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# Chimeric Antigen Receptor T Cell-Related Neurotoxicity: Mechanisms, Clinical Presentation, and Approach to Treatment

*Jessica Rice, MD*[1]
*Sarah Nagle, MD*[2]
*Julie Randall, BA*[3]
*Holly E. Hinson, MD MCR*[1,*]

**Address**
[*,1]Department of Neurology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, CR-127, Portland, OR, 97239, USA
Email: hinson@ohsu.edu
[2]Department of Medicine, Oregon Health & Science University, Portland, OR, USA
[3]Portland State University, Portland, OR, USA

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## Abstract

*Purpose of review* Chimeric antigen receptor T cell (CAR-T) adoptive cell therapy is an effective treatment for patients with refractory B cell malignancies. As its use has grown, there has been an increase in the incidence of a serious, potentially fatal neurotoxicity known as immune effector cell-associated neurotoxicity syndrome (ICANS). This review discusses the clinical manifestations of this neurotoxicity syndrome, current grading systems, management strategies, and proposed biologic mechanisms leading to neurotoxicity.
*Recent findings* Current research suggests that patients with a higher disease burden and higher CAR-T cell doses are positively associated with the development of ICANS, as are elevated serum levels of proinflammatory cytokines and the presence of cytokine release syndrome (CRS). While patterns observed on neuroimaging and electroencephalogram (EEG) are non-specific for the diagnosis of ICANS, each modality may provide helpful clinical information such as the detection of cerebral edema, the most serious of associated symptoms. Anti-epileptic medications and corticosteroids may ameliorate the symptoms of ICANS.

*Summary* The mechanism for ICANS is currently unknown; however, systemic inflammation and cytokine production triggering a cascade of endothelial activation and BBB disruption likely contribute. With limited treatment options available, further clinical research into the precise mechanism and treatment is urgently needed as the use of CAR-T and other adoptive cell therapies continues to grow.

## Introduction

Immunotherapy is emerging as a promising new cancer treatment modality, beyond conventional chemotherapy and radiation therapy. Chimeric antigen receptor T (CAR-T) cell therapy is one such adoptive cell transfer that utilizes the patient's own T cells to target and destroy tumor cells. Cluster of differentiation (CD) 19-specific CAR-T therapy has been particularly effective at treating refractory B cell malignancies. In 2017, the FDA approved the first two formulations of CD19-specific CAR-T therapy: tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta). Both are approved to treat relapsed/refractory diffuse large B cell lymphoma in adults. Additionally, tisagenlecleucel is approved to treat refractory B cell acute lymphoblastic leukemia (B-ALL) in pediatric and young adult patients [1•, 2•]. CAR-T cell therapy may be offered in cases refractory to conventional chemotherapy, with current commercial products resulting in high rates of prolonged remissions and potential cure in 40–60% of patients [1•, 2•, 3–9]. However, this efficacy may come at a cost, as serious neurotoxicities can occur in up to 87% [10] of patients depending on the disease and the product [1•, 2•, 3–9, 11••, 12•]. Symptoms vary from delirium, headache, aphasia, seizures, and cerebral edema. In rare circumstances, it can be fatal [12•, 13]. Treatment options are primarily limited to supportive care and corticosteroids. Known as "immune effector cell-associated neurotoxicity syndrome" or ICANS, this neurotoxicity syndrome is thought to be caused by systemic inflammation causing blood-brain barrier breakdown (BBBB), though its exact mechanism is under investigation. As the use of adoptive cell transfer therapies expands, it will be critical for neurologists to recognize ICANS in a timely manner in order to manage this potentially life-threating syndrome. In this review, we summarize the clinical manifestations of ICANS, recommendations on its management, and the available evidence on the underlying mechanism(s) of ICANS.

# CAR-T cell therapy

## Physiology

CD19 is a marker specific to B cells and is found on all normal B cells as well as the majority of malignant B cells found in B-ALL, chronic lymphocytic leukemia (CLL), and B cell non-Hodgkin lymphoma [14]. The production of CAR-T cells is a multistep process. First T cells are taken from the patient via apheresis. Subsequently, through adoptive cell transfer, a gene that encodes for a CD19-specific chimeric antigen receptor (CAR) is added to the T cells via viral transduction [14, 15]. Intracellularly, the CAR contains a costimulatory signaling domain that is important for anti-tumor activity, as well as CAR-T cell proliferation and persistence. Once the CAR-T cells have been manufactured, the patient undergoes a lymphodepleting chemotherapy regimen followed by a single infusion of the modified T cells. Once infused, CAR-T cells travel to sites of tumors where they identify and kill tumor cells. This process can initiate rapid proliferation of the CAR-T cells and recruitment of other components of

the immune system for tumor killing [15].

### Cytokine release syndrome (CRS)

The most common adverse event after CAR-T infusion is cytokine release syndrome (CRS), occurring in 58–94% of patients receiving CD19 CAR-T therapy, depending on the product [5, 16]. CRS results from a large release of cytokines into the peripheral circulation from activated CAR-T cells and the subsequent systemic inflammatory response [17] and occurs early (typically 48 h) after the CAR-T infusion. CRS can be accompanied by some neurologic symptoms such as confusion, agitation, and/or delirium. Despite this, CRS and ICANS are recognized as separate CAR-T-related toxicities given their differences in timing and treatment. However, CRS may still be implicated in the development of neurotoxicity. Several studies have shown that the majority of patients who develop ICANS after CAR-T infusion experienced at least grade 1 CRS with fever preceding the onset of neurologic symptoms [1•, 2•, 11••, 12•]. Additionally, investigators have found a strong correlation between severe ICANS and severe CRS [1•, 2•, 11••, 12•]. However, patients with ICANS who receive tocilizumab, an anti-IL-6 receptor monoclonal antibody used to treat CRS, have not shown improvement in neurologic symptoms [1•, 2•, 11••, 12•]. The limited response of ICANS to tocilizumab may indicate that targeting IL-6 alone is insufficient to treat or prevent neurotoxicity, likely due to the complex mechanism of ICANS. While it seems earlier and higher elevation of cytokines characteristic of CRS may play a role in the development of ICANS, it is evident that CRS alone does not lead to the development of neurotoxicity.

## Clinical manifestations of CAR-T cell neurotoxicity or ICANS

### Risk factors

ICANS appears to be positively associated with pre-treatment disease burden, in vivo CAR-T cell expansion [11••, 12•], severe cytokine release syndrome (CRS) [12•], and CAR-T cell dose [6, 12•, 18]. It is also dependent on the product, as patients who receive axicabtagene ciloleucel have an increased risk of neurotoxicity as compared with those who receive tisagenlecleucel [19]. Based on the autopsies of patients with fatal neurotoxicity, Gust et al. developed a classification model that suggested that fever, high serum IL-6, and high MCP1 concentrations within the first 36 h following CAR-T cell infusion indicated a higher risk of severe neurotoxicity [12•]. While such a model will require prospective validation, these observations may help with the early identification of patients for closer monitoring.

### Clinical signs and symptoms

The timing of onset, duration, and symptoms of ICANS vary. The median onset of ICANS is about 4–5 days [2•, 11••, 12•] after CAR-T cell infusion, while the average duration is approximately 5 days [12•]. In another study, the median time from the onset of neurotoxicity to the patient's highest neurotoxicity grade was 1 day [12•]. There is typically near complete resolution of symptoms within 1 month after infusion [1•, 11••, 12•]. The neurotoxicity associated with CAR-T cell therapy comprises a range of neurologic signs and symptoms,

**Table 1.** Published neurotoxicity grading systems. *ADL* activities of daily living, *CSF* cerebrospinal fluid, *EEG* electroencephalography. Reprinted from [19], with permission from Elsevier

| Grading system | Adverse event term/neurotoxicity domain | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|
| Common Terminology Criteria for Adverse Events v5.0 | Encephalopathy | Mild symptoms | Moderate symptoms, limiting instrumental ADL | Severe symptoms, limiting self-care ADL | Life-threatening consequences, urgent intervention indicated |
| | Seizure | Brief partial seizure and no loss of consciousness | Brief generalized seizure | New-onset seizures (partial or generalized), multiple seizures despite medical intervention | Life-threatening consequences |
| | Dysphasia | Awareness of receptive or expressive characteristics, not impairing ability to communicate | Moderate receptive or expressive characteristics, impairing ability to communicate spontaneously | Severe receptive or expressive characteristics, impairing ability to read, write, and communicate intelligibly | |
| | Tremor | Mild symptoms | Moderate symptoms, limiting instrumental ADL | Severe symptoms, limiting self-care ADL | |
| | Headache | Mild pain | Moderate pain, limiting instrumental ADL | Severe pain, limiting self-care ADL | |
| | Confusion | Mild disorientation | Moderate disorientation, limiting instrumental ADL | Severe disorientation, limiting self-care ADL | Life-threatening consequences, urgent intervention indicated |
| | Depressed level of consciousness | Decreased level of alertness | Sedation and slow response to stimuli, limiting instrumental ADL | Difficult to arouse | Life-threatening consequences and coma, urgent intervention indicated |
| | Cerebral edema | | | New onset, worsening from baseline | Life-threatening consequences, urgent intervention indicated |

most of which are non-specific. The most common and earliest symptoms are delirium or confusion, manifesting as transient impairment of cognition and/or attention, occurring in several trials [1•, 2•, 4, 7–9, 11••, 12•]. Delirium was reported in up to 66% [12•] of those patients with symptoms of neurotoxicity. Headache was also common, in up to 55% [12•] of patients with ICANS. Expressive aphasia, characterized by impaired naming and paraphasic errors, was also seen in up to 34%. Aphasia was often associated with delirium [12•] and impaired handwriting. Interestingly, expressive aphasia was noted to be the most characteristic feature of severe toxicity in one study, occurring in 21 of 22 patients with severe neurotoxicity, and was the first neurologic symptom in 19 of 22 patients [11••]. In these patients, expressive aphasia evolved to global aphasia over only a matter of hours. Other symptoms include decreased level of consciousness/somnolence (up to 25% [12•]), tremor (up to 29% [9]), seizures in up to 8%, and cerebral edema, with infrequent reports of focal neurologic deficits, ataxia, abnormal movements [12•], myoclonus, apraxia, frontal release signs, dysarthria, and facial automatisms [11••].

Initially, Neelapu et al. developed a neurotoxicity grading scale that incorporated clinical symptoms, the CARTOX 10. This 10-point scale was a neurological assessment of mental status and signs of more severe neurotoxicity such as increased intracranial pressure, seizures, and focal motor weakness. Grade 1 is defined as mild neurotoxicity, grade 2 is moderate, and grades 3–4 are severe neurotoxicity [20••]. Santomasso et al. observed that patients with mild neurotoxicity (11 of 53) most commonly display mild encephalopathy with disorientation, impaired attention or short-term memory with preserved alertness, and the ability to name and follow simple commands. Those with severe toxicity began as mild somnolence and expressive aphasia, but quickly progressed to global aphasia, myoclonus, somnolence/decreased level of consciousness, seizures, and cerebral edema [11••]. Recently, a group of experts assembled at a meeting sponsored by the American Society for Blood and Marrow Transplantation developed a set of consensus recommendations in an effort to standardize the grading scales and symptoms associated with CAR-T neurotoxicity and to make them applicable to other immune effector cell therapies. Among them was a proposal to replace the CARTOX 10 scoring scale with the Immune Effector Cell-Associated Encephalopathy (ICE) scale [19] (see Tables 1 and 2).

## Seizures and electroencephalography (EEG) findings

The frequency of seizures is variable. In Santomasso et al., of the 22 patients who experienced severe neurotoxicity (grades 3–4), 16 (72%) had at least one seizure. Most of the seizures were generalized tonic-clonic in nature, but 2 of 16 patients were found to be in non-convulsive status epilepticus (NCSE). Of note, both cases of NCSE were preceded by a clinical seizure [11••]. Other centers have noted NCSE in about 10% of patients treated with CAR-T cell therapy. Generally, seizures responded to first-line benzodiazepines and anti-epileptic drug (AED) titration. The most common pattern on EEG is diffuse slowing and frontal intermittent rhythmic delta activity (FIRDA), consistent with encephalopathy. FIRDA and generalized background slowing on EEG are both non-specific signs of diffuse cerebral dysfunction and can be caused by metabolic or toxic encephalopathy, central nervous system (CNS) infections, sedating

centrally acting medications, or neurodegenerative processes [21]. In a case series of patients with ICANS monitored with EEG, four patients developed generalized periodic discharges (GPD) that were not significantly improved by AED initiation or titration [22]. All patients in the series received 10 mg IV dexamethasone every 12 h and showed sustained overall clinical improvement. See below for the discussion of the use of steroids for treatment of ICANS.

### Cerebral edema and neuroimaging features

Cerebral edema is the most serious of the ICANS complications, and it can develop over a matter of hours. Cerebral edema has been reported in multiple clinical trials [8, 12•, 13, 23]. In the phase II ROCKET study, a clinical trial of CD19 CAR-T cell therapy in adults with ALL, five participants died of cerebral edema and severe neurotoxicity, leading to discontinuation of the study [13]. In another recent study [12•], four out of 133 participants died of neurotoxicity: 2 died from acute cerebral edema, 1 from brainstem hemorrhage and edema associated with disseminated intravascular coagulation (DIC), and 1 from cortical laminar necrosis with a minimally conscious state. MRI of patients with mild ICANS is often normal [5, 6, 11••, 12•]. Some patients with moderate to severe neurotoxicity also have normal brain MRI [11••], while others display patchy T2 hyperintensities throughout the white matter or T2 hyperintensities in the bilateral thalami and deep gray matter [11••, 12•]. Only about 30% of patients with ICANS had observable MRI abnormalities [12•]. A number of other abnormalities have been reported in ICANS patients including leptomeningeal enhancement, multifocal microhemorrhages, extensive cortical diffusion restriction indicative of cytotoxic edema [12•], and diffusion restriction in the splenium of the corpus callosum [11••]. In summary, no classic MRI pattern can easily be described in ICANS for the minority of patients who do have imaging abnormalities.

## Management of CAR-T neurotoxicity

The best approach to the management and treatment of ICANS is unknown, as no disease-modifying clinical trials have been completed to date. Most efforts have focused on symptom management, specifically to address cerebral edema and seizures. While grade 1 neurotoxicity may only require supportive care, ICANS can progress quickly and therefore requires close attention and may need rapid intervention [24]. For patients with severe (grade 3–4) neurotoxicity, monitoring in the ICU is recommended [20••].

### Cerebral edema

Neelapu et al. recommends a fundoscopic exam to rule out papilledema for all ICANS patients. When possible, lumbar puncture can allow for direct measure of intracerebral pressure and rule out intracranial infection via cerebrospinal fluid analysis. Neuroimaging, preferably with MRI, can help detect cerebral edema as well as rule out other acute intracranial abnormalities such as stroke [20••]. Hyperosmolar therapy with mannitol or hypertonic saline may be used to lower increased cranial pressure in symptomatic patients. Patients with cerebral edema are likely to benefit from intensive care unit admission, as

frequent neurologic checks may detect early or subtle changes in the exam that may prompt intervention [20••, 24, 25].

## Seizures

Prophylactic anti-epileptic drugs are frequently prescribed, but not universally [20••, 26]. Levetiracetam (Keppra) is the most common seizure prophylaxis used because of its favorable safety and drug-drug interaction profile, but the ideal dose and duration are unknown [20••, 25]. To treat seizures or status epilepticus, benzodiazepines remain the first-line therapy [20••] followed by a loading dose and maintenance of an appropriate anti-epileptic drug [11••]. Non-convulsive status epilepticus should be considered in patients whose mental status does not return to baseline. Long-term EEG should be considered for patients with waxing and waning mental status or in those patients who remain altered without a clear, compelling explanation.

**Table 2. ASBMT ICANS consensus grading for adults. ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as having grade 3 ICANS**

| Neurotoxicity domain | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| ICE score* | 7–9 | 3–6 | 0–2 | 0 (patient is unarousable and unable to perform ICE) |
| Depressed level of consciousness† | Awakens spontaneously | Awakens to voice | Awakens only to tactile stimulus | Patient is unarousable and requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma |
| Seizure | N/A | N/A | Any clinical seizure focal or generalized that resolves rapidly or non-convulsive seizures on EEG that resolve with intervention | Life-threatening prolonged seizure (> 5 min) or repetitive clinical or electrical seizures without return to baseline in between |
| Motor findings‡ | N/A | N/A | N/A | Deep focal motor weakness such as hemiparesis or paraparesis |
| Elevated ICP/cerebral edema | N/A | N/A | Focal/local edema on neuroimaging§ | Diffuse cerebral edema on neuroimaging, decerebrate or decorticate posturing, cranial nerve VI palsy, papilledema, or Cushing's triad |

*A patient with an ICE score of 0 may be classified as having grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as having ICANS if unarousable
†Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication)
‡Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading
§Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.
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### Disease modification

Corticosteroids are known to decrease inflammatory responses and have been used successfully for the treatment of neurotoxicity [11••, 12•, 20••, 24]. Dexamethasone is typically used for grades 1–3 whereas high-dose methylprednisolone is reserved for grade 4 ICANS [25]. However, corticosteroids affect T cell function [27] and therefore may reduce circulating CAR-T cell counts and decrease the efficacy of CAR-T cell therapy [25]. Fortunately, thus far, studies have not found that short courses of corticosteroids negatively affect the efficacy of CAR-T cell therapy [28]. However, given the theoretical risks as well as the toxicities of high-dose steroids (i.e., steroid myopathy, delirium, and opportunistic infections), brief courses are preferred [28]. Tocilizumab is effective for the treatment of CRS, but its use for the treatment of ICANS not associated with CRS is likely inadequate. Although limited to phase I and II clinical trials, the data thus far suggests tocilizumab to be of limited efficacy in ICANS [11••, 12•, 23, 28]. Furthermore, tocilizumab does not penetrate the CNS and some have suggested that it may increase CSF IL-6 levels and worsen neurotoxicity [12•]. Siltuximab, another anti-IL-6 monoclonal antibody, may be preferred for the treatment of neurotoxicity associated with CRS as it does not increase serum IL-6 levels [12•], but further investigations are needed. Another future treatment consideration is anakinra, a recombinant IL-1 receptor antagonist, which is currently used for the treatment of adult rheumatoid arthritis and systemic juvenile idiopathic arthritis [25, 29]. In a murine model of CAR-T-associated neurotoxicity, anakinra improved both rodent CRS and ICANS when given at the time of CAR-T cell infusion [30]. Rodent CRS was defined by serum IL-6 levels, fever, and weight loss, while rodent ICANS was characterized by meningeal inflammation and progressive lethal disease. Finally, other approaches such as plasma exchange, angiopeietin-1 augmentation, or platelet transfusion have been proposed to treat ICANS, but no studies have been performed in humans and no data supports their efficacy [25].

## Proposed mechanism of neurotoxicity

While the precise pathophysiology of CAR-T cell neurotoxicity remains unclear, some investigators believe it to be driven by systemic inflammation and cytokine production, initiating a cascade of endothelial activation, coagulopathy, and blood-brain barrier (BBB) disruption, with subsequent elevation of CSF cytokines, and, in severe cases, pan-encephalitis, hemorrhage, and cerebral edema [11••, 12•, 17, 31–33].

### Markers of systemic inflammation

Patients who develop severe ICANS after CAR-T cell infusion demonstrate earlier and higher fevers, significantly higher peak concentration of C-reactive protein (CRP), and higher early ferritin levels [1•, 2•, 11••, 12•], indicative of a systemic inflammation. Additionally, patients with severe neurotoxicity had higher levels of several proinflammatory cytokines in

the serum, such as IL-2, IL-15 [1•, 2•, 5, 11••, 12•], IL-10 [1•, 2•, 5, 11••], as well as cytokines known to activate endothelial cells [23, 24], such as IL-6 [1•, 2•, 5, 11••, 12•], IFNγ [1•, 2•, 11••, 12•], and TNFα [2•, 5, 12•]. There appears to be a correlation between early (< 6 days post-infusion) peak IL-6 serum concentration and the subsequent development of severe neurotoxicity [2•, 12•]. While these cytokines are non-specific and are also elevated in CRS, this evidence suggests that a more rapid rise in serum cytokine concentration and higher peak serum cytokine concentrations may influence the severity of ICANS, possibly via endothelial cell activation. Clinical observations support the idea that early development of systemic inflammation is a critical step in the pathogenesis of ICANS.

## Endothelial cell activation and coagulopathy

In patients with severe ICANS, endothelial cell dysfunction likely leads to vascular leak and coagulopathy. First, patients with both severe ICANS and CRS showed evidence of endothelial cell dysregulation via disruption of the angiopoietin (ANG)-TIE2 (a protein tyrosine kinase) axis, which controls the balance between endothelial cell activation and quiescence [11••, 12•, 16]. When ANG1 binds to the TIE2 receptor, it stabilizes the endothelial cells of the BBB, maintaining quiescence. Alternatively, ANG2 is a TIE2 antagonist stored in endothelial cells until inflammatory cytokines and other stimuli trigger endothelial cell activation. Once released, ANG2 displaces ANG1, leading to increased permeability of the BBB [11••, 12•]. Studies have found higher serum concentrations of ANG2 [12•], lower serum concentrations of ANG1 [11••], and higher ANG2 to ANG1 ratios [11••, 12•] in patients with severe neurotoxicity, all supporting the idea that endothelial cell activation and dysfunction play a role in the pathogenesis of ICANS (Fig. 1).

Next, patients with severe neurotoxicity demonstrate a higher incidence of a consumptive coagulopathy [3, 12•]. There is a correlation between severe ICANS and a higher frequency of laboratory markers of disseminated intravascular coagulation, including prolonged thrombocytopenia, higher prothrombin time, activated partial thromboplastin time, and d-dimer [11••, 12•]. Similarly, von Willebrand factor (vWF) is a protein involved in hemostasis and, like ANG2, is released into serum upon endothelial cell activation. Gust et al. found that those with severe neurotoxicity demonstrated very high concentrations of vWF in the serum, sometimes 4- to 5-fold higher than healthy donors [12•], further evidence of coagulopathy occurring during ICANS. Finally, the autopsy of 2 patients who died of severe neurotoxicity showed intravascular vWF binding and CD61+ platelet microthrombi consistent with endothelial activation, as well as vascular wall destruction and multifocal hemorrhage [12•]. Thus, endothelial cell activation and subsequent vascular leak and coagulopathy likely contribute to the neurotoxicity seen after CAR-T cell treatment.

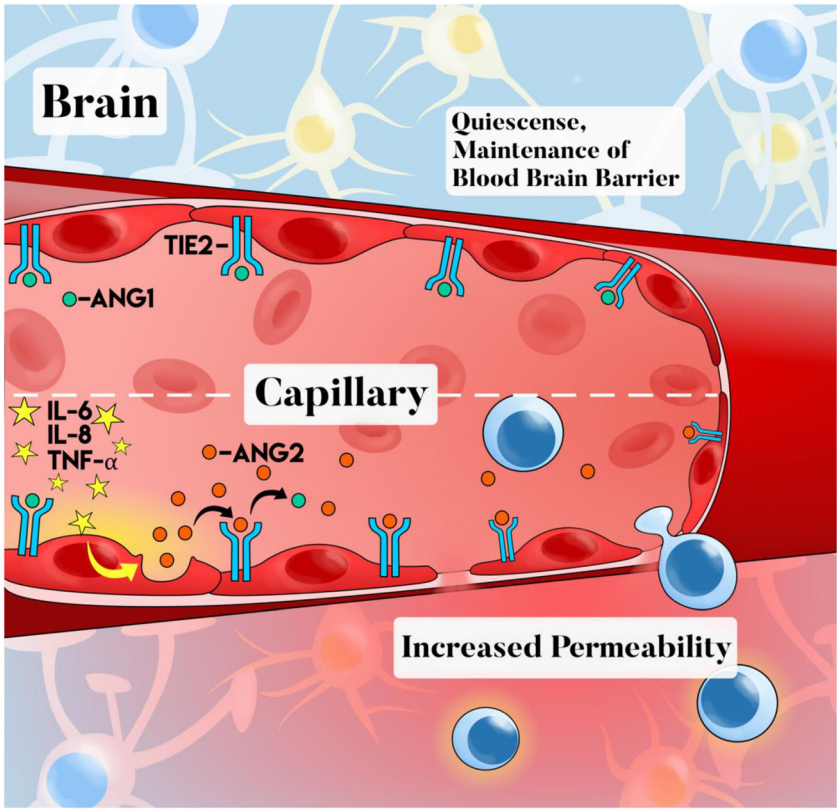


**Fig. 1.** Purported mechanism of vasogenic edema in ICANS. The top portion illustrates a capillary under usual conditions, and the bottom portion illustrates endothelial activation by systemic cytokines and a switch to an ANG2 phenotype resulting in increased capillary permeability.

## Blood-brain barrier disruption and inflammatory CSF cytokines

The BBB is a tightly regulated interface between the systemic circulation and the CNS. Disruption to the BBB can lead to CNS damage mediated by peripheral immune cells. Patients who develop ICANS display evidence of BBB disruption, likely due to endothelial cell dysfunction, as discussed above. Relative to serum, the CSF of patients with neurotoxicity demonstrates elevated protein levels and elevated white blood cell counts, supporting increased BBB permeability [11••, 12•]. While CSF cell count does not correlate with ICANS severity, higher CSF protein concentration does correlate with the grade of ICANS [11••]. The CSF of patients with severe ICANS also has elevated concentrations of cytokines (including IL-6, IL-10, IFNγ, and TNFα) [11••, 12•, 24], while other cytokines (IL-6, IL-8, IL-10, MCP1) were found to be disproportionately elevated in the CSF compared with serum [11••], suggesting CNS-specific production by activated microglia, macrophages, or astrocytes. It is still unclear whether the cytokines traveled from the serum into CSF as a result of increased BBB permeability and endothelial cell dysfunction or whether these cytokines were produced locally within the CNS.

### CNS penetration of CAR-T cells

There is evidence that CAR-T cells infiltrate the brain. Investigators in multiple human clinical trials have observed CAR-T cells in the CSF [6, 11••, 12•], and an autopsy of a patient who died of severe ICANS demonstrated that CAR-T cells comprised the majority of the T cell population in the brain [12•]. However, the quantity of CAR-T cells in the CSF does not always correlate with neurotoxicity [11••]. CAR-T cells have been observed in the CSF of patients without neurotoxicity [11••, 34], suggesting that infiltration of CAR-T cells alone is not sufficient for the pathogenesis of ICANS. Nonetheless, these observations are limited to the relatively few CSF samples from asymptomatic patients. Thus, the role of T cell infiltration into the CNS in the development of severe ICANS remains unclear.

There may be specific aspects to targeting CD19 receptors that predispose patients to the development of ICANS, but this has not been widely studied. Similar neurotoxicities have been reported in clinical trials of CAR-T cells directed against other targets, such as anti-CD22 and anti-CD30, but at much lower rates, affecting 25% or fewer of patients treated [18, 35, 36]. Compared with CD22 or CD30 CARs, CD19 CAR therapy produces more robust T cell activation, different levels of antigen expression, and greater affinity for antigen-target binding [12•]. It should be noted that ICANS rates are similar between studies with different CD19 CAR designs with different costimulatory domains [4, 5, 11••].

### Excitotoxicity

Increased CSF levels of excitatory neurotransmitters, glutamate and quinolinic acid (QA), during neurotoxicity have been observed [11••]. Glutamate is the most common excitatory neurotransmitter and can pay a role in neuronal damage and cell death via binding to receptors, including the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [37]. Increased CNS levels of TNFα have been observed in ICANS [11••, 12•], a cytokine known to induce the production of glutamate by microglia in the CNS, potentiating glutamate-mediated excitotoxicity [38]. Next, QA is an endogenous NMDA agonist [11••] produced in the CNS by activated microglia during CNS inflammation [39]. QA then induces astrocytes to express inflammatory cytokines [40], stimulates glutamate production and inhibits its reuptake [41], and can alter the integrity of the BBB [42], providing a feed-forward mechanism of CNS inflammation and neurotoxicity independent of T cells. Finally, both QA [43, 44] and glutamate [45] are implicated in epileptogenesis; the increased levels of these metabolites in the CSF of those with ICANS may also contribute to the seizures seen in severe ICANS.

## Conclusion

CD19-directed CAR-T cells have proven to be a promising therapy for refractory B cell malignancies, associated with durable remissions. Neurotoxicity, known as ICANS, is a common severe side effect, which has proven fatal in some cases.

A clinician's ability to predict the occurrence of ICANS and treat its manifestations is severely limited by the dearth of understanding of the pathophysiology and mechanism of ICANS. As CAR-T therapy becomes increasingly common, there is a critical need for the establishment of uniform criteria and standards for ICANS and its grades of severity and management. Investigation is urgently needed to determine the mechanism of ICANS and establish best practices for the prevention and treatment of this condition.

## Compliance with Ethical Standards

**Conflict of Interest**

Holly E. Hinson has consulted for Biogen on an ischemic stroke study unrelated to the current work. All other authors declare that they have no conflicts of interest.

**Human and Animal Rights and Informed Consent**

This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
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This was the pivotal clinical trial leading to the FDA approval of tisagenlecleucel.

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