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## Cytokine Release Syndrome After Chimeric Antigen Receptor T Cell Therapy for Acute Lymphoblastic Leukemia

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## Abstract

**Objective**—Initial success with chimeric antigen receptor–modified T cell therapy for relapsed/refractory acute lymphoblastic leukemia is leading to expanded use through multicenter trials. Cytokine release syndrome, the most severe toxicity, presents a novel critical illness syndrome with limited data regarding diagnosis, prognosis, and therapy. We sought to characterize the timing, severity, and intensive care management of cytokine release syndrome after chimeric antigen receptor–modified T cell therapy.

**Design**—Retrospective cohort study.

**Setting**—Academic children’s hospital.

**Patients**—Thirty-nine subjects with relapsed/refractory acute lymphoblastic leukemia treated with chimeric antigen receptor–modified T cell therapy on a phase I/IIa clinical trial (ClinicalTrials.gov number NCT01626495).

**Interventions**—All subjects received chimeric antigen receptor–modified T cell therapy. Thirteen subjects with cardiovascular dysfunction were treated with the interleukin-6 receptor antibody tocilizumab.

**Measurements and Main Results**—Eighteen subjects (46%) developed grade 3–4 cytokine release syndrome, with prolonged fever (median, 6.5 d), hyperferritinemia (median peak ferritin, 60,214 ng/mL), and organ dysfunction. Fourteen (36%) developed cardiovascular dysfunction treated with vasoactive infusions a median of 5 days after T cell therapy. Six (15%) developed acute respiratory failure treated with invasive mechanical ventilation a median of 6 days after T cell therapy; five met criteria for acute respiratory distress syndrome. Encephalopathy, hepatic, and renal dysfunction manifested later than cardiovascular and respiratory dysfunction. Subjects had a median of 15 organ dysfunction days (interquartile range, 8–20). Treatment with tocilizumab in 13 subjects resulted in rapid defervescence (median, 4 hr) and clinical improvement.

**Conclusions**—Grade 3–4 cytokine release syndrome occurred in 46% of patients following T cell therapy for relapsed/refractory acute lymphoblastic leukemia. Clinicians should be aware of expanding use of this breakthrough therapy and implications for critical care units in cancer centers.

## Keywords

cell therapy; leukemia; multiple organ failure; shock; tocilizumab

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Up to 90% of children with newly diagnosed B-precursor acute lymphoblastic leukemia (B ALL), the most common childhood malignancy, survive (1–3). However, survival remains poor for those with relapsed/refractory disease (4, 5). Newly reported strategies using chimeric antigen receptor (CAR)-modified T cells directed against the B cell antigen cluster

of differentiation 19 (CD19) effectively treat relapsed/refractory B ALL in children (6–8). CD19 CAR therapy involves genetically engineering a patient’s T cells to target CD19-expressing B cells (both normal and malignant) (9). CAR binding results in T cell activation, antigen-mediated cell killing, and T cell proliferation. Robust expansion and sustained proliferation of the modified T cell population can eradicate the cancer and appears to sustain remission in many patients (6–8, 10, 11). In a phase I/IIa trial of CD19 CAR T cells (CTL019) in relapsed/refractory B ALL, we observed a 90% complete remission rate with a 6-month event-free survival of 67% and overall survival of 78% (8).

One unique, significant toxicity following CAR therapy is cytokine release syndrome (CRS) (9, 12, 13). CRS is a systemic inflammatory response syndrome (SIRS) related to activated T cell proliferation with release of high levels of inflammatory cytokines (8, 12, 13). CRS typically peaks during maximal in vivo T cell proliferation (6, 13), which occurs days after CAR T cell infusion. CRS can result in profound shock and multiple organ dysfunction syndrome (MODS) with rapid evolution to life-threatening critical illness. Early recognition and appropriate therapy can control the risk of mortality due to CRS.

Due to promising results from early phase trials in pediatric relapsed/refractory B ALL and CTL019 receipt of the Food and Drug Administration Breakthrough Therapy status, CAR T cell therapy use is expanding through numerous multicenter trials. Thus, the occurrence of CRS is expected to rise. Unique therapeutic considerations are relevant for CRS to minimize toxicity while preserving the antileukemic activity of the CAR T cells. We characterized the time course, clinical phenotype, and intensive care management of severe (grade 3–4) CRS following CTL019 therapy in pediatric patients with relapsed/refractory B ALL. Since emerging evidence supports an important role for interleukin (IL)-6 in CRS (6–9, 12, 13), we report the effects of cytokine-directed immunotherapy with the humanized monoclonal anti-IL-6 receptor antibody, tocilizumab, on CRS clinical manifestations.

## METHODS

We conducted an analysis of CRS for 39 consecutive subjects treated with CTL019 through an institutional review board–approved phase I/IIa clinical trial (ClinicalTrials.gov number NCT01626495) at The Children’s Hospital of Philadelphia between April 2012 and September 2014. All subjects or their guardians provided written informed consent for the phase I/IIa trial, including approval for future data collection and analysis.

Efficacy data for the first 25 pediatric subjects treated in the CTL019 trial have been previously published (8), although details regarding time course, organ dysfunction severity, and response to critical care interventions have not been reported. Patients with relapsed/refractory CD19+ ALL who were ineligible for allogeneic hematopoietic stem cell transplant (HSCT) or who had relapsed after allogeneic HSCT were eligible for the phase I/IIa trial. Exclusion criteria and details of T cell therapy for the parent trial are provided in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>).

Data were abstracted from the medical record (Supplemental Methods, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>), and were anchored to the first day of CTL019 infusion (day 0). Subjects were followed for 28 days. We used published criteria to define organ dysfunction (14), modified as described in the Supplemental Methods (Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>). Severity of illness was measured by the Pediatric Index of Mortality (PIM)-2 (15). Inotrope scores were calculated using dosages of dopamine, epinephrine, and norepinephrine normalized to dopamine dosage in  $\mu\text{g}/\text{kg}/\text{min}$  (16). To characterize CRS severity, we have developed a descriptive grading scale (Table 1) (17).

Statistical analysis was performed using STATA (Version 12.1; StataCorp, College Station, TX). Descriptive data are presented as means  $\pm$  SD or medians with interquartile ranges (IQRs) for continuous variables and proportions for categorical variables. Differences between medians were analyzed using Wilcoxon signed rank test. Changes in vital signs over time were compared using Wilcoxon signed rank test. Kaplan-Meier and Cox regression analysis were used to evaluate the association of ferritin levels and time to resolution of organ dysfunction (Supplemental Methods, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>). Statistical significance was defined as a  $p$  value of less than 0.05.

## RESULTS

The initial 39 subjects with B ALL treated on the phase I/IIa trial of CTL019 were included in this analysis. The median age was 11 years (range, 5–22). Thirty-six subjects (92%) developed symptoms attributable to CRS: two with grade 1 (8%), 16 with grade 2 (41%), seven with grade 3 (18%), and 11 with grade 4 (28%). Subject characteristics by CRS grade were similar (Table 2).

Five of seven subjects with grade 3 and all subjects with grade 4 CRS were treated in the ICU for management of CRS-related organ dysfunction. The median time from CTL019 infusion to ICU admission was 5.6 (IQR, 3.7–6.2) days. Median PIM-2 score at ICU admission was 1.75 (IQR, 1.40–4.85), and median ICU length of stay was 7.8 (IQR, 2.9–14.9) days.

CRS was characterized by prolonged high fevers, tachycardia, and myalgias. Fever duration was longer in those with grade 3–4 CRS compared to those with grade 0–2 CRS: 7 (IQR, 4–9) versus 5 (IQR, 2–6) days ( $p = 0.04$ ). In subjects with grade 3–4 CRS, fever peaked a median of 5 days (IQR, 3–7) after CTL019 infusion, and the median peak heart rate was 170 beats/min (IQR, 156–186). All patients with fever underwent infectious evaluations, yet only one of 18 patients with grade 3–4 CRS had an identified infection within 1 week of CTL019 therapy.

The evolution of tachycardia and hypotension (Supplemental Fig. 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>) and laboratory and inflammatory marker abnormalities (Fig. 1) in the CRS grade 3–4 subjects were tracked. Subjects with grade 3–4 CRS developed a macrophage activation syndrome/hemophagocytic lymphohistiocytosis

(MAS/HLH)-like clinical picture: 12 subjects met MAS/HLH diagnostic criteria (with five of eight diagnostic criteria present), three had four criteria, and three had three criteria (Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>). All had fever, cytopenias, and peak ferritin levels greater than 1,123 pmol/L (500 ng/mL), 10 had fibrinogen less than 4.4  $\mu$ mol/L (150 mg/dL, measured in 16), and cryoprecipitate was administered to seven for coagulopathy. The median peak ferritin level was 135,300 pmol/L (60,214 ng/mL [IQR, 27,000–292,000 pmol/L or 12,000–130,000 ng/mL]).

The time course of organ dysfunction is presented in Figure 2. Subjects had a median of 15 (IQR, 8–20) days of organ dysfunction. Fourteen subjects developed MODS, with MODS onset a median of 5 days (IQR, 4–7) after CTL019 infusion. Time from organ dysfunction onset to resolution was longer for subjects with peak ferritin values greater than 11,200 pmol/L by day 5 after CTL019 infusion (hazard ratio, 0.17; 95% CI, 0.04–0.67;  $p = 0.011$ ; Supplemental Fig. 2, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>).

Fourteen subjects (36%) developed cardiovascular dysfunction: 13 had profound fluid-refractory vasoplegic shock treated with  $\alpha$ -agonist infusions and one had cardiomyopathy with diminished left ventricular systolic function supported with milrinone. Cardiovascular dysfunction developed a median of 5 days (IQR, 5–7) after CTL019 infusion, with a median duration of 4 days (IQR, 2–6). Hypotension was always preceded by fever and did not occur in the outpatient setting as we admitted all patients with fevers after CTL019. Shock was catecholamine resistant in 10 of 14 subjects, requiring more than one vasoactive agent. Vasopressin was used as a third or fourth agent in four subjects. Peak daily inotrope scores for those with grade 3 versus 4 CRS are shown in Supplemental Figure 3 (Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>). The total IV fluid volume administered in the 72 hours surrounding onset of shock was 270 mL/kg (IQR, 215–351). The median total output over the same time period was 127 mL/kg (IQR, 101–221), resulting in a substantial net positive fluid balance for most subjects.

The most common organ dysfunction was hepatic dysfunction (most commonly transaminitis), present in 25 subjects (64%) (Supplemental Table 2, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>), with a later onset than cardiovascular dysfunction (Fig. 2). Renal dysfunction was common and could be prolonged; acute kidney injury (AKI) was present in 18 subjects (46%), but was generally mild with only nine subjects developing stage 2 or 3 AKI. No subjects received renal replacement therapy. Five subjects (13%) developed pancreatitis.

Thirty-three percent developed encephalopathy, manifesting as profound confusion, delirium, hallucinations, aphasia, and/or seizure, and lasting a median of 7 days (IQR, 2–9). Encephalopathy typically occurred later in the CRS course while fever was resolving and was self-limited with return to baseline neurologic status in all subjects by day 18. Diagnostic evaluation consisted of head CT ( $n = 6$ ), brain MRI ( $n = 3$ ), and continuous electroencephalogram monitoring ( $n = 4$ ). Results of the diagnostic evaluation were nonspecific (Supplemental Results, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>).

Eight subjects developed respiratory failure: six supported with invasive mechanical ventilation (median duration, 8 d [IQR, 7–12]) and two supported only with noninvasive mechanical ventilation. Five subjects met criteria for pediatric acute respiratory distress syndrome (PARDS) (18), and two were supported with high frequency oscillatory ventilation. Respiratory variables for the six intubated subjects are presented in the Supplemental Table 3 (Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>).

Thirteen of fourteen subjects with cardiovascular dysfunction were treated with tocilizumab. Nine subjects received one dose, and four subjects received two or three doses of tocilizumab due to partial response or recurrence of symptoms after initial dose. The first dose of tocilizumab was administered a median of 5 days after CTL019 infusion. Fever and tachycardia improved rapidly after tocilizumab administration (Fig. 3). Subjects defervesced a median of 4 hours (IQR, 2–5) after tocilizumab administration. Concomitant with this, their overall clinical appearance markedly improved within hours of tocilizumab administration. Nevertheless, catecholamine-dependent shock resolved over days rather than hours (Fig. 3D), with complete resolution a median of 4 days (IQR, 2–4) after tocilizumab administration. Eight subjects were also treated with short courses of corticosteroids (median, 6.5 d) for refractory hypotension: five received hydrocortisone, two received methylprednisolone, and one received both. All four subjects treated with multiple doses of tocilizumab also received corticosteroids. All patients requiring tocilizumab and/or steroids for grade 4 CRS subsequently achieved disease remission and survived CRS.

## DISCUSSION

Severe CRS was common in pediatric subjects treated with CAR T cell therapy: 46% developed grade 3–4 CRS, 36% developed MODS, 26% had fluid-refractory/catecholamine-resistant vasoplegic shock, and 13% developed PARDS. Profound shock in grade 4 CRS occurred several days following fever onset. Subsequent progression to AKI, coagulopathy, and respiratory failure ensued, followed by delayed onset of hepatic dysfunction and encephalopathy. Treatment with the IL-6 receptor antibody, tocilizumab, resulted in dramatic improvement in fever, tachycardia, and overall clinical appearance within hours. Cardiovascular dysfunction subsequently improved, with more protracted recovery of other organ dysfunctions. CAR T cell therapy–associated CRS represents a novel critical illness syndrome, with similarities in phenotype to other shock states such as severe sepsis and MAS/HLH. However, the need to ensure survival of the infused T cells drives unique therapeutic considerations for this syndrome.

The profound cardiovascular and respiratory dysfunction in subjects with these toxicities was striking. Subjects with shock often had significant and refractory hypotension, and vascular leak with net positive fluid balance of over 140 mL/kg (14% fluid overload) over the 3 days around the time of shock onset. Rapid recognition and treatment of hypotension with crystalloid and colloid fluids and prompt transfer to the ICU with initiation of vasoactive infusion support early during fluid resuscitation were keystones in the supportive care of these patients.

Encephalopathy was a prominent feature of CRS. In contrast to septic shock, in which altered mental status is indicative of inadequate perfusion, or MAS/HLH, in which neurologic abnormalities are typically related to active CNS disease, the encephalopathy seen in CRS presented later than shock, when perfusion was adequately restored. This could be cytokine related, including IL-6 (12, 19, 20), or could be some direct action of the CAR T cells, which cross into spinal fluid (9). Prior administration of tocilizumab did not prevent encephalopathy. The late timing of encephalopathy raised suspicion for a new pathologic process other than CRS. However, no alternative etiology was identified in any subject, and it self resolved within days of onset.

In CAR T cell therapy, the degree of immune activation generated from the in vivo T cell expansion, which is necessary for clinical benefit, resulted in a clinical phenotype in many subjects with features similar to severe septic shock or MAS/HLH. Comprehensive cytokine profiling during CRS demonstrates a pattern that mirrors that seen in MAS/HLH (Teachey et al [21]). Despite phenotypic similarities to SIRS/septic shock and MAS/HLH, the severe CRS we observed was unique because of the overall favorable ICU outcomes, particularly in this high-risk relapsed/refractory leukemia population. The reported mortality in pediatric septic shock with MODS or SIRS with MAS/HLH is 18–50% (22–24), whereas the subjects in this cohort all survived to ICU discharge. Another important distinction between CRS and other forms of refractory shock is related to adjunctive shock therapy. Corticosteroid administration remains in the practice parameters for treatment of refractory pediatric shock (25) and is a mainstay of MAS/HLH therapy (26, 27); however, corticosteroid therapy may inhibit CAR T cell therapy efficacy (10, 12, 13, 28).

Tocilizumab, labeled for use in rheumatoid arthritis, is a monoclonal anti-IL-6 receptor antibody that blocks receptor binding of IL-6. IL-6 is a proinflammatory cytokine often elevated in patients with severe sepsis, particularly in nonsurvivors, and in MAS/HLH (29–31). Preclinical data suggests that IL-6 is not necessary for the efficacy of CAR T cell therapy, making this a better candidate for antibody therapy compared to therapies directed at interferon- $\gamma$  or soluble IL2 receptor  $\alpha$ , which could theoretically eliminate the CAR T cells (9). Thus far, treatment with tocilizumab at the time of grade 3–4 CRS does not seem to adversely impact the CTL019 cells or disease outcomes.

Given the rapid, dramatic clinical response to tocilizumab (6–8, 12) and lack of apparent side effects, tocilizumab has become a standard rescue therapy for CAR-mediated CRS within all ongoing clinical trials. However, a benefit to using tocilizumab to prevent CRS or as adjunctive therapy in mild CRS warrants specific trials. We administer tocilizumab as first line treatment for patients with grade 4 CRS and catecholamine-dependent shock to reverse shock and prevent worsening MODS (Supplemental Fig. 4, Supplemental Digital Content 1, <http://links.lww.com/CCM/C151>). The dose of tocilizumab we administer is the dose for treatment of rheumatoid arthritis: 12 mg/kg for patients less than 30 kg and 8 mg/kg for patients greater than or equal to 30 kg. Tocilizumab has a long half-life, but we have observed benefit with repeat dosing shortly after the first dose in some subjects, likely related to improved IL-6 receptor saturation. We use clinical signs and symptoms (especially refractory shock) to initiate tocilizumab therapy for CRS rather than cytokine levels. Cytokine levels are not rapidly available, nor is it known whether levels correlate with

illness severity. Four patients in this cohort were treated with multiple tocilizumab doses due to ongoing or recurrent symptoms, raising the possibility that some patients may be partial or nonresponders to tocilizumab. These patients were also treated concomitantly with steroids due to the refractory CRS.

We consider corticosteroid treatment second line to tocilizumab for patients with partial response or recurrent symptoms, as corticosteroid therapy potentially could decrease the effectiveness of the CTL019 cells (10). Our current protocol recommends methylprednisolone, although a brief trial of hydrocortisone to rule out adrenal insufficiency may be considered. Corticosteroid therapy should be weaned rapidly after the patient stabilizes.

As CAR and other T cell therapies (including bispecific T cell engaging antibody therapy) expand, it is crucial for centers employing these therapies to be prepared to treat the associated CRS, which appears to be a class-specific toxicity (21). Disease burden and rapidity of onset may help predict which patients will develop severe CRS (8), and higher ferritin levels may identify patients expected to have longer organ dysfunction. A plan regarding CRS surveillance and the clinical threshold to administer tocilizumab should be in place, and adequate pharmacy supply of this antibody confirmed, prior to initiating T cell therapies. Communication with the ICU team that will be managing severe CRS is essential, including pro-active preparation for rapidly progressive shock, the rationale for limiting corticosteroid exposure, and the potential use of tocilizumab adjunctive therapy.

This descriptive cohort study has several limitations. The reported laboratory and hemodynamic abnormalities are limited by sampling bias as the most severely ill subjects had more measurements. The small sample size of subjects treated with multiple tocilizumab doses ( $n = 4$ ) limited exploration of risk factors for ongoing refractory symptoms. The single-center design could limit generalizability, although the IL-6 blockade approach has been adopted in multiple centers. Growing clinical experience at other centers will allow for confirmation of our data, as well as future comparisons with different treatment strategies in larger, more diverse patient populations and may overcome some of these limitations.

## CONCLUSIONS

As use of novel CAR therapy expands, the data from this large pediatric clinical experience may help others anticipate the clinical course of severe CRS and manage shock. Future investigation should include optimizing protocols for cytokine and corticosteroid therapy for CRS that maximize CRS treatment while preserving the CAR cell efficacy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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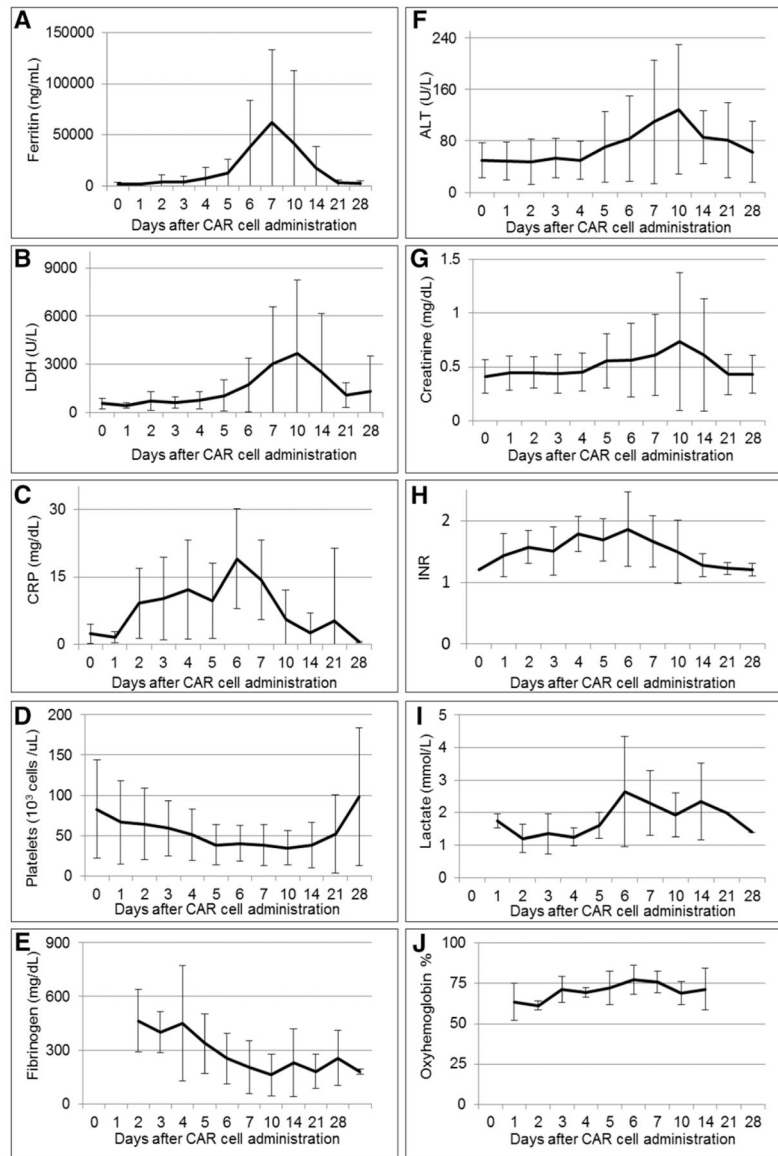


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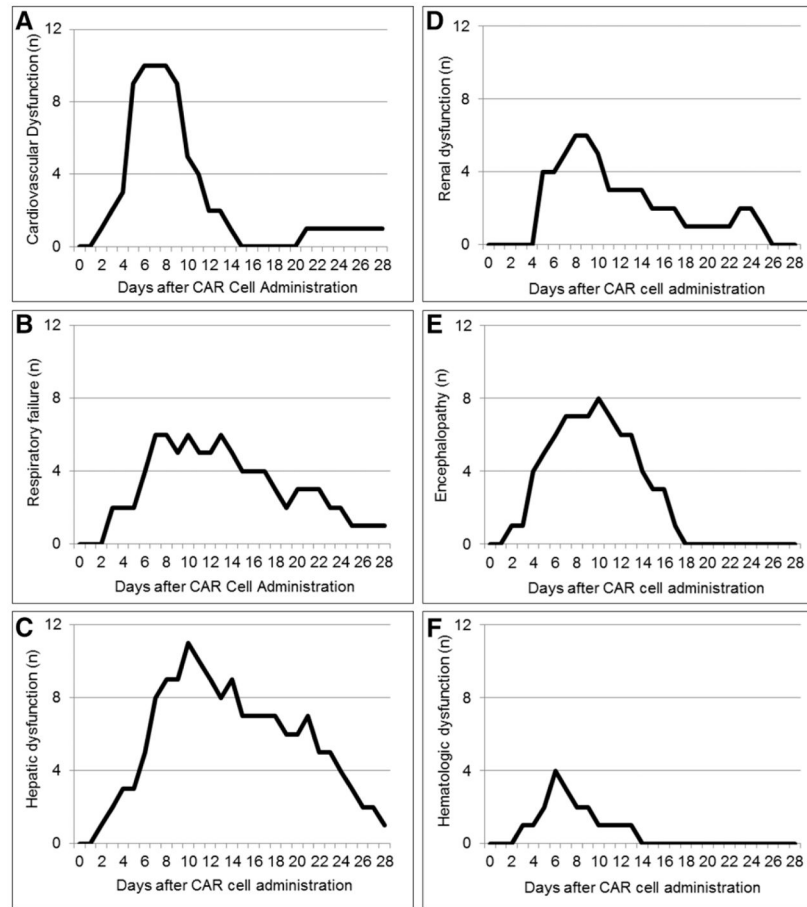
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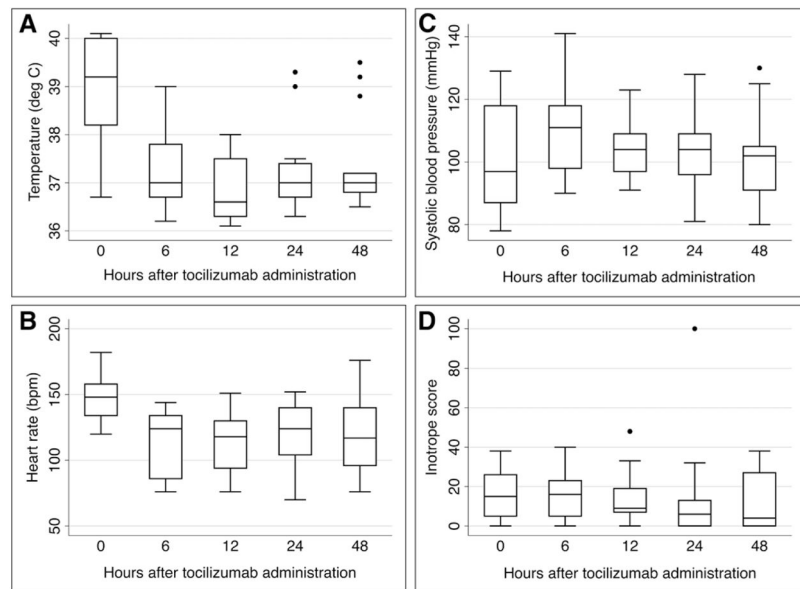
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**Figure 1.** Laboratory trends after chimeric antigen receptor (CAR) cell administration in grade 3 and 4 cytokine release syndrome patients. Means and SD are presented. **A**, Ferritin ( $n = 17$ ). **B**, Lactate dehydrogenase (LDH) ( $n = 18$ ). **C**, C-reactive protein (CRP) ( $n = 16$ ). **D**, Platelet count ( $n = 18$ ). **E**, Fibrinogen ( $n = 16$ ). **F**, Alanine aminotransferase (ALT) ( $n = 18$ ). **G**, Creatinine ( $n = 18$ ). **H**, International normalized ratio (INR) ( $n = 14$ ). **I**, Lactate ( $n = 15$ ). **J**, Central venous oxyhemoglobin saturation ( $n = 14$ ).



**Figure 2.** Time course of organ dysfunction after chimeric antigen receptor (CAR) cell administration in grade 3 and 4 cytokine release syndrome patients. The number of patients with each organ dysfunction on each day after CAR cell administration is presented. **A**, Cardiovascular dysfunction—use of vasoactive drug to support hemodynamic status (dopamine > 5  $\mu\text{g}/\text{kg}/\text{min}$  or any dobutamine, epinephrine, norepinephrine, vasopressin, or milrinone). **B**, Respiratory failure—invasive or noninvasive mechanical ventilation. **C**, Hepatic dysfunction—total bilirubin greater than or equal to 4 mg/dL or alanine aminotransferase greater than 2 $\times$  upper limit of normal for age. **D**, Renal dysfunction—two-fold increase in baseline creatinine. **E**, Encephalopathy—altered mental status or seizure. **F**, Hematologic dysfunction—international normalized ratio greater than 2.



**Figure 3.** Changes in **A**, temperature; **B**, heart rate; **C**, systolic blood pressure; and **D**), inotrope score after tocilizumab administration ( $n = 14$ ).  $p < 0.05$  for all changes in heart rate and temperature compared to time 0 (tocilizumab administration).

**TABLE 1**

## Definition of Cytokine Release Syndrome Grading System

<b>Grade</b>	<b>Description</b>
1	Mild: treated with supportive care (antipyretics, antiemetics)
2	Moderate: requiring hospitalization or IV therapies with some signs of organ dysfunction
3	Severe, requiring therapies such as IV fluid boluses or low dose vasopressors for hypotension, coagulopathy requiring cryoprecipitate or plasma transfusion, hypoxemia requiring high flow oxygen therapy or noninvasive mechanical ventilation
4	Life threatening: requiring therapies such as high dose vasopressors, hypoxemia requiring invasive mechanical ventilation

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**TABLE 2**

Demographic Comparison of Subjects With Grade 0–2 Versus Grade 3–4 Cytokine Release Syndrome

Characteristic	Grade 0–2 CRS ( <i>n</i> = 21)	Grade 3–4 CRS ( <i>n</i> = 18)
Age, median (IQR)	11 (8–15)	11 (9–16)
Gender (% male)	57	44
Weight, kg, median (IQR)	35.2 (22.5–52.3)	39.5 (34.5–48)
Height, cm, median (IQR)	141 (125–157)	142 (136–156)
Race, <i>n</i> (%)		
White	18 (86)	14 (78)
Black	2 (10)	1 (6)
Asian	0 (0)	2 (11)
Other	1 (5)	1 (6)
Ethnicity, <i>n</i> (%)		
Hispanic	3 (14)	2 (11)
Not Hispanic	18 (86)	16 (89)
Relapsed after allogeneic hematopoietic stem cell transplant, <i>n</i> (%)	13 (62)	10 (56)

CRS = cytokine release syndrome, IQR = interquartile range.

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