In the Eye of the Storm: Immune-mediated Toxicities Associated With CAR-T Cell Therapy

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Abstract
The success of chimeric antigen receptor (CAR)-T cell therapy with impressive response rates in hematologic malignancies but also promising data in solid tumors came along with the cognition of unexpected, potentially life-threatening immune-mediated toxicities, namely the cytokine release syndrome (CRS) and neurotoxicity recently referred to as “immune effector cell-associated neurotoxicity syndrome” (ICANS). These toxicities require urgent diagnostic and therapeutic interventions and targeted modulation of key cytokine pathways represents the mainstay of CRS treatment. However, as the underlying mechanisms of ICANS are not well understood, treatment options remain limited and further investigation is warranted.

Importantly, after the recent market approval of 2 CAR-T cell constructs, the application of CAR-T cell constructs will expand to nonacademic centers with limited experience in the management of CAR-T cell-associated toxicities.

Here, we review the current evidence of CRS and ICANS pathophysiology, diagnostics, and treatment.

Introduction
Impressive response rates including long-lasting remissions even in chemorefractory hematologic malignancies using CD19-directed T cells incorporating a chimeric antigen receptor (CAR) led to breakthrough therapy approval by both the Food and Drug Administration and European Medicines Agency. The CAR-T cell principle is based on genetically engineered autologous or allogeneic T cells that express a CAR which consists of an epitope-specific binding domain (most commonly an antibody-derived single-chain variable fragment), a hinge and transmembrane domain and finally signaling domains of the T cell receptor (TCR) (mostly consisting of the CD3\(\gamma\) chain). Current second-generation CAR-T constructs are combined with additional costimulatory domains such as CD28, 4-1BB, and OX40. This enables a strong antigen-specific T cell activation without the need of TCR-MHC interactions that are prone to coinhibitory signals.\textsuperscript{3}

Two CD19-targeting constructs have recently been approved for B lymphoblastic leukemia (B-ALL)\textsuperscript{2,3} and aggressive lymphoma (diffuse large B cell lymphoma [DLBCL], transformed follicular lymphoma [FL], primary mediastinal B cell lymphoma [PMBCL])\textsuperscript{4,5}. Early clinical data in multiple myeloma\textsuperscript{6} and glioblastoma\textsuperscript{7} are also promising. However, especially in B-ALL and aggressive lymphoma, severe toxicities with even fatal outcome have occurred, mainly due to 2 common CAR-T cell-mediated toxicities: the cytokine release syndrome (CRS) and CAR-T cell-related neurotoxicity, before referred to as CAR-T cell-related encephalopathy syndrome (CRES) and since the publication of consensus recommendations supported by the American Society for Blood and Marrow Transplantation (ASBMT) termed immune effector cell-associated neurotoxicity syndrome (ICANS). In addition to the above-mentioned CRS and ICANS, other severe toxicities like prolonged cytopenia, B cell depletion and febrile neutropenia have been frequently reported. Here we focus, review, and discuss the current understanding of CRS and ICANS disease pathophysiology, clinical presentation, and the toxicity management of CAR-T cell therapy.

History and epidemiology of CRS
CRS represents an immune-mediated toxicity characterized by an excessive immune reaction caused by immune-modulating drugs. Recently, T cell engaging therapies like T cell redirecting antibodies (eg, blinatumomab), immune-mobilizing monoclonal TCRs against cancer (ImmTAC), or other TCR-based strategies\textsuperscript{8,9}
and to an even larger extent CAR-T cell therapy have resulted in high CRS rates. With growing experience with CAR-T cell treatment, the rates of severe CRS have decreased in the more recent studies due to earlier intervention. Table 1 shows the reported incidences of CRS and ICANS in recent clinical trials.

Apart from CAR-T cell therapy, CRS has also been described with many T and B lymphocyte engaging therapies, namely murumomab-CD3 (OKT3),

antihuman globulin (ATG),

CD28 superagonist TGN1412,

rituximab,

chinomab,

dalotuzumab,

brentuximab,

nivolumab,

CD40 agonists but also in overwhelming viral infections like influenza

and in the hematopoietic stem cell transplant setting.

Unlike in CAR-T cells peak CRS symptoms occurring after these therapies usually start to develop shortly after antibody administration.

### CRS pathophysiology

CRS is thought to be mediated by an initial release of proinflammatory cytokines like interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) by activated or lysed effector cells including T cells activated by the tumor antigen recognizing CAR or T cell redirecting antibodies (eg, blinatumomab), lysed T and B lymphocytes in the case of, for example, ATG and rituximab. These cytokines lead to an activation of bystander immune cells and endothelial cells which in turn activate more immune cells culminating in a cytokine storm.

Macrophages seem to play an important role in CRS pathophysiology. When activated by secreted IFN-γ, they produce excessive amounts of cytokines such as interleukin 6 (IL-6), TNF-α, and interleukin 10 (IL-10). Depletion of macrophages in a humanized xenotolerant mouse model abated CRS. Macrophages were shown to be the major source of IL-1 and IL-6 production in this model while CAR-T cells only produced negligible levels. In addition, IL-6 seems to be central to CRS disease pathology since it is highly elevated in CRS patients and CRS mouse models.

These findings were recently emphasized by a study showing that an early increase in IL-6 and angiopoietin 2: angiopoietin 1 (Ang-2:Ang-1) ratio are associated with very severe CRS in patients treated with CAR-T cells. Moreover, blockade of the IL-6 pathway leads to rapid resolution of CRS symptoms in mice and humans. IL-6 has also been implicated in key pathogenetic aspects of CRS like vascular leakage, disseminated intravascular coagulation (DIC), and cardiomyopathy. Interestingly, blockade of IL-1 terminated CRS and ICANS symptoms in a mouse model underscoring the role of IL-1 in CRS pathogenesis. Another hallmark of CRS is the activation of endothelial cells. Serum levels of activation markers like Ang-2, von Willebrand factor and intercellular adhesion molecule were shown to be elevated in CRS patients and might lead to capillary leakage, hypotension, and coagulopathy. Moreover, the endothelium has been shown to be a major producer of IL-6 in a postmortem analysis of a patient who succumbed to CRS. Figure 1 shows the proposed pathomechanism of CRS.

In many aspects, severe CRS resembles hemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome (MAS). Clinical and laboratory findings and the cytokine profile including IL-18, IL-8, IP10, MCP1, MIG, and MIP1β representing a Th1 and myeloid-driven inflammatory responses are closely related between HLH/MAS and severe CRS and hemophagocytosis has been evident in clinical trials. Moreover, IL-6 can lead to dysfunctional cytokytic activity in T and NK cells and T-cell exhaustion.20,21 In addition, infection is a common underlying factor in both diseases.

**Table 1** Reported Data for CRS and ICANS in Recent Clinical Trials

| Author/Year | Disease | CAR Type | No. of patients | % CRS | % sCRS (°II) | % ICANS | % sICANS (°II) | Treatment-related deaths | Time to onset of CRS | ORR (°III–C0) | C0/C0 | % Infections | % MRD | % ORR | % CHLA/C0 | % ICANS (°III–C0) | % CHLA/C0 |
|-------------|---------|----------|----------------|-------|-------------|---------|--------------|------------------------|------------------|-------------|------|------------|-------|-------|----------|----------------|----------|
| Gardner et al, 2016 | B-ALL | CD19 CAR | 12 | 41 | 25 | 76 | 28 | 0 | 0-1 | 0 | 28 | N | 0 | 0 | 0 | 28 |
| Garfall et al, 2015 | MM | BCMA CAR | 11 | 76 | 18 | 100 | 3 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, et al, 2014 | B-ALL | CD19 CAR | 21 | 76 | 18 | 100 | 3 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Schuster et al, 2017 (a) | DLBCL, PMBCL | CD19 CAR | 28 | 83 | 62 | 93 (93) | 1 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Davila et al, 2017 | FL grade 3B, MCL | CD19 CAR | 24 | 62 | 33 | 23 (23) | 13 | 0 | 33 | 63 | (67) | 14 | 52 | 35 | 29 | 3 |
| Schuster et al, 2017 (b) | B-ALL | CD19 CAR (4-1BB) | 32 | 63 | 21 | 93 (93) | 1 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, 2016 | B-ALL | CD19 CAR (CD28) | 45 | 76 | 28 | 93 (93) | 1 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, 2015 | B-ALL | CD19 CAR (4-1BB) | 108 | 93 | 13 | 23 (23) | 13 | 0 | 33 | 63 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, 2014 | B-ALL | CD19 CAR (CD28) | 111 | 83 | 8 | 23 (23) | 13 | 0 | 33 | 63 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, 2013 | B-ALL | CD19 CAR (CD28) | 11 | 76 | 18 | 100 | 3 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |
| Lee et al, 2013 | B-ALL | CD19 CAR (CD28) | 53 | 76 | 18 | 100 | 3 | 0 | 60 | 83 | (67) | 14 | 52 | 35 | 29 | 3 |

Table 1: Reported incidences of CRS and ICANS in clinical trials. Schuster et al used a different grading system for CRS.

°Incidences reported for CRS and ICANS in clinical trials. Schuster et al used a different grading system for CRS.

°°B-lymphoblastic leukemia, CAR = chimeric antigen receptor, CLL = chronic lymphocytic leukemia, CR = chronic myeloid leukemia, CTCAE = common terminology criteria for adverse events, CTCAE = chronic myeloid leukemia, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, FL3B = follicular lymphoma grade 3B, ICANS = immune effector cell-associated neurotoxicity syndrome, IFN-γ = interferon gamma, IL = interleukin, ICANS = immune effector cell-associated neurotoxicity syndrome, IFN-γ = interferon gamma, IL = interleukin, ICANS = immune effector cell-associated neurotoxicity syndrome, MCL = mantle cell lymphoma, MM = multiple myeloma, MRD = minimal residual disease, ORR = overall response rate, Penn = Pennsylvania, TFL = transformed follicular lymphoma.
cells, a key driver of the dysregulated immune response in HLH/MAS. However, so far no link to genetic alterations found in familial HLH (eg, PRF1, STX11, STXBP2, and MUNC13-4) or MAS that trigger the inappropriate release of cytotoxic molecules (perforin, syntaxin) could be seen in a blinatumomab trial.27 Thus, CRS and MAS/HLH likely represent different syndromes characterized by a dysregulated immune response ending in a common pathway of hyperactivation.

The incidence of immunotoxities also seems to differ between the different CAR constructs. The CD28 costimulatory domain appears to confer a stronger antigen-specific stimulation of T cells due to a lower activation threshold thus potentially leading to higher rates of CRS.42 However, comparisons of the CRS rates between the pivotal NHL trials JULIET, ZUMA-1, and TRANSCEND NHL 001 are hampered by the differences in the patient population, dosing and cell composition, and the use of different grading systems (Table 1).5,43,44 The newly published consensus grading might help to compare the toxicity rates in future studies with different constructs.

Brudno et al compared the toxicities of 2 CAR constructs that only differed in the hinge and transmembrane domain as well as the origin of the CD19-binding moiety (CD8 alpha/human vs CD28/murine) and found a significantly lower rate of cytokine production and toxicity (neurotoxicity 5% vs 55%) in the

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Figure 1. Proposed pathomechanism of cytokine release syndrome. Activation of many T cells or lysis of immune cells induces a release of interferon gamma (IFN-γ) or tumor necrosis factor alpha (TNF-α). This leads to the activation of macrophages, dendritic cells, other immune cells, and endothelial cells. These cells further release proinflammatory cytokines. Importantly, macrophages and endothelial cells produce large amounts of interleukin 6 (IL-6) which in a positive feedback loop manner activates T cells and other immune cells leading to a cytokine storm. CAR = chimeric antigen receptor, FiO2 = fraction of inspired oxygen, IFN-γ = interferon gamma, IL-6 = interleukin 6, TNF-α = tumor necrosis factor alpha.
20 patients treated with the CD8 alpha/murine CAR-T cells with retained antilymphoma activity. Thus besides the costimulatory domain other parts of the CAR construct might play a role in the induction of immunotoxicities. Different target antigens might also result in differences of the extent of toxicities and initial reports on BCMA-directed CAR-T cell constructs implied a lower rate of CRS and ICANS compared with anti-CD 19 CAR-T cells. However, in a recent BCMA CAR-T cell trial of 16 multiple myeloma patients receiving the target dose of $9 \times 10^6$ CAR-T cell rates of severe CRS cases were comparable to the CD19-targeting constructs with 36% of the patients requiring vasopressor support but patients experienced less and milder neurotoxicity. New CAR constructs might mitigate the immunotoxic effects, for example, by targeted disruption of GM-cerebospinal fluid (CSF) through gene editing.

Disease burden has most consistently been associated with CRS severity after CAR-T cell therapy as shown in B-ALL, CLL, multiple myeloma, and B-NHL as well as for blinatumomab in B-ALL but also rituximab in CLL. Another important factor is the administered dose of the acting agent. This, along with the fact that a more intense lymphodepletion was associated with CRS in the CAR-T cell setting, probably reflects the notion that the strength of T cell activation and the degree of T cell expansion seem to correlate with the severity of CRS. However, a recent updated subgroup analysis of the Memorial Sloan Kettering Cancer Center trial analyzing 14 B-ALL patients suffering from severe CRS showed no correlation of CRS severity and the lymphodepletion or peak CAR-T cell expansion.

Although there are limited data available concerning the treatment of elderly patients with CAR-T cells, a subgroup analysis of the ZUMA-1 trial indicates that age is not a risk factor for the development of severe CRS and that the treatment of elderly patients is safe. Preexisting inflammation and endothelial activation seem to be associated with the severity of CRS and CAR-T cell administration is strongly discouraged in patients with preexisting inflammation and infection.

Clinical presentation of CRS

Early CRS presents with flu-like symptoms progressing to a condition resembling sepsis ultimately resulting in multiorgan failure. Fever is a hallmark and early sign of CRS, other mild symptoms include headache, rash, arthralgia, and myalgia. Within hours symptoms can progress to hypotension, vascular leakage, DIC, and respiratory failure ultimately affecting nearly every organ system. Typically, the first symptoms occur within hours up to 14 days after CAR-T cell therapy, probably depending on the administered cell dose, tumor burden, and costimulatory domain of the applied CAR-T cell construct. Of note, severe CRS has been linked to delayed hematologic recovery. Table 2 shows the common symptoms of CRS.

Differential diagnoses of CRS

It is vitally important to exclude sepsis and possibly treat infections prophylactically as cancer patients in general and CAR-T cell patients after lymphodepleting chemotherapy specifically are at great risk of infectious diseases, mostly bacterial infections. Moreover, severe CRS was associated with higher risk for infections, probably reflecting a state of immune paralysis after severe CRS. However, CRS cannot be safely distinguished from sepsis using established diagnostic criteria for sepsis (Sepsis-3). This underscores the necessity to perform a thorough infectiology workup and prophylactically treat infections using broad spectrum antibiotics and other anti-infective drugs.

As mentioned above, CRS shares many pathogenetic and clinical similarities with HLH/MA.S like high fever, very high ferritin levels, cytokine profiles, and the importance of macrophages in the pathogenesis. Whereas HLH can be triggered by virus infections, malignancy, autoimmune disease, and acquired immune deficiencies, MAS is most often associated with autoimmune and autoinflammatory diseases. Both conditions are characterized by a dysregulated immune response resulting in a cytokine storm. Hence, HLH/MAS are regarded as a consequence of CRS by some authors regarding CAR-T cells as a trigger of HLH/MAS. However, dysfunctional NK and T cell cytotoxic activity due to homozygous defects in cytolytic pathway genes (HLH) or acquired mutations in these genes (MAS) are critical to these conditions, but have not been shown for CRS suggesting that CRS and HLH/MAS are different pathologies. Nevertheless, CAR-T cells might trigger underlying MAS/HLH.

Tumor lysis syndrome might present with typical CRS symptoms such as fever, acute renal failure, cardiac arrhythmia, and seizures but is usually distinguishable by hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia but might occur concurrent with CRS.

Hypersensitivity reactions typically present with rash and urticaria, fever, dyspnea, hypotension, and gastrointestinal symptoms and eventually cardiorespiratory failure. Unlike in CRS symptoms of true type I reactions occur after repeated

| Symptoms of Cytokine Release Syndrome | General Symptoms | Cardiovascular | Pulmonary | Hematologic | Other Organs |
|--------------------------------------|-----------------|----------------|-----------|-------------|--------------|
| High fever                           | Tachycardia     | Hypoxia        | Pulmonary edema due to vascular leakage | Transaminitis/hepatitis | Acute renal injury |
| Malaise                              | Hypertension    |                |           |             |              |
| Arthralgia/myalgia                    | Cardiac arrhythmias |                |           |             |              |
| Nausea                               | Shock           | Pulmonary edema due to vascular leakage | Acute respiratory distress syndrome |                |
| Headaches                            |                 |                |           |             |              |
| Rash                                 |                 |                |           |             |              |
exposure to the causative agent\textsuperscript{65,66} and often require antihista-
mine or epinephrine therapy.\textsuperscript{67}

**CRS management**

The management of CRS follows a grading and risk-adapted approach.\textsuperscript{33,68} Low-grade CRS is treated symptomatically with antipyretics and fluids. Care should be taken with regard to excessive intravenous fluid replacement in light of the risk of vascular leakage with consecutive pulmonary edema. Screening for infections and antibiotic treatment is mandatory.

Due to its role in disease pathology, the mainstay of CRS therapy has been the selective blockade of IL-6 signaling by the IL-6 receptor antagonist tocilizumab or anti-IL-6 antibody siltuximab,\textsuperscript{11,44,50,69} leading to rapid resolution of CRS symp-
toms, typically within a few hours. Tocilizumab gained European Medicines Agency approval for the treatment of severe CRS in June 2018 and should be considered in moderate and definitively administered in severe CRS cases.

Of note, IL-6 blockade does not seem to negatively influence CAR-T cell function in mice\textsuperscript{26} and humans,\textsuperscript{70,71} nor does it influence prognosis.\textsuperscript{4,39,53,72} In a safety management study of the ZUMA-1 trial, prophylactic administration of tocilizumab at day 2 after CAR-T cells lowered grade \(\geq 3\) incidence of CRS in the 34 patients studied compared to the 101 patients in the main trial (3\% vs 13\%) while not influencing peak CAR-T cell numbers. However, the patients of the safety management cohort were younger and of lower disease stage and international prognostic index (IPI).\textsuperscript{76} Nevertheless, the use of tocilizumab could result in higher IL-6 levels and increase the risk of severe neurotoxicity. In addition, tocilizumab might increase the degree of long-term immunosuppression. Moreover, in rheumatic diseases, a higher incidence of lower intestinal perforations was reported after repetitive administration of tocilizumab\textsuperscript{77} which probably does not occur in the acute setting.

Corticosteroids should be reserved to severe, tocilizumab refractory cases since early studies showed reduced response rates in early case series,\textsuperscript{59,50,78} whereas it did not seem to impact response rates in more recent clinical trials.\textsuperscript{4,33,74}

In refractory cases, TNF-\(\alpha\) blocker, T cell depleting alemtu-
zumab and ATG, IL-1R-based inhibitors (anakinra)\textsuperscript{26} or cyclophosphamide,\textsuperscript{74} ibrutinib,\textsuperscript{79} and GM-CSF inhibition\textsuperscript{46} or cytokine adsorption as in the setting of severe HLH\textsuperscript{80,81} might be of benefit, although evidence is limited to single patient reports. Interestingly, a recent case report showed that hemofiltration could rapidly alleviate symptoms and decrease inflammatory parameters in a 10-year-old B-ALL patient with severe CRS and concurring neurotoxicity refractory to tocilizumab, high-dose steroids, and immunoglobulins. However, tocilizumab was just given once and half of the recommended dose.\textsuperscript{82} Figure 2 shows a grading-guided approach for the management of CRS.

**ICANS epidemiology and clinical features**

Neurotoxicity is one of the most common symptoms in patients treated with CAR-T cells. It was initially frequently referred to as CAR-T CRES. Since the publication of a new consensus grading system with the support of the ASBMT, it is recommended to refer to it as ICANS.\textsuperscript{83} The aim of the consensus was to define and summarize the symptoms and establish an easier grading system which can be applied to all patients regardless of the CAR-T cell product used in the treatment. The symptoms and the presentation of ICANS are varied and can progress from subtle signs as headaches, fatigue, and mild aphasia to more severe and potentially life-threatening presentations including seizures, raised intracranial pressure with cerebral edema, and coma.

![Figure 2. Grading and management of CRS. Considerations and approaches for the grading and management of CRS. The symptoms are divided into grade 1 to grade 4 according to the ASBMT consensus grading.\textsuperscript{83} Tocilizumab: a maximum of 800 mg per dose is recommended; a maximum of 3 doses in 24 hours are recommended. Low-dose corticosteroids: iv, 10 mg dexamethasone every 6 hours or equivalent. High-dose corticosteroids: 1000 mg methylprednisolone every 24 hours or equivalent. CRS = cytokine release syndrome. ASBMT = American Society for Blood and Marrow Transplantation.](image-url)
The onset of ICANS is usually later than CRS (in the ZUMA-1 trial—using CD19-CAR-T cells in DLBCL, PMBCL, transformed FL—the median onset was day 4 after transfusion) and the symptoms usually last longer than CRS symptoms (in the ZUMA-1 trial, the median time of duration was 11 days). The symptoms have been usually graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. Since the publication of the consensus grading, we recommend to use the new ASiMT grading to facilitate the comparison between different clinical centers and to develop standardized treatment procedures for patients developing ICANS throughout all clinical centers.

In the ZUMA-1 trial, 65 patients (64%) developed any neurological event and 37 (37%) developed grade 1 to 2 ICANS graded according to the CTC-AE criteria version 4.03.4 These findings are similar to the ones reported from another trial including patients with acute lymphoblastic leukemia where 33 patients (62%) developed any kind of neurological event and 11 (21%) patients had grade 1 to 2 ICANS (CTC-AE criteria version 4.03). In a trial including patients with relapsed/refractory DLBCL, CLL, and ALL, 53 patients (40%) had any grade of ICANS and 25 (19%) had grade 1 to 2 ICANS (CTC-AE criteria version 4.03). The primary analysis of the JULIET trial, which included patients with relapsed or refractory (r/r) DLBCL and transformed FL reported a lower event rate for any neurological events with just 21 patients (21%) developing any grade of ICANS and just 9 (9%) grade 1 to 2 ICANS (CTC-AE criteria version 4.03). Throughout all clinical trials we also find a high number of patients who developed grade 3 to 4 ICANS. The reported incidences for grade 3 to 4 ICANS are 28% in the ZUMA1 trial, 12% in the JULIET trial and 42% in the patients analyzed by Santomasso et al. A trial including patients with r/r DLBCL, B-ALL, and CLL reported 28 (21%) patients with grade 3 to 4 ICANS all of them graded according to the CTC-AE criteria version 4.03.5 These patients with more severe ICANS showed symptoms like aphasia, hallucinations, depressed level of consciousness, cranial nerve palsies, seizures, and coma requiring mechanical ventilation. Although almost all patients show a full resolution of the symptoms, some trials reported ICANS-associated fatalities. Data showing that patients with a DLBCL and FL developed less severe ICANS than patients with B-ALL indicates that neurotoxicity might differ with the used CAR construct and among different entities. For instance, patients treated by BCMA CAR-T cells for refractory multiple myeloma developed signs of CRS but only few patients showed mild ICANS. Moreover, earlier experiences with blinatumomab also showed significant rates of neurotoxicity indicating that targeting CD19 might be one of the major factors in the development of neurotoxicity. Thus further investigation with CARs targeting epitopes like BCMA or CD20 will be needed to underpin this theory. Patients who develop more severe and potentially life-threatening ICANS usually also show signs of severe CRS and there seems to be a strong correlation between the severity of CRS and the severity of ICANS. Nonetheless, patients can develop ICANS in the absence of CRS. The tumor burden prior to CAR-T cell therapy also seems to be a risk factor for a severe ICANS as well as higher CAR-T cell doses and higher CAR-T cell peak expansion values. A correlation between a systemic inflammation with elevated levels of cytokines and severe ICANS has also been shown. The presence of neurologic comorbidities in patients is also associated with a higher risk for ICANS. Nonetheless, no validated algorithm has been established in order to predict patients who will develop potentially life-threatening ICANS and benefit from an early intervention.

MRI of the brain in some of the patients with severe ICANS showed acute abnormalities like T2/FLAIR changes indicating vasogenic edema and sometimes contrast enhancement pointing toward blood-brain barrier (BBB) disruption. These abnormalities were only found in patients suffering from severe and not mild ICANS. These findings were reversible after resolution of the symptoms indicating that early imaging might be helpful to identify patients at risk for severe ICANS. Nonetheless, 64% of patients with severe ICANS showed normal MRI scans and therefore MRI should just be used as an additional tool to identify patients at risk for severe ICANS.

Continuous EEG (cEEG) showed generalized periodic discharges in patients who developed mild and severe ICANS. The clinical deterioration correlated with peak intensity of GDPs. These patients showed transient electroclinical improvement after treatment with antiepileptic drugs and especially dexamethasone. However, more robust data are needed to implement routine cEEG monitoring for the identification of patients at risk for severe ICANS.

Pathophysiology of ICANS

The pathophysiology of neurologic symptoms in CAR-T cell therapy is poorly understood, but the lack of a strict temporal association with CRS indicates that it might be independent from CRS. In addition, considering IL-6 pathway blockade does not seem to be beneficial in the treatment of neurologic symptoms unlike in CRS indicates a different pathomechanism.

The evaluation of larger patient collectives showed that a high tumor burden and a more severe CRS lead to a more severe ICANS. An analysis showed higher levels for cytokines which are usually associated with a systemic inflammation (i.e., IL-6, IL-10, and IFN-γ) in patients who develop severe ICANS indicating a correlation between systemic inflammation and ICANS. A recently reported trial using a CAR with an incorporated CD8 alpha hinge as compared to a CD28-derived hinge an transmembrane domain showed similar response rates but markedly less grade 3 or 4 ICANS (5% vs 35% in a previous trial) and significantly lower levels of proinflammatory effectors (IL-2, IL-15, INFγ, Granzyme A & B) indicating a major role of CAR design for the induced cytokine profile and the pathophysiology of ICANS.

Another important mechanism for ICANS could be an IL-1 triggered activation of by-standing monocytes which then produce IL-6 and lead to the systemic inflammation mentioned above. This possible mechanism was shown using a xenograft mouse model which also demonstrated amelioration of ICANS symptoms through blockade of IL-1 with anakinra but not through blockade of the IL-6 pathway by administration of tocilizumab. These results support the importance of IL-1 and monocytes in the pathogenesis of ICANS. The role of monocytes was also shown using a similar xenograft model in which the abrogation of GM-CSF signaling either using lenzilumab or GM-CSF knockout mice resulted in a significant reduction of ICANS while the antitumor effect was not suppressed.

Other findings strongly indicate a disruption of the BBB as a possible pathomechanism of ICANS. Higher protein levels in the CSF were found in patients with severe neurotoxicity indicating a dysfunction of the BBB. Some analyses also demonstrated higher CSF/blood ratios for cytokines like IL-6, IFN-γ, and
GM-CSF, indicating either a severe disruption of the BBB with consecutive trespassing of cytokines and leukocytes into the central nervous system (CNS) or intrathecal production of these cytokines which can lead to inflammation of the CNS.37,84,88 Early studies already reported that CAR-T cells could be detected in the CSF after infusion and expansion.50,53,89 A significant correlation with ICANS could just be found in one of these studies which showed higher levels of CAR-T cells in CSF in patients who developed ICANS when compared to patients without neurological symptoms.53 Nevertheless, the studies which did not show a significant correlation had a very small number of patients.50,89

Figure 3. Pathomechanism of ICANS. Shown are some of the discussed pathomechanism for ICANS. (A) Systemic inflammation and expression of IL-1 leads to activation of by-standing monocytes and an expression of different cytokines leading to an aggravation of the systemic inflammation by activation of T cells and macrophages.26 (B-C) A disruption of the blood-brain barrier (BBB) leads to migration of T cells (including CAR-T cells) in the brain parenchyma, and to elevated levels of cytokines and protein in the cerebrospinal fluid leading to an inflammation of the CNS.37,50,53,84,88,89 (D) Endothelial activation shown by elevated Ang-2: Ang-1 ratio aggravates the systemic inflammation and the BBB disruption.37,84 Ang-2 = angiopoetin, BBB = blood-brain barrier, CAR = chimeric antigen receptor, CNS = central nervous system, ICANS = immune effector cell-associated neurotoxicity syndrome, IFN-γ = interferon gamma, IL-1 = interleukin 1, IL-6 = interleukin 6, vWF = von Willebrand factor.
The disruption of the BBB and consequent neuroinflammation and infiltration of T cells into the CNS could also be shown through histopathological findings in a nonhuman primate model but also in an autopsy of a patient who died after developing severe ICANS. Figure 3 shows the proposed pathogenesis of ICANS.

A single-cell analysis of the preinfusion CD19 CAR-T cell products demonstrated a variety of polyfunctional T cell subsets. Applying a T cell polyfunctionality strength index (PSI) showed a statistically significant association of IL-17A PSI and high peaks of CAR-T cell expansion in patients who developed grade ≥ 3 ICANS. These findings suggest that a high number of CAR Th17 cells could lead to a higher incidence of ICANS underscoring the role of different T cell subsets in the response and toxicity mechanisms of CAR-T cell treatment.

Another frequently discussed mechanism that might lead to ICANS is the endothelial activation which might aggravate the BBB disruption. Several studies showed an increase in the Ang-2: Ang1 ratio in patients who develop severe ICANS.

ICANS management

The management of ICANS is based on a grading system. Similar to the management of CRS, patients with mild (grade ≤ 1) ICANS should be closely monitored and receive supportive measures, if needed. For patients who develop more severe ICANS (grade ≥ 2) the transfer to an intermediate care or intensive care unit should be considered. The consultation of a neurologist or neuro-intensivist as well as a lumbar puncture and radiological imaging is crucial for the detection of other causes of neurological symptoms including CNS involvement of the underlying malignancy, infection, intracranial hemorrhage, stroke, and others. As some patients showed GDPs during cEEG monitoring an early cEEG monitoring might be helpful.

The treatment is based on corticosteroids with escalating doses depending on the severity of the ICANS. In most cases, patients respond to steroids, although the median time to the resolution of symptoms is longer for ICANS (median 9 days, range 4–21 days) than it is for CRS. The recommended treatment consists of 10mg dexamethasone every 6 hours until the symptoms stop. In cases of grade 4 toxicities, high doses of methylprednisolone (eg, 1000mg every 24 hours) should be given.

A treatment with tocilizumab is definitely recommended if a concurrent CRS is present. Since many patients with severe ICANS also show symptoms of CRS, a treatment with steroids in combination with tocilizumab should be evaluated especially keeping in mind that the severity of CRS correlates with the presence of ICANS. Nonetheless, the treatment with tocilizumab alone was shown to be ineffective in ICANS in clinical studies as well as in animal models. Prophylactic treatment with tocilizumab had no effect on the incidence of a CRS but does seem to increase the incidence and severity of ICANS as shown in cohort 3 of the ZUMA-1 trial. This effect might be due to the higher blood levels of IL-6 caused by the blockade of the IL-6 receptor.

ICANS grading and management

ICANS grade 1
- awakens spontaneously
- fatigue
- ICE: 7-9 points

ICANS grade 2
- awakens to voice
- delirious/somnolent
- ICE: 3-6 points

ICANS grade 3
- awakens to tactile stimulus
- ICE: 0-2 points
- local edema on imaging
- seizure, that resolves with intervention

ICANS grade 4
- comatose
- ICE: 0
- cerebral edema
- life-threatening (>5min) seizure
- motor weakness

Figure 4. ICANS grading and management. Considerations and approaches for the grading and management of ICANS. The symptoms are graded according to the ASBMT consensus grading Immune Effector Cell-Associated Encephalopathy (ICE) score, is a nonvalidated neurological scoring system to easily assess the neurological status of CAR-T cell patients (items: orientation, naming 3 objects, following easy commands, writing, counting backwards; a maximum score of 10 points indicates no neurological impairment). Low-dose corticosteroids: ie, 10mg dexamethasone every 6 hours or equivalent. High-dose corticosteroids: 1000 mg methylprednisolone every 24 hours or equivalent. A concurrent CRS should be treated additionally as shown in Figure 2. Further treatment should be evaluated individually, that is, anakinra or other experimental approaches as reported previously. ASBMT = American Society for Blood and Marrow Transplantation, CAR = chimeric antigen receptor, CRS = cytokine release syndrome, ICANS = immune effector cell-associated neurotoxicity syndrome.
A prophylactic treatment with antiepileptic drugs can be considered, since some patients showed pathological EEG findings with no clinical signs of a seizure. However, the analysis of cohort 3 of the ZUMA-1 trial did not show any significant benefit for patients who received a prophylactic treatment with levetiracetam.

At this point there is no good clinical evidence for recommendations of treatments for patients unresponsive to high-dose steroids. Treatments with siltuximab (anti-IL-6 chimeric monoclonal antibody) might be effective due to its direct effect on circulating IL-6. Some authors also suggest the use of anakinra (recombinant human interleukin 1 receptor antagonist) since IL-1 seems to play an important role in the development of ICANS and anakinra was shown to be effective in a mouse model. A similar approach could be the inhibition of GM-CSF using lenzilumab to prevent a systemic inflammation caused by monocyte activation. Natalizumab, a humanized monoclonal antibody against the cell adhesion molecule a4-integrin that inhibits the infiltration of T cells into the CNS used in the treatment of multiple sclerosis has been suggested but efficacy data in the human setting is still lacking. Figure 4 shows a grading and management algorithm for ICANS.

Conclusion

Since CAR-T cells are rapidly entering clinical practice, a profound knowledge of the clinical presentation, early identification of patients at risk with appropriate monitoring as well as prompt therapeutic action is an essential requisite of the medical network surrounding cancer patients.

Although remarkable progress in the understanding of CAR-T cell-mediated immunotoxicities has been achieved within the last years, there still is a lack of knowledge especially in the field of CAR-T cell-related neurotoxicity. This is in part reflected by the scarcity of therapeutic alternatives in corticosteroid refractory ICANS cases and consecutively occurring mortality in clinical trials. Thus, clinical and preclinical studies are needed to further deepen our understanding of CAR-T cell-mediated immunotoxicities.

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