Basic Concepts and Indications of CAR T Cells

Jana van den Berg[1,2] Heinz Läubli[2,3,4] Nina Khanna[2,3,5] Lukas T. Jeker[2,3,6] Andreas Holbro[1,2,7]

[1] Division of Hematology, University Hospital Basel, Basel, Switzerland
[2] Innovation Focus Cell Therapies, University Hospital Basel, Basel, Switzerland
[3] Department of Biomedicine, University of Basel, Basel, Switzerland
[4] Division of Medical Oncology, University Hospital Basel, Basel, Switzerland
[5] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
[6] Transplantation Immunology and Nephrology, University Hospital Basel, Basel, Switzerland
[7] Regional Blood Transfusion Service, Swiss Red Cross, Basel, Switzerland

Address for correspondence Prof. Dr. med. Andreas Holbro, Division of Hematology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland (e-mail: andreas.holbro@usb.ch).

Hamostaseologie 2025;45:14–23.

Abstract

Chimeric antigen receptor (CAR) T cell therapy has revolutionized cancer immunotherapy, particularly for hematological malignancies. This personalized approach is based on genetically engineering T cells derived from the patient to target antigens expressed —among others—on malignant cells. Nowadays they offer new hope where conventional therapies, such as chemotherapy and radiation, have often failed. Since the first FDA approval in 2017, CAR T cell therapy has rapidly expanded, proving highly effective against previously refractory diseases with otherwise a dismal outcome. Despite its promise, CAR T cell therapy continues to face significant challenges, including complex manufacturing, the management of toxicities, resistance mechanisms that impact long-term efficacy, and limited access as well as high costs, which continue to shape ongoing research and clinical applications. This review aims to provide an overview of CAR T cell therapy, including its fundamental concepts, clinical applications, current challenges, and future directions in hematological malignancies.

Keywords
- chimeric antigen receptor T cell therapy
- adoptive cellular therapy
- immunotherapy
- lymphoma
- leukemia

Introduction

Chimeric antigen receptor (CAR) T cell therapy has revolutionized cancer treatment, particularly for hematological malignancies. By genetically engineering a patient's T cells to express receptors that target specific (e.g., tumor) antigens, CAR T cell therapy enables the immune system to effectively target and destroy cancer cells, often leading to long-term remissions in refractory or relapsed disease.

The concept of utilizing the immune system to fight malignancies dates back many decades but gained clinical traction in the 21st century with advancements in understanding the basis of the immune system, genetic engineering, and immune modulation. Early approaches to stimulate the immune system include Coley's therapy,[1] allogeneic stem cell transplantation,[2] the use of vaccines such as Bacillus Calmette-Guérin (BCG; Food and Drug Administration [FDA] approved in 1990 for bladder cancer), and cytokines like interleukin-2 (FDA-approved in 1998 for metastatic melanoma).[3] Tumor-infiltrating lymphocytes (TILs) were introduced in the 1990s of the last century and finally FDA-approved in 2024 for metastatic melanoma.[4,5] More recent developments include immune checkpoint inhibitors and early CAR T cell developments, offering a unique approach by directly engineering T cells to attack cancer cells. Since 2017, different CAR T cells targeting either CD19 or B cell maturation antigen (BCMA) in B cell malignancies have

received
October 16, 2024
accepted after revision
November 25, 2024

Georg Thieme Verlag KG,
Oswald-Hesse-Straße 50,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-2491-3652.
ISSN 0720-9355.

been approved as commercial products by the *FDA* and the *European Medicines Agency* (EMA). This led to significant improvements in outcomes for patients with acute B cell lymphoblastic leukemia (B-ALL), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), and multiple myeloma (MM). The impressive results of the pivotal phase II trials that led to their approval were later confirmed in randomized trials and real-world evidence studies.[6–8]

The clinical use of CAR T cell therapies in Europe has grown rapidly, with a 10-fold increase between the years 2018 and 2022.[9] However, despite their successes, CAR T cell therapy faces challenges such as prevention and management of toxicity, complex and time-consuming manufacturing, limited accessibility, and CAR T cell failure due to limited expansion, persistence, or the emergence of resistance mechanisms influencing clinical applications and further driving research in the field.

In this review, we aim to outline the fundamental concepts of CAR T cell therapy and clinical indications, discuss toxicity management, and raise awareness for the growing population of patients receiving these treatments.

History, Development, and Manufacturing of CAR T Cell Therapy

Conventional T cells play a critical role in the immune system by recognizing and destroying infected or malignant cells. Their T cell receptor (TCR) binds to antigens presented by the major histocompatibility complex (MHC) on target cells. T cell activation is driven by costimulatory receptors such as CD28 (►Fig. 1, bottom left). Cancer cells often evade detection by downregulating MHC molecules or an immunosuppressive tumor microenvironment, making them less accessible to T cells. To overcome this and to enable antigen-specific but HLA-independent targeting, T cells were engineered with artificial receptors, the so-called CARs, containing domains of a B cell receptor, i.e., the single-chain variable fragment (scFv) of an antibody (►Fig. 1, top left) to recognize target antigens independently of MHC presentation.[10]

The first generation of functional CARs was described as a proof of concept by Eshhar and colleagues in 1989[11] and combined the extracellular antigen-binding domain of an antibody with the intracellular CD3ζ signaling domain of the TCR (►Fig. 2). These CARs exhibited T cell response to target specific tumor antigens independently of MHC presentation, but displayed limited efficacy due to a relatively short persistence.[3,11,12] Second-generation (2G) CARs addressed these limitations by incorporating a costimulatory domain, such as CD28 or 4–1BB, alongside CD3ζ (►Fig. 2).[12–14] After their landmark clinical application by Carl June's group,[12,14] and subsequent clinical trials (discussed later), second-generation CARs have hence become the prototype of all six commercially available CAR T products to date. Building on second-generation CARs, third-generation (3G) CARs incorporate two costimulatory domains (►Fig. 2; e.g., CD28 and 4–1BB) to further enhance CAR T cell expansion and persistence. In one of the few clinical trials directly comparing 2G and 3G CAR T cells targeting CD19, Ramos and colleagues reported promising results in their first 16 patients of the still ongoing trial (NCT01853631).[15] However,

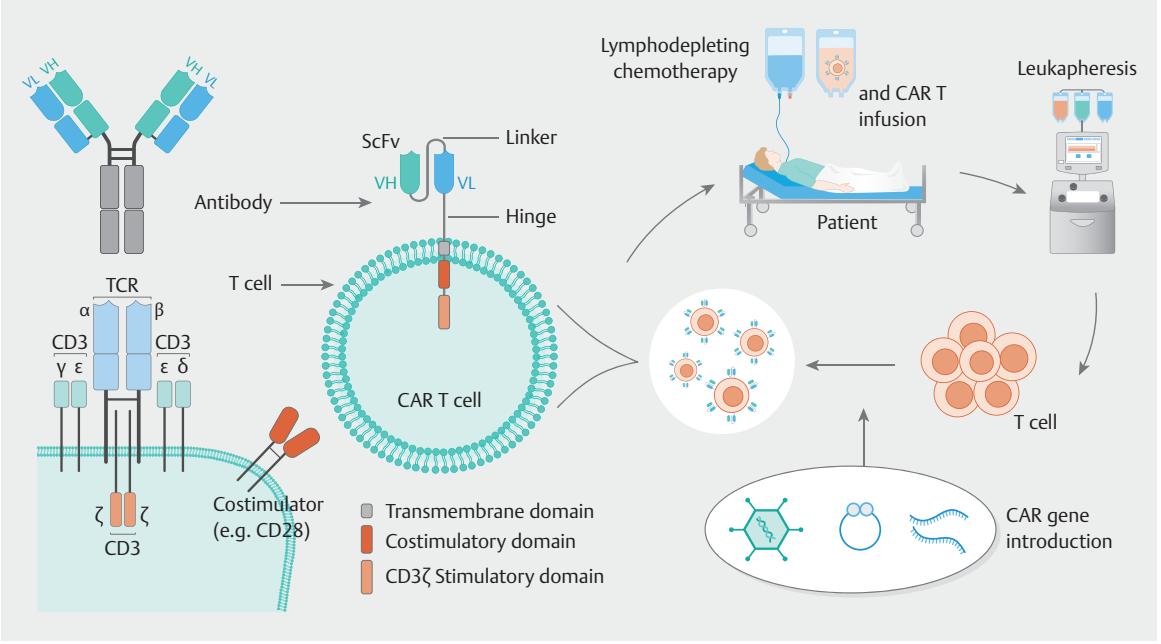


Fig. 1 CAR T structure and manufacturing. The general structure of CAR T cells (middle) integrates the single-chain variable fragment (scFv) of an antibody (top left) via a hinge and transmembrane domain with engineered intracellular domains derived from T cells (bottom left). The manufacturing of autologous CAR T cells starts with leukapheresis. Following selection and activation, the genetic material encoding the CAR is delivered into the T cells, by viral vectors, transposon systems, or mRNA transfection. After expansion and quality controls (not shown), the patient receives lymphodepleting chemotherapy followed by CAR T cell infusion. (Created with BioRender.com.)

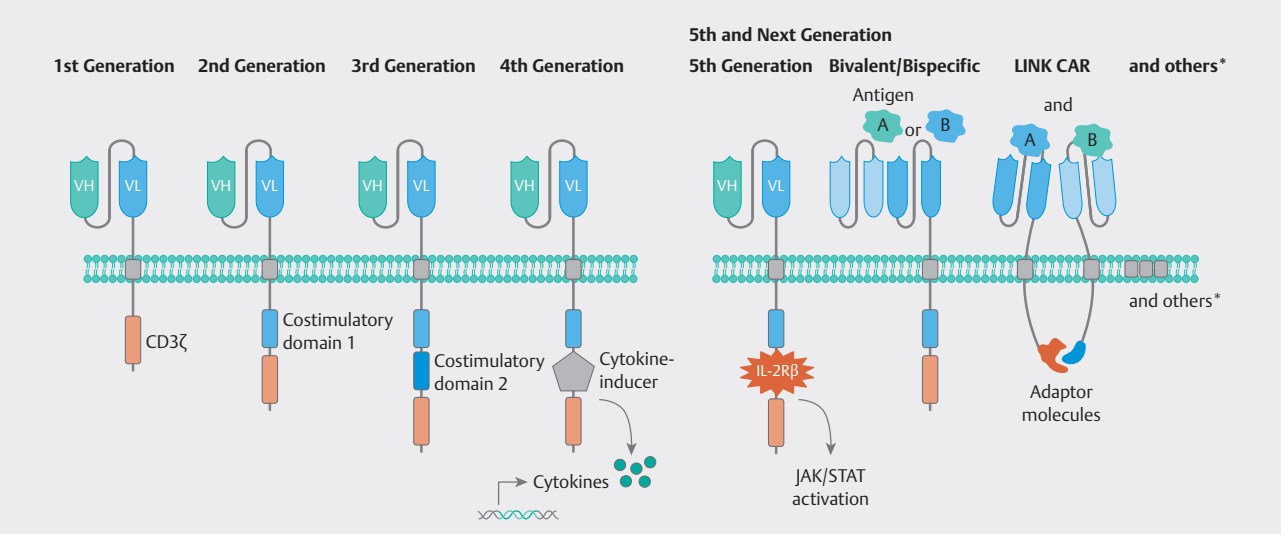


Fig. 2 Evolution and types of CARs. First-generation CARs (left) consisted of the antigen-binding single-chain variable fragment (scFv) linked to the CD3ζ signaling domain, allowing for basic T cell activation but with limited clinical efficacy. Second-generation CARs incorporate a costimulatory molecule (such as CD28 or 4–1BB) to enhance the expansion and persistence of CAR T cells, which has become the backbone for further and all approved products to date. Under clinical investigation, third-generation CARs include two costimulatory molecules, and the fourth, designed based on the second generation, is combined with cytokine expressors (e.g., IL-12). Next-generation CARs (many more than shown (*), reviewed by Labanieh and Mackall[21]) continue to innovate, introducing features such as bivalent CARs that target two different antigens simultaneously and LINK CARs that require two antigen signals for activation, increasing both specificity and safety. (Created with BioRender.com.)

while preclinical studies suggested a better efficacy, improved clinical outcomes of 3G CAR T cells over 2G have not yet been proved so far[16] (reviewed by Tomasik et al[10]). Meanwhile, advances in genetic engineering gave rise to further exciting generations of CARs. Fourth-generation CARs, also known as armored CAR Ts or TRUCKs (T cells redirected for universal cytokine-mediated killing), are engineered to not only target cancer cells but also secrete cytokines, such as IL-12, to modulate the tumor microenvironment with activation of further immune cells, especially seeking to improve outcomes in solid tumors[17] (reviewed by Tang et al[18]). Further (next-)generations of CARs focus on novel strategies to overcome existing challenges. For example, bispecific CARs target two distinct antigens to reduce the risk of tumor antigen escape (e.g., CD19 and CD20, Boolean, or Gate[19]) or LINK CARs to increase the specificity and reduce toxicity by requiring two antigens for activation (Boolean and Gate[20]). With several other innovative designs, a detailed review of these advancements is outside the scope of this article. For a more complete overview, we refer to the comprehensive review by Labanieh and Mackall.[21]

Patients deliver the starting material to manufacture these, to date personalized, living drugs. This is achieved through leukapheresis (►Fig. 1), a procedure where mononuclear cells are collected from the patients' peripheral blood.[22] In a GMP-compliant manufacturing facility, T-lymphocytes are selected and activated. Subsequently, in a crucial step, the genetic material encoding the CAR is delivered into the T cells (►Fig. 1). All currently approved CAR T cell products, as well as many under clinical investigation, utilize viral vectors for this purpose, but several other genomic engineering strategies, including non-viral approaches like mRNA transfection and Sleeping Beauty transposon systems, are currently under investigation to improve the process.[23] After quality controls, the final product is released and sent back to the clinic for infusion. This is done after a lymphodepleting chemotherapy (LD) aiming to improve expansion and persistence and thus efficacy through depletion of endogenous lymphocytes and modulation of the microenvironment. While optimal conditioning is still under investigation, the chemotherapeutic agents approved and most commonly used with all approved commercial products to date are fludarabine combined with cyclophosphamide.[24–27] Following conditioning and CAR T cell infusion, patients are closely monitored for adverse events and followed up for a minimum of 15 years (►Fig. 1).

Current Indications and Clinical Applications of CAR T Cells for Hematological Malignancies

Anti-CD19 CAR T Cells for Patients with Relapsed/Refractory (r/r) High-Grade and Indolent B Cell Lymphoma

The clinical breakthrough in the use of CAR T cell therapy was achieved in targeting CD19 in hematological malignancies. After early applications in adult patients with chronic lymphocytic leukemia[12,14] and two children with B cell acute lymphoblastic leukemia (ALL),[28] tisagenlecleucel (tisa-cel) was the first CAR T cell therapy to receive FDA approval in 2017 and EMA approval in 2018. To date, there are six FDA- and EMA-approved, commercially available CAR T products to treat B cell malignancies. Approval for pediatric ALL, DLBCL and primary mediastinal B cell lymphoma (PMBCL), occurred

after the impressive results of the single-arm phase 2 pivotal trials (Eliana, Juliet, Transcend, and ZUMA-1).[25–27,29]

The Eliana trial included 75 heavily pretreated pediatric and young adult patients with ALL, achieving an overall remission rate (CR or CRi) of 81% within 3 months of tisa-cel infusion and overall survival (OS) of 76% at 12 months.[29] Within the Juliet trial, 88 patients with r/r DLBCL (and 21 with transformed FL) received tisa-cel with an overall response rate of 52%. Tisa-cel targets CD19 and utilizes a 4–1BB costimulatory domain and is approved to date for young (< 26 years) patients with r/r B-ALL, DLBCL, and r/r FL (►Table 1).[25]

Lisocabtagene maraleucel (liso-cel), which also incorporates the 4–1BB costimulatory domain, demonstrated efficacy in the large TRANSCEND-NHL-001 multicohort trial, which included 269 patients primarily DLBCL (with 42% aged ≥65 years), but also PMBCL, DLBCL from indolent lymphoma, and FL grade 3B (►Table 1). Of 256 patients in the efficacy-evaluable set with a median follow-up of 19.9 months, an objective response rate (ORR) was achieved by 186 (73%) patients and a CR by 136 (53%) patients.[7]

Axicabtagene ciloleucel (axi-cel) also targets CD19 but utilizes a CD28 costimulatory domain. It was approved after demonstrating efficacy in the phase II ZUMA-1 trial, which included 111 patients with r/r DLBCL, PMBCL, and transformed FL, showing an ORR of 82% and CR rate of 54%.[26]

Brexucabtagene autoleucel (brexu-cel) has the same design as axi-cel (differing only in the manufacturing process) and was approved for the treatment of adult patients with r/r MCL based on the ZUMA-2 trial.[30] One year later, approval was expanded for adult (>25 years) patients with r/r B-ALL based on the results of the ZUMA-3 trial.[31] After initial approval in the third line, tisa-cel, axi-cel, and liso-cel were tested in three randomized phase 3 trials against standard-of-care salvage chemotherapy followed by autologous stem cell transplantation (ASCT) as second-line therapy in patients with r/r large B cell lymphoma.[6,7,32] Two studies (ZUMA-7 and Transform) met their primary endpoint, while the Belinda study showed no benefit of tisa-cel. A direct comparison of these products has not been performed so far and despite similarities, the three trials had major differences.[33] Only retrospective real-world data suggested a superior outcome but also higher toxicity of axi-cel over tisa-cel.[34] All three products were further tested in patients with FL.[35–37]

Anti-BCMA CAR T Cells for Patients with r/r Multiple Myeloma

Idecabtagene vicleucel (ide-cel), a second-generation CAR that targets BCMA and utilizes a 4–1BB costimulatory domain, was first approved based on the phase 2 KarMMa trial,[38] which demonstrated an overall response rate of 73% (with a 33% complete remission rate) in patients with heavily pretreated (median 6 lines) r/r multiple myeloma. The phase 3 KarMMa-3 trial found CAR Ts to be superior to the standard of care in patients who had received two to four prior treatment regimens.[39]

Ciltacabtagene autoleucel (cilta-cel) is also targeting BCMA and utilizing 4–1BB as a costimulatory domain but differs from the 2G CAR prototypic design by containing two heavy chains (VH) as a single-chain variable fragment (scFv), enabling it to bind two epitopes of BCMA.[40] In the CARTITUDE-4 trial, patients with lenalidomide-refractory multiple MM and one to three prior lines of therapy receiving CAR T cells showed a significantly better outcome compared with standard of care.[41] Cilta-cel is currently approved in

Table 1 EMA-approved CAR T therapies for B cell malignancies and multiple myeloma (*as of September 2024*)

Product:	Kymriah	Yescarta	Tecartus	Breyanzi	Abecma	Carvykti
Active substance	Tisagenlecleucel	Axicabtagene-ciloleucel	Brexucabtagene autoleucel	Lisocabtagene maraleucel	Idecabtagene-vicleucel	Ciltacabtagenum autoleucelum
Manufacturer	Novartis	Kyte/Gilead	Kyte/Gilead	BMS	BMS	Janssen
Approval (EMA)	2018	2018	2020	2022	2021	2022
Target	CD19	CD19	CD19	CD19	BCMA	BCMA
Costimulatory signal	4–1BB	CD28	CD28	4–1BB	4–1BB	4–1BB
Indication	r/r B-ALL (age ≤25, 3rd line) r/r DLBC (3rd line) r/r FL (3rd line)	r/r DLBCL, HGBCL (2nd line[a]) PMBCL (3rd line) r/r FL (4th line)	r/r MCL (3rd line incl. BTK-inhibitor) r/r B-ALL (age ≥26, 3rd line)	r/r DLBCL, PMBCL, HGBCL (2nd line[a]) FL3B (2nd line[a])	r/r MM (3rd line[b])	r/r MM (2nd line[c])

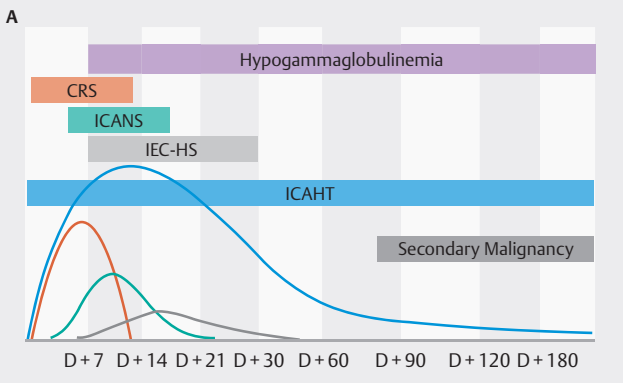
Abbreviations: B-ALL, acute B cell lymphoblastic leukemia; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HGBL, high-grade B cell lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; PMBCL, primary mediastinal B cell lymphoma; r/r, relapsed/refractory.
[a]Second line if refractory or early relapse (within 12 months after first-line chemoimmunotherapy); third line if later).
[b]After at least two lines including an immunomodulator, proteasome inhibitor, and anti-CD38 antibody.
[c]After first line including an immunomodulator, proteasome inhibitor, and refractory to lenalidomide.

the second line after treatment with a proteasome inhibitor and refractoriness to lenalidomide. It is further tested in the first line compared with ASCT (CARTITUDE-6; NCT05257083).

The current indications of EMA-approved CAR T therapies are summarized in ►Table 1.

Complications and Challenges of Current CAR T Cell Therapies

CAR T therapy is associated with a variety of possible short-, middle-, and long-term side effects (►Fig. 3). Some were recognized and described in early trials, such as cytokine release syndrome (CRS), whereas rarer ones were described only recently.[42] Severe forms of CRS and immune effector cell-associated neurotoxicity syndrome (ICANS) presented an initial limitation to the use of CAR T therapy. Awareness, early recognition, and management, as well as improvements in supportive care have significantly contributed to the safety and feasibility of these therapies.[26,42] With an outline of the most common and relevant complications described later, we refer to other reviews for a more in-depth description of CAR T cell-associated toxicities.[42,43]



B Risk factors for CRS & ICANS

CAR T-related factors
CAR T product
Type of co-stimulatory-domain (4-1BB < CD28)
CAR T cell dose
CD4/CD8 ratio of the CAR product (CD8 < CD4)
Higher peak/AUC levels of blood CAR T cells
Patient related factors
High tumor burden
Receipt of or requirement for bridging therapy
Elevated baseline inflammatory markers
Low baseline platelet count

Fig. 3 Toxicities and risk factors. (**A**) Approximate timing (*x*-axis) and frequency (*y*-axis) of specific CAR T-related toxicities. CRS typically occurs and resolves within 2 weeks of CAR-T infusion and ICANS may follow CRS. IEC-HS is rare and may be suspected with worsening inflammation after initial improvement of CRS. ICAHT and hypogammaglobulinemia may persist for weeks or months. (**B**) The box gives an overview (selection) of the risk factors for CRS and ICANS, which overlap significantly. Early and severe CRS was shown to correlate with the incidence and severity of ICANS.[49,68] (Created with BioRender.com.)

Cytokine Release Syndrome and Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome

CRS is an acute systemic inflammatory syndrome characterized by fever, chills, hypotension, tachycardia, hypoxia, and dyspnoea.[42,44] CRS is caused by a supraphysiologic response to immune therapy and the release of different proinflammatory cytokines (e.g., IL-6, IL-1, IL-2, and IFNγ) that engage T cells and other immune effector cells and can involve different organs.[45] Fever is typically the first sign of CRS and median time to onset is 2 to 7 days after infusion.[42]

CRS is graded based on clinical symptoms and the need for supportive measures to maintain blood pressure and oxygenation, currently following the ASTCT (American Society for Transplantation and Cellular Therapy) Consensus Grading System.[44] Grading is essential to guide clinical management, with more severe grades requiring interventions such as tocilizumab (an anti-IL-6 receptor antibody) or corticosteroids (►Table 2). Mild CRS (Grade 1) is defined by fever alone, while more severe grades (2–4) are characterized by hypotonia, hypoxia, or the need for vasopressors and/or respiratory support. While low-grade CRS is one of the most common side effects of CAR T cell therapy (>50% of patients), high-grade CRS is much less common (0–5% of patients, reviewed by Brudno and Kochenderfer and Ferreri and Bhutani[42,43]) also because of increased awareness and early intervention. Risk factors for the development of CRS include, among others, patient-related factors, such as type of lymphoma, a high tumor burden, elevated baseline inflammatory markers, and a low baseline platelet count, but also CAR T-related factors (►Fig. 3B). The different CAR T products and the types of costimulatory domains (4-1BB < CD28) are associated with different rates of CRS.[46] BCMA-directed products generally have lower rates compared with CD19-directed CAR T cells, and higher rates are observed in products utilizing CD28 costimulation (e.g., axi-cel) compared with those with 4–1BB costimulation (e.g., tisa-cel; ►Table 1, ►Fig. 3B).[25,26,34,42]

CRS in moderate to severe forms may overlap with the much rarer IEC-HS, defined by ASTCT as a "hyperinflammatory syndrome independent from CRS and ICANS." IEC-HS is characterized by hyperferritinemia, coagulopathy, cytopenia, transaminase elevation, and hypofibrinogenemia. Suspicion should arise with worsening inflammatory response after the initial improvement of CRS.[47]

Immune Effector Cell-Associated Neurotoxicity Syndrome

ICANS is a reversible encephalopathy with unclear pathophysiology and manifests with diverse neurological symptoms including dysphasia, encephalopathy, varying decreases in the level of alertness, to less common manifestations such as muscle weakness, myelitis, myoclonus, and seizures.[42,48] The earliest symptoms of ICANS are dysgraphia, mild difficulty with expressive speech (especially in naming objects), impaired attention, apraxia, and mild lethargy. ICANS can progress into severe encephalopathy with seizures, cerebral edema, and death. It may typically present concurrently with or soon

Table 2 Grading and suggested management of CRS

CRS grade	Diagnostic/Therapy
1 - Temperature ≥38 °C (no hypotension or hypoxia)	**Diagnostic** - Evaluate for/exclusion of infection, including imaging as indicated **Therapeutic** - Antibiotics, evaluate antifungal/antiviral therapy - Symptomatic measures: fluids, antipyretics - Consider tocilizumab 8 mg/kg IV (max. 800 mg), especially if fever persists despite antipyretics and antimicrobial treatment ≥ 3 d
2 - Temperature ≥38 °C ***and*** - Hypotension not requiring vasopressors ***and/or*** - Hypoxia requiring low-flow nasal cannula at ≤6 L/min	**Diagnostic as for grade 1** - Evaluation by intensive care specialist **Therapeutic** - Supportive therapy: fluids, antipyretics, and oxygen - Tocilizumab 8 mg/kg IV, • *No improvement*: repeat after 8–12 h, max. 4 doses in total • *Deterioration/no improvement* after 12–24 h despite tocilizumab: dexamethasone 10 mg IV every 6 h for 1–3 d
3 - Temperature ≥38 °C ***and*** - Hypotension requiring one vasopressor ***and/or*** - Hypoxia requiring high-flow >6 L/min, mask	**Diagnostic in addition to grade 1/2** - Echocardiography **Therapeutic in addition to grade 1/2** - Immediate transfer to ICU and continuous monitoring - Respiratory/volume/vasopressor support according to local ICU standards - Tocilizumab 8 mg/kg IV every 8–12 h, max. 2 doses, if not already done - Dexamethasone 10 mg IV every 6 h for 1–3 d • *Deterioration/no improvement*: after 12–24 h despite tocilizumab and dexamethasone 10 mg: increase dexamethasone dose to 20 mg IV every 6 h for 3 d, tapering within 3–7 d
4 - Temperature ≥38 °C ***and*** - Hypotension requiring multiple vasopressors ***and/or*** - Hypoxia requiring NIV or intubation and mechanical ventilation	**Diagnostic as for grade 3** **Therapeutic in addition to grades 1–3** - Respiratory/volume/vasopressor support according to local ICU standards - Tocilizumab 8 mg/kg IV every 12 h, max. 2 doses - Dexamethasone 20 mg IV every 6 h for 3 d, tapering within 3–7 d • *No improvement*: after 12–24 h despite tocilizumab and dexamethasone: rescue with high-dose steroids: Methylprednisolone 1 g IV for 3 d/250 mg every 12 h for 2 d/125 mg every 12 h for 2 d/60 mg every 12 h for 2 d, consider 3rd dose of tocilizumab - Anakinra (2–8 mg/kg IV or SC)

Notes: The table provides an overview of grading and management of CRS. We refer to other guidelines and local standards. Note that tocilizumab should not be used in patients with concomitant ICANS.
Abbreviations: NIV: non-invasive ventilation.
Source: Adapted from Hayden et al and hematool.ch.[56,67] Tocilizumab off-label.

after CRS, but delayed cases starting >3 weeks after CAR T cell infusion are also described.[42] Grading, according to ASTCT, includes a cognitive score (ICE, immune effector cell-associated encephalopathy score, that examines orientation, attention, the ability to follow commands, naming, and writing) as well as the level of alertness, evaluation for seizures, motor defects, elevated intracranial pressure, and cerebral edema.[44]

Interestingly, risk factors for both CRS and ICANS are similar (►**Fig. 3B**) and include patient- and product-related factors.[34] Additionally, early and severe CRS was shown to correlate with the incidence and severity of ICANS.[49]

While the rate of ICANS (and CRS) is lower in BCMA-directed CAR T therapy, these may cause specific movement disorders including parkinsonism and gait disturbance.[40] ICANS treatment is based on supportive care and corticosteroid therapy, depending on severity. Tocilizumab should be used with caution if ICANS is suspected because it may worsen neurological toxicity (►**Table 3**).[49]

Hematological Toxicities

Cytopenias, now termed "immune effector cell-associated hematotoxicity (ICAHT)," are the most common side effects after CAR T cell therapy and the most commonly reported grade ≥3 adverse events as defined by the Common Terminology Criteria for Adverse Events (CTCAE).[50,51]

Cytopenia is expected after LD and invariably evident early (<30 days, early ICAHT) after infusion. However, neutropenia recovers quickly in only one-quarter of patients, while the majority of patients show a biphasic course with intermittent recovery around week 3 followed by worsening 2 months after infusion. Cytopenias are therefore better assessed and classified using the EHA/EBMT consensus grading rather than CTCAE.[52] In contrast to the CTCAE, this grading better reflects the nature of post–CAR T cell hematopoietic reconstitution with delayed courses. In particular, some patients suffer prolonged aplasia, and late ICAHT is defined as neutropenia lasting beyond day 30.[51] Several risk

Table 3 Grading and suggested management of ICANS

ICANS grade	Diagnostic/Therapy
1 - Awakens spontaneously - ICE scores 7–9 - No higher-grade features[a]	**Diagnostic** - Evaluation/exclusion of alternative causes: infection, drugs, electrolytes; imaging as indicated; if not contraindicated, diagnostic lumbar puncture - Neurological consultation, EEG and neuroimaging **Therapeutic** - Supportive therapy, prevention of aspiration - No tocilizumab unless CRS $\geq$2 - Consider a single dose of dexamethasone 10 mg IV (in case of dysgraphia and/or neuropsychological abnormality and after exclusion of other causes) - Consider prophylactic levetiracetam - Correction electrolytes - Consider high-dose thiamine
2 - Depressed level of consciousness, but awakens to voice - ICE scores 3–6 - No higher-grade features[a]	**Diagnostic as for grade 1** **Therapeutic** - Consider transfer to ICU *In addition to grade 1:* - Target Na^+ 135–145 mmol/L - Suspend oral nutrition, oral drugs to IV - If seizure (clinically or EEG): antiepileptic drugs - Dexamethasone 10 mg IV and reassess: • No improvement: repeat every 6 h for 1–3 d. Consider anakinra 100 mg IV every 6 h • Improvement: continue every 12–24 h until ICANS grade $\leq$1, then quick taper
3 - Depressed consciousness, awakes only to tactile stimulus - ICE scores 0–2 - Seizures: focal or rapidly resolving - Neuroimaging: focal/local edema - No motor deficit	**Diagnostic as for grade 1** - Repeat brain imaging and EEG (every 2–3 d), lumbar puncture if not contraindicated **Therapeutic in addition to grade 1/2** - Immediate transfer to ICU - Target Na^+ 140–145 mmol/L - Upfront anakinra (100 mg every 6 h) in addition to dexamethasone
4 - Unarousable or requires vigorous or repetitive tactile stimuli - Unable to perform ICE - Seizures: life-threatening prolonged or repetitive - Neuroimaging/ICP: diffuse edema or signs of elevated intracranial pressure (ICP); e.g., papillary edema or Cushing's triad - Deep focal motor weakness, e.g., hemi- or paraparesis	**Diagnostic as for grade 3** **Therapeutic in addition to grades 1–3** - Anakinra 100 mg IV every 6 h (max. 10 mg/kg/d IV) - High dosage methylprednisolone 1 g/24 h IV for 3 d, 250 mg 2 $\times$/d for 2 d, 125 mg 2 $\times$/d for 2 d, 60 mg 2 $\times$/d for 2 d - Consider mechanical ventilation for airway protection - Treat increased ICP, cerebral edema, and seizures as per ICU standard

Note: The table provides an overview of the grading and management of ICANS. We refer to other guidelines and local standards.
[a]No higher-grade features: no seizures, motor deficits, or imaging and other abnormalities defining grades 3–4.
Source: Adapted from Hayden et al.[56] Tocilizumab and anakinra off-label.

factors have been identified (e.g., higher baseline levels of inflammatory markers, lower baseline blood counts, higher baseline tumor burden in the bone marrow, and a higher number of prior therapies[42,51]) leading to the development of a validated scoring system to predict ICAHT (CAR-HEMATOTOX).[51] The pathophysiology of prolonged myelosuppression remains unclear with hints toward a baseline inflammatory milieu and CAR T cell-induced inflammation.[42] Severe ICAHT significantly increases the risk for infectious complications and was associated with an increase in non-relapse mortality.[51,53] Therapy is mostly supportive, and in patients with severe and persistent cytopenias, further diagnostic steps, including bone marrow examinations, should be performed also to exclude secondary malignancies.[54–56]

B Cell Aplasia and Infections

B cell aplasia and hypogammaglobulinemia are common in patients after both CD19- and BCMA-directed CAR T cell therapy, can persist for years, and often require immunoglobulin replacement.[42,56]

Infections after CAR therapy are common, with higher grades ($\geq$3) being reported in 5 to 32% of patients across several studies and representing a leading cause of non-relapse mortality.[42] Prevention and management include a thorough screening for

latent infections before CAR T therapy, close monitoring of patients, and the use of antimicrobial prophylaxis until immune reconstitution. While the use of antibacterial agents is not routinely advised, anti-viral (e.g., (Val-)aciclovir) and anti-pneumocystis prophylaxis is generally recommended.[56,57]

Further Complications of CAR T Therapy

As CAR T cell therapy is relatively new, rare and previously unexpected side effects continue to emerge as its use expands. Local CRS, like cervical edema, was observed with and without systemic involvement and resolved after CRS-directed therapy.[58] Additionally, neurotoxicity beyond ICANS, including cases of myelopathy and encephalopathy,[42,48] underscores the complexity of managing the full spectrum of CAR T cell-related complications.

Secondary malignancies after CAR T cell treatment related to viral vector integration are of concern and, although likely similar to observations in patients treated with chemo- and/or radiotherapy, are under critical investigation.[42,54] A large analysis of 12,394 events from the FDA adverse event reporting system revealed that neoplasms were overrepresented, with 536 reports (4.3%).[59] In a large retrospective cohort (449 patients), projected over a 5-year period, Ghilardi and colleagues estimated a 15.2% risk of developing a secondary solid tumor and a 2.3% risk of a secondary hematologic malignancy.[54] Reporting on a cohort of 582 patients, Melody and colleagues reported a total rate of 8.2%.[55] Both studies found only one T cell lymphoma. With the FDA investigating the risk of T cell malignancies occurring after CAR T cell therapy, the rate is very low, with CAR T cell-derived T cell malignancies (i.e., the CAR transgene detected in the tumor) being extremely rare.[60] Hence, in most patients, otherwise facing a dire prognosis, the benefits of CAR T cell therapy outweigh this risk. The unclear relationship between CAR T cell therapy and secondary malignancies warrants further studies and underscores the need for long-term follow-up, currently mandatory for up to 15 years.

Relapse and Resistance

While CAR T cell therapies have achieved remarkable success in treating hematological malignancies, a significant proportion of patients eventually experience disease relapse.

Resistance characteristics differ by disease and product and several mechanisms have been identified (comprehensively reviewed by Ruella et al[61]). Secondary resistance (i.e., relapse after an initial response) is more common than primary refractoriness and was estimated to affect roughly 40 to 50% of cases with B-ALL and DLBCL, and at least 30% of patients with multiple myeloma.[6,38,61] Mechanisms involved include but are not limited to tumor-intrinsic mechanisms such as antigen loss (e.g., CD19 antigen-negative escape), an immunosuppressive tumor microenvironment, and CAR T cell dysfunction including exhaustion.[61] Strategies to overcome resistance are under active investigation and include—among others—patient selection, improved manufacturing, and improved CAR design like the use of dual-target CARs to overcome antigen-negative escape,[19] equipping the CARs to secrete cytokines (TRUCKs, ►Fig. 2), and allogenic CARs from healthy donors to prevent T cell dysfunction.[62] Recent approaches to overcome CAR T cell exhaustion include the addition of factors during the production to induce more stemness features in transferred cells.[63]

Conclusion and Outlook

CAR T cell treatment has revolutionized the treatment of hematological malignancies, offering substantial clinical benefits including cure, particularly for relapsed or refractory patients, and is expanding into earlier lines of therapy and broader indications (e.g., nonmalignant diseases). It is a complex process requiring an interprofessional and interdisciplinary team. Nonetheless, several challenges persist, including accessibility and cost, relapse, and the management of CAR T cell-related side effects. Additionally, the role of CAR T cells within growing therapeutic options (e.g., bispecific antibodies) remains dynamic. Certain toxicities, such as prolonged cytopenias or immunodeficiency, can persist and may be encountered by clinicians outside of specialized centers. Thus, it is crucial to maintain a high level of awareness for known toxicities and encourage the reporting of potentially novel side effects. Further innovations in adoptive cell therapies like optimized CAR designs, allogeneic CARs, the generation of in vivo CARs, and advances in cell engineering like shielded hematopoietic cells from chemo-immunotherapy[62,64] will further improve efficacy and reduce toxicity. Finally, early results showed rapid and sustained responses in patients with difficult-to-treat autoimmune diseases.[65,66]

Conflicts of Interest

L.T.J.: Founder, board member, holding equity of Cimeio Therapeutics AG (Cimeio); sponsored research agreement with Cimeio; inventor on granted patents and patent applications related to immune cell engineering; received speaker fees from Novartis; paid consultant for Kyowa Kirin.

The other authors declare no conflict of interest related to the work.

Acknowledgments

No specific financial support was received for the submitted work.

References

1 Coley WB. II. Contribution to the knowledge of sarcoma. Ann Surg 1891;14(03):199–220
2 Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med 2007;357(15):1472–1475
3 Mitra A, Barua A, Huang L, Ganguly S, Feng Q, He B. From bench to bedside: the history and progress of CAR T cell therapy. Front Immunol 2023;14:1188049
4 König D, Kasenda B, Sandholzer M, et al. Adoptive cell therapy with tumor-infiltrating lymphocytes in combination with nivolumab in patients with advanced melanoma. Immuno-Oncology and Technology 2024;24:100728 (Volume 24, Issue C; https://doi.org/10.1016/j.iotech.2024.100728)
5 Chesney J, Lewis KD, Kluger H, et al. Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after

progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study. J Immunother Cancer 2022;10(12):e005755
6 Locke FL, Miklos DB, Jacobson CA, et al; All ZUMA-7 Investigators and Contributing Kite Members. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. N Engl J Med 2022;386(07):640–654
7 Bishop MR, Dickinson M, Purtill D, et al. Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. N Engl J Med 2022;386(07):629–639
8 Kamdar M, Solomon SR, Arnason J, et al; TRANSFORM Investigators. Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial. Lancet 2022;399(10343):2294–2308
9 Passweg JR, Baldomero H, Ciceri F, et al. Hematopoietic cell transplantation and cellular therapies in Europe 2022. CAR-T activity continues to grow; transplant activity has slowed: a report from the EBMT. Bone Marrow Transplant 2024;59(06): 803–812
10 Tomasik J, Jasiński M, Basak GW. Next generations of CAR-T cells - new therapeutic opportunities in hematology? Front Immunol 2022;13:1034707
11 Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A 1989;86(24): 10024–10028
12 Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2011;365(08):725–733
13 Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. Nat Biotechnol 2002;20(01): 70–75
14 Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011;3 (95):95ra73
15 Ramos CA, Rouce R, Robertson CS, et al. In vivo fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. Mol Ther 2018;26(12):2727–2737
16 Enblad G, Karlsson H, Gammelgård G, et al. A phase I/IIa trial using CD19-targeted third-generation CAR T cells for lymphoma and leukemia. Clin Cancer Res 2018;24(24):6185–6194
17 Choi BD, Gerstner ER, Frigault MJ, et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. N Engl J Med 2024;390 (14):1290–1298
18 Tang L, Pan S, Wei X, Xu X, Wei Q. Arming CAR-T cells with cytokines and more: Innovations in the fourth-generation CAR-T development. Mol Ther 2023;31(11):3146–3162
19 Zurko JC, Fenske TS, Johnson BD, et al. Long-term outcomes and predictors of early response, late relapse, and survival for patients treated with bispecific LV20.19 CAR T-cells. Am J Hematol 2022; 97(12):1580–1588
20 Tousley AM, Rotiroti MC, Labanieh L, et al. Co-opting signalling molecules enables logic-gated control of CAR T cells. Nature 2023; 615(7952):507–516
21 Labanieh L, Mackall CL. CAR immune cells: design principles, resistance and the next generation. Nature 2023;614(7949): 635–648
22 Worel N, Holbro A, Vrielink H, et al. A guide to the collection of T-cells by apheresis for ATMP manufacturing-recommendations of the GoCART coalition apheresis working group. Bone Marrow Transplant 2023;58(07):742–748
23 Rossi M, Breman E. Engineering strategies to safely drive CAR T-cells into the future. Front Immunol 2024;15:1411393
24 Lickefett B, Chu L, Ortiz-Maldonado V, et al. Lymphodepletion - an essential but undervalued part of the chimeric antigen receptor T-cell therapy cycle. Front Immunol 2023;14:1303935
25 Schuster SJ, Bishop MR, Tam CS, et al; JULIET Investigators. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2019;380(01):45–56
26 Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377(26):2531–2544
27 Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet 2020;396(10254):839–852
28 Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013; 368(16):1509–1518
29 Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378(05):439–448
30 Wang M, Munoz J, Goy A, et al. Three-year follow-up of KTE-X19 in patients with relapsed/refractory mantle cell lymphoma, including high-risk subgroups, in the ZUMA-2 study. J Clin Oncol 2023; 41(03):555–567
31 Shah BD, Ghobadi A, Oluwole OO, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet 2021;398(10299):491–502
32 Abramson JS, Solomon SR, Arnason J, et al. Lisocabtagene maraleucel as second-line therapy for large B-cell lymphoma: primary analysis of the phase 3 TRANSFORM study. Blood 2023;141 (14):1675–1684
33 Westin J, Sehn LH. CAR T cells as a second-line therapy for large B-cell lymphoma: a paradigm shift? Blood 2022;139(18):2737–2746
34 Bachy E, Le Gouill S, Di Blasi R, et al. A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma. Nat Med 2022; 28(10):2145–2154
35 Fowler NH, Dickinson M, Dreyling M, et al. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med 2022;28(02):325–332
36 Jacobson CA, Chavez JC, Sehgal AR, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol 2022;23 (01):91–103
37 Morschhauser F, Dahiya S, Palomba ML, et al. Lisocabtagene maraleucel in follicular lymphoma: the phase 2 TRANSCEND FL study. Nat Med 2024;30(08):2199–2207
38 Munshi NC, Anderson LD Jr, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med 2021; 384(08):705–716
39 Rodriguez-Otero P, Ailawadhi S, Arnulf B, et al. Ide-cel or standard regimens in relapsed and refractory multiple myeloma. N Engl J Med 2023;388(11):1002–1014
40 Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet 2021;398(10297):314–324
41 San-Miguel J, Dhakal B, Yong K, et al. Cilta-cel or standard care in lenalidomide-refractory multiple myeloma. N Engl J Med 2023; 389(04):335–347
42 Brudno JN, Kochenderfer JN. Current understanding and management of CAR T cell-associated toxicities. Nat Rev Clin Oncol 2024; 21(07):501–521
43 Ferreri CJ, Bhutani M. Mechanisms and management of CAR T toxicity. Front Oncol 2024;14:1396490
44 Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated

with immune effector cells. Biol Blood Marrow Transplant 2019; 25(04):625–638
45 Fajgenbaum DC, June CH. Cytokine storm. N Engl J Med 2020;383 (23):2255–2273
46 Jacobson CA, Munoz J, Sun F, et al. Real-world outcomes with chimeric antigen receptor T cell therapies in large B cell lymphoma: a systematic review and meta-analysis. Transplant Cell Ther 2024;30(01):77.e1–77.e15
47 Hines MR, Knight TE, McNerney KO, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. Transplant Cell Ther 2023;29(07):438.e1–438.e16
48 Deschênes-Simard X, Santomasso BD, Dahi PB. Clinical features, pathophysiology, and management of acute myelopathy following CAR T-cell therapy. Blood 2024;144(20):2083–2094
49 Santomasso BD, Nastoupil LJ, Adkins S, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline. J Clin Oncol 2021;39(35):3978–3992
50 Fried S, Avigdor A, Bielorai B, et al. Early and late hematologic toxicity following CD19 CAR-T cells. Bone Marrow Transplant 2019;54(10):1643–1650
51 Rejeski K, Perez A, Sesques P, et al. CAR-HEMATOTOX: a model for CAR T-cell-related hematologic toxicity in relapsed/refractory large B-cell lymphoma. Blood 2021;138(24):2499–2513
52 Rejeski K, Subklewe M, Aljurf M, et al. Immune effector cell-associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. Blood 2023;142(10):865–877
53 Rejeski K, Wang Y, Hansen DK, et al. Applying the EHA/EBMT grading for ICAHT after CAR-T: comparative incidence and association with infections and mortality. Blood Adv 2024;8(08): 1857–1868
54 Ghilardi G, Fraietta JA, Gerson JN, et al. T cell lymphoma and secondary primary malignancy risk after commercial CAR T cell therapy. Nat Med 2024;30(04):984–989
55 Melody M, Epperla N, Shouse G, et al. Subsequent malignant neoplasms in patients previously treated with anti-CD19 CAR T-cell therapy. Blood Adv 2024;8(10):2327–2331
56 Hayden PJ, Roddie C, Bader P, et al. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). Ann Oncol 2022;33(03):259–275
57 Shahid Z, Jain T, Dioverti V, et al. Best practice considerations by the American Society of Transplant and Cellular Therapy: Infection Prevention and Management After Chimeric Antigen Receptor T Cell Therapy for Hematological Malignancies. Transplant Cell Ther 2024;30(10):955–969
58 Inoue Y, Fujino T, Chinen S, et al. Cervical local cytokine release syndrome following chimeric antigen receptor T-cell therapy in patients with relapsed or refractory diffuse large B-cell lymphoma. Cureus 2023;15(05):e38905
59 Elsallab M, Ellithi M, Lunning MA, et al. Second primary malignancies after commercial CAR T-cell therapy: analysis of the FDA adverse events reporting system. Blood 2024;143(20): 2099–2105
60 Verdun N, Marks P. Secondary cancers after chimeric antigen receptor T-cell therapy. N Engl J Med 2024;390(07):584–586
61 Ruella M, Korell F, Porazzi P, Maus MV. Mechanisms of resistance to chimeric antigen receptor-T cells in haematological malignancies. Nat Rev Drug Discov 2023;22(12):976–995
62 Bonini C, Chapuis AG, Hudecek M, Guedan S, Magnani C, Qasim W. Genome editing in engineered T cells for cancer immunotherapy. Hum Gene Ther 2023;34(17-18):853–869
63 Klysz DD, Fowler C, Malipatlolla M, et al. Inosine induces stemness features in CAR-T cells and enhances potency. Cancer Cell 2024;42 (02):266–282.e8
64 Garaudé S, Marone R, Lepore R, et al. Selective haematological cancer eradication with preserved haematopoiesis. Nature 2024; 630(8017):728–735
65 Schett G, Mackensen A, Mougiakakos D. CAR T-cell therapy in autoimmune diseases. Lancet 2023;402(10416):2034–2044
66 Müller F, Taubmann J, Bucci L, et al. CD19 CAR T-cell therapy in autoimmune disease - a case series with follow-up. N Engl J Med 2024;390(08):687–700
67 hematool.ch. Accessed December 4, 2024 at:(https://hematool.ch/therapy/immunotherapies-incl-immune-effector-cells/chimeric-antigen-receptor-car-t-cell-therapy
68 McNerney KO, Hsieh EM, Shalabi H, et al. INSPIRED Symposium Part 3: Prevention and management of pediatric chimeric antigen receptor T cell-associated emergent toxicities. Transplant Cell Ther 2024;30(01):38–55