Article Type: Article

Tisagenlecleucel Model-Based Cellular Kinetic Analysis of Chimeric Antigen Receptor (CAR)-T Cells

Andrew M. Stein,[1] Stephan A. Grupp,[2,3] John E. Levine,[4,5] Theodore W. Laetsch,[6,7] Michael A. Pulsipher,[8] Michael W. Boyer,[9] Keith J. August,[10] Bruce L. Levine,[11,12] Lori Tomassian,[13] Sweta Shah,[13] Mimi Leung,[13] Pai-Hsi Huang,[13] Rakesh Awasthi,[14] Karen Thudium Mueller,[14] Patricia A. Wood,[13] Carl H. June[11,12]

[1]Novartis Institutes for BioMedical Research, Cambridge, MA, USA; [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [3]Division of Oncology, Center for Childhood Cancer Research and Cancer Immunotherapy Program, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [4]University of Michigan, Ann Arbor, MI, USA; [5]Icahn School of Medicine at Mount Sinai, New York, NY, USA; [6]Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, TX, USA; [7]Pauline Allen Gill Center for Cancer and Blood Disorders, Children's Health, Dallas, TX, USA; [8]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, Keck School of Medicine of USC, Los Angeles, CA, USA; [9]Department of Pediatrics and Internal Medicine, University of Utah, Salt Lake City, UT, USA; [10]Children's Mercy Hospital Kansas City, Kansas City, MO, USA; [11]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [12]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [13]Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA; [14]Novartis Institutes for BioMedical Research, East Hanover, NJ, USA

Corresponding Author:
Andrew M. Stein
Novartis Institutes for BioMedical Research
Pharmacometrics, Modeling & Simulation
45 Sidney St
Cambridge, MA, 02139
Phone: +1 (617) 871-5006
Email: andrew.stein@novartis.com

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/PSP4.12388

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Keywords: Mixed effects model, oncology, pediatrics, translational medicine, systems pharmacology
Running Title: Cellular Kinetics of Tisagenlecleucel
Text body word count: 3971
Figures/Tables: 7

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Funding:
Novartis Pharmaceuticals Corporation provided funding for editorial assistance and sponsored the study. The authors also acknowledge Sebastian Jolivet for work on generating the Modeling dataset.

Conflict of Interest:
AMS: Employee of Novartis Institutes for BioMedical Research and owns equity in Novartis Pharmaceuticals Corporation
SAG: Received consultancy fees from Novartis Pharmaceuticals Corporation, Jazz Pharmaceuticals, Adaptimmune; research support from Novartis Pharmaceuticals Corporation
JEL: Received consultancy fees from Novartis Pharmaceuticals Corporation, Jazz Pharmaceuticals, Bluebird Bio, Therakos Inc. and holds patents, royalties or intellectual property with Viracor
TWL: Received consultancy fees from Novartis Pharmaceuticals Corporation, Loxo Oncology and Eli Lilly and received researching funding from Pfizer
MAP: Received consultancy fees from Novartis Pharmaceuticals Corporation, Jazz Pharmaceuticals; received travel accommodations or expenses from Medac and research funding from Adaptive Biotechnologies
MWB: Received honoraria from Novartis Pharmaceuticals Corporation
KJA: Participated in a speaker bureau for and received travel accommodations and expenses from Novartis Pharmaceuticals Corporation
BLL: Received consultancy fees from GE Health and Brammer Bio, has patents and royalties with and received research funding from Novartis Pharmaceuticals Corporation, and holds equity ownership in and received research funding from Tmunity Therapeutics
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CHJ: Received research support from Novartis Pharmaceuticals Corporation; received honoraria from and is a member on a board of directors or advisory committee for WIRB/Copernicus Group and Celldex, owns equity in and is a member on a board of directors or advisory committee for Immune Design, has patents and royalties with Novartis Pharmaceuticals Corporation, and received research funding from Tmunity Therapeutics

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Abstract

Tisagenlecleucel is a chimeric antigen receptor (CAR)-T cell therapy that facilitates killing of $CD19^+$ B cells. A model was developed for the kinetics of tisagenlecleucel and the impact of therapies for treating cytokine release syndrome (tocilizumab and corticosteroids) on expansion. Data from two phase 2 studies in pediatric and young adult relapsed/refractory B-cell acute lymphoblastic leukemia were pooled to evaluate this model and evaluate extrinsic and intrinsic factors that may impact the extent of tisagenlecleucel expansion. The doubling time, initial decline half-life, and terminal half-life for tisagenlecleucel were 0.78, 4.3, and 220 days respectively. No impact of (tocilizumab or corticosteroids) on the expansion rate was observed. This work represents the first mixed-effect model-based analysis of CAR-T cell therapy and may be clinically impactful as future studies examine prophylactic interventions in patients at risk of higher-grade cytokine release syndrome and the effects of these interventions on CAR-T cell expansion.

INTRODUCTION

Chimeric antigen receptor (CAR)-T cell therapy involves the adoptive transfer of autologous T cells genetically modified to facilitate antigen-specific cell killing through endogenous effector cell mechanisms of cytotoxicity.[1] Unlike canonical drug therapies that can be described by classical pharmacokinetics (PK), CAR-T cells undergo rapid expansion several logs beyond the infused cell dose and demonstrate long-term persistence that does not follow typical models of metabolism and clearance. Characterization of the cellular kinetics of CAR-T cells as well as factors impacting kinetics are important for understanding the efficacy, safety, and recommended dose ranges.

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Tisagenlecleucel (CTL019) is a CAR-T cell immunotherapy that produces durable responses in pediatric and young adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia (r/r B-ALL).[2, 3] This treatment paradigm genetically modifies autologous T cells to express a bioengineered CAR that will facilitate targeted killing of $CD19^+$ B cells. Following infusion, widespread distribution of tisagenlecleucel into various tissues occurs within a few hours.[4] Over the next several days, increases in tisagenlecleucel copy number reflect exponential growth, whereby tisagenlecleucel binding to its target antigen induces killing of the target cell and stimulates proliferation of the CAR-T cells. After the time of maximal expansion (T_{max}) is reached, the kinetics of tisagenlecleucel expansion are followed by a biexponential decline. The initial contraction occurs at a rapid rate and is thought to correspond to programmed cell death of activated CAR-T cells.[5] The second phase of tisagenlecleucel decline occurs more gradually over time and corresponds to the persistence of CAR-T cells that demonstrate an immune memory phenotype.[6]

The initial distribution of tisagenlecleucel after the administered cell dose depends largely on the complex interplay between T-cell trafficking, migration from peripheral blood to the bone marrow and other secondary lymphoid tissues, and the immune activation of tisagenlecleucel through binding of CD19 antigen on the surface of B cells. This unique pharmacology, including an initial characterization of the kinetics of tisagenlecleucel expansion, has previously been reported in single-center studies that included pediatric and adult patients with r/r B-ALL and patients with chronic lymphocytic leukemia (CLL).[7] Results from these prior studies demonstrated (1) higher expansion of tisagenlecleucel in peripheral blood in responding patients than in nonresponding patients, (2) higher grades of cytokine release syndrome (CRS) in patients with higher baseline tumor burden and greater expansion,[7] and (3) the significance of long-term tisagenlecleucel expansion and persistence in producing durable responses in patients with CLL and those with ALL.

Cytokine release syndrome typically occurs within 1 to 22 days of tisagenlecleucel infusion and is an on-target toxicity that results from tisagenlecleucel activation, expansion, and tumor-cell killing.[3] Because CRS is an on-target effect, CRS and efficacy are correlated. CRS is associated with increased serum levels of proinflammatory cytokines, including interleukin (IL)-6.[1] Although CRS has been successfully managed in some pediatric and young adult patients with r/r B-ALL using supportive care, patients with severe CRS have shown benefit from tocilizumab (anti–IL-6 receptor antibody) treatment and, in some instances, treatment with corticosteroids to further control CRS symptoms. Tocilizumab has been approved by the FDA for treating CRS and among patients receiving both tisagenlecleucel or axicabtagene ciloleucel and it was observed that 69% of patients were responders.[8] There is potential for these co-medications to impact the CAR-T cells as corticosteroids are known to reduce circulating T cells[9] and induce apoptosis[10] and tocilizumab is an anti-inflammatory medication that could also potentially impact T cells. Mueller and colleagues previously reported that tisagenlecleucel continues to expand and persist following administration of tocilizumab and/or steroids in patients experiencing severe CRS[7]; however, model-based methods characterizing the impact of tocilizumab on tisagenlecleucel expansion

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have not been previously described. A model-based approach allowed us to assess whether anti-inflammatory therapy would alters the expansion profile of the patient that receives it.

In this analysis, a nonlinear, mixed-effects modeling approach was used to characterize the impact of tocilizumab therapy on the kinetics of *in vivo* tisagenlecleucel expansion. The primary focus of this work was to investigate the differences in peak tisagenlecleucel levels and the rates of tisagenlecleucel expansion in patients who underwent tocilizumab or corticosteroid therapy compared with patients who did not require these treatments for CRS to assess whether anti-inflammatory therapy would alter the tisagenlecleucel expansion profile in the patients that receive it.

METHODS

Data

Data from two phase 2 studies of pediatric and young adult B-ALL (NCT02435849 [ELIANA] and NCT02228096 [ENSIGN]) were used for this analysis. ELIANA is an ongoing, global trial that included 62 patients from 10 countries at the time of data cutoff, August 17, 2016. ENSIGN is a US multicenter trial that enrolled a total of 29 patients at the time of data cutoff, February 1, 2016. Both clinical studies have near-identical enrollment and treatment protocols, allowing data to be pooled for analyses. Patients received a single dose of tisagenlecleucel. The median weight-adjusted dose was 3.1×10^6 CAR-positive viable T cells per kg (range, 0.2-5.4 $\times 10^6$ cells/kg) for patients weighing ≤ 50 kg, and the median total dose of CAR-positive viable T cells was 1.0×10^8 (range, 0.03-2.6 $\times 10^8$ cells) for patients weighing > 50 kg. The patient outcomes from interim analyses have been previously reported.[2, 11]

Both studies were approved by the institutional review board at each participating institution and conducted in accordance with the Declaration of Helsinki. ELIANA was sponsored and designed by Novartis Pharmaceuticals Corporation and ENSIGN was designed by the University of Pennsylvania and sponsored in conjunction with Novartis Pharmaceuticals Corporation. Patients or their guardians provided written informed consent or assent.

Sample analysis

Quantitative polymerase chain reaction

Tisagenlecleucel levels, reported as transgene copies/μg of genomic DNA, were measured in 90 patients (ELIANA, N = 61; ENSIGN, N = 29) using quantitative polymerase chain reaction (qPCR). Briefly, genomic DNA was isolated from samples of peripheral blood, and transgene copies were quantified using TaqMan technology (Applied Biosystems; Foster City, CA) and transgene-specific primers. A validated assay was used to detect integrated transgene sequences.[7, 12] To ensure quality control, a parallel amplification reaction was performed using a primer-probe combination specific to a nontranscribed sequence upstream of the cyclin-dependent kinase inhibitor 1A gene, *p21, CDKN1A*.[6] Amplification of the reference sequence was used to normalize the absolute quantification of the CD19 CAR transgene. The

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limit of quantification was 10 copies per reaction (≈ 50 transgene copies per µg of genomic DNA). Samples were collected at days 1, 2, 7, 11, 14, 21, and 28 followed by 3-month intervals from months 3 to 12 and then 6-month intervals until month 60. Day 1 corresponded to the day of infusion or first dose of tisagenlecleucel. In this study, Tisagenlecleucel levels were also measured by flow cytometry. Good correlation between flow cytometry and qPCR was observed and qPCR was selected for this analysis because it was a more sensitive assay[7] allowing for better characterization of long-term persistence.

Model-based analysis of tisagenlecleucel

Base model

The base structural model is adapted from a previously published empirical model used to describe the murine immune responses to *Listeria monocytogenes* or lymphocytic choriomeningitis virus, in which similar profiles of lymphocyte kinetics were observed; we use the analytical solution to equation 7 from DeBoer and Perelson.[4] The structural model captures exponential expansion of tisagenlecleucel with rate constant ρ up to time T_{max}, followed by biexponential contraction after T_{max} with rate constants α and β. The α slope corresponds to a rapid contraction due to programmed apoptosis of most activated lymphocytes following the initial immune response, and the β slope corresponds to a gradual decrease of T cells that can persist in the body for years or even decades.[13, 14, 15] A typical model profile is shown in **Figure 1a**. The model has 6 fixed parameters: C_{max}, T_{max}, $fold_x$, F_B, α, and β. C_{max} is the maximum transgene copies of tisagenlecleucel that occurs at time T_{max}; $fold_x$ is the fold expansion of tisagenlecleucel from baseline and is given by $fold_x = \exp(\rho \cdot T_{max})$, where $\rho = \log(fold_x)/T_{max}$. F_B describes the fraction of transgene copies present at peak expansion (T_{max}) that decline with gradual rate constant β, and $(1 - F_B)$ is the fraction of transgene copies present at peak expansion that decline with the rapid rate constant α.

For the base model, the terms R_0, A, and B in the equations were computed as follows to ensure continuity: $R_0 = C_{max}/fold_x$, R_0 is the baseline transcript levels in Equation 1; $A = C_{max} \cdot (1 - F_B)$; $B = C_{max} \cdot F_B$. A and B correspond to portion of Cmax that declines rapidly (at slope α) and more gradually (at slope β) at time Tmax, These parameters were employed in equation 1.

$$f(t) = \begin{cases} R_0 e^{\rho t} & , \ t < T_{\max} \\ A e^{-\alpha(t - T_{\max})} + B e^{-\beta(t - T_{\max})} & , \ t \geq T_{\max} \end{cases} \quad (1)$$

The above model equations are empirical in nature and describe the available data. The equations above are also described by the semi-mechanistic compartmental model in **Figure 2.**[4] Effector CAR-T cells can expand exponentially (ρ) up until time T_{max} and then decline (α – k) or transition to memory cells (k) such that the total rate constant for the decline of this population at time Tmax is α, which then persist and declines with a rate constant β.[6] The analytical solution to this compartmental model gives rise to equation 1 describing the PK of tisagenlecleucel (**Supplementary Material**). This is

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not the only model that can give rise to these kinetics and other semi-mechanistic models can also give rise to similar kinetics, as described in the Discussion

Comedication effect on expansion

As mentioned previously, CRS is an on-target toxicity associated with tisagenlecleucel activation and cellular expansion and, in some cases, requires the use of tocilizumab or corticosteroids to ameliorate severe symptoms and mitigate inflammatory organ damage. The key objective of this analysis was to study the impact of tocilizumab and corticosteroids on tisagenlecleucel expansion as used per the treatment algorithm[16] outlined in the study protocol. Two relationships between expansion and the comedications for treating CRS were explored: (1) treatment with tocilizumab and/or corticosteroids may slow the rate of expansion of tisagenlecleucel and (2) the requirement of treatment for CRS may be predictive of C_{max} because patients with higher exposure are more likely to have CRS and receive tocilizumab.[7, 17]

The effect of tocilizumab or corticosteroids on the expansion rate was explored by expanding the structural model to include a tocilizumab and corticosteroid impact on ρ in equation 2 (see also **Figure 1b**). Here, T_1 is the time of administration for the first comedication, and T_2 was the time of administration for the second comedication. F_1 and F_2 correspond to the impact of each comedication on the growth rate ρ, where $F < 1$ indicates a decrease in expansion rate due to the comedication. The CRS treatment algorithm specifies that tocilizumab be given first, followed by corticosteroids; thus, for most patients, $F_1 = F_{toci}$ (effect of tocilizumab) and $F_2 = F_{ster}$ (effect of steroids).[16] However, if the patient received corticosteroids for other clinical conditions unrelated to CRS, a corticosteroid dose could occur first. Although some patients received multiple doses of tocilizumab and corticosteroids, only the impact of the first dose was modeled. Because corticosteroids, when administered, was almost always after tocilizumab administration (with the exception of one patient that had an allergic reaction) an interaction term was not included in the model.

$$(2)\quad f(t) = \begin{cases} R_0 e^{\rho t} & , \quad t < T_1 \\ R_1 e^{F_1 \cdot \rho (t - T_1)} & , \ T_1 \le t < T_2 \\ R_2 e^{F_1 F_2 \cdot \rho (t - T_2)} & , \ T_2 \le t < T_{\max} \\ A e^{-\alpha (t - T_{\max})} + B e^{-\beta (t - T_{\max})} & , \quad t \ge T_{\max} \end{cases}$$

The new constants R_1 *and* R_2 are given by $R_1 = R_0 \cdot e^{\rho T1}$ and $R_2 = R_1 \cdot e^{\rho \cdot F1 (T2 \cdot T1)}$.

The relationship between comedications and C_{max} was explored by including binary covariate effects on C_{max}, described further below. Although either comedication could potentially impact any of the other model parameters, no other effects were explored, as explained in the **Discussion**. Other comedications for CRS (e.g., IL-6 and TNFα antagonists) were not modeled because only 10 patients received such drugs. The Monolix code for this model is given in the **Supplementary Material**.

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Random-effect model

Six random effects were included on the parameters: C_{max}, $fold_x$, T_{max}, F_B, α, and β (**Table 1**)**;** all parameters were assumed to be log-normally distributed. For the residual error model, log-transformed data was modeled with a proportional error model (constant error model in log-space), which is typical for the qPCR assay.[19]

Covariate model

C_{max} was chosen as the main covariate because it could be directly linked to area under the curve (AUC) by integrating equation 1, which gives the equation below (**Supplementary Material**).

(3) $$AUC_{0\text{-}\infty} = C_{max}\,[1/\rho + (1 - F_B)/\alpha + F_B/\beta]$$

This relationship indicates that intrinsic and extrinsic factors that impact C_{max} will also impact AUC. Factors affecting persistence were also of interest but were not explored for reasons explained in the Discussion section.

The categorical covariates explored in this model included sex, race, Down syndrome, prior lymphodepleting chemotherapy with fludarabine (when added to cyclophosphamide), and prior stem cell transplant. Continuous covariates included age (< 10, 10-17, ≥ 18 years), weight-adjusted dose, time to first tocilizumab dose, time to first corticosteroid dose, transduction efficiency of manufactured product, and patient weight. In some cases, there were very few patients in a particular category; therefore, this analysis should be treated as only exploratory. It is possible that differences in C_{max} may be related to other factors, such as differences in CD19 antigen density or variation in the capacity of a patient's autologous T cells to proliferate, though such data was not collected in these trials.
Covariates were explored by bootstrapping the full covariate model, where the data set was resampled and the full model refit 500 times.[20] In this analysis, the covariate analysis is done only for exploratory purposes and not to impact dose selection. Therefore, the full model is selected as the final model in this analysis. Further details on the motivation for choosing the covariates and how the covariate analysis was performed is provided in the **Supplementary Material**.

Analysis

The Monolix software system (version 4.3.2) was used to estimate population parameters, using the CENS column to denote data below the limit of quantitation (BLQ). BLQ assessments were treated as fixed-point censored data. The technical computing packages R and Matlab were used for some exploratory analysis, model building, and reporting of the final results.

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RESULTS

Analytical model of tisagenlecleucel cellular kinetics

The base model was applied to a patient cohort (n = 90) that included pediatric and young adult patients with r/r B-ALL who were treated with a single dose of tisagenlecleucel. There were 836 measurements with 43 below the limit of quantification. Patient characteristics are shown in **Table 2**. The base model characterized the data well, as shown by the visual predictive check and individual fits in **Figure 3**, with additional diagnostics included in the **Supplementary Material**. The model parameters are summarized in **Table 1**. The initial doubling time (ln 2/ρ) was 0.78 days, the half-life for the initial rate of decline (ln 2/α) was 4.3 days, and the terminal half-life (ln 2/β) was 220 days. Because the longest follow-up for this analysis was only 1 year (Figure 3a), this terminal half-life estimate must be interpreted with caution and will be updated as more data becomes available.

Because rich sampling was collected in all patients, the C_{max} and AUC from days 0 to 28 could be directly computed for most patients in this study by noncompartmental analysis (NCA). Good correlation between the NCA estimates and the model-computed NCA parameters was observed (**Supplementary Material**).

Impact of tocilizumab/corticosteroids on tisagenlecleucel expansion

The impact of tocilizumab and corticosteroids on the expansion rate constant is summarized in **Table 1**, which shows that F_{toci} and F_{ster} were both close to 1. Thus, neither was found to impact the rate of expansion. This can also be observed in **Figure 4**, which shows the individual fits for all patients who had rich qPCR data (≥ 6 data points in first 2 weeks) and who received their first tocilizumab dose on or before day 6. The rates of expansion (slope of qPCR curve) before and after tocilizumab or corticosteroids appear similar, suggesting that treatment with tocilizumab or corticosteroids may not impact the rate of tisagenlecleucel expansion. It should be noted that in some patients (eg, patient BB in **Figure 4**), the first dose of tocilizumab was given only a few days before C_{max} had been achieved; therefore, in some cases, the true impact of tocilizumab on expansion may be difficult to assess. Although most patients receive tocilizumab prior to corticosteroids, as specified by the CRS treatment protocol, one patient (patient EE in **Figure 4**) received corticosteroids first due to an allergic reaction.[16]

Covariate analysis

Exploratory plots were used to screen all covariates (**Supplementary Material**), and the results from bootstrapping the full covariate model showed no statistically significant impact of any covariate on the maximal concentration of tisagenlecleucel (**Table 1**). Because no covariates had a statistically significant impact on the cellular kinetic parameters of tisagenlecleucel expansion, we concluded that the final cellular kinetic model was the same as the base model. Mueller and colleagues demonstrated that a

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larger pre-infusion tumor burden was associated with increased tisagenlecleucel expansion,[7] however, pre-infusion tumor burden after lymphodepletion was not measured in these studies.

Although administration of tocilizumab or corticosteroids did not appear to impact the rate of expansion, we explored the possibility of a correlation between comedication treatment and C_{max} because patients with greater peak transgene levels were more likely to have grade 3 or 4 CRS and were therefore more likely to receive comedication treatment. The model-estimated C_{max} was 2-fold higher in patients who required tocilizumab and was similar to that in the previously published NCA.[7]

DISCUSSION

Classical pharmacological agents, such as small molecules or monoclonal antibodies, can be described using standard compartmental approaches to estimate the PK parameters. The distribution, metabolism, and excretion of small molecules and monoclonal antibodies are largely driven by the biophysical properties of the molecule and the plasma concentrations that result after absorption. However, the kinetics of cell-based therapies are much more complex and are influenced by the physiological functions of the targeted cell type and by cell-extrinsic factors, such as the abundance of the CAR target molecule in vivo. The major differences between the CAR-T cellular kinetic model and a traditional PK model are summarized in **Table 3** and listed below.[21, 22]

Applicability of standard PK parameters: Unlike a traditional drug, CAR-T cells have the capacity to proliferate. For this reason, traditional PK parameters such as clearance and volume of distribution are not applicable and were not computed as part of the NCA.[7]

Mechanism of biphasic decline: For a typical drug, the α and β slopes arise from the interplay of two processes, distribution of the drug from the blood to peripheral tissues and elimination of the drug from the body. However, elimination of T cells from the body is governed by fundamentally different mechanisms. The α slope corresponds to a rapid contraction due to programmed apoptosis of most activated lymphocytes following the initial immune response, and the β slope corresponds to a gradual decline of memory cells that can persist in the body for years or even decades.[13, 14] Although the persistence phase of tisagenlecleucel has kinetics similar to that of long-term memory cells, it may also be that the persistence is due to the continual production of B-cell precursors expressing CD19, with surrogate antigen acting as an endogenous vaccine. In this respect, the long persistence of CD19 CAR-T cells may be similar to T cells that are specific for latent viruses such as CMV.[23]

The model presented in Figure 2 is not the only semi-mechanistic model that gives rise to exponential growth followed by biexponential decay in Equation 1. If memory cells were produced during the expansion phase as well or if cells could transition back and forth from the effector to memory state, then the analytic solution to such a model would have the same functional form. It is also possible that terminal phase is not due to formation of memory cells, but rather the low level of constant proliferation in response to the continual production of CD19 antigen; the kinetics of this process would also look similar. DeBoer and Perelson[4] also showed in equations 8-10 of their paper that rather than have expansion stop

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at Tmax, one could use a "cascade" model of cell division, modeling each generation of expansion. While this model is more realistic, it behaves similarly to the simpler model used here.

Distribution: Like a traditional drug, tisagenlecleucel has the capacity to distribute to other tissues within the body. Lymphocytes can traffic to the bone marrow, gut, spleen, and lymph nodes, with peripheral blood containing only 2% of the total body lymphocyte population.[20] The bone marrow CAR-T cell population, where progenitor B-cells expressing CD19 are continually produced, may be the more important than blood for establishing relationships between the cellular kinetics of tisagenlecleucel and safety and efficacy.

Long-term persistence: In the current analysis, the longest follow-up time was approximately 1 year, thus limiting the accuracy of the estimate for the terminal half-life. For this reason, a covariate analysis of the terminal half-life was not performed. However, longer persistence for CAR-T cells has been observed.[7] Two of the first 3 patients treated with tisagenlecleucel in 2010 currently maintain low but stable levels of CAR-T cells and have B-cell aplasia now, 8 years later. In patients with HIV treated with CD4ζ CAR-T cells, the average half-life of the CAR-T cell in peripheral circulation has been reported to be 17 to 23 years.[15] As tisagenlecleucel-treated patients remain in follow-up in these trials, this analysis will be updated such that a better estimate of the terminal half-life can be obtained.

Model equations: Because CAR-T cells have the capacity to proliferate beyond the initial dose infused and the mechanism of elimination is different than that of traditional drugs, it is necessary to use a different structural model to describe the cellular kinetics than the standard compartmental models used for population PK of traditional drugs. Although the profile in **Figure 1a** may look similar to a standard 2-compartment PK model describing oral absorption of a small molecule, the kinetics of tisagenlecleucel is driven by fundamentally different processes that require different mathematical equations to model expansion and persistence. In particular, the cellular kinetic model describes exponential expansion of the tisagenlecleucel transgene levels with positive exponent ρ. This contrasts with the standard 2-compartment model with oral absorption, where all exponents are negative, as shown in the analytical solution in **Table 3**.

No relationship between dose and exposure: Unlike a traditional drug, no relationship was detected between tisagenlecleucel dose and C_{max} or any other model parameter (**Supplementary Figure S2a**). Although a dose-exposure relationship is generally expected for most drugs, the lack of a relationship here may be due to the capacity of tisagenlecleucel to proliferate in vivo and due to heterogeneity across a patient's product, native immune system, and tumor burden.

Differences between CAR-T kinetics and traditional immune kinetics: Unlike in natural immune responses, where thymic output contributes new cells into the peripheral compartment,[5] there is no de novo generation of CAR-T cells. However, there is de novo generation of the $CD19^+$ antigen, so it is not clear if the persistent cells are true memory cells or if they continually proliferate at a low rate in response to antigen.

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Effect of tocilizumab and corticosteroids on expansion: A 2-fold higher C_{max} was observed in patients that received tocilizumab. This is thought to be because patients with greater expansion are more likely to develop grade 3 and 4 CRS and are therefore more likely to require tocilizumab therapy. An impact of tocilizumab therapy on the rate of expansion was not detected. As shown in **Figure 4**, individual fits of patient data showed no changes in the rate of expansion of tisagenlecleucel before and after administration of tocilizumab and that some patients have a ≥ 10-fold increase in tisagenlecleucel levels even after receiving their first dose of tocilizumab for CRS. Given this observation, no further analysis was performed on patients who received a second dose of tocilizumab. These data suggest that greater tisagenlecleucel expansion (C_{max}) leads to an increased likelihood of CRS and, therefore, a correlation exists between tocilizumab administration and exposure.[7, 17] However, it is important to note that these analyses did not examine the impact of the prophylactic use of tocilizumab to treat CRS on C_{max} ; assessing prophylactic tocilizumab would need to be done in a carefully controlled clinical trial.

Although the analyses presented herein demonstrate that corticosteroids and tocilizumab did not impact the C_{max}, it is important to recognize that corticosteroids were administered at low doses, over a short duration, and that patients were weaned rapidly. The CRS treatment algorithm used in ELIANA and ENSIGN specified that corticosteroids be given only when the first dose of tocilizumab did not lead to an improvement in CRS. Furthermore, methylprednisolone doses were ≤ 2 mg/kg per day. Thus, the effect of giving corticosteroids at higher doses, before tocilizumab, without tocilizumab or before the development of severe CRS was not assessed.

None of the other covariates explored (including corticosteroid dosing) were confirmed to have an effect on C_{max}. Although it has been observed elsewhere that baseline tumor burden also correlates with an increased expansion[24], this was not assessed because in the ELIANA and ENSIGN trials, bone marrow biopsies were not collected after lymphodepletion but before tisagenlecleucel dosing.

This work represents the first mixed-effect model-based analysis of a CAR-T cell therapy and may have broad application to multiple CAR treatment platforms. Our cellular kinetic model may be adapted to characterize the expansion and persistence of genetically modified cells across multiple disease indications, within various cell types, and between different costimulatory domains. A fundamentally different model structure was needed to describe these data than is used for standard population cellular kinetic models because CAR-T cells can proliferate exponentially and expand > 1000-fold in most patients. The model consistently described aggregate data and individual patient data, and the cellular kinetic analysis provided similar results to those in the previous NCA.[7] Patients with higher peak transgene levels were more likely to receive tocilizumab, consistent with prior results, on the basis of NCA.[18] Finally, no effect of tocilizumab and corticosteroids on the rate of expansion was observed.

Study Highlights (less than 150 words)

- WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

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- Tisagenlecleucel is a CAR-T cell therapy that facilitates targeted cell killing of CD19+ B cells and provide robust responses in acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma. However, comprehensive cellular models that describe CAR-T cell kinetics are lacking.

- WHAT QUESTION DID THIS STUDY ADDRESS?
  - A model-based analysis was used to characterize the kinetics of tisagenlecleucel therapy and to assess the impact on expansion of intrinsic and extrinsic factors, with a focus on comedications for treating cytokine release syndrome (tocilizumab and corticosteroids)
- WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
  - This work represents the first mixed-effect model-based analysis of CAR-T cell therapy. No impact of tocilizumab or corticosteodis on the expansion rate was observed.
- HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

This work provides a methodology for future studies in patients at risk of severe adverse events for assessing the impact of earlier anti-CRS therapy earlier which may impede CAR-T cell kinetics or efficacy.

Acknowledgments

Editorial assistance was provided by Jennifer Gooch, PhD, and Allison Lytle, PhD (ArticulateScience LLC). Code was validated by Alison Margolskee. Creton Kalfoglou contributed to the bioanalytical assay development and bioanalytical data interpretation.

Author Contributions:

A.M.S. wrote the manuscript; A.M.S., S.A.G., J.E.L., T.W.L., M.A.P., M.W.B., K.J.A., B.L.L., L.T., S.S., M.L., K.T.M., P.A.W., and C.H.J. designed the research; A.M.S., S.A.G., J.E.L., T.W.L., M.A.P., M.W.B., K.J.A., B.L.L., L.T., S.S., M.L., P.H., R.A., K.T.M., P.A.W., and C.H.J. performed the research; A.M.S. analyzed the data.

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Figure Legends

Figure 1 (a) The graph depicts the mathematical model of tisagenlecleucel following expansion at a rate (ρ) up to time to maximal expansion (T_{max}), followed by a biphasic contraction at rates α and β. Because F_B is much less than 1, the initial decline rate is well estimated by α. **(b)** Mathematical model for tisagenlecleucel expansion concurrent with tocilizumab and corticosteroid administration given at times T_{toci} and T_{ster}, respectively. The model allows for a reduced rate of expansion with F_{ster}, effect of steroids, and F_{toci}, effect of tocilizumab.

Figure 2 Compartmental model describing T-cell kinetics. The model has exponential growth of effector cells (E) at rate ρ before time to maximal expansion (T_{max}). After T_{max}, effector cells rapidly decline at rate

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(α-k) and convert to memory cells at rate k; the memory cells then decline at a rate β. The rapid decline is most likely caused by programmed cell death following immune activation, and a slower decline rate is indicative of a longer persistence of the memory effector T-cell phenotype. The equations describing the rates of expansion and decline are shown. Additional information about the derivation can also be found in the **Supplementary Material**. C_{max}, maximal concentration; F_B, fraction of transgene copies present during the decline at the gradual rate β, starting from T_{max}; $fold_x$, fold expansion; F_{ster}, effect of steroids; F_{toci}, effect of tocilizumab; T_{ster}, time to maximal expansion with steroid therapy; T_{toci}, time to maximal expansion with tocilizumab therapy.

Figure 3 (a) Visual predictive check of the model simulation compared with the data. The blue dots show the transgene copies per µg of genomic DNA, and the red asterisks denote simulated data points for the measurements that were below the limit of quantification (BLQ) of 50 transgene copies per µg. The blue lines show the 10th, 50th, and 90th percentiles calculated directly from the population data, the blue-shaded areas denote the confidence intervals of the 10th and 90th percentiles from the model and the pink shaded area shows the 50th percentile. The empirical percentiles lie within the confidence intervals, except at day 5, indicating that overall the model describes the data well. **(b)** Individual fits for tisagenlecleucel transgene copies per µg of genomic DNA (y-axis) over time in months from CAR-T cell infusion (x-axis) observed in a representative set of patients. CAR, chimeric antigen receptor; emp, empirical; out, output; PI, predictive interval; prctile, percentile; qPCR, quantitative polymerase chain reaction.

Figure 4 Individual fits for a subset of all patients with rich quantitative polymerase chain reaction (qPCR) sampling who received tocilizumab and/or corticosteroids. The red dashed lines indicate treatment with corticosteroids, and the green lines indicate treatment with tocilizumab. Each dot represents a measurable qPCR value. Black horizontal lines indicate the limit of quantification equal to 50 transgene copies per µg of genomic DNA. BLQ, below the limit of quantification.

Supplementary Material

- **Supplementary Material**
- **Dataset**

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Table 1 Model parameters

Type	Parameter	Units	Estimate	RSE, %	Eta Shrinkage
Fixed effect	$fold_x$	—	3,900	30	—
Fixed effect	T_{max}	Days	9.3	4.2	—
Fixed effect	C_{max}	DNA copies/ µg	24,000	20	—
Fixed effect	F_{toci}	—	1.2	7.5	—
Fixed effect	F_{ster}	—	1	9	—
Fixed effect	α	1/day	0.16	11	—
Fixed effect	F_B	—	0.0079	15	—
Fixed effect	β	1/day	0.0032	23	—
Random effect	$fold_x$	—	2.4	9.5	0.39
Random effect	T_{max}	—	0.38	7.9	0.14
Random effect	C_{max}	—	0.65	10	0.29
Random effect	α	—	0.91	8.8	0.27
Random effect	F_B	—	0.8	15	0.53
Random effect	β	—	0.86	23	0.82
Residual error	a	—	0.56	3.3	—
Log C_{max} covariate effect	Female (vs male)	—	0.25	72	—
Log C_{max} covariate effect	Asian (vs White)	—	0.13	250	—
Log C_{max} covariate effect	Race other/unknown (vs White)	—	0.33	76	—
Log C_{max} covariate effect	Down syndrome	—	0.25	130	—
Log C_{max} covariate effect	Received HSCT	—	0.29	62	—
Log C_{max} covariate effect	No fludarabine received	—	−0.63	69	—

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Log C_{max} covariate effect	Study B2205J vs B2202	—	−0.11	190	—
Log C_{max} covariate effect	Transduction efficiency	—	0.22	72	—
Log C_{max} covariate effect	Dose normalized by body weight	—	0.093	140	—
Log C_{max} covariate effect	Received tocilizumab	—	0.44	59	—
Log C_{max} covariate effect	Received corticosteroids	—	−0.36	75	—

$Fold_x$ is computed by the equation $fold_x = \exp(\rho \cdot T_{max})$. Eta shrinkage for each parameter is calculated by the formula $(1 - var(\eta))/\omega^2$.

C_{max}, maximal concentration; Eta shrinkage, shrinkage of empirical Bayes estimates of the parameter; F_B, fraction of transgene copies present during the decline at the gradual rate β, starting from T_{max}; $fold_{x'}$ fold expansion of tisagenlecleucel from baseline; F_{ster}, effect of steroids; F_{toci}, effect of tocilizumab; HSCT, hematopoietic stem cell transplant; RSE, relative standard error of the parameter; T_{max}, time to maximal expansion.

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Table 2 Patient characteristics

Characteristic	Patients (N = 90)
Sex, male/female, %	50/50
Age, median (range), years	12 (3-25)
Weight, median (range), kg	39 (14-140)
Race, %	
White	77
Asian	9
Other/unknown	14
Down syndrome, %	8
Previous stem cell transplant, %	57
Lymphodepleting chemotherapy with fludarabine, %	94
Weight-adjusted dose of tisagenlecleucel, median (range), cells/kg	3.1×10^6 ($0.2\text{-}5.4 \times 10^6$)
Total dose of tisagenlecleucel, median (range), cells	1.0×10^8 ($0.03\text{-}2.6 \times 10^8$)
Transduction efficiency, median (range), %	19 (2.3-56)
Tisagenlecleucel cell viability, median (range), %	94 (55-99)
Received steroids, %	26
Timing of first steroid dose, median (range), days	7.5 (0.11-170)
Received tocilizumab, %	36
Timing of first tocilizumab dose, median (range), days	5.7 (1-27)

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Table 3 Difference between kinetics of a small molecule oral dose and CAR-T cell therapy

Property	Small Molecule	CAR-T Cell[17,18]
Ability to proliferate	No	Yes
Reason for α and β phase	Distribution and elimination	Contraction and persistence
Terminal half-life time scale	Hours, days, or weeks	Years
Applicability of traditional NCA parameters (clearance and volume of distribution)	Applicable	Not applicable due to the ability of CAR-T cells to proliferate
Variability in product from patient to patient	None	Variability exists due to variability in patient immune systems
Clear relationship between dose and exposure	Yes, although it may be nonlinear	No relationship between dose and exposure detected
Kinetic equation following small molecule oral dose or intravenous CAR T cell dose	$A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t) - (A + B) \cdot \exp(-k_a t)$	$f(t) = \begin{cases} R_0 e^{\rho t}, & t < T_{\max} \\ A e^{-\alpha(t-T_{\max})} + B e^{-\beta(t-T_{\max})}, & t \geq T_{\max} \end{cases}$
Sign of exponent for initial increase after dosing	Negative ($-k_a$)	Positive ($+\rho$)

CAR, chimeric antigen receptor; IV, intravenous; NCA, noncompartmental analysis; T_{max}, time to maximal expansion.

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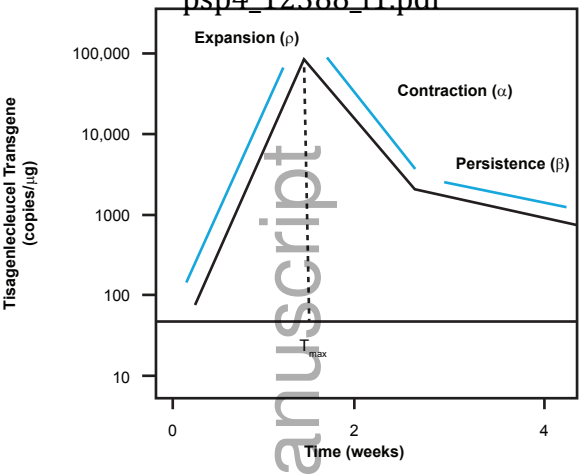

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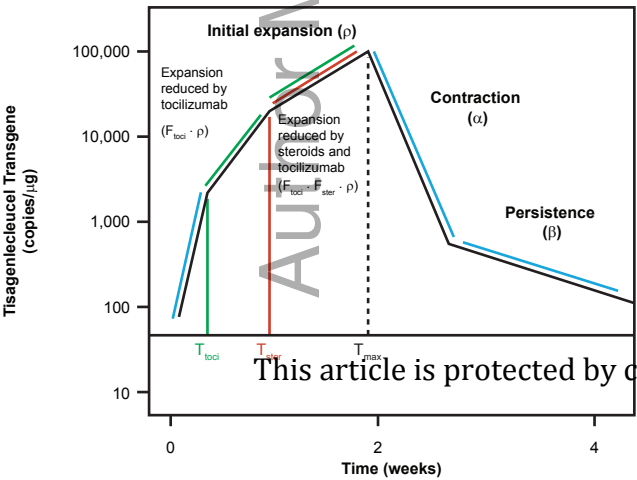

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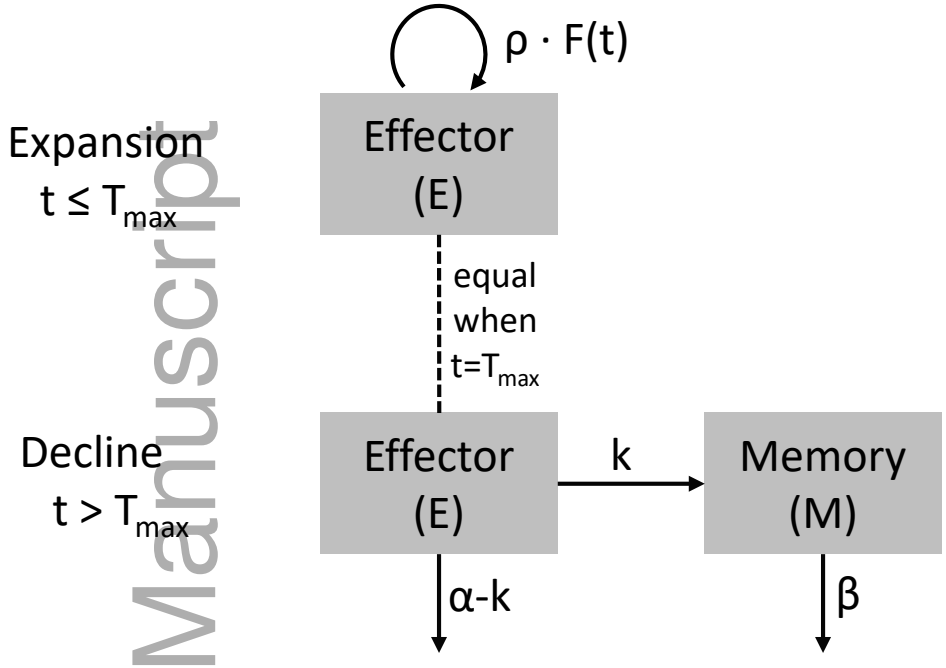
Compartmental Model



Definitions

$\rho = \log(fold_x)/T_{max}$

$k = F_B \cdot (\alpha - \beta)$

Equations

$$\frac{dE}{dt} = \rho \cdot F(t) \cdot E$$

$$\frac{dE}{dt} = -\alpha \cdot E$$

$$\frac{dM}{dt} = k \cdot E - \beta \cdot M$$

Initial Conditions

at t = 0: $E(0) = C_{max}/fold_x$

at t = T_{max}: $E(T_{max}) = C_{max}$

$M(T_{max}) = 0$

Comedication Effect: F(t)

$$F(t) = f_{toci}(t) \cdot f_{ster}(t)$$

$$f_{toci}(t) = \begin{cases} 1 & t \le T_{toci} \\ F_{toci} & t > T_{toci} \end{cases}$$

$$f_{ster}(t) = \begin{cases} 1 & t \le T_{ster} \\ F_{ster} & t > T_{ster} \end{cases}$$

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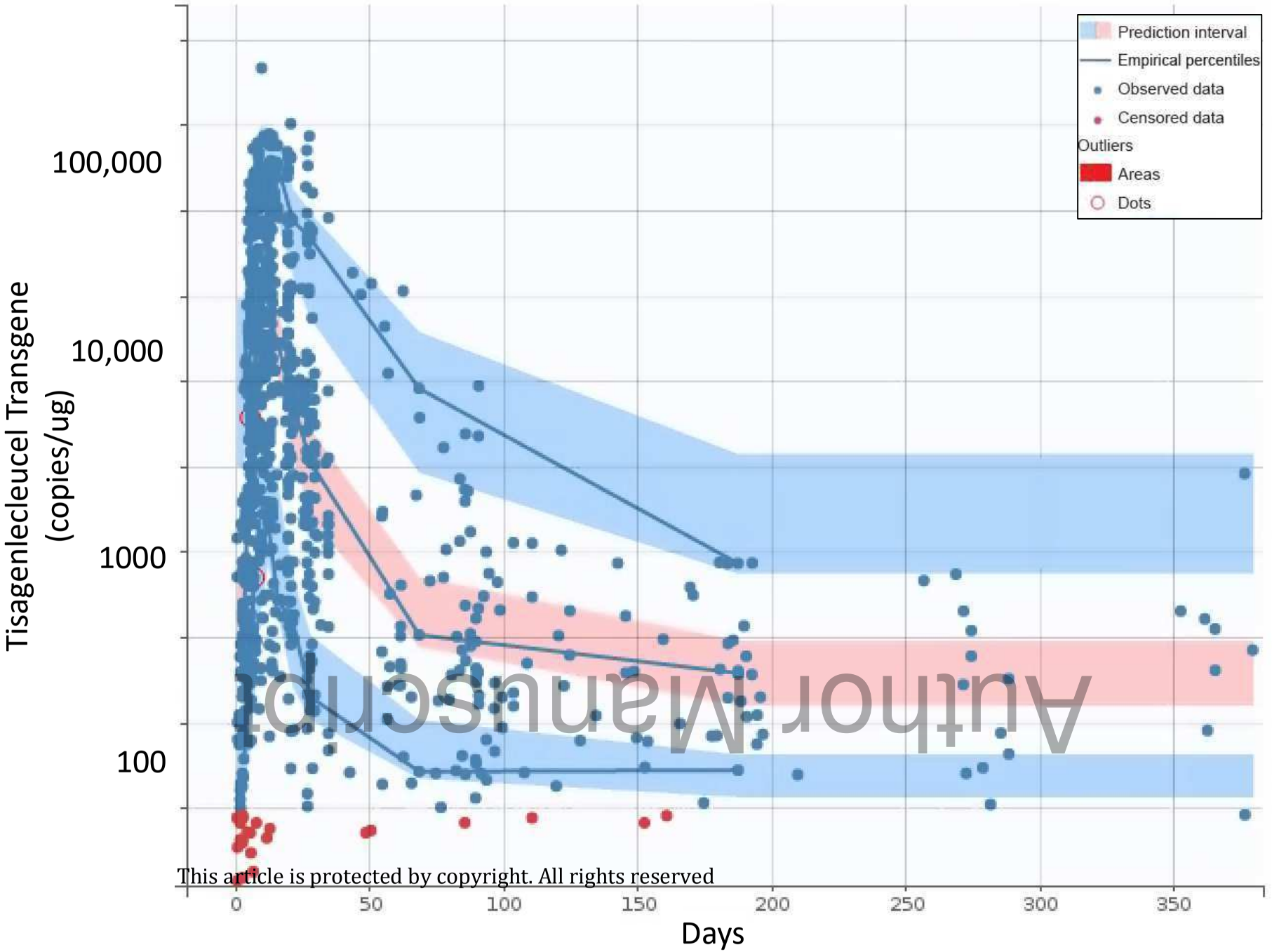

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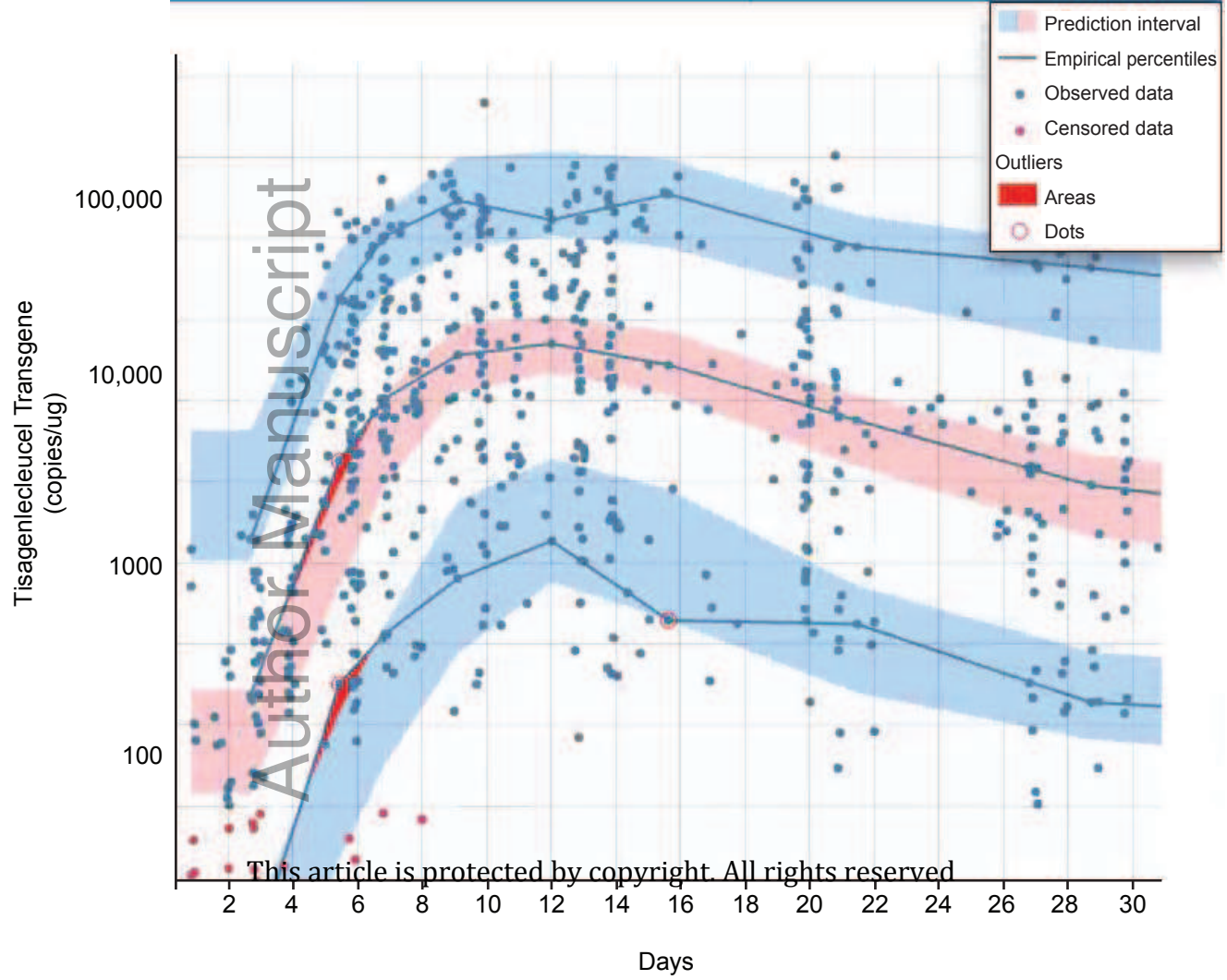

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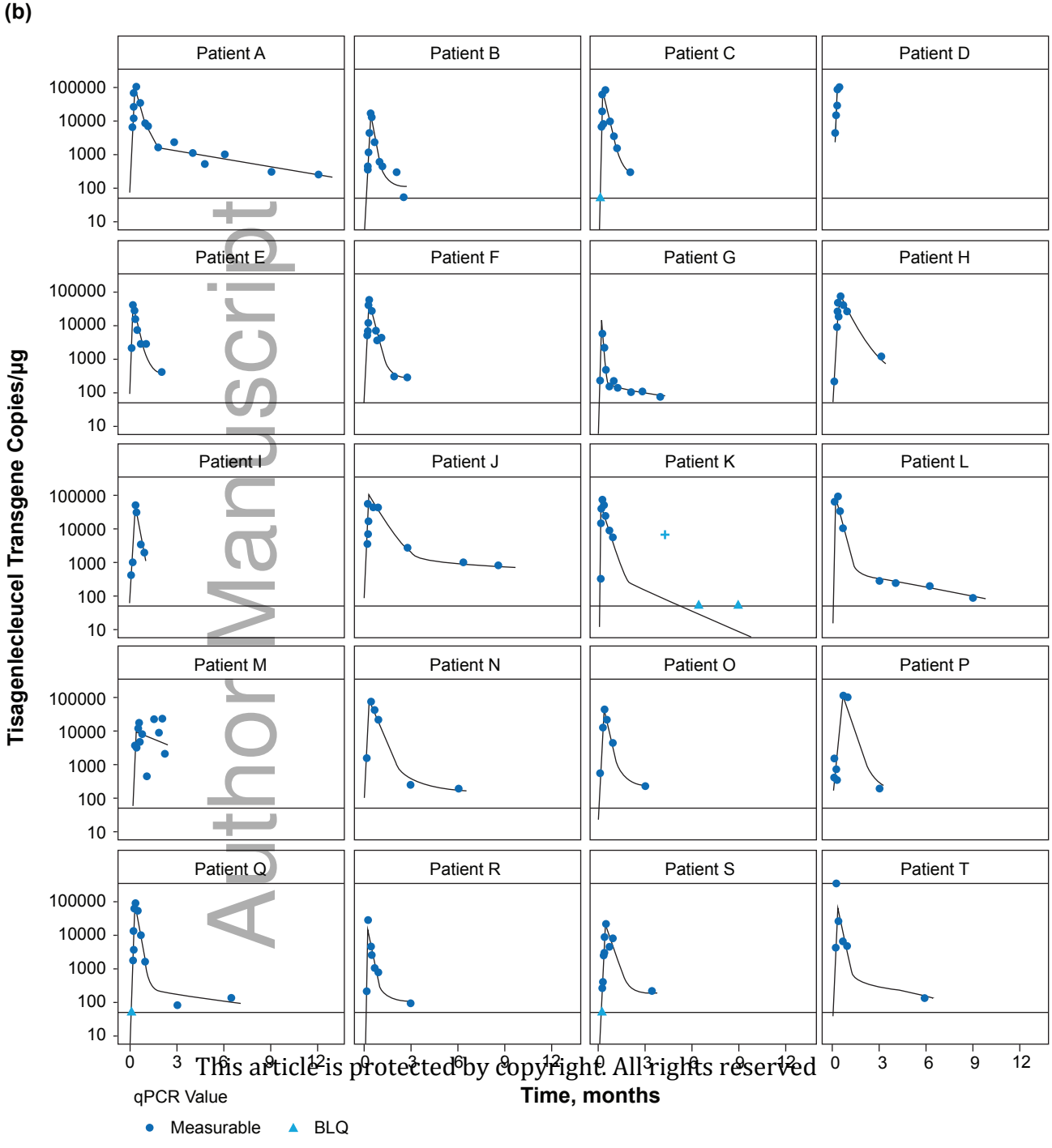

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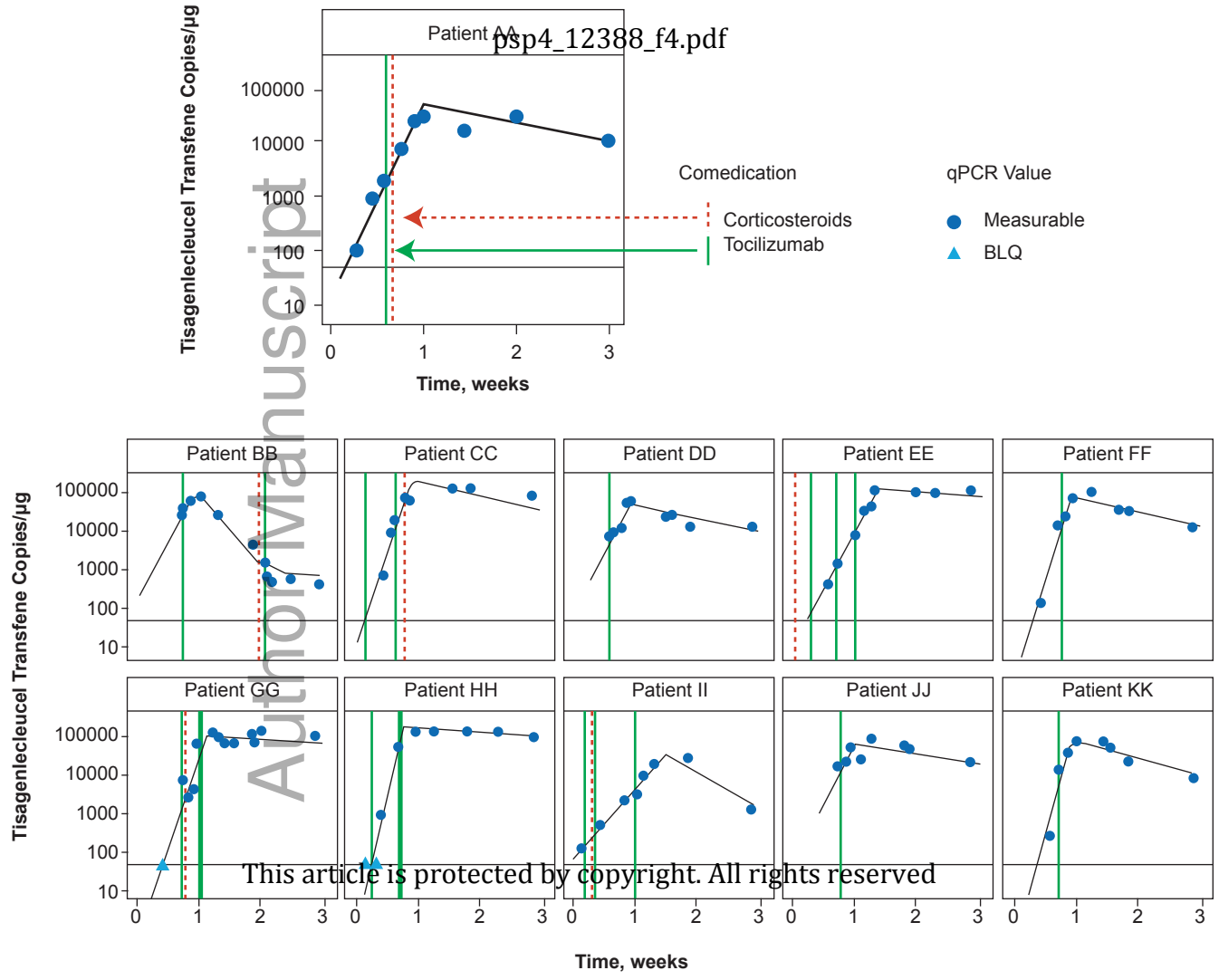

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