kinetics of tisagenlecleucel, the effect of patient factors, humoral immunogenicity, and manufacturing attributes on its kinetics, and exposure-response analysis for efficacy, safety and pharmacodynamic endpoints in 79 patients across two studies in pediatric B-ALL (ELIANA and ENSIGN).

Results: Using quantitative polymerase chain reaction to quantify levels of tisagenlecleucel transgene, responders ($N = 62$) had ≈ 2 -fold higher tisagenlecleucel expansion in peripheral blood than nonresponders ($N = 8$; 74% and 104% higher geometric mean Cmax and AUC_{0-28d}, respectively) with persistence measurable beyond 2 years in responding patients. Cmax increased with occurrence and

severity of cytokine release syndrome (CRS). Tisagenlecleucel continued to expand and persist following tocilizumab, used to manage CRS. Patients with B-cell recovery within 6 months had earlier loss of the transgene compared with patients with sustained clinical response. Clinical responses were seen across the entire dose range evaluated (patients ≤ 50 kg: 0.2 to 5.0×10^6 /kg; patients > 50 kg: 0.1 to 2.5×10^8 CAR-positive viable T cells) with no relationship between dose and safety. Neither preexisting nor treatment-induced antimurine CAR19 antibodies affected the persistence or clinical response.

Conclusions: Response to tisagenlecleucel was associated with increased expansion across a wide dose range. These results highlight the importance of cellular kinetics in understanding determinants of response to chimeric antigen receptor T-cell therapy. *Clin Cancer Res*; 24(24): 6175–84. ©2018 AACR.

¹Novartis Institutes for BioMedical Research, East Hanover, New Jersey. ²Novartis Pharmaceuticals Corporation, East Hanover, New Jersey. ³Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. ⁴Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania. ⁵University of Michigan, Ann Arbor, Michigan. ⁶Icahn School of Medicine at Mount Sinai, New York, New York. ⁷Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, Texas. ⁸Pauline Allen Gill Center for Cancer and Blood Disorders, Children's Health, Dallas, Texas. ⁹Division of Hematology Oncology/Blood and Marrow Transplant, Children's Hospital Los Angeles, USC Keck School of Medicine, Los Angeles, California. ¹⁰University of Utah, Salt Lake City, Utah. ¹¹Children's Mercy Kansas City, Kansas City, Missouri. ¹²Legend Biotech, Piscataway, New Jersey. ¹³Novartis Institutes for BioMedical Research, Cambridge, Massachusetts. ¹⁴Novartis Pharma AG, Basel, Switzerland. ¹⁵Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Corresponding Author: Karen Thudium Mueller, Novartis Institutes for BioMedical Research, One Health Plaza, East Hanover, NJ 07936. Phone: 862-217-9515; Fax: 973-781-7014; E-mail: karen.thudium@novartis.com

doi: 10.1158/1078-0432.CCR-18-0758

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Introduction

Chimeric antigen receptor (CAR) T-cell therapy is an autologous, adoptive cellular treatment strategy that uses genetic engineering to graft specificity into an immune effector cell (1). Unlike other small-molecule or antibody therapies, whose levels decrease over time, CAR-T cells are "living drugs" that undergo rapid exponential expansion and can persist for months or even years. Characterization of the cellular kinetics of CAR-T cells and factors impacting kinetics is of great importance for understanding the efficacy, safety, and recommended dose range.

Tisagenlecleucel (formerly CTL019) is a CAR-T-cell immunotherapy using patient-derived T cells that have been genetically engineered *ex vivo* via lentiviral transduction to express a CD19-targeted CAR (CAR19) that mediates T-cell activation in a major histocompatibility complex-independent manner. CAR19 is composed of a murine single-chain Fv antibody fragment that recognizes CD19 and is fused to a costimulatory domain from 4-1BB (CD137) and an intracellular signaling domain, CD3 ζ (2). The CD3 ζ component is critical for initiating T-cell activation and antitumor activity, while 4-1BB enhances the expansion and persistence of tisagenlecleucel

Translational Relevance

CD19-directed chimeric antigen receptor T-cell therapies (CAR-T) offer patients with hematologic cancers new treatment options. However, an in-depth characterization of CAR-T cellular kinetics, dose, and immunogenicity is lacking and yet is essential to understanding the safety and effectiveness of these therapies. Here, we present the first comprehensive analysis characterizing cellular kinetics and clinical pharmacology aspects of tisagenlecleucel, a CAR-T, to support the recommended dose range, safety, and efficacy profile in a pooled study of pediatric and young adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia. This work highlights the relationship between tisagenlecleucel persistence and on-target effects and demonstrates continued expansion and persistence following tocilizumab administration for cytokine release syndrome. Furthermore, we show that immunogenicity does not affect the safety or efficacy of tisagenlecleucel. This work advances the understanding of the clinical pharmacology aspects of CAR-T and the methodologies discussed may translate to new indications or other CAR-T targets.

(2, 3). Upon binding to CD19-expressing cells, the CAR transmits a signal to promote T-cell activation, expansion, target-cell elimination, and persistence of the tisagenlecleucel cells (2). Tisagenlecleucel represents an effective treatment modality in patients with specific B-cell malignancies for whom other therapies have failed (4, 5).

The unique pharmacology of CAR-T cells was reported with the initial characterization of the cellular kinetics of tisagenlecleucel in single-center studies in pediatric and adult patients with relapsed or refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL) and patients with chronic lymphocytic leukemia (CLL; ref. 6). The results from these prior studies demonstrate higher expansion of tisagenlecleucel cells in the responding patients compared with nonresponding patients across these multiple indications. Furthermore, an association between expansion, tumor burden, and cytokine release syndrome (CRS) was reported, whereby high grades of CRS were observed in patients with higher baseline tumor burden and higher expansion (6). The importance of long-term persistence of circulating tisagenlecleucel cells in producing durable responses in patients with CLL or ALL has been previously presented.

The current report extends the characterization of cellular kinetics to tisagenlecleucel with the first comprehensive analysis detailing the relationship of patient characteristics and clinical outcomes, including efficacy and safety, in regard to tisagenlecleucel dose, immunogenicity, B-cell recovery, and the impact of tocilizumab on the cellular kinetics in pediatric and young adult patients ages ≤ 25 years with r/r B-ALL treated on two multicenter trials.

Patients and Methods

Clinical trial design and patients

Data collected from patients enrolled in the studies ELIANA (ref. 5; $N = 50$) and ENSIGN (ref. 7; $N = 29$) were pooled for this analysis as these multicenter studies enrolled identical

patient populations and had similar study designs. Pooling the data allowed for a more robust analysis with an appropriate number of patients to evaluate clinical pharmacology endpoints. The global, multicenter ELIANA study was sponsored and designed by Novartis Pharmaceuticals Corporation and supported by Novartis manufacturing process. The US multicenter ENSIGN trial was codeveloped by Novartis and the University of Pennsylvania and was supported by the University of Pennsylvania manufacturing processes (5, 7). Comparability studies were performed between manufacturing sites as part of the technology transfer from the University of Pennsylvania to Novartis. The pivotal, single-arm, open-label, phase II ELIANA study was conducted at 25 sites and aims to determine the efficacy and safety of tisagenlecleucel in pediatric and young adult patients with r/r B-ALL ages between 3 years at the time of screening and 21 years at the time of initial diagnosis (5). The patients from the phase II ENSIGN study were treated at nine sites (7). For both studies, patients were required to have active bone marrow disease ($\geq 5\%$ blasts) at enrollment and no prior anti-CD19 therapy (5, 7). The protocol-specified dose ranges studied were 0.2 to 5.0×10^6 CAR19-positive viable T cells per kg body weight in patients who weighed ≤ 50 kg and 0.1 to 2.5×10^8 CAR19-positive viable T cells in patients who weighed > 50 kg, administered as a single infusion of 10 to 20 mL per minute.

Characterization of the clinical pharmacology includes summary statistics of the cellular kinetics by clinical response category; justification of the proposed dose range with dose-response, exposure-response, and dose-exposure analysis; evaluation of the impact of intrinsic and extrinsic factors on expansion; dose-safety analysis; and the evaluation of the impact of humoral immunogenicity on the cellular kinetics and effectiveness of tisagenlecleucel. Furthermore, the relationship between selected manufacturing characteristics and expansion has been evaluated. Additional insights on this unique living drug are presented here to enhance the understanding of the clinical relevance of the exposure data in terms of both efficacy and safety.

Bioanalytical methods

Cellular kinetic assays. A TaqMan-based qPCR assay was used for the detection of tisagenlecleucel transgene DNA (transgene copies/ μg of DNA) in peripheral blood and bone marrow samples, with a lower limit of quantitation of 50 copies/ μg of genomic DNA requiring 200 ng DNA/reaction. The assay tolerates $\pm 10\%$ variation in DNA input. This method was originally developed by the University of Pennsylvania and later validated by Navigate BioPharma Services for sample analysis. A 9-marker, 8-color flow cytometry assay was developed by Navigate BioPharma Services to detect and quantify tisagenlecleucel-positive CD3-positive T cells. Humoral immunogenicity was detected using a validated flow cytometry assay measuring mCAR19 antibodies in specimens collected before and after tisagenlecleucel infusion. Anti-mCAR19 IgM/IgG antibodies in human serum specimens were captured by Jurkat cells (an immortalized line of human T lymphocyte cells) transfected to express mCAR19. This method has a sensitivity of 100 ng/mL of positive control antibody in 100% human serum.

Cellular kinetics. The cellular kinetics of tisagenlecleucel were determined by measurement of transgene by both qPCR and the

presence of CAR19 protein expressed on the surface of CAR-positive T cells ($CD3^+$, CAR^+) as measured by flow cytometry. The cellular kinetic parameters (AUC0-28d, C_{max} , T_{max} , T_{last} , $T_{1/2}$) were derived from peripheral blood samples, and parameters were estimated by noncompartmental methods using Phoenix (Pharsight, Version 6.4). The area under the curve (AUC0-28d) of tisagenlecleucel represents the expansion and persistence of tisagenlecleucel during the first 28 days following infusion. C_{max} corresponded to the maximum (peak) expansion of tisagenlecleucel, while T_{max} corresponded to the time at which this maximum expansion was observed. Conventional methods were used to derive additional cellular kinetic parameters, such as the last observed quantifiable level of transgene (Clast), T_{last} , and $T_{1/2}$. Summary statistics of cellular kinetic parameters from peripheral blood were summarized by clinical response at day 28, and levels of transgene in bone marrow were summarized by time points (e.g., day 28; months 3, 6, 9, and 12). Due to the challenges in estimating $T_{1/2}$, a population cellular kinetic model was also used to characterize these data. It modeled tisagenlecleucel as having an exponential growth phase at rate ρ , followed by a biexponential decline at rates α and β . Further details are available in the Supplementary Material available online with this article. The day 28 clinical response assessment in both studies was based on the NCCN Clinical Practice Guidelines in Oncology—Acute Lymphoblastic Leukemia V1.2013 (8).

Patient characteristics and prior treatment on cellular kinetics

The impact of patient characteristics and prior clinical treatment factors was explored using linear models, scatter plots, box plots, and summary statistics and pharmacometric model (Supplementary Fig. S1; ref. 9). The impact on cellular kinetics of administration of tocilizumab and corticosteroids, which were administered based on a protocol-defined treatment algorithm for CRS management, was also evaluated.

Dose-exposure, exposure-response, and dose-response analysis

The relationships between dose-response, exposure-response, as well as between dose/exposure and safety events, were explored using logistic regression models and quartile analysis. Cox regression models and Kaplan–Meier analyses were used to explore the relationship between dose/exposure and time-to-event endpoints such as duration of response and resolution of hematopoietic cytopenias.

Relationship between cellular kinetics and B-cell aplasia

The relationship between cellular kinetics and B-cell aplasia was evaluated through event-free survival (EFS) graphical analysis. As part of the pharmacodynamic characterization, peripheral blood B cells were measured in all patients. B-cell recovery was defined as $>1\%$ B cells/WBCs or $>3\%$ B cells/lymphocytes (the lower limit of normal for blood B-cell levels).

Humoral immunogenicity

Antimurine CAR19 (mCAR19) antibodies were measured in serum collected before and after tisagenlecleucel infusion for the determination of humoral immunogenicity. Humoral immunogenicity was determined by measurement of anti-mCAR19 antibodies in serum by flow cytometry. Anti-mCAR19 antibodies were captured by Jurkat T cells transduced to express murine CAR19. To differentiate the anti-mCAR19 antibodies from those

specific to antigens on Jurkat T cells, specimens were also tested separately on wild-type Jurkat cells. After an incubation step, washing away any unbound substances, an anti-IgG/IgM F(ab')₂ fragment labeled with R-Phycoerythrin was added in addition to a viability dye (eFluor 780). After incubation, additional washing steps, and a fixating step, the samples were analyzed on a flow cytometer. The impact of anti-mCAR19 antibodies on cellular kinetics and efficacy was explored using box plots and summary statistics.

Study approval

The ELIANA and ENSIGN studies were conducted in accordance with the principles of the Declaration of Helsinki and were approved by the institutional review board at each participating institution; all patients or their guardians provided written informed consent (5, 7).

Results

Patient characteristics

Cellular kinetic data were pooled from two multicenter trials, ELIANA (a global study, $N = 50$) and ENSIGN (a US-based study, $N = 29$), in pediatric and young adult (age, 3–25 years; median, 12.0 years) patients with r/r B-ALL following a single infusion with tisagenlecleucel (ELIANA: NCT02435849; ENSIGN: NCT02228096). Baseline demographics and disease characteristics, which were previously reported (6), are summarized in Supplementary Table S1.

Cellular kinetics of tisagenlecleucel in peripheral blood

Following infusion of tisagenlecleucel, the cells distribute throughout the body. Upon binding to the CD19 antigen, tisagenlecleucel cells proliferate and the transgene levels undergo a rapid multi-log expansion beyond the initial infused dose (determined by quantitative polymerase chain reaction [qPCR]), followed by subsequent biexponential decline. A representative patient profile reflective of the expansion and persistence seen in responder patients is presented in Fig. 1. Median maximal expansion (C_{max}) and exposure during the first 28 days following infusion (AUC0-28d) were higher in patients who achieved complete remission (CR) or CR with incomplete blood count recovery (CRi) compared with nonresponding (NR) patients: 226% higher for AUC0-28d and 104% higher for C_{max} (Table 1). Although no statistically significant difference was observed due to the small population of NR patients ($n = 8$), CR/CRi patients tended to have a higher and earlier expansion compared with NR patients (Supplementary Fig. S2). Furthermore, for both the flow cytometry- and qPCR-based assessments, the C_{max} and AUC0-28d trend clearly supports this analysis, showing limited to no expansion in NR patients compared with high expansion and persistence in CR/CRi patients. These findings are consistent with previous reports describing the difference in expansion between responding and NR patients (6). In the ELIANA study, the surface expression of CAR19 (% $CD3^+CAR^+$ cells) was characterized by flow cytometry (Supplementary Fig. S3). Expansion and persistence of $CD3^+CAR^+$ cells were detected by flow cytometry in all patients. Flow cytometry-based measurements were used to quantify levels of CAR-expressing T cells as this method is able to measure the surface expression of the CD19 CAR in contrast to the qPCR assay that measures

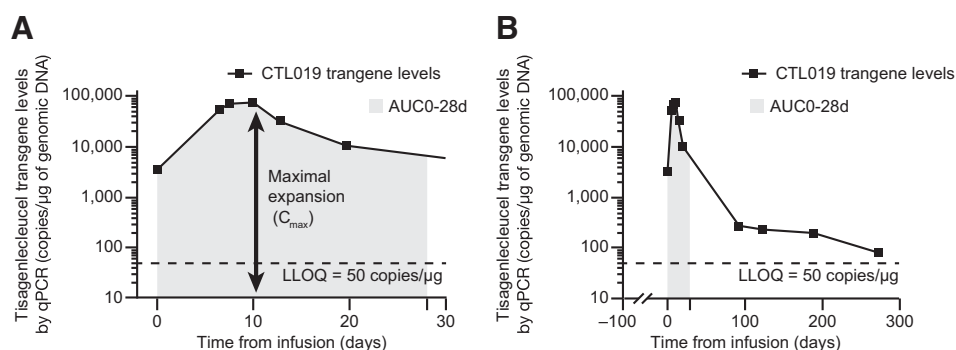


Figure 1. Tisagenlecleucel cellular kinetics. Tisagenlecleucel cellular kinetics from a representative profile of a patient with pediatric B-ALL in the ELIANA study showing the initial expansion phase to day 28 (A) and day 300 (B). LLOQ, lower limit of quantitation.

the bulk transgene level. Median peak expansion in responding patients was 31.6% CD3⁺CAR⁺ cells (range, 1.10–84.9) with persistence detected up to 9 months; with additional follow-up, the persistence of CD3⁺CAR⁺ cells is anticipated to lengthen. Importantly, in the ELIANA study, limited expansion with rapid clearance of surface CAR⁺ cells was detected in NR patients as determined by flow cytometry (0%, 0%, and 17.3%); only one of these NR patients achieved B-cell aplasia and all NR patients had measurable bone marrow blasts at day 28. Persistence was observed at low levels up to month 3 (12.1%) in a single NR patient with CD19-negative disease. The median number of days to reach maximum expansion (T_{max}) determined by qPCR was achieved at day 10 in responding patients with a delayed T_{max} of 20 days in NR patients (Fig. 2; Table 1).

We previously reported a significant difference in expansion between patients in CR/CRi and NR as detected by flow cytometry (6). In the present data, the maximum expansion determined from flow cytometry revealed a geometric mean maximum level of surface expression of 21.4 % (CV%, 145.2) in responding patients, although measurable in only one NR patient at a maximum expansion level of 17.3% with rapid clearance that was no longer detected by flow cytometry. Determination of AUC0-28d by flow cytometry requires at least three samples with detectable levels, which no NR patient achieved; therefore, this parameter could not be assessed in nonresponding patients. The

lack of expansion seen in NR patients represents the importance of performing flow cytometry analyses in clinical development for confirmation of functional CARs *in vivo*. In NR patients, CAR-T cells took longer to engraft and expand, a phenomenon observed in other trials as well (6). A trend toward a longer T_{1/2} was noted in patients with CR/CRi (16.8 days) compared with NR patients (2.5 days); however, the T_{1/2} estimates in NR patients were limited by low numbers and short follow-up of NR patients. Persistence of transgene (T_{last}) was detectable up to 380 days, with a median of 102 days (range, 17.8–380 days) in patients with CR/CRi, and 27.8 days (range, 20.9–83.9 days) in NR patients (Fig. 2). Notably, both parameters, T_{last} and apparent T_{1/2}, were dependent on the data cutoff date and length of assessment. A summary of these values is reported in Table 1. Due to the challenges of limited data in estimating individual-level T_{1/2} values using noncompartmental analysis, a pharmacometric approach was implemented. A nonlinear mixed-effect population cellular kinetic model was also used to characterize the exponential growth and biexponential decline by qPCR. The initial T_{1/2} of decline (after T_{max}) was estimated to be 4.3 days (coefficient of variation [CV], 91%), and the terminal T_{1/2} was estimated to be 220 days (CV 86%) in responding patients.

Tisagenlecleucel dose and cellular kinetics

Dose-exposure analysis showed a flat relationship between dose and the cellular kinetic parameters, with $r^2 < 0.001$

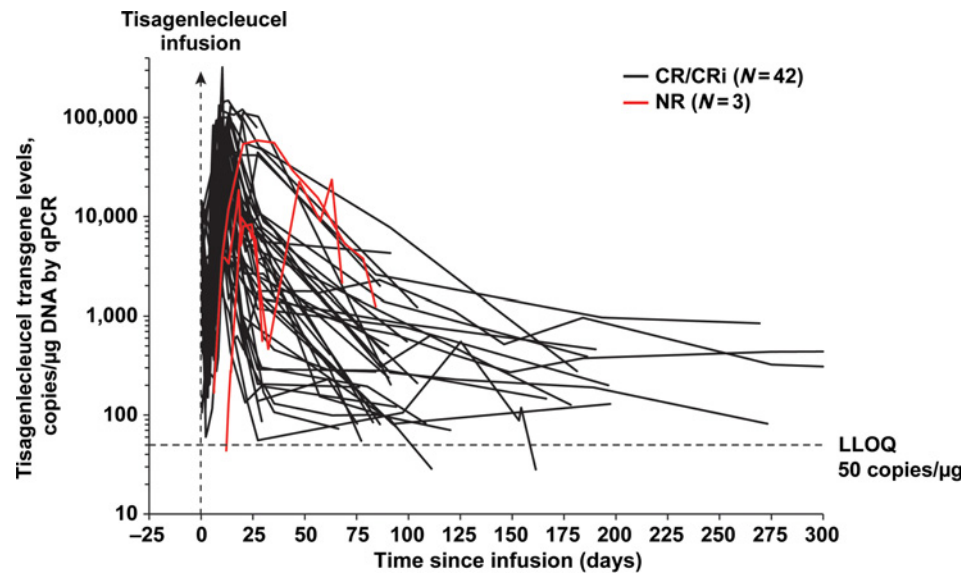
Table 1. Tisagenlecleucel cellular kinetic parameters in responding patients and nonresponding patients

Parameter	Statistic	CR/CRi (N = 62)	NR (N = 8)
AUC0-28d, copies/μg of DNA × days	n	61	6
	Geometric mean (CV%)	318,000 (177.8)	156,000 (99.4)
	Percent change (CR/CRi vs. NR)		104%
	Median (range)	420,000 (21,000–2,740,000)	129,000 (72,300–711,000)
C _{max} , copies/μg ^a	n	61	7
	Geometric mean (CV%)	34,700 (155.4)	20,000 (71.6)
	Percent change (CR/CRi vs. NR)		74%
	Median (range)	44,800 (2,210–316,000)	22,000 (9,700–57,700)
T _{max} , days	n	61	7
	Median (min-max)	9.91 (0.00764–27.0)	20.0 (0.0278–62.7)
T _{1/2} , days	n	54	3
	Geometric mean (CV%)	16.8 (155.9)	2.52 (171.9)
T _{last} , days	n	62	8
	Median (min-max)	102 (17.8–380)	27.8 (20.9–83.9)

Abbreviations: AUC, area under the curve; C_{max}, maximum (peak) expansion; CR, complete remission; CRi, complete remission with incomplete blood count recovery; CV%, coefficient of variation; max, maximum; min, minimum; NR, nonresponding response; T_{1/2}, half-life; T_{last}, time of last measurable in peripheral blood; T_{max}, time to reach maximum (peak) peripheral blood tisagenlecleucel transgene level.

^aSeven patients had a C_{max} determined from samples collected <1 day after infusion; these samples may represent the amount of tisagenlecleucel in the catheter rather than the amount of expanded tisagenlecleucel.

Figure 2.
Cellular kinetics of tisagenlecleucel transgene by response in the ELIANA study.



and = 0.002 for C_{max} and AUC_{0-28d}, respectively (Supplementary Table S2; Supplementary Fig. S4). Thus, there was no relationship between AUC_{0-28d} or C_{max} and total weight-adjusted tisagenlecleucel cell dose or total CAR-positive viable T-cell dose across a wide dose range of 0.2 to 5.0 × 10⁶ CAR19-positive viable T cells per kg of body weight in patients weighing ≤50 kg and 0.1 to 2.5 × 10⁸ CAR19-positive viable T cells in patients weighing >50 kg. In responding patients, high trafficking and penetration of tisagenlecleucel in bone marrow was detected with a profile consistent with that seen in peripheral blood. CAR19 transgene partitioning of blood to bone marrow ranged from 28% to 71% over 6 months. The blood to bone marrow partitioning suggests that transgene distribution in bone marrow was 44% of that present in blood at day 28 while at months 3 and 6, it distributed at 67% and 68.8%.

Impact of demographic, disease-specific characteristics, and prior therapies on cellular kinetics

Baseline tumor burden, cytogenetics, disease characteristics, patient demographics [age (Supplementary Fig. S5A), race, body weight, gender (Supplementary Fig. S5B)], and disease status did not affect cellular kinetic parameters (AUC_{0-28d} and C_{max}). We previously reported that patients with higher tumor burden have greater expansion (4–6); however, a similar analysis was not performed for the pooled data set because tumor burden was not assessed immediately prior to tisagenlecleucel infusion as it was in the single-center trial (NCT01626495).

We also assessed whether prior therapies (e.g., number of lines or prior stem cell transplant) affected cellular kinetics. Prior stem cell transplant (Supplementary Fig. S5C), the number of lines of prior therapies (Supplementary Fig. S5D), and the type of lymphodepletion regimen used did not affect expansion and persistence of the transgene. It should be noted, however, that the majority of patients received fludarabine-based lymphodepletion chemotherapy.

Tisagenlecleucel dosing and efficacy

Efficacy was observed across the entire dose range studied. A quartile analysis revealed consistent response rates across all dose

quartiles (Supplementary Table S3), and similar response rates were seen at the lowest and highest dose quartiles. Logistic regression analyses showed an increasing probability of response with dose, where a doubling in weight-adjusted dose was associated with a 97% increase in odds of response [odds ratio, 1.97 (95% CI, 1.183–3.293)]; however, the numbers of patients, especially those treated at low doses, were small (at dose < 1 × 10⁶ CAR19-positive viable T cells/kg), and the 95% CIs were wide (response rate, 5%–95%; Supplementary Fig. S6A). Similarly, for patients who weighed >50 kg, the dose-response showed decreased probability of response with doses <2.0 × 10⁶ CAR19-positive viable T cells/kg, and the probability of response plateaued with higher doses. The highest feasible dose is always attempted; however, due to individual apheresis and patient characteristics, infused doses may be on the lower end of the dose range. A logistic regression dose-response analysis was performed for the single-dose administration of tisagenlecleucel. A doubling in total dose was associated with a 78% increase in odds of response with a wide confidence interval due to limited data at the lower end of the dose range [odds ratio, 1.78 (95% CI, 1.098–2.883); Supplementary Fig. S6B]. The model-predicted probability of response for patients treated with the lowest administered dose for both weight categories was 43% and 30.9% for patients ≤50 kg and >50 kg, respectively. It should be noted that the studies were not designed to detect a dose–response relationship, and the exact dose given to an individual patient was not predefined but rather based on patient-specific characteristics and manufacturing feasibility.

Tisagenlecleucel dosing and safety

The most common adverse events (AEs) in ELIANA and ENSIGN were CRS, neurologic events, and cytopenias; therefore, the relationship between these AEs of interest and dose was evaluated. CRS occurred in 82.4% of patients. Logistic regression analysis of the relationship between tisagenlecleucel dose and CRS grade indicated that there was no impact of CAR-positive viable T-cell dose on the probability of grade 3 or 4 CRS (Supplementary Fig. S7). Transient neurologic events were reported in 37 patients (40.7%) within 8 weeks of

tisagenlecleucel infusion (10). Cytopenias not resolved by day 28 were reported in 35.2% of patients. Neither neurologic events nor cytopenias not resolved by day 28 were found to be affected by the CAR-positive viable T-cell dose (Supplementary Figures S8 and S9).

Tisagenlecleucel CD4:CD8 ratio in final product

The importance of the CD4:CD8 ratio is a relevant question within the field of CAR-T therapies. The CD4:CD8 ratio in the final product was determined for the ELIANA study, and the relationship between clinical response, safety, and *in vivo* expansion was evaluated (Supplementary Fig. S10A–S10F). The results of these analyses demonstrate a similar CD4:CD8 ratio between CR/CRi and NR patients based on clinical response assessments performed at day 28 (Supplementary Fig. S10A–S10B) with no differences in the CD4:CD8 ratio based on highest grade of CRS (Supplementary Fig. S10C–S10D). Finally, *in vivo* expansion (Cmax) is independent from the CD4:CD8 ratio (Supplementary Fig. S10E–S10F).

Impact of CRS severity and anticytokine therapy

The administration of anticytokine agents coincided with the peak of CRS, which was often concurrent with maximal expansion of tisagenlecleucel. Tocilizumab was administered to 42.7% of patients in accordance with the CRS treatment algorithm (11). Tisagenlecleucel continued to expand and persist following the administration of tocilizumab (Fig. 3). The tisagenlecleucel AUC0-28d and Cmax were 265% and 183% higher in responding patients treated with tocilizumab than in patients not treated with tocilizumab. The higher expansion observed in patients who received tocilizumab cannot be directly attributed to tocilizumab administration as it was used to treat those with higher-grade CRS, who showed higher expansion than patients with grade 1 or 2 CRS (Supplementary Fig. S11). Additionally, corticosteroids, in limited doses and duration, together with or after inadequate response to tocilizumab, were administered to 25.3% of patients for the management of CRS. AUC0-28d and Cmax were 89% and 55% higher, respectively, in responding patients treated with corticosteroids [geometric mean AUC0-28d, 509,000 (range, 39,300–2,100,000); geometric CV%, 196.8%; geometric mean Cmax, 47,800 (range, 4,040–146,000); geometric CV%, 140.9%] than those not treated with

corticosteroids [geometric mean AUC0-28d, 269,000 (range, 21,000–2,740,000); geometric CV%, 162.9%; geometric mean Cmax, 30,900 (range, 2,210–316,000); geometric CV%, 158.0%]. Although there was no relationship between tisagenlecleucel dose and CRS grade, or between dose and expansion as described above, patients with higher-grade CRS tended to have greater tisagenlecleucel expansion (Supplementary Fig. S11 and Supplementary Table S4); a doubling in Cmax was associated with a 59% increase in the odds of experiencing grade 3 or 4 CRS (odds ratio, 1.59; 95% CI, 1.091–2.302). There were an insufficient number of NR patients treated with tocilizumab and steroids to perform similar analyses.

Cellular kinetics and event-free survival

The cellular kinetics of tisagenlecleucel transgene were correlated with EFS. Patients with events prior to 6 months had rapid loss of CAR compared with patients with EFS \geq 6 months (Supplementary Fig. S12). Separation of the curves was most pronounced at approximately month 6 (day 183); however, the number of patients with EFS < 6 months was low ($n = 7$), and hence, there was no statistically significant difference.

Manufacturing attributes and cellular kinetics

The relationship between selected tisagenlecleucel product attributes, including cell viability, transduction efficiency, total T-cell number, and cellular kinetic parameters, was evaluated. There was no relationship between these select manufacturing attributes and the log-transformed cellular kinetic parameters (AUC0-28d, Cmax, and Tmax) determined by qPCR (Supplementary Table S5; Supplementary Figs. S13 and S14), within the observed data range.

B-cell pharmacodynamics

B-cell count in the peripheral blood is a pharmacodynamic marker for functional persistence of tisagenlecleucel cells as B-cell aplasia is an on-target effect of tisagenlecleucel. The relation between time to B-cell recovery and tisagenlecleucel persistence was determined. This analysis shows that patients with B-cell recovery occurring before 3 months or between 3 and 6 months had more rapid loss of transgene than patients who had sustained B-cell aplasia beyond 6 months (Fig. 4). Furthermore, a Kaplan–Meier analyses for time to

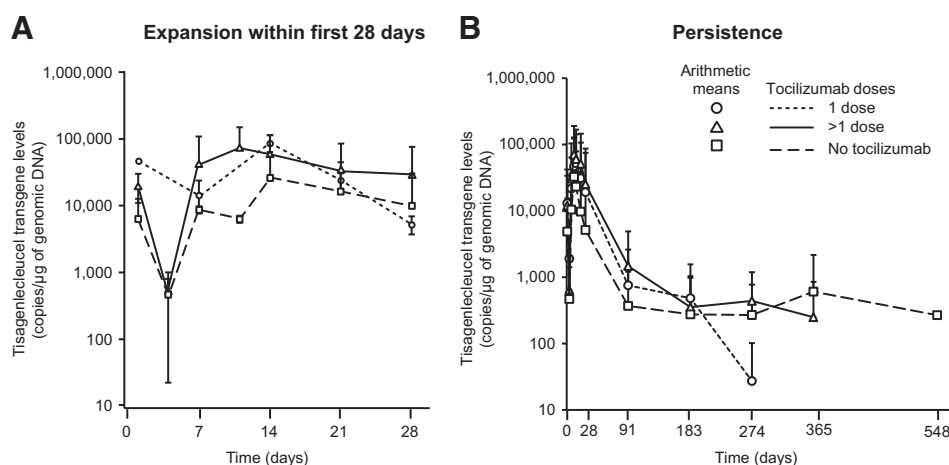


Figure 3. The impact of tocilizumab on tisagenlecleucel expansion and persistence. Days 0 to 28 (A) and days 0 to 600 (B) after tisagenlecleucel infusion. Persistence of tisagenlecleucel was observed for up to 457 days. Data presented are for an updated data cut with 68 patients who received tisagenlecleucel infusion. Day 548 corresponds with the month 18 study visit.

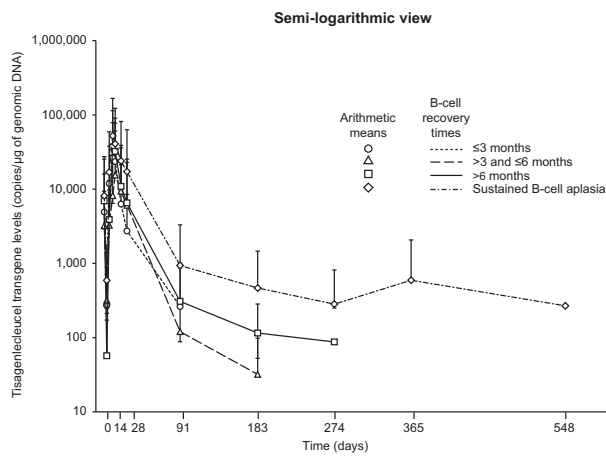


Figure 4. Tisagenlecleucel transgene levels by B-cell recovery time.

B-cell recovery shows the median time to recovery is around 11.4 months (Supplementary Fig. S15).

Cellular kinetics and MRD assessment

CD19-positive relapses were associated with lower expansion and more rapid loss of transgene than that seen in patients with sustained response (Fig. 5). Patients with a CD19-negative relapse had transgene levels comparable with those seen in patients with sustained responses with approximately 200 days of follow-up (Fig. 5).

Humoral immunogenicity

Before infusion, specific antimurine CAR19 (mCAR19) antibodies were detected in 84.8% of patients treated with tisagenlecleucel. Preexisting anti-mCAR19 antibodies and treatment-

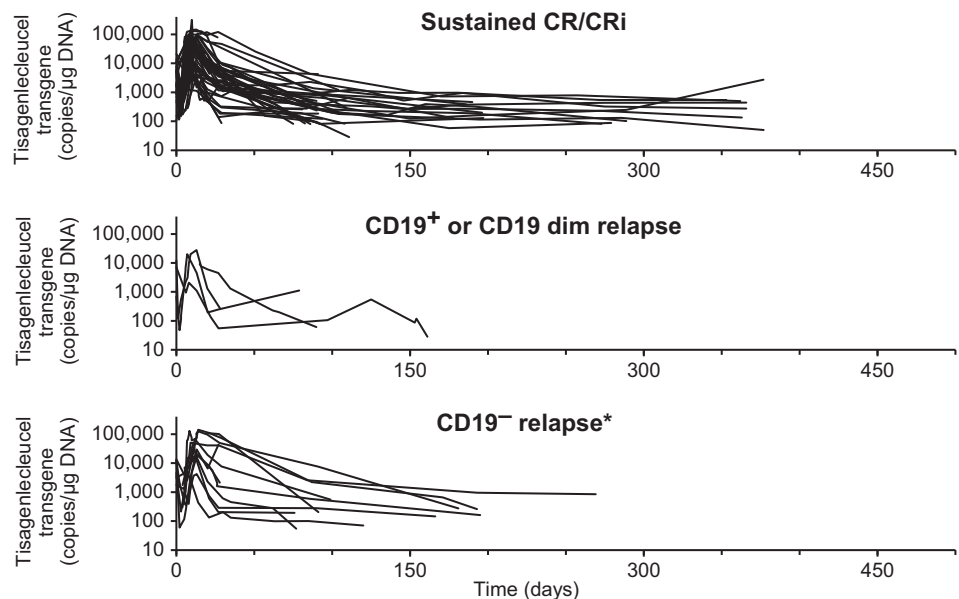
induced or treatment-booster anti-mCAR19 antibodies did not affect the expansion or cellular kinetics of tisagenlecleucel (Supplementary Fig. S16), nor did preexisting antibodies affect response or relapse (Supplementary Fig. S17).

After infusion, induced or boosted humoral immunogenicity was observed in 36.7% of patients treated with tisagenlecleucel. Treatment-induced immunogenicity was determined by a positive increase in measurable anti-mCAR19 antibodies from the baseline sample collected from an individual patient at enrollment and patient's specific cutoff point. No differences were observed in the AUC0-28d values in regard to baseline anti-mCAR19 antibodies (Supplementary Fig. S16A). A concentration-time profile of tisagenlecleucel transgene by presence or absence of treatment-induced immunogenicity showed consistent exposure between the two groups (Supplementary Fig. S16B). Furthermore, treatment-induced anti-mCAR19 antibodies did not affect efficacy, safety, or cellular kinetics of tisagenlecleucel (12). There was no impact of preexisting or treatment-induced immunogenicity on day 28 clinical response (Supplementary Fig. S18). The impact of treatment-induced immunogenicity on duration of remission was determined. The duration of response was comparable in patients with anti-mCAR19 antibody levels above and below the median level of detected anti-mCAR19 antibodies (Supplementary Fig. S17). Cox regression analyses show no statistically significant impact on DOR (Supplementary Table S6).

Discussion

Characterization of the clinical pharmacology of CAR-T therapies is fundamental to understanding the clinical efficacy and safety data to support the recommended dose for these novel therapies. In this pooled analysis of two multicenter trials of tisagenlecleucel, the cellular kinetic analyses showed that responding patients had higher expansion than NR patients, as determined by qPCR, with limited expansion detected in NR

Figure 5. Cellular kinetics of tisagenlecleucel by relapse category and sustained response.



* Three of 12 patients were classified as CD19^{+/-} relapse.

patients by flow cytometry. This finding underscores the lack of CAR expression on the cell surface, which is required for CAR function, in NR patients and is consistent with observations in the original study conducted by the Children's Hospital of Philadelphia (4, 6). Although the flow cytometry-based measurements can be more discriminating in accounting for proliferation and persistence of the surface CAR⁺ T cells, the high sensitivity of qPCR allowed tracking of tisagenlecleucel over a much longer period, furthering our understanding of the relationship between persistence and durability of remission with tisagenlecleucel. B-cell levels are a pharmacodynamic surrogate marker for functional persistence of tisagenlecleucel because of the on-target effect of tisagenlecleucel on normal and malignant CD19-positive B cells with resultant B-cell aplasia. Measurement of CAR transgene in patients treated with tisagenlecleucel is an important aspect for characterizing the *in vivo* activity of tisagenlecleucel in clinical trials; however, outside the clinical trial setting, this study suggests B cells are an appropriate marker to guide treatment of patients with B-ALL. The results of these analyses demonstrated that B-cell aplasia may be a useful surrogate to evaluate CAR persistence.

The time to B-cell recovery curve (Supplementary Fig. S15) closely resembles the duration of remission analyses previously reported (13). At this time, we hypothesize that a minimum of 6 months of B-cell aplasia is necessary to prevent CD19-positive relapses (Fig. 4 and Supplementary Fig. S15), but further follow-up data are required.

In previous studies, we have shown that tisagenlecleucel persistence is an important contributor to durable remission (4). We attempted to assess the impact of cellular kinetics on EFS; persistence of B-cell aplasia for a minimum of 6 months is necessary to prevent CD19-positive relapse; however, due to limited data surrounding early loss of CAR and short follow-up, more data are needed, and longer follow-up will be required to fully understand optimal duration of CAR-T-cell persistence.

CRS is the most common, and expected, AE observed in pediatric and young adult B-ALL patients treated with CAR-T-cell therapies (13). Consistent with previous studies of tisagenlecleucel, patients with higher tumor burden were at increased risk of higher-grade CRS and had greater *in vivo* tisagenlecleucel expansion. Patients with severe CRS (graded on the Penn grading scale; ref. 11) received tocilizumab and/or corticosteroids. In these analyses, we have shown that the administration of anticytokine agents or low-dose corticosteroids did not affect the *in vivo* expansion or persistence of the CAR; however, the impact of these agents on the cellular kinetics of tisagenlecleucel was not formally evaluated in a randomized study. These findings support the administration of tocilizumab and low-dose corticosteroids in patients with high-grade CRS or life-threatening emergencies without impacting *in vivo* kinetics or response. It is important to acknowledge that patients received low-dose corticosteroids (≤ 2 mg/kg) for a short duration and are weaned rapidly. Limited data exist on the impact of high doses of steroids over a long duration on CAR-T-cell expansion and persistence; therefore, we do not recommend this approach. Further studies will need to be performed to determine whether treating patients with tocilizumab prophylactically would mitigate the severity of CRS or interfere with tisagenlecleucel expansion and function. As more CAR-T therapies become available with varying CRS management algorithms/recommendations, it is important to fully characterize the impact of earlier

administration of anticytokine therapies on *in vivo* kinetics and clinical response of each product.

Patient characteristics often affect the pharmacokinetics and clinical response with cytotoxic chemotherapies used to treat pediatric and young adult patients with B-ALL, most likely because some of these attributes are associated with chemotherapy metabolism and resistance (14–16). This study found that tisagenlecleucel *in vivo* activity is not related to patient-specific factors, a finding that demonstrates that tisagenlecleucel has the potential to expand *in vivo* irrespective of prior therapies or prior hematopoietic stem cell transplantation in patients ages ≤ 25 years. Although this finding is specific to B-ALL, as tisagenlecleucel therapy expands into other indications, age ranges, or becomes an earlier line of treatment it will be important to confirm *in vivo* expansion is not affected by patient-specific factors that may ultimately affect efficacy. Furthermore, as more patients with B-ALL and non-Hodgkin lymphoma are treated with tisagenlecleucel, it will be important to look for insights into the characteristics of nonresponders.

The antibody-binding domain of tisagenlecleucel contains a murine scFv region responsible for recognizing CD19 on the surface of B cells; therefore, it poses a potential risk for eliciting an immunogenic response. The guidelines for immunogenicity assessment for therapeutic protein products (17) do not specifically outline the immunogenicity requirements for cell and gene therapies; however, similar assessments for characterizing the impact of preexisting and treatment-induced immunogenicity on clinical efficacy, safety, and cellular kinetics were performed for tisagenlecleucel. Characterizing immunogenicity is an important element for CAR-T therapies, specifically those that are composed of murine components. An important finding of this study was that preexisting and treatment-induced immunogenicity did not affect the expansion, persistence, safety, or efficacy of tisagenlecleucel. As the majority of patients included in this analysis were immune suppressed, the characterization of the impact of immunogenicity on efficacy and long-term safety in immune-competent patients post-CAR-T will be an important area for further investigation.

Traditional dose escalation studies were not performed for tisagenlecleucel. Tisagenlecleucel is a living drug and therefore, once infused, the cells expand beyond the initial infused dose. Importantly, no relationship between the doses administered in these studies and *in vivo* expansion was observed (Supplementary Fig. S4). The original protocol specified a dose range of 1.0 to 5.0×10^6 CAR-positive viable T cells/kg for patients ≤ 50 kg and 1.0 to 2.5×10^8 CAR-positive viable T cells for patients > 50 kg. The manufacturing specifications for tisagenlecleucel always targeted the high end of the dose range and treatment sites infused the highest achievable dose for each individual patient. However, during the course of the clinical trial, due to patient-specific characteristics, doses within the original protocol-specified dose range were not always able to be manufactured. For these cases, the FDA was consulted to allow infusion of tisagenlecleucel at doses outside the protocol-specified dose range. Each request was approved by the FDA and ultimately the original protocol-specified dose range was broadened to reflect the positive benefit-risk seen at the lower doses. The administration of tisagenlecleucel at lower doses has been shown to result in CRs in patients presented in this pooled analysis, thus supporting the approved dose range for tisagenlecleucel (18). Future studies should evaluate dosing in adult ALL patients beyond the age of 25 years.

Tisagenlecleucel offers pediatric and young adult patients with *t/r* B-ALL a new treatment option. These analyses show that cellular kinetics and clinical pharmacology aspects of tisagenlecleucel support the recommended dose range, safety, and efficacy profile in pediatric and young adult patients with *t/r* B-ALL. Similar analyses are being performed to characterize the dose and its safety and efficacy in each indication of tisagenlecleucel (19, 20).

Disclosure of Potential Conflicts of Interest

K.T. Mueller has ownership interests (including patents) at Novartis Pharmaceuticals Corporation. S.A. Grupp is a consultant/advisory board member for and reports receiving commercial research grants from Novartis. J.E. Levine is a consultant/advisory board member for Novartis. T.W. Laetsch is a consultant/advisory board member for Bayer, Eli Lilly, Loxo Oncology and Novartis. M.A. Pulsipher reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Novartis. K. August reports receiving speakers bureau honoraria from Novartis Pharmaceuticals. J. Hamilton has ownership interests (including patents) at Novartis. R. Awasthi has ownership interests (including patents) at Celgene. A. Chakraborty has ownership interests (including patents) at Novartis. B.L. Levine is a consultant/advisory board member for AVectas, Brammer Bio, CRC Oncology/Cure Genetics, Incysus, and Novartis, and reports receiving commercial research grants from Novartis and Tmunity. C.H. June has ownership interests (including patents) at and reports receiving commercial research grants from Novartis. S.Shah has ownership interests (including patents) at Novartis Pharmaceuticals. M. Leung is an employee of Novartis. T. Taran has ownership interests (including patents) in Novartis. S.L. Maude has ownership interests (including patents) in Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: K.T. Mueller, S.A. Grupp, M.A. Pulsipher, A. Chakraborty, B.L. Levine, C.H. June, L. Tomassian, T. Taran, P.A. Wood, S.L. Maude

Development of methodology: K.T. Mueller, A.M. Stein, D. Sickert, L. Tomassian, M. Leung

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.T. Mueller, S.A. Grupp, J.E. Levine,

T.W. Laetsch, M.A. Pulsipher, M.W. Boyer, K.J. August, D. Sickert, B.L. Levine, M. Leung, S.L. Maude

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.T. Mueller, E. Waldron, S.A. Grupp, J.E. Levine, T.W. Laetsch, M.A. Pulsipher, R. Awasthi, A.M. Stein, D. Sickert, A. Chakraborty, M. Leung, T. Taran, P.A. Wood, S.L. Maude

Writing, review, and/or revision of the manuscript: K.T. Mueller, E. Waldron, S.A. Grupp, J.E. Levine, T.W. Laetsch, M.A. Pulsipher, M.W. Boyer, K.J. August, J. Hamilton, R. Awasthi, A.M. Stein, D. Sickert, A. Chakraborty, B.L. Levine, C.H. June, L. Tomassian, S.S. Shah, M. Leung, T. Taran, P.A. Wood, S.L. Maude

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.T. Mueller, M.A. Pulsipher, B.L. Levine, L. Tomassian, S.S. Shah, M. Leung, T. Taran, P.A. Wood

Study supervision: K.T. Mueller, S.A. Grupp, M.A. Pulsipher, M.W. Boyer, L. Tomassian, S.S. Shah, M. Leung, T. Taran, P.A. Wood

Acknowledgments

The authors acknowledge and thank all the investigators in the ELIANA (NCT02435849) and ENSIGN (NCT02228096) trials, their medical staff and the patients who participated in these two trials. The authors acknowledge the Novartis teams supporting these studies, including the scientific and operational contributions provided by Creton Kalfoglou, statistical analyses provided by Yiyun Zhang, and Xia Han who supported MRD analyses. The authors recognize Margit Jeschke for her contributions to characterize the final product. The authors also recognize the scientists who contributed to the bioanalytical analyses of cellular kinetic data at Novartis (Fraser McBlane) and at Navigate BioPharma (Reinhold Pollner, Naveen Dakappagari, and Irene Ch'en). The authors acknowledge medical editorial assistance provided by Nicole Hjortland, PhD (ArticulateScience LLC), and funding by Novartis Pharmaceuticals Corporation.

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Received March 15, 2018; revised July 3, 2018; accepted August 31, 2018; published first September 6, 2018.

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