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Mechanisms and Management of Chimeric Antigen Receptor T-Cell Therapy Related Toxicities

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Abstract

Chimeric antigen receptor T-cell (CAR-T) therapy has proven to be a very effective cancer immunotherapy. Axicabtagene ciloleucel and Tisagenlecleucel are the first in class anti-CD19 CAR-T-cells currently available for relapsed-refractory adult large B-cell lymphoma. Tisagenlecleucel is also available for pediatric and young adult (up to age 25) patients with relapsed-refractory B-acute lymphoblastic leukemia. Cytokine release syndrome (CRS) and CAR T-cell associated encephalopathy syndrome (neurotoxicity) are the most common adverse effects associated CAR-T-cell therapy. They can lead to significant morbidity and preclude wide-spread use of this treatment modality. Treatment related deaths from severe CRS and cerebral edema have been reported. There is a significant heterogeneity in the side-effect profile of different CAR-T-cell products under investigation and there is a need to develop standardized guidelines for toxicity grading and management. Here, we summarize the current literature on pathogenesis, clinical presentation, and management of CRS and neurotoxicity. The different grading systems of CRS and management protocols used in different trials have made it difficult to compare the outcomes of different CAR-T-cell therapies. Several prevention strategies such as predictive biomarkers of CRS and neurotoxicity and modified CAR-T-cells with 'built-in' safety mechanisms are being studied, which has the potential to greatly expand the safety and applicability of CAR-T-cell treatment across various malignancies.

1. INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy is an immunotherapeutic approach that utilizes gene transfer to reprogram T-cells to recognize and eliminate cancerous cells by targeting and interacting with cell surface antigens specific to the tumor. The CAR consists of an extracellular domain that can bind to a target molecule expressed on the surface of tumor cells, a transmembrane domain, and an intracellular domain that provides an activation signal to T cells when the extracellular domain is engaged with its target. The

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extracellular domain usually comprises the antigen-recognition regions of an antibody, in the form of a single-chain variable fragment (scFv) or ligands of cell-surface receptors. The intracellular domain usually incorporates a region of the T-cell receptor (TCR) CD3ζ chain to provide an activation signal. First-generation CARs had very limited efficacy in pre-clinical models. Early clinical trials limited to solid organ malignancies without routine use of conditioning lymphoablative chemotherapy (i.e. fludarabine, cyclophosphamide) failed to show meaningful clinical responses and had minimal systemic toxicities.[1, 2] Second generation CARs have an additional domain from a co−stimulatory receptor, such as CD28, OX40 (CD134), and/or 4−1BB (CD137) to provide a second activation signal: axicabtagene ciloleucel (axi-cel) has a CD28 domain and tisagenlecleucel (tisa-cel) has a 4–1BB domain. An elegant clinical trial demonstrated the increased expansion capacity of second generation CD19 CAR-T as compared to first generation for lymphoma, however this was conducted without conditioning chemotherapy.[3] Addition of these co-stimulatory receptors and routine use of conditioning lymphoablative chemotherapy prior to CAR-T-cell infusion have greatly enhanced the efficacy of CAR T cell therapy, yet it also led to increased systemic toxicities.

The production of CAR-T-cells is a multistep process which includes collection of white blood cells (including T cells) via leukapheresis, introduction of CAR construct typically via a viral vector (replication-defective lentivirus or gammaretrovirus) followed by cell expansion and cryopreservation.[4] Multiple clinical trials are ongoing in both hematological and solid organ malignancies targeting various cell surface tumor antigens with autologous or allogenic CAR T-cells.[5] ZUMA-1, a phase 2 trial of axi-cel in refractory large B-cell lymphoma showed complete response rate (CR) of 54% with overall survival (OS) of 52% at 18 months median follow-up.[6] Another CD19 CAR-T-cell product from University of Pennsylvania (CTL019) showed similar responses in released/refractory DLBCL or follicular lymphoma with CR in 64% of the patients.[7] In the pivotal JULIET trial, tisa-cel showed overall remission rate of 81% after a single infusion in relapsed or refractory B-cell acute lymphoblastic lymphoma (B-ALL).[8] The recent approvals of tisa-cel for refractory pediatric and adolescent/young adult B-ALL and both tisa-cel and axi-cel for adult relapsed large B cell lymphoma by the United States FDA and European Commission (EC) are major advancements in the field of cancer immunotherapy.[9–11] These therapies offer a new hope to the patients who are refractory to conventional treatments with no effective salvage options.

CAR T cell therapy is associated with unique toxicities related to immune system activation. Cytokine release syndrome (CRS) and CAR-T-cell related encephalopathy syndrome (neurotoxicity) are two major complications which can lead to significant morbidity and mortality [12, 13]; Hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) is a rare and potentially fatal complication of CAR T-cell therapy.[6] These toxicities limit the application of CAR T-cell therapy to patients with good performance status and adequate organ function. Along with clinical development of CAR T-cell therapy, there have been significant efforts to understand the pathophysiology, and improve prevention and management strategies of CAR T-cell related toxicities. Prompt diagnosis and treatment is imperative for the management of CRS and neurotoxicity to prevent adverse outcomes. In this article, we provide a review of the clinical presentation, pathophysiology,

and management of CRS and neurotoxicity. We also consider potential future developments in CAR T-cells to prevent and effectively treat these complications.

We performed a thorough review using PubMed and meeting abstract databases from the American Society of Clinical Oncology (ASCO), the American Association of Cancer Research (AARC) and American Society of Clinical Oncology (ASH) updated through June 30, 2018. We narrowed our search with the following keywords and MeSH terms: chimeric antigen receptor T-cell therapy, cellular immunotherapy, high-grade B-cell lymphoma, diffuse large B-cell lymphoma, multiple myeloma, acute lymphoblastic leukemia, cytokine release syndrome, neurotoxicity. Studies reviewed here must have had at least preliminary results released before the date of the search. Two authors (B.D. and C.B.) reviewed the full text articles and meeting abstracts and summarized the clinical data. Senior author (F.L.) acted as a curator. Some observations regarding clinical presentations of CRS and neurotoxicity are based on our institutional experience. At the Moffitt Cancer Center we had infused 50 patients with commercial CAR T cell products and are approaching 100 additional patients with investigational products on clinical trials, as of November 1st, 2018.

2. CYTOKINE RELEASE SYNDROME

The most common toxicity associated with CAR T-cells is cytokine release syndrome (CRS) (Table 1). The precise pathophysiology behind CAR-T-cell associated CRS remains to be defined. CRS is a constellation of inflammatory symptoms caused by the activation of T cells and subsequent release of cytokines, as well as the recruitment and activation of other immune cells.[14] These cytokines include interleukin-6, interferon gamma, interleukin-10, and interleukin-2 and may be produced by the CAR T-cells directly or by other cells such as monocytes/macrophages in response to cytokines produced by the CAR T-cells. Recent reports demonstrated that host derived monocyte/macrophage and CAR-T-cell interactions play an important role in CRS pathophysiology. In a murine model, CAR-T cells promoted recruitment and proliferation of monocytes by direct cell contact between CD40 (dendritic cell/monocyte/macrophage)-CD40 ligand (T-cell), which in-turn produced IL-1, IL-6 and nitric oxide (NO).[15] It was also demonstrated that depletion of macrophages before CAR-T-cell infusion leads to elimination of CRS in xenograft human leukemia mouse models treated with CD19 CAR-T-cells.[15, 16] Additional investigation as to the interactions between CAR-T and other cells, whether it be cytokine driven, or cell contact mediated effects, is warranted.

2.1 Clinical and Laboratory Manifestations of CRS

CRS usually manifests with constitutional symptoms with the hallmark being fever; however, symptoms vary greatly and can affect any organ system, including cardiovascular, gastrointestinal, hepatic, renal, respiratory, hematological, and nervous systems. Hay et al examined 133 adult patients with relapsed/refractory B-cell malignancies who received CD 19 CAR-T-cells with a 4–1BB costimulatory domain. Patients with grade 4 CRS developed a fever earlier (median days after CAR-T-cell infusion= 3.9 vs 0.4 for grade 1–3 vs. those with grade 4) and the fever peaked sooner (median days after CAR-T-cell infusion= 5.7 vs 2.8 for grade 1–3 vs. those with grade 4) with a higher maximum temperature. Temperature ≥38.9°

C within 36 hours of CAR-T-cell infusion has 100% sensitivity and 84% specificity for grade ≥4 CRS[17]. Thus, the onset and peak time for fever after CAR-T-cell infusion may guide CRS prevention strategies.

The onset of CRS varies from hours to several days post CAR-T infusion with differences noted between CAR-T-cell products, disease state, and severity of CRS [12–14, 17]. Patients treated with anti-CD19-CD28-CD3ζ CARs may experience CRS earlier than those treated with anti-CD19-4-1BB-CD3ζ CARs[12]. This disparity may be due to differences in pharmacokinetic profiles as CD28 CAR constructs exhibit a greater peak expansion in vivo, while 4–1BB CAR T cells may exhibit greater longevity[18]. In ZUMA-1, the onset of CRS was a median of two days following infusion (range 1 to 12 days) [6]. In contrast, the median time to onset of CRS was 3 days (range 1 to 22 days) in the ELIANA trial with tisa-cel in pediatric and young adult acute B-ALL[19].

Commensurate with the immune mediated signs and symptoms of CRS, patients with severe CRS typically have higher levels of interleukin-6, interferon-gamma, CRP and ferritin than patients who did not experience severe CRS, although their ability to be predictive of impending toxicity is unverified.[20] In the previously mentioned report of 133 recipients of CD19 CAR T-cells biomarkers were evaluated to determine those associated with severe CRS. Patients who had grade ≥4 CRS demonstrated higher ferritin and CRP compared with those who experienced grade ≤3 CRS. Furthermore, the patients with grade ≥4 CRS exhibited higher concentration of interferon-γ, interleukin-6 (IL-6), IL-8, IL-10, IL-15, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor receptor p55 (TNFRp55), and macrophage inflammatory protein-1β (MIP-1β) within 36 hours of CAR-T-cell infusion compared to the degree of elevation of these cytokines in the patients with grade ≤3 CRS. Higher tumor burden and CAR-T-cell dose were also associated with severe CRS [17]. This is consistent with previously reported CD19 CAR-T-cell data [20–22]. In the pivotal ZUMA-1 trial, several biomarkers were significantly associated with grade 3 or higher CRS, including but not limited to IL-15, IL-6, interferon-γ, IL-10, IL-8, and granzyme B. Of note, it was also demonstrated that CAR T cell peak expansion and area under the curve were significantly associated with grade 3 or higher neurologic events, but not grade 3 or higher CRS [6].

Unfortunately, real-time rapid cytokine level measurement is not currently feasible or widely available necessitating the use of available surrogate indicators for CRS. CRP has demonstrated a correlation with CRS progression, increasing with the onset of CRS and returning to baseline with CRS resolution [22]. CRP is a useful daily monitoring tool for CRS in the CAR T cell therapy patients; however, this correlation is not detected in all patients and intervention for CRS should not be based on CRP alone [12]. Our experience with CAR T-cell treated patients on clinical trials and commercial Yescarta© indicates that CRP elevation does not always adequately precede CRS to be a reliable marker to guide an intervention as the elevation of CRP appears to occur concurrently with the clinical signs and symptoms of CRS. However, anecdotally we have seen its decrease herald resolution of CRS signs and symptoms and help guide the tapering of systemic therapy. The verification of predictive biomarkers for CRS remains incomplete and additional confirmatory research is necessary. The number one goal of CRS management is to prevent life-threatening

toxicities. However, any intervention to block cytokine release could in theory block CAR-T-cell activity and compromise anti-tumor activity. Current evidence suggests that anti-tumor activity can be preserved even in patients treated with tocilizumab and corticosteroids.[6]

2.2 CRS grading

The Common Terminology Criteria for Adverse Events version 4.03 (CTCAE v4.03)[23] was used for grading of organ toxicities throughout many CAR-T-cell trials. Typically, the organ toxicity associated with CRS was graded by this scale, but CRS requires grading of the entire syndrome. The CTCAE v4 contains grading criteria for cytokine release syndrome (Table 2); however, this was not created for cellular therapy and is directed towards management of infusion reactions with immunotherapies. This grading system is insufficient for CRS symptoms that occur days after the CAR-T cell infusion. Single center and pivotal trials of CAR T cell therapy have utilized differing criteria, making cross comparison of toxicity severities difficult. Lee et al. created the NCI consensus criteria to define mild, moderate, severe and life-threatening CRS associated with high risk immunotherapies and to guide treatment recommendations based on this grade. The Lee grading criteria is included in table 2 and is based on constitutional symptoms, oxygen requirement, indication for vasopressors and grade of organ toxicity [14]. The Lee grading system was used in the ZUMA-1 trial [6]. Of note, CTCAE version 5.0, released in November 2017, has modified the grading of cytokine release syndrome to correlate closely with the original Lee criteria (Table 3)[24].

A modification of the Lee grading system was proposed by the Neelapu and colleagues (CARTOX group). These recommendations expand upon Lee et al. grading system to develop a more consistent approach to monitoring, grading and management of CAR T-cell associated toxicities in adult patients. CRS grade was concordant with the Lee et al CRS grading, considering temperature, systolic blood pressure, oxygen requirement, and organ toxicity with only slight modification in relation to transaminase elevation. The CARTOX grading was then combined with additional CARTOX consensus management and intervention strategies [12, 25].

An alternative grading system based on clinical parameters created at by Porter, et al., was used in the ELIANA and JULIET trials [19]. This grading scale was originally created and used for CRS classification in chronic lymphocytic leukemia patients receiving CAR-T cells at the University of Pennsylvania. The PENN grading criteria is included in table 2 and, as with Lee scale is based on clinical features and organ toxicity as well as need for vasopressors and oxygen. Need for hospitalization is also included, although many patients have CAR T cells infused in the inpatient setting [26, 27].

When interpreting rates of reported cytokine release syndrome, it is important to be aware of the grading system utilized. The PENN grading scale created by Porter, et al. considers all patients with hypoxemia requiring oxygen grade 3 CRS, whereas the Lee scale considers patients with an oxygen requirement less than 40% a grade 2. Additionally, the Lee scale classifies hypotension responding to IV fluids or a low dose vasopressor grade 2; hypotension requiring intervention is grade 3 CRS under the PENN scale. Subsequently, hypotension requiring high dose or multiple vasopressors is a grade 4 under the PENN scale,

but a grade 3 when using the Lee scale. Given these differences the PENN CRS grading scale likely leads to a higher number of grade 3 and 4 CRS than if the Lee, et al system is used for the same subset of patients and graded in an otherwise consistent manner. Additional observational studies comparing these grading scales would be helpful to interpret pivotal trial data for different products. With a goal to unify CRS grading system The American Society of Blood and Marrow Transplantation (ASBMT) is in the process of developing both immune effector cell CRS and neurotoxicity grading systems for CAR-T and related therapies.[28]

2.3 Management of CRS

The clinical manifestations and severity of cytokine release syndrome varies greatly from mild constitutional symptoms to life-threatening severe toxicities. Adverse outcomes likely can be avoided with accurate and prompt evaluation and management of these patients. The management of these patients is complicated by overlapping conditions. The hallmark of CRS, fever, as well as other symptoms of CRS mirrors the presentation of infection. CRS symptoms typically resolve within two weeks of CAR-T infusion. This toxicity can be self-limiting requiring only symptomatic care or may require treatment with an IL-6 antagonist and/or glucocorticoids. The goal of treatment of CRS is to avoid harmful toxicities while maximizing the anti-tumor effect of the cellular therapy.

2.3.1 Location of Care—There is no consensus on need for hospitalization of patients receiving CAR-T cell therapy. In the ZUMA-1 study of adult patients receiving axicabtagene ciloleucel, all patients were hospitalized for the CAR-T infusion and for a minimum of 7 days afterwards [6]. In the pivotal JULIET trial that led to approval of tisa-cel for adult patients with DLBCL, 26% received CAR-T infusion in the outpatient setting and 77% of these patients remained outpatient for ≥3 days following infusion [29], Pediatric and young adult patients receiving tisa-cel on the ELIANA trial were also able to receive product infusions in the outpatient setting [6, 19]. Long-term follow-up data on the patients treated in outpatient settings is needed. Although this demonstrates that CAR T therapy can be administered in a well-controlled outpatient setting, it is important to note that the centers doing this had significant experience with CAR T cell therapy and already well-established robust outpatient hematopoietic stem cell transplant programs. Further development of predictive biomarkers is needed to identify patients at risk of severe early CRS requiring intensive monitoring and an inpatient admission before severe symptoms develop [30].

2.3.2 Supportive care—Supportive care measures begin following cell infusion and continue throughout all stages of CRS. Daily monitoring typically includes a complete blood count with differential and complete metabolic panel. At our center we perform daily CRP and ferritin as markers of inflammation associated with CRS. These can be particularly helpful in high risk patients or those experiencing severe CRS to trend the inflammatory state. Intravenous fluids are used to maintain hydration; however fluid balance (including daily bodyweight) must be monitored closely due to the risk of volume overload and pulmonary edema. Due to the risk of cardiac arrhythmias, telemetry monitoring should be considered from the time of CAR-T cell infusion until resolution of CRS, especially in patients with additional cardiac risk factors [12].

Acetaminophen may be administered for management of fever in patients with normal hepatic function; cooling blankets may also be used. Non-steroidal anti-inflammatory medications may be used as an alternative; however, caution must be taken in the setting of thrombocytopenia. Additionally, NSAIDs may contribute to hemorrhage, gastritis, and renal insufficiency[31]. As many of these patients are neutropenic, and all receive lymphodepletion, it becomes imperative to monitor for infection. Febrile patients should be assessed for infection including blood and urine cultures and chest radiography. Broad spectrum antibiotics should also be initiated.

2.3.3 Anti IL-6 therapy—Tocilizumab is a humanized monoclonal antibody (mAb) against the IL-6 receptor (IL-6R),recently FDA approved for CAR-T cell induced severe or life-threatening CRS.[32] Rapid resolution of CRS has been demonstrated following administration of tocilizumab in most patients. In addition, published reports suggest tocilizumab does not negatively impact CAR T cell expansion or persistence [6, 22, 33]. Tocilizumab is infused over 60 minutes at a dose of 12 mg/kg for patients with a body weight under 30 kg and 8 mg/kg for patients weighing 30 kg and over. The FDA approved dosing strategy includes the ability to administer up to 3 additional doses if there is no clinical improvement in the signs and symptoms of CRS, with a minimum of 8 hours between consecutive doses. Additional infusions may not be warranted if a patient has responded to the therapy and CRS symptoms are not recurring.

Although the approved indication is for severe or life-threatening CRS, there are no clear recommendations on the optimal timing for administration of tocilizumab. Lee and colleagues provide a treatment algorithm based on their CRS grading assessment; tocilizumab should be administered to patients experiencing CRS of grade 3 or greater and to patients with grade 2 CRS with comorbidities. On the ZUMA-1 trial 43% of patients received tocilizumab with no difference in response rate compared to patients that did not receive tocilizumab [6]. There was a decrease in the incidence of CRS and neurologic events of grade 3 or higher in ZUMA 1 over the course of the study (18 % ≥3 CRS at the interim analysis of n=62 patients to 13% ≥3 CRS at the final analysis of n=101 patients [6]). This decrease might be attributed to a protocol amendment allowing for earlier intervention for the treatment of CRS and neurologic toxicity partway through the trial. Essentially this amendment changed treatment guidelines from the Lee guidelines to the CARTOX guidelines, allowing for administration of tocilizumab for grade 2 CRS possibly leading to less patients progressing to grade 3 CRS [6, 25].

The grading system from the University of Pennsylvania was used in conjunction with management recommendations in the ELIANA trial of tisa-cel in pediatric and young adult ALL. Tocilizumab was administered as second line management for high fevers, hypoxia, and hypotension in response to any of the following: hemodynamic instability despite intravenous and fluids and vasopressor support, worsening respiratory distress or rapid clinical deterioration. Based on this algorithm, tocilizumab is given later in CRS progression than when utilizing the Lee algorithm or CARTOX guidelines for management of CRS. Forty percent of patients received tocilizumab for the management of cytokine release syndrome in this study [19]. The JULIET study of tisa-cel in adult DLBCL patients also

employed the PENN CRS grading scale and a similar algorithm with 15% of patients receiving tocilizumab [29].

The CARTOX group recommended earlier administration of tocilizumab, as compared to Lee guidelines, with consideration in both grade 1 and grade 2. In grade 1 this is specifically for patients with a refractory fever lasting over 3 days and in grade 2 CRS tocilizumab is recommended for hypotension that is refractory to fluid boluses. Repeat doses of tocilizumab can also be considered in grade 2 or higher. Anti-IL-6 therapy is also recommended for patients categorized as a grade 2 CRS due to persistent hypoxia at a fraction of inspired oxygen (FiO2) <40% and other grade 2 organ toxicities. Both tocilizumab and glucocorticoids are recommended for management of grades 3 and 4 CRS [12]. We suggest that additional doses of tocilizumab should be considered if CRS does not improve with initial dosing. Further studies are needed to determine optimal timing between doses and efficacy with repeat doses.

Siltuximab (anti-IL-6 chimeric mAb) is another drug being used off-label for CRS, especially in tocilizumab and steroid refractory cases. It can rapidly reverse CRS symptoms when used on different clinical studies.[12, 13, 20, 34] This drug is currently approved by the US FDA for Multicentric Castleman's disease [35]. It binds directly to IL-6 with a high affinity, so there is a theoretical advantage of more complete blockage of IL-6 activity over tocilizumab which blocks membrane bound and soluble IL-6R. There have been reports of transient increase in IL-6 levels after the first dose of tocilizumab, possibly due to decreased IL-6 clearance in peripheral tissue via IL-6R [33, 36]. Prospective randomized studies might be considered to compare efficacy of tocilizumab and siltuximab as therapeutics for CRS patients.

2.3.4 Glucocorticoids—Glucocorticoids have also demonstrated efficacy in ameliorating CRS due to the ability to suppress inflammatory responses. Published evidence suggests glucocorticoids dampen CAR-T cell expansion and anti-tumor effect in ALL patients post CD-19 directed CAR-T cell infusion [37], [22]. In contrast, data from the ZUMA-1 study suggests glucocorticoids used for treatment of CAR-T related toxicities do not impact objective response rates. The objective response rate for the 27 patients who received steroids was no different from patients that did not receive steroids [6]. Of note, this conflicting data is in patients with different disease states (ALL versus DLBCL). Due to concerns of CAR-T cell suppression, steroids remain a second-line treatment for CRS refractory to tocilizumab, except in extremely rapid onset cases of severe CRS, and should not be used for other non-life threatening indications.

The trigger to initiate steroids as well as the optimal steroid and dose is not clearly defined. Lee and colleagues recommend if the patient's condition does not improve or stabilize within 24 hours of the initial tocilizumab dose, a second dose of tocilizumab or glucocorticoid should be considered. This applies to patients with grade 3 and higher CRS, and elderly patients or those with significant comorbidities with grade 2 CRS. The CARTOX group includes glucocorticoids as an option of consideration for grade 2 CRS, specifically patients at high risk of severe CRS or if hypotension persists after 1–2 doses of anti-IL-6 therapy. Tocilizumab and steroids, specifically dexamethasone 10 mg IV every 6 hours, is

recommended in grade 3. Grade 4 CRS includes a recommendation for methylprednisolone 1000 mg IV per day followed by a rapid taper. Teachey et al. recommended restricting the starting dose of prednisone to 1 mg/kg based on their pediatric experience using CD19-4-1-BB CAR-T-cell product [38].

In summary, optimal timing and dosages of tocilizumab and glucocorticoids in the management of CRS need to be studied systemically across different CAR-T-cell products. Prospective intervention studies with standardize CRS grading scale are urgently needed at this point.

3. CAR T CELL ASSOCIATED NEUROTOXICITY

3.1 Clinical Manifestations and incidence of neurotoxicity

Neurotoxicity is the second most common toxicity related to CAR T cell therapy. Typical manifestations of neurotoxicity range from minor headache and diminished attention to seizure, severe encephalopathy, and death. The most characteristic manifestation is encephalopathy typified by confusion progressing to expressive aphasia and at the extreme obtundation. Early signs are language and handwriting impairment followed by confusion, agitation, hallucinations, tremors and headaches. Seizures, motor weakness, incontinence, mental obtundation, increased intracranial pressure, papilloedema, and cerebral edema can be seen in severe cases of neurotoxicity (grade >2) [12]. The manifestations may be biphasic with early confusion coinciding with high fevers and CRS, and later encephalopathy often following the resolution of CRS. The incidence and severity of neurotoxicity varies by different CAR constructs. Neurotoxicity may be more frequent in the patient with pre-existing neurological conditions, younger patients and heavily pretreated patients [39, 40].

Electroencephalography (EEG) findings in the patients with neurotoxicity induced encephalopathy are very non-specific with diffuse generalized slowing with or without triphasic waves [12]. The main utility of EEG is to rule out seizure activity which can occur in minority of these patients. Brain imaging is typically described as normal, even in the patients with severe neurotoxicity. Occasional T2/ fluid attenuated inversion recovery (FLAIR) MRI hyperintensity involving the thalami, mid-brain and cerebral edema have been reported [39, 41]. Elevated opening pressure (≥20 mmHg) during lumbar puncture is common in moderate to severe neurotoxicity. Elevated CSF protein and leukocyte count secondary to increase BBB permeability is seen in neurotoxicity patients; however, the cell count within the CSF does seem not to be correlated with the severity of neurotoxicity [39].

Turtle et al. studied 133 adults with B-ALL, NHL, or CLL who received CD19 CAR-T cells containing a 4–1BB costimulatory domain. A total of 53 of 133 patients (40%) had 1 or more grade ≥1 neurological adverse event; 7 (5%) developed grade ≥4 neurotoxicity and 4 patients (3%) died due to neurotoxicity within first 28 days of CAR T cell infusion. The median time of presentation of neurotoxicity was 4 days after CAR T cell infusion and the majority of these patients had preceding CRS (91%) before the onset of neurotoxicity.[39] In the phase 2 ZUMA 1 trial, CD19 CAR T cells with CD28 co-stimulatory domain were given to 101 patients with relapsed refractory large B-cell lymphoma. Neurologic events occurred in 65 patients (64%); 28% were grade 3 or higher and no deaths were attributed to

neurotoxicity. The median time to the onset of neurologic events occurred on day 5 (range, 1 to 17), with median resolution on day 17 after infusion. Median duration of neurotoxicity was around 5 days (range 1– 21 days) and typically lasted 2– 4 days.[6] Prolonged memory impairment and tremor beyond 3– 4 weeks after CAR-T-cell infusion have been reported [39, 42, 43]. In the JULIET trial testing tisa-cel (CD19-4-1BB) in 111 relapsed/refractory DLBCL, incidence of overall and grade 3–4 neurotoxicity was reported at 21% and 12%, respectively, however the FDA approved label reports (N=106) 58% and 18%, suggesting that different observers may attribute neurotoxicity to CAR T or other factors, thereby skewing summary neurotoxicity data [8, 44]. There seem to be a general trend of lower rate of neurotoxicity with 4–1BB compared to CD28 costimulatory CD19 CAR-T construct, however comparative studies controlling for confounding factors are needed.

Table 1 describes published clinical experience with different CARs tested in multi-center trials and rates of neurotoxicity. Due to difference in CAR construct, dose of infused CAR-T-cells, lymphodepleting chemotherapy, and target diseases, it is difficult to cross-compare rates of neurotoxicity among different clinical trials. Most of the currently available information about neurotoxicity is based on CD19 CAR-T-cell products and CNS toxicities associated with non-CD19 CAR-T-cell therapies are yet to be characterized. For DLBCL, the TRANSCEND NHL 001 study (CD19-4-1-BB, Juno therapeutics) reported a low rate of neurotoxicity.[45] Alternatively, 5 deaths in ROCKET trial (JCAR015, CD19−28−ζ) from cerebral edema have raised an alarm which lead to termination of the trial [40]. Again, comparing neurotoxicity rates across pivotal trials is fraught with complexities based upon attribution and definition of what exact CTCAE toxicities should be considered part of the spectrum of CAR T-cell therapy related encephalopathy and neurotoxicity. Improved consensus neurotoxicity definitions and grading scales are needed and must be prospectively validated so they could be utilized universally in CAR T cell clinical trials.

3.2 Pathophysiology of CAR T cell associated neurotoxicity

Precise underlying mechanisms behind neurotoxicity are not fully understood. Since the majority of neurotoxicity is preceded by CRS, one hypothesis is that neurotoxicity is a manifestation of passive diffusion of cytokines into the brain in presence of a permeable blood-brain barrier (BBB). Alternatively, a recent report by Santomasso, et al. demonstrated that there were disproportionately higher levels of IL6, IL8, MCP1 and IP10 in CSF compared to serum in the patients with severe neurotoxicity. There were also increased levels of endogenous excitatory neurotransmitters (glutamate, quinolinic acid) in CSF in those patients [46]. The data showing higher CSF cytokine levels as compared to peripheral blood, and the fact that CAR T cells can be found in the CSF of patients with or without severe neurotoxicity, suggests that active cytokine release from, or induced by, local CAR T cells within the CNS may be a driver [33, 46, 47]. In a study involving CD19 CARs, patients with severe neurotoxicity had evidence of endothelial activation and increased BBB permeability. The concentrations of IFNγ, TNFα, IL6, and TNFR p55 had increased significantly and were comparable between serum and CSF during the acute phase of neurotoxicity [39]. Autopsies on 2 patients who died from neurotoxicity followed by CD19 CAR-T-cells showed widespread vascular lesions, cerebral edema and necrosis with perivascular CD8+ T-cell infiltration, suggesting pervasive endothelial dysfunction and

destruction [39]. Another study of B-ALL patients treated with CD19 CAR T-cells showed elevated levels of IL1α, IL2, IL3, IL5, IL6, IL10, IL15, IP10, INF γ, GCSF, GMCSF, and MCP1 by day 3 in patients with severe neurotoxicity[46]. Pre-existing endothelial activation before CAR T cell infusion can also increase the risk for CRS and neurotoxicity. Angiopoietin-1 (ANG1) is an endothelial stabilizing cytokine and angiopoietin-2 (ANG2) promotes endothelial activation via ANG-TIE2 axis. [48, 49] A higher ANG2:ANG1 ratio was associated with severe neurotoxicity in a study by Santomasso, et. al [46]. High-intensity lymphodepletion with fludarabine before CAR-T cell infusion can lead to increased levels of IL-15 which is associated with greater peak CAR T-cell expansion and resultant neurotoxicity [50, 51]. High rates of cerebral edema in JCAR015 (phase II ROCKET trail) were attributed to rapid CAR T-cell expansion and elevated IL-15 levels before cell infusion [40].

It is unlikely that CNS invasion by CAR T-cells leads directly to neuronal cytotoxicity. Most patients exhibit a complete neurological recovery, which would be impossible with widespread neuronal destruction. CAR T-cells have been detected in the CSF of patients with neurotoxicity without CNS malignancy [52, 53]. However, there is no evidence of CD19 expression in neurological tissue and neurotoxicity has been reported in the patients who received CD22 CAR T-cells for B-ALL and BCMA CAR T-cell for multiple myeloma [54, 55]. It is unknown at this point how the incidence and severity of neurotoxicity differ between CD19 and non-CD19 CAR-T-cell therapies due to the paucity of data.

Most of the T-cells in the brain parenchyma (93%) and CSF (95%) were CAR T-cells. A higher fraction of the CD4+ CAR T-cell subset in the CSF compared with blood, suggests there might be difference in the migration pattern of CD4+ and CD8+ CAR T-cells across the BBB and their role in neurotoxicity [33, 39]. Similarly an association with higher CD4+ CAR T-cells in the CSF was seen in patients that later developed severe neurotoxicity as compared to those that did not, although the ratio of CD4:CD8 T-cells in the infused product did not predict the rates of CRS and neurotoxicity [6].

Only JCAR017 (Juno Therapeutics) has a 1:1 ratio of CD4+:CD8+ CAR T-cells in the final product, whereas other CAR T-cell therapies are not separately manufacturing and combining CD4 and CD8 T-cells [21]. More studies are needed with other CAR T-cell products in different diseases with exploratory immunophenotyping of blood, CSF, and brain tissue to validate findings by Guest et al.[39] Animal models of neurotoxicity have been developed, including a murine and a non-human primate model of CD20 CAR T-cell mediated CRS and neurotoxicity [16, 47]. These models will allow the study pathogenesis and test different therapeutic interventions for neurotoxicity. As with any animal model, species barrier will be a limiting factor when applying these pre-clinical findings in designing clinical trials.

3.3 Management of neurotoxicity

CAR T-cell therapy associated neurotoxicity management is guided by toxicity grading and may be informed by the severity of concurrent CRS. The CARTOX group coined the term CAR T-cell associated encephalopathy syndrome (CRES) which encompasses some of the neurotoxicity symptoms and signs, although does not address nonspecific neuropsychiatric

symptoms which may or may not have been attributed to CAR T in the pivotal trials. Table 3 shows CRES grading based on the CARTOX groups experience with CD19 CAR T-cell therapy in the adult patients with high-grade B cell lymphoma [12]. The CAR−T−cell-therapy-associated toxicity 10−point neurological assessment (CARTOX-10) is an easy to use clinical tool for bed-side assessment of patients at risk for neurotoxicity and it has been incorporated into the neurotoxicity grading score[42]. It can be easily performed multiple times per day by providers or nursing staff. It is important to consider that the CARTOX-10 and therefore the CRES neurotoxicity grading system have not been prospectively validated and that it is based primarily on axi-cel usage in adults with lymphoma. An additional shortcoming of the CRES grading scale is that it relies upon funduscopic exam and CSF opening pressures, tests which may not be universally available or performed correctly. Mini-mental state examination (MMSE)[56] and Glasgow Coma Scale (GCS)[57] are other tools for clinical evaluation of neurotoxicity. Additional updated grading systems will be helpful to further delineate the CAR related neurotoxicity from other likely unrelated symptoms like headache.

Neurotoxicity management involves frequent neurological and early involvement of neurology and critical care experts. The treatment is mainly supportive in grade 1/2 neurotoxicity without significant CRS with close monitoring, aspiration precautions, EEG, and CNS imaging. Lumbar puncture is ideal for opening pressure measurement but not always feasible in delirious patients with coagulopathy from CAR T-cell related disseminated intravascular coagulation.

3.3.1 Anti-IL-6 therapy and glucocorticoids—Neurotoxicity may occur early concurrent with CRS (approximately day 1–7) and/or later independently of CRS.[12] Patients experiencing grade ≥1 neurotoxicity with concurrent grade ≥2 CRS may benefit from anti-IL-6 therapy [10]. In contrast, neurotoxicity occurring independently of CRS should not be managed with tocilizumab as anti-IL6 therapy does not cross the BBB and has not been associated with resolution of CAR T cell related encephalopathy [12].

Tocilizumab can cause a transient increase in IL-6 levels after the initial administration, whether its use for CRS can initiate or exacerbate the neurotoxicity is unknown [36]. In the report by Gust et al. the peak grade of neurotoxicity occurred after the first dose of tocilizumab in 67% of the patients, and in 8 of those patients, the first presentation of neurotoxicity occurred after tocilizumab had been administered for CRS [39]. It is unknown if siltuximab has any advantage over tocilizumab in the patients with neurotoxicity with CRS, and prospective study is warranted.

In patients with grade ≥2 neurotoxicity without CRS, initial treatment with steroid should be considered over anti-IL-6 therapy. Optimal dose, duration and choice of steroid agent remain to be standardized. Our preferred regimens for grade 2–3 neurotoxicity are dexamethasone 10 mg IV every 6 hours or methylprednisolone 1 mg/kg IV every 12 hours with rapid taper over 7–10 days depending on clinical improvement. In refractory cases with grade 4 neurotoxicity, high-dose steroid therapy with methylprednisolone IV 1 g/day for 3 days may be considered followed by a rapid taper. Close monitoring for recurrence of neurotoxicity is needed during steroid taper. Anti-IL-6 therapy is added if a patient develops concurrent CRS

as described above [42]. The median time to resolution of neurotoxicity was 4 days (range 1–64 days) in CD19 CAR T-cell treated adult patients with relapsed B-cell malignancies [39]. Neurotoxicity treatment response is generally slower than response to CRS symptoms. The published clinical experience with different CD19 CAR T-cell therapies so far suggests that there may not be a co-relation between the use of anti-IL-6 therapy and steroids and the efficacy of CAR T-cell therapy [6, 22, 43, 51].

Of note, in the clinical trials evaluating the use of tisa-cel in both pediatric and adult B-ALL (ELIANA trial) and adult DLBCL (JULIET trial), glucocorticoids were not mandated for the treatment of neurotoxicity, and management may have consisted of supportive care only [8, 19].

3.3.2 Anti-epileptics and supportive care—Seizure prophylaxis with levetiracetam 750 mg orally or intravenously every 12 h for the first 30 days is commonly used in patients receiving CAR T-cell therapy with known risk of neurotoxicity/CRS, especially with CD19 CAR T-cell with CD28 co-stimulatory construct and BCMA CAR T-cell therapy.[12, 58] CD19 CAR T-cells with a 4–1-BB construct may have a lower rate of neurotoxicity in pediatric patients and routine seizure prophylaxis may not be required [38]. Ideally, neurotoxicity grade≥3 should be monitored in intensive care unit (ICU) as many of these patients require mechanical ventilation for air-way protection and permissive hypercapnia for cerebral edema. Non-convulsive and convulsive status epilepticus should be managed with benzodiazepines and additional antiepileptics as needed. Levetiracetam and phenobarbital are commonly used antiepileptics, in refractory cases endotracheal intubation and anesthesia is required.

4. Prediction and Prevention of CAR T-cell therapy associated toxicities

Based on risk factors defined above, different groups have started studying various preventative strategies to reduce the incidence and severity of CRS and neurotoxicity from CAR T-cell therapy. For lymphoma the amount of disease burden corresponds to severe neurotoxicity rates: more disease leads to higher risk.[59] Debulking chemotherapy before CAR T-cell infusion may reduce antigenic exposure and could be pursued. We have used bridging chemotherapy or pulse steroids between lymphocyte apheresis and CAR T-cell infusion with the goal to reduce disease burden and improve patient’s functional status and it appear safe in our experience. Bridging chemotherapy was allowed and was used in almost all patients treated on several of the pivotal trials for lymphoma. However, the effect of bridging therapy on CAR T-therapy efficacy is unknown at this point. Other approaches under investigation are early biomarker driven treatment with anti-IL6 and/or steroids and self-inactivating CAR T-cells which can be turned off in the patients with life-threatening CRS or neurotoxicity.

4.1 Predictive biomarkers of CRS and neurotoxicity

Given the significant morbidity and mortality attributed to CRS and neurotoxicity, the development of predictive biomarkers in the serum and CSF is an active area of investigation. Elevated cytokines in serum before and during CRS and neurotoxicity provides the logical basis for the approach as they likely play a central role in pathogenesis

to CAR T-cell therapy related toxicities [6, 39, 48, 51, 60]. The goal is to develop a validated cytokine profile to predict the occurrence and severity of these complications with a goal of prevention and reduction in the severity of these complete dictations by early cytokine directed therapy. Gardner et al. have developed an early intervention protocol with tocilizumab ± dexamethasone, which appear to reduce the severity of CRS without affecting the efficacy of CD19 CAR T-cell therapy [61]. Locke et al. presented their interim analysis findings of a ZUMA-1 safety expansion cohort of patients who received a prophylactic dose of tocilizumab on day 2 post axi-cel infusion. Only one patient (3%) experienced ≥ 3 (grade 4) CRS; however incidence of ≥3 neurotoxicity was 41% compared to 28% in original ZUMA-1 cohort [6, 33]. In one reported death from cerebral edema, the patient had very high levels of serum IL-15,IL-8, TNF-β, CVAM 1, TFN-γ, IL-1RA, CCL17, and IP-10, consistent with a presence of activated myeloid and lymphoid cells on the day of axi-cel infusion [33]. Other studies are ongoing to further explore the role of prophylactic anti-IL-6 therapy to prevent or reduce the severity of CAR T-cell therapy associated toxicities (,). Preclinical models showed a central role of host monocyte derived IL-1, IL-6, and NO in CRS and neurotoxicity. Treatment with anakinra (anti-IL-1 mAb) successfully prevented both CRS and neurotoxicity in human leukemia xenograft models treated with CD19 CAR-T-cells.[16] A simple correlation between elevated cytokine and CRS/neurotoxicity will not be adequate if it fails to predict the occurrence ahead of severe symptoms. CRP and ferritin are associated with CRS, but both biomarkers fail to predict development of severe CRS [34]. The group from University of Pennsylvania has developed an cytokine profile by measuring IFN-γ, IL-13, and MIP1α concentrations within 72 hours after CD19 CAR T-cell infusion in pediatric patients with B-ALL, which has a sensitivity of 100% and a specificity of 96% in predicting CRS [34]. Hay et al. showed that elevated serum monocyte chemoattractant protein-1 (MCP-1) in patients with fever ≥38.9 °C within 36 hours of CAR T-cell infusion has better sensitivity and specificity to predict grade ≥4 CRS than CRP and ferritin (sensitivity- 100%; specificity-95%). Adding IL-6 levels ≥ 16 pg/mL in the first 36 h after CAR T-cell infusion in this algorithm, it can predict grade ≥4 neurotoxicity with sensitivity of 100% and specificity of 94% [39, 48]. Gust et al. showed earlier peak of the IL-6 serum concentration was associated with a higher risk of grade ≥4 neurotoxicity [39]. It is important to note here that tocilizumab and siltuximab can interfere with the measurement of serum IL-6 and soluble IL-6R [62]. In ZUMA-1, patients developing grade ≥ 3 neurotoxicity had elevated IL-15 (P=0.0006) and decreased perforin (P=0.001) on day 0 before CAR T infusion and increased IL-15, MCP-1, and IL-6 on day 1 after [40]. Severe neurotoxicity correlated with higher peak concentration of C-reactive protein, ferritin, and multiple cytokines including IL-6, IFN-γ, and TNF alpha [39]. In the ZUMA-1 trial, elevated IL-2, GM–CSF, and ferritin were associated with neurological events without CRS [6]. In a Memorial Sloan Kettering Cancer Center (MSKCC) cohort of B-ALL patients treated with CD19 CAR, elevated IL-2 and IL-5 at day 3 were unique to neurotoxicity [63]. Measurement of endothelial activation biomarkers such as VWF, Ang-2 before and after CAR T-cell infusion can potentially be explored to predict permeability of the BBB to predict neurotoxicity (16). The MSKCC group has shown baseline clinical characteristics associated with severe neurotoxicity. Baseline platelet count <60 or mean corpuscular hemoglobin concentration>33.2% and morphologic disease (>5% blasts) predicted severe neurotoxicity with 95% sensitivity and 70% specificity [63]. Consumptive coagulopathy

with prolonged PT, aPTT, elevated D-dimer, and hyperfibrinogenemia was associated with grade ≥4 CRS [48].

4.2 Designing safer CAR T-cells

The balance between anti-tumor effect and toxicity reduction may be hard to achieve as most approaches to prevent CRS/neurotoxicity can potentially reduce CAR-T-cell proliferation and persistence, both of which are key to successful tumor elimination.[64] Pre-clinical studies have shown that optimizing the binding affinity of scFv in the CAR can improve anti-tumor activity and reduce associated toxicities. Excessive target affinity can lead to early exhaustion, poor persistence of CAR-T-cell, and increased toxicity.[65–68] Park et al. showed that ICAM-1 avid CAR-T-cells with micromolar affinity have improved efficacy and safety compared to the ones with nanomolar affinity.[66] Rapid advances in gene editing technology such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have made it possible to generate new CAR constructs with the goal to increase the efficacy and safety of the CAR T-cell product. The MSKCC group has developed a CD19-specific CAR inserted next to the T-cell receptor α constant (TRAC) locus using the CRISPER platform, which resulted in uniform CAR expression and enhanced potency of CAR T-cells [69]. This may allow a reduced dose of CAR T-cells with a goal to reduce the toxicity. Though, these findings are intriguing, gene editing is associated with a number of safety concerns, mainly insertional mutagenesis and acquired defects in DNA repair function[70].

Other modalities currently under investigation include the insertion of genetic constructs within CAR to selectively turn-off CAR T-cells when serious toxicity develops [71–75]. Tasian et al. from University of Pennsylvania developed anti-CD123-4-1BB-CD3ζ T-cells with co-expression of CD20. In pre-clinical models, it allowed successful eradication of CD123 acute myeloid leukemia cells with subsequent elimination of CAR T-cells with rituximab [74]. Similarly, Minagawa et al. showed that insertion of caspase9 allowed selective apoptosis of CAR T-cells with the administration of a non-therapeutic dimerizer, which activated the suicide gene [73]. Eliminating CAR-T-cells to control short-term toxicities will invariably compromise tumor control when long-term persistence of CAR-T-cell is important. This issue might be circumvented by insertion of an inducible gene-regulatory system which enables controlled expression of CARs upon drug administration. Sakemura et al. developed CD19 CAR with a tetracycline regulation system (Tet-on), which allowed controlled activation of CAR T-cells only in the presence of doxycycline. This approach allowed to turn the CAR T-cell “On” and “Off” by doxycycline administration [76]. Ma at al. showed that CAR T-cell activity can be controlled by soluble intermediary “switch” molecules. Modified CAR T-cells are dependent on these switch molecules to form a ternary complex between the CAR T-cell, switch, and tumor associated antigen. The theoretical advantage of this approach is that the activity of CAR T-cell could be titrated by adjusting the concentration of the switch molecule instead of completely turning it “On” or “Off”. These CAR T-cells could also be re-directed towards different tumor associated antigens based on specificity of the switch molecules, which can ultimately help to treat the disease relapse due to ‘antigen-escape’ [77]. At the same time, immunogenicity of these switch molecules and slower CAR-T-cell inactivation compared to the CAR-T-cells with a

suicide gene limits the usefulness of this approach in the setting of acute toxicity. Self-activating CAR-T-cells have been generated by fusing the oxygen sensing domain of hypoxia-inducing factor-1α (HIF-1α), which is only active in a hypoxic environment, commonly found in neoplastic tissue. These CAR-T-cells became inactive in normal tissue in the presence of normoxia and avoided off-target toxicities.[78] Roybal et al. constructed 'AND-gate' CAR T-cells using a synNotch receptor which required two antigen engagements to get activated. In vivo, T-cells engineered with dual-receptor circuits recognizing combinations of antigens can efficiently kill target tumor cells, while sparing bystander cells.[79] Giavridis et al. recently showed that the CAR-T-cell engineered to produce endogenous IL-1 receptor antagonist successfully prevented CRS without anti-leukemia efficacy in a human leukemia mouse model.[15] All these approaches are still in their infancy and will require robust clinical validation.

5. CONCLUSIONS

CAR T-cell therapy is the latest advance in cancer immunotherapy with promising results in various hematological malignancies. CAR T-cell therapy related toxicities, mainly CRS and neurotoxicity, remain major hurdles before its wide-spread use. Efforts are underway to understand host-tumor-CAR-T-cell interactions, which will lead to a better understanding of the pathophysiologies behind CRS and neurotoxicity. Timely diagnosis and multi-disciplinary management are the corner stones for optimal outcomes. Development of toxicity grading scales and protocol-based management are important advances, however, newer strategies are urgently needed to predict CRS/neurotoxicity and salvage those patients who are refractory to anti-IL-6 therapy and steroids. There is significant heterogeneity between the different CAR T-cell therapies (lymphodepleting chemotherapy, dose of CAR T-cell, co-stimulatory molecules, tumor types etc.), grading scales, and attributions of toxicities, which prevents generalization of CRS and neurotoxicity management protocols across disease types. Multi-institutional collaborations and standardized diagnosis and grading criteria are needed. Predictive biomarkers and next-generation CAR T-cells will guide the future treatment strategies of CAR T-cell associated toxicities. In the future, we may have the luxury select a particular CAR-T-cell construct based on the patient and disease characteristics to maximize efficacy and safety.

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Key Points

- Cytokine release syndrome (CRS) and neurotoxicity are two important chimeric antigen receptor T-cell (CAR-T) therapy associated toxicities.
- Incidence and severity of CRS and neurotoxicity varies by CAR-T-cell product, underlying malignancy, and patient characteristics.
- Tocilizumab (anti-IL-6 receptor antibody) and steroids are the current mainstays of CAR-T-cell therapy associated toxicities.
- Further study of the pathophysiology behind CAR-T-cell therapy associated toxicities will provide new insights into prevention and management strategies.

Table 1:

Incidence and treatment of CAR T related cytokine release syndrome and neurotoxicity in published clinical trials.

Study	CAR-T-cell construct	Diseases	Number of patients	Rate of cytokine release syndrome	Grad e III-IV cytokine release syndrome	Death from cytokine release syndrome	Rate of neurotoxicity	Grade III-IV neurotoxicity	Death from neurotoxicity	Treatment (CRS+ neurotoxicity)
Borchmann, et al. (2018)(JULIET) [8]	CD19-4-1BB (tisagenlecleucel;CTL019)	DLBCL	111	57 (58%)	23 (23%)	0	23 (21%)	12 (12%)	0	Tocilizumab-15%, Glucocorticoids-11%
Maude, et al (2018) (ELIANA)[19]	CD19-4-1BB	B-ALL	75	58 (77%)	35 (46%)	1 (1%)	30 (40%)	10 (13%)	0	
Neelapu et al. (2017)(ZUMA-1)[6]	CD19–28-ζ (axicabtagene ciloleucel; axi-cel,KTE-C19)	DLB CL	101	94 (93%)	13 (13%)	2 (2%)	65 (64%)	28 (28%)	0	Glucocorticoids-27%, Tocilizumab-43%
Schuster et al. (2017) (Novartis/ U.Penn)[7]	CD19-4-1BB	DLB CL	28	16 (57%)	5 (18%)	0	11 (39%)	3 (11%)	1 (4%)	Glucocorticoids-0%, Tocilizumab-4%
Abramson, et al. (2018) (TRANSCEND NHL 001)[45]	CD19-4-1BB (lisocabtagene maraleucel; liso-cell; JCAR017)	DLB CL	102	38 (37%)	1 (1%)	0	23 (23%)	13 (13%)	0	Glucocorticoids-21%, Tocilizumab-17%
Park et al. (2017)(MSKCC)[43]	CD19–28-ζ	B-ALL	53	45 (85%)	14 (26%)	1 (2%)	23 (43%)	22 (42%)	0	Glucocorticoids-7.5%, Tocilizumab-11%, Glucocorticoids+ Tocilizumab-25%
Maude et al. (2014) (U.Penn)[20]	CD19-4-1BB	B-ALL	30	30 (100 %)	8 (27%)	0	13 (43%)	N/A	0	Glucocorticoids-20%, Tocilizumab-30%
Ghorashian S et al. (2017)(NHS) [64]	CD19-4-1BB (low affinity)	B-ALL	8	8 (100 %)	0	0	3 (38%)	0	0	Tocilizumab-0%
Qasim et al. (2017) (NHS)[75]	CD19-4-1BB with RQR8 safety switch (allogeneic CAR-T)	B-ALL	5	5 (100 %)	1 (20%)	0	2 (40%)	0	0	Tocilizumab-20%
Gust et al. (2017) (Juno)[39,51]	CD19-4-1BB	B-ALL, NHL, CLL	133	93 (70%)	16 (12%)	5 (4%) *	53 (40%)	28 (21%)	4 (3%)	Glucocorticoids-11%, Tocilizumab-2%, Glucocorticoids+ Tocilizumab-38%
DeAngelo et al. (2017) (ROCKET) [40]	CD19–28-ζ (JCAR015)	B-ALL	38	NA	8 (21%)	0	N/A	20 (52%)	5 (13%)	NA
Cohen et al. (2017) (U.penn)[58]	BCMA-4–1BB	MM	21	18 (86%)	6 (29%)	0	3 (14%)	1 (5%)	0	Tocilizumab-19%
Raje et al. (2018)[55]	BCMA-4–1BB	MM	43	27 (63%)	2 (5%)	0	14 (33%)	1 (2%)	0	Glucocorticoids-9%, Tocilizumab-21%

* Combined deaths from CRS and neurotoxicity

Abbreviations: DLBCL- Diffuse Large B-cell Lymphoma; B-ALL- B-cell Acute Lymphoblastic Lymphoma; CLL- Chronic Lymphocytic Leukemia; MM- Multiple Myeloma, N/A- Not available, NHL- non-hodgkin lymphoma

Table 2:

Summary of cytokine release syndrome grading systems.

CRS Grading	CTCAE v4[23]	CTCAE v5[24]	Lee, et al. [14]	Porter, et al.[26]
Grade 1	Mild reaction; infusion interruption not indicated; intervention not indicated	Fever with or without constitutional symptoms	Symptoms are not life threatening and require symptomatic treatment only, eg, fever, nausea, fatigue, headache, myalgias, malaise	Mild reaction: Treated with supportive care such as anti-pyretics, anti-emetics
Grade 2	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours	Hypotension responding to fluids; hypoxia responding to <40% O2	Symptoms require and respond to moderate intervention Oxygen requirement <40% or Hypotension responsive to fluids or low dose of one vasopressor or Grade 2 organ toxicity	Moderate: Requiring IV therapies or parenteral nutrition; some signs of organ dysfunction (i.e. gr 2 Cr or gr 3 LFTs) related to CRS and not attributable to any other condition. Hospitalization for management of CRS related symptoms including fevers with associated neutropenia
Grade 3	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Hypotension managed with one pressor; hypoxia requiring ≥ 40% O2	Symptoms require and respond to aggressive intervention Oxygen requirement ≥40% or Hypotension requiring high dose or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis	More severe reaction: Hospitalization required for management of symptoms related to organ dysfunction including gr 4 LFTs or gr 3 Cr related to CRS and not attributable to any other conditions; excludes management of fever or myalgias. Includes hypotension treated with intravenous fluids or low-dose pressors, coagulopathy requiring FFP or cryoprecipitate, and hypoxia requiring supplemental O2 (nasal cannula oxygen, high flow O2, CPAP or BiPAP). Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS.
Grade 4	Life-threatening consequences; pressor or ventilatory support indicated	Life-threatening consequences; urgent intervention indicated	Life-threatening symptoms Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)	Life-threatening complications such as hypotension requiring “high dose pressors”, hypoxia requiring mechanical ventilation.

Abbreviations: NSAIDS: non-steroidal anti-inflammatory drugs, LFTs: liver function tests, CRS: cytokine release syndrome, Cr: creatinine, FFP: fresh frozen plasma, CPAP: continuous positive airway pressure, BiPAP: bilevel positive airway pressure

Table 3:

Comparison of different grading systems for cytokine release syndrome.

Clinical parameter	CTCAE v5[24]	Lee, et al. [14]	Porter, et al.[26]
Constitutional Symptoms	Grade 1	Grade 1	Grade 1
Hypotension Responsive to Fluids	Grade 2	Grade 2	Grade 3
<40% FiO2	Grade 2	Grade 2	Grade 3
High Dose Vasopressor requirement	Grade 3	Grade 3	Grade 4
Ventilator Support	Grade 4	Grade 4	Grade 4

Table 4:

Grading of CAR T cell-related neurotoxicity per CARTOX group[+]

Symptom or sign	Grade 1	Grade 2	Grade 3	Grade 4
Neurological assessment score (byCARTOX-10)	7–9 (mild impairment)	3–6 (moderate impairment)	0–2 (severe impairment)	Patient in critical condition, and/or obtunded and cannot perform assessment of tasks
Raised intracranial pressure	NA	NA	Stage 1–2 papilledema[*], or CSF opening pressure <20 mmHg	Stage 3–5 papilledema‡, or CSF opening pressure >20 mmHg, or cerebral edema
Seizures or motor weakness	NA	NA	Partial seizure, or non-convulsive seizures on EEG with response to benzodiazepine	Generalized seizures, or convulsive or non-convulsive status epilepticus, or new motor weakness

Abbreviations: CAR, chimeric antigen receptor; CARTOX−10, CAR−T−cell-therapy-associated toxicity 10−point neurological assessment CSF, cerebrospinal fluid; EEG, electroencephalogram; NA, not applicable.

[*] Papilledema grading is performed according to the modified Frisén scale.

[+] reprinted with permission from Neelapu et al. Nat Rev Clin Oncol.2017 [12]