Chimeric Antigen Receptor Cell Therapy Toxicity Assessment and Management

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Abstract

Immunotherapy with T cells genetically engineered to express a chimeric antigen receptor (CAR) is rapidly emerging as a promising new treatment for haematological and non-haematological malignancies. This therapy can induce rapid and durable clinical responses; however, it is also associated with unique acute toxicities, which can be severe or even fatal. Cytokine release syndrome (CRS), the most common toxicity observed with this therapy, may range in severity from low-grade constitutional symptoms to a high-grade syndrome associated with life-threatening multiorgan dysfunction. Neurotoxicity, termed CAR-related encephalopathy syndrome (CRES), is the second most common toxicity and can occur concurrently with CRS or after the CRS has subsided. Rarely, severe CRS has evolved into fulminant hemophagocytic lymphohistiocytosis (HLH). Intensive monitoring and prompt management of toxicities may minimize the morbidity and mortality associated with these potentially curative therapies. However, accurate and consistent grading and management algorithms of the toxicities are necessary in order to safely administer these therapies. We have formed a CAR T-cell therapy-associated TOXicity (CARTOX) Working Group with investigators from multiple institutions and multiple disciplines who have had experience in treating patients with various CAR T-cell therapy products, to develop recommendations for grading and management of toxicities. With collective experience from ongoing multicenter trials, approaches to grading and management of these toxicities continue to evolve. Herein, we describe the multidisciplinary approach adopted at our institutions and discuss our recommendations to monitor, grade, and manage the acute toxicities CRS, CRES, and HLH that may develop in patients treated with CAR T-cell therapy.

Keywords: CAR T-cell, cytokine release syndrome (CRS), CAR-related encephalopathy syndrome (CRES), neurotoxicity, hemophagocytic lymphohistiocytosis, cellular immunotherapy

Introduction

Cellular immunotherapy with autologous or allogeneic T cells that have been genetically altered to express chimeric antigen receptors (CARs) or T cell receptors (TCRs) to redirect their specificity against tumours is rapidly emerging as a promising treatment modality for a broad range of cancers.[1,2] The most advanced in clinical development is CAR T-cell therapy targeted against $CD19^+$ B-cell malignancies, including acute and chronic B-cell leukaemias and B-cell non-Hodgkin lymphomas. Multiple phase 1/2 clinical trials conducted at single institutions showed that CD19-specific CAR T-cell therapy frequently induces overall response rates of 50-90% in patients with B-cell malignancies refractory to standard therapies.[3-21] More importantly, durable remissions have been noted, suggesting that this therapy may be curative. The demonstration of the feasibility of central manufacturing and the safety of cryopreserved CAR T cells in early multicenter clinical trials with efficacy comparable to single institution trials suggests that this therapy may soon be broadly accessible.[19-21] Indeed, several phase 2 clinical trials with CD19-CAR T cells are currently ongoing with the intent of obtaining regulatory approvals for B-cell malignancies. In addition, novel targets are being explored with CAR and TCR re-directed cell therapies in preclinical and early phase clinical trials in both haematological and non-haematological malignancies.[1,2]

As adoptive T-cell therapies become more widely used, it is important to recognize their unique toxicities, which are distinct from those seen with traditional chemotherapies, monoclonal antibody (mAb), and small molecule targeted therapies. The two most commonly observed toxicities with CAR T-cell therapies are cytokine release syndrome (CRS), characterized by high fever, hypotension, hypoxia and/or multiorgan toxicity; and CAR-related encephalopathy syndrome (CRES) typically characterized by a toxic encephalopathic state with symptoms of confusion and delirium, and rarely, seizures and cerebral edema.[22-25] Rare cases of fulminant hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome (MAS) have

also been reported.[21,24,26,27] Such toxicities have also been observed after other redirected T-cell therapies such as TCR gene therapies, CAR NK-cell therapies, and bi-specific T-cell engaging antibody therapies.[28-33] In fact, both CRS and HLH/MAS have been observed after blinatumomab therapy, a CD19/CD3 bispecific T-cell receptor-engaging antibody.[34] Although these toxicities are manageable in most patients, some require monitoring and treatment in the intensive care setting, and fatalities may occur (**Table 1**). Accurate assessment and prompt management of toxicities may mitigate the adverse outcomes observed with these potentially curative therapies. The overall goal of management is to maximize the benefit from the cellular therapy while minimizing the risk for life-threatening complications of CRS and CRES. In order to have a consistent approach for monitoring, grading, and management of toxicities, we have formed a CAR T-cell therapy-associated TOXicity (CARTOX) Working Group with representatives from multiple institutions and multiple disciplines including hematologic oncology, solid tumour oncology, stem cell transplantation, neurology, critical care, immunology, and pharmaceutical sciences. These representatives were selected based on their extensive experience in treating patients with various CAR T-cell therapy products and other cellular therapies. Collectively, the authors have had experience in treating over 100 adult patients with leukemia or lymphoma with at least four different CD19 CAR-T cell therapy products[3,4,6,16,17] that were originally developed at academic institutions and subsequently licensed to commercial entities for further clinical development through multicenter clinical trials. The CARTOX Working Group discussed the available evidence in the literature and their collective experience in treating these patients over a period of six months and developed recommendations for monitoring, grading, and management of CRS, CRES, and HLH/MAS in adults. These recommendations incorporate and expand on the criteria proposed previously by Lee et al[22] for the diagnosis and management of CRS after cellular therapies. In addition, they provide a practical guide for monitoring, grading, and management of CRS, CRES, and HLH/MAS.

Herein, we review our management and treatment algorithms within the context of a clinical case.

Case

A 34 year-old female with refractory diffuse large B-cell lymphoma that previously failed rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy and subsequent salvage therapy using rituximab, ifosfamide, carboplatin, and etoposide followed by high-dose chemotherapy with autologous stem cell transplantation was treated with autologous CD19-CAR T-cell therapy with CD28 and CD3ζ signalling domains after conditioning chemotherapy with cyclophosphamide and fludarabine.[19] Within 24 hours of the CAR T cell infusion, she developed high-grade fever of up to 39.5^0C associated with tachycardia, fatigue, and decreased appetite that persisted for 6 days (**Figure 1A**). Fever (grade 1 CRS by Lee criteria[22]) was managed with acetaminophen, ibuprofen, and a cooling blanket. She received empiric broad-spectrum antibiotics and growth factor support for neutropenia; work-up was negative for infection. She also developed hypotension on day 1 with systolic blood pressure of 84 mmHg (grade 2 CRS[22]) and hypoxia on day 3 (grade 2 CRS[22]) and was treated with intravenous fluid bolus and supplemental nasal oxygen at 3 L/min, respectively. In addition, on days 1 and 3, she received intravenous (IV) tocilizumab (8 mg/kg), a humanized anti-IL-6 receptor (IL-6R) mAb that blocks IL-6 binding to its receptor, for management of hypotension and hypoxia, respectively and responded promptly. On day 5, she developed dysgraphia and subsequently became confused and disoriented (grade 2 by Common Terminology Criteria for Adverse Events (CTCAE) version 4.03).[35] Her handwriting impairment was the earliest sign of neurotoxicity and a mini-mental status examination done at the same time was only mildly decreased compared to baseline (**Figure 1B**). Neurotoxicity symptoms resolved 12h after treatment with tocilizumab on day 5. Corticosteroids were not administered. The C-reactive protein (CRP) level increased on day 2, the day after the onset of fever, and returned to baseline by the time the fever subsided (**Figure 1A**). She was discharged home on day 9. Restaging on day 30 revealed complete remission (**Figure 1C**) and she remains in remission 12

months later. Peak CAR T cell expansion in peripheral blood was observed within 2 weeks and circulating CAR T cells remained detectable at 12 months.[19]

Recommendations for grading and management of CRS

Cytokine release syndrome, the most common toxicity of cellular immunotherapy, is triggered by the activation of T cells when their TCRs or CARs engage the cognate antigens on tumour cells. The activation leads to proliferation of CAR T cells and release of cytokines and chemokines from antigen-redirected T cells (soluble IL2Rα, IFN-γ, IL-6, soluble IL6R, GM-CSF) as well as bystander immune cells such as monocytes/macrophages (IL-1RA, IL-10, IL6, IP-10, MIG, IFN-α, MIP-1α, MIP-1β, soluble IL6R), dendritic cells, and others.[9,11,18,19,21,26] CRS typically manifests with constitutional symptoms such as fever, malaise, anorexia, and myalgias but can affect any organ system in the body, including cardiovascular, respiratory, skin, gastrointestinal, hepatic, renal, haematological, and nervous system (**Table 2**).[22-24] Patients at high risk for severe CRS include subjects with bulky disease, co-morbidities, and those who develop early onset CRS within three days of infusion.[9,10,12] However, association of severe CRS with clinical parameters is imperfect and identification of biomarkers that predict severe toxicity are needed. Recent studies demonstrated that serum levels of IL-6, soluble gp130, IFN-γ, IL-15, IL-8, and/or IL-10 after CAR T cell infusion were associated with later development of severe CRS but the results need to be confirmed in prospective studies and seem to vary depending on the type of CAR T cell product used.[18,26]

Peak CRS toxicity after CAR T-cell therapy typically occurs within the first 7 days. Therefore, hospitalization with close monitoring is recommended for at least 7 days after CAR T cell infusion. Monitoring includes vital signs at least every 4 hours, and daily review of systems, physical exam, complete blood count with differential, complete metabolic profile, CRP, and ferritin (**Box 1**). Laboratory tests including blood counts and chemistry profile may need to be performed more than once daily especially in patients with risk of tumor lysis or in patients at risk of severe CRS or CRES. Due to high risk of arrhythmias, cardiac monitoring by telemetry is advised from the time of CAR T cell infusion until resolution of CRS symptoms. Additional

investigations such as chest x-ray, electrocardiogram, echocardiogram, electroencephalogram (EEG), and imaging studies may be performed as needed depending on toxicities. Fluid balance and daily weights should be strictly monitored and maintenance IV hydration is suggested for all patients. We recommend central venous access preferably with a double or triple lumen catheter prior to CAR T cell infusion. Packed red blood cells and platelets may be transfused per standard institutional guidelines. Corticosteroids should be avoided for management of fever or for premedication prior to blood transfusions. Growth factor support with filgrastim may be provided for neutropenia. Tumour lysis precautions should be used for patients with high tumour burden as per standard institutional guidelines. Patients who develop fever should be assessed for infection with blood and urine cultures and chest x-ray and additional tests such as cytomegalovirus PCR, reparatory viral screen, and computed tomography of chest as indicated. Such tests should also be performed prior to initiation of CAR T-cell therapy when there is suspected infection. Therapy with CAR T cells should be delayed until the infection has been controlled or ruled out, as undiagnosed infections may have catastrophic consequences in the setting of CRS (**Table 1**). We recommend conditional orders for fever and hypotension for all patients receiving CAR T-cell infusion so that appropriately trained nursing staff can act quickly in the event of toxicities minimizing wait times for intervention (**Box 1**). This should include empiric broad spectrum antibiotic therapy including gram-negative bacterial coverage, as sepsis and CRS have overlapping features and the absence of positive cultures cannot rule out pathogenic infection in immunocompromised cancer patients.

Peak CAR T cell levels and serum IL-6 levels have strongly correlated with the severity of CRS after CAR T-cell therapy.[9,10,18,21] IL-6 may signal directly by binding to membrane-bound IL-6R and gp130 (cis signalling) or by trans signalling where the IL-6-soluble IL6R complex binds to membrane-bound gp130 and leads to activation of JAK/STAT pathway.[36] While the expression of membrane-bound IL-6R is restricted to hematopoietic cells such as macrophages,

neutrophils, and T cells as well as hepatocytes, membrane-bound gp130 is expressed widely on all types of cells.[37] Thus, while cis signalling, which occurs at low levels of IL-6 affects only a few cell types and mediates anti-inflammatory effects; trans signalling, which predominates at higher levels of IL-6 as seen with CRS can affect most cell types in the body and mediate pro-inflammatory effects.[37] Therefore, tocilizumab, an anti-IL6R mAb, and siltuximab, a chimeric anti-IL-6 mAb that potently binds IL-6, have become the drugs of choice for management of moderate to severe CRS.[9,10,22,23,38] Tocilizumab is approved for treatment of rheumatoid arthritis[39] and siltuximab is approved for management of multicentric Castleman disease.[40] Both have been used off-label for management of CRS and have induced rapid reversal of CRS symptoms in most patients.[9,10,22,23,26,41] To date, tocilizumab has been used more commonly for management of CRS and its use does not appear to affect the efficacy of CAR T-cell therapy.[9,10,12,19,21] It is unclear whether tocilizumab offers an advantage over siltuximab for management of CRS. While IL-6 binds to IL-6R with an affinity of around 1 nM, siltuximab inhibits IL-6 with a Kd of 1pM and tocilizumab binds to IL-6R with a Kd of ~2.54 nM (**Box 2**).[42,43] Therefore, IL-6 could compete with the binding of tocilizumab to IL-6R. For this reason, it is possible that siltuximab might be more effective than tocilizumab to control CRS. In addition, IL-6 levels were shown to increase in serum after administration of tocilizumab.[44] There is theoretical concern that this might increase passive diffusion of IL-6 into the central nervous system (CNS) and increase the risk of neurotoxicity, whereas, this is unlikely to occur with siltuximab as it binds directly to IL-6. Direct comparison of tocilizumab and siltuximab is needed for comparison of effectiveness against CRS in prospective clinical studies.

Corticosteroids are also effective in the management of toxicities after cellular therapies since they suppress inflammatory responses.[22-24] However, they should be avoided for other indications after cellular therapies as they can suppress T cell function and/or induce apoptosis of T cells.[45-47] Studies of allogeneic stem cell transplant recipients have demonstrated that

cytomegalovirus-specific T cells may persist despite corticosteroid therapy but may have impaired cytokine production.[48] In the setting of immunotherapy, these data suggest that corticosteroids will likely impair the function, if not the persistence, of infused tumour-directed T cells. In a recent clinical trial, corticosteroid use for management of toxicities after CAR T-cell therapy did not impact objective response rate, however, the impact on long-term efficacy is unknown at this point.[21] Given these concerns, corticosteroids are generally considered when the toxicities are refractory to anti-IL-6 therapy.

We propose a 3-step approach involving assessment, grading, and management for CRS and CRES after CAR T-cell therapy (**Figure 2**). A subject may have CRS if at least one of the following four symptoms or signs is present within the first 3 weeks of cellular therapy: fever $\geq 38^0$C, hypotension with systolic blood pressure <90 mmHg, hypoxia with oxygen saturation of <90% on room air, and/or evidence of organ toxicity as outlined in **Table 2**.[22,23] Some of these symptoms and signs may be caused by other concurrent conditions or therapies; therefore, the provider should use clinical judgment to determine whether they are due to CRS or other causes.

The CRS grade should be determined at least twice daily and any time there is a change in the patient's status. We recommend the CRS grading proposed by Lee et al[22] with some modifications. It is based on four parameters; three of which are vital signs (temperature, blood pressure, and oxygen saturation) and the fourth is the grade of any organ toxicity that may be present (**Table 2**). Grading of organ toxicities is performed according to CTCAE version 4.03.[35] The definition of high-dose vasopressors that is used to distinguish between grades 2 and 3 CRS was previously reported by Lee et al.[22] However, it is important to evaluate shock as a dynamic parameter and not based on static dose requirements for vasopressors. A patient requiring a rapid increase in the dose of vasopressors or exhibiting evidence of end-organ hypo-perfusion should be treated aggressively irrespective of the dose of vasopressors required.

We recommend management of CRS in accordance with the grade as previously reported by Lee et al[22] with some modifications (**Table 3)**. Grade 1 CRS is primarily managed by supportive care. Maintenance IV fluids are recommended to keep patients well hydrated with special attention to fluid balance to avoid pulmonary vascular congestion. For grade 2 CRS, hypotension should be treated promptly with IV fluid boluses of normal saline. In addition, tocilizumab/siltuximab is recommended and may be repeated if needed for hypotension refractory to fluid boluses (**Table 3**). If hypotension persists after two fluid boluses and anti-IL-6 therapy, vasopressors should be initiated and transfer to intensive care unit (ICU) should be considered. A bedside echocardiogram is recommended for persistent or repeated episodes of hypotension to determine ejection fraction as left ventricular dysfunction can occur with CRS.[22] Moreover, non-invasive monitoring of hemodynamic parameters such as inferior vena cava filling pressures, passive leg raise, pulse pressure, and stroke volume variation can help guide the management of hypotension regarding need for IV fluids, vasopressors, or inotropic agents. Hypoxia associated with either non-cardiogenic pulmonary edema or pleural effusions should be managed with supplemental oxygen and diuresis or thoracentesis if indicated. Tocilizumab/siltuximab is recommended and may be repeated as needed for persistent hypoxia with FiO_2 <40% and other grade 2 organ toxicities. In patients at high-risk for severe CRS (grade 3 or 4) or persistent grade 2 CRS despite anti-IL-6 therapy, corticosteroids may be considered (**Table 3**).

Patients with grades 3 and 4 CRS are treated in the ICU for continuous monitoring, management of life-threatening arrhythmias, non-invasive positive pressure ventilation, and invasive support such as vasopressors or inotropes, mechanical ventilation, and/or dialysis. Both anti-IL-6 therapy and corticosteroids should be used for the management of grades 3 and 4 CRS and their associated organ toxicities. Corticosteroid taper should be individualized depending on response and toxicity, but it is generally recommended to be as rapid as possible.

It is important for the critical care team to be aware of all CAR-T treated patients in the hospital to facilitate prompt transfer and management in the ICU when needed. At our institutions, the ICU team communicates with the primary team daily and assesses any CAR T cell patients that may require ICU transfer.

CRP may be a useful marker to monitor in patients undergoing cellular therapies as IL-6 induces production of CRP by liver hepatocytes.[49-51] Therefore, a rise in CRP level is typically seen after the onset of CRS and correlates with IL-6 levels.[10,12,18,22,41] The return of CRP levels to baseline indicate that the CRS phase has ended and the patient can be considered for discharge from the hospital, assuming other toxicities that require monitoring and/or intervention have resolved. However, the correlation between CRP levels and CRS may vary and may not always be observed. Correlation between ferritin levels and CRS is even less consistent. Nevertheless, monitoring ferritin levels could be useful for diagnosis of CAR-related HLH/MAS.

Recommendations for grading and management of CRES

CRES typically manifests as a toxic encephalopathy with the earliest signs being diminished attention, language disturbance, and impaired handwriting. Other symptoms and signs may include confusion, disorientation, agitation, aphasia, somnolence, and tremors. In more severe cases, seizures, motor weakness, incontinence, mental obtundation, increased intracranial pressure, papilledema, and cerebral edema may also occur. The onset of CRES may be biphasic; the first phase typically occurs concurrently with high fever and other CRS symptoms within the first five days after cellular therapy, and the second phase may occur after the fever and other CRS symptoms subside, often after five days. Delayed neurotoxicity with seizures or episodes of confusion occurring in the third or fourth week after CAR-T therapy has also been noted. In our experience, anti-IL-6 therapy may reverse CRES during the first phase but is generally not effective during the second phase when corticosteroids are the preferred agents. It is possible that the differential benefit of anti-IL-6 therapy between the two phases is the result of a more permeable blood-brain barrier during CRS, allowing the diffusion of the mAbs. CRES typically lasts 2-4 days but may vary in duration from a few hours to weeks and the grade may fluctuate rapidly thus necessitating close monitoring. CRES may be disturbing to patients, families, and the medical staff, but is generally reversible, although rare fatal cases have occurred.[17,18,26,52]

The pathophysiology of CRES remains unclear. Two potential explanations include passive diffusion of cytokines as higher levels of serum IL-6 and IL-15 levels were associated with more severe neurotoxicity[12,21] or alternatively trafficking of T cells into CNS as CAR T cells have been detected in cerebrospinal fluid (CSF) of patients with neurotoxicity in the absence of malignant CNS disease and tend to be higher in patients that develop neurotoxicity vs. those that do not.[5,9,12,25] Other organ dysfunction (hepatic, renal, hypoxemia, and infection) could contribute to the encephalopathy as well. Secondary cortical irritation is suggested by EEG

findings of epileptiform discharges or non-convulsive electrographic seizures. The most common finding on EEG is diffuse generalized slowing with and without triphasic waves at 1-2 Hz in keeping with an encephalopathic state. Non-convulsive electrographic seizures are defined according to published EEG guidelines and response to benzodiazepines.[53] Criteria include repetitive epileptiform discharges with frequency of >2.5 Hz or multifocal frequent epileptiform discharges responding to intravenous benzodiazepines with background organization. Incidence of non-convulsive status epilepticus after CAR T cell therapy in our experience is approximately 10%. Some patients may develop non-convulsive status epilepticus after convulsive status epilepticus. Response to benzodiazepine in some patients has been quick with improvement of both the EEG and mental status. Protein is usually elevated in the CSF, suggesting impaired blood brain barrier. Magnetic resonance imaging and computerized tomography of the brain are usually negative for any anatomical pathology to account for neurotoxicity symptoms, although cerebral edema has been rarely observed.[25] Patients with malignant cerebral edema while rare tend to have a very rapid course with ensuing brain death. In fact, five deaths due to cerebral edema were recently reported with one of the CD19 CAR T-cell products in a multicenter clinical trial.[54] It is unclear why these deaths due to cerebral edema have been observed with one CD19 CAR-T product but not others. Additional investigations are needed to understand the pathophysiology of this fatal complication. Seizure prophylaxis with levetiracetam 750 mg oral/IV every 12h is recommended for 30 days starting on the day of infusion for CAR T-cell therapies known to cause CRES. Levetiracetam is the preferred agent for seizure prophylaxis as it has better drug-drug interaction profile, lower risk of cardiotoxicity compared to other anti-epileptics, and may be administered safely in the setting of hepatic dysfunction. Dose adjustments may be needed for renal dysfunction. Furthermore, cytokine levels are not affected by levetiracetam.[55]

Like organ toxicities, CRES has been graded according to CTCAE[35] with respect to level of consciousness, orientation, ability to perform activities of daily living in the setting of encephalopathy, speech, tremors, seizures, incontinence, and motor weakness. However, the CTCAE grading does not adequately quantify the acute neurologic deficits that appear to be unique to CAR T-cell therapies. Thus, we have developed a new grading system for CRES along with a "CARTOX 10-point neurological assessment" tool (**Table 4**). The CARTOX-10 was created based on observation and treatment of over 50 patients with neurotoxicity from CAR T-cell therapy. CARTOX-10 takes into consideration the predominant alterations in concentration, speech, and writing ability observed in patients with CRES. It takes some of the key elements from the 30-point Mini-Mental Status Exam (MMSE) to assess the acute neurotoxic events seen in patients treated with CAR T cells on a 10-point scale. Compared with MMSE that is used to screen patients for dementia (not delirium), the CARTOX-10 is simple to use, can be administered rapidly, and used repeatedly several times a day by all healthcare providers including nurses and physicians involved in the care of the patients. The tasks used for neurological assessment by CARTOX-10 could be simplified depending on the education level of the patient but need to be documented prior to CAR T-cell infusion to be reliable and consistent during subsequent follow up. In addition to CARTOX-10, parameters such as papilledema and CSF opening pressure were included to detect early signs of raised intracranial pressure and cerebral edema. The advantages of this grading system over the CTCAE grading include greater objectivity and ease of application as the CARTOX 10-point neurological assessment can be performed by all providers involved in the care of the patient. We recommend that this 10-point neurological assessment be performed every 8 hours while hospitalized. Any change from a normal score should prompt thorough investigation as described below. Patients who are aphasic but awake and without other neurological symptoms or signs such as motor weakness, seizures and papilledema are considered to have grade 3 CRES.

Similar to CRS, the management of CRES is based on the grade as summarized in **Table 5**. Grade 1 CRES is primarily managed with supportive care. The head-of-bed should be elevated by at least 30 degrees to minimize aspiration risks and to improve cerebral venous flow. A neurology consultation should be requested for thorough neurological evaluation, including EEG and fundoscopic exam to rule out papilledema for all patients with CRES regardless of grade. Assessment for papilledema can be difficult in restless patients with non-dilated pupils. Neuroimaging and CSF opening pressure, when available, are much better surrogates of increased intracranial pressure and possible cerebral edema. However, lumbar puncture may not always be feasible if patients are restless or have coagulopathy. In patients with ommaya reservoir, opening pressure can be measured in the supine position with the base of the manometer placed at heart level. Combinations of the above techniques as well as ophthalmological evaluation for assessing papilledema should be considered to diagnose increased intracranial pressure and cerebral edema. Repeated neuroimaging is recommended to diagnose early cerebral edema for patients with grade 3 and 4 CRES and in patients with rapid changes in the CRES grade (Δ grade change by 2 (for example, a patient with grade 1 CRES worsening to grade 3)). Review of imaging studies with a neuroradiologist to detect early signs of cerebral edema is also recommended. Clinical status of the patient may dictate the neuroimaging study. While magnetic resonance imaging (MRI) of brain is preferred computed tomography (CT) scan can be pursued in unstable or agitated patients. The development of cerebral edema in our experience was associated with other acute and significant neurological changes such as lower CRES score and/or seizures. Anti-IL-6 therapy is recommended for ≥ grade 2 CRES with concurrent CRS. If CRES is not associated with CRS, corticosteroids are the preferred agents for management (**Table 5**). Corticosteroids may be tapered after improvement of CRES to grade 1. While the optimal duration of corticosteroid therapy remains unclear, in our experience, short courses of steroids have been associated with resolution of neurologic toxicities without impairing antitumor responses.[21] Patients should be monitored for

recurrence of neurotoxicity symptoms during the taper. Monitoring in the ICU is recommended for grade 3 CRES. All patients with grade 4 CRES should be monitored in the ICU as they may require mechanical ventilation for airway protection. Non-convulsive and convulsive status epilepticus are managed with benzodiazepines and additional anti-epileptics as needed (**Box 3**). Phenobarbital is the preferred second anti-epileptic for management of seizures as phenytoin and lacosamide have higher risk of cardiovascular adverse effects, precluding use in patients with concurrent CRS to avoid arrhythmias and hypotension. Cerebral edema should be managed promptly with corticosteroids and acetazolamide for mild cerebral edema, and high-dose corticosteroids, hyperventilation, and hyperosmolar therapy for more severe cases (**Box 4**).

Recommendations for grading and management of CAR-related HLH

HLH/MAS is a syndrome of severe immune activation characterized by hyperactive macrophages and lymphocytes, proinflammatory cytokine production, lymphohistiocytic tissue infiltration, and immune mediated multiorgan failure. This clinical syndrome is similar irrespective of the underlying cause of HLH/MAS. Patients with CRS after CAR T-cell therapy have clinical and laboratory features that resemble HLH/MAS including high fever, multiorgan dysfunction, CNS disturbances, elevated ferritin, elevated lactate dehydrogenase, high soluble CD25, low fibrinogen, and increased serum cytokines such as IFN-γ and IL-6.[21,24,26,27,56-58] It is possible that CRS and HLH/MAS represent a similar spectrum of systemic hyperinflammatory disorders. While CRS usually responds to supportive care, anti-IL-6 therapies, and corticosteroids, fulminant and refractory HLH/MAS observed in ~1% of patients treated with CAR T-cell therapy may require additional therapy and has a high mortality rate if not treated promptly.[59,60] However, the diagnosis of HLH/MAS can be difficult in the setting of CRS. Many of the diagnostic criteria traditionally used for HLH/MAS such as fever, splenomegaly, cytopenias in at least two of three cell lineages, hypertriglyceridemia or hypofrinogenemia with elevated D-dimers, hemophagocytosis in bone marrow, hyperferritinemia, high soluble CD25, and low or absent NK cell activity are not specific and are frequently present in patients with even low-grade CRS and among patients with advanced hematologic malignancies even in the absence of CAR T-cell therapy.[61] Therefore, new criteria are needed for diagnosis of HLH/MAS in patients with CRS after CAR T-cell therapy.

We propose that a diagnosis of HLH/MAS should be considered in patients treated with CAR T-cell therapy if 1) the subject has had a peak ferritin of >10,000 ng/mL during the CRS phase, and 2) has developed any two of the following: ≥ grade 3 organ toxicities involving liver, kidney, or lung, or hemophagocytosis in bone marrow or organs (**Box 5**). Patients with suspected HLH/MAS should be managed with anti-IL-6 therapy and corticosteroids for ≥ grade 3

organ toxicities as per CRS algorithm (**Table 3, Figure 3**).[34] If there is no improvement clinically or serologically within 48h, additional therapy with etoposide 75-100 mg/m^2 should be considered for management of HLH/MAS as available evidence suggests that this is the preferred choice of therapy for refractory HLH (**Figure 3**).[56,59,62] Etoposide may be used in HLH patients with liver and kidney dysfunction. Indeed, it is imperative to initiate etoposide therapy rapidly in spite of organ dysfunction in patients with high degree of suspicion for HLH.[61] Etoposide may be repeated in 4-7 days as clinically or serologically indicated to achieve adequate disease control. Intrathecal cytarabine with or without hydrocortisone should also be considered in patients with HLH with associated neurotoxicity. Although etoposide and cytarabine are often used for HLH associated with other causes,[56,59,62] direct evidence to support their use in patients with CAR T cell-associated HLH is lacking at this time. The goal of therapy in HLH is to suppress overactive CD8$^+$ T-lymphocytes and macrophages. However, cytokines that play a central role in HLH/MAS may be targetable in the near future using agents in clinical development such as NI-0501, a humanized anti-IFN-γ mAb that neutralizes IFN-γ and has produced responses in 9 of 13 children with refractory primary HLH with good tolerability.[63]

Conclusions and Future Directions

CAR T-cell therapies offer promise to improve clinical outcomes and induce remissions in refractory malignancies. However, their unique acute toxicities, which may be fatal, require intensive monitoring and prompt management. Many factors likely affect the onset, peak, duration, and type of acute toxicity after various CAR T-cell therapies, which should be considered when monitoring and treating each patient. These may include: 1) the nature of the conditioning chemotherapy; 2) the design of the CAR construct; 3) CAR T cell dose; 4) the cellular composition of the CAR T cell product; 5) the manufacturing process for CAR T cells; and 6) host characteristics including the type of malignancy, tumour burden, patient age, and sites of disease.

Systematic investigation will be necessary to define predictors of efficacy and toxicity, and to determine whether current interventions such as anti-IL-6 therapies and corticosteroids affect efficacy. Such studies may also identify novel biomarkers of severe toxicity and lead to development of prophylactic strategies to further improve safety. Indeed, peak IFN-γ level after CAR T-cell therapy also correlated with severity of CRS[12,18,26] and may be another important target for management of CRS in the future although there is concern whether blocking IFN-γ might affect anti-tumour efficacy. Other approaches currently being tested in preclinical and clinical studies to improve safety include CAR T-cell therapies with “safety (suicide) switches” or “elimination genes” that can be activated or targeted to eliminate the CAR T cells prior to the development of life-threatening toxicities.[41,64-70] An alternative approach is to use “remote-controlled” CARs using an inducible system that controls the expression of CARs upon drug administration.[71]

While the above approaches may enhance the safety of CAR T-cell therapies, combination strategies with immune checkpoint blockade may increase their efficacy and

persistence but also have the potential to increase their toxicity. Furthermore, CAR T-cell therapies may be associated with on-target, off-tumour effects if the target antigen is expressed on normal tissues. This has been well recognized with CD19 CAR T-cell therapy, which may cause prolonged B-cell aplasia and hypogammaglobulinemia.[3,9,11,19,72] However, normal B cells are expendable as hypogammaglobulinemia may be easily corrected with IV immunoglobulin replacement therapy. In contrast, if the target antigen for CAR T cells is shared with vital normal tissues, it may lead to severe and possibly fatal toxicity.[73] Indeed, this possibility of on-target, off-tumour toxicity is the greatest obstacle for successful development of CAR T-cell therapy approaches for solid malignancies. A patient treated with HER-2/neu-specific CAR developed fatal respiratory failure and multiorgan dysfunction that was thought to be due to expression of the target antigen in lung tissue.[74] However, it is possible that this death may have been related to the higher dose of CAR T cells used[74] as another trial targeting HER-2/neu with a lower dose of CAR T cells was tolerable.[75] In other studies, CAR T cells targeting carcinoembryonic antigen have caused colitis[76] and CAR T cells targeting carbonicanhydrase-IX have resulted in cholestasis.[77,78] An acute hypersensitivity reaction with anaphylaxis was also described in one patient treated with multiple infusions of mesothelin-specific CAR T cells.[79] This presumably occurred due to development of human anti-mouse antibodies against the murine mAb used in the CAR construct.[79] Therefore, vigilant monitoring and expecting the unexpected is necessary for all CAR T-cell and transgenic TCR therapies evaluating novel conditioning regimens, novel constructs, novel targets, and novel combinations. We also advocate for the development of customized tracking tools in electronic health record systems to monitor and grade these toxicities (**Table 6**).

In conclusion, the recommendations provided here are meant to serve as a framework for assessment and management of toxicities associated with CAR T-cell therapies but may also be used for TCR gene therapies, CAR NK-cell therapies, and potentially bi-specific T-cell

engaging antibody therapies that may also cause similar toxicities.[28-34] While appropriate given present knowledge and experiences to date, these recommendations are expected to evolve, as we increase our understanding of the pathophysiology of these toxicities, the determinants of durable antitumor responses, and the effects of interventions used to manage toxicities of these promising novel therapies.

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Authorship

SSN wrote the first draft of the manuscript. All authors contributed to the development of the proposed guidelines and reviewed and edited the manuscript.

Conflict of interest disclosures

SSN has received research support and honoraria from, and served as a consultant and Scientific Board Member for Kite Pharma; FLL has served as a scientific advisory board attendee for Kite Pharma; KVK has served as a scientific advisor to Kite Pharma and Juno Therapeutics, and has received research funding from Kite Pharma and Juno Therapeutics. YL has received research funding from Janssen. NJ has received research support form Pfizer and Cellectis, and received research support and honoraria from, and served as an Advisory Board member for Servier.

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Table 1. Reported causes of death after CAR T-cell therapies.

Age (yrs)	CAR T cell product	CAR T cell dose (per kg)	Malignancy	Day of death post-CAR T	Cause of death	Reference
48	CD19-137-ζ	$CD4^+$ (11.6×10^6)+ $CD8^+$ (8.4×10^6)	B-ALL	3	CRS	Turtle et al.[17]
>18	CD19-137-ζ	$CD4^+$ (10×10^6)+ $CD8^+$ (10×10^6)	NHL	30	CRS (+GI Bleed)	Turtle et al.[18]
>18	CD19-137-ζ	6.5×10^6	B-ALL	5	CRS (+Influenza B)	Frey et al.[80]
>18	CD19-137-ζ	6.7×10^6	B-ALL	15	CRS (+Pseudo-monas sepsis, pneumonia)	Frey et al.[80]
>18	CD19-137-ζ	8.4×10^6	B-ALL	15	CRS (+Steno-trophomonas sepsis, pneumonia)	Frey et al.[80]
69	CD19-28-ζ	$1.2\text{-}3.0\times10^7$	CLL	2	CRS	Brentjens et al.[81]
52	CD19-137-ζ	$CD4^+$ (1×10^6)+ $CD8^+$ (1×10^6)	B-ALL	122	Neurotoxicity	Turtle et al.[17]
>18	CD19-137-ζ	$CD4^+$ (10×10^6)+ $CD8^+$ (10×10^6)	NHL	13	Neurotoxicity (+CNS bleed)	Turtle et al.[18]
>18	CD19-137-ζ	N/A	FL	N/A	Encephalitis	Chong et al.[82]
>18	CD19-28-ζ	2×10^6	DLBCL	N/A	HLH	Neelapu et al.[21]
39	ERBB2-28-137-ζ	1×10^{10} total cells	Colon cancer	5	ARDS	Morgan et al.[74]
>18	CD19-28-ζ	2×10^6	NHL	N/A	Cardiac arrest	Locke et al.[83]
30	CD19-28-ζ	2.5×10^6	PMBCL	16	Unknown (possibly cardiac arrhythmia)	Kochenderfer et al[11]
N/A	CD19-28-ζ	N/A	ALL	N/A	Cerebral edema (5 cases)	Johnson et al.[54]

CAR – chimeric antigen receptor; 137 – CD137 (4-1BB); 28 – CD28; ζ – CD3ζ; ALL – acute lymphoblastic leukemia; NHL – non-Hodgkin lymphoma; CLL – chronic lymphocytic leukemia; FL – follicular lymphoma; DLBCL – diffuse large B-cell lymphoma; PMBCL – primary mediastinal B-cell lymphoma; CRS – cytokine release syndrome; GI – gastrointestinal; CNS – central nervous system; HLH – hemophagocytic lymphohistiocytosis; ARDS – acute respiratory distress syndrome; N/A – not available.

Table 2. Grading of cytokine release syndrome (CRS) (adapted from Lee et al).[22]

Category	Symptom/Sign	CRS Grade 1[a]	CRS Grade 2[b]	CRS Grade 3[b]	CRS Grade 4[b]
Vital signs	Temperature ≥38 ^{0}C	Yes	Any	Any	Any
	SBP <90 mmHg	No	Responds to IV fluids or low-dose vasopressor	Needs high-dose or multiple vasopressors[d]	Life- threatening
	Needing oxygen for O_2 sat >90%	No	FiO_2 <40%	FiO_2 ≥40%	Needing ventilator support
Organ toxicity[c]	See below	Grade 1	Grade 2	Grade 3 or grade 4 transaminitis	Grade 4 except grade 4 transaminitis

Symptoms or signs of organ toxicity

i. Cardiac – tachycardia, arrhythmias, heart block, low ejection fraction
ii. Respiratory – tachypnea, pleural effusion, pulmonary edema
iii. Gastrointestinal – nausea, vomiting, diarrhea
iv. Hepatic – increased aspartate transaminase, alanine transaminase, or bilirubin
v. Renal – acute kidney injury (increased creatinine), decreased urine output
vi. Skin – rash (less common)
vii. Coagulopathy – disseminated intravascular coagulation (less common)
viii. Neurologic – confusion, disorientation, agitation, dysphasia, aphasia, tremors, seizures, motor weakness, incontinence, increased intracranial pressure, papilledema, cerebral edema

[a] Grade 1 CRS may manifest as fever and/or grade 1 organ toxicity

[b] For Grades 2, 3, or 4 CRS, any one of the criteria other than temperature is sufficient

[c] Grading of organ toxicities is performed according to Common Terminology Criteria for Adverse Events, version 4.03[35]

[d] See Reference Lee et al.[22] for definition of high-dose vasopressors

SBP – systolic blood pressure; FiO_2 – fraction of inspired oxygen

Table 3. Recommendations for management of cytokine release syndrome (CRS)[a].

CRS Grade	Symptom or Sign	Management
Grade 1	Fever or grade 1 organ toxicity	• Acetaminophen and hypothermia blanket for fever • Ibuprofen may be used as second option for fever if not contraindicated • Assess for infection with blood and urine cultures, and chest x-ray • Empiric broad-spectrum antibiotics and filgrastim if neutropenic • Maintenance IV fluids for hydration • Symptomatic management of constitutional symptoms and organ toxicities • Consider tocilizumab 8 mg/kg or siltuximab 11 mg/kg IV for persistent (>3 days) and refractory fever
Grade 2	Hypotension	• IV fluid bolus of 500 – 1000 mL normal saline • May give a second IV fluid bolus if SBP remains <90 mmHg • Tocilizumab 8 mg/kg[b] IV or siltuximab 11 mg/kg IV for hypotension refractory to fluid boluses; may be repeated if needed • If hypotension persists after two fluid boluses and anti-IL-6 therapy, start vasopressors, consider transfer to ICU, obtain echocardiogram and initiate other methods of hemodynamic monitoring • In patients at high-risk[c] or if hypotension persists after 1-2 doses of tocilizumab/siltuximab, may use dexamethasone 10 mg IV every 6h • Manage fever and constitutional symptoms as in grade 1
	Hypoxia (FiO_2<40%)	• Supplemental oxygen • Tocilizumab/siltuiximab +/- corticosteroids and supportive care as in hypotension
	Grade 2 organ toxicity	• Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab/siltuximab +/- corticosteroids and supportive care as in hypotension
Grade 3	Hypotension	• IV fluid boluses as needed as in grade 2 • Tocilizumab/siltuximab as in grade 2 if not administered previously • Vasopressors as needed • Transfer to ICU, echocardiogram and hemodynamic monitoring as in grade 2 • Dexamethasone 10 mg IV every 6h; increase to 20 mg IV every 6h if refractory • Manage fever and constitutional symptoms as in grade 1
	Hypoxia (FiO_2≥40%)	• Supplemental oxygen including high flow oxygen delivery and non-invasive positive pressure ventilation • Tocilizumab/siltuximab + corticosteroids and supportive care as above
	Grade 3 organ toxicity or grade 4 transaminitis	• Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab/siltuiximab + corticosteroids and supportive care as above
Grade 4	Hypotension	• IV fluids, anti-IL-6 therapy, vasopressors, and hemodynamic monitoring as in grade 3 • Methylprednisolone 1 gram/day IV may be used in place of dexamethasone • Manage fever and constitutional symptoms as in grade 1
	Hypoxia	• Mechanical ventilation • Tocilizumab/siltuximab + corticosteroids and supportive care as above
	Grade 4 organ	• Symptomatic management of organ toxicities as per standard

	toxicity excluding transaminitis	guidelines • Tocilizumab/siltuximab + corticosteroids and supportive care as above

[a] All medication doses indicated are for adults.
[b] Tocilizumab – maximum per DOSE is 800 mg
[c] High risk patients include subjects with bulky disease, co-morbidities, and those who develop early onset CRS within three days of infusion

IV – intravenous; SBP – systolic blood pressure; ICU – intensive care unit; FiO_2 – fraction of inspired oxygen;

Table 4. Grading of CAR-Related Encephalopathy Syndrome (CRES).

Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
Neurological assessment score (see below)	Mild (7-9)	Moderate (3-6)	Severe (0-2)	Critical / Obtunded
Raised intracranial pressure	-	-	Stage 1 or 2 papilledema[a]; or CSF opening pressure <20 mmHg	Stage 3, 4, or 5 papilledema; CSF opening pressure ≥20 mmHg; or cerebral edema
Seizures or motor weakness	-	-	Partial seizure; non-convulsive seizures on EEG responding to benzodiazepine	Generalized seizures; convulsive or non-convulsive status epilepticus; new motor weakness
CARTOX 10-point neurological assessment **(Assign one point for each task performed correctly; Score of 10 = normal)** • Orientation to year, month, city, hospital, President: 5 points • Name 3 objects (point to clock, pen, button): 3 points • Ability to write a standard sentence (e.g. Our national bird is the bald eagle): 1 point • Count backwards from 100 by ten: 1 point				

[a]Papilledema grading is performed according to Modified Frisén scale.[84]

CSF – cerebrospinal fluid; EEG – electroencephalogram; CARTOX – CAR T-cell therapy-associated TOXicity

Table 5. Recommendations for management of CAR-Related Encephalopathy Syndrome (CRES)[a].

Grade	Management
Grade 1	• Vigilant supportive care; aspiration precautions; IV hydration • Withhold oral intake of food, medicines, and fluids and assess swallowing • Convert all oral medications and/or nutrition to IV if swallowing is impaired • Avoid medications that cause central nervous system depression • Low doses of lorazepam (0.25-0.5 mg IV every 8h) or haloperidol (0.5 mg IV every 6h) may be used for agitated patients with careful monitoring • Neurology consultation • Fundoscopic exam to assess for papilledema • MRI brain with and without contrast; diagnostic lumbar puncture with opening pressure; MRI spine if focal peripheral neurological deficits; CT scan of brain may be performed if MRI brain is not feasible • Daily 30 min EEG until toxicity symptoms resolve; if no seizures on EEG, continue levetiracetam 750 mg every 12h • If EEG shows non-convulsive status epilepticus, treat as per algorithm in Box 2 • Consider tocilizumab 8 mg/kg[b] or siltuximab 11 mg/kg IV if associated with concurrent CRS
Grade 2	• Supportive care and neurological work-up as per grade 1 • Tocilizumab 8 mg/kg[b] or siltuximab 11 mg/kg IV if associated with concurrent CRS • Dexamethasone 10mg IV every 6h or methylprednisolone 1 mg/kg IV every 12h if refractory to anti-IL-6 therapy or for CRES without concurrent CRS • Consider ICU transfer if associated with grade 2 or greater CRS
Grade 3	• Supportive care and neurological work-up as per grade 1 • ICU transfer is recommended • Tocilizumab/siltuximab if associated with concurrent CRS as per grade 2 and if not administered previously • Corticosteroids as above for worsening symptoms despite anti-IL-6 therapy or for CRES without concurrent CRS; Continue corticosteroids until improvement to grade 1 and then taper • Stage 1 or 2 papilledema with CSF opening pressure < 20 mmHg, treat as per algorithm in Box 3 • Consider repeat neuro-imaging (CT or MRI) every 2-3 days if persistent ≥ grade 3 CRES
Grade 4	• Supportive care and neurological work-up as per grade 1 • ICU monitoring; Consider mechanical ventilation for airway protection • Tocilizumab/siltuximab and repeat neuro-imaging as per grade 3 • High-dose corticosteroids (e.g. methylprednisolone IV 1 g/day x 3 days followed by rapid taper at 250 mg every 12h x 2 days, 125 mg every 12h x 2 days, and 60 mg every 12h x 2 days); Continue corticosteroids until improvement to grade 1 and then taper • For convulsive status epilepticus, treat as per algorithm in Box 2 • Stage 3, 4, or 5 papilledema, CSF opening pressure ≥ 20 mmHg, or cerebral edema, treat as per algorithm in Box 3

[a] All medication doses indicated are for adults
[b] Tocilizumab – maximum per DOSE is 800 mg

IV – intravenous; MRI – magnetic resonance imaging; CT – computed tomography; EEG – electroencephalogram; CRS – cytokine release syndrome; ICU – intensive care unit; CSF – cerebrospinal fluid

Table 6. An example of a customized tracking tool for monitoring CAR T cell therapy toxicity in electronic health records.

Toxicity category	Date & Time	Date & Time	Date & Time
CRS symptoms and signs			
Temperature			
Heart rate			
Blood pressure			
Oxygen saturation			
FiO2			
Cardiac			
Sinus tachycardia			
Arrhythmia			
Hear block			
Ejection fraction			
Vasopressors			
Respiratory			
Pleural effusion			
Pulmonary edema			
Gastrointestinal			
Nausea			
Vomiting			
Diarrhea			
Hepatic			
AST			
ALT			
Total bilirubin			
Renal			
Urine output			
Creatinine			
Coagulopathy			
PT			
PTT			
D-dimers			
Skin rash			
Other toxicities			
CRS Grade			
CRES symptoms and signs			
CARTOX10			
Orientation x 5			
Name 3 objects			
Count backwards			
Ability to write			
CARTOX10 score			
Increased intracranial pressure			
CSF opening pressure			
Papilledema			
Cerebral edema			
Seizures			
Convulsive			
Non-convulsive			
Motor weakness			
Alertness			
Other toxicities			
CRES Grade			
Miscellaneous			
CRP level			
Ferritin level			
Hemophagocytosis			
HLH/MAS			

Box 1. Supportive care considerations for CAR T-cell therapy.

Prior to CAR T cell infusion

- Baseline brain magnetic resonance imaging to rule out any central nervous system disease
- Central venous access with double or triple lumen catheter
- Cardiac monitoring by telemetry starting on the day of CAR T cell infusion and continued until CRS resolves
- Tumour lysis prophylaxis for patients with bulky tumours
- Seizure prophylaxis with levetiracetam at 750 mg orally q12h for 30 days starting on the day of infusion for CAR T-cell therapies known to cause CRES
- Hospitalization recommended for at least 7 days after CAR T-cell therapy

Monitoring after CAR T cell infusion

- Vitals q4h, strict input and output, daily weights
- Daily history and physical examination
- Daily blood counts and complete metabolic profile
- C-reactive protein and ferritin levels daily starting on day 0
- Assessment and grading of CRS should be done at least twice daily and whenever there is a change in patient's status
- Assessment and grading for CRES using the CARTOX 10-point neurological assessment should be done at least every 8 hours and should include writing a sentence twice daily
- Maintenance IV fluids with normal saline to ensure adequate hydration

Notifications and contingency orders

- Notify physician
  - ✓ SBP >140 or <90 mmHg
  - ✓ Heart rate >120 or <60 / min or arrhythmia
  - ✓ Respiratory rate >25 or <12 / min
  - ✓ Oxygen Saturation <92% on room air
  - ✓ Urine output <1500 mL/24h
  - ✓ Upward trends in creatinine or liver function tests
  - ✓ Tremors or jerky movements in extremities
  - ✓ Change in mental status (alertness, orientation, speech, and ability to write a sentence)
- For temperature greater than 38.3 ^{0}C, send blood cultures (central and peripheral) and urine for urinalysis and culture, obtain portable chest x-ray, and notify physician
- For patients with neutropenia and fever, start empiric broad-spectrum antibiotics
- Do not administer corticosteroids unless approved by physician
- If patient develops CRES, withhold oral intake and notify physician
- PRN medications
  - ✓ Acetaminophen (1st choice) or ibuprofen (2nd choice if not contraindicated) for fever > 38.3 ^{0}C
  - ✓ Cooling blanket prn fever > 38.3 ^{0}C
  - ✓ Normal saline 500 to 1000 mL bolus prn SBP <90 mmHg; may repeat once if SBP <90 mmHg after 1st bolus
  - ✓ PRN tocilizumab or siltuximab to be activated on physician order

CRS – cytokine release syndrome; CRES – CAR-Related Encephalopathy Syndrome; CARTOX – CAR T-cell therapy-associated TOXicity; IV – intravenous; SBP – systolic blood pressure; PRN – pro re nata (as needed)

Box 2. Comparison of tocilizumab and siltuximab.

Tocilizumab

- Humanized anti-IL-6 receptor IgG1κ monoclonal antibody
- Binds to both soluble and membrane-bound human IL-6 receptors and inhibits IL-6-mediated signalling through these receptors
- Kd for binding of tocilizumab to IL-6R is ~2.54 nM
- FDA approved for treatment of rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, and systemic juvenile idiopathic arthritis
- Off-label use for management of cytokine release syndrome
- Dose – 4 to 8 mg/kg infused over 1 hour
- Maximum dose is 800 mg
- Pre-medication not required
- Terminal half-life is 6.3 days
- Serum IL-6 levels increase after administration of tocilizumab

Siltuximab

- Chimeric anti-IL-6 IgG1κ monoclonal antibody
- Binds to human IL-6 and prevents the binding of IL-6 to both soluble and membrane-bound IL-6 receptors
- Kd for binding of siltuximab to IL-6 is 1 pM
- FDA approved for treatment of multicentric Castleman disease who are human immunodeficiency virus (HIV) negative and human herpesvirus-8 (HHV-8) negative
- Off-label use for management of cytokine release syndrome
- Dose – 11 mg/kg infused over 1 hour
- Complete infusion within 4 hours of reconstitution
- Pre-medication not required
- Terminal half-life is 20.6 days
- Serum IL-6 levels not expected to increase after administration of siltuximab

Box 3. Recommendations for management of non-convulsive and convulsive status epilepticus after CAR T-cell therapy[a].

Non-convulsive status epilepticus

- Assess airway, breathing, and circulation; check blood sugar
- Lorazepam 0.5 mg IV × 1 with additional 0.5 mg IV every 5 min up to a total of 2 mg to control electrographical seizures
- Levetiracetam 500 mg IV bolus
- If seizures persist, transfer to ICU and add phenobarbital loading dose 60 mg IV
- Maintenance doses after resolution of non-convulsive status epilepticus
  - ✓ Lorazepam 0.5 mg IV every 8h × 3 doses
  - ✓ Increase levetiracetam to 1000 mg IV every 12h
  - ✓ Phenobarbital 30 mg IV every 12h

Convulsive status epilepticus

- Assess airway, breathing, and circulation; check blood sugar
- Transfer to ICU
- Lorazepam 2 mg IV × 1 with additional 2 mg IV to a total of 4 mg to control seizures
- Levetiracetam 500 mg IV bolus
- If seizures persist, add phenobarbital loading dose 15 mg/kg IV
- Maintenance doses after resolution of convulsive status epilepticus
  - ✓ Lorazepam 0.5 mg IV every 8h × 3 doses
  - ✓ Increase Levetiracetam to 1000 mg IV every12h
  - ✓ Phenobarbital 1-3 mg/kg IV every 12h
  - ✓ Continuous EEG monitoring, if seizures are refractory

[a] All medication doses indicated are for adults

IV – intravenous; ICU – intensive care unit; EEG – electroencephalogram

Box 4. Recommendations for management of cerebral edema after CAR T-cell therapy[a].

Stage 1 or 2 papilledema[b] with CSF opening pressure < 20 mmHg without cerebral edema

- ✓ Consider acetazolamide 1000 mg IV followed by 250 mg to 1000 mg IV every 12h (adjust dose based on renal and acid/base balance)

Stage 3, 4, or 5 papilledema[b], any cerebral edema on imaging studies, or CSF opening pressure ≥ 20 mmHg

- ✓ Use high-dose steroids as per grade 4 CRES along with the following measures for management of cerebral edema
- ✓ Elevate head end of bed to 30 degrees
- ✓ Hyperventilation to achieve target $PaCO_2$ of 28-30 mmHg for no greater than 24h
- ✓ Hyperosmolar therapy with either mannitol 20% or hypertonic saline (3% or 23.4%)
  - Mannitol: initial dose 0.5 to 1 g/kg, maintenance at 0.25 to 1 g/kg every 6h while monitoring metabolic profile and serum osmolality every 6h; hold mannitol if serum osmolality ≥ 320 mOsm/kg or osmolality gap ≥40
  - Hypertonic saline: initial 250 mL of 3% hypertonic saline, maintenance at 50-100 mL/h while monitoring electrolytes every 4h; hold infusion if serum Na ≥155 mEq/L
  - Imminent herniation: Initial 30 mL of 23.4% hypertonic saline (may repeat in 15 min)
- ✓ If patient has ommaya reservoir, drain CSF to target opening pressure < 20 mmHg
- ✓ Consider neurosurgery consultation, IV anesthetics for burst-suppression EEG
- ✓ Metabolic profile every 6h, daily computed tomography scan of head and adjust above medications to prevent rebound cerebral edema, renal failure, electrolyte abnormalities, hypovolemia, and hypotension

[a] All medication doses indicated are for adults
[b] Papilledema grading is performed according to Modified Frisén scale.[84]

CSF – cerebrospinal fluid; IV – intravenous; CRES – CAR-Related Encephalopathy Syndrome; $PaCO_2$ – partial pressure of arterial carbon dioxide; EEG – electroencephalogram

Box 5. Diagnostic criteria for CAR-related hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome (MAS).

If a subject that had peak ferritin >10,000 ng/mL during the cytokine release syndrome phase developed any two of the following organ toxicities after CAR T-cell therapy, the subject may have HLH/MAS

- ≥ Grade 3 increase in bilirubin, aspartate transaminase, or alanine transaminase[a]
- ≥ Grade 3 oliguria or increase in creatinine[a]
- ≥ Grade 3 pulmonary edema[a]
- ≥ Presence of hemophagocytosis by morphology and/or CD68 immunohistochemistry in bone marrow or organs

[a]Grading as per Common Terminology Criteria for Adverse Events, version 4.03[35]

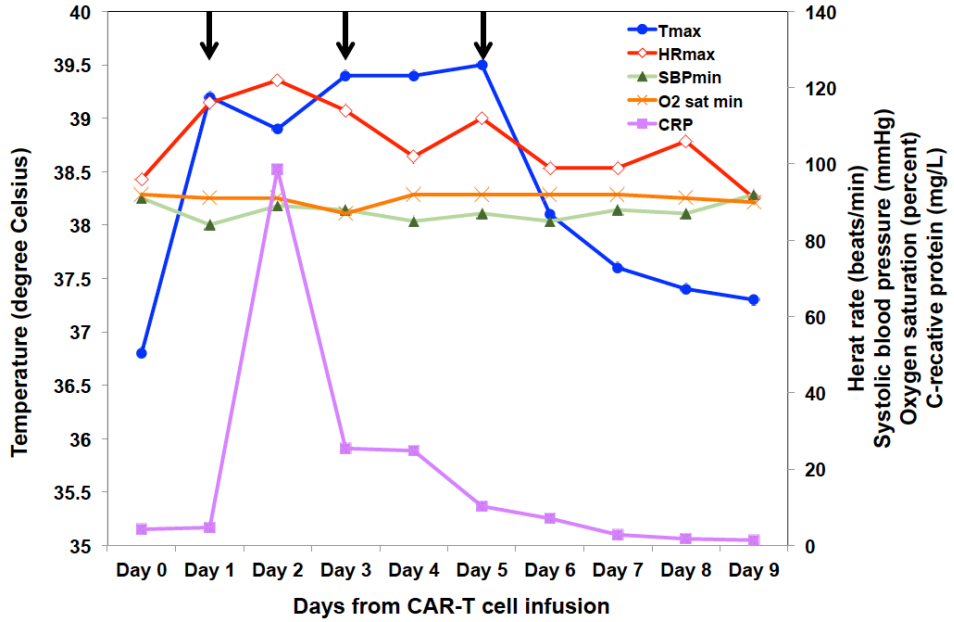
Figure Legends

Figure 1. Representative patient with cytokine release syndrome and CAR-related encephalopathy syndrome after CD19-CAR T-cell therapy for refractory diffuse large B-cell lymphoma. A) Maximum temperature (Tmax), maximum heart rate (HRmax), minimum systolic blood pressure (SBPmin), minimum oxygen saturation (O2 sat min), and C-reactive protein (CRP) level in serum recorded on each day after CD19-CAR T-cell therapy is shown. Tocilizumab (black arrows) was administered on days 1, 3, and 5 for hypotension, hypoxia, and encephalopathy, respectively. B) Handwriting samples and mini-mental status exam (MMSE) scores on days 4, 5, and 6 after CD19-CAR T-cell therapy. Handwriting was markedly impaired on day 5. C) Positron emission tomography-imaging showing retroperitoneal lymph nodes and ileo-colic region involved with lymphoma at baseline (highlighted in red circle) and induction of remission 30 days after infusion of CD19-CAR T cells.

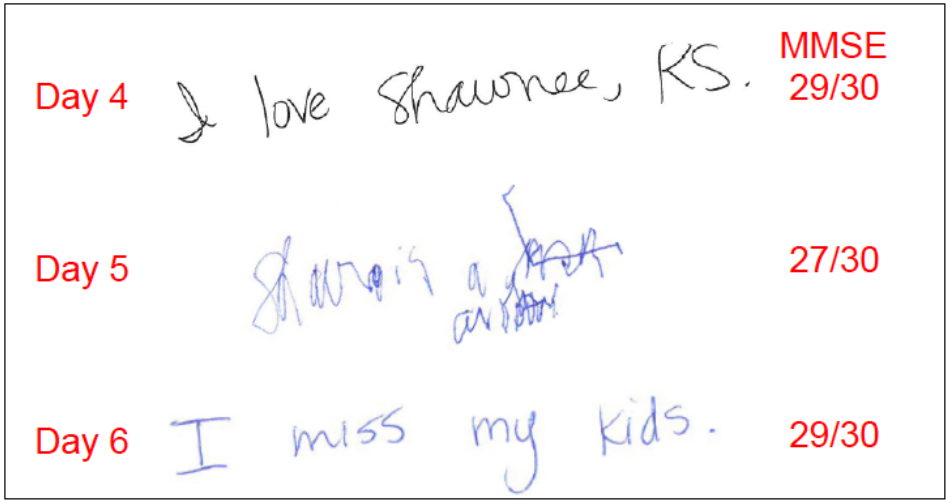
Figure 2. Three-step approach for assessment and management of acute CAR T-cell therapy toxicity. The symptoms and signs monitored to determine the nature of the CAR T-cell toxicity for Cytokine Release Syndrome (CRS), CAR-Related Encephalopathy Syndrome (CRES), and Hemophagocytic Lymphohistiocytosis (HLH) or Macrophage Activation Syndrome (MAS) are shown under Step 1. References to the grading system and the management algorithms used for each of the toxicity categories are provided under Steps 2 and 3, respectively. (ICP – intracranial pressure; CTCAE – Common Terminology Criteria for Adverse Events, version 4.03).

Figure 3. Recommendations for management of CAR-related hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome (MAS).

A


Tmax
HRmax
SBPmin
O2 sat min
CRP
Temperature (degree Celsius)
Herat rate (beats/min)
Systolic blood pressure (mmHg)
Oxygen saturation (percent)
C-recative protein (mg/L)
40
39.5
39
38.5
38
37.5
37
36.5
36
35.5
35
140
120
100
80
60
40
20
0
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Days from CAR-T cell infusion

B


MMSE
Day 4
I love Shawnee, KS.
29/30
Day 5
27/30
Day 6
I miss my kids.
29/30

C

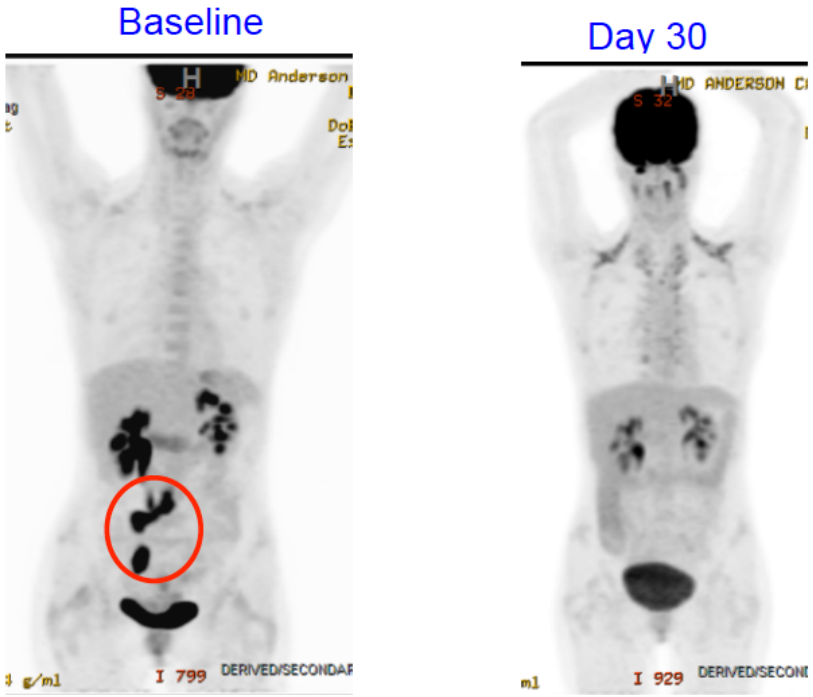

Baseline
Day 30
MD Anderson
I 799
I 929

Figure 1

Figure 2

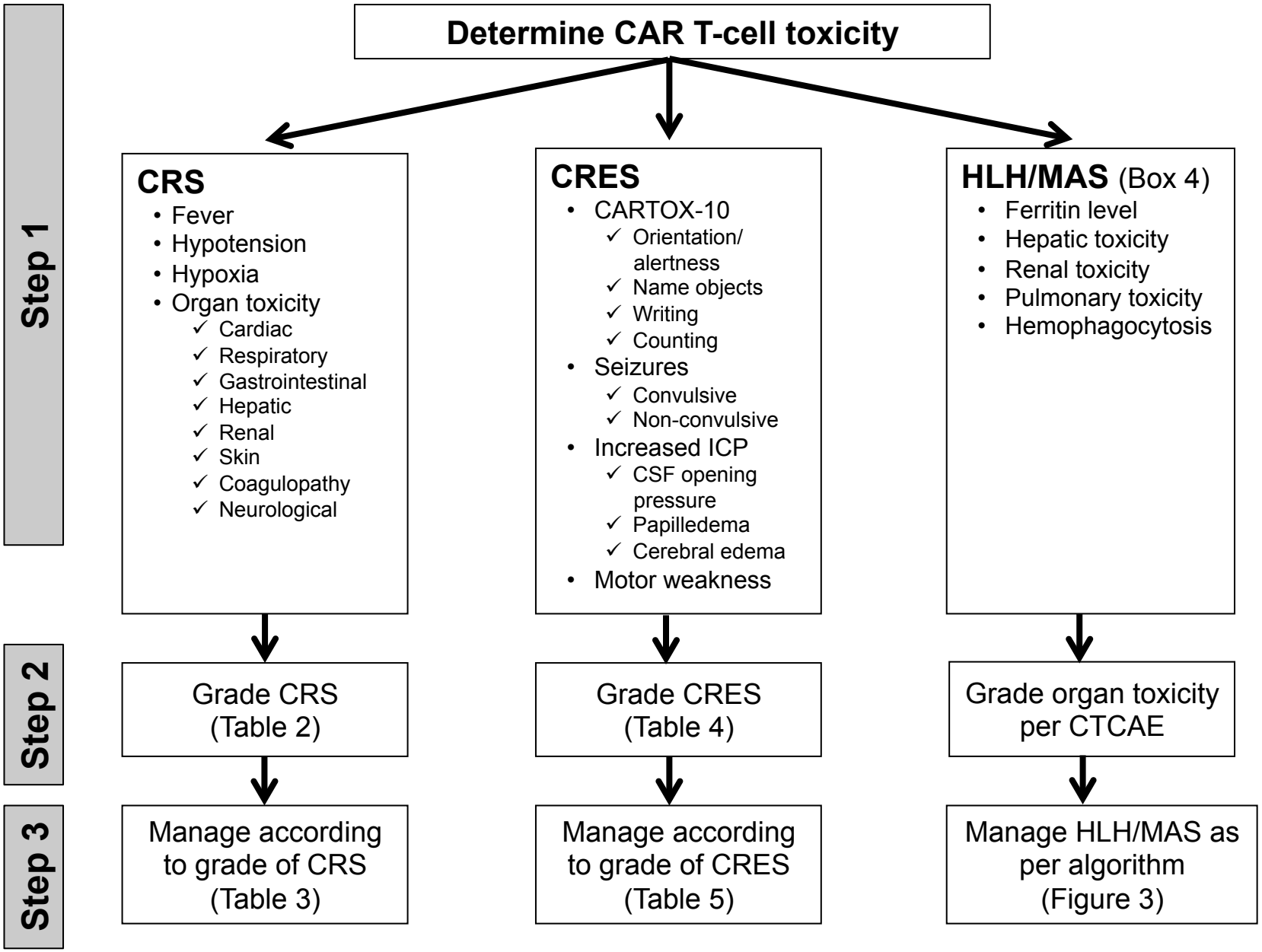
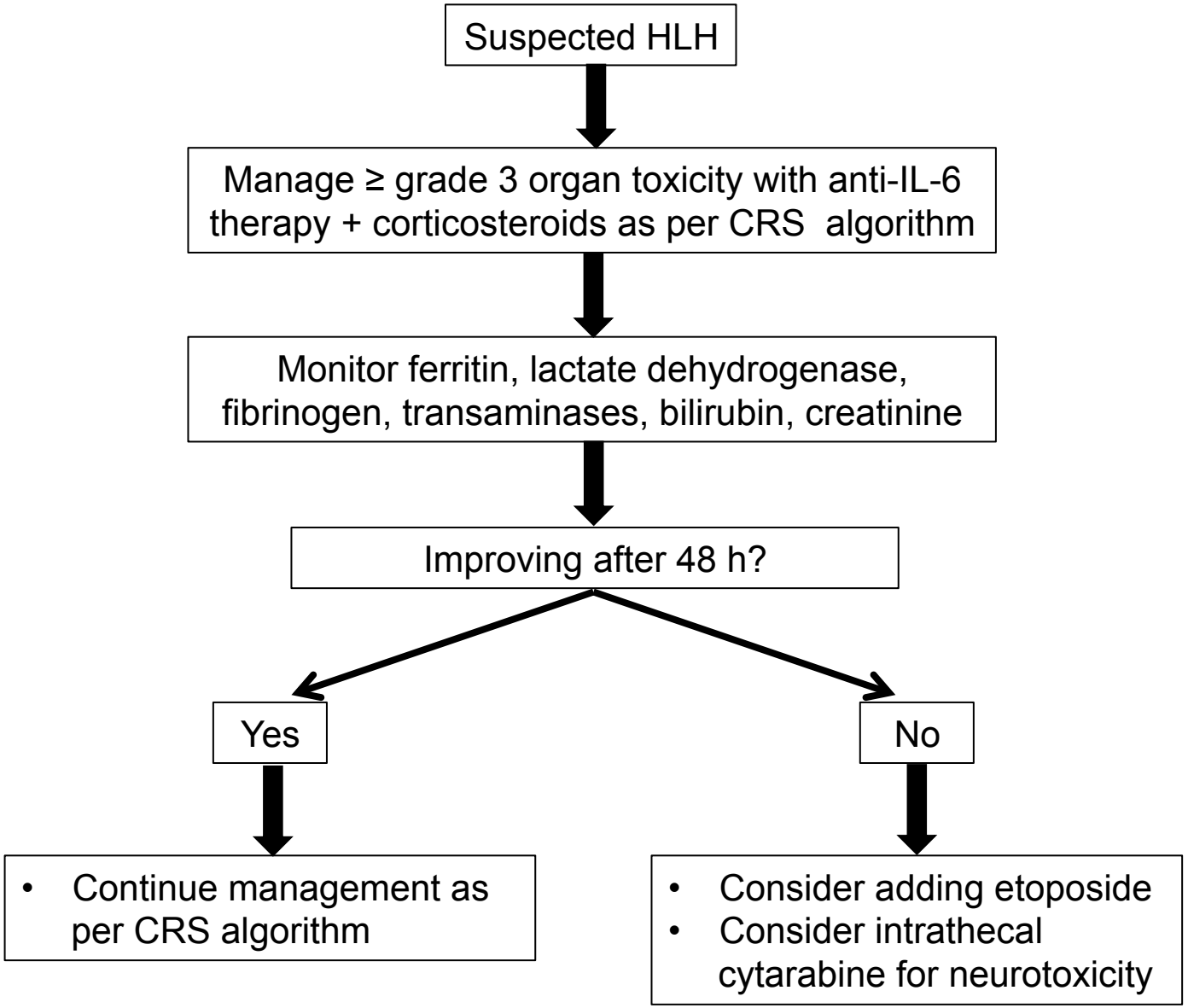

Step 1
Step 2
Step 3
Determine CAR T-cell toxicity
CRS
• Fever
• Hypotension
• Hypoxia
• Organ toxicity
✓ Cardiac
✓ Respiratory
✓ Gastrointestinal
✓ Hepatic
✓ Renal
✓ Skin
✓ Coagulopathy
✓ Neurological
CRES
• CARTOX-10
✓ Orientation/ alertness
✓ Name objects
✓ Writing
✓ Counting
• Seizures
✓ Convulsive
✓ Non-convulsive
• Increased ICP
✓ CSF opening pressure
✓ Papilledema
✓ Cerebral edema
• Motor weakness
HLH/MAS (Box 4)
• Ferritin level
• Hepatic toxicity
• Renal toxicity
• Pulmonary toxicity
• Hemophagocytosis
Grade CRS (Table 2)
Grade CRES (Table 4)
Grade organ toxicity per CTCAE
Manage according to grade of CRS (Table 3)
Manage according to grade of CRES (Table 5)
Manage HLH/MAS as per algorithm (Figure 3)

Figure 3


Suspected HLH
Manage ≥ grade 3 organ toxicity with anti-IL-6 therapy + corticosteroids as per CRS algorithm
Monitor ferritin, lactate dehydrogenase, fibrinogen, transaminases, bilirubin, creatinine
Improving after 48 h?
Yes
No
• Continue management as per CRS algorithm
• Consider adding etoposide
• Consider intrathecal cytarabine for neurotoxicity