

## Management of CAR T-cell related toxicities: What did the learning curve teach us so far?

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## REVIEW ARTICLE

# Management of CAR T-cell Related Toxicities: What did the Learning Curve Teach us so Far?

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### Abstract

Chimeric antigen receptor T cell (CAR-T) therapy is an immunotherapy, which represents a therapeutic breakthrough in the treatment of B-cell malignancies and multiple myeloma. Since the first CAR T-cell approval in 2017, there have been five FDA approved CAR-T products, more approved disease indications for CAR-T therapy, and investigational trials launched for other cancers, including solid organ malignancies. CAR-T therapy possesses unique toxicities. Better understanding of these toxicities over time has helped in more efficient diagnosis, management, and treatment strategies. This review will focus on CAR-T-related toxicities including cytokine release syndrome, immune effector cell associated neurotoxicity syndrome (ICANS), cytokine release syndrome (CRS), and hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome in terms of assessment, grading, and current management strategies. Additionally, this review will cover future directions and research on CAR-T-related toxicities.

**Keywords:** Chimeric antigen receptor T-cell therapy, Cytokine release syndrome, Immune effector cell associated neurotoxicity syndrome, Hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome, Toxicities

## 1. Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy is a breakthrough T cell engineered immunotherapy, which changed the therapeutic landscape of various B-cell lymphoid malignancies. Since 2017, there are six CAR-T products approved by the U.S. Food and Drug Administration (FDA) for a number of disease indications including non-Hodgkin lymphoma (NHL) (diffuse large B cell lymphoma (DLBCL), follicular lymphoma, mantle cell lymphoma, primary mediastinal B-cell lymphoma), adult and pediatric acute lymphoblastic leukemia (ALL), and multiple myeloma [1–8]. Pivotal phase 3 clinical trials have demonstrated benefits of CAR-T compared to standard of care at first relapse in DLBCL [9,10]. Trials are ongoing for both newly diagnosed NHL and myeloma and relapsed/refractory solid organ malignancies.

CAR-T therapies have toxicities that are unique to this treatment modality, specifically cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS). CRS, the most common non-hematologic adverse event observed after CAR T-cell therapy, is an escalated immune response and on rare occasions can evolve into a fulminant hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS). ICANS is the second most common non-hematologic adverse event characterized by a toxic encephalopathic state with early manifestations including tremor, dysgraphia, and expressive dysphasia; in severe states, it can manifest with seizures and life-threatening cerebral edema.

These toxicities are potentially fatal if not recognized and treated promptly. From clinical trials and real-world experience, toxicities are better understood and outpatient administration of CAR-T

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therapy is more feasible than before. With increasing availability and administration of CAR-T therapy among older patients and for new disease indications, the understanding and management of CAR-T toxicities will likely continue to evolve. In this review, CAR-T toxicities, including grading and management are reviewed. Additionally, novel investigational treatments are reviewed to highlight future directions in management of CAR-T-related toxicities.

## 2. Cytokine release syndrome

### 2.1. Evolution of assessment and management

CRS is a hyper-inflammatory state resulting from high-level immune activation following the interaction of CAR T-cells with the targeted antigen [11,12]. The release and expansion of inflammatory cytokines causes clinical signs and symptoms ranging from a mild febrile state with or without flu-like symptoms to life-threatening manifestations and multi-organ failure [13,14].

The assessment of CRS severity post CAR-T has constantly evolved over the past decade. Initially, the National Institutes of Health (NIH) Common Terminology Criteria for Adverse Events (CTCAE) was utilized [15]; however, there were limitations observed. First, CTCAE did not require fever as a CRS prerequisite. Second, CTCAE was designed for monoclonal antibody infusions; grading was largely dependent on infusion interruption. CAR T-cell infusions being single-dose administrations reduced CTCAE application for CAR-T-associated CRS. New grading criteria were developed, with different assessments used for different CAR-T clinical trials (Table 1).

In the ZUMA-1 study evaluating axicabtagene ciloleucel for R/R DLBCL [16], CRS grading was performed using modified CTCAE v4.0 later known as the Lee scale or NIH consensus criteria [11]. CRS-specific symptoms in ZUMA-1 included fever, hypotension, tachycardia, acute kidney injury, heart failure, headache, hypoxia, metabolic acidosis, and neurotoxicity [16]. The Lee scale was widely adopted as it recognized CRS-associated organ specific toxicities and correlated CRS grade to the recommended clinical management [11]. The JULIET trial investigating tisagenlecleucel in R/R DLBCL [7] used the Penn grading scale for CRS assessment [17]. The Penn grading system relied on hemodynamic instability and vasopressor requirements as determinants for CRS severity grading and removal of vasopressor support as the criterion for CRS resolution, using easily accessible clinical features allowing more consistency in grading and global

applicability [17]. A third grading system from the group at Memorial Sloan Kettering Cancer Center (MSKCC) was also introduced into clinical practice. Initial versions relied on serum cytokine levels [18,19] and further modifications used duration of vasopressor support and oxygen (FIO<sub>2</sub>) requirements as criteria for CRS severity [19].

Given these differences in cytokine release syndrome (CRS) grading and benefits of each grading criterion, consensus CRS grading criteria were developed. First the CAR T-cell-therapy-associated Toxicity (CARTOX) Working Group comprising investigators and representatives from multiple institutions, published consensus guidelines for management of CAR-T-related toxicities including CRS [20]. The CARTOX grading included grade 1 organ toxicity under grade 1 CRS and used fever, hypotension, and hypoxia as grading criteria (Table 1). More recently, the American Society for Transplantation and Cellular Therapy (ASTCT), following a consensus conference attended by experts from all aspects of the field [21] offered a simplified grading scale focused on fever, hypotension, hypoxia, and early symptoms detected during the first 14 days following CAR T-cell infusion.

The management of CRS continues to evolve with experience. Initially, as CAR-T was considered a “living drug,” there was early apprehension of dampening CRS except in life threatening situations. This likely influenced the current day management of low-grade CRS, comprising supportive care and including the use of intravenous fluids, antipyretics, antihistamines, and empiric antibiotics considering symptom overlap between CRS and the possibility of early sepsis.

Higher grade CRS, defined as > grade 1 CRS by ASTCT criteria, typically requires a prompt and aggressive approach. Interleukin-6 (IL-6) significantly correlated with CRS incidence and severity. Importantly, IL-6 levels did not appear to impact CAR T-cell function or efficacy, thus, targeting IL-6 became an attractive therapeutic strategy [22,23]. Tocilizumab, a humanized monoclonal antibody that targets the IL6 receptor, initially approved for certain rheumatologic conditions, emerged as one of the first treatment options for severe CRS [14]. Tocilizumab's impact on CRS was effective and fast with responses appreciated within hours of administration [24,25]. More importantly, tocilizumab usage did not to adversely affect CAR-T efficacy [22]. Tocilizumab was FDA-approved for CRS treatment in patients aged ≥2 years in August 2017 [26,27].

The management of CRS beyond tocilizumab remains less clear. While CRS may resolve following a

Table 1. Comparison of CRS grading systems.

CRS Grade	CTCAE v4.03 [15]	Lee Scale [11]	Penn Scale [17]	MSKCC [19]	CARTOX [20]	ASTCT [21]
Grade 1	<ul style="list-style-type: none"> <li>Mild reaction</li> <li>No intervention required</li> </ul>	<ul style="list-style-type: none"> <li>Constitutional symptoms</li> <li>Symptomatic treatment</li> </ul>	<ul style="list-style-type: none"> <li>Mild reaction</li> <li>Supportive care</li> </ul>	<ul style="list-style-type: none"> <li>Mild symptoms</li> <li>Supportive care</li> </ul>	<ul style="list-style-type: none"> <li>Fever <math>\geq 38</math> °C</li> <li>Grade 1 organ toxicity</li> </ul>	<ul style="list-style-type: none"> <li>Fever <math>\geq 38</math> °C</li> <li>No hypotension</li> <li>No hypoxia</li> <li>Fever <math>\geq 38</math> °C</li> <li>Hypotension not requiring vasopressors</li> <li>Hypoxia requiring low-flow oxygen</li> </ul>
Grade 2	<ul style="list-style-type: none"> <li>Infusion interruption</li> <li>Prompt response to symptomatic treatment</li> <li>Prophylactic medication</li> </ul>	<ul style="list-style-type: none"> <li>Moderate intervention</li> <li>Hypotension responsive to fluids</li> <li>Hypoxia responsive to <math>&lt;40\%</math> oxygen</li> <li>Grade 2 organ toxicity</li> </ul>	<ul style="list-style-type: none"> <li>Moderate reaction</li> <li>IV therapies</li> <li>Organ dysfunction: Cr (grade 2), LFTs (grade 3)</li> <li>Hospitalization for febrile neutropenia</li> </ul>	<ul style="list-style-type: none"> <li>Hypotension requiring vasopressors <math>&lt;24</math> h</li> <li>Hypoxia requiring <math>&lt;40\%</math> oxygen</li> </ul>	<ul style="list-style-type: none"> <li>Hypotension responsive to IV fluids or low-dose vasopressors</li> <li>Hypoxia requiring <math>&lt;40\%</math> oxygen</li> </ul>	<ul style="list-style-type: none"> <li>Fever <math>\geq 38</math> °C</li> <li>Hypotension requiring vasopressors</li> <li>Hypoxia requiring high-flow oxygen</li> </ul>
Grade 3	<ul style="list-style-type: none"> <li>Not rapidly responsive to infusion interruption or symptomatic management</li> <li>Hospitalization indicated</li> </ul>	<ul style="list-style-type: none"> <li>Aggressive intervention</li> <li>High dose/multiple vasopressors</li> <li>Oxygen requirement <math>\geq 40\%</math></li> <li>Grade 3 organ toxicity or grade 4 transaminitis</li> </ul>	<ul style="list-style-type: none"> <li>Severe reaction</li> <li>Hospitalization</li> <li>Cr (grade 3), LFTs (grade 4)</li> <li>Hypotension requiring multiple fluid boluses</li> <li>Coagulopathy</li> <li>Hypoxia requiring supplemental oxygen</li> </ul>	<ul style="list-style-type: none"> <li>Hypotension requiring vasopressors <math>\geq 24</math> h</li> <li>Hypoxia requiring <math>\geq 40\%</math> oxygen</li> </ul>	<ul style="list-style-type: none"> <li>High dose/multiple vasopressors</li> <li>Hypoxia requiring <math>\geq 40\%</math> oxygen</li> <li>Grade 3 organ toxicity or grade 4 transaminitis</li> </ul>	<ul style="list-style-type: none"> <li>Fever <math>\geq 38</math> °C</li> <li>Hypotension requiring vasopressors</li> <li>Hypoxia requiring high-flow oxygen</li> </ul>
Grade 4	<ul style="list-style-type: none"> <li>Life-threatening sequelae</li> <li>Ventilatory support and pressors indicated</li> </ul>	<ul style="list-style-type: none"> <li>Life-threatening</li> <li>Mechanical ventilation</li> <li>Grade 4 organ toxicity</li> </ul>	<ul style="list-style-type: none"> <li>Life-threatening</li> <li>High-dose vasopressors</li> <li>Mechanical ventilation</li> </ul>	<ul style="list-style-type: none"> <li>Life-threatening</li> <li>Hypotension refractory to multiple vasopressors</li> <li>Mechanical ventilation</li> </ul>	<ul style="list-style-type: none"> <li>Life-threatening</li> <li>Mechanical ventilation</li> <li>Grade 4 organ toxicity</li> </ul>	<ul style="list-style-type: none"> <li>Fever <math>\geq 38</math> °C</li> <li>Hypotension requiring multiple vasopressors</li> <li>Hypoxia requiring positive pressure</li> </ul>

Abbreviations: CRS: cytokine release syndrome; CTCAE v4.03: Common Terminology Criteria for Adverse Events version 4.03; ASTCT: The American Society for Transplantation and Cellular Therapy; IV: intravenous; Cr: creatinine; LFTs: liver function tests.

Table 2. Selected ongoing clinical trials to prevent or treat CRS.

Trial Number	Study Title	Phase	Disease/condition	Intervention	Country
NCT05306080	Tadekinig Alfa (IL-18BP) Rescue Therapy for CAR T Cell Related CRS and HLH-like Syndrome	Early Phase 1	<ul style="list-style-type: none"> <li>• CAR T-related CRS</li> <li>• HLH</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Tadekinig alfa (IL-18BP)</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT04048434	Extracorporeal Cytokine Adsorption (Cytosorb) as Additive Treatment of CAR-T Associated CRS	Not Applicable	<ul style="list-style-type: none"> <li>• CAR T-related CRS</li> </ul>	<ul style="list-style-type: none"> <li>• Device: Cytosorb</li> </ul>	<ul style="list-style-type: none"> <li>• Germany</li> <li>• Switzerland</li> </ul>
NCT04082910	Metoprolol Treatment for CRS in Patients Treated with CAR-T	Phase 1 Phase 2	<ul style="list-style-type: none"> <li>• CAR T-related CRS</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Metoprolol</li> <li>• Drug: metoprolol, infliximab, etanercept, tocilizumab and/or other agents</li> </ul>	<ul style="list-style-type: none"> <li>• China</li> </ul>
NCT04975555	Study to Evaluate the Role of Siltuximab in Treatment of CRS and ICANS Related to CAR-T	Phase 2	<ul style="list-style-type: none"> <li>• CRS</li> <li>• ICANS</li> <li>• NHL</li> <li>• MM</li> <li>• ALL</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Siltuximab</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT04314843	Study of Lenzilumab and Axicabtagene Ciloleucel in Participants with Relapsed or Refractory Large B-Cell Lymphoma (ZUMA-19)	Phase 1	<ul style="list-style-type: none"> <li>• Relapsed/Refractory Large B-cell Lymphoma</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Cyclophosphamide</li> <li>• Drug: Fludarabine</li> <li>• Biological: Lenzilumab</li> <li>• Biological: Axicabtagene Ciloleucel</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT04359784	Anakinra for the Prevention of CRS and Neurotoxicity in Patients with B-Cell NHL Receiving CD19-Targeted CAR-T	Phase 2	<ul style="list-style-type: none"> <li>• B-cell NHL</li> </ul>	<ul style="list-style-type: none"> <li>• Biological: Anakinra</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT03275493	Humanized CD19 CAR-T Cells with CRS Suppression Technology for r/r CD19+ ALL	Phase 1 Phase 2	<ul style="list-style-type: none"> <li>• ALL</li> <li>• CD19 Positive</li> <li>• Relapse</li> <li>• Refractory</li> </ul>	<ul style="list-style-type: none"> <li>• Biological: Humanized CD19 CAR-T cells</li> <li>• Biological: Humanized CD19 CAR-T cells with CRS suppression technology</li> </ul>	<ul style="list-style-type: none"> <li>• China</li> </ul>
NCT04071366	A Study of Itacitinib for the Prevention of CRS Induced by Immune Effector Cell Therapy	Phase 2	<ul style="list-style-type: none"> <li>• CRS</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Itacitinib</li> <li>• Drug: Immune effector cell therapy</li> <li>• Drug: Placebo</li> <li>• Biological: Yescarta</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT04148430	A Study of Anakinra to Prevent or Treat Severe Side Effects for Patients Receiving CAR-T	Phase 2	<ul style="list-style-type: none"> <li>• B-cell ALL</li> <li>• B-cell lymphoma</li> <li>• B-cell NHL</li> </ul>	<ul style="list-style-type: none"> <li>• Drug: Anakinra</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT03016377	Administration of Autologous CAR-T CD19 Antigen with Inducible Safety Switch in Patients with Relapsed/Refractory ALL	Phase 1 Phase 2	<ul style="list-style-type: none"> <li>• ALL</li> <li>• Immune System Diseases</li> <li>• Immunoproliferative Disorders</li> </ul>	<ul style="list-style-type: none"> <li>• Biological: iC9-CAR19 cells</li> <li>• Drug: Rimiducid</li> <li>• Drug: Cyclophosphamide</li> <li>• Drug: Fludarabine</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>
NCT03696784	Anti-CD19 CAR-T Cells with Inducible Caspase 9 Safety Switch for B-cell Lymphoma	Phase 1	<ul style="list-style-type: none"> <li>• Lymphoma</li> <li>• B-cell lymphoma</li> <li>• Immune System Diseases</li> <li>• Immunoproliferative Disorders</li> <li>• Lymphatic Diseases</li> </ul>	<ul style="list-style-type: none"> <li>• Biological: iC9-CAR19 T cells</li> <li>• Drug: Bendamustine</li> <li>• Drug: Fludarabine</li> <li>• Drug: AP1903</li> <li>• Drug: Cyclophosphamide</li> </ul>	<ul style="list-style-type: none"> <li>• USA</li> </ul>

Source: [ClinicalTrials.gov](https://www.clinicaltrials.gov) (accessed on April 13, 2022).

Abbreviations: CRS: cytokine release syndrome; HLH: hemophagocytic lymphohistiocytosis; ICANS: immune effector cell-associated neurotoxicity syndrome; NHL: non-Hodgkin lymphoma; MM: multiple myeloma; ALL: acute lymphocytic leukemia.



single dose of tocilizumab, persistent or worsening CRS may require repeated dosing, typically every 8 h for 3–4 total doses. For patients not responding to tocilizumab, corticosteroids could be administered to reduce the overactive immune response associated with CAR T-cell expansion. There was considerable concern with early corticosteroid usage and its perceived effect in dampening the efficacy of CAR T-cell therapy [18]. However, with recent studies showing the benefit of steroids in early CRS management and prevention without adverse effects on the anti-tumor potency of CAR-T therapy [28,29], these concerns are assuaged considerably. Beyond tocilizumab and corticosteroids, there are reports of agents effective against CRS, including siltuximab, anakinra, and inhibitors of JAK-STAT pathway [30–32]. These agents are considered only in severe, refractory cases or investigational as part of a clinical trial.

## 2.2. Future directions in managing CRS: prevention and novel therapeutics

The clinical use of tocilizumab and corticosteroids for CRS management is based on the biological understanding of mechanisms underlying CRS; treatment algorithms are developed based mostly on expert opinion and retrospective observational studies. Our current CRS management strategies in CAR-T recipients require further improvement [26]. These can be divided into those preventing and effectively treating CRS.

Preventive strategies independent of therapeutic agents for CRS management include limiting the maximum CAR T-cell dose in patients with bulky disease or adopting a fractionated schedule, as bulky disease prior to CAR-T infusion is a notable predictor of severe CRS [33,34]. Other effective ways to prevent CRS include identifying and incorporating predictive biomarkers with high sensitivity and specificity in CRS treatment algorithms [35]. In one study, the combination of fever  $\geq 38.9$  °C within 36 h of CAR-T infusion and serum monocyte chemoattractant protein-1 (MCP-1) concentration  $\geq 1343.5$  pg/mL could identify patients with grade  $\geq 4$  CRS with 100% sensitivity and 95% specificity [36].

Modifying CAR construct represents another approach to limit CRS. Various methodologies are underway and a better understood one is the introduction of suicide genes as a safety switch into the CAR using the caspase 9 (iCasp9)/AP1903 suicide system [37,38]. Other safety switches that are effective include the herpes simplex thymidine kinase, epidermal growth factor receptor, and

CD20, inducing T-cell death upon exposure to ganciclovir, cetuximab, and rituximab, respectively [39–41]. Prophylactic or early use of corticosteroids and tocilizumab reduces the incidence and severity of CRS [28,29,42]. Based on long-term follow-up data from cohort 6 of ZUMA-1, which evaluated the use of prophylactic and earlier corticosteroids and/or tocilizumab, the regulatory approval of axicabtagene ciloleucel is updated to include prophylactic corticosteroids across all approved indications [43].

Various therapeutic agents act against CRS (Table 2). Ibrutinib is a Bruton tyrosine kinase inhibitor with established clinical activity in B-cell malignancies; it also modulates T-cell response [44]. Studies in pre-clinical models of mantle cell lymphoma, chronic lymphocytic leukemia, and acute lymphoblastic leukemia demonstrated that when ibrutinib was administered with CD19 directed CAR-T, the antitumor responses were enhanced and there was less CRS incidence [45].

Macrophages contribute to CRS through production of nitric oxide, IL-1, and granulocyte-macrophage colony-stimulating factor (GM-CSF), among other cytokines and chemokines [46]. Blockade of IL-1 and GM-CSF through anakinra or engineering CAR T-cells to produce IL-1 receptor antagonist and lenzilumab, a GM-CSF neutralizing antibody, decreases the risk of CRS-related mortality [47,48]. Clinical trials with these agents and strategies are ongoing (NCT04314843).

Other therapeutic agents that have clinical activity by dampening the cytokine storm in CRS include siltuximab, a chimeric monoclonal antibody that binds directly to IL-6; etanercept and infliximab that target tumor necrosis factor alpha (TNF $\alpha$ ) [49] and kinase inhibitors including dasatinib and ruxolitinib, which limit CRS by suppressing cytokine production [50,51].

## 3. ICANS/Neurotoxicity

### 3.1. Evolution of assessment, grading, and management

Neurotoxicity has a biphasic presentation where neurologic symptoms first overlap with CRS, and later re-appear after CRS resolution, typically around 7 days post-CAR-T [20]. Like CRS, neurotoxicity assessment has evolved since the first application of CAR-T; however, the main grading system used in clinical trials was CTCAE (versions v.4.03 and later v.5) [1,4,7,15,52]. In this system, grades 1–2 of neurotoxicity are associated with mild and moderate neurological symptoms. Grade

2 mostly results in moderate impairment of instrumental activities of daily living (ADL). Grade $\geq$ 3 toxicities comprise significant cognitive impairment, usually accompanied by more severe symptoms of seizures, altered mental status, motor weakness, increased intracranial pressure (ICP) or papilledema, and a more pronounced impact on self-care ADL (Table 3) [53]. However, the CTCAE system did not adequately address the acute neurological symptoms resulting from CAR-T. Subsequently, the CARTOX group proposed new criteria, i.e., CAR T-cell-related encephalopathy syndrome (CRES). This tool incorporates an encephalopathy assessment, CARTOX-10, which is a measure of cognitive function over a 10-point scale based on key components of the Mini-Mental State Examination such as orientation, naming, writing ability, and attention (Table 4) [20,53]. However, the need for a lumbar puncture for ICP evaluation made the CRES assessment cumbersome. Later, the ASTCT group introduced the term ICANS and proposed the immune effector cell-associated encephalopathy (ICE) score, which is a modified version of CARTOX [21]. The ICE score revised the definition of increased ICP and eliminated the need for lumbar puncture; it integrated evaluation of the level of consciousness, motor weakness, and seizures, with any seizure prompting a grade $\geq$ 3 ICANS [21]. It also added the ability to follow commands in the encephalopathy score (Table 4) [21]. The ICE score was quickly adapted in clinical practice owing to its easy applicability and is recommended at least daily for patients receiving CAR-T. Table 3 provides a comparison of the neurotoxicity grading systems.

The standard management of isolated CAR-T-related neurotoxicity is mainly supportive as ICANS is usually self-limited. Measures include intravenous hydration, seizure prophylaxis and control, aspiration precaution, and control of increased ICP and cerebral edema, among others [54]. Corticosteroids are the first-line therapy for grade $\geq$ 3 ICANS [55]. The choice of corticosteroids is dependent on institutional standards, but dexamethasone is the most commonly prescribed agent due to its good CNS penetration [56]. High doses of methylprednisolone are warranted for management of severe cases of ICANS [20]. Tocilizumab is not recommended for treatment of isolated ICANS, as it was shown to worsen the severity of neurotoxicity by increasing IL-6 levels [30,57]. ICANS management takes precedence over low grade CRS when they coexist simultaneously, to allow for subsequent use of tocilizumab for CRS treatment, if needed.

### 3.2. Future directions for understanding the mechanism(s) of ICANS and its management

#### 3.2.1. Understanding the pathophysiology of ICANS

The pathophysiology ICANS is poorly understood [58,59] and is a focus of ongoing research. CRS and ICANS share similarities as both their developments are linked to production of pro-inflammatory cytokines by CAR T-cells and innate immune responses in the tumor microenvironment. Patients with severe ICANS can have high serum levels of C-reactive protein, ferritin, IL-6, IL-15, interferon  $\gamma$  (IFN- $\gamma$ ), granulocyte-macrophage colony-stimulating factor, IL-2, IL-5, IL-1a, granzyme B, tumor necrosis factor  $\alpha$ , CXCL10, and monocyte chemoattractant protein 1 (MCP-1) [55]. Severe ICANS including CRS, is associated with disseminated intravascular coagulation and endothelial cell activation and dysfunction, as evidenced by elevated levels of von Willebrand factor and angiopoietin 2, causing increase vascular permeability and blood brain disruption [59,60]. This could partially explain similar cytokine profiles in the cerebrospinal fluid of those with severe ICANS as is the case with CRS [61].

Besides cytokine profiles in serum and CSF, other factors are implicated and some more importantly, do not correlate with development of ICANS. Tumor or tumor antigen presence in the CNS is not linked with ICANS, as evidenced in early studies of CAR-T in glioblastoma multiforme [62]. Additionally, the presence of CAR-T in the CNS (e.g., CD19-directed products) has not correlated with ICANS development or severity [61]. One study found that CD19 expression in human brain mural cells could explain ICANS with CD19 CAR-T [63]. However, similar incidence and severity of ICANS with other CAR T-cell products such as BCMA [6], and similar CD22 expression in brain mural cells without significant increase in ICANS seen with CD22 CAR T-cell therapy [64], question this explanation.

#### 3.3. Prevention and novel therapeutics

The pathophysiology of ICANS is less understood compared to CRS; consequently, progress with optimal treatment and prevention has lagged. Many current clinical trials are not specific for ICANS but for both CRS and ICANS, which is understandable given the similar cytokine profiles. There are some approaches under investigation, which are considered as promising early interventions to prevent development of severe ICANS.

Anakinra is effective for ICANS based on the role of IL-1 in the pathogenesis of ICANS [47] and IL-1 inhibition prevented severe ICANS in preclinical



models [46]. Clinically, there are conflicting reports regarding its efficacy in the treatment of ICANS [31,65]. One phase II clinical trial of CD19 CAR T-cell recipients with relapsed or refractory B-cell lymphoma found that the subcutaneous injection of 100 mg anakinra every 12 h for a minimum of 10 days was safe for severe ICANS observed in only 6% of patients [66]. Another study with 14 patients who received commercial CD19-targeting CAR T-cell

therapy found that three daily subcutaneous doses of anakinra for steroid-refractory ICANS were largely ineffective [65], while a small retrospective series from MD Anderson Cancer Center showed that anakinra use for 12 days (median) was effective in four out of six patients with severe ICANS [31]. Several trials using anakinra for ICANS prevention are ongoing (NCT04150913, NCT04205838, NCT04359784, NCT04148430, and NCT04432506).

Table 3. Comparison of neurotoxicity grading systems.

Neurotoxicity Grade	CTCAE v5.0 [52]	CARTOX [20]	ICE [21]
Grade 1	<ul style="list-style-type: none"> <li>• Mild encephalopathy</li> <li>• Brief partial seizure</li> <li>• Awareness of receptive or expressive dysphasia</li> <li>• Mild tremor</li> <li>• Mild headache</li> <li>• Mild disorientation</li> <li>• Decreased level of alertness</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 7–9</li> <li>• Mild impairment</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 7–9</li> <li>• Awakens spontaneously</li> </ul>
Grade 2	<ul style="list-style-type: none"> <li>• Moderate encephalopathy limiting instrumental ADL</li> <li>• Brief generalized seizure</li> <li>• Moderate receptive or expressive dysphasia – impaired communication</li> <li>• Moderate tremor limiting instrumental ADL</li> <li>• Moderate headache limiting instrumental ADL</li> <li>• Moderate disorientation limiting instrumental ADL</li> <li>• Sedation</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 3–6</li> <li>• Moderate impairment</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 3–6</li> <li>• Awakens to voice</li> </ul>
Grade 3	<ul style="list-style-type: none"> <li>• Severe symptoms limiting self-care ADL</li> <li>• New-onset seizures</li> <li>• Severe receptive or expressive dysphasia – impaired communication, reading and writing</li> <li>• Severe tremor limiting self-care ADL</li> <li>• Severe headache limiting self-care ADL</li> <li>• Severe disorientation limiting self-care ADL</li> <li>• Difficult to arouse</li> <li>• New onset/worsening cerebral edema</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 0–2</li> <li>• Severe impairment</li> <li>• Stage 1–2 papilledema</li> <li>• Partial or non-convulsive seizures</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 0–2</li> <li>• Awakens to tactile stimulus</li> <li>• Seizure</li> <li>• Focal cerebral edema</li> </ul>
Grade 4	<ul style="list-style-type: none"> <li>• Life-threatening</li> <li>• Urgent intervention indicated</li> </ul>	<ul style="list-style-type: none"> <li>• Critical condition</li> <li>• Stage 3–5 papilledema or cerebral edema</li> <li>• Generalized seizures</li> <li>• New motor weakness</li> </ul>	<ul style="list-style-type: none"> <li>• Score<sup>a</sup> 0</li> <li>• Stupor or coma</li> <li>• Prolonged/repetitive seizures</li> <li>• Focal weakness</li> <li>• Diffuse cerebral edema/papilledema/decerebrate or decorticate posturing</li> </ul>

Abbreviations: CTCAE v5.0: Common Terminology Criteria for Adverse Events version 5.0; ICE: immune effector cell-associated encephalopathy; ADL: activities of daily living.

<sup>a</sup> Encephalopathy assessment score, see Table 4.

Table 4. Encephalopathy assessment tools for neurotoxicity grading.

Function	CARTOX-10 [20]	ICE [21]
Orientation	<ul style="list-style-type: none"> <li>To year, month, city, hospital, president/prime minister of country of residence</li> <li>5 points<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>To year, month, city, hospital</li> <li>4 points<sup>a</sup></li> </ul>
Naming	<ul style="list-style-type: none"> <li>Ability to name 3 objects</li> <li>3 points<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>Ability to name 3 objects</li> <li>3 points<sup>a</sup></li> </ul>
Writing	<ul style="list-style-type: none"> <li>Ability to write a standard sentence</li> <li>1 point<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>Ability to write a standard sentence</li> <li>1 point<sup>a</sup></li> </ul>
Attention	<ul style="list-style-type: none"> <li>Ability to count backwards from 100 by 10</li> <li>1 point<sup>a</sup></li> </ul>	<ul style="list-style-type: none"> <li>Ability to count backwards from 100 by 10</li> <li>1 point<sup>a</sup></li> </ul>
Following commands		<ul style="list-style-type: none"> <li>Ability to follow simple commands</li> <li>1 point<sup>a</sup></li> </ul>

Abbreviations: ICE: immune effector cell–associated encephalopathy; ICANS: Immune effector cell-associated neurotoxicity syndrome.

<sup>a</sup> Scoring: 10: no impairment, 7–9: grade 1 ICANS, 3–6: grade 2 ICANS, 0–2: grade 3 ICANS, 0: patient unarousable, grade 4 ICANS.

Lenzilumab is an inhibitory antibody of the macrophage-activating and monocyte-activating cytokine GM-CSF that increased CAR T-cell activity and decreased CRS and neuro-inflammation in a preclinical study [48]. This agent is being tested in the ZUMA-19 clinical trial, which combines lenzilumab with axicabtagene ciloleucel in patients with relapsed and/or refractory large B-cell lymphoma as a preventative strategy for ICANS associated with CAR T-cell therapies (NCT04314843).

GM-CSF secretion contributes to pro-inflammatory myeloid cells, which are associated with ICANS occurrence. This has led to investigating ICANS prevention strategies with GM-CSF depletion, through use of the anti-GM-CSF antibody lenzilumab (NCT04314843) and GM-CSF gene knockout [48]. Another approach is to directly target myeloid cells. Given the marked increase in the number of

myeloid cells in the CSF of patients with severe ICANS [61], intrathecal chemotherapy is considered for its direct cytotoxic effects and is reportedly beneficial in severe ICANS [67,68]. Finally, given the role of endothelial dysfunction as a key component in ICANS development, defibrotide is also being investigated for ICANS prevention [69] (NCT03954106).

#### 3.4. CAR T HLH/MAS: a new entity

HLH is a rare, life threatening hyper-inflammatory syndrome caused by pathologic T-cell activation and is associated with natural killer (NK) cell dysfunction. HLH is characterized by hyperactivation of macrophages and lymphocytes, pro inflammatory cytokine production, lymphohistiocytic tissue infiltration, and immune-

Table 5. Comparison of HLH criteria to the proposed CAR-T associated sHLH/MAS criteria.

HLH- 2004 diagnostic criteria [78]	CAR-T associated sHLH/MAS proposed criteria [20]
A molecular diagnosis consistent with HLH	Ferritin >10,000 µg/L
OR	OR
Five out of 8 diagnostic criteria:	Two of the following:
<ul style="list-style-type: none"> <li>Fever</li> <li>Splenomegaly</li> <li>Cytopenia in 2/3 lineages (Hb &lt; 9, plt &lt;100, ANC &lt;1000)</li> <li>Hypertriglyceridemia (&gt;264 mg/dL) or hypofibrinogenemia (≤1.5 g/L)</li> <li>Hemophagocytosis in bone marrow, spleen, or lymph nodes (no evidence of malignancy)</li> <li>Low or no NK cell activity</li> <li>Ferritin ≥500 µg/L</li> <li>sCD25 (soluble IL-2 receptor) ≥ 2400 U/mL</li> </ul>	<ul style="list-style-type: none"> <li>Grade 3 or higher serum transaminitis or bilirubinemia</li> <li>Grade 3 or higher oliguria or increase in serum creatinine</li> <li>Grade 3 or higher pulmonary edema</li> <li>Histological evidence of hemophagocytosis in bone marrow or organs</li> </ul>

Abbreviations: HLH: hemophagocytic lymphohistiocytosis; sHLH: secondary hemophagocytic lymphohistiocytosis; MAS: macrophage activation syndrome; Hb: hemoglobin; plt: platelets; ANC: absolute neutrophil count; NK: natural killer.

mediated multiorgan failure [70]. Secondary HLH (sHLH), also known as macrophage activation syndrome (MAS), is reportedly associated with rheumatologic disorders including systemic lupus erythematosus, hematopoietic cell transplantation, and more recently in CAR-T therapy [71–73]. Patients with HLH typically present with unremitting fever, cytopenias, hepatosplenomegaly and often have hyperferritinemia, coagulopathy, hypertriglyceridemia, and transaminitis.

The incidence of sHLH/MAS is rare with CD19 CAR-T products, ranging from 3 to 6% [73]. CAR T-cell associated sHLH/MAS negatively affects efficacy and is associated with inferior overall survival [74]. This low incidence may reflect the rare nature of CAR-T-associated sHLH/MAS but could also represent difficulty during diagnosis. Higher incidence of sHLH/MAS is seen in clinical trials of CAR-T targeting CD22. Lichtenstein and colleagues at the NIH reported 59 patients infused with CD22 CAR T-cells with 21 patients developing CAR-T-associated sHLH/MAS, described as featuring hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, coagulopathy, transaminitis, hyperbilirubinemia, severe neutropenia, elevated lactate dehydrogenase, and occasionally hemophagocytosis [75]. Other early phase clinical trials with anti-CD22 CAR-T also reported HLH/MAS development [76,77].

Diagnosis and management of sHLH/MAS can be difficult. The traditional diagnostic criteria for HLH/MAS are not specific (Table 5) [78]. CRS and sHLH/MAS share several clinical features and laboratory findings making differentiating severe CRS from sHLH/MAS difficult. CRS and sHLH/MAS may also share similarities in the genesis of hyperinflammatory response to CAR-T therapy, raising questions regarding the diagnostic criteria and optimal management of CAR-T-associated sHLH/MAS. Neelapu and colleagues proposed CAR T-cell-related HLH/MAS criteria (Table 5) [20]. These criteria consider clinical and laboratory findings associated with sHLH/MAS and CRS and allow for a prompt diagnosis.

Management of sHLH/MAS currently remains uncertain. Neelapu and colleagues recommended etoposide and intrathecal cytarabine for patients unresponsive to tocilizumab and corticosteroids in sHLH/MAS with associated neurotoxicity [20]. Concerns are raised regarding the effect of CAR-T function with etoposide administration [79]. Anakinra reportedly elicits some response in CAR-T related sHLH/MAS although these are reported in small numbers [31,80]. More attention and scientific advancement are needed for diagnosis, grading, and management of CAR-T-associated sHLH/MAS.

#### 4. Other late effects

With increasing use of CAR-T across multiple disease indications, we are beginning to have a better understanding of late effects in recipients of CAR-T. Grade  $\geq 3$  cytopenia across multiple cell lines is the most common adverse event in CAR-T recipients; while most patients recover counts by 30 days post CAR-T, a notable percentage of patients have persistent cytopenia at  $\geq 3$  months. On long term follow-up, 17% of patients that enrolled in ZUMA-1 had grade  $\geq 3$  cytopenia at  $\geq 3$  months and in TRASCEND-NHL-001 trial, 37% of the patients had cytopenias that had not resolved at 29 days [2,81]. The understanding of factors impacting late or persistent cytopenia is limited currently, but studies have suggested that patients who are heavily pretreated prior to CAR T-cell infusion are more likely to experience persistent cytopenia [82]. Recently, the CAR-HEMATOTOX model was published where baseline hematopoietic reserve and inflammation prior to CAR-T was predictive of delayed cytopenia [83].

Complete responses with CD19-directed CAR-T in B-cell malignancies are associated with B-cell aplasia and subsequent hypogammaglobinemia [84]. A variable incidence of hypogammaglobinemia is observed with CD19-directed CAR T-cell therapy with a higher rate noted in B-cell acute lymphoblastic leukemia in comparison to diffuse large B-cell lymphoma [81,85]. Hypogammaglobinemia can be persistent and can last for years resulting in increased infections. Management involves routine surveillance of immunoglobulin G (IgG) levels with intravenous (IV) or subcutaneous IgG replacement to  $\geq 400$  mg/dL [86]. The use of CAR-T is expected to increase in near future with the results of recent studies showing safety and efficacy of CAR-T in the second line [9,10]. Longer follow-up will inform other important late effects such as secondary malignancies and their association with CAR T-cell therapy.

#### 5. Discussion

CAR-T therapy changed the therapeutic landscape of B-cell lymphoma, ALL, and multiple myeloma. Since the first FDA approval in 2017, we witnessed approvals of six CAR T-cell products and clinical trials are ongoing for newly diagnosed lymphoma and myeloma, relapsed refractory acute myeloid leukemia, and solid tumor malignancies. With increased utilization of CAR T-cells, new potential indications including solid organ malignancies demonstrated feasibility of outpatient CAR-T administration [2,87–89], early recognition, management, and prevention of CAR-T-associated toxicities.

Despite considerable progress, there exist unanswered questions. Regarding CRS, the ideal management of CRS that is not responsive to tocilizumab and corticosteroids is unclear. With promising agents such as anakinra, JAK inhibitors, and others, determining the ideal agents and timing is certainly needed. Similarly, management of ICANS that does not respond to steroids is unclear. Case reports have suggested resolution with intrathecal chemotherapy [67,68]. Steroid sparing strategies or early steroid discontinuation for both CRS and neurotoxicity is another area requiring research. sHLH/MAS remains a significant severe CAR-T toxicity wherein management is uncertain due to its rarity; however, considering reports of sHLH/MAS potentially increased with anti-CD22 CAR-T, significant progress in grading and management, similar to the progress with CRS, will be required. Finally, better understanding of late toxicities, particularly post-CAR T-cell cytopenias is needed.

CAR T-cell therapies have thus far and likely will continue to change the landscape of cancer therapeutics. With better refinement of existing strategies and development of new therapies to prevent and treat CAR T-cell related toxicities, more patients, through new disease indications or various clinical settings, would safely benefit from this novel treatment modality.

### Conflict of interest

HSM reports consultancy and advisoryboard with CRISPR Therapeutics.

MAK-D declares no financial conflicts of interest.

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