Mechanisms of immune effector cell-associated neurotoxicity syndrome after CAR-T treatment

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Abstract
Chimeric antigen receptor T-cell (CAR-T) treatment has revolutionized the landscape of cancer therapy with significant efficacy on hematologic malignancy, especially in relapsed and refractory B cell malignancies. However, unexpected serious toxicities such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) still hamper its broad application. Clinical trials using CAR-T cells targeting specific antigens on tumor cell surface have provided valuable information about the characteristics of ICANS. With unclear mechanism of ICANS after CAR-T treatment, unremitting efforts have been devoted to further exploration. Clinical findings from patients with ICANS strongly indicated existence of overactivated peripheral immune response followed by endothelial activation-induced blood–brain barrier (BBB) dysfunction, which triggers subsequent central nervous system (CNS) inflammation and neurotoxicity. Several animal models have been built but failed to fully replicate the whole spectrum of ICANS in human. Hopefully, novel and powerful technologies like single-cell analysis may help decipher the precise cellular response within CNS from a different perspective when ICANS happens. Moreover, multidisciplinary cooperation among the subjects of immunology, hematology, and neurology will facilitate better understanding about the complex immune interaction between the peripheral, protective barriers, and CNS in ICANS. This review elaborates recent findings about ICANS after CAR-T treatment from bed to bench, and discusses the potential cellular and molecular mechanisms that may promote effective management in the future.

This article is categorized under:
Cancer > Biomedical Engineering
Immune System Diseases > Molecular and Cellular Physiology
Neurological Diseases > Molecular and Cellular Physiology

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INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) treatment as a major success among adoptive cellular immunotherapy has revolutionized the landscape of cancer therapy, especially in hematological malignancies (Ding et al., 2021; Grupp et al., 2013; Locke et al., 2017; Maude et al., 2014; Mohty et al., 2019; Neelapu et al., 2017). By utilizing synthetic antigen recognition receptor implanted into T cells to facilitate targeting specific tumor-associated antigens (TAAs) on cell surface, such as CD19, CD20, CD22 and BCMA (CD269), CAR-T cells exert dramatic cytotoxicity on tumor cells independent of major histocompatibility complex-restricted T-cell receptor signaling (Ali et al., 2016; Larson & Maus, 2021; Maude et al., 2015; Ying et al., 2019). Among various antigen targets, CD19-directed CAR-T cell therapy is most widely used, with paramount efficacy of a nearly 70% overall response rate and a 52% complete response rate from real-world post-marketing data (Greenbaum et al., 2021). Despite making exciting progress in maintaining long-term durable remission, concomitant adverse reactions have been reported as well, raising challenges to safe and broad application of CAR-T treatment (Larson & Maus, 2021; Lynn et al., 2019; O’Rourke et al., 2017). Among those, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are severe and life-threatening (Miao et al., 2021; Morris et al., 2021; Shao et al., 2021; Zhu et al., 2021). Yet a detailed understanding about the mechanism and management of ICANS after CAR-T treatment is extremely lacking.

ICANS manifests mainly as toxic encephalopathy with receptive aphasia, dysregulated motor function, and altered consciousness (Herr et al., 2020; Lee et al., 2019). Severe cases presented with seizures, coma, and cerebral edema that caused high mortality (Torre et al., 2018). Peripheral blood and cerebrospinal fluid (CSF) analysis revealed dramatically elevated cytokines with similar patterns in patients with ICANS (Hunter & Jacobson, 2019; Taraseviciute et al., 2018). Electroencephalogram (EEG) monitoring and imaging examination indicated extensive neurologic abnormality along with signs of interstitial or cytotoxic edema (Danish & Santomasso, 2021; Gust et al., 2021). FDG PET-CT has a diagnostic value for predicting neurotoxicity after CAR-T treatment (Wang et al., 2019). Postmortem pathologic biopsy also discovered perivascular lymphocyte and monocyte exudation with microhemorrhage (Gust et al., 2017; Hunter & Jacobson, 2019). Existing clinical findings and animal models all suggested peripheral immune overactivation and dysfunctional blood–brain barrier (BBB) with increased permeability in the development of ICANS (Giavridis et al., 2018; Larson & Maus, 2021; Pennell et al., 2018; Staedtke et al., 2018; Taraseviciute et al., 2018). However, the cellular and molecular mechanisms still remain vague due to lack of available experimental models and analytic methods to decipher complex interactions in both the peripheral blood and the central nervous system (CNS).

Building on continuously updated discoveries, we focus on detailed elaboration of potential mechanisms of ICANS after CAR-T treatment particularly in hematologic malignancies. Furthermore, we summarize novel management strategies to improve the safety without impairing efficacy according to related mechanisms.

CLINICAL CHARACTERISTICS OF ICANS

2.1 Incidence and risk factors

The reported incidence of ICANS varied, ranging from 15% to 70% (Table 1). Various factors including patient characteristics and specific features of CAR products could account for the relative high incidence (Gust et al., 2017). Current research illuminated that younger age, higher tumor burden, preexisting neurological symptoms, and factors related to CAR-T cell therapy, such as preadministrated lymphodepletion agents, severe CRS, and high CSF protein level after treatment, were all risk factors of ICANS (Hunter & Jacobson, 2019). Particularly, the incidences of ICANS in pediatric and young adult B-ALL range from 28.6% to 49%, which seems to be higher than that in grown adults (DiNofia & Maude, 2019; Gardner et al., 2017; Gofshteyn et al., 2018). Studies also found evidence of compromise of the neurovascular unit (NVU) and astrocyte injury in pediatrics (Gust et al., 2019). Indeed, in rats with the age comparable to human, astrocyte gradually becomes mature after birth, which is slower than other components like endothelial cell...
| Trial registration | Disease | Target | CRS (%) | sCRS (%) | ICANS (%) | sICANS (%) | Time  | Journal                  |
|--------------------|---------|--------|---------|----------|-----------|------------|-------|--------------------------|
| Cilta-cel NCT03548207 | MM      | BCMA   | 95      | 4        | 21        | 9          | 2021  | The Lancet               |
| Relma-cel NCT04089215 | LBCL    | CD19   | 47.50   | 5.10     | 20.30     | 5.10       | 2021  | Cancer Medicine          |
| 4SCAR19 ChiCTR-OOC-16007779 | NHL    | CD19   | 14      | 0        | 4.76      | 4.76       | 2020  | Frontiers in Immunology  |
| UCART19 NCT02808442 and NCT02746952 | B-ALL  | CD19   | 91      | 14       | 38        | 0          | 2020  | The Lancet               |
| LV20.19 NCT03019055 | B cell malignancy | CD19/CD20 | 64     | 5        | 32        | 14         | 2020  | Nature Medicine          |
| HuCART19 NCT02374333 | T-ALL   | CD7    | 90      | 10       | 15        | 0          | 2021  | Journal of Clinical Oncology |
| KTE-X19 NCT02614066 | B-ALL   | CD19   | 89      | 24       | 33        | 25         | 2021  | The Lancet               |
| KTE-X19 NCT01593696 | B-ALL   | CD19   | 100     | 30       | 20        | 8          | 2021  | Journal of Clinical Oncology |
| Ide-cel NCT03361748 | MM      | BCMA   | 84      | 5        | 18        | 3          | 2021  | The New England Journal of Medicine |
| Liso-cel NCT02631044 | LBCL    | CD19   | 42      | 2        | 30        | 10         | 2020  | The Lancet               |
| Axi-cel non-trial | B-NHL   | CD19   | 93      | 16       | 70        | 35         | 2020  | Journal of Clinical Oncology |
| Axi-cel NCT03153462 | LBCL    | CD19   | 91.20   | 6.80     | 32.80     | 1.70       | 2020  | Journal of Clinical Oncology |
| NCT01747486 | CLL      | CD19   | 63      | 24       | 40.63     | 9.38       | 2020  | Journal of Clinical Oncology |
| NCT0231561 | B-ALL   | CD22   | 86.20   | 20.30    | 32.80     | 1.70       | 2020  | Journal of Clinical Oncology |
| KTE-X19 NCT02601313 | MCL     | CD19   | 91      | 15       | 63        | 31         | 2020  | The New England Journal of Medicine |
| Tisagenlecleucel | B-ALL   | CD19   | 94      | 18       | 40        | 6          | 2020  | Journal of Clinical Oncology |
| Bb2121 NCT02658929 | MM      | BCMA   | 76      | 6        | 42        | 3          | 2019  | The New England Journal of Medicine |
| Axi-cel NCT02348216 | LBCL    | CD19   | 91.74   | 11       | 66.01     | 32         | 2019  | The Lancet Oncology      |
| Tisagenlecleucel NCT02445248 | DLBCL  | CD19   | 58      | 22       | 21        | 12         | 2019  | The New England Journal of Medicine |
| NCT01044069 | B-ALL   | CD19   | 85      | 26       | 44        | 42         | 2018  | The New England Journal of Medicine |
| Axi-cel NCT02435849 | B-ALL   | CD19   | 77      | 47       | 40        | 13         | 2018  | The New England Journal of Medicine |
| Axi-cel NCT02348216 | LBCL    | CD19   | 93      | 13       | 64        | 28         | 2017  | The New England Journal of Medicine |
| CTL019 NCT02030834 | B cell lymphoma | CD19  | 57      | 18       | 39        | 11         | 2017  | The New England Journal of Medicine |
| NCT01865617 | CLL      | CD19   | 83      | 8        | 33        | 25         | 2017  | Journal of Clinical Oncology |
(EC) and pericyte, reflecting more susceptibility and less regulatory capacity of BBB in younger individuals (Bors et al., 2018; Delaney & Campbell, 2017). On the other hand, CAR structure like hinge, costimulatory domains, and transmembrane regions might also contribute (Brudno et al., 2020). Higher rates of ICANS after receiving CAR-T cell infusion were observed in hematological malignancies, compared to that almost none was reported in solid tumor (Ahmed et al., 2015; Brown et al., 2016; Johnson et al., 2015; Kershaw et al., 2006). The recent clinical data of ICANS during CAR-T cell therapy in hematological malignancies are listed below (Table 1).

### 2.2 Clinical manifestation

The symptoms of ICANS resembled that of toxic encephalopathy, as receptive aphasia, dysregulation of fine motor function, and reduced level of attention (Herr et al., 2020; Lee et al., 2019). In mild cases, symptoms started early and patients tolerated well. While in severe cases, elevated intracranial pressure (ICP), seizures, and cerebral edema occurred with high mortality (Greenbaum et al., 2021; Rice et al., 2019). Though in general, ICANS is self-limited and reversible, very few patients still suffer from long-term sequelae (Maillet et al., 2021; Pensato et al., 2021). ICANS presents in a biphasic pattern. In one phase, ICANS concurrently happens with CRS (Brudno & Kochenderfer, 2019; Neelapu et al., 2018). In the other, ICANS presents after CRS remission but with high severity and long persistence. Extremely few cases with seizure or confusion took place 3–4 weeks after CAR-T cells infusion as “delayed neurotoxicity” (Danish & Santomasso, 2021). With rapid onset and gradual remission, ICANS usually happens at 4–6 days after CAR-T cell infusion, peaks at 7–9 days, and lasts for 5–13 days (Brown et al., 2021; Schuster et al., 2017).

### 2.3 Clinical examination

Laboratory examination, imaging, and EEG analysis are necessary for diagnosis. The CAR-T cell kinetics and serum cytokines were connected with ICANS in a sequential time-dependent manner (Morris et al., 2021), which also showed tightly correlation with CRS (Gust et al., 2020; Hong et al., 2021). In consideration of CNS symptoms as the main character of ICANS, the CSF analysis is thought to be a better diagnostic tool. The presence of CNS inflammation was indicated by cerebral infiltration CAR-T cell and myeloid cell with elevated CSF level of interleukin (IL)-1, IL-6, IL-10, Interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), CCL2, CXCL10, and granzyme B (Brown et al., 2021; Hu et al., 2016; Miao et al., 2021). In addition, both GFAP (marker of astrocyte injury) and S100b (marker of astrocyte activation) significantly increased in the CSF compared to patients without ICANS, which strongly suggested local inflammatory response in the CNS (Gust et al., 2019). Furthermore, endothelium-related factors showed a correlation with ICANS. For instance, decreased level of angiopoietin 1 (Ang1), increased level of Ang2, vWF, and CXCL8, were presented as unique markers in patients with severe ICANS (Mackall & Miklos, 2017; Miao et al., 2021).

Imaging results often exhibit no abnormal signs for patients with mild ICANS (Strati et al., 2020). Nevertheless, interstitial or cytotoxic edema with a particular susceptibility for certain brain regions like bilateral thalami, supratentorial white matter, and brainstem were often seen in severe cases (Gust et al., 2020; Rubin et al., 2019). Serving as a real-time measure for brain function, EEG could help detect brain dysfunction especially for delirium and seizure, and provide evidence for diagnostic criteria and evaluation of therapeutic response. A study indicated that EEG background abnormalities were detected in all patients with ICANS, of which 8/19 developed clinical seizures (Gust et al., 2017; Santomasso et al., 2018). Additionally, abnormal EEG patterns graded by Synek scale were positively correlated with ICANS severity (Maziarz et al., 2020; Riegler et al., 2019).

### 2.4 Clinical diagnosis and grading

Early published grading systems for assessing encephalopathy after CAR-T cell therapy include Common Terminology Criteria for Adverse Events (CTCAE), CAR-T cell-therapy-associated TOXicity (CARTOX), and immune effector cell-associated encephalopathy (ICE) score (Neelapu et al., 2018; Schmidts et al., 2021). In 2018, consensus was reached by the American Society for Blood and Marrow Transplantation (ASBMT), elucidating that neurotoxicity caused by CAR-T cell therapy was re-defined as ICANS and re-graded into five levels mainly based on clinical symptoms (Lee et al., 2019).
3 | POTENTIAL MECHANISMS OF ICANS

So far, diverse theories have been proposed to decipher the mechanisms of ICANS, but the precise pathophysiology still lacks an integral interpretation. Currently, CRS is considered as a crucial “initiating event” or cofactor for subsequent ICANS, with numerous overlapping aspects including timescale, cytokine profile, and immunocyte involvement (Miao et al., 2021; Morris et al., 2021; Teachey et al., 2016). Massive pro-inflammatory cytokines are released from both activated CAR-T cells and other host immune cells after engagement with tumor cells (Norelli et al., 2018), which induce endothelial activation with increased permeability and BBB dysfunction (Gust et al., 2017). Further infiltration of cytokines and immunocytes into the CNS triggers noninfectious neuroinflammation and corresponding symptoms (Gallic et al., 2012; Turtle et al., 2016; Wendeln et al., 2018). The schematic illustration of ICANS is presented below (Figure 1).

3.1 | CAR-T cell-induced peripheral immune overactivation

Significantly elevated circulating cytokines including IL-1, IL-6, IFN-γ, TNF-α, and GM-CSF have been observed in patients with ICANS (Gust et al., 2020), which indicated overactivated peripheral immune system. Due to prior lymphodepletion, those excessive pro-inflammatory mediators may lead to great damage on blood vessel and other normal organs in absence of normal immunosuppressive compartments (Kang & Kishimoto, 2021).

![Schematic illustration of immune effector cell-associated neurotoxicity syndrome (ICANS) pathogenesis after chimeric antigen receptor T-cell (CAR-T) treatment and potentially relevant management.](attachment:image.png)
3.1.1 | CAR-T cell-induced monocyte/macrophage activation

With significant CAR-T cell proliferation in patients responsive to the therapy (Deng et al., 2020; Maude et al., 2014), CAR-T cell itself is considered as a causative factor associated with occurrence and progression of ICANS. Clinical observations have demonstrated higher tumor burden, prior lymphodepletion, larger CAR-T cell infusion doses, and faster CAR-T cell expansion in vivo to be related to the onset and severity of ICANS (Brown et al., 2021; Gust et al., 2017; Holtzman et al., 2021; Karschnia et al., 2019). Activated CAR-T cells release IFN-γ, TNF-α, and granulocyte-macrophage colony stimulating factor (GM-CSF) (Spear et al., 2012). Those factors not only exert cytotoxicity on tumor cells directly, but also activate monocyte/macrophage in a manner of cascade amplification.

Monocyte and macrophage are important innate immune cells in defending foreign pathogens and removing aging cells to maintain homeostasis (Mosser & Edwards, 2008). “Classically activated macrophages” respond to elevated CAR-T cell-derived cytokines including IFN-γ, TNF-α, and GM-CSF (Norelli et al., 2018; Patel et al., 2017). Macrophages could also be activated by damage associated molecular patterns (DAMPs) released from lysed tumor cells, such as ATP, HMGB1, and histone H3, through binding of Toll-like receptor (TLR) (Harada-Shirado et al., 2020; Mosser & Edwards, 2008). In response to above mediators, activated macrophages enhance functional capacity and secrete IL-1, IL-6, and TNF-α that act as both products and effectors throughout the whole immune activity (Hao et al., 2020).

3.1.2 | Peripheral cytokines

Activated CAR-T cell and macrophage secrete large amounts of cytokines into peripheral blood. IFN-γ is a broad-spectrum effector that exerts fundamental anti-tumor and pro-inflammatory functions by mediating activation of macrophage (Elinav et al., 2013). TNF-α, a key mediator and regulator of immune activity (Bradley, 2008; Teachey et al., 2016), exerts different signal transduction pathways depending on expression patterns of TNF receptors on effector cells (Hopkins, 2013). In specific, TNF-α activates monocyte/macrophage to promote proliferation, migration, and cytokine production depending on MAPK/NF-κB pathway (Bradley, 2008). GM-CSF predominantly promotes macrophage activation by enhancing its responsiveness to CSF-1, a regulator of myeloid progenitor differentiation that enhances macrophage migration, proliferation, function, and survival (Becher et al., 2016; Hao et al., 2020; Xue et al., 2017).

IL-1, known as human leukocytic pyrogen, is released from activated innate immune cells through ligand stimulation, and further promotes monocyte activation, neutrophil infiltration, and macrophage recruitment (Gottschlich et al., 2021). Emerging evidences also suggest that IL-6 plays a central role in promoting cellular functions between CAR-T cell and macrophage (Chen et al., 2020; Jiang et al., 2021; Norelli et al., 2018; Tanaka et al., 2016). IL-6 is a multifunctional factor that regulates immune response, inflammation, and hematopoiesis (Hunter & Jones, 2015). IL-6 initiates downstream signaling in effector cells in two alternative patterns, the cis-activating pathway and the trans-activating pathway (Rose-John et al., 2006). In cis-pathway, IL-6 binds to membrane-bound IL-6 receptor (mIL-6R), transduces signaling by dimerization of the membrane protein gp130, and activates JAK1/JAK2-STAT3 and MAPK pathways (Zegeye et al., 2018). In trans-pathway, soluble IL-6 receptor (sIL-6R) is produced from mIL-6R by catalyzed of cellular protease, and then binds IL-6. The IL-6/sIL-6R complex further interacts with gp130 on target cell surface and induces similar signalings. To determine the origin, researchers used T cells derived from HPSC-humanized SGM3 mice to generate CAR-T cell-mediated CRS and ICANS mouse model (Norelli et al., 2018). They found that IL-1 and IL-6 primarily originated from monocytes by single-cell RNA-sequencing (scRNA-seq) analysis.

Following CAR-T cell infusion, IL-1 elevation precedes IL-6 up to 24 h in both the serum and CSF from patients with CRS and/or ICANS, indicating that IL-1 might serve as a potential initiative factor (Gottschlich et al., 2021; Norelli et al., 2018). Intravenous application of anakinra, an IL-1 blockade monoclonal antibody that can cross BBB, significantly relieved symptoms of CRS and ICANS at the same time (Giavridis et al., 2018). In addition, multifocal brain meningeal thickening accompanied by macrophage infiltration in lethal neurotoxicity could be effectively prevented by anakinra but not the IL-6 blocker tocilizumab. However, the exact relationship between CAR-T cell and IL-6 is still controversial. Some indicated that IL-6 from monocyte lineage had no effect on CAR-T cell function in vitro (Singh et al., 2017), while others confirmed IL-6 overexpression in CAR-T cell could enhance expansion and antitumor activity via trans-signaling pathway in xenograft models (Jiang et al., 2021). Another group discovered that IL-6 knock-down in CAR-T cell significantly reduced IL-6 secretion in monocytes, while maintained comparable antitumor efficacy (L. Kang et al., 2020). Further investigations should be conducted to determine the effective roles and exact source of IL-6 in ICANS.
In ZUMA-1 clinic trial, GM-CSF along with IL-2 and ferritin were proposed as biomarkers for CAR-T cell-mediated neurotoxicity by correlative analysis of pro-inflammatory mediators (Locke et al., 2017), indicating GM-CSF as a valuable therapeutic target. As for TNF-α, although diverse TNF-blocking agents showed great efficacy in inflammatory diseases, such as rheumatoid arthritis, psoriasis, and Crohn’s disease (Adegbola et al., 2018; Bek et al., 2017; Tokuyama & Mabuchi, 2020), application of such strategies in ICANS have not been conducted yet due to concerns about impairing CAR-T cell function.

3.1.3 | Adrenal system activation

Elevated level of catecholamines were detected in patients with ICANS (Staedtke et al., 2018). Targeting adrenal signaling loop might attenuate ICANS toxicity. Catecholamines is a major group of effectors in adrenal system, including nor-epinephrine (NE), epinephrine (E), and dopamine (DA), with overall effect on immune activities among CAR-T cells and other host immunocytes (Riddell, 2018). When challenged by stimulus, macrophages and neutrophils in host body produce catecholamines in a self-amplifying loop, along with elevated levels of α1/α2-adrenergic receptor on T cells, leading to immune dysregulation and injury (Bao et al., 2007; Bergquist et al., 1994). However, how immunocyte activation drives catecholamines production and how catecholamines boost cytokine production are currently unknown. The metyrosine is a tyrosine hydroxylase inhibitor that specifically inhibits catecholamines production (Staedtke et al., 2018). The atrial natriuretic peptide (ANP), a regulator of water–electrolyte balance, also possesses anti-inflammatory function by inhibiting cytokine release. As application of ANP and metyrosine in patients with CRS significantly reduced cytokine secretion and improved survival, future exploration about the interactions among adrenal system, CAR-T cell, and other components in host immune system will facilitate our understanding about catecholamines and improve ICANS management.

In conclusion, activated CAR-T cell interacting with host monocyte/macrophage contributes to the peripheral immune overactivation. Adrenal system and tumor lysates, such as catecholamines and DAMPs, respectively, also exacerbate toxicity by cascade amplification (Figure 2).

3.2 | Endothelial activation-induced BBB dysfunction

Excessive circulating cytokines and inflammatory mediators establish positive feedbacks through interactions with ECs, and further induce BBB dysfunction (Jiang et al., 2021; Norelli et al., 2018).

3.2.1 | EC function and homeostasis

BBB function largely depends on the functional integrity of EC. EC lines the inner layer of blood vessel as a permeable barrier, and supports vascular integrity together with smooth muscle cell and pericyte in outer layer to regulate substances exchange (Sturtzel, 2017). EC maintains immune system homeostasis but also mediates pathological change when under uncontrolled stimulation. Particularly, EC regulates cytokine secretion, immunocytes migration, and other inflammatory mediators during immune responses.

Sophisticated pathways regulate endothelial function and permeability through specific ligand-receptor interaction, of which vascular endothelial growth factor (VEGF)/VEGF receptor axis and Ang/Tie axis contribute profoundly (Apte et al., 2019; Leligdowicz et al., 2018) (Figure 3). VEGF regulates vasculature formation and function, while the balance between Ang1 and Ang2 is crucial to the vascular homeostasis. Ang1 predominates in vascular quiescence by forming a Tie1/Tie2 complex in a β1-integrin-dependent manner (Brindle et al., 2006). The Tie1/Tie2 heterodimer contains high affinity to fibronectin and collagen, which anchor EC to the extracellular matrix. Tie2 activation also induces downstream signaling pathways to prevent phosphorylate Forkhead box protein O1 (FOXO1) translocating into the nucleus (Daly et al., 2004). Upon inflammatory stimulation, Ang2, which is originally synthesized and stored within the Weibel–Palade bodies (WPB) in EC, is significantly released in an autocrine regulatory pattern (Fiedler et al., 2004). Large amount of Ang2 competes with Ang1 binding to Tie2 receptor, and exerts antagonistic effect on Tie2 signaling (Mandriota & Pepper, 1998). Interestingly, reduced Tie2 expression in EC under inflammation is mediated by NF-κB-dependent transcriptional modulation and proteolytic
cleavage-induced post-translational regulation (Kurniati et al., 2013). Inactivation of Tie2 signaling restores FOXO1 activity, promotes subsequent nuclear translocation, and drives Ang2 expression in a positive feedback loop (Ghosh et al., 2012).

Clinical observations have discovered that low serum level of Ang1, high serum concentration of Ang2, high ratio of Ang2 to Ang1 along with significantly elevated levels of cytokines in both serum and CSF were all associated with severe CRS and ICANS (Gust et al., 2017). From another perspective, Ang1 overexpression in mouse model showed preserved endothelial nitric oxide synthase (eNOS), decreased nitric oxide synthesis, reduced microvascular permeability, and decreased leukocyte infiltration into interstitial spaces (Witzenbichler et al., 2005).

3.2.2 | Cytokine-mediated endothelial overactivation and injury

Elevated peripheral cytokines significantly alter the endothelium homeostasis in ICANS. IL-6 and GM-CSF are both considered as biomarkers in CRS and ICANS after CAR-T treatment. TNF-α, IL-6, and other pro-inflammatory mediators contribute to endothelial activation and increased permeability (Blecharz-Lang et al., 2018; Gust et al., 2017;
Kang & Kishimoto, 2021), participating in cerebrovascular and neurotoxic disease like cerebral ischemia, hemorrhage, and edema (S. Kang et al., 2020; Marcos-Ramiro et al., 2014; Figure 4). TNF-α influences endothelium homeostasis in a dose-dependent manner by stimulating matrix metalloproteinase (MMP) production in ECs (Hosomi et al., 2005), which degrades extracellular matrix (Rosenberg & Navratil, 1997). Although low level of TNF-α is seemed to be neuroprotective in certain conditions, excessive TNF-α induces MMP-2 and MMP-9 production from ECs, which disrupt normal cell-matrix adhesion and induce cell swelling and death, compromising microvascular integrity and permeability (Hosomi et al., 2005). TNF-α also promotes leukocyte adhesion, diapedesis, and vascular leak through additional TFNR2-mediated PI3K/Akt pathway (Bradley, 2008). TNF-α neutralization by monoclonal antibody significantly reduced cerebral edema, supporting its essential participation.

Serum IL-6 maintains at a very low level under physiological conditions but increases rapidly upon inflammation (Hunter & Jones, 2015). Evidences have shown that EC additionally secretes cascade-amplified IL-6 in response to existing soluble IL-6 (Dalal et al., 2020; Garbuzova-Davis et al., 2018; Kang & Kishimoto, 2021; Marin et al., 2001). IL-6R and trans-signaling pathway were both induced in peripheral and cerebral EC under inflammation (Kang & Kishimoto, 2021; Nishimoto et al., 2008). Endogenous inhibition of IL-6 or GM-CSF in CAR-T cell reduced serum IL-6 level and restored endothelium permeability via downregulating interactions with myeloid cells (Sachdeva et al., 2019; Sterner et al., 2019). Further investigation is encouraged to explore their contribution to cerebral EC injury and dysfunction.

3.2.3 | Thrombotic microangiopathy

Elevated serum level of vWF and D-dimer, and decreased fibrinogen and thrombocyte were accompanied by cerebral multifocal hemorrhage in patients with CRS and/or ICANS, indicating an involvement of thrombotic microangiopathy.
that compromised BBB function (Gust et al., 2017; Hunter & Jacobson, 2019; Schuster et al., 2017). Tumor lysates like HMGB1, histone H3, and ATP continuously activate and injure EC, exposing TF and collagen fibers that trigger extrinsic and intrinsic coagulation pathways, which threaten endothelium integrity and induce thrombotic microangiopathy (Miao et al., 2021; Figure 4). vWF, a marker of endothelium injury, is originally stored within WPB in ECs and released upon stimulation, participating in coagulation process by connecting platelet to collagen (Schwameis et al., 2015). The unbalance of these factors increases the risk of disseminated intravascular coagulation (Gust et al., 2017; Schuster et al., 2017).

3.2.4 | BBB dysfunction

Post mortem examination discovered local inflammation with cellulosic exudation and even microhemorrhage around cerebral capillaries, indicating a participation of BBB dysfunction in ICANS (Hunter & Jacobson, 2019). BBB is a selective border formed by EC, pericyte, and astrocyte endfoot process that regulates immunocyte trafficking, transports necessary materials, and prevents entrance of noxious substances to protect the brain (Banks, 2015; Munji et al., 2019; Profaci et al., 2020). Pericyte predominantly regulates BBB permeability by stabilizing EC via direct contact or paracrine
Due to endothelium dysfunction, exposed pericyte produces IL-6 and VEGF in response to excessive IFN-γ (Dalal et al., 2020; Siegler & Kenderian, 2020), which exacerbate BBB disruption by disarraying tight junctions (TJs) between brain microvascular ECs (Capaldo & Nusrat, 2009). Fibrous glia is a specific type of astrocyte that surrounds cerebral microvascular wall and regulates fluid exchange with endfoot process (Abbott et al., 2006). Astrocyte swelling and clasmatomendrosis restrains the ability of fluid control, leading to further BBB dysfunction and cerebral edema (Hulse et al., 2001; Rama Rao et al., 2010). Immunocyte migration and infiltration into the CNS is commonly observed in the CNS inflammation due to compromised BBB permeability. T cell trafficking through vessel is mediated by interaction of surface adhesion molecules between T cell and EC (Redondo-Munoz et al., 2019). Under inflammation, expressions of VCAM-1 and ICAM-1 are elevated in cerebral EC to facilitate leukocyte infiltration (Savarin et al., 2015). Enrichment of peripheral immunocytes and their effector cytokines increase the risk of cerebral thrombosis, and intensify local immune response in the CNS by activating neuroglial cells.

In conclusion, cerebral EC overactivation and injury due to excessive pro-inflammatory cytokines and mediators ultimately lead to increased permeability of cerebral capillary and BBB dysfunction, which facilitate the CNS infiltration of peripheral cytokines and immunocytes (Figure 5).

### 3.3 Central nervous system inflammation

Normal CNS function relies on the BBB integrity to be free from peripheral stimulus. Endothelial activation-induced BBB disruption with increased permeability initiates subsequent CNS noninfectious inflammation in ICANS after CAR-T treatment. CNS infiltration of immunocytes with functional plasticity along with excessive cytokines may convert local immune landscape and induce subsequent astrocyte injury, microglia activation, and neuron dysfunction with corresponding symptoms.

#### 3.3.1 CNS infiltration

CAR-T cells and CD14+ myeloid cells were detected in CSF from patients with ICANS (Locke et al., 2017; Siegler & Kenderian, 2020). Infiltrative CAR-T cells contained Th1, Th17, and Treg subsets (Norelli et al., 2018). scRNA-seq discovered diverse features in infiltrative CAR-T cells, including TCR, cytokine receptor, immune activation, trafficking, and differentiation (Li et al., 2021; Sterner et al., 2019; Xue et al., 2017). Interestingly, an unusual subgroup with monocyte-like transcriptional characters correlated with severe ICANS (Martin-Antonio, 2021). Substantial white matter infiltration and expansion of macrophages in perivascular space were found in brain tissues from lethal cases (Danish & Santomasso, 2021; Torre et al., 2018). A rhesus macaque model using autologous CD20-specific CAR-T cell demonstrated comparable results (Taraseviciute et al., 2018). Pathologic biopsy discovered CAR-T cell, host T cell, and monocyte/macrophage in the brain parenchyma, with multifocal perivascular infiltration in the basal ganglia and parietal lobe. Upregulated VCAM-1 and ICAM-1 were found on the cerebral endothelium, together with significantly increased VLA-4 on CAR-T cell, indicating a potential adhesion-mediated CAR-T cell migration into the CNS. CSF cytokine analysis showed elevation of IFN-γ, TNF-α, GM-CSF, IL-1, IL-6, and IL-10, similarly in serum (Hunter & Jacobson, 2019; Taraseviciute et al., 2018). GM-CSF neutralization reduced CNS infiltration of CAR-T cells and myeloid cells in xenograft mouse models (Sterner et al., 2019).

#### 3.3.2 Astrocyte activation, injury, and dysfunction

GFAP and S100b, markers of astrocyte activation and injury, increased along with the elevated cytokines in the CSF (Brown et al., 2021; Hunter & Jacobson, 2019). MRI analysis presented bilateral thalami swelling showing an interstitial or vasogenic edema (Gust et al., 2019, 2020; Santomasso et al., 2018). Post mortem examination found perivascular astrocyte clasmatomendrosis that indicated cytoplasmic swelling and vacuolation with extensive astrocyte loss (Torre et al., 2018). In severe cases, astrocyte clasmatomendrosis was presented throughout the cortex and white matter.

Astrocyte shares unique structural and functional properties in regulating substances exchange with endfoot process, and participates in neuron excitation by producing excitatory neurotransmitter glutamate (Hosomi et al., 2005). Astrocyte activation and injury present in inflammatory or degenerative neurological diseases, including multiple sclerosis (MS), Alzheimer disease (AD), and Parkinson’s disease (PD) (Wolf et al., 2017). IL-6 and GM-CSF secreted by
activated astrocytes contribute to disease progression in MS and PD (Sofroniew, 2020). Under physiological state, low level of IL-6 promotes differentiation of oligodendrocytes to facilitate myelin repair, and maintains neuronal survival and regeneration. BBB dysfunction causes IL-6 penetration into CNS from both peripheral blood and EC secretion. GM-CSF is predominantly secreted by activated astrocytes (Linnerbauer et al., 2020). GM-CSF receptors are generally expressed on brain macrophages, astrocytes, and microglia, suggesting broad downstream effects upon astrocyte activation. Intracranial ejection of IFN-γ induced direct toxicity on astrocytes and exacerbated disease progression with CNS inflammation and immunocyte infiltration in a stage-specific manner (Ottum et al., 2015; Savarin et al., 2015). Astrocyte injury and swelling further compromised the function in regulating fluid exchange, and resulted in subsequent cerebral edema (Sepehrinezhad et al., 2020).

3.3.3 | Microglia activation

Microglia activation showed diverse patterns of location in fetal ICANS. One patient with CAR-T cell-related cerebral edema showed extensive distribution, while another presented perivascular localization (Torre et al., 2018). These
differences might be attributed to individual heterogeneity with differential susceptibility to inflammatory stimulus in certain brain areas.

Microglia are CNS resident macrophages with highly heterogenic features including complement responsiveness, phagocytic ability, and neurodegeneration involvement (Han et al., 2020; Wendeln et al., 2018; Wolf et al., 2017). Microglia show distinct plasticity in activating diverse metabolic and immune pathways to modulate normal neuron functions (Zheng et al., 2021). Microglia express OX-6 and OX-42 as markers of activation (Jiang et al., 2009). During vasogenic edema, activated microglia secrete IL-1 and TNF-α that caused increased BBB permeability and neuron injury (Clark & Vissel, 2016; Conroy et al., 2004; Holmin & Mathiesen, 2000). IL-1 induces temporary and reversible increased permeability by opening endothelial TJs, while TNF-α causes long-term effect due to endothelial cytotoxicity (Capaldo & Nusrat, 2009; Marcos-Ramiro et al., 2014). IL-6 also induces microglia activation and additional IL-6 production, leading to neuron damage and astrogliosis (Sofroniew, 2015). IL-6 neutralization upregulated expression of galectin-1 on astrocytes and dramatically reduced microglia activation (Sirko et al., 2015). Another special microglia subset, the IFN-M, was identified in AD models with multiple IFN-stimulated transcriptional profiles, indicating its potential regulation of cognitive function (Olah et al., 2020). Microglia were also involved in cerebral malaria-induced neurotoxicity with BBB disruption and cerebral edema (Dorovini-Zis et al., 2011).

3.3.4 Abnormal neuronal function

Abnormal neuron function is related to the dysregulation of neuroglial cells and neurotransmitters by overwhelmed CNS inflammation. Significantly, high level of glutamate and quinolinic acid in the CSF may explain EEG background abnormalities and symptoms in patients with varying degrees of ICANS (Gust et al., 2021; Santomasso et al., 2018; Tallantyre et al., 2021). Glutamate regulates memory and learning process. Astrocytes exposed to IL-1β showed decreased glutamate transporters with impaired function of glutamate re-uptake (Rama Rao et al., 2010). Physiologic TNF-α maintains normal neuron function, while excessive amount causes extracellular accumulation of glutamate by both increased release and inhibited re-uptake (Clark & Vissel, 2016). Moreover, TNF-α induces long-term excitability alteration by glutamate receptor upregulation and GABA receptor endocytosis. Quinolinic acid, a stimulator for extracellular glutamate accumulation, induces synaptosome depolarization through exogenous Ca²⁺ influx (Hosomi et al., 2005). High level of IL-6 altered neuronal excitability in Ca²⁺-dependent manner with lower firing rate, oscillatory firing patterns, and prolonged inhibitory phase (Conroy et al., 2004), enhanced neurotoxicity, and blocked neurogenesis (Gruol & Nelson, 2005).

In conclusion, increased BBB permeability leads to neuroinflammation with astrocyte dysfunction, microglia activation, and abnormal neuronal function during CNS inflammation (Figure 6).

4 OTHER POTENTIALLY RELEVANT FACTORS

4.1 CNS targeting by CAR-T cell

4.1.1 CNS involvement

The CNS involvement is a severe situation in tumor progression with dismal prognosis in pediatrics and adults (del Principe et al., 2018). The existence of tumor cells in the CNS may contribute to a secondary neurotoxicity after CAR-T cell targeting (Wang et al., 2021). Tumor cell may infiltrate into the CNS independent of BBB, followed by CAR-T cell in the same routes (Yao et al., 2018). Patients with CNS leukemia experienced varying degrees of ICANS with detectable CAR-T cell in the CSF (Santomasso et al., 2018). However, it is hard to distinguish whether increased CAR-T cell in the CSF is due to local expansion in the CNS or massive infiltration from peripheral blood.

4.1.2 On-target off-tumor effect

On-target off-tumor effect is a common adverse reaction mostly seen in CAR-T cell therapy and other antibody-based target therapy. Normal tissues expressing the same TAAs may suffer additional cytotoxicity (Ahmed et al., 2015; Beatty et al., 2014; Brown et al., 2016; Johnson et al., 2015; Kershaw et al., 2006).
CD19 is an appropriate TAA candidate with confined expression on B cells, while B cell dysplasia usually happens due to normal B cells targeted by CD19 CAR-T cells (Cordeiro et al., 2020). To our surprise, recent studies discovered that human mural cells also expressed CD19 (Parker et al., 2020; Figure 7). scRNA-seq analysis of human prefrontal cortex cells found CD19 epitope on mural cells that could be recognized by clinical CAR-T cells, which was further confirmed by perivascular staining at protein level. Mural cells are classified into vascular smooth muscle cell (vSMC) and pericyte in regard of its different localization along larger vessels or capillaries, respectively. The absence of pericyte impairs normal BBB development. Infusion of murine CD19-directed CAR-T cells into NSG mice induced BBB disruption with increased permeability. However, human brain pericytes are more susceptible to clinical CD19-directed CAR-T cells due to relative higher abundance of CD19 expression than mouse brain pericytes (Han et al., 2018).

CD22 substitutes for CD19 as a second target after tumor relapse (Fry et al., 2018). Increased incidences of inflammatory toxicities were reported in patients receiving CD22 CAR-T product (Baird et al., 2021; Shah et al., 2020). scRNA-seq data confirmed CD22 expression in human microglia, whose upregulation inhibited phagocytosis and impaired

**Figure 6** The Central nervous system (CNS) inflammation. Peripheral immunocytes and cytokines infiltration into the CNS contribute to the CNS inflammation. Activated astrocyte releases IL-6 and GM-CSF that induce microglia activation to produce additional IL-1β, IL-6, and TNF-α, which further influence neuron function. Increased GFAP and S100b suggest astrocyte activation and injury, while downregulation of galectin-1 indicates reduced control of microglia activation by astrocyte. Upregulated OX-6 and OX-42 on microglia indicate activated status. Astrocyte activation and injury induce glutamine accumulation and decomposition on mitochondria membrane, which lead to ATP depletion and mitochondria permeability transition. Energy insufficiency induces compromised water control by APQ4 and leads to astrocyte swelling. Dysfunctional astrocyte promotes extracellular glutamate accumulation by increased released and inhibited re-uptake. Accumulative glutamate along with neuron GABA receptor endocytosis caused by TNF-α increase neuronal excitability through NMDA and AMPA receptor in Ca2+ dependent manner. Abnormal neuron functions present with corresponding symptoms of immune effector cell-associated neurotoxicity syndrome (ICANS) after chimeric antigen receptor T-cell (CAR-T) treatment.
clearance of toxic metabolites like myelin debris (Olah et al., 2020) (Figure 7). Interestingly, infusing CD22 CAR-T cells did not induce more frequent or severer ICANS than CD19 CAR-T cells. The correlation between CD22 and ICANS remains to be elucidated.

4.2 Tocilizumab application

Tocilizumab is effective to manage patients with severe CRS after CAR-T treatment by inhibiting both downstream cis and trans-signaling in effector cells via competitively binding to membrane-bound and soluble IL-6R (Kotch et al., 2019; Le et al., 2018; Nishimoto et al., 2008). However, tocilizumab has an opposite effect on ICANS. Studies observed secondary neurotoxicity with serum IL-6 elevation after CRS symptoms remission in patients receiving tocilizumab (Chen et al., 2016; Karschnia et al., 2019). Massive dissociated IL-6 penetrates disrupted BBB into the CNS and enhances IL-6 signaling cascades (Tanaka et al., 2016) (Figure 7). However, tocilizumab with large molecular size fails to reach into the CNS to exert inhibitory effect on microglia and neurons. Delayed ICANS occurred in very few patients with undetermined mechanism (Neelapu et al., 2018; Schuster et al., 2017). One explanation is that cerebral capillary remained integral until later disrupted by tocilizumab-induced serum IL-6 accumulation. Others include individual heterogeneity, previous medicine administration, and pharmacokinetics. Correlation between the use of tocilizumab and the onset of ICANS should be elucidated concretely for its safety to manage concurrent CRS.

**FIGURE 7** Other potentially relevant factors in immune effector cell-associated neurotoxicity syndrome (ICANS) after chimeric antigen receptor T-cell (CAR-T) treatment. Previous cerebral disease may potentiate neuronal response to stimulus with increased neuronal excitability. CD19 expression on pericyte and CD22 expression on microglia may induce on-target off-tumor effect by CAR-T cell that contributes to blood–brain barrier (BBB) dysfunction and CNS inflammation. Tocilizumab application in patients with cytokine release syndrome (CRS) after CAR-T treatment may promote peripheral free IL-6 accumulation and infiltration into the central nervous system (CNS) in absence of tocilizumab penetrating through BBB. Astrocyte swelling with endfoot process disruption and clasmadendrosis compromises its fluid exchange between the peripheral blood and CNS.
4.3 | Previous cerebral disease

Previous poor cerebral conditions such as epilepsy history and other pathological changes in focal lesions may potentiate ICANS occurrence after CAR-T treatment. Epileptic seizure is a paroxysmal depolarizing shift caused by abnormal, excessive, and synchronized neuronal activities (Gavvala & Schuele, 2016). BBB disruption participates in epileptogenesis (Oby & Janigro, 2006). Dysfunctional ion channels and imbalanced excitatory or inhibitory circus result in abnormal neuron activation (Tian et al., 2012). Prolonged influx of Ca\(^{2+}\) and Na\(^{+}\) lead to long-term depolarization and repetitive action potentials. Loss of inhibitory neuron and abnormal GABAergic signaling also induce neuron hyperexcitability (Figure 7). Previous epilepsy may create persistent sublethal injury in neurons with potentiated responsiveness to extracellular ionic and other neurotransmitters such as GABA, quinolinic acid, and glutamate (Gallic et al., 2012). Other pathological changes in focal lesions also influence neuronal electrophysiologic conditions (O'Rourke et al., 2017; Sepehrinezhad et al., 2020).

5 | MANAGEMENT OF ICANS

According to the consensus from ASTCT, proper management should be based on ICANS grades (Lee et al., 2019). Supportive treatment with intravenous fluid infusion, aspiration support, and prophylactic anti-seizure agents are recommended in mild ICANS. High-dose corticosteroids or biological agent like siltuximab and anakinra should be considered in severe ICANS (Davis et al., 2015; Giavridis et al., 2018). Potential biological agents with relevant mechanisms (Table 2) and CAR-T cell modulation are promising strategies to manage or prevent ICANS (Figure 1).

| Table 2 | Potential biological agents with relevant mechanisms in immune effector cell-associated neurotoxicity syndrome (ICANS) |
|---------|------------------------------------------------------------------------------------------------------------------|
| **Drug** | **Mechanism** | **Stage** | **Approved applications** | **Research related to CAR-T** |
| Corticosteroids | Anti-inflammatory effect in nonspecific way | FDA approved and widely used for ICANS | Abundant applications concerning inflammation | Widely used to treat CRS and ICANS |
| Siltuximab | IL-6 antagonist | FDA approved | Castleman disease | Clinical trials in treating CRS and ICANS |
| Anakinra | IL-1 receptor antagonist | FDA approved | RA/CAPS/deficiency of interleukin-1 receptor antagonist | Clinical trials in treating CRS and ICANS |
| Lenzilumab | Anti-GM-CSF antibody | Under clinical investigation | | Clinical trials for combination of lenzilumab and CART19 cell therapy |
| Ruxolitinib | JAK inhibitor | FDA approved | MF/PV/aGVHD | Several cases reported for CRS treatment |
| Itacitinib | JAK inhibitor | Under clinical investigation | | Clinical trials in treating CRS |
| Ibrutinib | BTK inhibitor | FDA approved | MCL/CLL/SLL/WM/MZL/cGVHD | Clinical trials for combination of ibrutinib and CART cell therapy |
| Natalizumab | Anti-VLA4 antibody | FDA approved | MS/Crohn’s disease | No; possibly related to progressive multifocal leukoencephalopathy |
| Etanercept | TNF-α inhibitor | FDA approved | RA/polyarticular JIA/PsA/AS/PsO | Preclinical study for CRS |

Abbreviations: aGVHD, acute graft-versus-host disease; AS, ankylosing spondylitis; cGVHD, chronic graft versus host disease; CAPS, cryopyrin-associated periodic syndromes; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; JIA, juvenile idiopathic arthritis; MCL, mantle cell lymphoma; MF, myelofibrosis; MS, multiple sclerosis; MZL, marginal zone lymphoma; PsA, psoriatic arthritis; PsO, plaque psoriasis; PV, polycythemia vera; RA, rheumatoid arthritis; WM, Waldenstrom’s macroglobulinemia.
5.1  Corticosteroids

Corticosteroids exert profound anti-inflammatory effects on immune cells through rapid nongenomic alteration and gene transcriptional regulation to rebalance pro and anti-inflammatory mediator production, block cytokine signaling or adhesion molecules interaction, and induce apoptosis (Timmermans et al., 2019). The application of high-dose corticosteroids achieved excellent remission in severe CRS and ICANS (Brudno & Kochenderfer, 2019). However, corticosteroids may influence CAR-T cell function and efficacy. Pioneer studies have observed CAR-T cell functional deficiency after applying high-dose or long-period corticosteroids in patients with severe ICANS (Brentjens et al., 2013; Davila et al., 2014). Recently, investigators found that higher dose of corticosteroids, and earlier or longer-period use was associated with significantly shorter progression-free survival and overall survival in a retrospective study evaluating the efficacy of CAR-T cell therapy among 100 patients with large B-cell lymphoma (Strati et al., 2021). These results highlighted a cautious consideration before applying corticosteroids to manage ICANS.

5.2 Biological agents

5.2.1 IL-6 signaling blockade

Siltuximab is an IL-6 neutralization antibody different from tocilizumab (Chen et al., 2016; Davis et al., 2015). Intravenous administration of siltuximab did not increase the concentration of IL-6 in the CSF (Neelapu et al., 2018), therefore to be suitable for managing CRS and ICANS simultaneously.

5.2.2 IL-1 signaling blockade

Anakinra is an IL-1 receptor antagonist with ability to penetrate BBB into the CNS for the treatment of both systemic and cerebral autoinflammatory disease, including Still's disease, rheumatoid arthritis, and macrophage activation syndrome (MAS) (Ramirez & Canete, 2018; Sonmez et al., 2018; Vastert et al., 2019). As discussed above, IL-1 positively regulates both the peripheral and CNS inflammation. Therefore, targeting IL-1 might prevent ICANS progression and avoid severe outcomes. Recent preclinical studies demonstrated that anakinra could alleviate severe neurotoxicity better than tocilizumab without impairing CAR-T cell function in mice (Gottschlich et al., 2021; Norelli et al., 2018). Several clinical trials are currently evaluating the combinational strategy with CAR-T cell and IL-1 blockade (NCT04432506, NCT04359784, NCT04150913, NCT04148430, NCT04205838, and NCT03430011).

5.2.3 Targeting other cytokines

In preclinical study, GM-CSF neutralization in xenograft mouse models by lenzilumab, an anti-GM-CSF antibody, showed reduced CNS infiltration of CAR-T cells and myeloid cells, relieved signs of neurotoxicity, and improved overall survival without impairing CAR-T cell function (Sterner et al., 2019). Inhibition of IFN-γ production and signaling by using JAK inhibitors such as ruxolitinib or itacitinib (Elli et al., 2019; Huarte et al., 2020; Pan et al., 2021), and BTK inhibitor ibrutinib all successfully achieved ICANS remission, but doing great damage on CAR-T cell function and efficacy (Gauthier et al., 2020; Ruella et al., 2017).

5.2.4 Adhesion molecules blockade

Alternative strategy to target specific surface molecules on CAR-T cells and lymphocytes is also being developed recently. VLA-4 plays a key role in lymphocytes trafficking into extravascular tissues through interactions with VCAM-1 and fibronectin on EC (Redondo-Munoz et al., 2019). As CAR-T cells expressed more VLA-4 than non-CAR T cells, blocking VLA-4 by natlizumab may prevent the CNS infiltration of CAR-T cells and reduce CNS inflammation (Khoy et al., 2020; Taraseviciute et al., 2018).
5.3 CAR-T modulation

CAR-T cell modulation by redesigning CAR construct has drawn much attention into improving efficacy and reducing toxicities (Feucht et al., 2019; Hu et al., 2021; Wang et al., 2020; Ying et al., 2019). Specific tag or ON/OFF switch could be embedded into CAR construct in order to control CAR-T cell biological functions in space and time (Feucht et al., 2019; Larson & Maus, 2021). Additional cell surface proteins such as EGFR or CD20 that are artificially introduced into CAR-T cell could be targeted by exogenous monoclonal antibodies to induce specific elimination, such as cetuximab or rituximab, respectively (Paszkiewicz et al., 2016). However, such strategy remains risks of on-target off-tumor effect. Incorporation of inducible apoptosis signaling combined with specific receptor serves as a safety switch in CAR-T cell. In a pioneer study, modified human caspase-9 and FK506 binding protein (FKBP) were embedded into CD19-targeted CAR construct to prevent severe ICANS progression via inducing CAR-T cell apoptosis upon specific stimulation by chemical inducer of dimerization (CID) (Diaconu et al., 2017). Similarly, split CAR design facilitates selective and reversible modulation of CAR-T cell function under certain circumstances (Mestermann et al., 2019). CAR-T cells with further endogenous knock-out of key cytokines like GM-CSF by gene-editing technology sufficiently reduced host monocyte/macrophage activation and cytokine release in mouse models, while preserving CAR-T cell efficacy (Sachdeva et al., 2019; Sterner et al., 2019).

6 CONCLUSION

CAR-T cell therapy provides with a promising strategy in cancer treatment, while specific toxic adverse events like CRS and ICANS still hinder its broad clinical application, with the precise mechanisms remaining largely unknown.

In our conclusion, CAR-T cell-induced overactivated peripheral immune activity with excessive systemic cytokines and pro-inflammatory mediators contribute to endothelial activation and injury during the early stage of ICANS. Endothelial activation-induced BBB dysfunction with increased cerebral microvascular permeability facilitates the CNS infiltration of peripheral cytokines and immunocytes, which result in subsequent CNS inflammation and neuron dysfunction. On-target off-tumor effect promotes pericyte-mediate BBB disruption and induces microglia activation. Previous cerebral diseases and tocilizumab application might also increase CNS sensitivity to inflammatory stimulus and accelerate ICANS progression. Targeting key cytokines involved in ICANS induces rapid remission without impairing CAR-T cell efficacy. Much effort should also be devoted to CAR-T modulation and optimization with safety control to avoid rather than treat ICANS.

Challenges still remain despite existing progress. First, ICANS presents with diverse CNS symptoms with lack of universally adopted comprehensive description and category. Next, the absence of ideal experimental animal models and appropriate analytic technologies hinder our deeper understanding about ICANS. Single-cell RNA sequencing might facilitate to decipher complex cellular and molecular interactions in the brain. Finally, discovering specific cytokines or adhesion molecules involved in the pathogenesis of ICANS might contribute to developing novel therapeutic target. Digging into the depth of mechanisms of ICANS will shed more light on the safe application of CAR-T cell therapy in the future.

AUTHOR CONTRIBUTIONS

Tianning Gu: Conceptualization (lead); writing – original draft (lead); writing – review and editing (lead). Kejia Hu: Conceptualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). Xiaohui Si: Conceptualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). Yongxian Hu: Conceptualization (lead); supervision (lead). He Huang: Conceptualization (lead); supervision (lead).

ACKNOWLEDGMENT

Thanks to Xia Li for useful discussion.

CONFLICT OF INTEREST

The authors have declared no conflict of interest for this article.
DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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