Toxicities of Chimeric Antigen Receptor T cells: Recognition and Management

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Off-label use: The use of tocilizumab for indications not approved by the Food and Drug Administration will be discussed.
Abstract

Chimeric antigen receptor T cells (CAR T cells) can produce durable remissions in hematologic malignancies that are not responsive to standard therapies. Yet the use of CAR T cells is limited by potentially severe toxicities. Early case reports of unexpected organ damage and deaths following CAR T-cell therapy first highlighted the possible dangers of this new treatment. CAR T cells can potentially damage normal tissues by specifically targeting a tumor-associated antigen that is also expressed on normal tissue. Cytokine release syndrome (CRS), a systemic inflammatory response caused by cytokines released by infused CAR T cells can lead to widespread reversible organ dysfunction. CRS is the most common type of toxicity caused by CAR T-cells. Neurologic toxicity due to CAR T cells might in some cases have a different pathophysiology than CRS manifestations such as hypotension and requires different management. Aggressive supportive care is necessary for all patients experiencing CAR T-cell toxicities, with early intervention for hypotension and treatment of concurrent infections being essential. Interleukin-6 receptor blockade with tocilizumab remains the mainstay pharmacologic therapy for CRS, though indications for administration vary among centers. Corticosteroids should be reserved for neurologic toxicities and CRS not responsive to tocilizumab. Pharmacologic management is complicated by the risk of immunosuppressive therapy abrogating the anti-malignancy activity of the CAR T cells. This review describes the toxicities caused by CAR T-cells and reviews the published approaches used to manage toxicities. This review also includes guidelines for treating patients experiencing CRS and other adverse events following CAR T-cell therapy.
Anti-malignancy Activity of Chimeric Antigen Receptor T Cells

Human T cells can be genetically modified to express chimeric antigen receptors (CARs), fusion proteins containing both an antigen recognition moiety and T-cell activation domains. CAR T cells targeting the B-cell antigen CD19 have been studied extensively in relapsed or chemotherapy-refractory acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and Non-Hodgkin lymphoma (NHL). CAR T-cell therapies are also being developed for solid tumors, but these studies are in early stages.

Reported Chimeric Antigen Receptor T-cell Toxicities

Introduction to CAR T-cell Toxicities

CAR T cells can cause toxicity by several mechanisms. If the tumor-associated antigen to which the CAR is targeted is expressed on normal tissues, those tissues may be damaged, as is the case with normal B cells being depleted by anti-CD19 CAR T cells. CAR T cells may damage normal tissues by unexpectedly cross-reacting with a protein that is not expressed on tumor cells. Acute anaphylaxis and tumor lysis syndrome (TLS) have occurred following infusion of CAR T cells. The most prominent and well-described toxicity of CAR T cells is cytokine release syndrome (CRS) a constellation of symptoms including fever and hypotension that is caused by cytokines released by the infused T cells. Neurologic toxicities due to CAR T-cell therapy may occur concurrently with CRS or occur in absence of CRS. Hypothetically, the gene-therapy vector could be capable of autonomous viral replication or cause a secondary malignancy through insertional mutagenesis. Importantly, neither of these...
toxicities involving the gene-therapy vector have been reported in clinical trials of genetically-modified T cells.42-45

**Toxicities caused by CAR T cells damaging cells that express the targeted antigen**

CAR T cells could damage tissues that express the antigen recognized by the CAR. This mechanism of toxicity can be minimized but not eliminated by an exhaustive search for expression of a targeted antigen on normal tissues during preclinical development of a CAR.46-48 Examples of this mechanism of toxicity have been reported in the literature. In one study, three patients with metastatic renal cell carcinoma who received infusions of autologous T cells transduced with a CAR targeting carboxy-anhydrase-IX (CAIX) experienced grade 3-4 increases in alanine aminotransferase, aspartate aminotransferase, or total bilirubin.20,49-51 Liver biopsies of affected patients revealed a cholangitis with a T-cell infiltration surrounding the bile ducts, and bile duct epithelial cells were unexpectedly found to express CAIX.20,49

A patient with metastatic colorectal cancer who received an infusion of autologous CAR T cells directed against the antigen ERBB2 (Her-2/neu) experienced acute respiratory distress and pulmonary edema requiring mechanical ventilation. She subsequently died. The pulmonary toxicity and subsequent death of the patient is hypothesized to be due to expression of ERBB2 on normal lung tissue.32

**Cross-reactivity of a CAR with a non-targeted protein**

Organ damage could hypothetically occur when CAR T cells cross-react with an antigen expressed on normal tissue that is similar to the target antigen expressed by the malignancy. This toxicity has not been documented in clinical trials of CARs, but it has been observed in clinical trials of T cells genetically modified to express T-cell receptors (TCRs).33,52,53
Allergic Reactions and tumor lysis syndrome

Allergic reactions to CAR T cells have been reported. A patient with pleural mesothelioma received multiple infusions of autologous T cells transduced with an anti-mesothelin CAR. While he tolerated his first two cell infusions well, he experienced anaphylaxis and cardiac arrest one minute following completion of his third infusion, with dramatically elevated serum tryptase levels. He received cardiopulmonary resuscitation and recovered.34

While chemotherapy may have caused TLS in some cases, the infusion of CAR T cells in the absence of prior conditioning chemotherapy has led to tumor lysis syndrome.8,13

CRS

The most common acute toxicity of CAR T cells is CRS. The cytokines implicated in CRS may be directly produced by the infused CAR T cells, or other immune cells such as macrophages might produce cytokines in response to cytokines produced by the infused CAR T cells. A wide variety of cytokines including interleukin (IL)-6, interferon-γ (IFN-γ), tumor necrosis factor (TNF), IL-2, IL-2-receptor-α, IL-8, and IL-10 are elevated in the serum of patients experiencing fever, tachycardia, hypotension and other toxicities after CAR T-cell infusions.4,7-9,11,12,35,54 In one report, the severity of toxicity experienced by patients receiving anti-CD19 CAR T cells correlated with serum IFNγ and TNF levels.16 Increased CRS grade was associated with increased sIL-2R levels,5,11 peak IL-6 levels,5,6,9,11 peak ferritin,5,9 peak CRP,5,9 and higher levels of blood CAR T cells.5,6,11 In some reports, the severity of CRS and elevation of serum cytokines have been related to disease burden, with higher disease burden predicting more toxicity.4-7,9,11 Predictive models of CRS based on cytokine profiles are in development.7,55 Figure 1 summarizes the organ toxicities caused by CRS.
CRS-Related Toxicities by Organ System

Constitutional signs and symptoms

Fever is usually the first symptom of CRS. The time of onset of fever can be quite variable, ranging from a few hours to more than a week after CAR T-cell infusion. Temperatures frequently exceed 40° C,5,7,8,14,35 and grade 3-4 fevers occurred in 40-80% of patients in three reports.6,14,15 Rigors, malaise, headaches, myalgias, arthralgias, and anorexia occur frequently.

Cardiovascular

Cardiovascular toxicities include tachycardia, which often occurs with fever. With more severe CRS, hypotension, arrhythmias and decreased cardiac ejection fraction can occur. Grade 3-4 hypotension has been reported in 22-38% of patients.5-7,15 The pathophysiology of the decreased cardiac output that can occur in CRS is not well understood, but is thought to be similar to the stress-cardiomyopathy that can be seen in sepsis.35,56 Cardiac arrest has been reported 7 days following CAR T-cell infusion in a patient with ALL whose left ventricular ejection fraction fell to less than 25% from a normal baseline.6 In addition to this dramatic case, reversible reduced cardiac ejection has been reported in multiple other patients.13,14,54 Reversible increases in serum troponin can occur.13,14 Asymptomatic prolongation of the QTc interval of the ECG,6 and atrial fibrillation,7 have also been reported.

Pulmonary

CRS can lead to pulmonary edema, hypoxia, dyspnea, and pneumonitis, which can be severe enough to require mechanical ventilation.4,6-8,13-16,35 In four reports, Grade 3-4 hypoxia was reported in 6-15% of patients.6,7,14,15
Renal

Acute renal injury following CAR T-cell infusion is multifactorial and almost always reversible. Reduced renal perfusion is often the most important cause of renal injury. Reduced renal perfusion can be caused by cytokine-mediated vasodilation, decreased cardiac output, or intravascular dehydration due to insensible losses from high fevers. TLS and drug effect from medications such as antibiotics are other possible causes of renal injury. Electrolyte disturbances, such as hyponatremia, hypokalemia, and hypophosphatemia are not uncommon.

Hepatic and Gastrointestinal

Elevations in serum transaminases and bilirubin can occur during CRS. As with other laboratory abnormalities in CRS, these changes are almost always reversible, with return to baseline values following CRS resolution. Diarrhea, colitis, nausea, and abdominal pain have been reported following CD19 CAR T cell infusions.

Hematologic

Cytopenias are a common occurrence following CAR T-cell infusion. Grade 3-4 anemia, thrombocytopenia, leukopenia, neutropenia, and lymphopenia are frequently reported. There is often difficulty in determining the etiology of cytopenias occurring after CAR T-cell infusions, because chemotherapy that causes cytopenias is often given before CAR T-cell infusions. Patients not receiving conditioning chemotherapy have also experienced cytopenias following CAR T-cell infusion, demonstrating that the CAR T cells cause myelosuppression by a cytokine-mediated mechanism or some other mechanism.
Derangements of coagulation following CAR T-cell infusion include prolongation of the prothrombin time (PT) and partial thromboplastin time and (PTT),\textsuperscript{5,6,14} D-Dimer elevation,\textsuperscript{8} low fibrinogen,\textsuperscript{5,8} disseminated intravascular coagulation,\textsuperscript{9} and macrophage activation syndrome.\textsuperscript{5,11} Hemorrhage is infrequent but possible.\textsuperscript{5} Prolonged B-cell aplasia is an expected and common toxicity of anti-CD19 CAR T cells.\textsuperscript{5,6,8-16,31,54} B-cell aplasia and hypogammaglobulinemia may last 2 months to over 2 years following CAR T-cell infusion.\textsuperscript{4,5,16,31}

Infectious Disease

Patients on CAR T-cell clinical trials frequently become neutropenic and lymphopenic following administration of chemotherapy followed by CAR T-cells. Such immune compromise predisposes these patients to opportunistic infection. In this setting, the fevers, tachycardia, and hypotension associated with CRS can be difficult to differentiate from sepsis. In an early report, a patient with CLL who received chemotherapy and anti-CD19 CAR T cells died with fever, hypotension, and renal failure. It was later found that this patient had elevated serum levels of inflammatory cytokines before CAR T-cell infusion, suggesting that the patient had a prior infection.\textsuperscript{57} Bacteremia,\textsuperscript{15,16,35} salmonella,\textsuperscript{5} urinary tract infections,\textsuperscript{15} and viral infections such as influenza,\textsuperscript{16} respiratory syncytial virus,\textsuperscript{13} and herpes zoster,\textsuperscript{16} have also occurred following CAR T-cell infusion.

Musculoskeletal

Elevated creatine phospho-kinase (CPK) has been reported in a patient receiving anti-CD19 CAR T cells for ALL\textsuperscript{6} and in two patients who received donor-derived allogeneic anti-CD19 CAR T cells.\textsuperscript{6,14} One of these patients also experienced myalgias and weakness.\textsuperscript{14} Similar muscle
weakness and pain accompanied by elevations of CPK has been reported in a patient with multiple myeloma who received anti-BCMA CAR T cells. (J. Kochenderfer, unpublished data)

**Neurologic Toxicities**

Neurologic toxicities have been reported with other therapies in which serum cytokine levels are increased. Exogenous high-dose IL-2, when administered for solid tumor malignancies, can cause a global encephalopathy.\(^{58}\) Blinatumomab, a bispecific antibody that both targets CD19 and activates T lymphocytes, can cause both a global encephalopathy as well as more localized defects such as aphasia, tremor, ataxia, hemiparesis, and cranial nerve palsies.\(^{59,60}\) The neurologic toxicities associated with anti-CD19 CAR T-cells are in many cases similar to the neurologic toxicities of blinatumumab can also be diverse, and do not localize to one specific area of neuroanatomy. The incidence of neurologic toxicity is quite variable, with published reports of 0-50%.\(^{5-7,9,11,14,15}\) Neurologic events may occur at different times than CRS or in the absence of CRS toxicities,\(^{5}\) which suggests that at least in some cases the neurologic toxicity might have a different mechanism than many of the other toxicities such as hypotension and fever. Reported neurologic toxicities include headaches, confusion, alterations in wakefulness, hallucinations, dysphasia, ataxia, apraxia, facial nerve palsy, tremor, dysmetria, and seizures.\(^{4-9,11,15,16,35}\) Neurologic toxicities may also necessitate intubation and mechanical ventilation for airway protection in absence of respiratory failure.\(^{7}\) Central nervous system involvement of leukemia has not been shown to be associated with neurologic toxicity.\(^{5,6}\) Multiple groups have found anti-CD19 CAR T cells in the cerebrospinal fluid (CSF) of patients,\(^{5-8,15}\) and elevated IL-6 levels in the CSF have been observed in patients experiencing neurotoxicity.\(^{35}\) In one series, higher levels of anti-CD19 CAR T cells were seen in the cerebral spinal fluid (CSF) of patients experiencing neurologic toxicities compared to patients without neurologic toxicities.\(^{6}\)
Graft Versus Host Disease (GVHD)

Allogeneic hematopoietic stem cell transplant recipients have received infusions of anti-CD19-CAR-transduced allogeneic T cells from their original transplant donors. In one report, donor-derived virus-specific T cells transduced with an anti-CD19 CAR did not cause any GVHD in 8 post-transplant patients. In another series of 20 patients receiving donor-derived allogeneic anti-CD19 CAR T cells, the only GVHD that occurred was slowly worsening chronic GVHD in a patient with pre-existing chronic GVHD and mild eye GVHD more than a year after CAR T-cell infusion in another patient.

Grading CRS

The Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) includes a grading scale of CRS-related adverse events caused by immunotherapies, but it was created for toxicity grading of acute infusional toxicities of monoclonal antibodies rather than T-cell therapies.

Another published rating scale for CRS integrates lab findings with clinical features. A category of severe CRS (sCRS) is defined as CRS requiring pharmacologic and medical intervention. Criteria for sCRS are fevers of 38°C or greater for at least three consecutive days and elevation of two serum cytokines by 75-fold or of at least one serum cytokine by at least 250 fold, as well as one clinical sign of severe toxicity. While this rating scale reliably identifies patients who will need intensive monitoring and intervention for CRS, obtaining real-time cytokine levels may not be possible at some facilities. Elevation of C-reactive protein (CRP) greater than 20 mg/dL correlates with severe CRS with a specificity of 100%, but the predictive value of this biomarker is unknown. CRP may be helpful for identifying the peak point of toxicity and predicting toxicity resolution.
A widely referenced grading mechanism is a modification of the CTCAE to make it suitable for grading CRS due to T-cell therapies.35 Grade 1 symptoms require only symptomatic management. Grade 2 symptoms respond to moderate intervention. These include oxygen requirement < 40%, grade 2 organ toxicity, or hypotension responding to IV fluids or low doses of one vasopressor, for example, < 20 μg/min of norepinephrine. Grade 3 CRS includes oxygen requirement greater than 40%, hypotension requiring high-dose or multiple vasopressors, grade 4 transaminitis, and grade 3 organ toxicity at other sites. Grade 4 CRS is defined as life-threatening symptoms requiring ventilator support or grade 4 organ toxicity other than transaminitis.35

A third system of grading has been reported.11 In this system, Grade 1 CRS requires only supportive care. Grade 2 CRS includes requirement for IV therapies, grade 2 creatinine elevation, grade 3 transaminitis, neutropenic fevers, and other indications for hospitalization. Grade 3 CRS criteria include grade 3 creatinine elevation, grade 4 transaminitis, hypotension responding to IV fluids or low-dose vasopressors, hypoxia requiring supplemental oxygen, or coagulopathy requiring fresh frozen plasma or cryoprecipitate. Grade 4 CRS includes life-threatening complications, such as hypoxia requiring mechanical ventilation or hypotension requiring high-dose vasopressors.

Preventing and Managing Toxicities

Prior to Cell Infusion

Patients receiving CAR T-cell therapies should have limited comorbidities so that they are able to tolerate potentially severe CRS. Criteria for study participation for patients enrolled on clinical trials of CAR T cells at the Experimental Transplantation and Immunology Branch of the National Cancer Institute are summarized in Table 1. Patients must have normal cardiac ejection
fraction, no history of myocardial infarction, and no cardiac arrhythmias, including atrial fibrillation. Good baseline bone marrow function with minimal cytopenias is important because of the possibility of cytopenias caused by either the conditioning chemotherapy or the CAR T cells. Patients who have undergone allogeneic stem cell transplant are not treated unless they have grade 1 or less acute GVHD or mild global score or less chronic GVHD.

For patients with significant disease burden, especially ALL with extensive marrow infiltration or NHL with bulky adenopathy, many groups start allopurinol for TLS prophylaxis prior to conditioning chemotherapy or prior to cell infusion.\textsuperscript{13,57}

**Following Cell Infusion: Hemodynamic Management and Supportive Care**

Our practice for supportive care following cell infusion is summarized in Table 2. Close hemodynamic monitoring is imperative following CAR T-cell therapy. At our center, patients remain hospitalized for at least 9 days after CAR T-cell infusion. At other centers, CAR T-cell infusions are performed on an outpatient basis. Vital signs are checked every 4 hours during the inpatient stay. If patients have heart rates persistently above 115 beats/minute, vital signs are checked every 2 hours. A complete blood count with differential and a comprehensive metabolic panel are drawn twice daily; uric acid and CRP are checked daily.

Fever is most often the first sign of CRS. Acetaminophen and cooling blankets can be used for fever management. We avoid corticosteroids for fever management because of the risk of inhibiting the CAR T cells. Non-steroidal anti-inflammatory medications are also not used for fever management because these agents could contribute to hemorrhage, gastritis, and renal insufficiency. If a patient is neutropenic and febrile, blood cultures should be drawn, and broad spectrum antibiotic therapy should be initiated. Infectious diagnoses should be aggressively
pursued by imaging and cultures to avoid missing infections in the setting of patients who are expected to be febrile secondary to CRS.

Hypotension must be recognized early and managed aggressively. At our center, systolic blood pressures (SBP) are used to guide hypotension management. The volume of fluid given for resuscitation varies greatly, and the approach must be tailored for each individual patient. For each patient, the benefit of volume resuscitation is weighed against the risk of vascular leak and pulmonary edema. Patients with hypotension that is not fluid responsive should receive vasopressors. We prefer norepinephrine as the first-line vasopressor.

The threshold for transfer to an intensive care unit will clearly vary among institutions. Indications for transfer to the ICU at our center include SBP less than 75% of a patient’s baseline and less than 100 mm Hg following a 1 Liter normal saline bolus, SBP less than 90 mm Hg following a 1 Liter normal saline bolus if 90 mm Hg is less than the patient’s baseline systolic blood pressure, continuous tachycardia with heart rate higher than 125 beats per minute for at least 4 hours, oxygen requirement of more than 4 Liters flow by nasal cannula, and neurologic toxicity greater than grade 2 by the CTCAE version 4.0.

Cardiac arrhythmias and decrease in cardiac ejection fraction may occur during CRS and may be asymptomatic. Patients with other symptoms of CRS should be monitored with ECGs and echocardiograms. Our approach is to obtain an ECG, serum troponin, and echocardiogram for all patients who require more than one fluid bolus for hypotension, who are transferred to the ICU for hemodynamic management, or who require any dose of vasopressor for hypotension. For patients maintained on vasopressors, repeated cardiac echocardiograms should be performed at least every 2 to 3 days.
Cytopenias following cell infusion are managed with transfusion support and growth factors. Our practice is to initiate filgrastim when the ANC decreases to less than 500/mm$^3$ and to stop it when the ANC is greater than 1500/mm$^3$. Transfusion support is provided to keep the hemoglobin at least 8.0 mg/dL and platelet count at least 20,000/mm$^3$. Fresh frozen plasma is given for any grade 2 PTT prolongation, and cryoprecipitate is given when the fibrinogen is less than 100 mg/dL.

**Tocilizumab**

Tocilizumab is an IL-6 receptor antagonist that is used to treat rheumatologic disorders.$^{63,64}$ It has effectively treated CRS-related toxicities in clinical trials and is now widely used off-label for toxicity following CAR T-cell infusions. Tocilizumab can effectively lessen or abrogate the CRS-related toxicities following CAR T-cell infusions.$^5$-$^8,^{11}$ Resolution of CRS following administration of tocilizumab is demonstrated in Figure 2.

Experience with treating ALL patients with tocilizumab demonstrated that complete remissions still occur when patients receive tocilizumab to treat CRS caused by CAR T cells.$^5$-$^6$ Some concern still exists that tocilizumab might subtly impair the depth or duration of anti-malignancy responses caused by CAR T cells; formal studies of the impact of tocilizumab on anti-malignancy outcomes have not been performed. In addition, most published experience with tocilizumab is with ALL. Tocilizumab might impair the efficacy of CAR T cells against lymphoma or other malignancies even if it does not impair the activity of CAR T cells against ALL. The approach used in the set of guidelines published by Lee and colleagues is to administer tocilizumab to all patients experiencing CRS of grade 3 or greater and to patients with CRS of grade 2 or greater and comorbidities.$^{35}$ The goal of these guidelines is to avoid life-
threatening grade 4 toxicity. Tocilizumab dosing varies among centers, and the agent may be
given at a dose of 4 mg/kg or 8 mg/kg. There is general consensus that if CRS has not improved
with initial tocilizumab administration, an additional dose of tocilizumab should be given or
another immunosuppressive agent such as corticosteroids should be considered.7,35

Our practice is to give tocilizumab when specific hemodynamic and organ function
thresholds are crossed, rather than for a certain grade of CRS. These criteria are listed in Table 3.
We have used a dose of tocilizumab of 4 mg/kg infused over 1 hour, at a dose not to exceed 800
mg. If necessary, we administer a second dose of 4 mg/kg of tocilizumab. An initial dose of 8
mg/kg of tocilizumab might be optimal in some cases. We generally do not administer
tocilizumab for neurologic toxicity because of concerns about the ability of tocilizumab to cross
the blood brain barrier and experience in an admittedly very small number of patients that
tocilizumab did not ameliorate neurologic toxicity.35

Corticosteroids and other agents

Systemic corticosteroids have been used effectively to abrogate CRS related toxicities.4,6-8,11,54
Some evidence suggests that corticosteroids may inhibit CAR T-cell persistence and anti-
malignancy efficacy, as reported previously in ALL patients following anti-CD19 CAR T-cell
infusion.4,7 For this reason, corticosteroid therapy has been reserved for use following failure of
tocilizumab to ameliorate CRS. Indications for giving corticosteroids differ among centers. At
our center, corticosteroids are considered for CRS that does not improve following tocilizumab
(Table 3). Other immunosuppressive agents that have been used or considered in CRS
management include siltuximab,65 etanercept,8,9,35 infliximab,35 and anakinra.35 Due to paucity of
data, no one second-line agent can be recommended over another.
Management of Neurologic Toxicities

As neurologic toxicities may occur concurrently with or following resolution of CRS, it follows that management of these toxicities may differ from that of CRS alone. It is unclear if tocilizumab has any beneficial effect on neurologic toxicities. Because tocilizumab is a monoclonal antibody, its size makes efficient blood-brain barrier penetration unlikely. The smaller molecule IL-6 is known to cross the blood-brain barrier and has been shown to cause neurologic defects. Saturation of IL-6 receptors following systemic tocilizumab administration may increase serum IL-6 levels, which could theoretically lead to an increase in cerebrospinal fluid IL-6 levels, which might worsen neurologic toxicity. Similar to other groups, it is our practice to treat severe neurologic toxicities with systemic corticosteroids rather than tocilizumab as the first line agent. Dexamethasone is often chosen in this context because of its excellent central nervous system penetration (Table 3). We give dexamethasone for grade 3 neurologic toxicities other than headaches lasting more than 24 hours, grade 4 neurologic toxicities of any duration, and for any seizures. Our practice is for all patients with grade 2 or greater neurologic toxicity to be evaluated by the neurology consult service. For patients with seizures, standard anti-epileptic therapy is given. The management algorithm used for CRS and neurologic toxicity in adult patients at our center is delineated in Figure 3.

Outpatient Monitoring

Hypogammaglobulinemia is common with the profound and prolonged B-cell aplasia that may occur following anti-CD19 CAR T cell infusions. Multiple groups have administered replacement therapy with intravenous immunoglobulins (IV IgG). Our practice is to administer IV IgG when the serum IgG level is less than 400 mg/dL. The utility of repeating
vaccine series in patients who have achieved B-cell recovery is an important area for future research.

**Recombinant DNA Advisory Committee Symposium**

On June 10, 2015, the NIH Recombinant DNA Advisory Committee (RAC) held a symposium, “Cytokine Release Syndrome after T-cell Immunotherapy,” in Bethesda, Maryland. Members of the RAC and researchers in the field of CAR T-cell therapy met to discuss the grading and management of toxicity. It was agreed that a single system for grading CRS toxicity would benefit the field as a whole.

The importance of vigilant monitoring and supportive care for patients experiencing CRS was discussed. Specifically, the importance of recognizing and treating concurrent infections early in the course was emphasized. The criteria used for tocilizumab infusion vary greatly among institutions, with some groups using a pre-emptive approach, giving tocilizumab during grade 1 toxicities, while other groups prefer to reserve the agent for high vasopressor requirement or impending need for mechanical ventilation. Small randomized trials of a pre-emptive versus reactive approach were posited as an avenue of further investigation. The use of corticosteroids to treat CRS was discussed. Some attendees stated that corticosteroids should be the first-line agent for severe neurologic toxicities, although a uniform threshold for their use has not been established.

**Conclusion**

Toxicities caused by CAR T cells are diverse and not fully understood. Management requires vigilant monitoring, aggressive supportive treatments, and, in some cases, intensive care.
Administering immunosuppressive agents to decrease toxicity is an evolving practice. Consensus guidelines for grading and managing toxicity will facilitate administration of CAR T cells at more centers. Improving the management of CAR T-cell toxicity is one of the most important avenues for overall improvement in the field of CAR T-cell therapies.

Acknowledgements

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Author Contributions

JNB wrote the first draft of the review. JNB and JNK participated extensively in revising the review and in determining the content contained in the review. JNB has no conflicts-of-interest. JNK is Principle Investigator of Cooperative Research and Development Agreements between the NCI and Kite Pharma and bluebird bio. JNK has multiple patents covering CAR technology. Funding for preparation of this review came from NCI intramural funds.

References


Table 1: Safety-related Eligibility Criteria for Adult CAR T-cell Clinical Trials at the National Cancer Institute

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<tr>
<th>Clinical Category</th>
<th>Eligibility Criteria</th>
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<tr>
<td>Patient Characteristics</td>
<td>• ECOG 0-1&lt;br&gt;• Not pregnant of breastfeeding</td>
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<tr>
<td>Pulmonary</td>
<td>• No active obstructive or restrictive pulmonary disease</td>
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<tr>
<td>Hematologic*</td>
<td>• Hemoglobin &gt; 8.0 g/dL&lt;br&gt;• Platelets ≥ 45,000/mm³ without transfusion support&lt;br&gt;• Absolute neutrophil count ≥ 1000/mm³ without growth factor support&lt;br&gt;• No active hemolytic anemia&lt;br&gt;• No active coagulopathy</td>
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<tr>
<td>Other End Organ Function</td>
<td>• Serum creatinine ≤ 1.4 mg/dL&lt;br&gt;• Total bilirubin ≤ 2.0 mg/dL&lt;br&gt;• Serum AST and ALT ≤ 3 times the institutional upper limit of normal unless liver involvement by malignancy is demonstrated&lt;br&gt;• No active seizure disorder&lt;br&gt;• No current central nervous system involvement with malignancy</td>
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<tr>
<td>Infectious Disease</td>
<td>• No history or serologic evidence of HIV, hepatitis B or hepatitis C&lt;br&gt;• No active uncontrolled systemic infection</td>
</tr>
<tr>
<td>Immunologic</td>
<td>• No active autoimmune disease&lt;br&gt;• No history of primary immunodeficiency</td>
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Abbreviations: ALT: alanine aminotransferase. AST: aspartate aminotransferase. ECOG: Eastern Cooperative Oncology Group. *Hematologic criteria must be met for enrollment regardless of bone marrow involvement with malignancy.
<table>
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<th>Toxicity</th>
<th>Preventive and Supportive Care Interventions</th>
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| Constitutional| • Administer acetaminophen for symptomatic management of fevers in patients with normal hepatic function.  
• Provide cooling blankets for fevers > 40°C.  
• Avoid corticosteroids and non-steroidal anti-inflammatory agents.  
• Avoid meperidine.                                                                                                                                                                                                                               |
| Cardiovascular| • Stop or taper antihypertensive medications prior to cell infusion.  
• Monitor vital signs at least every 4 hours on an inpatient unit for at least 9 days following infusion.  
• Monitor vital signs every 2 hours in patients with fevers and tachycardia.  
• Initiate replacement intravenous fluids for patients with poor oral intake or high insensible losses to maintain net even fluid balance.  
• Administer intravenous fluid boluses for patients with systolic blood pressure less than their pre-infusion baseline.  
  o Patients with a systolic blood pressure less than 80% of their pre-infusion baseline and less than 100 mm Hg receive a 1 Liter normal saline bolus.  
  o Patients with a systolic blood pressure less than 85 mm Hg receive a 1 Liter normal saline bolus regardless of baseline blood pressure.  
• Patients receiving more than 1 IV fluid bolus for hypotension or patients in the ICU for toxicity management have a serum troponin drawn and an electrocardiogram and an echocardiogram performed to evaluate for cardiac toxicity.  
• Patients with hypotension are initiated on vasopressor support. Norepinephrine is the preferred first-line vasopressor.                                                                                                                                 |
| Infectious disease| • Initiate prophylactic antimicrobials, such as trimethoprim-sulfamethoxazole, for Pneumocystis prophylaxis prior to conditioning chemotherapy.  
• Initiate prophylactic antimicrobials, such as acyclovir or valacyclovir, for herpes virus prophylaxis prior to conditioning chemotherapy.  
• All patients with fevers and neutropenia have blood cultures drawn and broad-spectrum antibiotic coverage initiated.                                                                                                                                                                          |
| Hematologic   | • Initiate allopurinol for tumor lysis syndrome prophylaxis in patients without a contraindication prior to conditioning chemotherapy.  
• Transfuse packed red cells for goal hemoglobin of ≥ 8.0 g/dL.  
• Transfuse platelets for a goal platelet count of ≥ 20,000/µL.  
• Monitor complete blood count with differential twice daily. When absolute neutrophil count (ANC) decreases to < 500/µL, initiate filgrastim support. Continue until ANC increases to > 1,500 µL.  
• Transfuse fresh frozen plasma with a goal of normalization of partial thromboplastin time (PTT) in patients with a PTT 1.5-fold above the upper limit of normal or higher.  
• Transfuse cryoprecipitate to maintain fibrinogen of ≥ 100 mg/dL. If patient is bleeding, a higher level of fibrinogen should be maintained.                                                                                                                                 |
<table>
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<th>Neurologic</th>
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<tr>
<td>• The nursing staff conducts focused neurologic exams every 8 hours in patients experiencing neurologic toxicity.</td>
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<td>• Perform brain MRI in any patient experiencing neurologic toxicity.</td>
</tr>
<tr>
<td>• Perform lumbar puncture to evaluate for infectious pathogens, cytokine levels and CAR T-cell levels in patients experiencing neurologic toxicity whenever feasible.</td>
</tr>
<tr>
<td>• Request neurology consultation for any patient experiencing neurologic toxicity.</td>
</tr>
<tr>
<td>• Standard anti-epileptic medications are used for patients having active seizures. We do not use prophylactic anti-epileptic medications.</td>
</tr>
</tbody>
</table>

*These are the current treatment guidelines used for adult patients at the National Cancer Institute Experimental Transplantation and Immunology Branch.*
Table 3: Pharmacologic Management of CRS and Neurological Toxicities*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocilizumab</td>
<td>• Left ventricular ejection fraction less than 40% by echocardiogram</td>
<td>4 to 8 mg/kg infused over 1 hour, dose not to exceed 800 mg</td>
</tr>
<tr>
<td></td>
<td>• Creatinine greater than 2.5-fold higher than the most recent level prior to CAR T-cell infusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Norepinephrine requirement at a dose greater than 2 mg/minute for 48 hours since the first administration of norepinephrine, even if administration is not continuous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Systolic blood pressure of 90 mm Hg that cannot be maintained with norepinephrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Oxygen requirement of fraction of inspired oxygen (FiO2) greater than 50% or more for more than 2 continuous hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Dyspnea that is severe enough to potentially require mechanical ventilation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Activated partial thromboplastin time&gt;2X upper limit of normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Clinically-significant bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Creatine kinase greater than 5x upper limit of normal for greater than 2 days</td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>• CRS toxicity refractory to tocilizumab</td>
<td>1-2 mg/kg IV every 12 hours</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>• Grade 3 neurological toxicities, with the exception of headaches, that last continuously for 24 hours or longer</td>
<td>10 mg IV q 6 hours until either</td>
</tr>
<tr>
<td></td>
<td>• Grade 4 neurological toxicity of any duration</td>
<td>• Toxicities improved to Grade 1 or less</td>
</tr>
<tr>
<td></td>
<td>• Any generalized seizure</td>
<td>• At least 8 doses have been given</td>
</tr>
</tbody>
</table>

*These are the current treatment guidelines used for adult patients at the National Cancer Institute Experimental Transplantation and Immunology Branch.
Legends for Figures:

**Figure 1: Cytokine release syndrome toxicities by organ system.** After infusion of CAR T cells, CRS toxicities affecting a wide variety of organs can occur.

**Figure 2: Response to tocilizumab in 2 patients.** Patient 1 is a 54-year-old male who received T-cells transduced with a CAR targeting B-cell maturation antigen (BCMA). He experienced severe cytokine release syndrome starting 4 hours following T-cell infusion, with development of fevers, tachycardia, tachypnea, hypoxia, and hypotension requiring vasopressors. (A) Patient 1 received tocilizumab 25 hours after his cell infusion, which was followed by a transient decrease in temperature and heart rate. He experienced worsening CRS and received a second dose of tocilizumab on Day 5 following cell infusion, which was followed by a sustained decrease in temperature and heart rate. (B) The respiratory rate of patient 1 decreased following his first dose of tocilizumab, and intubation was avoided. (C) Patient 2 is a 20-year-old woman with a history of ALL with a past history of a matched related donor stem cell transplant. She received donor-derived T cells transduced with a CAR targeting CD19 for progressive ALL after transplant. She experienced CRS toxicity with fevers, tachycardia, tachypnea, hypoxia, left ventricular systolic dysfunction, prolonged aPTT, and increased creatine kinase. She received tocilizumab on Day 4 following CAR T-cell infusion. Her respiratory rate decreased following tocilizumab, and intubation was avoided. (D) The heart rate of patient 2 decreased following tocilizumab. (E) Following tocilizumab, Patient 2’s C-reactive protein decreased over a period of days.

**Figure 3: General treatment algorithm for CRS and neurologic toxicities.**
A general algorithm used for treatment of CAR T-cell toxicity occurring in patients at the National Cancer Institute Experimental Transplantation and Immunology Branch is shown.
Neurological:
- headaches
- changes in level of consciousness
- delirium
- aphasia
- apraxia
- ataxia
- hallucinations
- tremor
- dysmetria
- myoclonus
- facial nerve palsy
- seizures

Constitutional:
- fevers
- rigors
- malaise
- fatigue
- anorexia
- arthralgias

Cardiovascular:
- tachycardia
- widened pulse pressure
- hypotension
- arrhythmias
- decreased left ventricular ejection fraction
- troponinemia
- QT prolongation

Pulmonary:
- tachypnea
- hypoxia

Gastrointestinal:
- nausea
- emesis
- diarrhea

Hepatic:
- transaminitis
- hyperbilirubinemia

Renal:
- acute kidney injury
- hyponatremia
- hypokalemia
- hypophosphatemia
- tumor lysis syndrome

Hematologic:
- anemia
- thrombocytopenia
- neutropenia
- febrile neutropenia
- lymphopenia
- B-cell aplasia
- prolonged prothrombin time
- prolonged activated thromboplastin time
- elevated D-Dimer
- hypofibrinogenemia
- disseminated intravascular coagulation
- hemophagocytic lymphohistiocytosis

Musculoskeletal:
- myalgias
- elevated creatine kinase
- weakness
Figure 2  Response to Tocilizumab in Two Patients

A: Temperature and Heart Rate over Days after cell infusion.
B: Respiratory Rate over Hours after tocilizumab.
C: Respiratory Rate over Hours after tocilizumab.
D: Heart Rate over Hours after tocilizumab.
E: C-reactive protein over Days after cell infusion.
Patient experiences toxicity following CAR T-cell infusion.

- Neurologic toxicity meeting criteria for corticosteroids as listed in Table 3?
  - Yes: Administer dexamethasone until significant improvement and continue supportive care per Table 2.
  - No: Continue supportive care as per Table 2.

- CRS with indication for tocilizumab as listed in Table 3?
  - Yes: Administer tocilizumab and continue supportive care as per Table 2.
  - No: Continue supportive care as per Table 2.

- Response within 12 hours?
  - Yes: Redose tocilizumab and consider second-line corticosteroids. Continue supportive care per Table 2.
  - No: Continue supportive care per Table 2.
Toxicities of chimeric antigen receptor T cells: recognition and management

Jennifer N. Brudno and James N. Kochenderfer