Supplemental Material

Methods

Tisagenlecleucel

Tisagenlecleucel is manufactured ex vivo by genetically modifying autologous peripheral blood T cells (harvested by leukapheresis) using a lentiviral vector to introduce the chimeric antigen receptor (CAR) transgene. The CAR consists of an extracellular murine single-chain anti-CD19 antibody fragment and the CD3-ζ intracellular signaling and 4-1BB costimulatory domains.[1,2] After infusion, tisagenlecleucel undergoes expansion in vivo and targets CD19–expressing malignant B-cells.[3] As anti-CD19 CAR-T cells are unable to discriminate malignant from normal CD19–expressing B-cells, it can lead to long-term B-cell aplasia.

Study design, patients, and treatment

The JULIET study included patients who had previously received ≥2 lines of chemotherapy and had either relapsed after autologous hematopoietic stem cell transplant (HSCT) or were ineligible for autologous HSCT. Patients were excluded if they received prior CD19-directed therapy, had prior allogeneic HSCT, or had active central nervous system involvement. All patients underwent leukapheresis, and the cryopreserved cells were shipped to the manufacturing facility. Following leukapheresis, enrolled patients received investigator's choice of optional bridging therapy for disease stabilization and lymphodepleting chemotherapy consisting of fludarabine/cyclophosphamide or bendamustine before tisagenlecleucel infusion; lymphodepleting chemotherapy was not required for patients whose white blood cell count was ≤1000 cells/μL within 1 week before tisagenlecleucel infusion. Tisagenlecleucel administered during the JULIET study was primarily manufactured at the Novartis US manufacturing facility

(Morris Plains, NJ, USA); some batches were also manufactured at the EU manufacturing facility (Fraunhofer Institut für Zelltherapie, Leipzig, Germany).[4]

All patients who received tisagenlecleucel were included in the full analysis set and safety analysis set (for all analyses related to safety endpoints). All patients who had ≥1 sample with evaluable cellular kinetics data were included in the pharmacokinetic analysis set. Patients who had ≥3 months of follow-up after tisagenlecleucel infusion were included in the efficacy analysis set.

Clinical assessments

The primary endpoint was best overall response rate determined by independent review using the Lugano Classification[5] in patients with ≥3 months follow-up (efficacy analysis set as described previously).[4] Duration of response, event-free survival, progression-free survival, overall survival, safety, and cellular kinetics were secondary endpoints. Adverse events occurring during the study were recorded and graded according to MedDRA version 20.1 and CTCAE version 4.03. Cytokine-release syndrome grades were determined using the University of Pennsylvania grading scale and managed using a protocol-specific algorithm.[6,7]

Summary of bioanalytical methods

Baseline CD19 expression and tumor burden

Quantitative immunofluorescent staining for CD19 by automated quantitative analysis (AQUA), performed at Navigate BioPharma Services (Carlsbad, CA), was performed in formalin-fixed paraffin-embedded biopsies collected before tisagenlecleucel infusion; the majority of biopsies were archival and collected months before tisagenlecleucel infusion. Relative expression levels of CD19 protein in all cells (AQUA score) were obtained by dividing the total pixel intensity of CD19 divided by the total area of nucleated cells (4′,6-diamidino-2-phenylindole, dichloride

[DAPI]- positive). An analytical cutoff at an AQUA score of 10,000 was utilized to distinguish strong positives from low/negative samples based on a set of 24 DLBCL samples benchmarked to standard chromogenic IHC and interpretation by board certified pathologist. The AQUA score cutoff of 10,000 separated the group of samples with high H score (>130) from those with negative/low H scores (<10).

For tumor burden assessments, baseline positron emission tomography (PET) scans for each subject were quantitatively evaluated by delineating the appropriate tumors that demonstrated focal fluorodeoxyglucose (^{18}F-FDG) uptake by a single independent radiologist at Parexel International. Total metabolic tumor volume was computed with the 41% maximum standardized uptake value (SUV_{max}) threshold automatically derived by MIM software (version 5.1.2, MIM Software Inc., Cleveland, OH).

Rituximab levels

An enzyme-linked immunosorbent assay (ELISA) was developed and validated for the determination of rituximab concentration in serum samples. Samples were analyzed using a validated assay at WuXi Laboratories, China.

Immunogenicity

Humoral immunogenicity was evaluated by measurement of anti-murine anti-CD19 CAR (anti-mCAR19) antibodies in serum before and after tisagenlecleucel infusion using a flow cytometry assay.

Anti-mCAR19 antibodies in human serum samples were captured using Jurkat cells that were transfected to express mCAR19; untransfected cells were used as the reference standard. This method measures bound IgG and IgM on viable cells. The validation experiments were conducted with a surrogate positive control (humanized anti-CART19 antibody) with the

determined assay parameters being specific to this antibody. The assay sensitivity was 100 ng/mL of positive control antibody in 100% human serum. If a humoral immune response against mCAR19 was induced or boosted after a patient received tisagenlecleucel, a patient-specific cut off point was calculated. Details related to methodology for humoral immunogenicity have been previously published.[8,9]

Typical cellular kinetic profile in ALL and DLBCL patients

A representative profile demonstrating maximal CAR transgene expansion and persistence by qPCR (primary y-axis) along-side a theoretical estimation of these parameters by CAR-positive T cells/µL of blood (secondary y-axis) in a patient with DLBCL is presented in **Figure S2A**. The theoretical estimation of CAR-positive T cells/µL is for illustrative purposes only, and was derived by multiplication of the following factors: WBC counts (cells/µL), percentage of CAR-positive cells among CD3-positive T cells (flow cytometry), and percentage of CD3-positive cells among WBC (flow cytometry). Representative profiles for patients with pediatric B-ALL (from ELIANA[7,8]) and DLBCL by qPCR (**Figure S2B**) demonstrate higher initial expansion in B-ALL versus DLBCL, with similar patterns in the elimination/persistence phase.

References

1. Milone MC, Fish JD, Carpenito C, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol Ther*. 2009;17(8):1453-1464.
2. Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med*. 2011;3(95):95ra73.
3. Buechner J, Kersten MJ, Fuchs M, Salmon F, Jäger U. Chimeric antigen receptor-T cell therapy: practical considerations for implementation in Europe. *HemaSphere*. 2018;2(1):e18.
4. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45-56.
5. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3068.
6. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303):303ra139.
7. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378(5):439-448.
8. Mueller KT, Waldron E, Grupp SA, et al. Clinical pharmacology of tisagenlecleucel in B-cell acute lymphoblastic leukemia. *Clin Cancer Res*. 2018;24(24):6175-6184.

9. Potthoff B, McBlane F, Spindeldreher S, Sickert D. A cell-based immunogenicity assay to detect antibodies against chimeric antigen receptor expressed by tisagenlecleucel. *J Immunol Methods*. 2019:112692.

Table S1. Multivariate Analaysis of Impact of Expansion (C_{max}), Dose, and Tumor Burden on Probability of Grade 3/4 CRS and Nuerologic Events

	Grade 3/4 CRS		Grade 3/4 Neurologic Events	
	Parameter estimate	***P* value**	**Parameter estimate**	***P* value**
Intercept	−8.6311	0.0001	-0.0197	0.9868
log(Dose)	0.3433	0.6916	-0.2522	0.5673
log(C_{max})	0.687	0.0036	0.2084	0.1483
Tumor burden	0.00441	0.0047	0.00161	0.2268

Table S2. Rituximab Administration Prior to Tisagenlecleucel Infusion

	All Patients N=111
Patients who received rituximab prior to tisagenlecleucel infusion, n (%)	109 (98.2)
Within 0–1 month prior	16 (14.4)
Within 1–2 months prior	34 (30.6)
Within 2–6 months prior	28 (25.2)
Within 6–12 months prior	14 (12.6)
Beyond 1 year prior	17 (15.3)

Figure S1. Pictorial representation of relationships between tisagenlecleucel cellular kinetics and key endpoints in JULIET. The impact of select product characteristics, intrinsic and extrinsic factors, CD19 expression, and baseline tumor burden on cellular kinetics; of cellular kinetics on efficacy, safety, and B-cell aplasia; of dose on expansion, efficacy, and safety; and of immunogenicity on exposure and efficacy were evaluated. CRS, cytokine-release syndrome; DOR, duration of response.

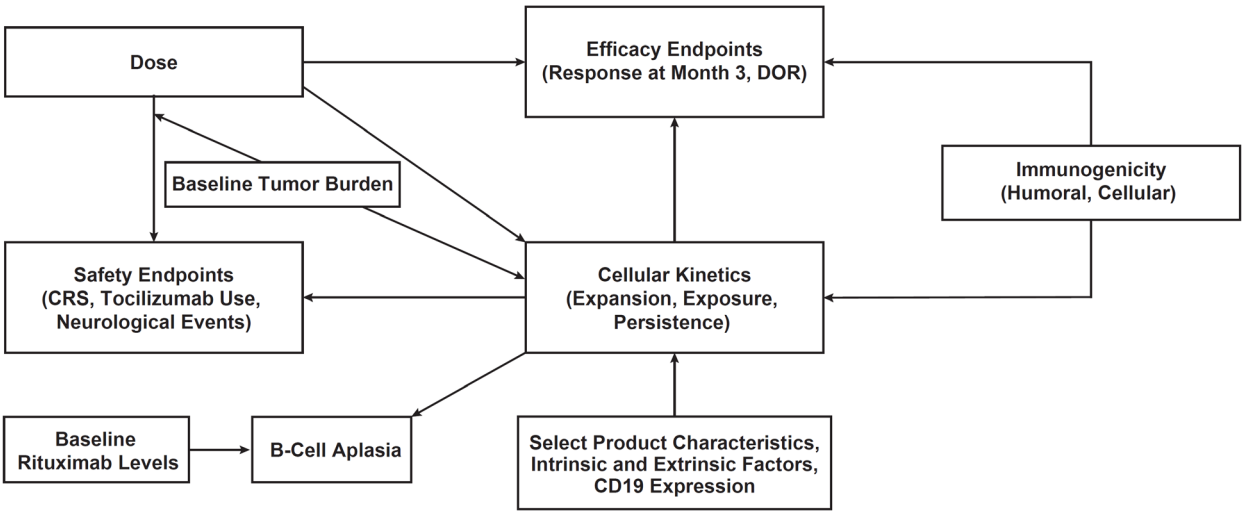


Figure S2. Correlation between expansion and exposure in transgene levels by qPCR and CAR-positive cells by flow cytometry. Correlation between **(A)** C_{max} and **(B)** $AUC_{0\text{-}28d}$ by qPCR and flow cytometry. *Percentage of CAR-positive cells among CD3-positive T cells. $AUC_{0\text{-}28d}$, area under the curve from time zero to 28 days post-infusion; CAR, chimeric antigen receptor; C_{max}, observed maximum plasma concentration following infusion; qPCR, quantitative polymerase chain reaction.

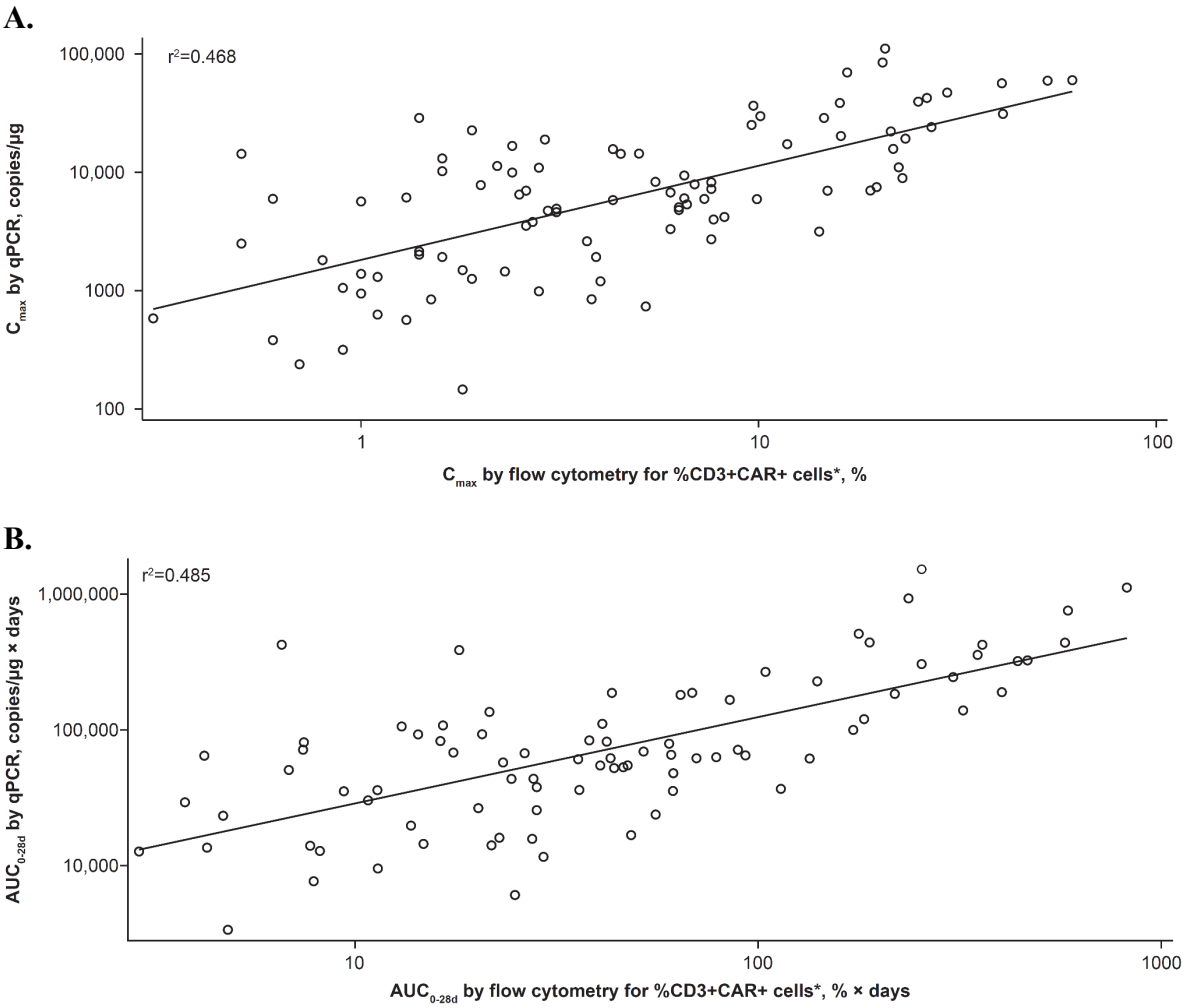
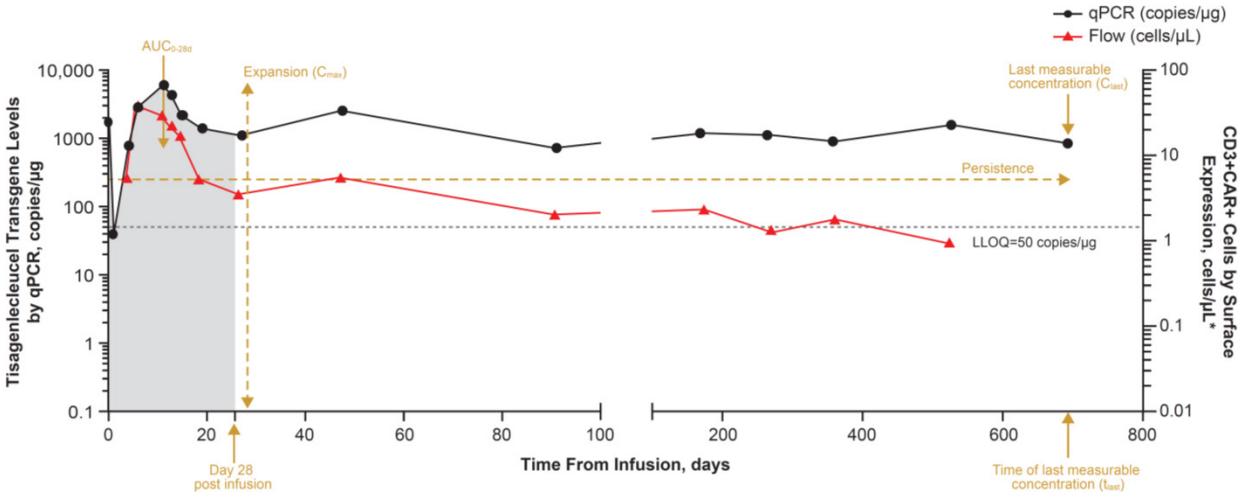


Figure S3. Cellular kinetics profile of tisagenlecleucel assessed by qPCR in patients with relapsed/refractory DLBCL. (A) Typical cellular kinetics profile in an individual patient with DLBCL with a complete response at month 3. Transgene levels by qPCR, expressed as copies/µg, are presented on the primary y-axis; theoretical estimates* for surface expression of CAR-positive cells among CD3-positive T cells, expressed as cells/µL are presented on the secondary y-axis. An initial rapid expansion phase following tisagenlecleucel infusion with C_{max} (geometric mean among responders in efficacy analysis set, 6210 copies/µg) achieved at approximately day 9 was followed by biexponential decline and persistence of transgene levels above the LLOQ (50 copies/µg) for up to 2 years. **(B)** Typical cellular kinetic profiles for responding pediatric B-ALL (from ELIANA[7,8]) and adult DLBCL patients, showing similar patterns in expansion and biexponential decline. *Theoretical estimates from flow cytometry data (CD3+CAR+ T cells) and WBC counts. †Patients who had ≥1 sample with evaluable cellular kinetics data and received tisagenlecleucel manufactured in the US were included. $AUC_{0\text{-}28d}$, area under the curve from time zero to 28 days post-infusion; B-ALL, B-cell precursor acute lymphoblastic leukemia; CAR, chimeric antigen receptor; C_{last}, last observed plasma concentration; C_{max}, observed maximum plasma concentration following infusion; CR, complete response; DLBCL, diffuse large B-cell lymphoma; LLOQ, lower limit of quantification; PD, progressive disease; PR, partial response; qPCR, quantitative polymerase chain reaction; SD, stable disease; t_{last}, time of last quantifiable concentrations; UNK, unknown; WBC, white blood cell.

A.


qPCR (copies/µg)
Flow (cells/µL)
AUC0-28d
Expansion (Cmax)
Last measurable concentration (Clast)
Persistence
LLOQ=50 copies/µg
Tisagenlecleucel Transgene Levels by qPCR, copies/µg
CD3+CAR+ Cells by Surface Expression, cells/µL*
Time From Infusion, days
Day 28 post infusion
Time of last measurable concentration (tlast)

B.

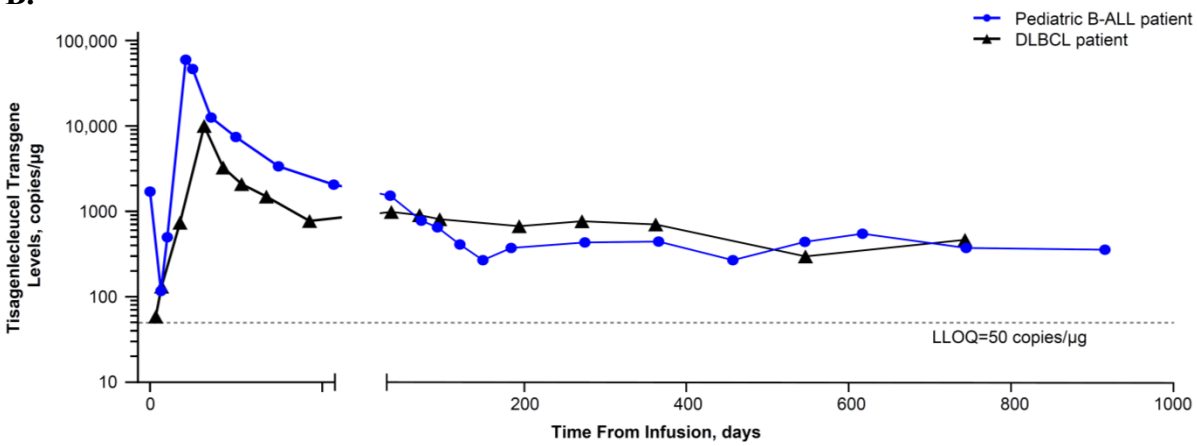
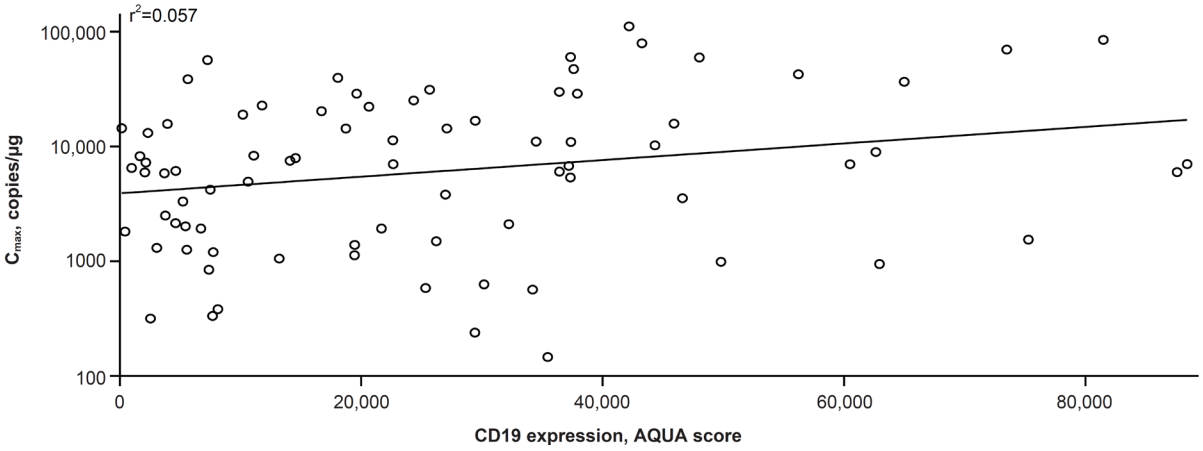

Pediatric B-ALL patient
DLBCL patient
Tisagenlecleucel Transgene Levels, copies/µg
LLOQ=50 copies/µg
Time From Infusion, days

Figure S4. Relationship between disease characteristics and tisagenlecleucel cellular kinetics.

Relationship between **(A)** baseline CD19 expression and **(B)** baseline tumor burden vs C_{max}.

AQUA, automated quantitative analysis; C_{max}, observed maximum plasma concentration following infusion.

A.



B.

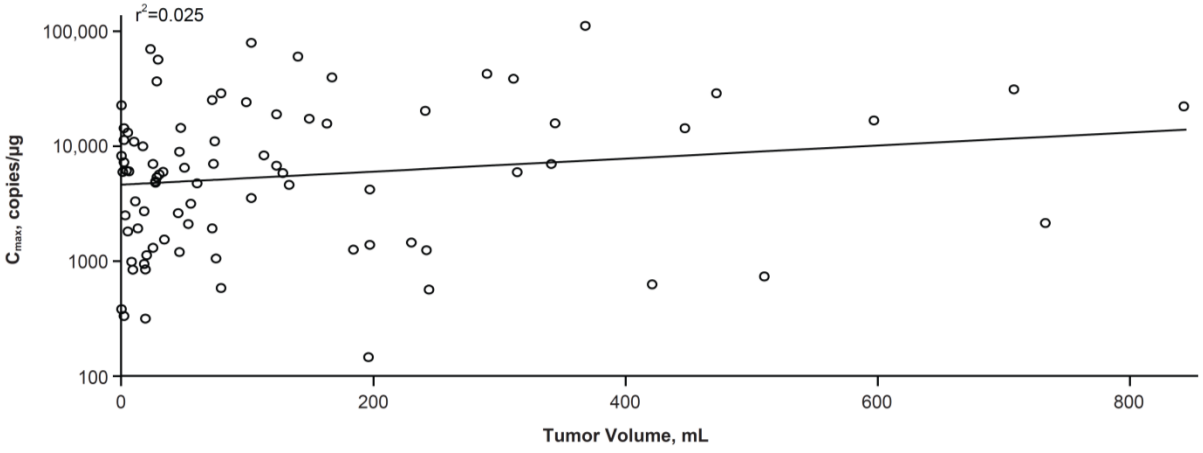
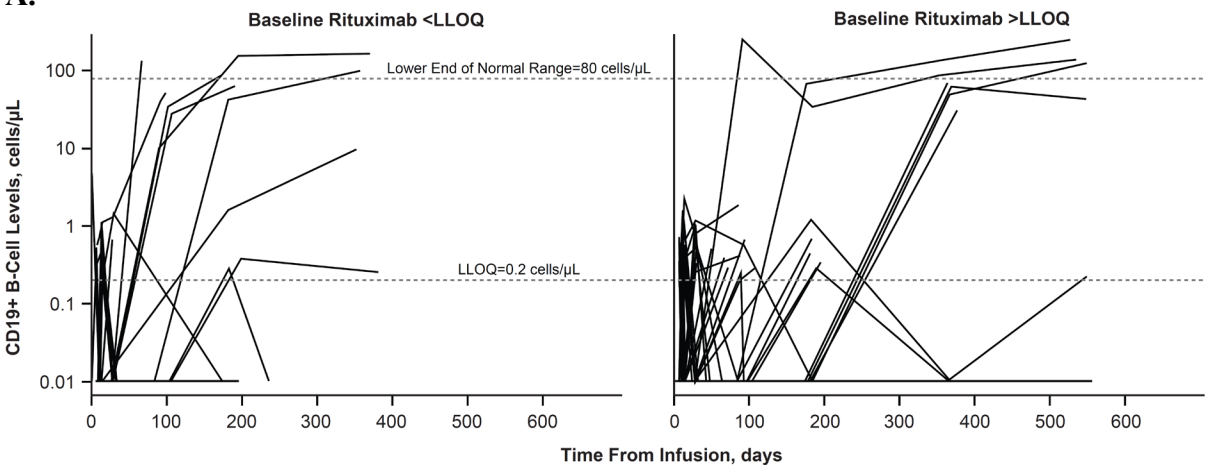
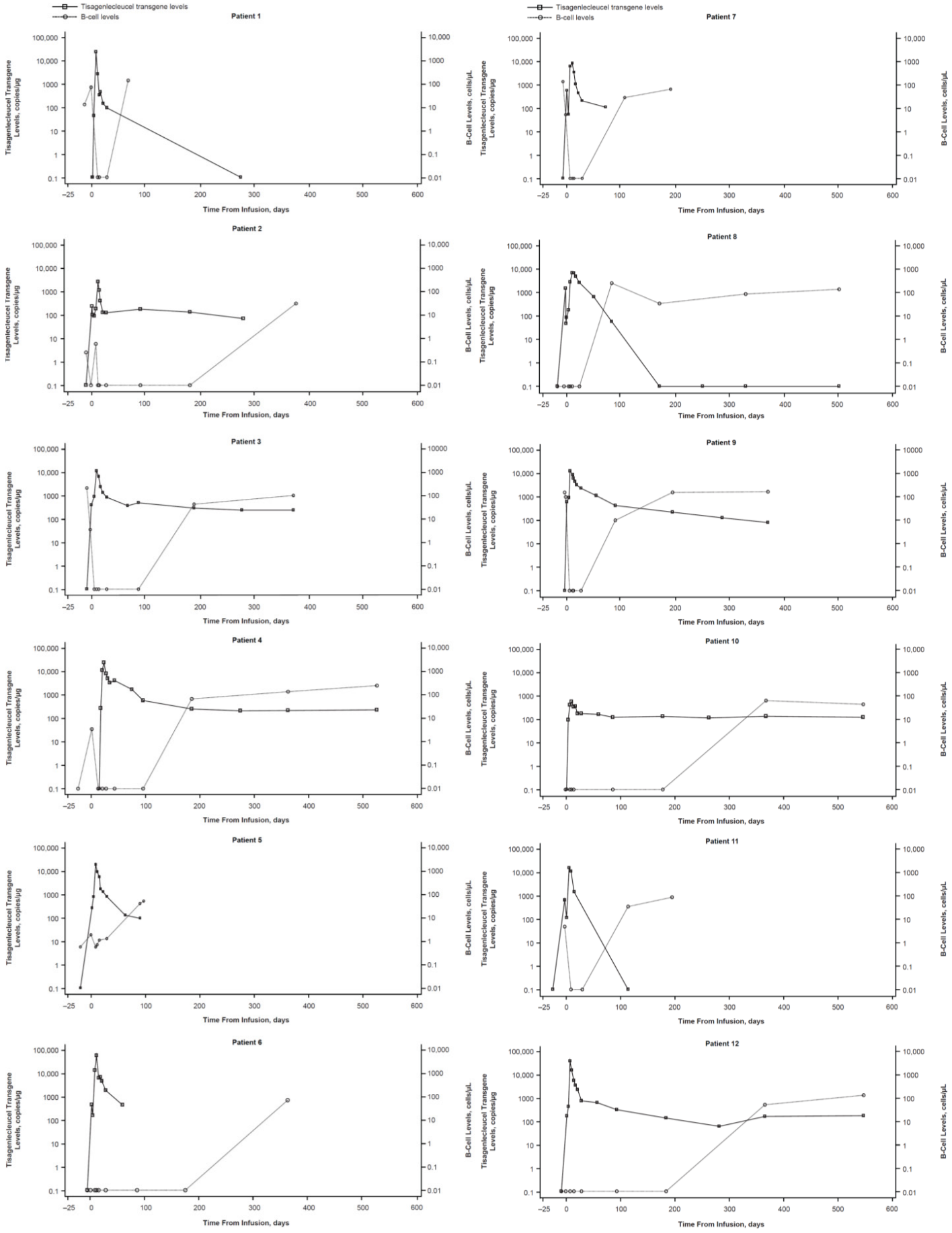


Figure S5. Association between B cell aplasia, rituximab, and tisagenlecleucel persistence. (**A**) CD19-positive B-cell levels over time in patients with rituximab levels <LLOQ and >LLOQ at baseline. (**B**) Individual plots of tisagenlecleucel transgene levels and B-cell levels over time following tisagenlecleucel infusion in patients with B-cell levels >30 cells/µL before infusion. LLOQ, lower limit of quantification.

A.



B.


Tisagenlecleucel transgene levels
B-cell levels
Patient 1
Patient 7
Patient 2
Patient 8
Patient 3
Patient 9
Patient 4
Patient 10
Patient 5
Patient 11
Patient 6
Patient 12
Tisagenlecleucel Transgene Levels, copies/µg
B-Cell Levels, cells/µL
Time From Infusion, days