Severe cytokine release syndrome is associated with hematologic toxicity following CD19 CAR T-cell therapy

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Krishna Juluri (University of Washington, United States) Qian Wu (Fred Hutchinson Cancer Research Center, United States) Jenna Voutsinas (Fred Hutchinson Cancer Research Center, United States) Jue Hou (Harvard T.H. Chan School of Public Health, United States) Alexandre Hirayama (Fred Hutchinson Cancer Research Center, United States) Erin Mullen (Seattle Cancer Care Alliance, United States) Nancy Miles (Fred Hutchinson Cancer Research Center, United States) David Maloney (University of Washington, United States) Cameron Turtle (University of Washington, United States) Merav Bar (University of Washington, United States) Jordan Gauthier (University of Washington, United States)

Abstract:
CD19-targeted chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable efficacy in patients with relapsed/refractory B-cell malignancies, however, is associated with toxicities including cytokine release syndrome (CRS), neurotoxicity, and impaired hematopoietic recovery. The latter is associated with high grade cytopenias requiring extended growth factor or transfusional support, potentially leading to additional complications such as infection or hemorrhage. To date, the factors independently associated with hematologic toxicity have not been well characterized. To address this, we retrospectively analyzed 173 patients who received defined-composition CD19 CAR T-cell therapy on a phase I/II clinical trial (NCT01865617), with primary endpoints of absolute neutrophil count (ANC) and platelet count at day-28 following CAR T-cell infusion. We observed cumulative incidences of neutrophil and platelet recovery of 81% and 75% respectively, at 28 days post-CAR T-cell infusion. Hematologic toxicity was noted in a significant subset of patients with persistent neutropenia in 9% and thrombocytopenia in 14% at last follow-up. Utilizing debiased LASSO regression analysis for high-dimensional modeling and considering patient-, disease-, and treatment-related variables, we identified increased CRS severity as an independent predictor for decreased platelet count and lower pre-lymphodepletion platelet count as independent predictors for both decreased neutrophil and platelet counts following CD19 CAR T-cell infusion. Furthermore, multivariable models including CRS-related cytokines identified associations between higher peak serum concentrations of IL-6 and lower day-28 counts; in contrast, higher serum concentrations of TGF-β were associated with higher counts. Our findings suggest that patient selection and improved CRS management may improve hematopoietic recovery following CD19 CAR T-cell therapy.

Conflict of interest: COI declared – see note

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Krishna R. Juluri¹, Qian Vicky Wu¹, Jenna Voutsinas⁴, Jue Hou⁵, Alexandre V. Hirayama¹,², Erin Mullane¹, Nancy Miles², David G. Maloney¹,²,³, Cameron J. Turtle¹,²,³, Merav Bar¹,³, Jordan Gauthier¹,²,³

¹Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA
²Integrated Immunotherapy Research Center, Fred Hutchinson Cancer Research Center, Seattle, WA
³Division of Medical Oncology, University of Washington, Seattle, WA
⁴Public Health Services Division, Fred Hutchinson Cancer Research Center, Seattle, WA
⁵Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

Corresponding author:
Jordan Gauthier, MD, MSc
Phone: (206) 667-2713
Fax: (206) 667-7983
Email: jgauthier@fredhutch.org
Clinical Research Division,
Fred Hutchinson Cancer Research Center
1100 Fairview Ave N, Seattle WA 98109, USA

Key Points

- Hematologic toxicity in observed in approximately 20% of patients receiving anti-CD19 CAR T-cell therapy
- Higher CRS severity and CRS-related cytokine levels and lower pre-lymphodepletion platelet count predicted hematologic toxicity

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Abstract

CD19-targeted chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable efficacy in patients with relapsed/refractory B-cell malignancies, however, is associated with toxicities including cytokine release syndrome (CRS), neurotoxicity, and impaired hematopoietic recovery. The latter is associated with high grade cytopenias requiring extended growth factor or transfusional support, potentially leading to additional complications such as infection or hemorrhage. To date, the factors independently associated with hematologic toxicity have not been well characterized. To address this, we retrospectively analyzed 173 patients who received defined-composition CD19 CAR T-cell therapy on a phase I/II clinical trial (NCT01865617), with primary endpoints of absolute neutrophil count (ANC) and platelet count at day-28 following CAR T-cell infusion. We observed cumulative incidences of neutrophil and platelet recovery of 81% and 75% respectively, at 28 days post-CAR T-cell infusion. Hematologic toxicity was noted in a significant subset of patients with persistent neutropenia in 9% and thrombocytopenia in 14% at last follow-up. Utilizing debiased LASSO regression analysis for high-dimensional modeling and considering patient-, disease-, and treatment-related variables, we identified increased CRS severity as an independent predictor for decreased platelet count and lower pre-lymphodepletion platelet count as independent predictors for both decreased neutrophil and platelet counts following CD19 CAR T-cell infusion. Furthermore, multivariable models including CRS-related cytokines identified associations between higher peak serum concentrations of IL-6 and lower day-28 counts; in contrast, higher serum concentrations of TGF-β were associated with higher counts. Our findings suggest that patient selection and improved CRS management may improve hematopoietic recovery following CD19 CAR T-cell therapy.
Introduction

CD19-targeted chimeric antigen receptor (CD19 CAR) T-cell therapy has demonstrated promising efficacy in patients with relapsed or refractory (R/R) B-cell malignancies with high objective or overall (ORR) and complete response/remission (CR) or complete remission with incomplete hematologic recovery (CRi) rates in acute lymphoblastic leukemia (ALL) (68-93% CR/CRi), chronic lymphocytic leukemia (CLL) (57-74% ORR, 21% CR)\(^2,3\), mantle cell lymphoma (59% CR), and large B-cell lymphoma (LBCL) (52-88% ORR, 40-59% CR), the latter including patients with diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBCL), and transformed follicular lymphoma (FL). A subset of these patients has demonstrated durable responses with CR of greater than two years without the need for further treatment.\(^5,7\). Consequently, the FDA approved four CD19 CAR-T products: axicabtagene ciloleucel (Yescarta) for the treatment of R/R LBCL and PMBCL, tisagenlecleucel (Kymriah) for R/R LBCL and ALL in children and young adults, and most recently brexucabtagene autoleucel (Tecartus) for the treatment of R/R MCL and lisocabtagene maraleucel (Breyanzi) for patients with R/R LBCL.

CD19 CAR T-cell therapy is however associated with significant toxicities impeding its development and wide dissemination; namely, cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), B-cell aplasia, and early and late infections.\(^11-13\). Furthermore, hematologic toxicity, including delayed hematopoietic recovery with persistent high-grade neutropenia, anemia, and thrombocytopenia, has been observed in a subset of patients undergoing CAR-T therapies targeting CD19\(^5,7,9,11,14\) and other antigens.\(^15,16\)

We have previously reported prolonged cytopenia requiring transfusions or growth factor support on a phase I/II trial of defined-composition CD19 CAR-T therapy either starting or persisting beyond 90 days post-CAR-T infusion in 16% of patients with ongoing CR for at least one year and without MDS following treatment.\(^11\). In the ZUMA\(^5\) and ELIANA\(^17\) studies,
investigators reported 17% of patients with grade ≥ 3 cytopenia at three months or greater following CAR-T infusion in the former and grade ≥ 3 neutropenia (11%) and thrombocytopenia (12%) at median follow-up of 13.1 months in the latter. Prolonged cytopenias were also noted in the recent TRANSCEND NHL 001 study\(^9\) with 37% of evaluable patients with grade ≥ 3 cytopenia at day 29, and prolonged neutropenia (7%), thrombocytopenia (26%), and anemia (9%) by day 180.

Prolonged cytopenia may result in increased frequency of infections\(^{13,18}\), hemorrhagic events, extended growth factor administration, and blood product transfusions. Persistent transfusion requirements are associated with risks of iron overload, transfusion-associated reactions, circulatory overload, and lung injury. This ultimately may contribute to treatment-related morbidity and mortality, increased resource utilization, and impaired quality of life.

Studies from our group\(^{19}\) and others\(^{20}\) have previously suggested an association between CRS severity and lower hematologic nadirs, delayed hematopoietic recovery, and increased transfusion dependence. Yet, the factors independently influencing hematopoietic recovery after CD19 CAR T-cell therapy remain poorly understood; recent analyses have been limited by small cohort sizes leading to low statistical power, precluding robust multivariable modeling. In addition, the methodology used in these studies did not account for competing events.\(^{14,20-22}\)

Here, we performed a retrospective analysis of a cohort of 173 patients treated at our institution on a phase I/II clinical trial of defined-composition CD19 CAR T-cell therapy for B-cell malignancies. By applying the debiased LASSO (least absolute shrinkage and selection operator) to draw inference of effects of multiple variables, we identified key factors independently associated with prolonged hematopoietic toxicity after CD19 CAR T cell therapy.

**Methods**

**Patients and study design**
We performed a retrospective analysis of patients with R/R B-cell malignancies including ALL, non-Hodgkin lymphoma (NHL), and CLL, who were treated with CD19-targeted CAR T-cells on a phase 1/2 clinical trial at our institution (NCT01865617)\textsuperscript{1,2,8}. The CAR construct was comprised of a CD19-targeting single-chain variable fragment (scFv) derived from the FMC63 monoclonal antibody fused to an immunoglobulin-G4 (IgG4) hinge region, CD28 transmembrane domain, 4-1BB costimulatory domain, and a CD3ζ signaling sequence. The CAR construct was separated by a T2A ribosomal skip sequence from a truncated human epidermal growth factor receptor (EGFRt), which served as a marker of transgene expression as previously described\textsuperscript{1,8}. Patients received lymphodepleting chemotherapy with cyclophosphamide and fludarabine at high (60 mg/kg or >1500 mg/m\textsuperscript{2} cyclophosphamide with fludarabine 75-125 mg/m\textsuperscript{2}) or low (30 mg/kg or ≤1500 mg/m\textsuperscript{2} cyclophosphamide with fludarabine 75-90 mg/m\textsuperscript{2}) intensities or other regimens (Supplementary Table S2). Lymphodepletion was followed two to four days later by infusion of CD19 CAR T-cells formulated in a 1:1 ratio of CD4+:CD8+ CAR T-cells at one of three dose levels (DL1, 2 x 10\textsuperscript{5} EGFRt+ cells/kg; DL2, 2 x 10\textsuperscript{6} EGFRt+ cells/kg; DL3, 2 x 10\textsuperscript{7} EGFRt+ cells/kg).

For this analysis, we included 173 of the 195 patients who received treatment following enrollment in the study (Figure 1). Five patients with missing or incomplete data were excluded as were 17 patients with NHL treated on a pilot “dose-dense” cohort who received a planned second CAR T-cell infusion without additional lymphodepleting therapy 15 days after the first CAR T-cell infusion. The study was conducted according to the principles of the Declaration of Helsinki and with the approval of the Fred Hutchinson Cancer Research Center Institutional Review Board.

Our primary endpoint for hematologic toxicity was ANC and platelet count at day-28 following CAR T-cell infusion. As secondary endpoints, we assessed the cumulative incidence of hematopoietic recovery accounting for competing risks.
Evaluation of hematologic toxicity

**Hematopoietic recovery**

Criteria for neutropenia, thrombocytopenia, and recovery of neutrophil and platelet counts were defined as per the established Center for International Blood and Marrow Transplant Research (CIBMTR)\(^{23}\) reporting guidelines for cellular therapy: neutropenia, absolute neutrophil count (ANC) ≤ 0.5 x 10\(^9\)/L; thrombocytopenia, platelet count ≤ 20 x 10\(^9\)/L; neutrophil recovery, ANC >0.5 x 10\(^9\)/L for three consecutive laboratory values obtained on different days, irrespective of growth factor administration; platelet recovery, platelet count > 20 x 10\(^9\)/L for three consecutive values obtained on different days, in the absence of platelet transfusion for the preceding seven days.

**Grading of cytopenias**

Cytopenias were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.3 at a median of 14, 28, 60, 90, 120, and 180 days following CAR-T infusion (range 13-17, 23-33, 55-65, 85-95, 110-130, 160-200 days, respectively) in evaluable patients, with minimum value for ANC, hemoglobin, or platelet count within each date range selected. To grade pre-infusion cytopenias, the minimum value between the date of infusion and 7 days prior to infusion was selected.

**Evaluation and grading of CRS and ICANS, serum biomarkers, cytokines, and additional clinical laboratories**

Severity of CRS was graded according to the 2014 Lee criteria\(^{24}\). ICANS was graded according to CTCAE 4.0.3 for neurologic adverse events, with overall ICANS grade defined as the highest grade in each patient. Collection and analysis of serum biomarkers, cytokines, and additional laboratory parameters were performed as previously described\(^2,19\). The following serum
cytokines were evaluated in this study: TGFβ-1, IFNγ, IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, IL-22, IL-2Rα, MCP-1, MIP-1β, Fas, IL-6R, sTNFR1 (p55), sTNFR1 (p75), TNFα, TIM-3.

Statistical analysis

In evaluable patients, descriptive statistics excluded blood count data at time points following initiation of subsequent anti-tumor therapy, second CAR T-cell infusion, relapsed disease, or loss of follow-up.

Primary endpoint: day-28 ANC and platelet count

For linear regression the primary endpoints were ANC and platelet counts at day-28 following CAR T-cell infusion. Using a window of +/- 7 days, 164 of the 173 patients were evaluable for this analysis (Figure 1). The following patient-, disease-, and treatment-related variables were considered for univariate and multivariable analyses: age, sex, disease type (ALL, CLL, NHL), number of prior non-transplant treatments, prior autologous or allogeneic hematopoietic stem cell transplant (HSCT), CAR-T infusion dose level, CRS grade, pre-lymphodepletion neutrophil, lymphocyte, and platelet counts, pre-lymphodepletion D-dimer, prothrombin time (PT), partial thromboplastin time (PTT), ferritin, C-reactive protein (CRP), and fibrinogen levels.

A second analysis was performed to assess the contribution of CRS-related cytokines in which the CRS grade variable was replaced by each of the cytokines listed above. For this analysis, we selected peak serum concentrations after CAR T-cell infusion, with the exception of TGFβ-1, for which we used the trough serum concentration. This was determined by inspection of the serum concentration kinetics after CAR T-cell infusion categorized by CRS grade (Supplemental Figure S1), and by applying a non-parametric smoother (LOESS) to the longitudinal data.

A univariate linear regression model was used to assess for each risk factor with neutrophil and platelet recovery. For the joint analysis, a novel debiased LASSO model was applied. First, regression coefficients were penalized using the LASSO approach. Next, the penalized
coefficient estimates were debiased to allow for statistical inference (confidence intervals and null hypothesis testing). Multiple imputation using chained equations and predictive mean matching was used for selected variables which were missing for <25% of patients included in analysis. We conducted univariate analysis on three variables (pre-lymphodepletion C-reactive protein, fibrinogen, and D-dimer) with >25% missing values before and after imputation (number and proportion of patients with missing data shown in Supplementary Table S5). As none were statistically significant at the $p = 0.05$ level, these were removed from the covariate list. Regression beta coefficients were reported with 95% confidence intervals (CIs).

Secondary endpoints: cumulative incidence of ANC and platelet recovery

For our secondary endpoints, we chose a method accounting for competing risks, since clinical events unrelated to CAR T-cells may preclude hematopoietic recovery after CD19 CAR T-cell therapy. An event was defined as neutrophil or platelet recovery by the CIBMTR criteria as described above, while the following events were considered as competing events: death, new anti-tumor therapy, or disease relapse with marrow involvement in the absence of neutrophil or platelet recovery. As noted above, patients receiving a second CAR-T infusion as part of the "dose-dense" expansion cohort were excluded from analysis. For patients included in the analysis who received more than one CAR T-cell infusion, second lymphodepletion (if administered) or second CAR-T infusion (if administered without second lymphodepletion) were also considered as competing events. Patients who never met the CIBMTR criteria for neutropenia or thrombocytopenia were considered as having recovered at time = 0. Median follow-up time was estimated using reverse Kaplan-Meier analysis. The cumulative incidences of neutrophil and platelet recovery were estimated using the Kalbfleisch and Prentice method, and univariate comparisons across categories performed using Gray’s test.
Data analysis was performed using R (version 3.6.3), RStudio (version 1.4.1106), and the following packages: cmprsk, ggplotly, ggpubr, gsummary, hdi, mice, rms, scales, survival, survminer, and tidyverse.

Results

Patient characteristics

One hundred seventy-three patients with relapsed/refractory B-cell malignancies (ALL, n = 62; CLL, n = 48; NHL, n = 63) were included, with a median age of 55 years (range, 20-76). Patients were heavily pre-treated with a median of 4 prior therapies (range, 1-11) and with 61 (35%) having undergone hematopoietic stem cell transplantation (autologous, 23 [13%]; allogeneic, 35 [20%]; both autologous and allogeneic 3 [2%]). One hundred forty-six (85%) received a lymphodepletion regimen containing both cyclophosphamide and fludarabine at either high (80, 47%) or low (66, 39%) intensity and 25 (15%) received an alternative regimen (see Supplemental Table S2). Median ANC and platelet counts prior to lymphodepletion were 2.41 x 10^9/L (range 0-23) and 123 x 10^9/L (range 7-448), respectively. Median pre-lymphodepletion abnormal bone marrow B-cell percentage was 8% (range 0-98%). Patients received CAR T-cells infused at one of three dose levels. Patient, disease, and treatment characteristics are summarized in Table 1.

Hematologic toxicity after CD19 CAR T-cell therapy

Severity of hematologic toxicities

A significant proportion of patients presented with severe cytopenia after CAR T-cell therapy. We observed grade ≥ 3 neutropenia at day 0, 14, and 28 in 58.7% (95% CI 51.0-66.1), 49.1% (95% CI 41.3-56.9), and in 45.9% (95% CI 37.9-54.0), respectively (Figure 2A). We observed grade ≥ 3 thrombocytopenia at day 0, 14, and 28 in 36.6% (95% CI 29.4-44.3), 44.3% (95% CI 36.6-52.2), and in 33.8% (95% CI 26.4-41.7) of patients, respectively (Figure 2B). Grade 3
Anemia was noted at day 0, 14, and 28 in 14.5% (95% CI 9.6-20.7), 16.2% (95% CI 10.9-22.6), and in 14.6% (95% CI 9.5-21.2) of patients, respectively (Figure 2C). The observed frequencies of cytopenia up to 180 days after CAR T-cell infusion are shown in Figure 2. As faster hematopoietic recovery could reflect response to treatment, we assessed the relationship between day 28 ANC and platelet count and response at first assessment in evaluable patients, typically performed at day 28 post-CAR T-cell infusion. Patients were categorized as responders (complete remission, complete remission with minimal residual disease, or partial response) or non-responders (stable disease or progression of disease). No statistically significant difference (Wilcoxon unpaired test) was noted for either ANC or platelet count (Supplemental Figures S2A and S2C). When stratified by disease cohort, day 28 ANC was lower with statistical significance in non-responders within the ALL cohort (p = 0.037), however no significant differences were noted for day 28 platelet count in ALL or for either measure in CLL and NHL cohorts. (Supplementary Figure S2B and S2D).

**ANC and platelet kinetics post CAR T-cell infusion**

To visualize trends in ANC and platelet counts and to assess recovery at our primary endpoint of day-28 post-CAR T-cell infusion, we applied a non-parametric smoother to longitudinal data (Figure 3) grouping subjects by CRS grade. Mean ANC (x 10⁹/L) at day-28 was 1.54, 1.21, 1.42, and 0.65 and mean platelet count (x 10⁹/L) was 70.8, 63.1, 44.7, and 26.1 for CRS grades 0, 1, 2, and 3-5 respectively. Mean time to ANC recovery by CRS grade was 4.2, 5.9, 6.5, and 12.8 days for CRS grades 0, 1, 2, and 3-5 respectively. ANC and platelet counts (median and interquartile range) at day-28 post-CAR T-cell infusion for evaluable patients overall and by baseline characteristics are shown in Table 2, with continuous variables stratified by quartile.

**Cumulative incidence (CI) of hematopoietic recovery**
After a median follow-up time of 40.8 months (ALL, 45.7 months; CLL, 26.6 months; NHL 47.3 months), the proportion of evaluable patients demonstrating ANC and platelet recovery according to the CIBMTR criteria after CD19 CAR T-cell therapy were 91% (ALL, 85%; CLL, 95%; NHL, 98%) and 86% (ALL, 82%; CLL, 83%; NHL, 90%), respectively. Median time to ANC recovery was 8 days (range 0 – 146) and the cumulative incidence of ANC recovery at day 28, 60, and 90 were 81% (95% CI, 75-87), 88% (95% CI, 83-93) and 89% (95% CI, 85-94), respectively (Figure 5A). Median time to platelet recovery was 0 days (range 0 – 173) and the cumulative incidence of platelet recovery on the day of CAR T-cell infusion was 58% (95% CI, 50-65); rising to 76% (95% CI, 69-82), 84% (95% CI, 78-89), and 84% (95% CI, 79-90) at day 28, 60, and 90, respectively (Figure 5B). When excluding patients who never met criteria for neutropenia or thrombocytopenia (Supplemental Figure S3), median time to ANC recovery was 8.75 days (range 1 – 146) and median time to platelet recovery was 36.5 days (range 3 – 173). Cumulative incidence of hematopoietic recovery stratified by disease type is shown in Supplemental Figure S4.

**Multivariable analyses of day-28 ANC and platelet count**

We applied debiased LASSO in a high-dimension linear regression model to identify independent predictors of ANC (Figure 4A) and platelet (Figure 4B) recovery. We considered patient, disease, and treatment-related predictors in our models as described in the Methods section. Univariate analyses are shown in the Supplementary Material (Table S2A and S2B).

Higher CRS grade was associated with lower day-28 platelet count (beta -0.09 per CRS grade, 95% CI -0.16 – -0.014, p = 0.019) and suggested a similar trend for ANC but we could not reject the null with a similar but non-significant trend for ANC (beta -0.07, 95% CI -0.17 – 0.04, p = 0.24). In contrast, higher pre-lymphodepletion platelet count was associated with higher day-28 ANC (beta 0.22 per log_{10} platelet x 10^{9}/L, 95% CI 0.127 – 0.32, p < 0.00001) and higher day-28 platelet count (beta 0.21 per log_{10} platelet x 10^{9}/L, 95%CI 0.150 – 0.279). Confirming our
observations, the univariate cumulative incidence of ANC recovery at day-28 by pre-LD platelet was 58% (95%CI 43 –72), 84% (95%CI 72 – 95), 90% (95%CI 81 – 100), and 93% (95%CI 85 – 100) for pre-LD platelet count of 7 – 66, 67 – 124, 125 – 197, and 198 – 448 x 10^9/L, respectively (Figure 5C) and the univariate CI of platelet recovery at day 28 by pre-LD platelet count was 44% (95% CI 30 – 59), 86% (95% CI 75 – 97), 90% (95% CI 81 – 100), and 84% (95% CI 72 – 95) for the same quartiles (Figure 5D).

We could not confirm associations between several patient, disease, and treatment factors and hematopoietic recovery that were suggested in other studies. Notably, these included bone marrow disease burden prior to lymphodepletion (ANC: beta -0.01, 95%CI -0.145 – 0.123, p = 0.87; platelet: beta 0.08, 95%CI -0.009 – 0.17, p = 0.08) and number of prior treatment regimens (ANC: beta 0.00 per additional treatment, 95%CI -0.10-0.10, p = 0.96; platelet: beta -0.03, 95%CI -0.099 – 0.036, p = 0.36). Similarly, the association between CAR-T dose level and hematopoietic recovery was undetermined (ANC: beta 0.05 per dose level, 95%CI -0.053-0.148, p = 0.36; platelet: beta -0.02, 95%CI -0.09-0.04, p = 0.49).

Cytokine levels and day-28 ANC and platelet count

Since our findings suggested that CRS grade was a key factor impacting hematopoietic recovery, we included a panel of selected CRS-related cytokines in our high-dimensional linear regression models, in addition to cytokines known to have a role in hematopoiesis (Figures 4C and D), and retaining key baseline variables, pre-infusion ANC and platelet counts.

Higher trough serum concentrations of TGFβ-1 were associated with higher day-28 ANC (beta 0.23 per log_{10} pg/mL increase, 95% CI 0.127 – 0.325, p < 0.00001) and higher day-28 platelet count (beta 0.18, 95% CI 0.116 – 0.238, p < 0.00001). In contrast, higher peak IL-6 serum concentration was associated with lower day-28 ANC (beta -0.18 per log_{10} pg/mL increase, 95% CI -0.31 – -0.05, p = 0.006).
Discussion

CAR T-cell therapy has been associated with significant cytopenia responsible for prolonged transfusion requirements and associated with increased risk of infections and bleeding events. Since this complication remains poorly characterized, we studied the kinetics of hematopoietic recovery and the factors associated with hematologic toxicity after CD19 CAR T-cell therapy in a large cohort of 173 patients.

Importantly, the day-28 CI of neutrophil and platelet recovery in all patients were 81% and 89%, respectively, indicating a significant proportion of patients have impaired hematopoiesis after CD19 CAR T-cell therapy. Using the debiased LASSO for high-dimensional linear regression modeling, we identified key factors independently associated with hematopoietic recovery. Our approach achieves robust variable selection in multivariable analysis as it can account for large numbers of covariates and highly correlated covariates. Importantly, it provides statistical inference with robust estimates for coefficients and allows for the computation of confidence intervals for all coefficients, which cannot be achieved by other approaches such as stepwise regression or linear regression using non-debiased LASSO. CRS severity was independently predictive of day-28 platelet count and the pre-LD platelet count was independently predictive of both day-28 ANC and platelet count, though prior work from our group also noted an association between baseline platelet count and severity of subsequent CRS. The identification of pre-LD platelet count suggests poor bone marrow “reserve” or treatment-related bone marrow injuries may be a key factor associated with impaired hematopoietic recovery after CD19 CAR T-cell therapy. Other studies suggested that the intensity of lymphodepletion regimen and prior treatment regimens may impact hematopoietic recovery following CAR T-cell therapy. Our study did not determine an association with either number of prior treatment regimens or status of prior autologous or allogeneic transplant and the day-28 ANC or platelet count. Because
selection of lymphodepletion regimen intensity was influenced by patients’ pretreatment baseline counts and the disease type, we did not include this variable in our analysis.

Since our multivariable modeling suggested a strong detrimental effect of CRS severity on hematopoietic recovery, we sought to investigate the impact of twenty serum cytokines associated with CRS on ANC and platelet recovery. Higher peak serum concentrations of IL-6 known to be strongly associated with CRS severity, was independently associated with impaired hematopoietic recovery in our multivariable analysis, while higher peak concentration of TGF-β was associated with improved recovery.

In our study, we noted an association between higher peak IL-6 serum concentration and slower hematopoietic recovery. IL-6 is a pleotropic cytokine known to have pleiotropic effects on the hematologic system, including megakaryocytic maturation and platelet release from the bone marrow, myeloid differentiation during neutropenia, T-cell differentiation, and stimulation of antibody production from B-cells. Further studies are needed to clarify the role of IL-6 in hematologic toxicity after CAR T-cell therapy. We hypothesize high IL-6 serum concentrations may reflect a homeostatic increase to stimulate hematopoiesis in response to cytopenia, though we acknowledge that IL-6 serum concentrations in the bone marrow were not measured in our study. Furthermore, the effects of therapeutic IL-6R blockade with tocilizumab on hematopoietic precursors and its contribution to hematologic toxicities of CAR T-cells remain unknown. We could not identify an independent association between the use of tocilizumab and hematopoietic recovery (Supplementary Table S4).

Higher serum concentrations of TGF-β were associated with improved hematopoietic recovery. TGF-β is a pleotropic cytokine expressed in a variety of tissues and stored in high concentrations within α granules of platelets. TGF-β has complex effects on hematopoietic system both on mature cells and stem cell progenitors. While TGF-β is known to mediate cell-cycle arrest in CD34+ cells, more recent evidence suggest differential response to TGF-β
signaling by different subsets of HSC, with proliferation of myeloid producing HSCs and inhibition of lymphoid producing HSCs,\textsuperscript{39} and our findings may reflect this activity. Alternatively, lower TGF-β serum concentrations may be reflective of decreased platelet production or CRS-related platelet consumption; we and other groups have shown that severe CRS is associated with consumptive coagulopathy.\textsuperscript{19,40,41} Maintenance of higher TGF-β serum concentrations may also reflect its homeostatic immunomodulatory effects during CAR T-cell activation\textsuperscript{37}.

While further studies are needed to characterize the biological effects of CRS-related cytokines under these conditions on hematopoiesis, our findings suggest interventions that may have immediate clinical impact. In our cohort, patients with pre-lymphodepletion platelet counts falling into the lowest quartile exhibit prolonged hematopoietic recovery. This suggests that risk stratification taking pre-lymphodepletion platelet count into consideration when enrolling patients in CAR T-cell clinical trials, selecting lymphodepletion regimen intensity, number of CAR T-cell infusions, or CAR T-cell dose may be beneficial. The identification of CRS and CRS-related cytokines as predictors of delayed hematopoietic recovery in our study suggests that both early identification, through frequent monitoring of cytokines and inflammatory markers, and early interventions to prevent high-grade CRS, may be beneficial to mitigate hematopoietic toxicity.

In summary, we characterized the kinetics and pattern of hematopoietic recovery in 173 patients treated with CD19 CAR T-cell therapy for R/R B-cell malignancies. Pre-lymphodepletion blood counts, CRS severity and CRS-related cytokines independently impacted hematopoietic recovery after CD19 CAR T-cell therapy. Our study suggests that both patient selection and advancing CRS management may improve hematopoietic recovery after CD19 CAR T-cell therapy.
Data sharing statement

Dataset will be made available upon request to the corresponding author: jgauthier@fredhutch.org. CBC data from a subset of patients was reported in Hay et. al., *Blood*. 2017;130(21):2295-2306.

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Author contributions

*Conception and design:* Krishna R. Juluri, Merav Bar, Jordan Gauthier

*Collection and assembly of data:* Krishna R. Juluri, Jordan Gauthier, Jenna Voutsinas, Qian “Vicky” Wu

*Data analysis and interpretation:* Krishna R. Juluri, Merav Bar, Jordan Gauthier, Jenna Voutsinas, Qian “Vicky” Wu

*Manuscript writing:* All authors

*Final approval of manuscript:* All authors
Accountable for all aspects of the work: All authors

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Figure Legends

Figure 1. Inclusion and exclusion criteria for analysis.

*Patients excluded from analysis at time of first competing event following CAR T-cell infusion, defined as relapse with marrow involvement, new cytotoxic therapy, second lymphodepletion or CAR T-cell infusion, death, or loss to follow-up.

Abbreviations: CAR, chimeric antigen receptor; NHL, non-Hodgkin lymphoma

Figure 2. Percentage of patients with neutropenia (A), thrombocytopenia (B), and anemia (C) by post-CAR T-cell infusion day, stratified by CTCAE grade.

Due to variability in collection dates, for day = n, the minimum cell count falling within an arbitrary range of n was selected for each patient (i.e. Day 0 = day -7 and 0, Day 14 = day 12 – 16, Day 28 = day 23 – 33, Day 60 = day 55 – 65, Day 90 = day 85 – 95, Day 120 = day 110 – 130, Day 180 = day 160 – 200. Patients were no longer included in this analysis pending receipt of of subsequent line of therapy, second CAR T-cell infusion, or if discontinued off study)

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events

Figure 3. Kinetics of Hematopoietic Recovery

Longitudinal ANC and platelet counts (log scale) are depicted on a spaghetti plot with the application of a non-parametric (LOESS) smoother to subjects grouped by CRS grade.

Figure 4. Forest plots of regression coefficients for day-28 neutrophil (A and C) or platelet (B and D) counts determined by high dimensional inference for selected patient, disease, and treatment characteristics (A and B) or serum cytokine concentrations (C and D).

Regression coefficient and associated 95% CI denoted by circles and lines from a linear regression model, respectively. P-value of regression coefficient denoted by color gradient. CRS and ICANS variables are stratified by grade. Disease cohorts (CLL and NHL) and sex (female) are compared against a reference variable, ALL and male, respectively. All other variables are modelled as continuous variables.

Abbreviations: ALC, absolute lymphocyte count; LD, lymphodepletion; PT, prothrombin time; PTT, partial thromboplastin time; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TGF, transforming growth factor; TIM, T-cell immunoglobulin and mucin domain-containing protein; TNF, tumor necrosis factor; sTNFR, soluble TNF-receptor

Figure 5. Cumulative incidence (CI) of neutrophil (A and C) and platelet (B and D) recovery as defined by CIBMTR criteria.

CI estimated using Kalbfleisch and Prentice method with univariate comparisons across categories using Gray’s test and stratified by entire cohort (A, B) or by pre-lymphodepletion platelet count (C, D, grouped by quartiles). Shaded areas represent 95% confidence intervals.

Abbreviations: CAR, chimeric antigen receptor
References

1. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+ :CD8+ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126(6):2123-2138.
2. Turtle CJ, Hay KA, Hanafi LA, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. *J Clin Oncol*. 2017;35(26):3010-3020.
3. 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.
4. Wang M, Munoz J, Goy A, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. *N Engl J Med*. 2020;382(14):1331-1342.
5. Port 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.
6. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
7. 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.
8. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin’s lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med*. 2016;8(355):355ra116.
9. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396(10254):839-852.
10. Hirayama AV, Gauthier J, Hay KA, et al. High rate of durable complete remission in follicular lymphoma after CD19 CAR-T cell immunotherapy. *Blood*. 2019;134(7):636-640.
11. Costa MD, Bezerra ED, Hirayama AV, et al. Late Events after Treatment with CD19-Targeted Chimeric Antigen Receptor Modified T Cells. *Biol Blood Marrow Transplant*. 2020;26(1):26-33.
12. Hirayama AV, Turtle CJ. Toxicities of CD19 CAR-T cell immunotherapy. *Am J Hematol*. 2019;94(S1):S42-s49.
13. Hill JA, Li D, Hay KA, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. *Blood*. 2018;131(1):121-130.
14. Logue JM, Zucchetti E, Bachmeier CA, et al. Immune reconstitution and associated infections following axicabtagene ciloleucel in relapsed or refractory large B-cell lymphoma. *Haematologica*. 2020;105:238634.
15. Brudno JN, Maric I, Hartman SD, et al. T Cells Genetically Modified to Express an Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor Cause Remissions of Poor-Prognosis Relapsed Multiple Myeloma. *Journal of Clinical Oncology*. 2018;36(22):2267-2280.
16. Shalabi H, Shah NN, Fry TJ, Yates B, Delbrook C. Chimeric Antigen Receptor Induced Cytopenia Differs from Chemotherapy Induced Myelosuppression. *Blood*. 2017;130(Supplement 1):5048-5048.
17. Moade 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.
18. Wudhikarn K, Palomba ML, Pennisi M, et al. Infection during the first year in patients treated with CD19 CAR T cells for diffuse large B cell lymphoma. *Blood Cancer Journal*. 2020;10(8):79.
19. Hay KA, Hanafi LA, Li D, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood*. 2017;130(21):2295-2306.
20. Fried S, Avigdor A, Bielorai B, et al. Early and late hematologic toxicity following CD19 CAR-T cells. Bone Marrow Transplant. 2019;54(10):1643-1650.

21. Nahas GR, Komanduri KV, Pereira D, et al. Incidence and risk factors associated with a syndrome of persistent cytopenias after CAR-T cell therapy (PCTT). Leukemia & Lymphoma. 2019;1-4.

22. Jain T, Knezevic A, Pennisi M, et al. Hematopoietic recovery in patients receiving chimeric antigen receptor T-cell therapy for hematologic malignancies. Blood Adv. 2020;4(15):3776-3787.

23. Center for International Blood & Marrow Transplant Research. Cellular Therapy Manuals - Peripheral Blood Count Recovery.

24. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-195.

25. Tibshirani R. Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Series B (Methodological). 1996;58(1):267-288.

26. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17(4):343-346.

27. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: John Wiley & Sons, Inc.; 1980.

28. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.

29. Gray RJ. A Class of $K$-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. Ann Statist. 1988;16(3):1141-1154.

30. Fu Z, Parikh CR, Zhou B. Penalized variable selection in competing risks regression. Lifetime Data Analysis. 2017;23:353-376.

31. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321-3330.

32. del Carmen Rodriguez Ma, Bernad A, Aracil M. Interleukin-6 deficiency affects bone marrow stromal precursors, resulting in defective hematopoietic support. Blood. 2004;103(9):3349-3354.

33. Feng X, Scheinberg P, Wu CO, et al. Cytokine signature profiles in acquired aplastic anemia and myelodysplastic syndromes. Haematologica. 2011;96(4):602-606.

34. Tie R, Li H, Cai S, et al. Interleukin-6 signaling regulates hematopoietic stem cell emergence. Experimental & Molecular Medicine. 2019;51(10):1-12.

35. Zhao JL, Ma C, O'Connell RM, et al. Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell. 2014;14(4):445-459.

36. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. J Biol Chem. 1983;258(11):7155-7160.

37. Oh SA, Li MO. TGF-β: guardian of T cell function. J Immunol. 2013;191(8):3973-3979.

38. Blank U, Karlsson S. TGF-β signaling in the control of hematopoietic stem cells. Blood. 2015;125(23):3542-3550.

39. Challen GA, Boles NC, Chambers SM, Goodell MA. Distinct hematopoietic stem cell subtypes are differentially regulated by TGF-beta1. Cell Stem Cell. 2010;6(3):265-278.

40. Santomasso BD, Park JH, Salloum D, et al. Clinical and Biological Correlates of Neurotoxicity Associated with CAR T-cell Therapy in Patients with B-cell Acute Lymphoblastic Leukemia. Cancer Discovery. 2018;8(8):958.

41. Teachey DT, Lacey SF, Shaw PA, et al. Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia. Cancer Discovery. 2016;6(6):664.
**Table 1. Patient and treatment characteristics by disease cohort**

| Disease Cohort (n, %) | ALL (n=62, 36%) | CLL (n=48, 28%) | NHL (n=63, 36%) | Total (N = 173) |
|-----------------------|-----------------|-----------------|-----------------|----------------|
| **Age (years) – median (IQR) [range]** | 40 (28, 54) [20-76] | 61 (55, 66) [40-73] | 58 (52, 64) [28-71] | 55 (43, 64) [20-76] |
| **Sex – n (%)** | | | | |
| Female | 26 (42) | 15 (31) | 17 (27) | 58 (34) |
| Male | 36 (58) | 33 (69) | 46 (73) | 115 (66) |
| **Race – n (%)** | | | | |
| White | 50 (80.6) | 43 (90) | 58 (92) | 151 (87) |
| Non-white | 12 (19.4) | 4 (8.3) | 5 (7.9) | 21 (12) |
| Unknown | 0 (0) | 1 (2.1) | 0 (0) | 1 (0.6) |
| **Number of prior therapies – median (IQR) [range]** | 3 (2, 4) [1 - 11] | 5 (4, 7) [1 - 10] | 4 (4, 6) [1 - 11] | 4 (3, 6) [1 - 11] |
| **Prior HSCT – n (%)** | | | | |
| Autologous | 0 (0) | 1 (2) | 22 (35) | 23 (13) |
| Allogeneic | 24 (39) | 7 (15) | 4 (6.3) | 35 (20) |
| Both | 0 (0) | 0 (0) | 3 (4.8) | 3 (1.7) |
| None | 38 (61) | 40 (83) | 34 (54) | 112 (65) |
| **Pre-LD marrow abnormal B-cells (%) – median (IQR) [range]** | 22.2 (1.71, 0.98) [0 - 98] | 52 (20, 74) [0 - 96] | 0 (0, 0) [0 - 92] | 8 (0, 57) [0 - 98] |
| **Pre-lymphodepletion ANC (x 10^3/L) – median (IQR) [range]** | 1.86 (0.88, 3.06) [0 - 7.62] | 2.13 (0.94, 4.30) [0 - 13.65] | 3.24 (1.98, 5.50) [0.23 - 23.17] | 2.41 (1.25, 4.23) [0.23 - 23.2] |
| **Pre-lymphodepletion ALC (x 10^3/L) – median (IQR) [range]** | 0.7 (0.4, 1.3) [0 - 5.1] | 1.9 (1.0, 7.6) [0.2 - 58.9] | 0.6 (0.4, 1.1) [0 - 8.8] | 0.9 (0.5, 1.7) [0 - 5.9] |
| **Pre-lymphodepletion hemoglobin (g/dL) – median (IQR) [range]** | 10.6 (9.8, 11.7) [7.3 - 14.8] | 10.6 (9.8, 12.35) [7.4 - 16] | 11.1 (9.8, 12.7) [7.2 - 15] | 10.8 (9.8, 12.2) [7.2 - 16] |
| **Pre-lymphodepletion platelet (x 10^9/L) – median (IQR) [range]** | 110 (48, 200) [9 - 339] | 114 (86, 146) [7 - 434] | 143 (80, 214) [9 - 448] | 123 (64, 196) [7 - 448] |
| **Lymphodepletion – n (%)** | | | | |
| High-intensity CyFlu | 31 (50) | 15 (31) | 34 (54) | 80 (46) |
| Low-intensity CyFlu | 19 (31) | 29 (60) | 18 (29) | 66 (38) |
| Non-CyFlu | 12 (19) | 4 (8.3) | 11 (17) | 27 (16) |
| **CAR T-cell dose – n (%)** | | | | |
| DL1 | 38 (61) | 5 (10) | 4 (6) | 47 (27) |
| DL2 | 22 (35) | 42 (88) | 50 (79) | 114 (66) |
| DL3 | 2 (3.2) | 1 (2.1) | 9 (14) | 12 (6.9) |
| **CRS grade – n (%)** | | | | |
| 0 | 14 (23) | 8 (17) | 30 (48) | 52 (30) |
| 1 | 12 (19) | 15 (31) | 13 (21) | 40 (23) |
| 2 | 23 (37) | 18 (38) | 14 (22) | 55 (32) |
| 3-5 | 13 (21) | 7 (15) | 6 (9.5) | 26 (15) |
| **Neurotoxicity grade – n (%)** | | | | |
| 0-1 | 39 (63) | 32 (67) | 50 (79) | 121 (70) |
| 2-3 | 19 (31) | 15 (31) | 11 (17) | 45 (26.0) |
| 4-5 | 4 (6.5) | 1 (2.1) | 2 (3.2) | 7 (4.0) |

High-intensity CyFlu, cyclophosphamide (Cy) 60 mg/kg or >1500 mg/m² with fludarabine (Flu) 75-125 mg/m²; low-intensity CyFlu, Cy 30 mg/kg or ≤1500 mg/m² with Flu 75-90 mg/m²; Non-CyFlu, any conditioning regimen other than as noted above including single agent Cy or Flu. CAR-T cell dose level (DL) - DL1 = 2 x 10^5 cells/kg, DL2 = 2 x 10^6 cells/kg, DL3 = 2 x 10^7 cells/kg. CRS grade as defined by Lee criteria. Neurotoxicity grade as defined by CTCAE 4.03. ALC, absolute lymphocyte count; HSCT, hematopoietic stem cell transplant; IQR, interquartile range; LD, lymphodepletion.
Table 2. ANC and platelet Count at day-28 post-CAR T-cell infusion

| Variable                        | ANC (x 10^9/L) Median (IQR) | Platelet (x 10^9/L) Median (IQR) |
|---------------------------------|-----------------------------|-----------------------------------|
| **Disease Cohort**              |                             |                                   |
| Total (n = 164)                 | 1.52 (0.87, 2.46)           | 82 (46, 149)                      |
| ALL (n = 59)                    | 1.27 (0.62, 2.02)           | 77 (32, 146)                      |
| CLL (n = 46)                    | 1.61 (0.88, 2.82)           | 84 (43, 121)                      |
| NHL (n = 59)                    | 1.64 (1.06, 2.51)           | 88 (60, 179)                      |
| **Sex**                         |                             |                                   |
| Female (n = 52)                 | 1.50 (0.92, 2.20)           | 72 (42, 130)                      |
| Male (n = 112)                  | 1.55 (0.84, 2.54)           | 91 (51, 153)                      |
| **Race**                        |                             |                                   |
| White (n = 143)                 | 1.53 (0.88, 2.41)           | 79 (44, 150)                      |
| Non-white (n = 20)              | 1.35 (0.84, 2.61)           | 112 (58, 154)                     |
| Unknown (n = 1)                 | 3.60 (3.60, 3.60)           | 140 (140, 140)                    |
| **Number of prior therapies**   |                             |                                   |
| <Q1 (1 - 2)                     | 1.71 (0.95, 2.23)           | 126 (51, 171)                     |
| Q1-Q2 (3 - 4)                   | 1.31 (1.07, 2.50)           | 78 (43, 162)                      |
| Q2-Q3 (5 - 6)                   | 1.41 (0.78, 3.10)           | 66 (46, 116)                      |
| >Q3 (7 - 11)                    | 1.60 (0.54, 2.44)           | 78 (42, 114)                      |
| **Prior HSCT**                  |                             |                                   |
| Autologous (n = 22)             | 1.42 (1.02, 2.34)           | 70 (58, 149)                      |
| Allogeneic (n = 34)             | 1.36 (0.49, 1.74)           | 76 (28, 112)                      |
| Both (n = 3)                    | 3.91 (3.56, 4.96)           | 88 (80, 152)                      |
| None (n = 105)                  | 1.58 (0.91, 2.53)           | 94 (50, 155)                      |
| **Pre-LD marrow abnormal B-cells (%)** |                     |                                   |
| <Q1 (0-0.006)                   | 1.61 (1.02, 2.58)           | 91 (61, 178)                      |
| Q1-Q2 (0.006 - 7.99)            | 1.72 (1.22, 2.55)           | 104 (56, 149)                     |
| Q2-Q3 (8.00 - 60.0)             | 1.68 (1.24, 2.65)           | 78 (49, 147)                      |
| >Q3 (60.1 - 98.5)               | 0.80 (0.26, 1.93)           | 55 (27, 115)                      |
| **Pre-lymphodepletion ANC (x 10^9/L)** |                     |                                   |
| <Q1 (0 - 1.35)                  | 1.00 (0.43, 1.90)           | 50 (24, 90)                       |
| Q1-Q2 (1.36 - 2.43)             | 1.47 (1.14, 2.13)           | 101 (60, 147)                     |
| Q2-Q3 (2.44 - 4.34)             | 1.74 (1.10, 2.84)           | 128 (62, 156)                     |
| >Q3 (4.35 - 23.17)              | 2.14 (1.17, 3.20)           | 86 (49, 201)                      |
| **Pre-lymphodepletion ALC (x 10^9/L)** |                     |                                   |
| <Q1 (0.00 - 0.51)               | 1.58 (0.79, 2.00)           | 61 (42, 113)                      |
| Q1-Q2 (0.52 - 0.89)             | 1.36 (0.93, 2.45)           | 116 (45, 174)                     |
| Q2-Q3 (0.90 - 1.94)             | 1.77 (1.24, 2.48)           | 83 (60, 147)                      |
| >Q3 (1.95 - 58.92)              | 1.35 (0.72, 2.31)           | 100 (41, 133)                     |
| **Pre-lymphodepletion hemoglobin (g/dL)** |                     |                                   |
| <Q1 (7.2 - 9.8)                 | 1.37 (0.58, 2.51)           | 64 (32, 107)                      |
| Q1-Q2 (9.9 - 10.8)              | 1.69 (0.56, 2.22)           | 74 (37, 143)                      |
| Q2-Q3 (10.9 - 12.1)             | 1.52 (1.03, 2.20)           | 83 (52, 142)                      |
| >Q3 (12.2 - 16.0)               | 1.70 (1.24, 2.71)           | 137 (70, 188)                     |
| **Pre-lymphodepletion platelet (x 10^9/L)** |                     |                                   |
| <Q1 (7 - 66)                    | 0.66 (0.24, 1.41)           | 33 (20, 64)                       |
| Q1-Q2 (67 - 126)                | 1.53 (1.10, 2.84)           | 79 (55, 115)                      |
| Q2-Q3 (127 - 197)               | 1.68 (1.17, 2.23)           | 125 (62, 164)                     |
| >Q3 (198 - 448)                 | 2.20 (1.24, 3.42)           | 174 (76, 215)                     |
| **Lymphodepletion**             |                             |                                   |
| High-intensity CyFlu (n = 77)   | 1.58 (1.07, 2.53)           | 107 (55, 161)                     |
| Low-intensity CyFlu (n = 61)    | 1.53 (0.82, 2.39)           | 62 (31, 113)                      |
| Non-CyFlu (n = 26)              | 1.23 (0.88, 2.09)           | 104 (60, 196)                     |
| **CAR T-cell dose**             |                             |                                   |
| DL1 (n = 46)                    | 1.21 (0.46, 2.10)           | 86 (36, 155)                      |
| DL2 (n = 108)                   | 1.63 (1.02, 2.70)           | 80 (54, 138)                      |
| DL3 (n = 10)                    | 1.53 (1.03, 2.15)           | 144 (61, 217)                     |
| **CRS grade**                   |                             |                                   |
| 0 (n = 50)                      | 1.62 (1.01, 2.31)           | 89 (57, 172)                      |
| 1 (n = 39)                      | 1.97 (1.12, 3.11)           | 105 (58, 144)                     |
High-intensity CyFlu, cyclophosphamide (Cy) 60 mg/kg or >1500 mg/m² with fludarabine (Flu) 75-125 mg/m²; low-intensity CyFlu, Cy 30 mg/kg or ≤1500 mg/m² with Flu 75-90 mg/m²; Non-CyFlu, any conditioning regimen other than as noted above including single agent Cy or Flu. CAR-T cell dose level (DL) - DL1 = 2 x 10⁵ cells/kg, DL2 = 2 x 10⁶ cells/kg, DL3 = 2 x 10⁷ cells/kg. CRS grade as defined by Lee criteria²¹. Neurotoxicity grade as defined by CTCAE 4.0.3. ALC, absolute lymphocyte count; HSCT, hematopoietic stem cell transplant; IQR, interquartile range; LD, lymphodepletion; Q1, 25% quantile (1st quartile); Q2, 50% quantile (median); Q3, 75% quantile (3rd quartile).

| Neurotoxicity grade – n (%) | 2 (n = 53) | 3-5 (n = 22) | 84 (32, 147) | 50 (26, 98) |
|---------------------------|-----------|--------------|--------------|------------|
| 0-1 (n = 117)             | 1.27 (0.81, 2.14) | 1.44 (0.42, 2.22) | 1.64 (0.99, 2.55) | 82 (50, 155) |
| 2-3 (n = 44)              | 1.28 (0.76, 2.04) | 1.68 (1.17, 3.35) | 29 (28, 47) |
| 4-5 (n = 3)               | 1.44 (0.42, 2.22) | 85 (48, 130) |

1.27 (0.81, 2.14) 1.44 (0.42, 2.22) 84 (32, 147) 50 (26, 98)
Patients with relapsed/refractory B-cell malignancies screened on phase I/II trial of CD19-targeted CAR T-cell therapy (n=226)

Enrolled in study (n=198)

Received at least one infusion of CD19 CAR T-cells (n=195)

NHL dose-dense cohort expansion receiving second CAR T-cell infusion (n=17)

Incomplete data (n=5)

Excluded from analysis n=22

Cohort included in descriptive and competing risk analyses (n=173)

Death prior to day 28 or incomplete data (n=9)

Patients evaluable at day 28 post-CAR T-cell infusion included in multivariable analyses (n=164)
Figure 2. Severity of Hematologic Toxicities

A. Neutropenia Grade by Post-Infusion Day

B. Thrombocytopenia Grade by Post-Infusion Day

C. Anemia Grade by Post-Infusion Day
Figure 3. Kinetics of Hematopoietic Recovery

A) ANC Post-CAR T-cell Infusion

B) Platelet Count Post-CAR T-cell Infusion
Figure 4. Multivariable Analysis of Factors Associated with Day-28 ANC/Platelet Count

A

Factors Associated with Day-28 ANC

B

Factors Associated with Day-28 Platelet Count

C

Cytokines Associated with Day-28 ANC

D

Cytokines Associated with Day-28 Platelet Count

Pre-LD platelet -0.20 -0.10 0.00 0.10 0.20 0.30
Pre-LD PT
CRS grade
Pre-LD ALC
Female sex
CAR-T dose level
CLL
Age
NHL
Prior allogeneic HSCT
Pre-LD ferritin
Pre-LD ALC
Pre-LD PT
Bone marrow disease burden
Prior autologous HSCT
ICANS grade
Number of prior treatments

Pre-LD platelet -0.20 -0.10 0.00 0.10 0.20 0.30
CRS grade
Bone marrow disease burden
ANC Pre-LD
Pre-LD PT
Pre-LD ferritin
CAR-T dose level
Prior autologous HSCT
Age
Number of prior treatments

Pre-LD platelet -0.20 -0.10 0.00 0.10 0.20 0.30
Pre-LD ALC
IL-7
TNF-alpha
IL-6
IL-10
sTNFR1 (p75)
IL-2R-alpha
IL-5
IL-15
IL-18
Pre-LD platelet

Pre-LD platelet -0.20 -0.10 0.00 0.10 0.20 0.30
CRS grade
Bone marrow disease burden
ANC Pre-LD
Pre-LD PT
Pre-LD ferritin
CAR-T dose level
Prior autologous HSCT
Age
Number of prior treatments

Pre-LD platelet -0.20 -0.10 0.00 0.10 0.20 0.30
Pre-LD ALC
IL-2R-alpha
sTNFR1 (p75)
IL-7
TNF-alpha
IL-6
sTNFR1 (p75)
IL-2R-alpha
Pre-LD platelet

p-value
0.050
0.001

Regression Beta Coefficient (95% CI)
