

Chimeric antigen receptor T-cell therapy — assessment and management of toxicities

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Abstract | Immunotherapy using T cells genetically engineered to express a chimeric antigen receptor (CAR) is rapidly emerging as a promising new treatment for haematological and non-haematological malignancies. CAR-T-cell therapy can induce rapid and durable clinical responses, but is associated with unique acute toxicities, which can be severe or even fatal. Cytokine-release syndrome (CRS), the most commonly observed toxicity, can range in severity from low-grade constitutional symptoms to a high-grade syndrome associated with life-threatening multiorgan dysfunction; rarely, severe CRS can evolve into fulminant haemophagocytic lymphohistiocytosis (HLH). Neurotoxicity, termed CAR-T-cell-related encephalopathy syndrome (CRES), is the second most-common adverse event, and can occur concurrently with or after CRS. Intensive monitoring and prompt management of toxicities is essential to minimize the morbidity and mortality associated with this potentially curative therapeutic approach; however, algorithms for accurate and consistent grading and management of the toxicities are lacking. To address this unmet need, we formed a CAR-T-cell-therapy-associated TOXicity (CARTOX) Working Group, comprising investigators from multiple institutions and medical disciplines who have experience in treating patients with various CAR-T-cell therapy products. Herein, we describe the multidisciplinary approach adopted at our institutions, and provide recommendations for monitoring, grading, and managing the acute toxicities that can occur in patients treated with CAR-T-cell therapy.

Cellular immunotherapy with autologous or allogeneic T cells genetically engineered to express chimeric antigen receptors (CARs) or T-cell receptors (TCRs), in order to redirect their cytotoxic specificity towards tumour cells, is emerging as a promising new treatment modality for a broad range of cancers^{1,2}. CARs consist of an extracellular domain that can bind specifically to a target molecule expressed on the surface of tumour cells, a transmembrane domain, and an intracellular domain that provides an activation signal to T cells when the extracellular domain is engaged with its target. The extracellular domain usually comprises the antigen-recognition regions of an antibody, in the form of a single-chain variable fragment (scFv); however, other molecules, such as ligands of cell-surface receptors, can

also be used. The intracellular domain usually incorporates a region of the TCR CD3 ζ chain to provide 'signal 1' and one or more domains from co-stimulatory receptors, such as CD28, OX40 (CD134), and/or 4-1BB (CD137), to provide 'signal 2' for T-cell activation. The adoptive T-cell-therapy approach that is most advanced in clinical development is the use of anti-CD19 CAR T cells for the treatment of CD19⁺ B-cell malignancies, including acute and chronic B-cell leukaemias and B-cell non-Hodgkin lymphomas (NHLs). The results of numerous phase I/II clinical trials conducted at single institutions indicate that this approach is typically associated with an overall response rate of 50–90% in patients with B-cell malignancies refractory to standard therapies^{3–20}. More importantly, durable remissions have been noted,

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Key points

- Chimeric antigen receptor (CAR)-T-cell therapy is a promising approach for the treatment of refractory malignancies, but is associated with unique acute toxicities that need specialized monitoring and management
- Cytokine-release syndrome (CRS) and CAR-T-cell-related encephalopathy syndrome (CRES) are the most-common toxicities observed after CAR-T-cell therapy and, rarely, CRS can evolve into fulminant haemophagocytic lymphohistiocytosis (HLH)
- Intensive monitoring, accurate grading, and prompt management of toxicities with aggressive supportive care, anti-IL-6 therapy, and/or corticosteroids for severe cases could reduce the morbidity and mortality associated with CAR-T-cell therapy
- The guidelines proposed could also be used for grading and management of toxicities associated with other redirected-T-cell therapies, such as TCR-gene therapies and bispecific T-cell-engaging antibody (BiTE) therapies

suggesting that this therapeutic strategy can be curative^{3,11,20–22}. The feasibility of central manufacturing of CAR-T-cell therapies and the safety of treatment with cryopreserved CAR-T-cell products has been demonstrated in multicentre clinical trials^{23–28}, with efficacy comparable to that observed in single-institution trials, suggesting that these therapies might soon be broadly available. Several multicentre phase II clinical trials of anti-CD19 CAR T cells are ongoing, with the intent of obtaining regulatory approvals for the treatment of B-cell malignancies. Indeed, on 30th August 2017, the FDA approved the first anti-CD19 CAR-T-cell product, tisagenlecleucel, for the treatment of paediatric and young adult patients with relapsed and/or refractory B-cell precursor acute lymphoblastic leukaemia. In addition, novel targets, such as CD20, NY-ESO-1, and B-cell maturation antigen, are being explored with CAR-based and TCR-redirected cell therapies in preclinical studies and early phase clinical trials, in both haematological and non-haematological malignancies^{1,2,29}.

As adoptive T-cell therapies become more widely used, recognition of their unique toxicities, which are distinct from those seen with traditional chemotherapies, monoclonal antibodies (mAbs), and small-molecule targeted therapies, is of the utmost importance. The two most commonly observed toxicities with CAR-T-cell therapies are cytokine-release syndrome (CRS), characterized by

high fever, hypotension, hypoxia, and/or multiorgan toxicity; and a CAR-T-cell-related encephalopathy syndrome (CRES), typically characterized by a toxic encephalopathic state with symptoms of confusion and delirium, and occasionally seizures and cerebral oedema^{30–33}. Rare cases of fulminant haemophagocytic lymphohistiocytosis (HLH) (also known as macrophage-activation syndrome (MAS)), which is characterized by severe immune activation, lymphohistiocytic tissue infiltration, and immune-mediated multiorgan failure, have also been reported^{24,32,34,35}. Such toxicities have also been observed in patients treated with other redirected-T-cell therapies, such as TCR-gene therapies and bispecific T-cell-engaging antibodies (BiTEs), and preclinically with CAR natural killer (NK) cells^{36–41}. Indeed, both CRS and HLH/MAS have been observed in patients treated with blinatumomab, an anti-CD19/CD3 BiTE⁴². These toxicities are manageable in most patients, although some require monitoring and treatment in the intensive-care setting, and fatalities can occur, as emphasized by the clinical trial experiences reported to date (TABLE 1).

Accurate assessment and prompt management of toxicities can mitigate the adverse outcomes associated with these potentially curative immunotherapies. The overall goal of management is to maximize the benefit from the cellular therapy while minimizing the risk of life-threatening complications, particularly CRS and CRES. In order to develop a consistent approach to the monitoring, grading, and management of toxicities, we have formed a CAR-T-cell-therapy-associated TOXicity (CARTOX) Working Group, with representatives from multiple institutions and multiple medical disciplines, including haematological oncology, solid-tumour oncology, stem-cell transplantation, neurology, critical care, immunology, and pharmaceutical sciences. These investigators were selected based on their extensive experience in treating patients with various CAR-T-cell-therapy products and with other cellular therapies. Collectively, the authors have been involved in treating more than 100 adult patients with leukaemia or lymphoma^{3,4,6,16,17}, using at least four different anti-CD19 CAR-T-cell platforms^{16,23,24,26–28} that were originally developed at academic institutions and subsequently licensed to commercial entities for further clinical development in multicentre trials. Over a period of 6 months, the CARTOX Working Group discussed the available evidence in the literature and their collective experience in treating these patients, and collectively developed recommendations and a practical guide for monitoring, grading, and management of CRS, CRES, and HLH/MAS in adult patients. These recommendations incorporate and expand on the criteria proposed previously by Lee *et al.*³⁰ for the diagnosis and management of CRS arising after treatment with cellular therapies. Herein, we present and discuss the CARTOX management and treatment algorithms, with reference to a representative clinical case.

Clinical case study

A 34-year-old female presented with refractory diffuse large-B-cell lymphoma (DLBCL) that had progressed following standard rituximab, cyclophosphamide,

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Table 1 | Reported causes of death after CAR-T-cell therapies (excluding progressive disease)

Study	Malignancy	Patient age (years)	CAR-T-cell product* (designation or name)	CAR-T-cell dose (per kg)	Day of death after CAR-T-cell infusion	Cause of death [‡]
Morgan <i>et al.</i> (2010) ⁸⁸	Metastatic colon cancer	39	HER2-28-137-ζ	1 × 10 ¹⁰ total cells	5	ARDS
Brentjens <i>et al.</i> (2010) ⁹⁴	CLL	69	CD19-28-ζ (19-28z)	1.2–3.0 × 10 ⁷	2	CRS
Frey <i>et al.</i> (2014) ⁴⁴	B-ALL	>18	CD19-137-ζ (tisagenlecleucel, previously known as CTL019)	6.5 × 10 ⁶	5	CRS (+ Influenza B)
				6.7 × 10 ⁶	15	CRS (+ Pseudomonas sepsis, pneumonia)
				8.4 × 10 ⁶	15	CRS (+ Stenotrophomonas sepsis, pneumonia)
Kochenderfer <i>et al.</i> (2015) ¹¹	PMBCL	30	CD19-28-ζ	2.5 × 10 ⁶	16	Unknown (possibly cardiac arrhythmia)
Chong <i>et al.</i> (2016) ⁹⁵	FL	>18	CD19-137-ζ (tisagenlecleucel)	NA	NA	Encephalitis
Neelapu <i>et al.</i> (2016) ²⁴ (ZUMA-1)	DLBCL	>18	CD19-28-ζ (axicabtagene ciloleucel; axi-cel, also known as KTE-C19)	2 × 10 ⁶	NA	HLH
Locke <i>et al.</i> (2016) ⁹⁶ (ZUMA-1)	NHL	>18	CD19-28-ζ (axi-cel)	2 × 10 ⁶	NA	Cardiac arrest
Turtle <i>et al.</i> (2016) ¹⁷	B-ALL	48	CD19-137-ζ	11.6 × 10 ⁶ CD4 ⁺ + 8.4 × 10 ⁶ CD8 ⁺	3	CRS
		52	CD19-137-ζ	1 × 10 ⁶ CD4 ⁺ + 1 × 10 ⁶ CD8 ⁺	122	Neurotoxicity
Turtle <i>et al.</i> (2016) ¹⁸	NHL	>18	CD19-137-ζ	10 × 10 ⁶ CD4 ⁺ + 10 × 10 ⁶ CD8 ⁺	30	CRS (+ GI bleed)
				10 × 10 ⁶ CD4 ⁺ + 10 × 10 ⁶ CD8 ⁺	13	Neurotoxicity (+ CNS bleed)
ROCKET (2017) ^{64,69}	B-ALL	NA	CD19-28-ζ (JCAR015)	NA	NA	Cerebral oedema (5 cases)
ZUMA-1 (2017) ⁷⁰	NHL	>18	CD19-28-ζ (axi-cel)	NA	NA	Cerebral oedema
Turtle <i>et al.</i> (2017) ¹⁹	CLL	62	CD19-137-ζ	1 × 10 ⁶ CD4 ⁺ + 1 × 10 ⁶ CD8 ⁺	11	Cerebral oedema

ζ, T-cell receptor CD3ζ chain; 137, CD137 (4-1BB); 28, CD28; ARDS, acute respiratory distress syndrome; B-ALL, B-cell acute lymphoblastic leukaemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CNS, central nervous system; CRS, cytokine-release syndrome; DLBCL, diffuse large-B-cell lymphoma; FL, follicular lymphoma; GI, gastrointestinal; HLH, haemophagocytic lymphohistiocytosis; NA, not available; NHL, non-Hodgkin lymphoma; PMBCL, primary mediastinal B-cell lymphoma. *In the format: target antigen/co-stimulatory domain/T-cell-receptor activation domain. ‡Single case, unless otherwise noted.

doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy, and after subsequent salvage therapy consisting of rituximab, ifosfamide, carboplatin, and etoposide (R-ICE), followed by high-dose chemotherapy with autologous stem-cell transplantation (ASCT). After conditioning chemotherapy with cyclophosphamide and fludarabine, the woman was treated with autologous T cells transfected to express an anti-CD19-CAR construct incorporating CD28 and CD3ζ signalling domains²³. Within 24 h of the CAR-T-cell infusion, she developed high-grade fever of up to 39.5 °C, which was associated with tachycardia, fatigue, and decreased appetite that persisted for 6 days (FIG. 1a). The fever was classified as grade 1 CRS according to the criteria proposed by Lee *et al.*³⁰, and was managed with acetaminophen, ibuprofen, and a cooling blanket. She received empiric broad-spectrum antibiotics and growth-factor support for neutropenia — the work-up was negative for

infection. She also developed hypotension with a systolic blood pressure of 84 mmHg (classified as grade 2 CRS³⁰) on day 1 after CAR-T-cell infusion, and hypoxia on day 3 (grade 2 CRS³⁰), which were treated with an intravenous fluid bolus and supplemental nasal oxygen at 3 l/min, respectively. In addition, on days 1 and 3, she received intravenous tocilizumab (8 mg/kg) — a humanized anti-IL-6-receptor (IL-6R) mAb that blocks IL-6 binding to and signalling through its receptor — for the management of hypotension and hypoxia suspected to be caused by CRS, and responded promptly to this treatment. On day 5, she developed handwriting impairment (dysgraphia) and subsequently became confused and disoriented (grade 2 confusion according to the Common Terminology Criteria for Adverse Events version 4.03 (CTCAE v4.03)⁴³. Her dysgraphia was the earliest sign of neurotoxicity, and her contemporaneous mini-mental status examination (MMSE) score was

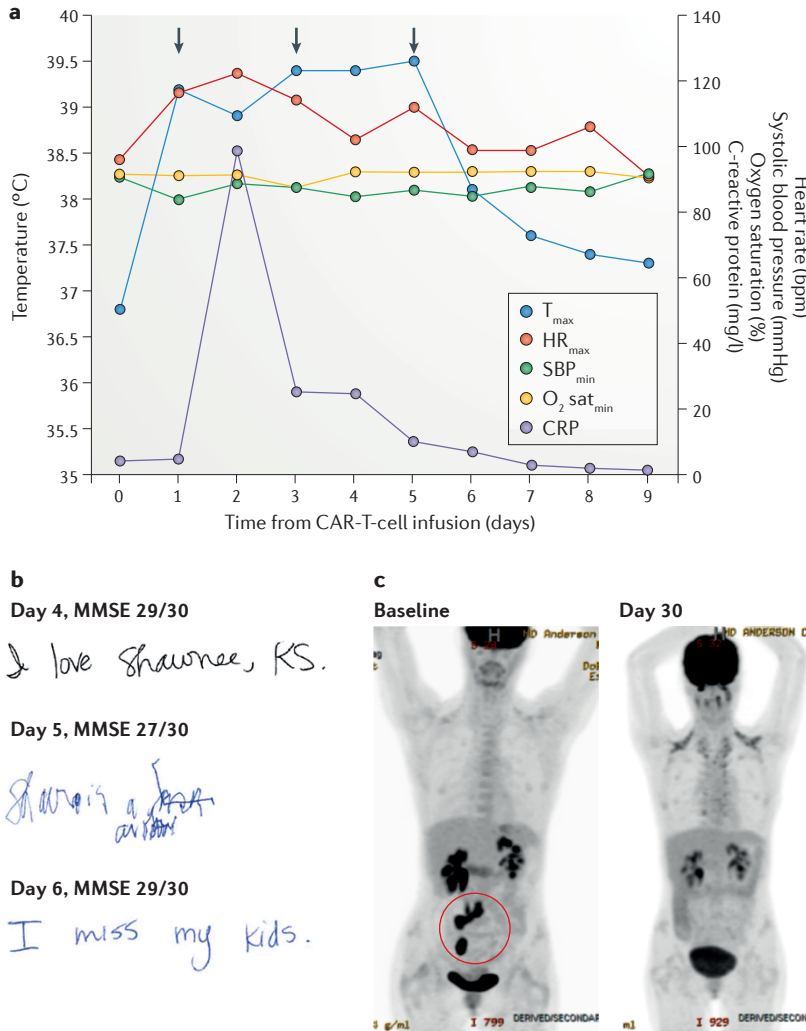


Figure 1 | Clinical case study. The findings of key clinical assessments are shown for a representative patient with cytokine-release syndrome and chimeric antigen receptor (CAR)-T-cell-related encephalopathy syndrome after anti-CD19 CAR-T-cell therapy for refractory diffuse large-B-cell lymphoma. **a** | The graph shows the patient's maximum temperature (T_{max}), maximum heart rate (HR_{max}), minimum systolic blood pressure (SBP_{min}), minimum oxygen saturation ($O_2 sat_{min}$), and serum C-reactive protein (CRP) level recorded on each day after anti-CD19 CAR-T-cell therapy. The anti-IL-6 receptor antibody tocilizumab was administered on days 1, 3, and 5 (arrows) for the treatment of hypotension, hypoxia, and encephalopathy, respectively. **b** | Handwriting samples and mini mental status exam (MMSE) scores obtained on days 4, 5, and 6 after CAR-T-cell therapy; note how the patient's handwriting was markedly impaired on day 5, despite only a small decrease in their MMSE score. **c** | ^{18}F fluoro-2-deoxy-D-glucose PET images showing the retroperitoneal lymph nodes and ileocolic region harbouring lymphoma at baseline (highlighted in red circle; bottom left), and loss of tracer uptake indicative of induction of disease remission at 30 days after infusion of CAR T cells (bottom right).

only mildly decreased compared with that obtained at baseline (FIG. 1b). The neurotoxicity symptoms had resolved by 12 h after treatment with tocilizumab on day 5. Corticosteroids were not administered; tocilizumab was the first-choice treatment for the management of neurotoxicity because she had concurrent CRS symptoms with fever. The patient's serum C-reactive protein (CRP) level increased on day 2, a day after the onset of fever, and returned to baseline levels by the time the fever subsided (FIG. 1a). She was discharged on day 9.

Disease restaging on day 30 revealed that the patient was in complete remission (FIG. 1c), and she remains in remission 12 months later. Peak CAR-T-cell expansion in peripheral blood was observed within 2 weeks of cell infusion and circulating CAR T cells remained detectable at 12 months²³. This case study does not illustrate the most-severe toxicities observed after CAR-T-cell therapy, although it does reflect the most commonly observed clinical scenario. The incidence and severity of toxicities reported varied in different multicentre trials. Whether this variation is because of differences in trial designs, patient populations, disease types, the toxicity grading systems used, or the CAR-T-cell platform is currently unclear. In the pivotal multicentre ZUMA-1 trial of axicabtagene ciloleucel (an anti-CD19-CD28-CD3 ζ CAR also known as axi-cel or KTE-C19) in 101 patients with refractory aggressive B-cell NHL, the rates of grade ≥ 3 CRS and neurological toxicities were 13% and 28%, respectively²⁷. Conversely, in an interim analysis of the JULIET trial of tisagenlecleucel (an anti-CD19-4-1BB-CD3 ζ CAR previously known as CTL019) in 51 patients with relapsed or refractory DLBCL, these rates were 26% and 13%²⁶. Of note, the grading systems for CRS differed between these two trials.

Grading and management of CRS

Pathophysiology of CRS. CRS, the most-common toxicity of cellular immunotherapy, is triggered by the activation of T cells on engagement of their TCRs or CARs with cognate antigens expressed by tumour cells. The activated T cells release cytokines and chemokines (including IL-2, soluble IL-2R α , IFN γ , IL-6, soluble IL-6R, and GM-CSF), as do bystander immune cells, such as monocytes and/or macrophages (which secrete IL-1RA, IL-10, IL-6, IL-8, CXCL10 (IP-10), CXCL9 (MIG), IFN α , CCL3 (MIP-1 α), CCL4 (MIP-1 β), and soluble IL-6R), dendritic cells, and others^{9,11,18,23,24,34}. CRS typically manifests with constitutional symptoms, such as fever, malaise, anorexia, and myalgias, but can affect any organ system in the body, including cardiovascular, respiratory, integumentary, gastrointestinal, hepatic, renal, haematological, and nervous systems³⁰⁻³² (TABLE 2). Patients at high risk of severe CRS include those with bulky disease, comorbidities, and those who develop early onset CRS within 3 days of cell infusion^{9,10,12}; however, the correlation between the development of severe CRS and clinical parameters is imperfect, and identification of predictive biomarkers for severe toxicity is needed. Findings have demonstrated that high serum levels of IL-6, soluble gp130, IFN γ , IL-15, IL-8, and/or IL-10 either before or 1 day after CAR-T-cell infusion are associated with subsequent development of severe CRS^{18,34}, but these results need to be confirmed in prospective studies; notably, the predictive values of these biomarkers seem to vary depending on the type of CAR-T-cell product used^{18,34}.

General precautions and supportive care. The onset of CRS toxicity usually occurs within the first week after CAR-T-cell therapy, and typically peaks within 1-2 weeks of cell administration. In our experience,

Table 2 | Grading of cytokine-release syndrome (CRS)

Symptom or sign of CRS	CRS grade 1*	CRS grade 2*	CRS grade 3*	CRS grade 4*
Vital signs				
Temperature $\geq 38^\circ\text{C}$ (fever)	Yes	Any	Any	Any
Systolic blood pressure < 90 mmHg (hypotension)	No	Responds to IV fluids or low-dose vasopressors	Needs high-dose or multiple vasopressors [§]	Life-threatening
Needing oxygen for $\text{SaO}_2 > 90\%$ (hypoxia)	No	$\text{FiO}_2 < 40\%$	$\text{FiO}_2 \geq 40\%$	Needing ventilator support
Organ toxicities				
<ul style="list-style-type: none"> • Cardiac: tachycardia, arrhythmias, heart block, low ejection fraction • Respiratory: tachypnoea, pleural effusion, pulmonary oedema • GI: nausea, vomiting, diarrhoea • Hepatic: increased serum ALT, AST, or bilirubin levels • Renal: acute kidney injury (increased serum creatinine levels), decreased urine output • Dermatological: rash (less common) • Coagulopathy: disseminated intravascular coagulation (less common) 	Grade 1	Grade 2	Grade 3 or grade 4 transaminitis	Grade 4 except grade 4 transaminitis

Adapted from Lee *et al.*³⁰ The CRS grade should be determined at least twice a day, and whenever a change in the patient's status is observed. ALT, alanine aminotransferase; AST, aspartate aminotransferase; FiO_2 , fraction of inspired oxygen; GI, gastrointestinal; IV, intravenous; SaO_2 , arterial oxygen saturation. *Grade 1 CRS can manifest as fever and/or grade 1 organ toxicity. [†]For grades 2, 3, or 4 CRS, any one of the criteria other than fever is sufficient. [§]High-dose vasopressors are defined as any of the following (as reported by Lee *et al.*³⁰): noradrenaline ≥ 20 $\mu\text{g}/\text{min}$; dopamine ≥ 10 $\mu\text{g}/\text{kg}/\text{min}$; phenylephrine ≥ 200 $\mu\text{g}/\text{min}$; adrenaline ≥ 10 $\mu\text{g}/\text{min}$; if on vasopressin, vasopressin plus noradrenaline equivalent of ≥ 10 $\mu\text{g}/\text{min}$; and if on combination vasopressors (not including vasopressin), noradrenaline equivalent of ≥ 20 $\mu\text{g}/\text{min}$. The noradrenaline equivalent dose is calculated using the VASST trial³⁷ vasopressor equivalent equation: [noradrenaline ($\mu\text{g}/\text{minute}$)] + [dopamine ($\mu\text{g}/\text{kg}/\text{minute}$)/2] + [adrenaline ($\mu\text{g}/\text{minute}$)] + [phenylephrine ($\mu\text{g}/\text{minute}$)/10]. ^{||}Grading of organ toxicities is performed according to Common Terminology Criteria for Adverse Events, version 4.03 (REF. 43).

CRS tends to occur earlier in patients treated with anti-CD19–CD28–CD3 ζ CARs than in those treated with anti-CD19–4-1BB–CD3 ζ CARs. Patient hospitalization with close monitoring is recommended for at least 7 days after CAR-T-cell infusion. Monitoring should include assessment of vital signs at least every 4 h, and daily review of organ systems, physical exam, complete blood count with differential, complete metabolic profile, coagulation profiles, and measurement of serum CRP and ferritin levels (BOX 1). Laboratory tests, including blood counts and chemistry panel, might need to be performed more than once daily, especially for patients at high risk of severe CRS and/or CRES, or those with a high tumour burden, who are at risk of tumour lysis; for the latter group, precautions to avoid tumour lysis should be used, as per standard institutional guidelines. Owing to a high risk of arrhythmias, cardiac monitoring by telemetry is advised from the time of CAR-T-cell infusion until resolution of any emergent CRS symptoms. Additional investigations, such as chest radiography, electrocardiography, echocardiography, electroencephalography (EEG), and imaging studies, can be performed as needed, depending on the toxicities that arise. Daily fluid balance and bodyweights should be strictly monitored, and maintenance intravenous hydration is recommended for all patients with, or at risk of developing, CRS. Indeed, we recommend central venous access, preferably with a double or triple lumen catheter, before CAR-T-cell infusion, to facilitate the timely delivery of any medications needed to manage toxicities. Packed red blood cells and platelets can be transfused according to standard institutional guidelines. Corticosteroids

should be avoided for the management of fever or for premedication before blood transfusions, in order to avoid limiting the effectiveness of CAR-T-cell therapy, but growth-factor support with filgrastim can be provided for neutropenia. Patients who develop fever should be assessed for infection using blood and urine cultures, chest radiography, and additional tests, such as cytomegalovirus PCR, respiratory viral screening, and CT of the chest, as indicated. Such tests should also be performed before initiation of CAR-T-cell therapy when infection is suspected. Therapy with CAR T cells should be delayed until infection has been controlled or ruled out; undiagnosed infections can have catastrophic consequences in patients with CRS, probably owing to exacerbation of systemic inflammation associated with the underlying infection, with deaths reported in clinical trials⁴⁴ (TABLE 1). We recommend conditional orders for fever and hypotension should be put in place for all patients receiving CAR-T-cell infusion, in order that appropriately trained nursing staff can act quickly in the event of toxicities, thereby minimizing delays in intervention (BOX 1). These contingency orders should include empiric broad-spectrum antibiotic therapy, including Gram-negative bacterial coverage, because sepsis and CRS have overlapping symptoms and the absence of positive cultures cannot rule out pathogenic infection in immunocompromised patients with cancer.

IL-6 and IL-6R antagonists. Strong positive correlations of peak blood CAR-T-cell levels and serum IL-6 levels with the severity of CRS after CAR-T-cell therapy have been reported^{9,10,18,24}. IL-6 can signal by

'*cis*-signalling', via direct binding to membrane-bound IL-6R and gp130 complexes, or by '*trans*-signalling', whereby IL-6 binds to soluble IL-6R and the resultant ligand–receptor complex interacts with membrane-bound gp130; both routes lead to activation of JAK/STAT pathway signalling⁴⁵. The expression of membrane-bound IL-6R is restricted to haematopoietic cells, such as macrophages, neutrophils, and T cells, as well as hepatocytes, whereas membrane-bound gp130 is expressed abundantly on all cell types⁴⁶. Thus, *cis*-signalling, which is activated at low levels of

IL-6, affects only a few cell types and mediates anti-inflammatory effects. By contrast, *trans*-signalling predominates at higher levels of IL-6 (as occur in patients with CRS), can affect most cell types, and mediates pro-inflammatory effects⁴⁶. Hence, tocilizumab or the chimeric anti-IL-6 mAb siltuximab have become the drugs of choice for the management of moderate-to-severe CRS^{9,10,30,31,47}. Tocilizumab is approved for the treatment of rheumatoid arthritis⁴⁸, and siltuximab is approved for the management of multicentric Castleman disease⁴⁹; however, both agents have been used off-label for the management of CRS and induce rapid reversal of CRS symptoms in most patients^{9,10,30,31,34,50}. In August 2017, together with the approval of tisagenlecleucel, the FDA also approved tocilizumab for the treatment of CRS occurring after CAR-T-cell therapy. To date, tocilizumab has been used more commonly than siltuximab for the management of CRS, and use of this agent does not seem to affect the efficacy of CAR-T-cell therapy, in terms of overall response rates, complete response rates, or the durability of responses^{9,10,12,23,24,27}. Nevertheless, whether the use of tocilizumab offers advantages over siltuximab treatment for the management of CRS remains unclear. Interestingly, IL-6 binds to IL-6R with an affinity (K_d) of around 1 nM, whereas tocilizumab binds to IL-6R with a K_d of 2.54 nM (REFS 51,52) (Supplementary information S1 (table)); therefore, IL-6 might compete with tocilizumab for binding to IL-6R. By contrast, siltuximab inhibits IL-6 with a K_d of ~1 pM and, thus, IL-6R is unlikely to compete favourably with siltuximab for IL-6 binding. For this reason, siltuximab might be a more-effective treatment than tocilizumab for controlling CRS. In addition, serum IL-6 levels have been shown to increase after administration of tocilizumab, presumably by preventing the IL-6R-mediated uptake of IL-6 into peripheral tissues⁵³; thus, a theoretical concern is that this effect might increase passive diffusion of IL-6 into the central nervous system (CNS) and thereby increase the risk of neurotoxicity. This scenario is unlikely to occur with siltuximab because it binds directly to IL-6. Prospective clinical studies are needed to directly compare the effectiveness of tocilizumab and siltuximab in the treatment of CRS.

Corticosteroids usage. Corticosteroids also suppress inflammatory responses and are, therefore, effective in the management of CRS, CRES, and HLH/MAS associated with cellular therapies^{30–32}. However, because corticosteroids suppress T-cell function and/or induce T-cell apoptosis^{54–56}, use of these drugs should be avoided for other indications (such as premedication for blood transfusions) after adoptive stem-cell therapy. Of note, data from studies in allogeneic stem-cell transplant recipients have demonstrated that cytomegalovirus-specific T cells can persist despite corticosteroid therapy, but have impaired cytokine production⁵⁷. In the setting of cell-based immunotherapy, these findings suggest that corticosteroids will impair the function, if not the persistence, of the infused tumour-directed T cells. Preliminary data from one clinical trial suggest, however, that corticosteroid use for the management of toxicities resulting

Box 1 | Supportive-care considerations for CAR-T-cell therapy

Before and during CAR-T-cell infusion

- Baseline brain MRI to rule out any central nervous system (CNS) disease
- Central venous access, preferably with double or triple lumen catheter, for intravenous fluid and other infusions in case of toxicities
- Cardiac monitoring by telemetry starting on the day of CAR-T-cell infusion and continued until cytokine-release syndrome (CRS) resolves, in order to detect arrhythmias
- Tumour lysis precautions for patients with bulky tumours, as per standard institutional guidelines
- Seizure prophylaxis with levetiracetam at 750 mg orally every 12 h for 30 days, starting on the day of infusion for CAR-T-cell therapies known to cause CAR-T-cell-related encephalopathy syndrome (CRES)
- Hospitalization recommended for at least 7 days after CAR-T-cell therapy

Patient monitoring after CAR-T-cell infusion

- Assess vital signs every 4 h, close monitoring of oral and intravenous fluid input and urine output, and daily measurement of bodyweight
- Daily review of patient history and physical examination
- Daily blood counts, complete metabolic profiling, and coagulation profiling
- C-reactive protein and ferritin levels measured daily, starting on the day of infusion
- Assessment and grading of CRS should be done at least twice daily, and whenever the patient's status changes
- Assessment and grading of CRES using the CAR-T-cell-therapy-associated toxicity 10-point neurological assessment (CARTOX-10; TABLE 4) should be done at least every 8 h
- Maintenance intravenous fluids with normal saline to ensure adequate hydration

Notifications and contingency orders

- The physician should be notified on detection of any of the following: systolic blood pressure (SBP) >140 mmHg or <90 mmHg; heart rate >120 bpm or <60 bpm, or arrhythmia; respiratory rate >25 breaths per min or <12 breaths per min; arterial oxygen saturation <92% on room air; urine output <1,500 ml per day; upward trend in blood creatinine levels or the results of liver function tests; tremors or jerky movements in extremities; change in mental status (alertness, orientation, speech, ability to write a sentence, or CARTOX-10 score)
- For patients with a temperature $\geq 38.3^\circ\text{C}$, order blood cultures (central and peripheral), urinalysis and urine cultures, portable chest radiography, and notify physician
- For patients with neutropenia and fever, start empiric broad-spectrum antibiotics
- Corticosteroids should not be administered unless approved by physician
- If patient develops CRES, withhold oral intake of food, fluids, and medicine, and notify physician
- *Pro re nata* (as needed) medications, acetaminophen (first choice) or ibuprofen (second choice, if not contraindicated), and cooling blanket for fever $\geq 38.3^\circ\text{C}$; normal saline 500–1,000 ml bolus for SBP <90 mmHg, with one repeat if SBP remains <90 mmHg after first bolus
- Anti-IL-6 therapy with tocilizumab or siltuximab to be initiated only on physician order CAR, chimeric antigen receptor.

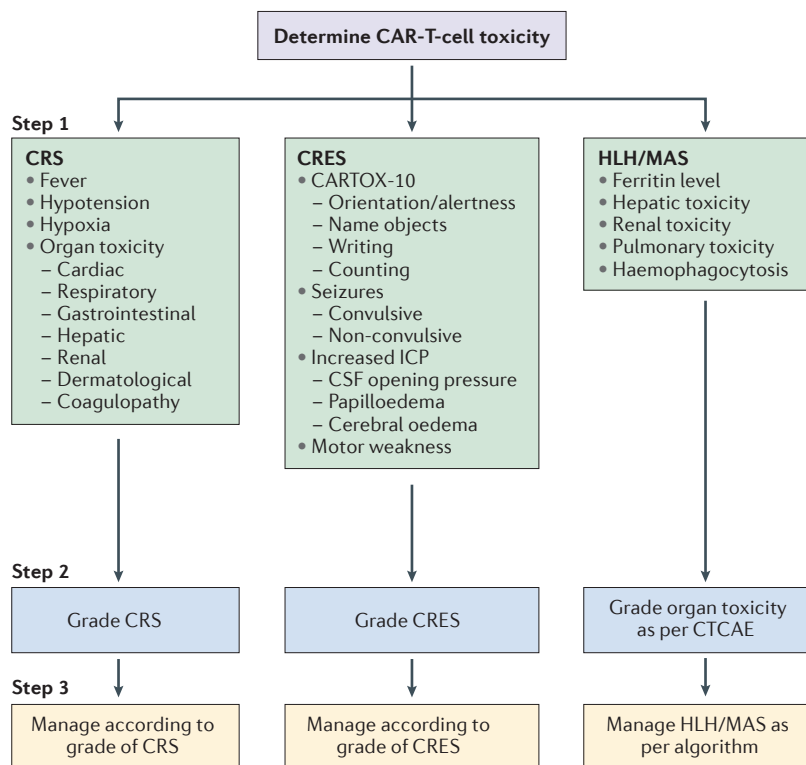


Figure 2 | Three-step approach to the assessment and management of acute toxicities associated with chimeric antigen receptor (CAR)-T-cell therapy. Step 1: the patient’s clinical and biological symptoms should be monitored to determine the nature of the CAR-T-cell-related toxicity, in order to diagnose cytokine-release syndrome (CRS), CAR-T-cell-related encephalopathy syndrome (CRES), and haemophagocytic lymphohistiocytosis/macrophage-activation syndrome (HLH/MAS; BOX 5). Step 2: the severity of CRS, CRES, and HLH/MAS should be graded using the criteria provided in TABLE 2, TABLE 4, and the Common Terminology Criteria for Adverse Events, version 4.03 (CTCAE)⁴³, respectively. Step 3: the toxicities should be treated according to the management algorithms we have provided for CRS (TABLE 3), CRES (BOX 2), and HLH/MAS (FIG. 3). CARTOX-10, CAR-T-cell-therapy-associated toxicity 10-point neurological assessment; CSF, cerebrospinal fluid; ICP, intracranial pressure.

from CAR-T-cell therapy does not affect objective and complete response rates, nor the durability of responses, although whether long-term efficacy is affected remains unknown^{24,27}. Given these concerns, the use of corticosteroids is generally considered only when the toxicities of CAR-T-cell therapy are refractory to anti-IL-6 therapy.

CRS grading. We propose a three-step approach to the management of the major toxicities associated with CAR-T-cell therapy, including CRS: assessment, grading, and treatment (FIG. 2). CRS should be suspected if at least one of the following four symptoms or signs is present within the first 3 weeks of cellular immunotherapy: fever $\geq 38^\circ\text{C}$; hypotension with systolic blood pressure < 90 mmHg; hypoxia with an arterial oxygen saturation of $< 90\%$ on room air; and/or evidence of organ toxicity^{30,31} (TABLE 2). Some of these symptoms and signs can be caused by other concurrent conditions or therapies; therefore, the health-care provider should use careful clinical judgment to determine whether they are attributable to CRS.

The CRS grade should be determined at least twice daily, and at any time when a change in the patient’s status is observed. We recommend that CRS grading is performed using the classification proposed by Lee *et al.*³⁰, with some modifications (TABLE 2). This system is based on four parameters, three of which are vital signs — temperature, blood pressure, and oxygen saturation — and the fourth is the grade of any organ toxicity detected. Grading of organ toxicities is performed according to CTCAE v4.03 (REF. 43). The need for low-dose versus high-dose vasopressor therapy to control hypotension can be used to distinguish grade 2 from grade 3 CRS, according to the definitions of ‘high-dose’ vasopressors previously reported by Lee and colleagues³⁰. Importantly, however, haemodynamic shock in the setting of CRS should be evaluated as a dynamic parameter, and not based on static dose requirements for vasopressors; a patient requiring a rapid increase in the dose of vasopressors, or exhibiting evidence of end-organ hypoperfusion should be treated intensively for grade 3 CRS, even if the vasopressor therapy required is low-dose by definition.

Management of CRS. We recommend management of CRS in accordance with the grade of this toxicity, according to a modified version of the scheme suggested by Lee *et al.*³⁰ (TABLE 3). Grade 1 CRS is primarily managed with supportive care; the use of maintenance intravenous fluids is recommended to keep patients well-hydrated, with special attention to fluid balance in order to avoid pulmonary vascular congestion. In patients with grade 2 CRS, hypotension should be treated promptly with intravenous fluid boluses of 0.9% normal saline. In addition, anti-IL-6 therapy with tocilizumab or siltuximab is recommended for hypotension that is refractory to fluid boluses (with response rates $> 95\%$ based on our experience), and can be repeated if needed (TABLE 3). If hypotension persists, low-dose vasopressors should be initiated and titrated to achieve a systolic blood pressure of > 90 mmHg, and transfer of the patient to an intensive-care unit (ICU) should be considered. Bedside echocardiography to determine ejection fraction is recommended for patients with persistent or repeated episodes of hypotension, because left ventricular dysfunction can occur in patients with CRS³⁰. Moreover, non-invasive monitoring of haemodynamic parameters, such as inferior vena cava filling pressures, passive leg raise, pulse pressure, and stroke volume variation, can help guide the management of hypotension, in terms of the need for intravenous fluids, vasopressors, or inotropic agents. Hypoxia associated with either non-cardiogenic pulmonary oedema or pleural effusions should be managed with supplemental oxygen and diuresis, or thoracentesis, if indicated. Anti-IL-6 therapy is recommended, and can be repeated as needed, for patients with persistent hypoxia at a fraction of inspired oxygen (FiO_2) $< 40\%$ and other grade 2 organ toxicities. Other organ toxicities should be managed symptomatically according to standard guidelines. For patients at high risk of severe CRS (grade 3 or 4), or those with persistent grade 2 CRS despite anti-IL-6 therapy, the use of corticosteroids can be considered (TABLE 3).

Table 3 | Recommendations for the management of cytokine-release syndrome (CRS)

CRS grade	Symptom or sign	Management
Grade 1	Fever or organ toxicity	<ul style="list-style-type: none"> • Acetaminophen and hypothermia blanket for the treatment of fever • Ibuprofen can be used as second treatment option for fever, if not contraindicated • Assess for infection using blood and urine cultures, and chest radiography • Empiric broad-spectrum antibiotics and filgrastim if neutropenic • Maintenance intravenous (IV) fluids for hydration • Symptomatic management of constitutional symptoms and organ toxicities • Consider tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV for persistent (lasting >3 days) and refractory fever
Grade 2	Hypotension	<ul style="list-style-type: none"> • IV fluid bolus of 500–1,000 ml of normal saline • Can give a second IV fluid bolus if systolic blood pressure (SBP) remains <90 mmHg • Tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV for the treatment of hypotension that is refractory to fluid boluses; tocilizumab can be repeated after 6 h if needed • If hypotension persists after two fluid boluses and anti-IL-6 therapy, start vasopressors, consider transfer to intensive-care unit (ICU), obtain echocardiogram, and initiate other methods of haemodynamic monitoring • In patients at high-risk[†] or if hypotension persists after 1–2 doses of anti-IL-6 therapy, dexamethasone can be used at 10 mg IV every 6 h • Manage fever and constitutional symptoms as in grade 1
	Hypoxia	<ul style="list-style-type: none"> • Supplemental oxygen • Tocilizumab or siltuximab ± corticosteroids and supportive care, as recommended for the management of hypotension
	Organ toxicity	<ul style="list-style-type: none"> • Symptomatic management of organ toxicities, as per standard guidelines • Tocilizumab or siltuximab ± corticosteroids and supportive care, as indicated for hypotension
Grade 3	Hypotension	<ul style="list-style-type: none"> • IV fluid boluses as needed, as recommended for the treatment of grade 2 CRS • Tocilizumab and siltuximab as recommended for grade 2 CRS, if not administered previously • Vasopressors as needed • Transfer to ICU, obtain echocardiogram, and perform haemodynamic monitoring as in the management of grade 2 CRS • Dexamethasone 10 mg IV every 6 h; if refractory, increase to 20 mg IV every 6 h • Manage fever and constitutional symptoms as indicated for grade 1 CRS
	Hypoxia	<ul style="list-style-type: none"> • Supplemental oxygen including high-flow oxygen delivery and non-invasive positive pressure ventilation • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
	Organ toxicity	<ul style="list-style-type: none"> • Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
Grade 4	Hypotension	<ul style="list-style-type: none"> • IV fluids, anti-IL-6 therapy, vasopressors, and haemodynamic monitoring as defined for the management of grade 3 CRS • Methylprednisolone 1 g/day IV • Manage fever and constitutional symptoms as in grade 1 CRS
	Hypoxia	<ul style="list-style-type: none"> • Mechanical ventilation • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
	Organ toxicity	<ul style="list-style-type: none"> • Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above

All medication doses indicated are for adults. *Maximum amount of tocilizumab per dose is 800 mg. [†]High-risk patients include those with bulky disease, those with comorbidities, and those who develop early onset CRS within 3 days of CAR-T-cell infusion.

Patients with grade 3 or 4 CRS should be treated in the ICU to enable continuous monitoring, management of life-threatening arrhythmias, haemodynamic shock, non-invasive positive pressure ventilation, mechanical ventilation, and/or dialysis (TABLE 3). Both anti-IL-6 therapy and corticosteroids should be used for the management of grades 3 and 4 CRS, and the associated organ toxicities. Corticosteroid tapering should be individualized depending on the patient’s response and any adverse effects, but is generally recommended to be as rapid as possible. Importantly, the critical care team should be aware of all CAR-T-cell-treated patients in the hospital, in order to facilitate prompt transfers to the ICU, when needed.

Serum CRP levels are a useful marker to monitor in patients undergoing cellular immunotherapy because IL-6 induces the production of CRP by hepatocytes^{58–60}. Thus, an increase in serum CRP level is typically

detected after the onset of CRS (FIG. 1a), and correlates with increased levels of IL-6 (REFS 10,12,18,30,50). Moreover, the return of CRP levels to baseline indicates that the CRS phase of CAR-T-cell therapy has ended (FIG. 1a), and the patient can be considered for discharge from the hospital, assuming other toxicities that require monitoring and/or intervention have resolved. Of note, the correlation between CRP levels and CRS is variable, and is not observed in all patients. The correlation between serum ferritin levels and CRS is even less consistent. Nevertheless, monitoring ferritin levels can be useful for diagnosis of CAR-T-cell-related HLH/MAS.

Grading and management of CRES

Symptoms and signs of CRES. CRES typically manifests as a toxic encephalopathy, with the earliest signs being diminished attention, language disturbance,

Table 4 | Grading of CAR-T-cell-related encephalopathy syndrome (CRES)

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

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

*In the CARTOX-10, one point is assigned for each of the following tasks that is performed correctly (normal cognitive function is defined by an overall score of 10): orientation to year, month, city, hospital, and President/Prime Minister of country of residence (total of 5 points); name three objects — for example, point to clock, pen, button (maximum of 3 points); write a standard sentence, for example, 'our national bird is the bald eagle' (1 point); count backwards from 100 in tens (1 point).

[‡]Papilloedema grading is performed according to the modified Frisén scale⁹⁸.

and impaired handwriting; other symptoms and signs include confusion, disorientation, agitation, aphasia, somnolence, and tremors^{11,17–20,24,26,27,61–63}. In severe cases of CRES (grade >2), seizures, motor weakness, incontinence, mental obtundation, increased intracranial pressure, papilloedema, and cerebral oedema can also occur^{11,17–20,23,61–64}. In our experience, the manifestation of CRES can be biphasic; the first phase occurs concurrently with high fever and other CRS symptoms, typically within the first 5 days after cellular immunotherapy, and the second phase occurs after the fever and other CRS symptoms subside, often beyond 5 days after cell infusion. Notably, delayed neurotoxicity with seizures or episodes of confusion occurred during the third or fourth week after CAR-T-cell therapy in approximately 10% of our patients. We have also observed that anti-IL-6 therapy can reverse CRES during the first phase, but is generally not effective in the second phase, when corticosteroids are the preferred treatment. The differential benefit of anti-IL-6 therapy between the two phases could potentially reflect greater permeability of the blood–brain barrier (BBB) during CRS than at the later post-CRS phase, enabling increased diffusion of the mAb therapeutics into the brain. CRES typically lasts for 2–4 days, but can vary in duration from a few hours to weeks. In general, CRES occurring concurrently with CRS tends to be of shorter duration and lower grade (grade 1–2) than CRES occurring post-CRS, which is more commonly grade ≥3 and protracted. Moreover, the severity of CRES can fluctuate rapidly, thus, necessitating close patient monitoring. CRES is often disturbing to the patient, their families, and the medical staff, but is generally reversible; although, rare fatal cases have occurred^{17,18,34,61} (TABLE 1).

Pathophysiology of CRES. The pathophysiological mechanism underlying CRES remains to be determined. Two potential explanations can be postulated. Firstly, passive diffusion of cytokines into the brain, supported by the finding that high serum levels of IL-6 and IL-15 are associated with severe neurotoxicity in patients treated with CAR-T-cell therapy^{12,24}. Secondly, trafficking of T cells into the CNS, as indicated by the detection

of CAR T cells in cerebrospinal fluid (CSF) from patients with neurotoxicity, in the absence of malignant CNS disease^{5,9,12,33}. Indeed, in one study¹², CAR-T-cell numbers were found to be significantly higher in CSF from patients with versus those without neurotoxicity ($P=0.0039$); the numbers of circulating CAR T cells also tend to be higher in patients who develop neurotoxicity than in those who do not^{5,9,12,33}. Of note, protein levels in the CSF are usually elevated in patients with CRES, compared with baseline measurements, suggesting disruption of the BBB^{62,63}. Other organ dysfunction (hepatic and renal), as well as hypoxaemia, and infection, might also contribute to the encephalopathy.

In patients with CRES, secondary cortical irritation is indicated by EEG findings of epileptiform discharges or non-convulsive electrographic seizures. The most-common finding on EEG, in our experience, is diffuse generalized slowing with or without triphasic waves at 1–2 Hz, in keeping with an encephalopathic state. Non-convulsive electrographic seizures should be defined according to published EEG guidelines and on the basis of response to benzodiazepine treatment^{65,66}; criteria include repetitive epileptiform discharges with a frequency of >2.5 Hz, or multifocal frequent epileptiform discharges responding to intravenous benzodiazepines with organized background activity. The incidence of non-convulsive status epilepticus (NCSE) in patients treated with CAR-T-cell therapy is, in our experience, approximately 10%, with some patients (<5%) developing NCSE after convulsive status epilepticus. Seizure prophylaxis with levetiracetam 750 mg orally or intravenously every 12 h is recommended for 30 days, starting on the day of infusion for patients undergoing CAR-T-cell therapies that are known to cause CRES (BOX 1). Levetiracetam is the preferred agent for seizure prophylaxis because it has a better drug–drug interaction profile and lower risk of cardiotoxicity compared with those of other antiepileptic agents, and can be administered safely to patients with hepatic dysfunction; although, dose adjustments might be needed for those with renal dysfunction⁶⁷. Furthermore, cytokine levels are not affected by levetiracetam treatment⁶⁸. Of note, not all CAR or TCR-engineered T-cell products have

Box 2 | Recommendations for management of CAR-T-cell-related encephalopathy syndrome (CRES)**Grade 1**

- Vigilant supportive care; aspiration precautions; intravenous (IV) hydration
- Withhold oral intake of food, medicines, and fluids, and assess swallowing
- Convert all oral medications and/or nutrition to IV if swallowing is impaired
- Avoid medications that cause central nervous system depression
- Low doses of lorazepam (0.25–0.5 mg IV every 8 h) or haloperidol (0.5 mg IV every 6 h) can be used, with careful monitoring, for agitated patients
- Neurology consultation
- Fundoscopic exam to assess for papilloedema
- MRI of the brain with and without contrast; diagnostic lumbar puncture with measurement of opening pressure; MRI spine if the patient has focal peripheral neurological deficits; CT scan of the brain can be performed if MRI of the brain is not feasible
- Daily 30 min electroencephalogram (EEG) until toxicity symptoms resolve; if no seizures are detected on EEG, continue levetiracetam 750 mg every 12 h
- If EEG shows non-convulsive status epilepticus, treat as per algorithm in BOX 3
- Consider anti-IL-6 therapy with tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV, if CRES is associated with concurrent cytokine-release syndrome (CRS)

Grade 2

- Supportive care and neurological work-up as described for grade 1 CRES
- Tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV if associated with concurrent CRS
- Dexamethasone 10 mg IV every 6 h or methylprednisolone 1 mg/kg IV every 12 h if refractory to anti-IL-6 therapy, or for CRES without concurrent CRS
- Consider transferring patient to intensive-care unit (ICU) if CRES associated with grade ≥ 2 CRS

Grade 3

- Supportive care and neurological work-up as indicated for grade 1 CRES
- ICU transfer is recommended
- Anti-IL-6 therapy if associated with concurrent CRS, as described for grade 2 CRES and if not administered previously
- Corticosteroids as outlined for grade 2 CRES if symptoms worsen despite anti-IL-6 therapy, or for CRES without concurrent CRS; continue corticosteroids until improvement to grade 1 CRES and then taper
- Stage 1 or 2 papilloedema with cerebrospinal fluid (CSF) opening pressure < 20 mmHg should be treated as per algorithm presented in BOX 4
- Consider repeat neuroimaging (CT or MRI) every 2–3 days if patient has persistent grade ≥ 3 CRES

Grade 4

- Supportive care and neurological work-up as outlined for grade 1 CRES
- ICU monitoring; consider mechanical ventilation for airway protection
- Anti-IL-6 therapy and repeat neuroimaging as described for grade 3 CRES
- High-dose corticosteroids continued until improvement to grade 1 CRES and then taper; for example, methylprednisolone IV 1 g/day for 3 days, followed by rapid taper at 250 mg every 12 h for 2 days, 125 mg every 12 h for 2 days, and 60 mg every 12 h for 2 days
- For convulsive status epilepticus, treat as per algorithm in BOX 3
- Stage ≥ 3 papilloedema, with a CSF opening pressure ≥ 20 mmHg or cerebral oedema, should be treated as per algorithm in BOX 4

All medication doses indicated are for adults. CAR, chimeric antigen receptor. *Maximum amount of tocilizumab per dose is 800 mg

been associated with CRES. Thus, for patients undergoing treatment with new agents that have an unknown risk of CRES, seizure prophylaxis can be omitted until data from initial clinical trials have been analysed.

MRI and CT scans of the brain are usually negative for any anatomical pathology that would account for the neurotoxicity symptoms observed in patients treated with CAR-T-cell therapy, although rare cases of reversible T2/fluid attenuated inversion recovery (FLAIR) MRI hyperintensity involving the thalami, dorsal pons, and medulla⁶², and cerebral oedema have been

reported^{19,33,64,64,69,70}. Of note, life-threatening cerebral oedema, although very rare in patients treated with cellular immunotherapy, tends to have a very rapid course with ensuing brain death within 24 h^{19,33,64,64,69,70}. Notably, in March 2017, five deaths attributed to cerebral oedema were reported following treatment of the patients with one anti-CD19 CAR-T-cell product (JCAR015) as part of a multicentre clinical trial^{64,69}. The sponsor has now halted development of this agent^{64,69}. Why deaths relating to cerebral oedema have been observed with certain anti-CD19 CAR-T-cell products^{19,33,64,64,69,70} (TABLE 1),

Box 3 | Recommendations for the management of status epilepticus after CAR-T-cell therapy**Non-convulsive status epilepticus**

- Assess airway, breathing, and circulation; check blood glucose
- Lorazepam* 0.5 mg intravenously (IV), with additional 0.5 mg IV every 5 min, as needed, up to a total of 2 mg to control electrographical seizures
- Levetiracetam 500 mg IV bolus, as well as maintenance doses
- If seizures persist, transfer to intensive-care unit (ICU) and treat with phenobarbital loading dose of 60 mg IV
- Maintenance doses after resolution of non-convulsive status epilepticus are as follows: lorazepam 0.5 mg IV every 8 h for three doses; levetiracetam 1,000 mg IV every 12 h; phenobarbital 30 mg IV every 12 h

Convulsive status epilepticus

- Assess airway, breathing, and circulation; check blood glucose
- Transfer to ICU
- Lorazepam* 2 mg IV, with additional 2 mg IV to a total of 4 mg to control seizures
- Levetiracetam 500 mg IV bolus, as well as maintenance doses
- If seizures persist, add phenobarbital treatment at a loading dose of 15 mg/kg IV
- Maintenance doses after resolution of convulsive status epilepticus are: lorazepam 0.5 mg IV every 8 h for three doses; levetiracetam 1,000 mg IV every 12 h; phenobarbital 1–3 mg/kg IV every 12 h
- Continuous electroencephalogram monitoring should be performed, if seizures are refractory to treatment

All indicated doses of medication are for adult patients. CAR, chimeric antigen receptor. *Lorazepam is the recommended benzodiazepine because it is short-acting, compared with diazepam, and has been widely used in the management of seizures.

but not others, remains unclear. Additional investigations are needed to better understand the pathophysiology of this fatal complication, for which anti-IL-6 therapy is not effective^{9,64}.

Grading of CRES. Similar to other organ toxicities, CRES has been graded according to the CTCAE v4.03 (REF. 43), in terms of level of consciousness, orientation, ability to perform activities of daily living (in the context of encephalopathy), speech, tremors, seizures, incontinence, and motor weakness. The CTCAE grading system does not, however, adequately quantify the acute neurological deficits that seem to be unique toxicities of CAR-T-cell therapies. Thus, we have developed a new grading system for CRES, together with the CARTOX 10-point neurological assessment (CARTOX-10) tool (TABLE 4). We formulated this tool based on our experiences in the observation and treatment of more than 50 adult patients with grade 1–5 neurotoxicity from CAR-T-cell therapy, approximately 50% of whom developed grade ≥ 3 neurological adverse events. The CARTOX-10 incorporates some of the key elements of the 30-point MMSE, encompassing the predominant alterations in concentration, speech, and writing ability that are associated with CRES, to enable evaluation of the acute neurotoxic events observed in patients treated with CAR T cells using a 10-point scale. In the CARTOX-10, one point is assigned for each of the following tasks that is performed correctly: orientation to year, month, city, hospital, and President/Prime Minister of country of residence (total of 5 points); naming three objects (maximum of 3 points); writing a standard sentence (1 point); counting backwards from 100 in tens (1 point). Normal cognitive function is defined by an overall score of 10. In comparison with the MMSE, which is used to screen patients for dementia (not delirium), the CARTOX-10 is simple to use, and can be performed rapidly and

repeatedly several times a day by all health-care providers, including nurses and physicians. The tasks used in the CARTOX-10 can be simplified depending on the education level of the patient, but need to be documented, together with a baseline score, before CAR-T-cell infusion, to ensure that follow-up assessments are reliable and consistent; however, this tool is primarily designed for the assessment of adult patients, and alternative tools need to be developed to assess children. We recommend that the 10-point neurological assessment be performed every 8 h while the patient is hospitalized after CAR-T-cell therapy. Any change from a normal score should prompt thorough investigation as described in the following section of this manuscript. Patients who are aphasic (CARTOX-10 score of 0), but awake/arousable and without other neurological symptoms or signs (such as motor weakness, seizures and papilloedema), are considered to have grade 3 CRES.

In addition to the CARTOX-10, parameters including papilloedema, CSF opening pressure, and imaging assessment were incorporated into our CRES grading system (TABLE 4), in order to detect signs of raised intracranial pressure and cerebral oedema. In contrast with the CTCAE v4.03 (REF. 43), seizures are upgraded to a grade 3 or 4 adverse event in the proposed CRES grading system (TABLE 4). Thus, the advantages of this grading system over the CTCAE include greater objectivity and ease of application.

Management of CRES. Similar to CRS, the management of CRES is based on the toxicity grade (BOX 2). Grade 1 CRES is primarily managed with supportive care. The head of the patient's bed should be elevated by at least 30 degrees to minimize aspiration risks and to improve cerebral venous flow. A neurology consultation should be requested for thorough neurological evaluation, including EEG and fundoscopic

examination to rule out papilloedema, of all patients with CRES, regardless of grade. Assessment for papilloedema can be difficult in restless patients with non-dilated pupils. Neuroimaging and CSF opening pressure, if available, are much better surrogates of increased intracranial pressure and possible cerebral oedema than papilloedema; however, lumbar puncture might also be infeasible when patients are restless or have coagulopathy. In patients with an ommaya reservoir, opening pressure can be measured in the supine position with the base of the manometer placed at heart level. Combinations of these techniques should be considered to diagnose increased intracranial pressure and cerebral oedema. In particular, repeated neuroimaging, preferably with review of the results by a neuroradiologist, is recommended to detect early signs of cerebral oedema in patients with grade 3 or 4 CRES, and in patients with rapid changes in the CRES grade (increase in grade by two levels, for example, grade 1 CRES worsening to grade 3). The clinical status of the patient often dictates the choice of neuroimaging modality: MRI of the brain is preferred, but cannot be performed for unstable or agitated patients, whereas CT can be. In our experience (relating to four cases in ~50 patients with CRES), the development of cerebral oedema in patients treated with CAR T cells is associated with other acute and clinically significant neurological changes, such as a low CARTOX-10 score and/or seizures.

Anti-IL-6 therapy is recommended for patients with grade ≥ 1 CRES with concurrent CRS; if not associated with CRS, corticosteroids are the preferred treatment for grade ≥ 2 CRES, and can be tapered after improvement of CRES to grade 1 (BOX 2). The optimal

duration of corticosteroid therapy remains unknown, although in our experience, short courses of steroids have been associated with resolution of neurological toxicities without impaired antitumour responses^{24,27}. Patients should be monitored closely for recurrence of neurotoxicity symptoms during corticosteroid tapering. Monitoring in the ICU is recommended for patients with grade 3 CRES, and is required for all patients with grade 4 CRES because they might need mechanical ventilation for airway protection (BOX 2). Non-convulsive and convulsive status epilepticus in these patients should be managed with benzodiazepines and additional antiepileptics (preferably with levetiracetam), as needed (BOX 3). The response of some patients to benzodiazepine is rapid, with improvements in both EEG findings and mental status. After levetiracetam, phenobarbital is the preferred second antiepileptic for the management of CRES-related seizures: phenytoin and lacosamide are associated with higher risks of cardiovascular adverse effects, therefore, their use in patients with concurrent CRS should be excluded to avoid arrhythmias and hypotension. Grade 3 CRES with raised intracranial pressure (TABLE 4) should be managed promptly with corticosteroids and acetazolamide (BOX 4); patients who develop grade 4 CRES with cerebral oedema (TABLE 4) should receive high-dose corticosteroids, hyperventilation, and hyperosmolar therapy (BOX 4).

CAR-related HLH grading and management

HLH/MAS encompasses a group of severe immunological disorders characterized by hyperactivation of macrophages and lymphocytes, proinflammatory cytokine production, lymphohistiocytic tissue infiltration,

Box 4 | Recommendation for management of raised intracranial pressure (ICP) after CAR-T-cell therapy

Stage 1 or 2 papilloedema* with cerebrospinal fluid (CSF) opening pressure of <20 mmHg without cerebral oedema

- Acetazolamide 1,000 mg intravenously (IV), followed by 250–1,000 mg IV every 12 h (adjust dose based on renal function and acid–base balance, monitored 1–2 times daily)

Stage 3, 4, or 5 papilloedema*, with any sign of cerebral oedema on imaging studies, or a CSF opening pressure of ≥ 20 mmHg

- Use high-dose corticosteroids with methylprednisolone IV 1 g/day, as recommended for grade 4 CAR-T-cell-related encephalopathy syndrome (CRES; BOX 2)
- Elevate head end of the patient's bed to an angle of 30 degrees
- Hyperventilation to achieve target partial pressure of arterial carbon dioxide (PaCO_2) of 28–30 mmHg, but maintained for no longer than 24 h
- Hyperosmolar therapy with either mannitol (20 g/dl solution) or hypertonic saline (3% or 23.4%, as detailed below)
 - Mannitol: initial dose 0.5–1 g/kg; maintenance at 0.25–1 g/kg every 6 h while monitoring metabolic profile and serum osmolality every 6 h, and withhold mannitol if serum osmolality is ≥ 320 mOsm/kg, or the osmolality gap is ≥ 40
 - Hypertonic saline: initial 250 ml of 3% hypertonic saline; maintenance at 50–75 ml/h while monitoring electrolytes every 4 h, and withhold infusion if serum Na levels reach ≥ 155 mEq/l
 - For patients with imminent herniation: initial 30 ml of 23.4% hypertonic saline; repeat after 15 min, if needed
- If patient has ommaya reservoir, drain CSF to target opening pressure of <20 mmHg
- Consider neurosurgery consultation and IV anaesthetics for burst-suppression pattern on electroencephalography
- Metabolic profiling every 6 h and daily CT scan of head, with adjustments in usage of the aforementioned medications to prevent rebound cerebral oedema, renal failure, electrolyte abnormalities, hypovolemia, and hypotension

All medication doses indicated are for adults. CAR, chimeric antigen receptor. *Papilloedema grading should be performed according to the modified Frisén scale⁹⁶.

Box 5 | Diagnostic criteria for CAR-T-cell-related HLH/MAS

A patient might have HLH/MAS if he/she had a peak serum ferritin level of >10,000 ng/ml during the cytokine-release syndrome phase of CAR-T-cell therapy (typically the first 5 days after cell infusion) and subsequently developed any two of the following:

- Grade ≥3 increase in serum bilirubin, aspartate aminotransferase, or alanine aminotransferase levels*
- Grade ≥3 oliguria or increase in serum creatinine levels*
- Grade ≥3 pulmonary oedema*
- Presence of haemophagocytosis in bone marrow or organs based on histopathological assessment of cell morphology and/or CD68 immunohistochemistry

CAR, chimeric antigen receptor; HLH, haemophagocytic lymphohistiocytosis; MAS, macrophage-activation syndrome. *Grading as per Common Terminology Criteria for Adverse Events, version 4.03 (REF. 43)

and immune-mediated multiorgan failure^{71,72}. These disorders have similar clinical manifestations, irrespective of the underlying cause. Patients with CRS after CAR-T-cell therapy have clinical features and laboratory findings that resemble those of HLH/MAS, including high fever; multiorgan dysfunction; CNS disturbances; high serum levels of ferritin, lactate dehydrogenase, soluble CD25, and cytokines (such as IFN γ and IL-6); and low serum levels of fibrinogen^{24,32,34,35,71–73}. Thus, CRS and HLH/MAS might belong to a similar spectrum of systemic hyperinflammatory disorders. Whereas patients with CRS usually respond to supportive care, anti-IL-6 therapies, and corticosteroid treatment, fulminant and refractory HLH/MAS, in our experience, is observed in ~1% of all patients treated with CAR-T-cell therapy, and necessitates additional therapy. Indeed, refractory HLH/MAS is associated with high mortality if not treated promptly^{74,75}; however, the diagnosis of HLH/MAS can be difficult in the context of CRS. Many of the traditional diagnostic criteria for HLH/MAS — fever, splenomegaly, cytopenias in at least two of three haematopoietic cell lineages (red blood cells, white blood cells, and platelets), hypertriglyceridemia or hypofibrinogenemia with elevated D-dimers, haemophagocytosis in bone marrow, hyperferritinaemia, high levels of soluble CD25, and low or absent NK-cell activity — are not specific. Indeed, these features are frequently present in patients with even low-grade CRS and also in those with advanced-stage haematological malignancies in the absence of CAR-T-cell therapy⁷⁶. Thus, new criteria are needed for the diagnosis of HLH/MAS in patients with CRS after CAR-T-cell therapy.

We propose that a diagnosis of CAR-T-cell-related HLH/MAS should be made if the patient has had peak ferritin levels of >10,000 ng/ml during the CRS phase (typically within the first 5 days after cell infusion), and has developed any two of the following: grade ≥3 organ toxicities involving the liver, kidney, or lung, or haemophagocytosis in the bone marrow or other organs (BOX 5). Patients with suspected HLH/MAS should be managed with anti-IL-6 therapy and corticosteroids for grade ≥3 organ toxicities as per the CRS recommendations (TABLE 3; FIG. 3). Cytokine-directed therapy has been demonstrated to

reverse HLH/MAS arising in a patient after blinatumomab⁴². If the patient has no improvement clinically or serologically within 48 h, additional therapy with etoposide 75–100 mg/m² should be considered, as the available evidence in contexts other than CAR-T-cell therapy indicates that this agent is the preferred treatment for refractory HLH^{71,74,77}. Moreover, this agent can be used in patients with liver and kidney dysfunction^{71,74,77}. Indeed, rapid initiation of etoposide therapy, in spite of organ dysfunction, is imperative for patients with high probability of a HLH diagnosis⁷⁶, owing to the high risk of death^{74,75}. Etoposide can be repeated after 4–7 days, as indicated clinically or serologically, to achieve adequate disease control. Intrathecal cytarabine, with or without hydrocortisone, should also be considered for patients with HLH-associated neurotoxicity (FIG. 3). Although etoposide and cytarabine are often used in the treatment of familial and malignancy-associated HLH^{71,74,77}, at present, direct evidence to support their use in patients with CAR-T-cell-associated HLH is lacking.

Broadly, the goal of therapy for HLH is to suppress overactive CD8⁺ T cells and macrophages that orchestrate this immunological syndrome; however, the current treatments do not specifically target these cell types. In the near future, specific cytokines that play a central part in HLH/MAS, such as IFN γ , will

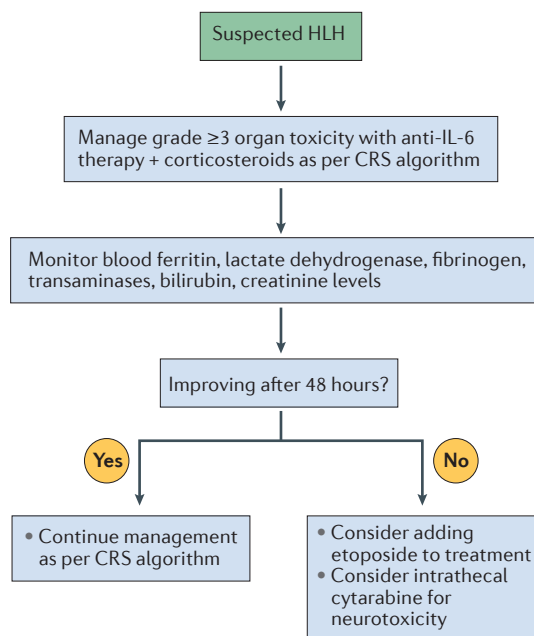


Figure 3 | Recommendations for the management of Chimeric antigen receptor (CAR)-T-cell-related haemophagocytic lymphohistiocytosis/macrophage-activation syndrome (HLH/MAS). HLH/MAS should initially be managed according to the guidelines for cytokine-release syndrome (CRS; TABLE 3), with appropriate subsequent laboratory testing to monitor response to treatment. If the results of these tests reveal no improvement within 48 h, escalation of treatment should be considered.

probably be targetable using agents in clinical development. For example, a humanized anti-IFN γ mAb, NI-0501, produced responses in nine of 13 children with refractory primary HLH (69%), with good tolerability⁷⁸.

Future directions

CAR-T-cell therapies offer the promise to improve clinical outcomes and induce remissions in patients with refractory cancers; however, the unique acute toxicities of these agents, which can be fatal, require intensive monitoring and prompt management. Many factors probably affect the onset, peak level, duration, and type of acute toxicity that will occur after treatment with various CAR-T-cell products, and this variability should be considered when monitoring and treating each patient. Such factors might include: the nature of the conditioning chemotherapy; the design of the CAR construct; CAR-T-cell dose; the cellular composition of the CAR-T-cell product; the manufacturing process used to generate the CAR T cells; and host characteristics, including the type of malignancy, tumour burden, patient age, and sites of disease.

Systematic investigations are necessary to define predictors of efficacy and toxicity, and to determine whether current interventions, such as anti-IL-6 therapies and corticosteroids, affect the efficacy of CAR-T-cell therapy. Such studies might also identify novel biomarkers of severe toxicity and lead to the development of prophylactic strategies to further improve the safety of treatment. Indeed, the peak level of serum IFN γ after CAR-T-cell therapy is positively correlated with the severity of CRS^{12,17,18,34}; therefore, IFN γ could potentially be another important therapeutic target for the management of CRS in the future, although the possibility that blocking this cytokine will affect antitumour efficacy is a concern. Similarly, peak levels of serum IL-6 are associated with severe CAR-T-cell-related neurotoxicity^{12,17,18,24,63}; as IL-6 antagonists are not expected to cross the BBB, however, investigation of intrathecal administration of these agents is warranted for the management of CRS. Other approaches that are currently being tested in preclinical and clinical studies to improve the safety of CAR-T-cell therapies include the integration of genetic constructs containing 'safety (suicide) switches' or 'elimination genes' that can be activated or targeted in order to eliminate the CAR T cells when life-threatening toxicities develop^{50,79–85}. An alternative strategy is to use 'remote-controlled' CARs, whereby an inducible gene-regulatory system enables controlled expression of CARs upon drug administration⁸⁶.

Whereas such approaches might enhance the safety of CAR-T-cell therapies, combination strategies with immune-checkpoint blockade have the potential to increase the antitumour activity and persistence CAR T cells, but also to increase their toxicity. Furthermore, CAR-T-cell therapies will probably be associated with 'on-target, off-tumour' effects if the target antigen is expressed on non-malignant cells. This situation has been well recognized with anti-CD19 CAR-T-cell therapy, which

can cause protracted B-cell aplasia and hypogammaglobulinemia^{3,9,11,22,23}. Non-malignant B cells are expendable, however, because hypogammaglobulinaemia is easily corrected with intravenous immunoglobulin replacement therapy. By contrast, if the target antigen for CAR T cells is present in non-malignant tissues of vital organs, treatment with these agents might lead to severe and possibly fatal toxicity⁸⁷. Indeed, this possibility of on-target, off-tumour toxicity is the greatest obstacle for the successful development of CAR-T-cell therapies for solid malignancies. This limitation has been underscored by the report of fatal respiratory failure and multiorgan dysfunction in a patient treated with HER2-specific CAR T cells (TABLE 1), which was hypothesized to be an on-target, off-tumour effect resulting from expression of the target antigen in lung tissue⁸⁸; however, this death might have been related, at least in part, to the high dose of CAR T cells used (a total of 1×10^{10} cells were infused)⁸⁸, because lower-dose HER2-target CAR-T-cell therapy (1×10^4 – 1×10^8 /m² cells) has been demonstrated to be tolerable in another trial⁸⁹. Other factors, such as the omission of lymphodepleting chemotherapy, non-inclusion of supplementary IL-2, the absence of a 4-1BB co-stimulatory domain in the CAR molecule, or the use of a different anti-HER2 scFv, might also have contributed to the lower toxicity observed in the latter trial⁸⁹. In other clinical studies, the treatment of patients with CAR T cells targeting carcinoembryonic antigen caused colitis⁹⁰, and the use of CAR T cells targeting carbonicanhydrase-IX resulted in cholestasis^{91,92}. An acute hypersensitivity reaction with anaphylaxis has also been described in one patient who received multiple infusions of mesothelin-specific CAR T cells⁹³; however, the researchers concluded that this adverse effect was attributable to the development of human anti-mouse antibodies targeting the murine mAb components of the CAR construct⁹³. Together, these observations emphasize that vigilant monitoring and expectation of the unexpected is necessary when evaluating any novel CAR-T-cell or transgenic TCR-based therapy, including evaluations of novel conditioning regimens, novel CAR or TCR constructs, novel targets, and novel treatment combinations. We also advocate the development of customized tracking tools in electronic health-record systems to monitor and grade these toxicities (Supplementary information S2 (table)).

Conclusions

The recommendations provided herein are meant to serve as a framework for the assessment and management of toxicities associated with CAR-T-cell therapies, but can also be used for TCR-gene therapies, CAR-NK-cell therapies, and potentially BiTE therapies that can cause similar adverse events^{36–42}. While appropriate given our current knowledge and experience, these recommendations are expected to be modified and evolve as we increase our understanding of the pathophysiology of these toxicities, the determinants of durable antitumour responses, and the effects of interventions used to manage toxicities of these promising novel therapies.

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Author contributions

S.S.N. and S.T. contributed equally to this article, and wrote the manuscript. S.S.N., S.T., N.J., N.D., A.M.G., and S.A. contributed to researching data for article. All authors contributed to discussions of content, to the development of the proposed guidelines, and to review/editing of the manuscript before submission.

Competing interests statement

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