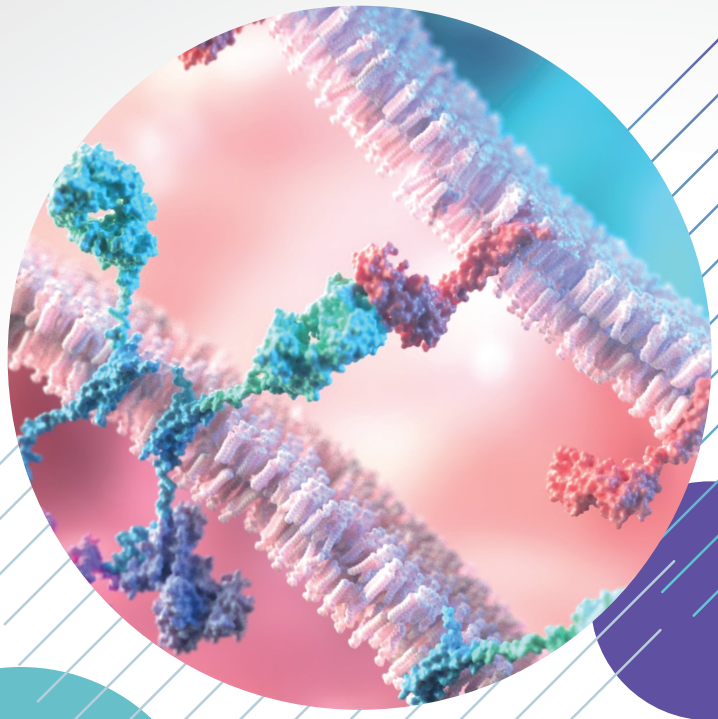

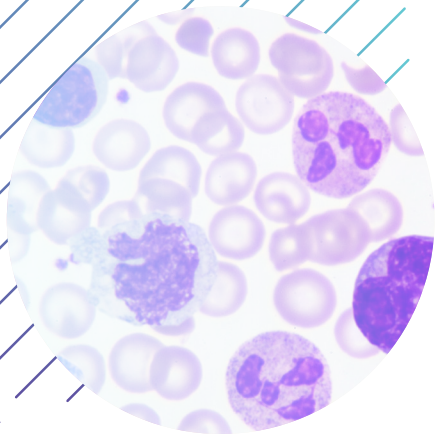

Taiwan CAR-T Cell Therapy Handbook

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Pre CAR-T Cell Therapy

Chapter 1

Pre CAR-T Cell Therapy

Chimeric antigen receptor T (CAR-T) cell therapy is a new form of immunotherapy that uses the mechanisms of the immune system to fight tumor cells and achieve anticancer effects. CAR-T cell therapy products must be manufactured by pharmaceutical manufacturers that have obtained approval from the competent authorities in compliance with the Good Manufacturing Practice (GMP) grades, and must pass stringent quality checks. To ensure cell quality in raw materials and finished products and reduce the care risks posed by the treatment itself and adverse effects, medical institutions performing cell therapy must also obtain relevant certification as legally required. [1-3]

Medical teams performing CAR-T cell therapy comprise members from different professional specialties and provide CAR-T cell therapy treatment courses with a patient- and family-centered approach (**Figure 1**). The course of treatment may be divided into three phases:[1-3]

1. **Pre-treatment Phase**
   This is the phase before infusing CAR-T cells and includes pretreatment evaluation, cell collection, cell preservation, cell production, delivery procedures, bridging therapy, lymphodepletion evaluation, pre-infusion prophylactic care, and patient and family evaluation.
2. **Treatment Phase**
   This runs from the day the CAR-T cell infusion is initiated until within 30 days or after the patient is discharged, and includes cell thawing, cell infusion, assessment for infusion reactions, assessment for post-infusion complications and their medical interventions, and operating procedures for transfers to an intensive care unit (ICU).
3. **Post-treatment Follow-up Phase**
   Subsequent management after CAR-T cell therapy and discharge, including assessment of post-treatment complications and long-term follow-up.

1.1 CAR-T Cell Therapy Multidisciplinary Team

Roles and Responsibilities of Team Members[4]

1. **Cell Therapy Unit**
   It is responsible for obtaining relevant certifications and ensuring patient identification and consistency throughout the overall process. In addition, it is also responsible for overseeing the receipt of medicinal products, establishing storage management mechanisms, performing cell freezing and handling procedures and monitoring, overseeing delivery (transport) processes, and possibly aiding/executing cell thawing procedures and handling.

   Required education and training include:
   - Having ample professi3onal knowledge related to cell freezing/thawing
   - Understanding cell processing procedures and obtaining certifications through simulation testing
   - Understanding critical points and anomaly handling for process risk management

2. **Physicians**
   They are responsible for evaluating patients' treatment needs and understanding CAR-T cell therapy procedures, including mechanisms of action; the status of cell collection, preservation, and manufacture; bridging therapy; evaluation and management of cell therapy complications; and long-term follow-up after treatment.
   Required education and training include:
   - Providing timely updates of new developments and continuing education information on cell immunotherapy
   - Establishing continuing education and training activity programs for health care providers administering CAR-T cell therapy

3. **Medical Technicians and Cell Processing Technicians**
   They are responsible for assisting in the collection, testing, and quality management of autologous T lymphocytes from individual patients, and must also perform cryopreservation and thawing of collected cells.
   Required education and training include:
   - Understanding relevant information and techniques on apheresis, cell processing, cryopreservation, and thawing
   - Receiving training in double lumen care
   - Receiving training in aseptic operations, cell counting, and operation of flow cytometers
   - Understanding test quality control and laboratory certifications
   - Participating in continuing education and training activity programs

4. **Pharmacists**
   They are responsible for verifying patients' identity and handling recognition and verification as well as understanding the mechanisms, transport, preservation, thawing, and pre-, intra-, and post-infusion precautions for relevant medicinal products (CAR-T cells and related therapeutic medications in the course of treatment). In addition, they must also observe patients for medication infusion reactions and establish relevant safety reporting mechanisms, as well as inventorying and stocking relevant medications for CAR-T cell therapy, bridging therapy, and management of complications.
   Required education and training include:
   - Understanding the selection of CAR-T cell therapy and confirming whether patients fulfill the indications and usage criteria

- Checking and confirming whether there are additional concomitant medications
- Understanding the procedures and steps in ordering medicinal products and receiving and confirming personalized medicinal products
- Participating and assisting in updates to continuing education and training activity programs related to CAR-T cell therapy

❺ Clinical Nurses/Nurse Specialists

They are responsible for evaluating patients' physical status and psychological, social, and familial support systems. In addition, they must have professional expertise in central venous catheter care, cell collection, and thawing operations, so that they are capable of performing these technical operations during cell infusion. They must be able to use grading scales to evaluate, monitor, and manage possible post-treatment complications, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). They also need to evaluate the needs of patients and their families, know when to refer them to specialist care teams, and carry out long-term case follow-up.

Required education and training include:

- Understanding cell immunotherapy mechanisms and the evaluation and monitoring of complications
- Learning how to manage complications in a timely manner
- Participating and assisting in updates to continuing education training activities associated with CAR-T cell therapy for health care providers

❻ Other Potential Participating Healthcare Providers

① Case Managers

They are responsible for evaluating patients' physical status and psychological, social, and familial support systems before, during, and after treatment. At the same time, they must establish relevant databases to assess the needs of patients and their families, and refer them to the pertinent specialist care team when necessary. In addition, establishing CAR-T cell therapy support groups and long-term case follow-up mechanisms is essential.

Required education and training include:

- Understanding relevant governmental policies, laws, and regulations
- Understanding the relevant therapeutic mechanisms of cell immunotherapy and evaluation and monitoring for complications
- Learning how to manage complications and communicate the relevant matters in a timely manner
- Participating and assisting in the timely update of continuing education and training activity programs for health care providers administering CAR-T cell therapy
- Establishing a program for long-term case follow-up

② Psychologists

They are responsible for conducting psychosocial evaluations of patients and their family members, as well as family support evaluations before, during, and after treatment, and providing patients with appropriate mental health interventions.

Required education and training include:

- Understanding relevant governmental policies, laws, and regulations
- Understanding the therapeutic mechanisms of cell immunotherapy, evaluation and monitoring for complications, and managing complications and relevant communications in a timely manner
- Participating and assisting in the timely update of continuing education and training activity programs for health care providers administering CAR-T cell therapy
- Establishing long-term follow-up programs for the mental health of the case patient and family members

③ Social Workers

They are responsible for conducting family evaluation of patients and their family members, as well as family support evaluations before, during, and after treatment, and providing appropriate social support and financial aid.

Required education and training include:

- Understanding relevant governmental policies, laws, and regulations
- Participating and assisting in the timely update of continuing education and training activity programs for health care providers administering CAR-T cell therapy

1.2 Patient Selection

Appropriate patient selection for CAR-T cell therapy may be considered from three levels:

❶ Indication

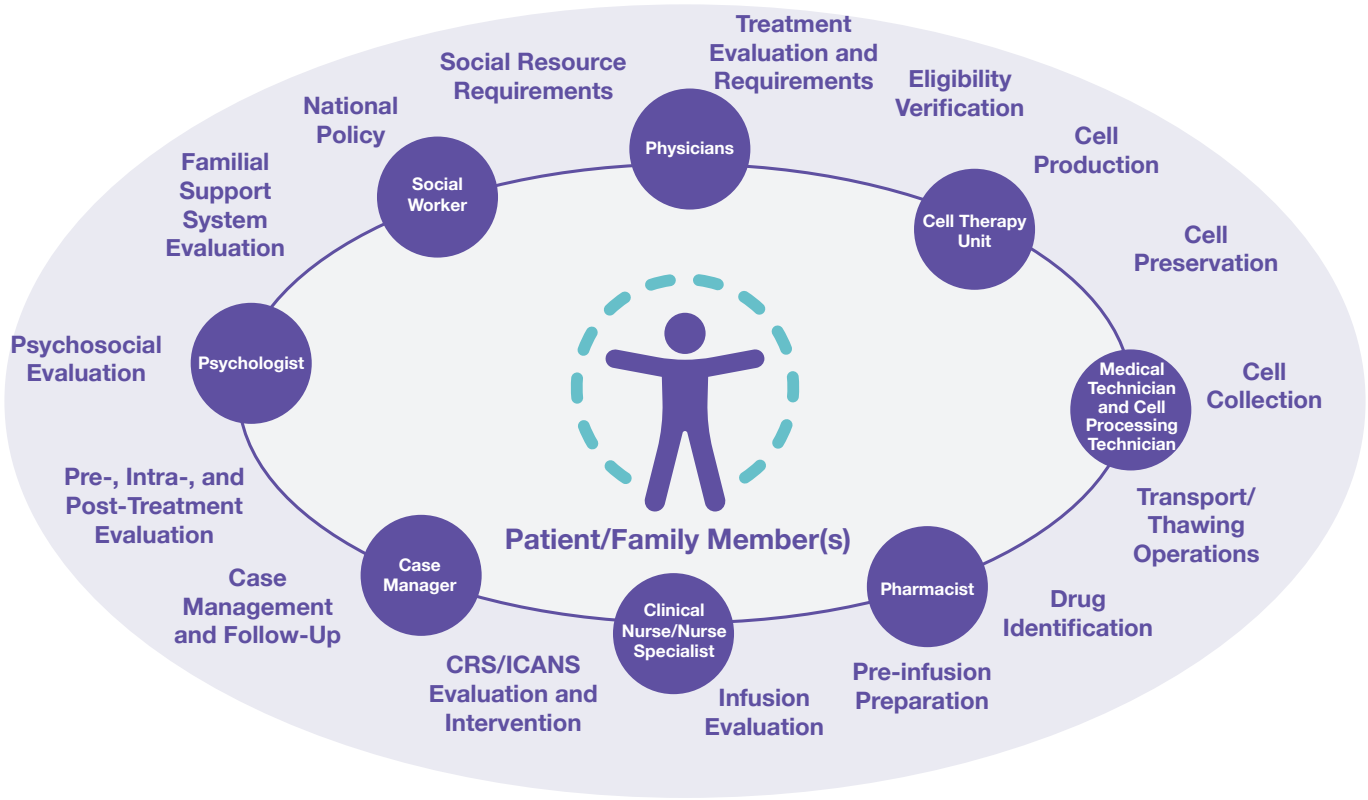
❷ Evaluation of Patient's Status and Organ Function

❸ CAR-T Cell Therapy Efficacy in Different Diseases

1.2.1 Indication

The only CAR-T cell therapy product that has obtained marketing approval from the Food and Drug Administration, Ministry of Health and Welfare, is tisagenlecleucel (brand name in English: Kymriah®, brand name in Chinese: 祈萊亞®). The therapeutic

Figure 1. CAR-T Cell Therapy Care Team[4]



CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

target of this CAR-T cell therapy is CD19. Therefore, it may only be used to treat CD19 expressing malignancies. Its indications include the following three disease conditions:[5]

1. Children and young adult patients under age of 25 years with B-cell acute lymphoblastic leukemia (ALL) that is refractory or in post-transplantation, second, or later relapse.
2. Adult patients with recurrent or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy.
3. Adult patients with recurrent or refractory follicular lymphoma (FL) after two or more lines of systemic therapy.
   These indications received accelerated approval due to tumor response rate and response duration, and still require confirmatory studies to demonstrate clinical benefits.

1.2.2 Evaluation of Patient's Status and Organ Function

Before a patient receives CAR-T cell therapy, lymphocyte collection must first be arranged. There is a wait time for CAR-T cell production (production times vary for different CAR-T cell products). Therefore, the European Society for Blood and Marrow Transplantation (EBMT) has recommended that patients with an estimated survival of at least 6-8 weeks are more eligible for CAR-T cell therapy. In addition, research has shown that 30-100% of patients who received CD19 CAR-T cell therapy will develop CRS of varying severity, and approximately 10-30% of patients will develop severe (Grade 3 and over) CRS. Consequently, it is recommended that patients receiving CAR-T cell therapy should not have poor organ function and must be confirmed to have sufficient cardiopulmonary function to tolerate at least mild CRS.To ensure that patients can tolerate fludarabine-based lymphodepleting chemotherapy (LDC), it is recommended that a marker of the patient's renal function, creatinine clearance rate (CCr), is at least 30 mL/min. [6] Furthermore, consider that ALL patients using CAR-T cell therapy may have received multiple allogeneic hematopoietic stem cell transplantations, or allo-HCT; therefore, post-transplantation status must also be evaluated, particularly whether graft-versus-host disease (GvHD) is present and whether immunosuppressant drugs are currently being used.In general, CAR-T cell therapy and allo-HCT should be separated by an interval of at least 100 days,[7] preferably with cessation of immunosuppressant use before this time. If the patient is still using immunosuppressants, it is not an absolute contraindication to CAR-T cell therapy. However, there may be a higher risk of post-treatment GvHD, and immunosuppressant drugs may affect CAR-T cell function. [8] Please refer to **Table 1**, EBMT/European Hematology Association (EHA) Recommendations from 2021 for other criteria.[8]

Table 1. Patient Eligibility Criteria for CAR-T Cell Therapy[8]

Eligibility Criterion	EBMT/EHA Recommendations	Explanation
Age	No age limit	• Although it is more difficult to collect sufficient cells through apheresis for infant or pediatric patients, CAR-T cell therapy should be indicated based on the patient's physiological status rather than age • Real-world evidence (RWE) on CAR-T cell therapy indicates that 5.9% of B-ALL patients who received treatment were under 3 years of age, whereas 53.5% of non-Hodgkin lymphoma (NHL) patients who received treatment were over 65 years of age. Both studies demonstrated that the percentage achieving complete response (CR) was comparable to that in patients at other ages
Performance status	ECOG <2 Karnofsky >60% or Lansky >60%	• Previously, patients with ECOG >1 received treatment in the setting of a non-clinical study, but results showed that the overall survival (OS) and progression-free survival (PFS) were markedly reduced
Life expectancy	>6-8 weeks	• The risk-benefit ratio must be carefully considered
High tumor burden	Risks and benefits must be evaluated	• High tumor burden in B-ALL and DLBCL is a risk factor for treatment failure and development of higher toxicity, so the individual patient's risk-benefit evaluation must be carefully considered
History of malignancy	No active malignancy requiring treatment, except for non-melanoma skin cancer or carcinoma in situ (as in cervical cancer, bladder cancer, breast cancer)	• The risk-benefit ratio must be carefully considered
Previous allogeneic hematopoietic stem cell transplantation (allo-HCT)	Not a contraindication	• Not a contraindication provided that the patient does not require immunosuppressants • Studies in ALL have shown that allo-HCT may increase the risk of toxicities associated with CAR-T cell therapy
Previous targeted therapy directed against CAR-T antigens, such as bispecific antibody/ previous CAR-T cell therapy	Not a contraindication, but antigen-negative escape should be. ruled out before administering CAR-T cell therapy in the case of B-ALL relapse after targeted therapy	• Diminished CD19 expression may not reduce the efficacy of CD19 CAR-T cell therapy in B-ALL • Previous blinatumomab treatment may reduce or impair the efficacy of CAR-T cell therapy • Reinfusion of CD19 CAR-T cells may also induce disease alleviation in a subset of patients
Immunosuppressive therapy	Relative contraindication	• Any systemic immunosuppressive therapy may impair the efficacy of CAR-T cell therapy. However, intermittent, topical, inhaled, or intranasal corticosteroids are not subject to such limitations
Bacterial or fungal infection	An active infection is a contraindication	• Treatment should be administered to achieve adequate control of the infection, e.g., stable control of infection should be achieved before the patient undergoes leukapheresis • In most cases, the occurrence of active infection requires postponing CAR-T cell therapy

Eligibility Criterion	EBMT/EHA Recommendations	Explanation
Viral infection	Viremia is a contraindication If COVID-19 PCR testing returns positive results, treatment should be postponed	• During an active viral infection, CAR-T cell therapy should be immediately postponed, with treatment to recommence only after the infection is controlled • Some latent infections (such as human immunodeficiency virus [HIV]) are contraindicated with certain CAR-T cell therapy products; be sure to refer to the package insert • If a patient with a latent hepatitis B virus, hepatitis C virus, or HIV infection undergoes CAR-T cell therapy, he or she must be given prophylactic antiviral therapy • Asymptomatic patients testing positive for COVID-19 via quantitative real-time polymerase chain reaction (qPCR) may still be able to continue with CAR-T cell therapy procedures, but certain risks are involved; doctors should determine eligibility on a case-by-case basis and confirm feasibility with the CAR-T cell production center as soon as possible before leukapheresis
Previous central nervous system (CNS) involvement	Relative contraindication	• The risk-benefit ratio must be carefully considered • **LBCL:** CNS involvement was an exclusion criterion for the ZUMA-1 and JULIET studies, but the TRANSCEND study allowed the inclusion of patients with controlled secondary central nervous system lymphoma (SCNSL) • **MCL**: CNS involvement was an exclusion criterion in the ZUMA-2 study • **B-ALL:** Ongoing CNS involvement was an exclusion criterion for the ELIANA study • Current RWE indicates that CAR-T cell therapy is well-tolerated and potentially useful in treating DLBCL patients with CNS involvement

Adapted from: Table 1, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
ALL, acute lymphoblastic leukemia; allo-HCT, allogeneic hematopoietic cell transplantation; B-ALL, B-cell acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T; CNS, central nervous system; COVID-19, coronavirus disease 2019; CR, complete response; DLBCL, diffuse large B-cell lymphoma; EBMT, European Society for Blood and Marrow Transplantation; ECOG, Eastern Cooperative Oncology Group; EHA, European Hematology Association; HIV, human immunodeficiency virus; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; qPCR, quantitative PCR; RWE, real-world evidence; SCNSL, secondary central nervous system lymphoma.

1.2.3 CAR-T Cell Therapy Efficacy in Different Diseases

Acute lymphoblastic leukemia (ALL)

According to current research, factors that influence the efficacy of CAR-T cell therapy in treating ALL include:[9]

❶ Genetic changes in leukemia

According to available research, three gene mutations may contribute to poor outcomes with CAR-T cell therapy. These are *KMT2A* rearrangement, *TP53* mutations, and hypodiploidy. Other gene mutations do not influence the efficacy of CAR-T cell therapy. Notably, although the gene mutation in Philadelphia-positive (Ph+) ALL does not affect prognosis *per se*, the recommended targeted agent for such patients, tyrosine kinase inhibitors (TKI), may affect CAR-T cell activity. Therefore, it is recommended that Ph+ ALL patients planning to receive CAR-T cell therapy avoid concomitant use of TKI drugs.

❷ Prior treatments

There is literature to suggest that previous use of blinatumomab, an immunotherapy drug that also has CD19 as its therapeutic target, may affect the efficacy of subsequent CAR-T cell therapy, but this idea is still controversial. Other literature asserts that a poor response to blinatumomab can predict a poor response to CAR-T cell therapy as well, but a good response to blinatumomab does not affect the efficacy of subsequent CAR-T cell therapy. Furthermore, ALL patients who previously received more lines of treatment may also exhibit a lesser response to CAR-T cell therapy.

3 Bone marrow tumor burden
Tumor burden is currently the most significant factor in predicting the efficacy of CAR-T cell therapy. If tumor cells are more than 5% of bone marrow nucleated cells at the time of CAR-T cell therapy, therapeutic efficacy will be significantly diminished.

4 Extramedullary disease (EMD)
Patients with active non-CNS extramedullary disease at the time of CAR-T cell therapy have worse treatment efficacy; if a patient had EMD during relapse that subsequently resolved at the time of CAR-T cell infusion, this will not affect prognosis as much. Notably, although the majority of literature indicate that prognosis does not differ significantly whether or not an ALL patient has CNS involvement, CNS involvement was excluded in the earliest ELIANA study enrollment.

5 CD19 expression levels
Low CD19 expression levels (<15%) in patient bone marrow, including leukemia cells and healthy B-cells, may affect CAR-T cell expansion, which in turn affects treatment efficacy.

The aforementioned prognostic factors influencing CAR-T efficacy may vary by study. Please refer to **Table 2** for further details on these prognostic factors:

Table 2. Factors Affecting ALL Treatment Efficacy[9]

Risk Factors	Abstract
Disease Characteristics	
Cytogenetics of leukemia	• In principle, cytogenetic pathologies in leukemia do not have a significant impact on the outcomes of CD19 CAR-T cell therapy. However, some rare specific lesions may require attention: ▸ *KMT2Ar*: Although its recurrence risk is similar to that of other groups, there is a higher incidence of myeloid lineage switch, lower survival, and worse OS ▸ *TP53* mutations: In small studies, TP53 mutations may lead to worse leukemia-free survival (LFS) and OS ▸ Hypodiploidy: Several small studies have indicated that treatment response is poor in these patients
Molecular targets	• In summary, CD19 CAR-T cells are therapeutically effective against multiple molecular subtypes of B-ALL • However, high-dose dasatinib is toxic to T cells, so CAR-T cells in combination with dasatinib is not recommended for patients with Philadelphia-positive (Ph^+) or certain Philadelphia-like B-ALL
Previous treatments	
Blinatumomab	• Two single/dual-center studies have suggested that prior use of blinatumomab may influence the CAR-T therapy response rate and recurrence risk • In contrast, the results of a different large multicenter study demonstrated that it was not the previous use of blinatumomab monotherapy, but the lack of treatment response to blinatumomab that was associated with a lower response rate and event-free survival (EFS)
Number of previous treatments	• Two multicenter analyses revealed that a higher number of previous treatments may reflect the disease's refractoriness and lead to a poorer prognosis
Risk Factors for Infusion	
Bone marrow tumor burden	• Multiple clinical trials and multicenter analyses have shown that, compared to low tumor burden, high tumor burden (generally defined as ≥5% tumor cells) is positively correlated with the risk of recurrence
Extramedullary disease (EMD)	• If active non-central nervous system extramedullary disease (non-CNS EMD) emerges during CD19 CAR-T cell infusion, EFS will be worse; however, previous EMD or CNS disease now under control does not affect the prognosis
$CD19^+$ antigen load	• Bone marrow CD19+ antigen load is associated with CAR persistence, thus affecting recurrence risk

Adapted from: Table 1, Myers RM, et al. *Blood* 2023;141:1251-1264.
ALL, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T; CNS, central nervous system; EFS, event-free survival; EMD, extramedullary disease; *KMT2Ar*, *KMT2A* rearrangement; LFS, leukemia-free survival; OS, overall survival.

Diffuse large B-cell lymphoma (DLBCL)

The results of past research indicate that factors affecting the efficacy of CAR-T cell therapy can be discussed from three aspects:

1 Patient-related factors

- Patients are more prone to recurrence after CAR-T cell therapy if they had greater tumor burden or accompanying extranodal disease before CAR-T cell infusion. [10]
- Patients with increased lactate dehydrogenase (LDH, over twice the reference value), decreased platelets (PLT, <50,000/μL) before CAR-T cell infusion have worse treatment outcomes. [11,12]
- Treatment outcomes are worse for patients who were unable to receive the full dose of fludarabine for lymphodepleting chemotherapy before CAR-T cell infusion. [12,13]
- CAR-T cell therapy efficacy and survival were worse in patients who previously underwent bendamustine treatment (with less than 9 months intervening between treatments) before lymphocyte collection. [14]

2 T cell-related factors

- When there are more naive T cells and stem cell-like memory T cells in the apheresis material from the patient's body, the prepared CAR-T cells tend to exhibit a higher capacity for expansion and better treatment efficacy. [15]
- The fewer regulatory T cells (Treg cells) present, the better the long-term treatment response. [16]

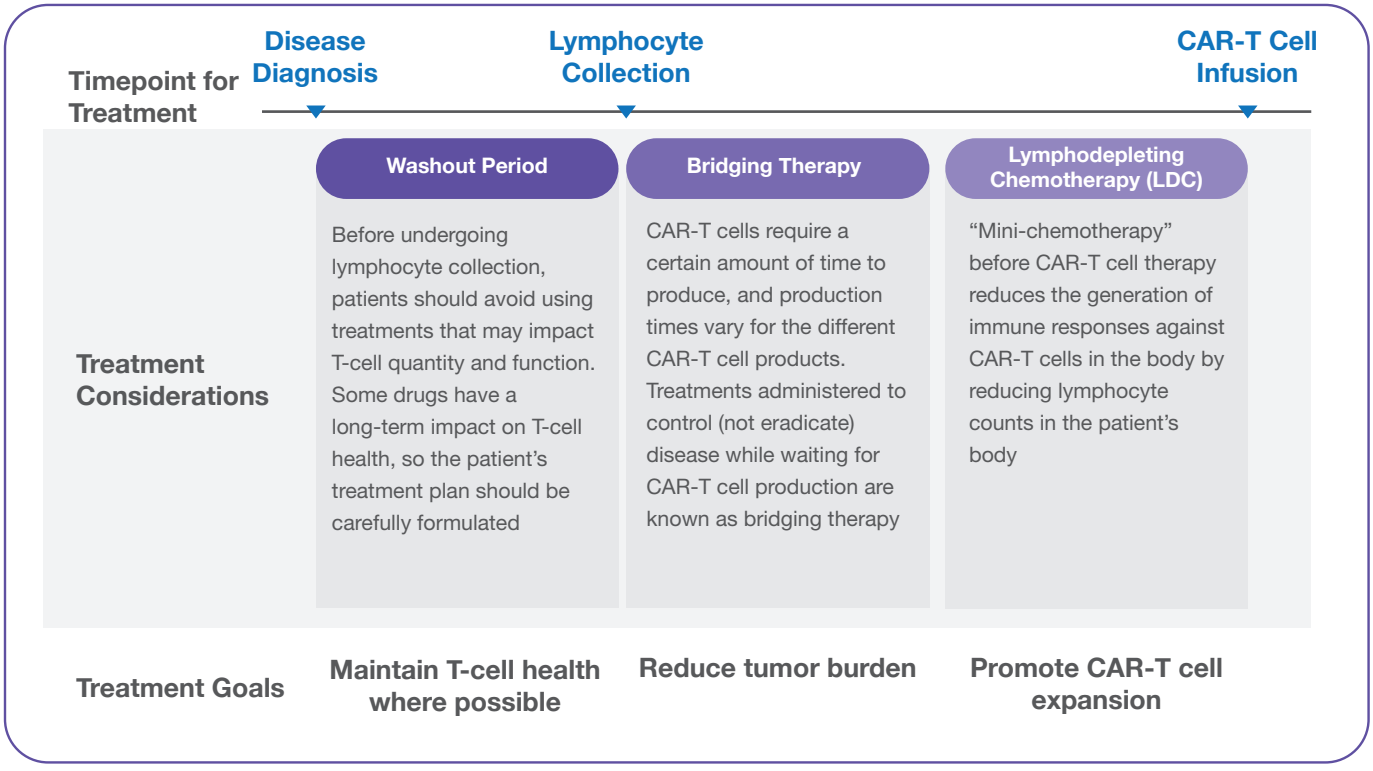
3 Tumor and tumor microenvironment factors

- *MYC* overexpression, *TP53* mutations, and a more inhibitory tumor microenvironment (TME) tend to lead to worse patient response. [17,18]

The effects of CAR-T cell therapy differ with the condition of each case and must be determined individually by physicians

1.3 Washout Period before Leukapheresis

Figure 2. Treatment Timepoints, Considerations, and Goals for the Washout Period, Bridging Therapy, and Lymphodepleting Chemotherapy



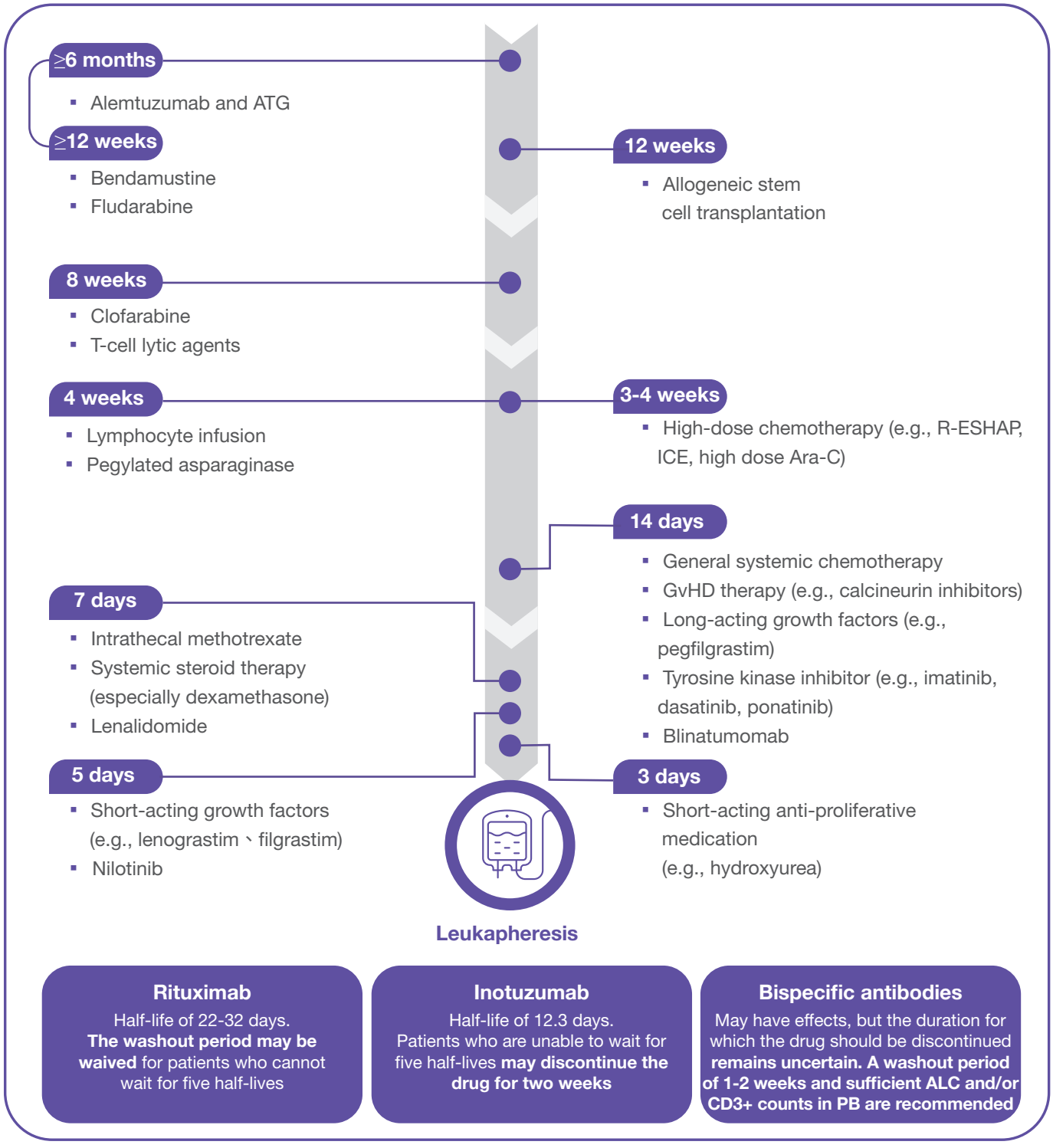
CAR-T, chimeric antigen receptor T.

The use of certain medications will have an adverse impact on T lymphocytes. For better T lymphocyte quality, it is recommended to examine previous medications prior to leukapheresis and designate a washout period, the time from disease diagnosis to CAR-T cell infusion, according to the different medications. Please refer to **Figure 2** for the timing, therapeutic considerations, and treatment goals for the washout period. As for the effects of the washout period on T cells, broadly speaking, medications with definite adverse effects on T lymphocytes require **waiting for five of their half-lives**. The washout periods in **Figure 3** are only recommendations; clinicians may make adjustments for each patient's condition.

1.4 Leukapheresis

❶ **Purpose**
Using leukapheresis, collect appropriate numbers f T lymphocytes to facilitate the subsequent CAR-T cell therapy preparation process.

Figure 3. Recommended Washout Periods for Different Drugs[19]



Adapted from: Figure 2, Qayed M, et al. *Cytotherapy* 2022;24:869-878.
ALC, absolute lymphocyte count; Ara-C, cytarabine; ATG, anti-thymocyte globulin; GvHD, graft-versus-host disease; ICE, ifosfamide + carboplatin + etoposide; PB, peripheral blood; R-ESHAP, rituximab + etoposide + methylprednisolone + cytarabine + cisplatin.

❷ Perform the pre-apheresis evaluation[8,20]

Assessment Items	Recommended Reference Values	Explanation
Physical Status	• ECOG <2, Karnofsky >60%	
Blood Oxygen Levels	• Without provision of supplementary oxygen, SaO_2 >92%	
Review of Past Medical History	• Women of childbearing age must be confirmed not to be pregnant at the time of lymphocyte collection • The pregnancy test performed on blood or urine collected within 7 days must be negative	
Infectious Disease Testing	• There should at least be laboratory results for HBsAg, HBcAb, HBsAb, anti-HCV, and anti-HIV within the past 30 days	• Tested per the requirements of the manufacturer and local competent health authorities • If HBsAg or anti-HCV is positive, viral load must also be tested
Peripheral Blood Count	• Hemoglobin >8.0 g/dL, HCT >24% • Platelet >30,000/μL	• Assist in establishing an interface for apheresis • Apheresis may consume 30% of the circulating platelets. Transfusion may increase platelet count and reduce the risk of bleeding
	• Lymphocyte count >200/μL (optional)	• Low lymphocyte counts may lead to collection failure • Tested per the requirements of the manufacturer and local competent health authorities
Standard Biochemistry Values	• Total bilirubin <1.98 mg/dL • ALT and AST <4x ULN • Creatinine clearance >30 mL/min • Cardiac function LVEF >40% • Baseline ECG • Baseline cardiac biomarkers (troponin and NT-proBNP) • Serum Ca^{2+}, Mg^{2+} within normal range	• Physicians may adjust this according to the patient's clinical condition

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; HBcAb, hepatitis B core antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HCT, hematocrit; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; ULN, upper limit of normal; SaO_2, arterial oxygen saturation.

❸ Estimate the total blood volume processed during apheresis (using Kymriah® as an example)

Lymphocyte Count	$CD3^+$ Cell Count	Total Blood Volume Processed
<100/μL	<100/μL	Consider sampling on two separate days
<300/μL	<150/μL	3 to 4 times
>300/μL	>150/μL	2 to 3 times
Significantly higher	Significantly higher	Consider two smaller-scale collections

4 Perform leukapheresis

Action Item	Recommended Reference Values	Explanation
Establish venous access	Options include a central venous catheter or a double lumen tube	
Collection rate	1.0 mL/min, which may be adjusted down to 0.8 mL/min in patients with lower lymphocyte or $CD3^+$ counts	
Set collection preferences	If collected via Spectra Optia, keeping the collection tubing at a pale salmon color may be preferable, as shown in the figure below: 	
Anticoagulant use	Acid-citrate-dextrose solution A (ACD-A) is recommended as the anticoagulant	• Heparin may be used in combination per institutional guidelines, but heparin may not be used alone
Electrolyte monitoring	Monitoring of calcium in blood or other electrolytes is recommended; supplement calcium as needed	• In accordance with institutional requirements
Priming	Approximately 200 cc of blood must be drawn from the patient's blood vessel to prime the entire line in the Spectra Optia system. If this exceeds 15% of the patient's total blood volume, leukocyte-reduced packed red blood cells (PRBC) should be used to prime the disposable line of the apheresis device before performing apheresis	• Different access lines may require different priming volumes • Suitable for low body weight or pediatric patients • Children may need to have the procedure performed in the pediatric ICU ward

ACD-A, anticoagulant citrate dextrose solution A; PRBC, packed red blood cells.

5 Documentation and related sample collection

Action Item	Recommended Reference Values	Explanation
Vital Signs	Vital signs should be measured and recorded during apheresis and until its completion	
Documentation	It is recommended to record the following information • Processed Blood Volume • Collection Volume of Leukapheresis Product • Collection Time/Date • Manufacturer/Supplier Lot Number and Effective Period	
Product Sampling (with Kymriah® as an example)	Apheresis material should be inspected for at least • Cell counts (CBC/DC) • Total nucleated count: ≥2 x 10^9 • Total $CD3^+$ count: ≥1 x 10^9 • Proportion of T-cell/total nucleated counts (TNC): >3% • Overall cell viability	In accordance with institutional requirements

CBC, complete blood count; DC, differential count; TNC, total nucleated cell.

6 Transport of apheresis materials

① Use a certified, temperature-controlled, insulated container to transport the leukapheresis product to the cell processing unit for processing. Such processing may take the form of cryopreservation, storage, and transport or may proceed directly to the production facility for production, depending on manufacturer guidelines.

② If the apheresis product cannot be frozen within 4 hours, it should be placed in a 2-8°C environment.

③ Cryopreservation must be completed within 24 hours of completing the leukapheresis product.

7 Cell product processing and cryopreservation

Action Item	Recommended Reference Values	Explanation
Action setting	Aseptic technique as required in an ISO 5 environment (e.g., a Class II biosafety cabinet)	
Cryopreservative preparation (using Kymriah® as an example)	• Dimethyl sulfoxide (DMSO): Final product concentration: 5–10% • Protein: ▸ Human serum albumin (HSA): 2.5–5% ▸ Autologous plasma: any percentage ▸ Human type AB plasma: <5%	• The cryopreservative may be pre-cooled to 2-8°C
Cryopreserved leukocyte levels (using Kymriah® as an example)	0.5 x 10^8–2.5 x 10^8 WBC/mL	• Concentrate cells via plasma removal according to actual needs • Cells may be pre-cooled • Cell concentrations after addition of cryopreservative may differ according to the requirements of each manufacturer
Quality control (QC) vials (with Kymriah® as an example)	QC vials must contain at least 1 mL of cryopreserved leukocyte product	• The content of the QC vial should come directly from the leukocyte concentrate from the final cryopreservation • The QC vial must be placed into the programmed cooling system at the same time as the cryopreserved leukocyte concentrate, and the two must be placed into the liquid nitrogen container at the same time • May vary according to the requirements of each manufacturer
Microbiological examination	After adding the cryopreservative, samples must be collected to perform microbiological examination (blood/fungus cultures)	• If samples test positive during microbiological examination, the manufacturer and institutional supervisor must be notified promptly

DMSO, dimethyl sulfoxide; HSA, human serum albumin; ISO, international organization for standardization; QC, quality control; WBC, white blood cells.

8 Programmed cooling (with Kymriah® as an example)

① After the DMSO-containing cryopreservative has been added to the cell material, it must be placed in a low-temperature environment (<4°C) with rapid initiation of the programmed cooling step, preferably performed within 20 minutes.

② The quality control (QC) vial must undergo the programmed cooling step together with the cell material in cryopreservative.

③ Example of programmed cooling operations: Each manufacturer may have different requirements, so the cooling procedure must be detailed in a graph. Please refer to **Table 3** for an example.

Table 3. Programmed Cooling Process (with Kymriah® as an example)

Steps	Mode	Rate (°C/min)	Target Temperature (°C)	Duration (min)	Explanation
1	Wait Before Start		0	0	Wait for equilibrium temperature
2	Cool	-1	-5	15	Cool at a rate of -1°C/min
3	Cool	-25	-47	1	Eutectic compensation
4	Rewarm	+10	-9	0	Eutectic compensation
5	Cool	-1	-40	0	Cool at a rate of -1°C/min
6	Cool	-10	-90	10	Cool at a rate of -10°C/min
7	End				

❾ Cryogenic Transport to Storage in a Liquid Nitrogen Container

Cell materials that have completed programmed cooling must be conveyed using a cryogenic transport container (device) to a liquid nitrogen container for storage. Temperature control documentation is required during transport. Operators must wear personal protective equipment.

❿ Other precautions

① Before performing apheresis, verify the patient's identity with at least 2-3 forms of identification (it must be confirmed that the patient has signed a consent form for the usage of their personal data).

② Only one patient's leukapheresis material may be handled at a time in the biosafety cabinet.

③ When printing labels or other chain of identity (COI) documentation, it is recommended to have two staff members check and confirm the information.

1.5 Bridging Therapy

Bridging therapy refers to therapy undertaken during the period of waiting for the completion CAR-T cell preparation after the patient's T-cells have been collected by leukapheresis, before lymphodepleting chemotherapy. Clinical studies have shown that approximately 7% of patients do not survive the period of waiting for CAR-T cell preparation to be completed,[20] highlighting the importance of reduced cell preparation time and bridging therapy. The goal of bridging therapy is to prevent rapid disease progression, reduce disease burden, increase the efficacy of CAR-T cell therapy, and simultaneously reduce the risk of possible accompanying adverse immune reactions while awaiting CAR-T cell infusion. [7,8] Bridging therapy may be omitted in case of shorter time from "material collection to infusion" (also known as vein-to-vein time) of CAR-T cell therapy and lower disease burden. [8]

For rapidly deteriorating patients, the choice of bridging therapy is determined by the specific clinical situation; this includes considering tolerance and response to prior treatment(s), the site and mass of neoplasm growth, complications/present abnormalities in organ function, and the patient's current physical status. For patients with greater tumor burden, the ideal state is successful tumor debulking or maintenance of stable disease; however, this is not also feasible, particularly when the disease develops tolerance to chemotherapy. Therefore, selection of suitable bridging therapy and preventing excessive toxicity in patients to prevent serious adverse events helps prevent delays in CAR-T cell infusion, which in turn reduces possible CRS-related impairments in organ function, or reduces chronic cytopenia and infection risk. [7]

Caution must also be taken to prevent bridging therapy from adding to the toxicity of lymphodepleting chemotherapy. Therefore, experts recommend the need for a washout period separate from lymphodepleting chemotherapy, as shown in **Table 4**. [8]

In addition, a study by S. Shahid and colleagues showed the impact of bridging therapy on CAR-T cell therapy outcomes[21] by dividing the core bridging therapy into high and low potency groups. High potency bridging therapy is defined as being expected to cause more than 7 days of myelosuppression. This retrospective study found that patients using stronger bridging therapy with more numerous treatment cycles tend to be patients with more severe disease; the study results indicate that pre-treatment tumor burden, pre-lymphodepleting chemotherapy tumor burden, and tumor reduction from bridging therapy do not affect the efficacy of CAR-T cell therapy. Conversely, patients who use more than two cycles of bridging therapy exhibit not only a higher risk of infection, but also worse overall survival.

Table 4. Recommended Intervals Between Bridging Therapy and Lymphodepleting Chemotherapy (Expert Opinion)[8]

Treatment Method	EBMT/EHA Recommendations	Explanation
High-dose chemotherapy	3-4 weeks	Avoid additional toxicity and chronic cytopenia
Intrathecal chemotherapy	1 week	Avoid additional toxicity
Short-acting cell-mediated cytotoxicity/ anti-proliferative medication	3 days	Avoid additional toxicity
Radiotherapy	1 week (2 weeks for the lungs)	Avoid additional toxicity
Tyrosine kinase inhibitor (TKI)	3 days	Avoid additional toxicity

Adapted from: Table 5, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
EBMT, European Society for Blood and Marrow Transplantation; EHA, European Hematology Association; TKI, tyrosine kinase inhibitor.

Finally, this may be administered at either the original referring hospital or CAR-T cell therapy providing hospital provided that the original referring hospital and the CAR-T cell therapy providing hospital discuss the bridging therapy strategy in detail.

1.5.1 Bridging Therapy Strategy for ALL

Recommendations proposed by the EBMT/EHA for possible strategies for the bridging therapy used by ALL patients before CAR-T cell therapy include the following:[22]

1. **Treatment not required**
   smoldering disease.
2. **Low-intensity chemotherapy**
   Patients with low tumor burden and/or slowly progressing ALL (Ph^+ ALL patients may consider targeted therapy, such as tyrosine kinase inhibitor therapy).
   ① Weekly administration of vincristine in combination with oral mercaptopurine (6-MP) and methotrexate
   ② Weekly administration of vincristine in combination with dexamethasone 6 mg/m^2, 2 days/ week
3. **Intermediate-intensity chemotherapy**
   Patients with tumor burden and/or progressing ALL.
   ① Consolidation therapy (6-MP, cytarabine, cyclophosphamide)
   ② Weekly administration of vincristine in combination with dexamethasone, bortezomib, and asparaginase
4. **High-intensity chemotherapy**
   Patients with invasive or extramedullary disease.
   ① High-dose cytarabine, etoposide (VP16) in combination with cyclophosphamide and hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone)
   ② If CNS is involved, administer high-dose methotrexate
5. **Very high-intensity chemotherapy**
   Patients with rapidly progressing disease.
   ① Sequential approach such as administering high-dose cytarabine treatment followed by lymphodepleting chemotherapy
   ② The use of immunotherapy (such as blinatumomab and inotuzumab ozogamicin) in bridging therapy may still be effective. However, blinatumomab may theoretically cause selection of CD19-negative clones, and if inotuzumab ozogamicin treatment achieves minimal residual disease (MRD), the absence of residual B-cells may affect subsequent CAR-T cell expansion and persistence[22]

1.5.2 Bridging Therapy Strategy for Lymphoma

For lymphoma patients, bridging therapy options include:[20]

1. **Chemotherapy**
   Such as gemcitabine, etoposide, or platinum. Avoid high-toxicity treatment methods that may cause serious side effects or hinder CAR-T cell infusion.
2. **Radiotherapy**
   This may be considered for disease with prominent focal swelling/symptoms and may be continued until 1 day before lymphodepleting chemotherapy.
3. **Steroids**
   Before lymphodepleting chemotherapy, perform pulse steroid therapy or appropriate tapering; discontinue steroid use before lymphodepleting chemotherapy.
4. **Targeted therapy**
   ① Ibrutinib: Clinical data show increased durability and response to CAR-T cell therapy; this may be used until 1 day before lymphodepleting chemotherapy provided that there is no drug-associated cytopenia
   ② Lenalidomide: Clinical data suggests increased efficacy of CAR-T cell therapy; discontinuation one week before lymphodepleting chemotherapy is recommended for blood cell recovery to facilitate lymphodepleting chemotherapy
   ③ Rituximab, obinutuzumab
   ④ Polatuzumab + bendamustine + rituximab (pola-BR)
5. **Anti-CD19 therapy**
   In studies, tafasitamab in combination with lenalidomide had unknown effects on the efficacy of CAR-T cell therapy.

02

CAR-T Cell Therapy

Chapter 2

CAR-T Cell Therapy

2.1 Lymphodepleting Chemotherapy (LDC)

Lymphodepleting chemotherapy (LDC) or a lymphodepleting conditioning regimen is key to the success of CAR-T cell therapy; therefore, LDC will typically be given before CAR-T cell therapy. Its primary aim is to reduce lymphocyte numbers and the immune response to CAR-T cells in the human body, thus promoting CAR-T cell expansion. Furthermore, LDC can also reduce the cells in the human body responsible for inhibiting immune responses, such as regulatory T cells and regulatory cytokines, and may even enhance disease control. [22] Available research all indicate that LDC plays an indispensable role in CAR-T cell therapy; in the absence of LDC administration, CAR-T cell therapy exhibits worse efficacy. [7] LDC is highly important for the expansion and persistence of CAR-T cells. Please refer to **Table 5** for related mechanisms. [22]

Table 5. Mechanisms and Effects of LDC Regimens[22]

Lymphodepletion	Reduce overall NK, B-, and T-cell counts
Immune response to CAR-T cells	Reduce the immune response of the patient's immune system to anti-transgene cells
Eradicate immune suppressor cells	Treg cells and myeloid-derived suppressive cells (MDSC)
Modulate tumor-suppressing actions	Reduce IDO expression and increase costimulatory molecular expression
Eliminate homeostatic cytokine sink	Increase IL-2, IL-7, IL-15, and MCP-1 expression
Enhance cell expansion, function, and persistence	Better, more durable tumor suppression

Adapted from: Table 25.1, The EBMT/EHA CAR-T Cell Handbook.
CAR-T, chimeric antigen receptor T; IDO, indoleamine deoxygenase; IL, interleukin; LDC, lymphodepleting chemotherapy; MCP-1, monocyte chemoattractant protein-1; MDSC, myeloid derived suppressor cell; NK, natural killer; Treg, regulatory T cell.

Current LDC medications types, doses, and schedules all vary somewhat according to the different CAR-T cell therapy products and diseases treated, but most are based primarily on fludarabine and cyclophosphamide (see the **following table**). [7,22] Complications potentially arising from LDC include neutropenia, anemia, thrombocytopenia, and risk of infection due to immunosuppression. Specific toxicities related to lymphodepleting medications include:[8]

- **Fludarabine:** fever and neurotoxicity.
- **Cyclophosphamide:** hemorrhagic cystitis, pericarditis, and neurotoxicity.

In addition, the dose should be adjusted appropriately for insufficient renal or hepatic function.

2.1.1 LDC for ALL

Recommendations for CAR-T cell therapy use in the LDC of ALL (temporarily using Kymriah® as an example):[5,7]

- **Fludarabine** (intravenous infusion 30 mg/m^2 30 minutes x 4 days) and **cyclophosphamide** (initiated with the first dose of fludarabine, intravenous infusion 500 mg/m^2 60 minutes x 2 days).
  Current research showed that insufficient doses of fludarabine may lead to poor CAR-T cell efficacy.
- **Cytarabine** (intravenous infusion 500 mg/m^2 x 2 days) and **etoposide** (initiated with the first dose of cytarabine, intravenous infusion 150 mg/m^2 x 3 days): If the patient previously developed Grade 4 hemorrhagic cystitis due to cyclophosphamide administration or has demonstrated chemotherapy refractoriness to cyclophosphamide-based therapy, cytarabine and etoposide therapy should be used.
  To prevent LDC from directly affecting CAR-T

Day	-6	-5	-4	-3	-2	-1	0
Fludarabine x 4	✓	✓	✓	✓			
Cyclophosphamide x 2	✓	✓					
CAR-T Cell Infusion							✓

CAR-T, chimeric antigen receptor T.

cells, it is currently recommended to perform CAR-T cell infusion 48 hours after completing LDC. It is also because of this that LDC is recommended to be administered 2-14 days before CAR-T cell infusion. [7] If CAR-T cell infusion cannot be performed for some reason after administering LDC, infusion may be delayed for at most four weeks; this means that if CAR-T cell infusion is not performed for four weeks after LDC administration, LDC must be re-administered. [8]

Possible risks of LDC include: cytopenia, infection, hemorrhagic cystitis, and neurotoxicity[22]. Consequently, whether the patient can receive LDC and the subsequent CAR-T cell therapy must be re-evaluated before administering LDC. Please refer to **Table 6** for assessment items. Key points for evaluation include infection status; cardiac, hepatic, and renal function; and the progress of CAR-T cell preparation;[8] if the patient's lymphocyte count has already dropped below 200/μL, some literature recommend considering not administering LDC. [22]

Table 6. Assessment Items for ALL Patients Before LDC[8]

Criterion	EBMT/EHA Recommendations	Explanation
CAR-T cell product	LDC should be initiated after receiving the CAR-T cell product	• In specific cases, LDC may be initiated once successful CAR-T cell product preparation has been confirmed, but before receiving the product
Clinical status	Active infection should be ruled out before performing LDC	• The patient's health status should be appropriate for receiving LDC
Blood oxygen saturation	≥92%	
WBC count	All patients must undergo LDC, regardless of their WBC count or absolute lymphocyte count (ALC)	• Tisagenlecleucel's product summary explains: Patients with a lower WBC count (<1x10^9/L) one week before CAR-T cell infusion may not need to receive LDC • Some experts will perform LDC with caution in patients with idiopathic neutropenia before CAR-T cell infusion. • Given the importance of LDC to CAR-T cell activity, it is generally not recommended to perform CAR-T cell infusion without LDC
C-reactive protein, ferritin, LDH, metabolic profiling, fibrinogen	Ongoing infection must be excluded	• Evaluate the incidence risk of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome based on baseline status
Bilirubin	• <2 mg/dL • Patients with Gilbert's syndrome have a higher acceptable upper limit (>2.5 mg/dL)	• No clinical data is available to support values beyond those provided here
AST/ALT	These should satisfy ≤4 ULN or specific test standards	• Attempts should be made to identify the cause of hepatic function abnormality, such as infection, drug toxicity (including antifungal medications), veno-occlusive disease (VOD), GvHD, or other diseases
Creatinine clearance	>30 mL/min	• For creatinine clearance rate <60 mL/min, the physician should consider appropriate dose reductions for cyclophosphamide and fludarabine, and may increase the time interval between LDC and CAR-T cell reinfusion, so that there is ample time for fludarabine metabolites to be cleared from the body
Cardiac function	When heart failure or cardiotoxicity during bridging therapy occurs, repeated cardiac examinations are required	• When necessary, transesophageal echocardiography (TEE), electrocardiography (ECG), cardiac biomarker (cardiac troponin and NT-proBNP) studies may be performed again; a cardio-oncology assessment is also mandatory

Criterion	EBMT/EHA Recommendations	Explanation
Evaluation of tumor burden	Baseline assessment	• Use PET-CT/other imaging modalities; perform bone marrow tests; perform lumbar puncture (LP) if necessary

Adapted from: Table 7, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
ALC, absolute lymphocyte count; ALL, acute lymphoblastic leukemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; EBMT, European Society for Blood and Marrow Transplantation; ECG, electrocardiogram; EHA, European Hematology Association; GvHD, graft-versus-host disease; ICANS, immune effector cell-associated neurotoxicity syndrome; LDC, lymphodepleting chemotherapy; LDH, lactate dehydrogenase; LP, lumbar puncture; NT-proBNP, N-terminal pro-brain natriuretic peptide; PET-CT, positron emission tomography-computed tomography; TTE, transthoracic echocardiography; ULN, upper limit of normal; VOD, veno-occlusive disease; WBC, white blood cells.

2.1.2 LDC for DLBCL/FL

Recommendations for LDC during the use of CAR-T cell therapy in patients with recurrent or refractory DLBCL and FL patients (temporarily using Kymriah® as an example):[5]

- **Fludarabine** (intravenous infusion 25 mg/m^2 x 3 days) and **cyclophosphamide** (initiated after the first dose of fludarabine, intravenous infusion 250 mg/m^2 x 3 days).
- **Bendamustine** (intravenous infusion 90 mg/m^2 x 2 days): If the patient previously developed Grade 4 hemorrhagic cystitis not long before receiving LDC or has demonstrated chemotherapy refractoriness to cyclophosphamide-based therapy, bendamustine therapy should be used.

Intervals between LDC and CAR-T cell therapy infusion for DLBCL/FL patients are, respectively:

- For patients with recurrent or refractory DLBCL, performing CAR-T cell infusion 2 to 14 days after completing LDC is recommended.
- For patients with recurrent or refractory FL, performing CAR-T cell infusion 2 to 6 days after completing LDC is recommended.

If the patient develops significant cytopenia, such as a leukocyte count <1,000/mL one week before infusion, LDC may be omitted. If the interval between LDC completion and CAR-T cell infusion is **over 4 weeks and WBC is >1,000/mL**, the patient should undergo LDC again before CAR-T cell infusion. [7]

2.2 Product Receipt, Thawing, and Infusion

Purpose
Receive the CAR-T cell product produced by the manufacturer, undertake COI checks as part of the standard biobank entry registration, perform thawing at the patient's bedside, and complete CAR-T cell infusion.

2.2.1 Product Receipt

Product Receipt Process

Assessment Items	Recommended Reference Values	Explanation
Product Receipt	Confirm the product and check the patient's COI records	• At least two people should be checking this at the same time, in accordance with the institutional operating guidelines; pharmacist participation may be mandatory
Review of release and acceptance documents	Release documents from the manufacturer may include the following: • CAR-T cell viability • Volume • Documentation related to temperature control during the transport process • Microbiological testing results (sterility testing) • Quality control reports	• Handle per the manufacturer and institutional operating guidelines • Out-of-specification (OOS) CAR-T cell therapy products may still be used (exceptions include microbial/hazardous material contamination) provided that the certificate of release specifies OOS details, the manufacturer's risk assessment form and the clinician's usage assessment are both available in writing (the manufacturer and physician both bear liability), and the patient also provides written consent[8]
Warehousing operations	Perform warehousing operations according to the unique blood bag code	• Handle per the institutional operating guidelines

Assessment Items	Recommended Reference Values	Explanation
Storage in liquid nitrogen containers	After removal from the cryogenic transport container, the chain of identity documents and package quantity and integrity must be re-confirmed to have no leakage or damage, and the product should be placed into a liquid nitrogen container for storage as soon as possible	• Handle per the institutional operating guidelines

CAR-T, chimeric antigen receptor T; COI, chain of identity; OOS, out of specification.

2.2.2 Thawing

Operations Before Thawing

Action Item	Explanation
Excluding emergency complications	• Before thawing and infusing CAR-T cells, the following situations must be ruled out: acute infection, heart failure, uncontrolled cardiac arrhythmia, shock requiring vasopressors, or recent organ failure
Establish venous access for infusion	• Tubing must be tested to be sterile and unblocked to ensure that CAR-T cells can be infused intact into the body
Pre-infusion medications	• 30-60 minutes before infusion, acetaminophen/paracetamol and diphenhydramine may be administered to reduce acute infusion reactions • Systemic corticosteroid injections should be avoided
Establish a physiological monitoring system	• Monitor blood oxygen, electrocardiography, and blood pressure (3-in-1 physiological monitor) to evaluate vital signs
Prepare a heater	• Dry or wet heaters may be set to a temperature of 37±2°C

CAR-T, chimeric antigen receptor T.

Thawing operations

Action Item	Explanation
Confirm the doctor's order for infusion and release criteria	• Confirm that the patient's identity matches that on the CAR-T cell product • Confirm that the CAR-T cell product meets clinical or local regulatory release criteria
Withdrawal operations and transfer of CAR-T cells to a cryogenic transport container	• Temperature control is required during the process of delivering the cryogenic transport container to the patient's bedside
Perform thawing	• Place the CAR-T cell product into the heater for thawing and ensure that it receives even heating • After thawing, the product should be removed from the heater and placed at room temperature (20-25°C) • If there is more than one bag of CAR-T cell product, the second bag must be thawed only after completing the infusion of the first bag

CAR-T, chimeric antigen receptor T.

2.2.3 Infusion

Infusion Operations

Action Item	Explanation
Reinfusion	• After receipt of the thawed CAR-T cell product, have two people verify the patient's name and date of birth and the product's expiration date together • Connect an IV administration set without a leukocyte removal filter for the infusion. Complete the infusion as quickly as possible, within 20-30 minutes of thawing. Normal saline solution may be used to rinse the IV bag to reduce cell residue • Healthcare providers must participate for the full duration of the infusion to monitor blood oxygen saturation, electrocardiography, and blood pressure
Monitor vital signs	• Evaluate the patient's vital signs every 15 minutes during the infusion process • After completing reinfusion, evaluate the patient's vital signs once every 30 minutes, for at least 1-2 hours, to observe whether there are any adverse reactions
Completing documentation	• The code of the product infused into the patient and the infusion time (the time from start to finish) must be recorded • Common adverse reactions: nausea, vomiting, abdominal pain, perception of a garlic-like odor • Rare but serious adverse reactions: hypotension, hypertension, bradycardia, cardiac arrest, chest tightness, skin rash, anaphylactic shock

CAR-T, chimeric antigen receptor T.

03

Chapter 3

Post CAR-T Cell Therapy

3.1 Short-term Complications

3.1.1 Tumor Lysis Syndrome (TLS)

Tumor lysis syndrome (TLS) refers to the situation in which large numbers of tumor cells die off within a short time and release high quantities of intracellular substances into the blood, causing electrolyte abnormalities and renal impairment.

Clinical Symptoms

Symptoms that may be induced by TLS include cardiac arrhythmia (hypocalcemia or hyperkalemia), acute kidney injury, or seizures; these symptoms may be classified based on disease progression and severity into TLS with only blood test abnormalities (laboratory TLS) and clinically symptomatic TLS (clinical TLS). [23] Prophylactic administration or early treatment after laboratory abnormalities can reduce the likelihood of symptoms and complications after cell infusion.

TLS due to CAR-T cell therapy is less frequent than that caused by conventional treatment of other hematological cancers. [24] CAR-T cell therapy may cause TLS through LDC or the destruction of large numbers of tumor cells. Consequently, lymphoma patients with a bulky tumor or lymphoblastic leukemia patients with a high tumor burden experience a higher incidence of TLS,[25] requiring careful attention. Because of this, literature and clinical guidelines all recommend administering prophylactic TLS such as allopurinol or febuxostat from the start of LDC. According to reports in literature, CAR-T cell therapy patients who do not receive LDC may also develop TLS; furthermore, delayed onset of TLS may occur at Day 28 to 100 after CAR-T cell therapy.[26] This may be similar in cause to delayed CRS, as both may be caused by continued destruction of tumor cells by CAR-T cells.

Clinical Management

TLS induced by CAR-T cell therapy is similar to TLS caused by other treatments. In general, this may be managed according to standard hospital protocols for TLS.

The TLS management guidelines for adult and pediatric hematological cancers proposed by the British Committee for Standards in Haematology (BCSH) in 2015[27] stratify patients by risk factors into low-, intermediate-, and high-risk groups for prophylaxis and treatment. [28] Under the BCSH guidelines, diseases for which CAR-T cell therapy is currently indicated are all considered moderate- or high-risk. Older patients or those with less than ideal renal function have a higher chance of developing TLS. The BCSH-recommended strategies for TLS prevention and treatment are as follows:[27]

Preventing TLS

1. Patients in the moderate-risk group should be given hydration or 7 days of allopurinol (or until there is no risk of TLS).
2. Unless the patient has glucose-6-phosphate dehydrogenase (G6PD) deficiency, it is recommended that high-risk patients be given prophylactic hydration and rasburicase. Fixed dose administration: 3 mg for adults, 0.2 mg/kg for children, or adjust according to the situation.
3. Urine alkalinization is not recommended.
4. Patients given rasburicase do not require concomitant use of allopurinol; this is to prevent reduced efficacy of rasburicase.

Treatment Strategies for TLS

1. Transfer to an intensive care facility is recommended.
2. It is recommended that hydration volumes must maintain urine output of >4 mL/kg/hr (>100 mL/m^2/hr for infants), and the infusion must not contain potassium ions.
3. Unless the patient has G6PD deficiency, rasburicase therapy should be given at a dose of 0.2 mg/kg/day for 3-7 days.
4. Treatment is not recommended in asymptomatic hypocalcemia.
5. Indications for renal dialysis:
   ① Intractable fluid overload.
   ② Hyperkalemia ≥7 mmol/L.
   ③ Hyperuricemia, hyperphosphatemia, hypocalcemia, or worsening renal function after administering the treatments above.
6. Peritoneal dialysis is not recommended.

3.1.2 Cytokine Release Syndrome (CRS)

Pathogenesis[29]

After CAR-T cells enter the human body, they recognize tumor cells bearing specific antigens. Using the CD19 CAR-T cell as an example, CAR-T cells recognize CD19-expressing tumor cells. Once tumor cells are discovered, the CAR-T cells begin to activate and expand, secreting a series of cytokines, including IFN-γ, TNF-α, and IL-2. These cytokines will not only further activate CAR-T cells and stimulate the secretion of other cytokines such as IL-1, IL-6, and IL-10, but also simultaneously stimulate other peripheral immune effector cells such as macrophages, which will begin to attack tumor cells and thus cause the symptoms of CRS. As CAR-T cells continue their activation and expansion, cytokine secretion continues to increase, inducing a systemic inflammatory response. This causes endothelial damage and vascular leakage in multiple organs, leading in turn to hypotension, hypoxemia, and other forms of organ damage. These inflammatory responses will become attenuated and ultimately disappear after the CAR-T cells clear away the tumor cells, and the patient will also achieve relief from CRS. CRS incidence varies by clinical study and cell product, with reports ranging from 30% to nearly 100%. Incidence of severe CRS is approximately in the range of 10-30%. [8]

Clinical Symptoms

CRS is a systemic inflammatory response. Fever is its earliest symptom, and other possible symptoms include tachycardia, nausea, headaches, and rashes. As the inflammatory response grows in strength, tachypnea and hypoxemia may occur. More severe cases may present with hypotension, respiratory failure, and multiple organ failure, and may even be life-threatening. [30] CRS may occur within 1-14 days of CAR-T cell infusion, with a median time of approximately 7 days after infusion. However, there have been cases of delayed CRS occurring over 14 days after infusion; this indicates the importance of continued observation within 30 days. CRS symptoms may persist for 4-7 days. [8]

CRS Classification

The American Society for Transplantation and Cellular Therapy (ASTCT) classifies CRS into four grades based on the clinical severity of three indicators: fever, hypotension, and hypoxia, as shown in **Table 7**. [31]

Clinical Examination

All patients who develop CRS must undergo blood culture, urine culture, chest X-rays, and other related studies to rule out the possibility of infection, and virological studies such as those for cytomegalovirus (CMV) or Epstein-Barr virus (EB virus, EBV) may

Table 7. ASTCT CRS Classification[31]

	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C
		and	and	and
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors, but excluding vasopressin
		and/or†	and/or†	and/or†
Hypoxia	None	Must receive ≤6 L/min low-flow‡ or blow-by oxygen therapy	Must receive >6 L/min high-flow oxygen, mask, non-rebreathing mask, or Venturi mask oxygen therapy	Must receive positive-pressure oxygen therapy (such as continuous positive airway pressure [CPAP], bilevel positive airway pressure [BiPAP], intubation, and mechanical ventilation)

CRS-related organ toxicities may be graded according to CTCAE v5.0, but they do not affect CRS grading

* Fever is defined as a body temperature ≥38°C in the absence of other causes (e.g., infection). For CRS patients who have already received antipyretic or anti-cytokine therapy (such as tocilizumab or steroidal treatment), subsequent grading no longer needs to take fever into account and may be determined based on hypotension and hypoxia

† Hypoxia requiring one type of vasopressor and low-flow oxygen is classified as Grade 3

‡ Low-flow oxygen is defined as an oxygen delivery rate ≤6 L/minute, which also includes blow by oxygen delivery (sometimes used for pediatric patients); high-flow oxygen is defined as an oxygen delivery rate >6 L/minute

Adapted from: Table 2, Lee DW, et al. *Biol Blood Marrow Transplant* 2019;25:625-638.

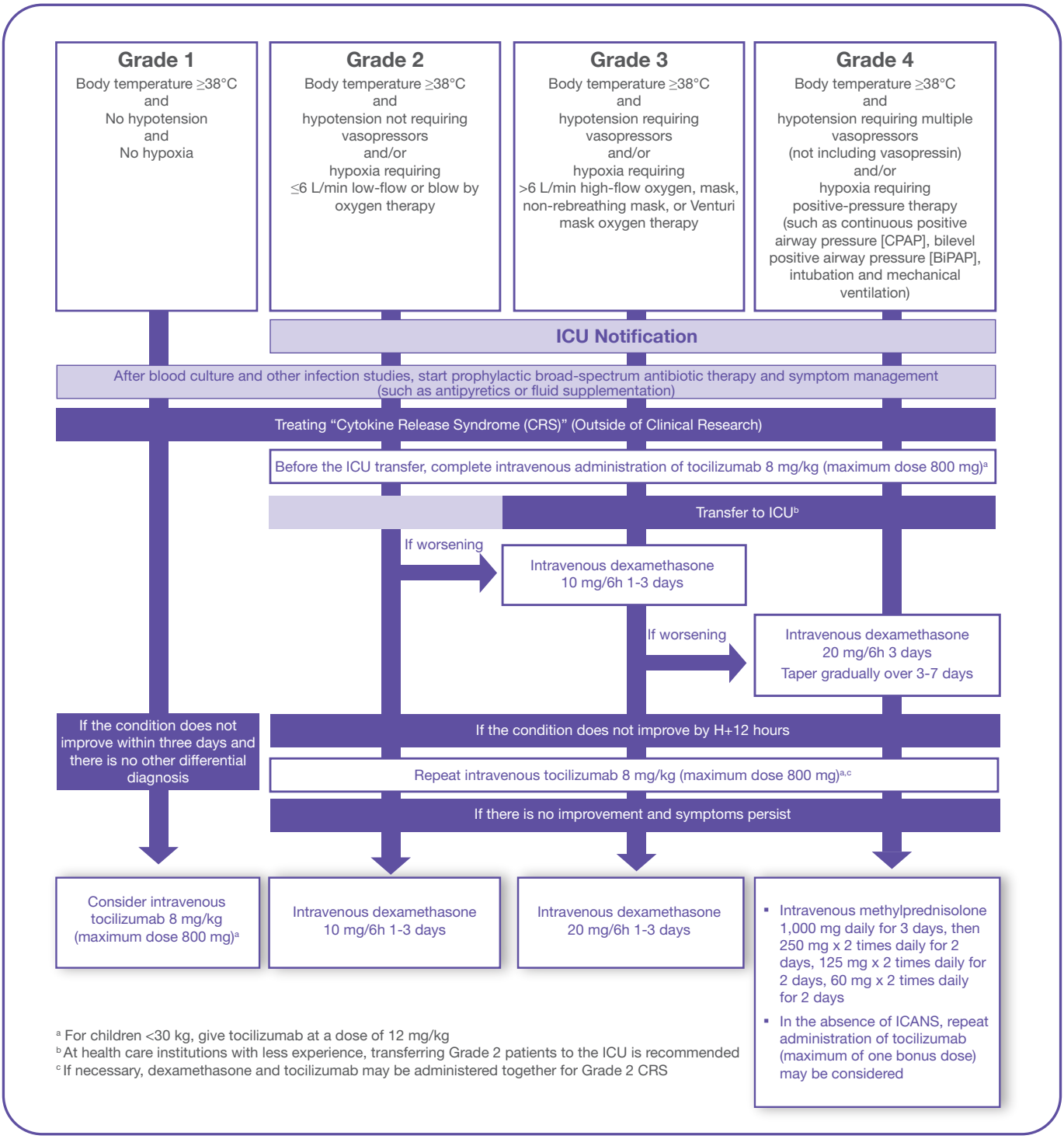
ASTCT, American Society for Transplantation and Cellular Therapy; BiPAP, bi-level positive airway pressure; CPAP, continuous positive airway pressure; CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events.

also be considered. If a patient develops severe CRS, performing an echocardiogram may be considered to assess cardiac function. Supportive therapy plays an important role in CRS treatment. Antipyretics, intravenous fluids, oxygen, broad-spectrum antibiotics, and other medications for symptom relief must be administered in a timely manner. If the patient develops Grade 2 or higher CRS, a continuous vital signs monitor must be used; if a patient's vital signs become unstable, transfer to an intensive care unit for active monitoring must be considered early on. [30]

Clinical Management

Fever, hypotension, hypoxemia, or organ damage developing after CAR-T cell infusion should be suspected as symptoms of CRS. Clinical management of CRS differs by severity and grading. Please refer to **Figure 4** for further details. [8]

Figure 4. Grading and Management of CRS[8]



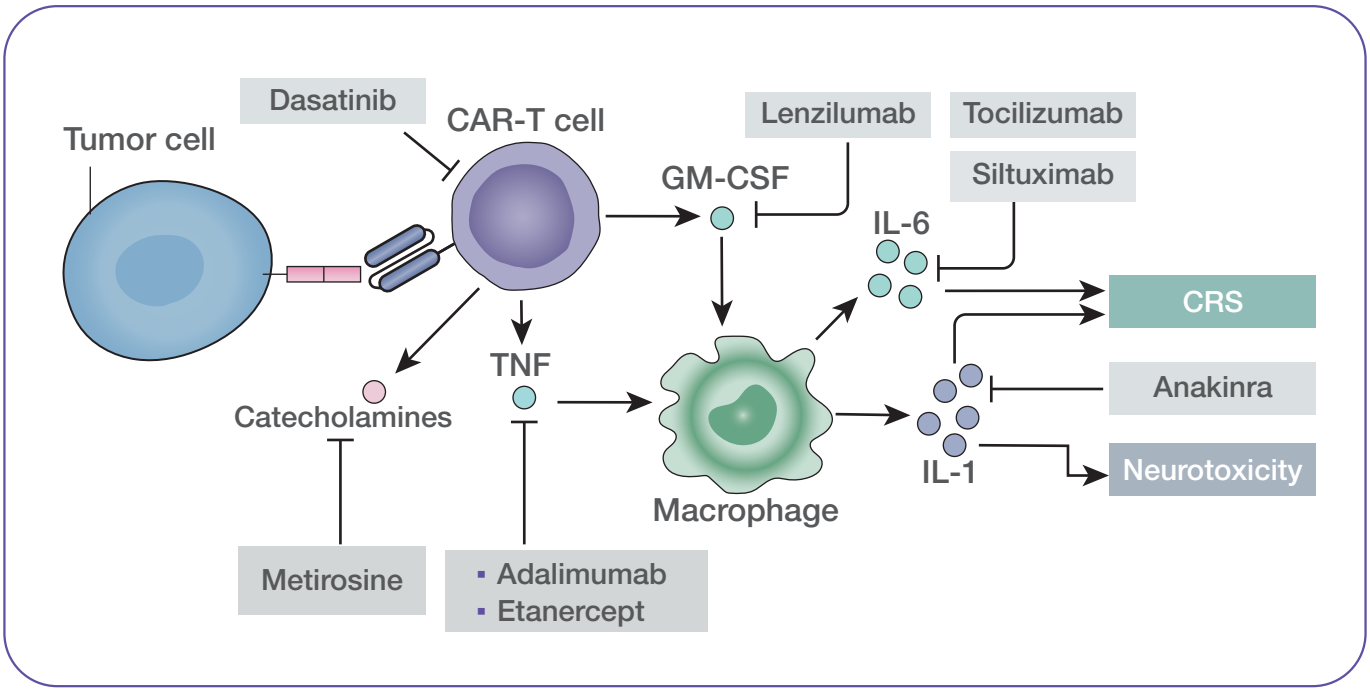
Adapted from: Figure.1 Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
BiPAP, bi-level positive airway pressure; CPAP, continuous positive airway pressure; CRS, cytokine release syndrome; h, hours; ICANS, immune effector cell-associated neurotoxicity syndrome; ICU, intensive care unit.

Mechanisms and Medications for CRS

Cytokines play an important role in the pathogenesis of CRS, and blocking cytokines is the core strategy for managing CRS. Currently, this strategy primarily takes the form of tocilizumab (8 mg/kg/dose for adults, 12 mg/kg/dose for children <30 kg) use in IL-6 blockade. If tocilizumab elicits no significant response after a single administration, it may be repeated every eight hours, typically no more than three times within 24 hours, for a total of four times. [30] Notably, if clinical symptoms fail to improve after two administrations of tocilizumab, besides continuing to administer tocilizumab, it is even more important to consider administering steroids (dexamethasone, 10 mg/dose for adults, 0.5 mg/kg/dose for children). It may be inferred from available evidence that short-term steroid use does not affect long-term CAR-T cell efficacy and may still be used if clinically necessary. However, once CRS improves, steroids should be tapered as soon as possible. [8,30]

If the patient develops resistant CRS that does not respond to any of the above treatments, other IL-6 blockade drugs such as siltuximab may also be considered. [24] Otherwise, cytokines other than those involved in IL-6 blockade may also be considered, including: IL-1 blockers such as anakinra may be considered for CRS and ICANS that either are refractory or respond poorly to tocilizumab and steroids. Please refer to **Figure 5** for related drugs and targets. [29]

Figure 5. Mechanisms and Medications for CRS[29]



Adapted from: Figure 3, Morris EC, et al. *Nat Rev Immunol* 2022;22:85-96.
CRS, cytokine release syndrome; CAR-T, chimeric antigen receptor T; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; TNF, tumor necrosis factor.

3.1.3 Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Approximately 20-60% of patients receiving CD19 CAR-T cell therapy develop ICANS (Grade ≥3 in 12-30%). Neurotoxicity typically occurs in the 4-10 days after CAR-T cell infusion and may continue for 14-17 days. ICANS typically occurs not long after CRS or simultaneously with CRS; 10% of patients will manifest "delayed ICANS" over 3 weeks post-infusion.

The pathophysiology of ICANS may be attributed to a combination of multiple etiological factors, including inflammatory cytokines causing increased vascular permeability, activation of endothelial cells causing loss of blood-brain barrier function, and increased cytokines in the cerebrospinal fluid, which may cause cerebral edema under certain circumstances. Pharmacokinetic findings show that earlier, more numerous CAR-T cell proliferation in the body is associated with a higher risk of ICANS. Risk factors for Grade ≥3 ICANS include: CD28 CAR-T cell products, higher therapeutic doses in CAR-T cell therapy, higher burden of disease, pre-existing neurological disease, low platelet counts, and previous early, serious CRS. The appearance of fever (≥38.9°C) and hemodynamic instability within 36 hours after CAR-T cell infusion may predict the occurrence of severe ICANS with high sensitivity.

Clinical Symptoms and Imaging Studies

Clinical symptoms of ICANS include tremors, confusion, agitation, and seizures. Other significant symptoms include dysphasia, hesitant speech, and deterioration in handwriting. These symptoms may progress to expressive and receptive aphasia. However, it is not recommended to administer routine anti-convulsant prophylaxis except in the case of high-risk patients. In addition, ICANS patients have previously developed fatal cerebral edema, and there are also reports of late psychiatric manifestations.

ICANS is primarily a clinical diagnosis, and its differential diagnosis is aided by magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) studies. Electroencephalogram (EEG) results may appear normal, but may also show diffuse slowing, frontal intermittent rhythmic delta activity, or general periodic discharges with triphasic morphology. Patients may exhibit other non-seizure EEG abnormalities, including generalized asynchronous slow activity and focal slowing. Patients with focal EEG abnormalities often have focal neurological symptoms such as aphasia. For a small subset of patients, spike waves and other epileptiform discharges may be present. If patients continue to manifest related conditions, they should undergo continuous or intermittent EEG studies and be administered anti-convulsant medications/corticosteroids based on EEG results to control their condition. The CSF of ICANS patients often reveals mild leukocytosis and elevation in protein levels, reflecting damage to and increased permeability of the blood-brain barrier. Elevated protein levels in CSF are associated with the severity of neurotoxicity, but normal or diminished protein levels cannot be used to rule out ICANS.

Patients should be examined by MRI. If they cannot undergo an MRI examination, they should undergo examination by computed tomography (CT) instead. MRI studies should include T1W, T2W, fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI), and susceptibility-weighted imaging (SWI) with and without contrast; of these, SWI displays considerable sensitivity in diagnosing intracranial hemorrhage. However, the neuroimaging studies of patients with significant neurotoxicity often appear normal. Normal CT images may be used to help exclude other lesions (such as acute ischemic stroke or hemorrhage). Nonetheless, occasionally patients with cerebral edema, even those with Grade 3-4 ICANS, may have normal-appearing MRI results. One study indicated that the T2/FLAIR images of such patients have hyperintense signals in the white matter, sometimes involving the bilateral thalami, external capsule, or corpus callosum as well. Other MRI changes may also include vasogenic edema, microhemorrhages, and leptomeningeal enhancement. According to the treatment guidelines of the National Comprehensive Cancer Network (NCCN), cerebral edema concurrent with intracranial hemorrhage (ICH) does not reflect neurotoxicity induced by CAR-T cells, and it is also not listed in the ASTCT ICANS classification. ICH may also be classified and documented using the CTCAE v5.0 system.

ICANS may be classified using the ASTCT ICANS consensus grading scale, as shown in **Table 8**. This grading scale uses a standardized evaluation scale for encephalopathy and scores immune effector cell-associated encephalopathy (ICE) and the severity of the following four aspects of neurotoxicity, including depressed consciousness, seizures, activity, increased intracranial pressure (ICP)/cerebral edema as its classification criteria. The pediatric version adopts the Cornell Assessment of Pediatric Delirium (CAPD) and may be used to evaluate children under 12 years of age or with developmental delays. Notably, the ICANS scale regards any single clinically apparent seizure or subclinical EEG seizure as Grade 3; chronic or recurrent clinical or subclinical seizures without recovery to baseline levels are considered Grade 4.

Grading of CAR-T Cell-Related Neurotoxicity[31,32]

Assessment tools for ICANS include ICE and CAPD:

Immune effector cell-associated encephalopathy (ICE) assessment tools (for children ≥12 years and adults)[31]

• **Explanation:** Ability to name the current year, month, city, and hospital: 4 points	**ICE scores** ▸ 7-9, Grade 1 ▸ 3-6, Grade 2 ▸ 0-2, Grade 3 ▸ A patient who is unarousable for ICE scoring may simply be considered to have Grade 4 ICANS
• **Naming:** Able to name three objects (e.g., the physician can point to a clock, pen, and button and have the patient answer): 3 points	
• **Following commands:** Ability to follow simple orders (e.g., please hold up "two fingers" or "close your eyes and stick out your tongue"): 1 point	
• **Writing:** Ability to write a complete sentence (e.g., "I will win against cancer"): 1 point	
• **Attention:** Ability to count backwards from 100 by 10: 1 point	

Modified from: Table 5, Lee DW, et al. *Biol Blood Marrow Transplant* 2019;25:625-638.
ICE, immune effector cell-associated encephalopathy; ICANS, immune effector cell-associated neurotoxicity syndrome.

Cornell Assessment of Pediatric Delirium (CAPD) (applicable for children <12 years of age)[31]

	Never 4 points	Rarely 3 points	Sometimes 2 points	Often 1 point	Always 0 points
1. Can the child make eye contact with his or her caregiver?					
2. Are the child's actions purposeful?					
3. Is the child aware of his or her surroundings?					
4. Can the child communicate needs and wants?					
	Never 0 points	**Rarely 1 point**	**Sometimes 2 points**	**Often 3 point**	**Always 4 points**
5. Is the child restless?					
6. Is the child inconsolable?					
7. Is the child underactive, with little movement even when awake?					
8. Does it take the child a long time to respond to interactions?					

Modified from: Table 7, Lee DW, et al. *Biol Blood Marrow Transplant* 2019;25:625-638.
CAPD, Cornell Assessment of Pediatric Delirium.

The ICANS grade is determined by the most severe event of the 5 assessed parameters (the CAPD score substitutes the ICE score as the neurotoxicity assessment for children under age 12), but these events cannot be secondary to other causes.

Clinical Management

Please refer to **Figure 6**. It should be noted that, unlike CRS, ICANS typically exhibits no therapeutic response to tocilizumab, as intravenous tocilizumab cannot cross the blood-brain barrier. Therefore, corticosteroid use, careful monitoring, and supportive care are fundamental to ICANS care. Treatment of neurotoxicity should take into account the ICANS grade:

1. **Grade 1 neurotoxicity**
   Supportive care alone is recommended and should suffice. If ICANS develops within 72 hours of infusion, consider intravenous dexamethasone 10 mg (children: 0.5 mg/kg) every 12-24 hours, and reassess after administration of two doses.
2. **Grade 2 neurotoxicity**
   Patients should receive supportive care and intravenous dexamethasone 10 mg, and be reassessed after these treatments. If there is no improvement, repeat one dose of dexamethasone every 6-12 hours.

Table 8. ASTCT Consensus Grading for ICANS in Patients[31]

	Grade 1	Grade 2	Grade 3	Grade 4
ICE scores for children ≥12 years of age and adults*	7–9	3–6	0–2	0 (Unarousable and unable to assess ICE)
CAPD scores for children <12 years of age	1–8	1–8	≥9	Unable to assess CAPD
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimuli	The patient is stuporous or comatose and either cannot be awakened at all or requires strong or repeated tactile stimuli to awaken
Seizure	N/A	N/A	Any clinically apparent generalized seizure that resolves rapidly or non-convulsive generalized seizure that resolves with intervention	Life-threatening seizure of long duration (>5 minutes) or repeated seizures that are apparent either clinically or on an EEG, and the patient does not recover to baseline within the period
Mobility‡	N/A	N/A	N/A	Deep focal motor weakness, such as hemiplegia or paraplegia
Increased intracranial pressure (ICP)/ cerebral edema	N/A	N/A	Focal lesions or edema found on neuroimaging§	Decerebrate or decorticate rigidity; sixth cranial nerve palsy; papilledema; Cushing's triad; or a finding of diffuse cerebral edema on neuroimaging

* An ICE 0 patient who cannot speak may be classified as Grade 3; however, an ICE 0 patient who is unarousable is considered Grade 4
† The depressed level of consciousness should not be secondary to other causes (e.g., no sedative use)
‡ Tremors and myoclonus associated with immune effector cell therapy may be stratified using CTCAE v5.0, but this result does not affect the ICANS grading
§ Intracranial hemorrhage (with or without associated edema) is not considered a characteristic of neurotoxicity and is excluded from the ICANS grading. Nonetheless, it can be stratified based on CTCAE v5.0
Modified from: Table 6 and 8, Lee DW, et al. *Biol Blood Marrow Transplant* 2019;25:625-638.
ASTCT, American Society for Transplantation and Cellular Therapy; CAPD, Cornell Assessment of Pediatric Delirium; CTCAE, Common Terminology Criteria for Adverse Events; ICANS, immune effector cell-associated neurotoxicity syndrome; EEG, electroencephalogram; ICE, immune effector cell-associated encephalopathy; ICP, intracranial pressure; N/A, not applicable.

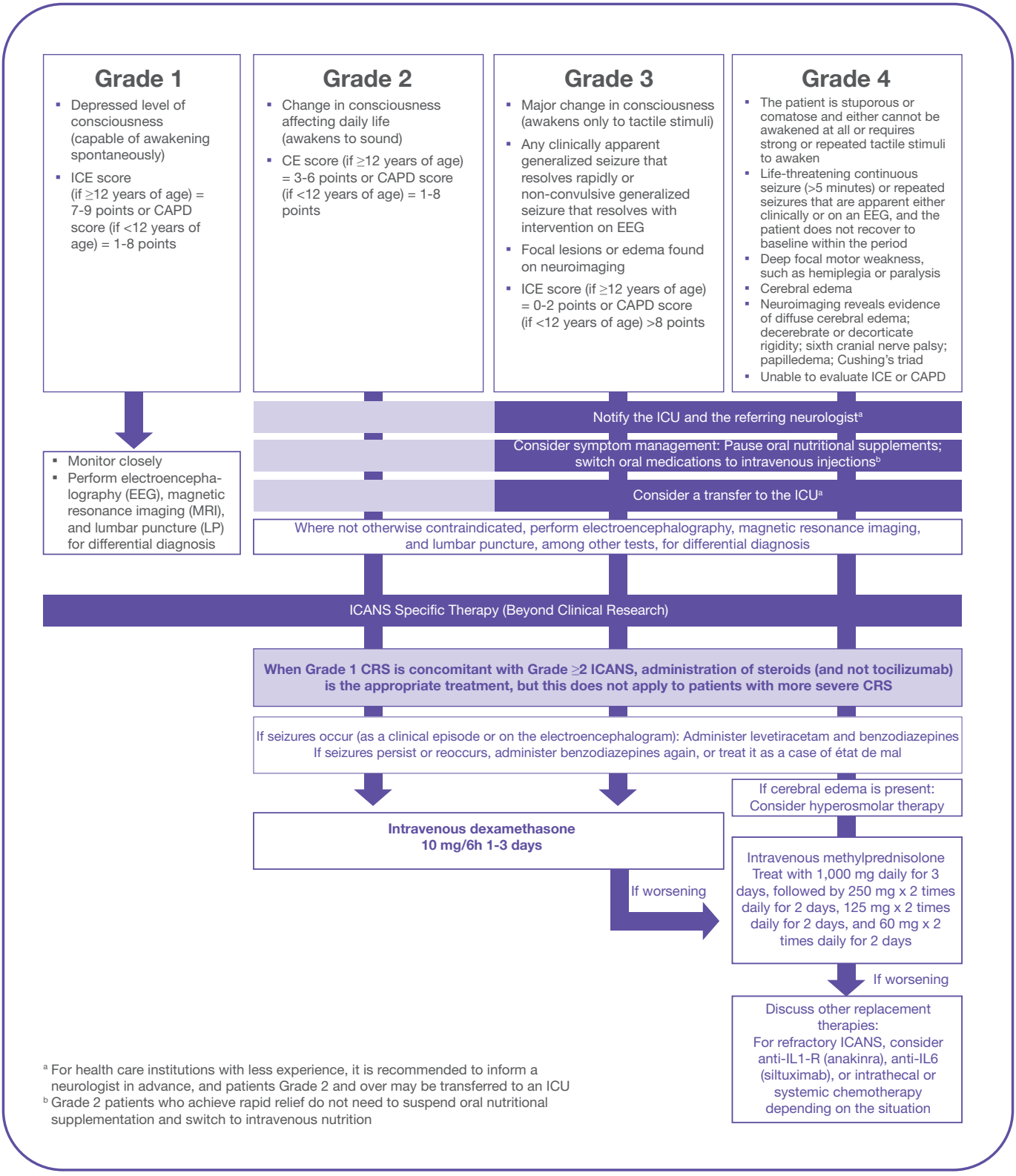
3. **Grade 3 neurotoxicity**
   IV dexamethasone (10 mg every 6 hours) or methylprednisolone (1 mg/kg every 12 hours).
4. **Grade 4 neurotoxicity**
   The recommended therapeutic option is high-dose corticosteroids, administered intravenously as 1,000 mg methylprednisolone (children: 30 mg/kg) with reassessment after administration of 2 doses for 3 days. Afterwards, rapidly taper to 250 mg, 125 mg, and 60 mg every 12 hours, with each dose adjustment maintained for 2 days before the next dose reduction. Convulsive status epilepticus should be treated according to the hospital guidelines. Patients with Grade ≥3 neurotoxicity should receive ICU care. Antifungal prophylaxis for patients undergoing steroid treatment for CRS or neurotoxicity is strongly recommended. Steroids for managing ICANS should be tapered as soon as possible after symptom improvement.

3.1.4 Hemophagocytic Lymphohistiocytosis (HLH)

Classical Definition of HLH

Hemophagocytic lymphohistiocytosis (HLH) refers to a cascade-induced hyperinflammatory syndrome. Symptoms include fever and hepatosplenomegaly. Characteristic laboratory findings are hyperferritinemia, coagulopathy, multiple organ dysfunction, and cytopenia. HLH may be primary or secondary, and immunotherapy-related secondary HLH has been added now as well.

Figure 6. Process for ICANS Grading and Management[8]



Adapted from: Figure 3, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
CAPD, Cornell Assessment of Pediatric Delirium; CRS, cytokine release syndrome; EEG, electroencephalogram; ICANS, immune effector cell-associated neurotoxicity syndrome; ICE, immune effector cell encephalopathy; ICU, intensive care unit; IL, interleukin; MRI, magnetic resonance imaging; LP, lumbar puncture; R, receptor.

The clinical manifestations of CAR-T cell therapy complicated with HLH are very similar to those of primary and other secondary HLH. Early on, a subset of patients with the CAR-T cell therapy CRS comorbidity was observed to have clinical manifestations that were highly similar to those in HLH/macrophage activation syndrome (MAS). SS Neelapu proposed diagnostic criteria for "CAR-T cell therapy-induced HLH" in 2018. The criteria[33] are ferritin over 10,000 ng/mL accompanied by two or more of these anomalies: hepatotoxicity Grade 3 and above, nephrotoxicity, or pulmonary edema, and the presence of hemophagocytosis in biopsies of bone marrow or other organs. As patients who meet the criteria for Grade 3 CRS

also tend to meet the diagnostic criteria for HLH, it is still unclear whether HLH is a severe form of CRS or a distinct comorbidity, and the calculated incidence rates are correspondingly imprecise. HLH incidence has been reported to be approximately 1%, but this is quite likely an underestimate, as the symptoms overlap with those of severe CRS and may have been attributed to CRS instead.

An HLH-Like Comorbidity Occurring After CAR-T Cell Therapy: Immune Effector Cell-Associated HLH-Like Syndrome (IEC-HS)

With advances in CAR-T cell therapy, people are becoming more aware of this HLH-like comorbidity, which may not be merely a more severe version of CRS; increasing numbers of reports show that the onset of HLH-like symptoms is typically dissociated from CRS, and may frequently have a more delayed onset compared to CRS. More importantly, HLH-like syndrome may cause multiple organ failure. In response, the ASTCT established a task force expressly to formulate management guidelines for this comorbidity and **renamed it the immune effector cell-associated HLH-like syndrome (IEC-HS)**,[24] thus differentiating it from primary HLH. Additionally, although CRS and IEC-HS are similar in their pathogenesis and clinical manifestations, IEC-HS is independent of CRS. [24] IEC-HS often has a delayed onset and may emerge only during or after resolution of CRS. Therefore, IEC-HS should not be regarded as a comorbidity of severe CRS after the CRS fails to improve under treatment;[24] the two are different toxic entities. [34]

Clinical Symptoms

Please refer to **Table 9** for the ASTCT definition and identifying criteria of IEC-HS. [34]

Table 9. Definition and Identifying Criteria of IEC-HS[34]

Definition of IEC-HS	IEC-HS is a hyperinflammatory syndrome with pathological and biochemical findings distinct from CRS and ICANS. Findings: 1. Has the clinical characteristics of HLH/MAS 2. Occurs after IEC treatment 3. Has new onset or progression of the following laboratory anomalies, including cytopenia, hyperferritinemia, coagulopathy with hypofibrinogenemia, and transaminitis
Identifying Criteria of IEC-HS*	Clinical Manifestations/Laboratory Findings
Common Manifestations†	• Required: Elevated (>2 x ULN or >2 x baseline at CAR-T cell infusion) or aberrant, rapidly increasing blood ferritin (based on clinical assessment) • At onset, CRS is resolving, has resolved, or had shown initial improvement under CRS-directed therapy, but is continuing to worsen‡ • Elevated hepatic transaminase >5 x ULN (if the baseline was normal) or >5 x baseline value (if the baseline was abnormal) • Hypofibrinogenemia (<150 mg/dL or <LLN) • Hemophagocytosis in the bone marrow or other tissues • Cytopenia (whether new-onset, worsening, or refractory)
Other Possible Manifestations	• Elevated LDH (>ULN) • Other coagulopathy (e.g., increased PT/PTT) • Direct hyperbilirubinemia • New-onset splenomegaly • Fever (new-onset or persistent fever) • Neurotoxicity • Lung manifestations (such as hypoxia, pulmonary infiltration, or pulmonary edema) • Renal insufficiency (new-onset renal insufficiency) • Hypertriglyceridemia (fasting level, >265 mg/dL)

* This diagnosis can only be made when it is not attributable to alternative etiologies (e.g., CRS, infection, and/or disease progression)
† This constellation of manifestations typically occurs simultaneously (typically all occurring within 72 hours)
‡ Although most cases of IEC-HS appear after CRS, there may also be exceptions. Further clinical experience will clarify the possible manifestations of IEC-HS
Adapted from: Table 1, Hines MR, et al. *Transplant Cell Ther* 2023;2029:438.e1-e16.
CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; HLH, hemophagocytic lymphohistiocytosis; ICANS, immune effector cell-associated neurotoxicity syndrome; IEC, immune effector cell; IEC-HS, immune effector cell-associated HLH-like syndrome; LDH, lactate dehydrogenase; LLN, lower limit of normal; MAS, macrophage activation syndrome; PT, prothrombin time; PTT, partial prothrombin time; ULN, upper limit of normal.

For differences compared to other hematological toxicities, please refer to Section 3.1.5, Hematological Toxicities, **Table 14** (Comparison of HLH, CRS/MAS, IEC-HS).

Highlights of the ASTCT IEC-HS identifying criteria are as follows:[34]

1. **Not including fever among the identifying criteria**
   Although fever is an important parameter in the HLH-2004 diagnostic criteria, IEC-HS must be distinguished from suspected recurrent or delayed-onset CRS for disambiguation, so fever has been excluded from the recommended identifying criteria for IEC-HS.
2. **Not establishing a definite criterion for blood ferritin levels**
   "The presence of elevation and the rapidity of the elevation" serve as the identifying criteria. Rapid elevation is determined by individual care providers to prevent delays in treatment when waiting for levels to reach the standard criterion.
3. **Not including cytokine profiling among the identifying criteria**
   This is because test results are not always available in real-time and may be confounded by prior anti-cytokine therapy for CRS, which is typically antecedent.

The ASTCT identifying criteria for IEC-HS emphasizes holistic assessment, clinical manifestations, and bedside evaluation of severity, as opposed to relying on any single test result. Additional tests must be performed to rule out other etiologies that may cause HLH-like syndrome; in addition to distinguishing the condition from CRS, **active infection such as that caused by HSV, VZV, CMV, EBV, HHV-6, adenovirus, enterovirus, or parvovirus must also be ruled out**.

In addition, it is also necessary to monitor for progression of the original disease, Epstein-Barr virus-induced posttransplant lymphoproliferative disease (EBV-PTLD), or secondary cancer.

IEC-HS Grading

Please refer to **Table 10** for the ASTCT grading of IEC-HS severity. This classification divides IEC-HS into 5 grades based on the description of immune system disorders in the Common Terminology Criteria for Adverse Events (CTCAE) proposed by the National Cancer Institute (NCI).

Clinical Management

The treatment strategies recommended by the ASTCT primarily combine experts' opinions, past CRS/IEC-HS study results, and experience in HLH treatment.

ASTCT Recommendations:

1. **Initiate treatment for symptomatic patients with mild disease using steroid or anakinra as either monotherapy or combination therapy**
   Please note that evaluation for a certain period of time (such as 48 hours) is recommended before determining a second-line treatment; this is to prevent overtreatment from causing drug toxicity and compromising CAR-T cell efficacy.
2. **Consider ruxolitinib as second-line treatment**
   As IEC-HS increases in severity, patient survival and the benefit-risk analysis for the CAR-T cell response change dynamically; making adjustments to preserve the patient's life is the ultimate goal. Therefore, to avoid serious, even life-threatening symptoms, commencing treatment as soon as possible is the most important strategy.

Table 10. IEC-HS Classification[34]

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
IEC-HS*	Absent or mild symptoms that do not require intervention.	Mild to moderate symptoms that require intervention (such as, immunosuppressants for IEC-HS or transfusions for asymptomatic hypofibrinogenemia)	Severe or clinically significant symptoms that are not immediately life-threatening (for example, coagulopathy accompanied by bleeding that requires transfusion or hypotension or respiratory caused by new-onset acute renal failure requiring inpatient admission for treatment)	Life-threatening symptoms that require emergency intervention (such as, life-threatening bleeding or hypotension, respiratory distress requiring intubation, acute kidney injury requiring dialysis)	Death

*Not secondary to other causes. IEC-HS is defined as the occurrence of pathological and biochemical abnormalities characteristic of MAS/HLH that can be attributed to IEC treatment and associated with new-onset or worsening cytopenia, hyperferritinemia, coagulopathy with hypofibrinogenemia, and transaminitis (> 5 x ULN). Although according to the ASTCT definition, patients with serious CRS often present manifestations similar to HLH, but IEC-HS often occurs after a delay and has an onset after or during improvement from CRS

Adapted from: Table 2, Hines MR, et al. *Transplant Cell Ther* 2023;2029:438.e1-e16.

ASTCT, American Society for Transplantation and Cellular Therapy; CRS, cytokine release syndrome; HLH, hemophagocytic lymphohistiocytosis; IEC, immune effector cell; IEC-HS, immune effector cell-associated HLH-like syndrome; ULN, upper limit of normal.

3. **Third-line treatment is etoposide or emapalumab**. Because there was no historical evidence that anti-IL-6 therapy was effective against HLH and the patient was likely to have used the medication already, administration of anti-IL-6 therapy is not recommended, particularly when managing IEC-HS with no CRS manifestations.

Selection and Usage of Pharmacological Treatments for IEC-HS[34]

The number of treatment days references the 2021 EBMT/EHA recommendations:[8]

1. **Anakinra** – an IL-1 receptor antagonist
   Adults: 100-200 mg/day sc/iv q6-12h.
   Children: 5-7 mg/kg/day (or a higher dose of 8-10 mg/kg/day) q8-12h, or 4 mg/kg q6h.Administer for 2-4 days.

2. **Steroids**
   Adults: Dexamethasone 10-40 mg/day (most commonly 10 mg q6h).
   Children: Dexamethasone 10 mg/m^2/day or methylprednisolone 1-2 mg/kg q6-12h. Administer for 4 days.
   In the 2021 EBMT/EHA recommendations, ineffective dexamethasone treatment may be switched to methylprednisolone 1,000 mg/day for 3 days, followed by 250 mg x 2/day for 2 days, followed by 125 mg x2/day for 2 days, followed by 60 mg x 2/day for 2 days.

3. **Ruxolitinib** – a JAK1/JAK2 inhibitor
   Adults (≥14 years of age):
   10 mg q12h (may be increased to 20 mg q12h).
   Children (<14 years of age) weighing >25 kg:
   5 mg q12h.
   Children (<14 years of age) weighing <25 kg:
   2.5 mg q12h, and the dose may subsequently be increased depending on the response.

4. **Etoposide** – a chemotherapy agent
   Adults and children: 50-100 mg/m^2/dose, given on Days 1 and 4 and, if necessary, again on Day 7.

5. **Emapalumab** – a IFN-γ monoclonal antibody
   Starting dose: 1 mg/kg, with subsequent adjustments based on the recommendations in the instructions. In general, those with a poor response may be given 3 mg/kg starting on Day 3, 6 mg/kg starting on Day 6, and 10 mg/kg starting on Day 9.

Clinical Monitoring and Other Supportive Care[34]

1. Monitor CBC/DC, PT/aPTT, fibrinogen, and hepatic and renal function daily.
2. Consider monitoring biochemistry parameters in HLH diagnostic criteria, such as soluble CD25, NK cell function, triglycerides, IFN-γ, CXCL9 ratio, CXCL10, IL-10, and IL-18.
3. Maintain Hb >7 g/dL, with PLT ≥50 x 10^9/L and fibrinogen >150 mg/dL if bleeding is present and fibrinogen >100 mg/dL when there is no bleeding. Administer vitamin K when INR >1.5 and fresh frozen plasma (FFP) plus cryoprecipitate when INR >2.0.
4. In the acute phase, use of granulocyte colony-stimulating factor (G-CSF) may exacerbate a cytokine storm.
5. Eltrombopag or romiplostim may be used to increase platelet count, but outcomes are unknown.
6. When administering immunosuppressive agents, be alert to the infection risks of each drug; consider monitoring the presence of bacteria, viruses, or fungi in the blood, urine, and sputum or administering prophylactic or preemptive antimicrobial agents.

3.1.5 Hematological Toxicities

Any target of CAR-T cell therapy may give rise to short-term hematological toxicities, a phenomenon known as immune effector cell-associated hematotoxicity (ICAHT). ICAHT has the following characteristics:

1. Cytopenia may persist after resolution of CRS, sometimes for as long as several months or years.
2. Recovery of blood cell counts may be intermittent, as in a transient recovery followed by one or more reductions.
3. It may progress to serious bone marrow aplasia and respond poorly to treatment with growth factors.
4. The pathogenesis is unknown but may involve inhibition of hematopoietic cells by severe inflammatory responses and cytokines.

Risk Factors

Please refer to **Table 11** for risk factors that increase hematological toxicities.

ICAHT Grading

ICAHT grading has a cut-off at 30 days after CAR-T cell infusion; events occurring 0-30 days post-infusion are classified as early ICAHT, whereas events occurring after 30 days are classified as late ICAHT. Please refer to **Table 12**.

Table 11. Risk Factors Associated with Cytopenia After CAR-T Cell Therapy[35]

	Risk Factors	Explanation
Disease-related factors	• Disease status	The risk is higher in B-cell acute lymphoblastic leukemia (B-ALL) than in B-cell non-Hodgkin lymphoma (B-NHL)
	• Tumor burden before CAR-T cell infusion (disease progression, high LDH)	Particularly in disease with bone marrow invasion
Treatment history	• Prior lines of treatment	Related to baseline hematopoietic system function
	• Prior hematopoietic stem cell transplantation	
	• Bridging therapy	
Status of hematopoietic system and bone marrow	• Tumor cell infiltration of bone marrow	
	• Existing cytopenia	Particularly in existing thrombocytopenia
	• Clonal hematopoiesis of indeterminate potential (CHiP)	Related to the inflammatory response; one of the potential risk factors
Inflammatory status	• Elevated serum C-reactive protein	
	• Elevated serum ferritin	
CAR-T Cell Therapy Product-Related and Post-Infusion Risk Factors	• Costimulatory molecules (risk is higher with CD28 than with 41BB)	Differences in lymphodepleting chemotherapy doses (cyclophosphamide doses)
	• Type of chimeric antigen receptor construct (risk is higher with tandem antigen receptor constructs than with single-target antigen receptor constructs)	
	• Severe CRS	
	• Persistent elevation of inflammatory biomarkers	
	• Active infection	Primarily in viral infections or accompanying sepsis
	• CRS/MAS or IEC-HS	Cytopenia is an overlapping symptom

Adapted from: Table 2, Rejeski K, et al. *Blood* 2023;142:865-877.
B-ALL, B-cell acute lymphoblastic leukemia; B-NHL, B-cell non-Hodgkin lymphoma; CAR-T, chimeric antigen receptor T; CHiP, clonal hematopoiesis of indeterminate potential; CRS, cytokine release syndrome; CRS/MAS, cytokine release syndrome with macrophage activation syndrome; IEC-HS, immune effector cell-associated HLH-like syndrome; LDH, lactate dehydrogenase.

Table 12. ICAHT Grading[35]

	Grade 1	Grade 2	Grade 3	Grade 4
Early ICAHT (0-30 days)				
● ANC ≤500/μL	<7 days	7-13 days	≥14 days	Never exceeding 500/μL
● ANC ≤100/μL	—	—	≥7 days	≥14 days
Late ICAHT (after Day +30)*				
● ANC	≤1,500/μL	≤1,000/μL	≤500/μL	≤100/μL

*Must be measured at ≥2 points in time to rule out non-transient neutropenia
Adapted from: Table 1, Rejeski K, et al. *Blood* 2023;142:865-877.
ANC, absolute neutrophil count; ICAHT, immune effector cell-associated hematotoxicity.

Risk Assessment

After CAR-T cell infusion, the CAR-HEMATOTOX score may be used for risk assessment. Please refer to **Figure 7** or proceed to the German Lymphoma Alliance website to perform the assessment.

Evaluation of the CAR-HEMATOTOX score may be performed five days before lymphodepleting chemotherapy (day-5). This uses the patient's current test results for the absolute neutrophil count (ANC), hemoglobin (Hb), C-reactive protein, and ferritin to evaluate the prognosis within 60 days of CAR-T cell infusion.

Although the CAR-HEMATOTOX score applies to large B-cell lymphoma (LBCL), mantle cell lymphoma (MCL), and multiple myeloma (MM), it nonetheless serves as a valuable reference in adult or pediatric B-cell acute lymphoblastic leukemia (B-ALL).

It should be noted that the CAR-HEMATOTOX score has high sensitivity, but lower specificity, and clinical evaluations still require careful interpretation.

Figure 7. Using the CAR-HEMATOTOX Score as a Risk Stratification Tool[35]

Before lymphodepleting chemotherapy (Day -5)

Using the **CAR-HEMATOTOX score** to evaluate the risk that an individual patient will develop hematotoxicity and infection

- Acceptable period for laboratory test values: 3 days

Characteristics	0 points	1 point	2 points
Platelet count	>175,000/μL	75,000–175,000/μL	<75,000/μL
ANC	>1,200/μL	≤1,200/μL	-
Hemoglobin	>9.0 g/dL	≤9.0 g/dL	-
C-reactive protein	<3.0 mg/dL	≥3.0 mg/dL	-
Ferritin	<650 ng/mL	650–2,000 ng/mL	>2,000 ng/mL

Low risk: 0-1 point High risk: ≥2 points

Low risk (CAR-HEMATOTOX score 0-1)

Risk Factors	LBCL (n=235)	MCL (n=103)	MM (n=113)
Median duration of severe neutropenia (ANC <500/μL, Days 0-60)	5.5 days (95% CI 5-8 days)	6 days (95% CI 5-7 days)	3 days (95% CI 2-5 days)
Aplastic phenotype	2.6%	0%	3%
Incidence of serious infection	8%	5%	5%
Incidence of serious infection from bacterial causes	0.9%	5%	3%

High risk (CAR-HEMATOTOX score 2-7)

	LBCL (n=235)	MCL (n=103)	MM (n=113)
Median duration of severe neutropenia (ANC <500/μL, Days 0-60)	12 days (95% CI 10-16 days)	14 days (95% CI 9-18 days)	9 days (95% CI 7-13 days)
Aplastic phenotype	36%	47%	32%
Incidence of serious infection	40%	30%	40%
Incidence of serious infection from bacterial causes	27%	28%	34%

Adapted from: Figure 1, Rejeski K, et al. *Blood* 2023;142:865-877.
ANC, absolute neutrophil count; CI, confidence interval; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma.

Response Measures

1. If the assessment results show that the patient has a high risk of ICAHT, consider performing bone marrow studies before lymphocyte collection or lymphodepleting chemotherapy in order to verify the status of the bone marrow and further assess risks.
2. If there is unexpected cytopenia, such as failure of blood cells to recover two to three weeks after CAR-T cell infusion, please refer to **Table 13** for conducting basic examinations and stepwise differential diagnosis.

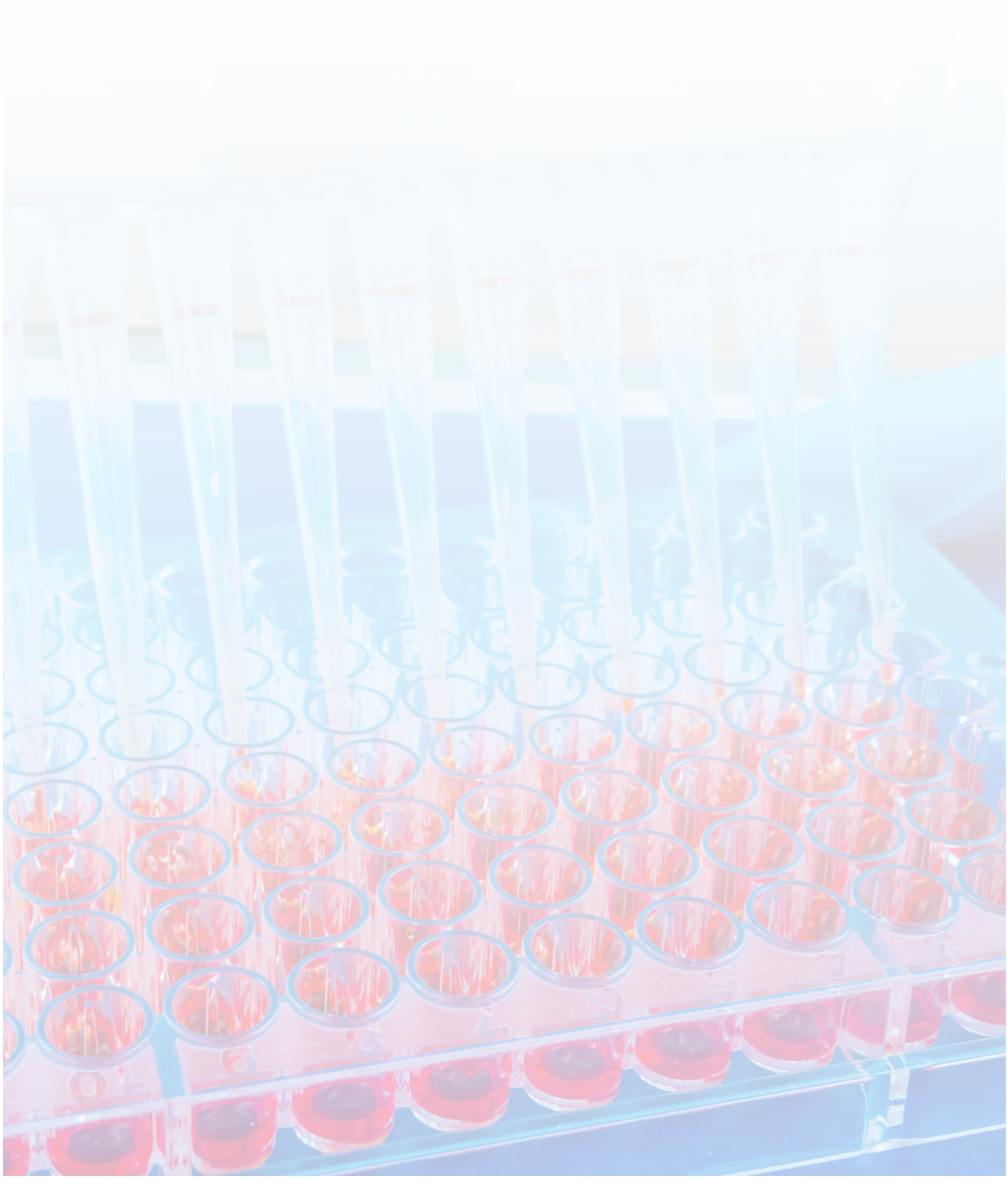


Table 13. Stepwise Diagnosis Based on ICAHT Severity[35]

Category	Putative Cause	Test	Timepoint for Testing
Phase 1 | Basic workup with a lower threshold			
Inadequate hematopoietic capacity of the bone marrow	• Related to prior treatment: allo-HCT, use of fludarabine, tumor cell infiltration of the bone marrow	CBC, RPI, peripheral blood smear	Routinely performed
Drug side effects	• Check for concomitant myelosuppressive agents		Routinely performed
Vitamin deficiency	• Vitamin B12, folic acid	Measurement of serum levels	Routinely performed
Rule out infections	• Infection caused by bacteria/ viruses/fungi	Blood culture, CMV PCR, blood procalcitonin, $CD4^+$ T cells, immunoglobulin G (IgG), B-cell count	Routinely performed
Rule out macrophage activation syndrome (MAS)	• CRS/MAS or IEC-HS	Ferritin and triglyceride levels	Routinely performed
Phase 2 | Subsequent workup - performed when G-CSF therapy is ineffective (no response after administration for five consecutive days), phase 1 test results are all negative, and/or other risk factors are present			
Category	**Putative Cause**	**Test**	**Timepoint for Testing**
Depending on clinical manifestations, consider using PCR for viral detection	• Parvovirus	Parvovirus B19 PCR	During chronic anemia
	• HHV6, JCV	Perform HHV6 and JCV PCR on blood/cerebrospinal fluid specimens	Appearance of nervous system symptoms
	• EBV, adenovirus, HSV	PCR	In case of HLH
Bone marrow diseases	• (MDS/AML/myelofibrosis) or relapse	Bone marrow aspirate and biopsy, flow cytometry, immunohistochemistry (IHC), cytogenetics, next-generation sequencing (NGS)	In case of prolonged cytopenia
	• Leukemia/lymphoma relapse	Flow cytometry of peripheral blood/ bone marrow specimens, with a B-cell panel	Routinely performed
Other causes	• Other rare hematological diseases, bone marrow diseases, paroxysmal nocturnal hemoglobinuria (PNH), autoimmune diseases	Myeloid panel, GPI-linked structures, direct antiglobulin test (DAT)	Suspected MPN, PNH, or autoimmune disease

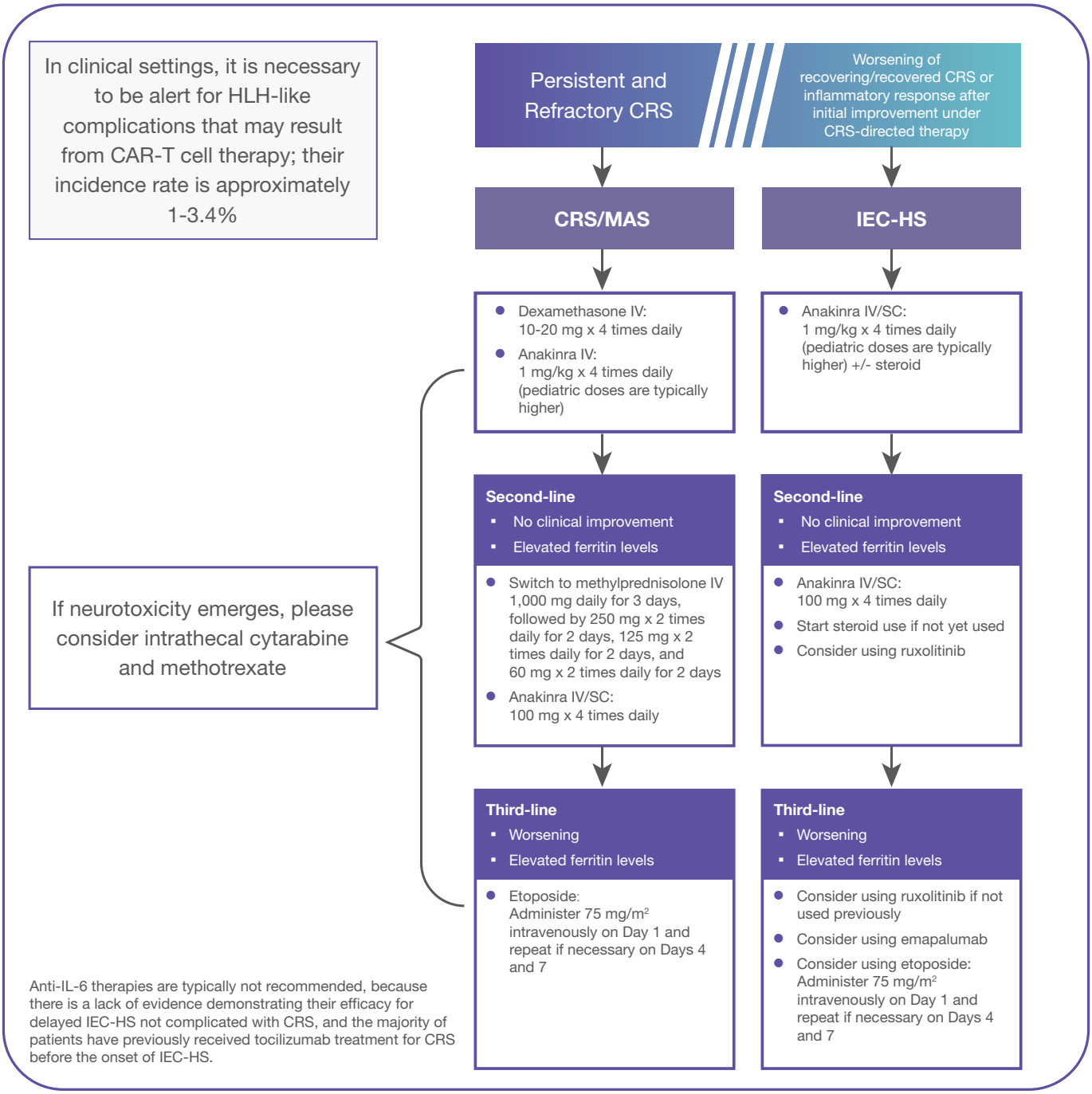
Adapted from: Figure 2, Rejeski K, et al. *Blood* 2023;142:865-877.
allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; CBC, complete blood count; CMV, cytomegalovirus; CRS/MAS, cytokine release syndrome with macrophage activation syndrome; DAT, direct antiglobulin test; EBV, Epstein-Barr virus; G-CSF, granulocyte colony stimulating factor; GPI, glycosylphosphatidylinositol; HHV6, human herpesvirus 6; HLH, hemophagocytic lymphohistiocytosis; HSV, herpes simplex virus; ICAHT, immune effector cell-associated hematotoxicity; IEC-HS, immune effector cell-associated HLH-like syndrome; IgG, immunoglobulin G; IHC, immunohistochemistry; JCV, John Cunningham virus; MAS, macrophage activation syndrome; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; PCR, polymerase chain reaction; PNH, paroxysmal nocturnal hemoglobinuria; RPI, reticulocyte production index.

Hemophagocytic Syndrome and Cytopenia Caused by CAR-T Cell Therapy

CAR-T cell therapy may cause HLH-like complications, approximately at a rate of 1-3.4%. Such complications may be classified based on the other symptoms and manifestations present into cytokine release syndrome with macrophage activation syndrome (CRS/MAS) and IEC-HS. Please refer to the HLH-2004 criteria, H-score, and optimized HLH inflammatory index or **Table 14** for diagnostic criteria and **Figure 8** for treatment options.

If cytopenia arises as a result of HLH, the theory behind HLH treatment may serve as a reference. This includes the use of anti-IL-1 anakinra, anti-IFN-γ emapalumab, or attempted cytokine adsorption to reduce cytokine levels in the body. If the above elicit a poor response or cannot be obtained, the chemotherapeutic agent etoposide may also be considered to kill upstream CAR-T cells causing the severe inflammatory response.

Figure 8. Management of CRS/MAS and IEC-HS[8,35]



Modified from: Hayden PJ, et al. *Ann Oncol* 2022;33:259-275 and Rejeski K, et al. *Blood* 2023;142:865-877.
CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; CRS/MAS, cytokine release syndrome with macrophage activation syndrome; HLH, hemophagocytic lymphohistiocytosis; IEC-HS, immune effector cell-associated HLH-like syndrome; IL, interleukin; IV, intravenous; SC, subcutaneous.

Table 14. Comparison of HLH, CRS/MAS, and IEC-HS

	Classical Definition of HLH		Definition of Hematological Toxicities Caused by CAR-T Cell Therapy			Comments on CRS/MAS and IEC-HS
	HLH-2004 (for fHLH)	H-score (for all sHLH/MAS)	MD Anderson[33]	CRS/MAS[8]	IEC-HS[34]	
CRS				Refractory CRS	Persistent progression during/after CRS resolution or after improvement of the inflammatory response	
Fever	✓	✓		✓ (++)	✓	• May not appear during steroid use and in patients with IEC-HS
New-onset organomegaly	✓	✓		✓ (+/-)	✓	• Useful in distinguishing between CRS and CRS/MAS
Clinical pulmonary symptoms			✓		✓	
Renal dysfunction			✓		✓	
Central nervous system involvement					✓	• Coexistent with ICANS
Severe cytopenia	✓	✓		✓ (++)	✓	• Commonly occurring • New-onset or progressive cytopenia may be observed in IEC-HS patients
Serum transminases and/or bilirubin		✓	✓	✓ (+)	✓	• May be found in CRS, albeit with fluctuating values
Ferritin	✓	✓	✓	✓ (++)	✓ (A two-fold or rapid elevation)	• May be found in CRS, albeit with fluctuating values
LDH					✓	• Nonspecific
Hypofibrinogenemia	✓	✓		✓ (+/-)	✓	• Often occurs because of hepatic dysfunction
Hypertriglyceridemia	✓	✓		✓(+)	✓	• Often occurs because of hepatic dysfunction
Hemophagocytosis in the bone marrow or other tissues	✓	✓	✓	✓ (+++)	✓	• An important diagnostic tool in CRS/MAS, used to differentiate between other potential overlapping diseases
Coagulopathy				✓ (+/-)	✓	• Often caused by hepatic dysfunction
Depressed activity or deficiency of NK cells	✓					• Non-routine test items
sIL-2r (CD25)	✓					• Non-routine test items

+ means severity.

CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; CRS/MAS, cytokine release syndrome with macrophage activation syndrome; fHLH, familial HLH; HLH, hemophagocytic lymphohistiocytosis; ICANS, immune effector cell-associated neurotoxicity syndrome; IEC-HS, immune effector cell-associated HLH-like syndrome; LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; NK, natural killer; sHLH, secondary HLH; sIL-2r, soluble interleukin-2 receptor.

Recommended Interventions for Cytopenia (Refer to Table 15 and Figure 9)

❶ Reinfusion of Previously Cryopreserved Hematopoietic Stem Cells

① Patients with no response to G-CSF therapy by Day 14 after CAR-T cell infusion typically have a higher risk of developing fatal infections. Although evidence is limited, use of thrombopoietin (TPO) agonists may be considered, particularly for patients with pre-existing thrombocytopenia.

② If the thrombocytopenia was caused by severe CRS, anti-inflammatory agents such as pulse-dose corticosteroids or anti-proliferative agents (tocilizumab or anakinra) should be administered as early as possible.

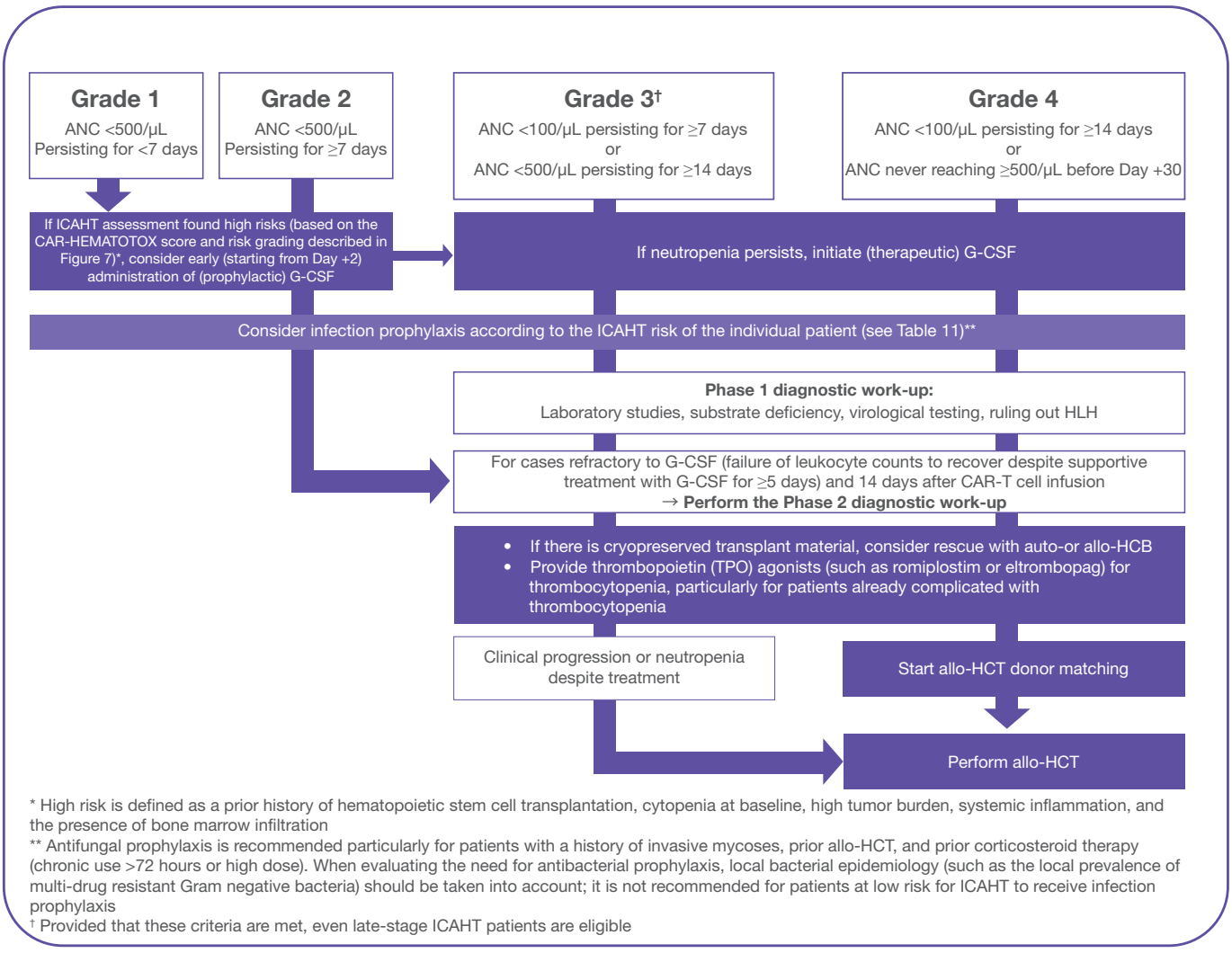
③ If the patient still has previously cryopreserved CD34$^+$ hematopoietic stem cells, whether autologous or allogeneic, reinfusion of these CD34$^+$ hematopoietic stem cells may be an effective management method. Patients with prolonged bone marrow failure may see gradual blood cell recovery after reinfusion of hematopoietic stem cells, but be alert to the possible development of immune reconstitution inflammatory syndrome.

④ 14 days after receiving CAR-T cell infusion, if a patient develops Grade 3 ICAHT or above that is confirmed to be G-CSF-refractory, the patient's previously cryopreserved CD34$^+$ hematopoietic stem cells may be used for management; some experts recommend bypassing conditioning chemotherapy and proceeding to reinfusion of the hematopoietic stem cells for a better prognosis.

❷ Allogeneic Hematopoietic Stem Cell Transplantation

① If there is no improvement after applying the various methods described above and ICAHT remains at Grade 4 or above 30 days after infusion of CAR-T cells, allogeneic hematopoietic stem cell transplantation may be considered.

Figure 9. ICAHT Treatment Process[35]



* High risk is defined as a prior history of hematopoietic stem cell transplantation, cytopenia at baseline, high tumor burden, systemic inflammation, and the presence of bone marrow infiltration

** Antifungal prophylaxis is recommended particularly for patients with a history of invasive mycoses, prior allo-HCT, and prior corticosteroid therapy (chronic use >72 hours or high dose). When evaluating the need for antibacterial prophylaxis, local bacterial epidemiology (such as the local prevalence of multi-drug resistant Gram negative bacteria) should be taken into account; it is not recommended for patients at low risk for ICAHT to receive infection prophylaxis

† Provided that these criteria are met, even late-stage ICAHT patients are eligible

Adapted from: Figure 3, Rejeski K, et al. *Blood* 2023;142:865-877.

allo-HCB, allogeneic hematopoietic cell boost; allo-HCT, allogeneic hematopoietic cell transplantation; ANC, absolute neutrophil count; auto-, autologous; CAR-T, chimeric antigen receptor T; G-CSF, granulocyte-colony stimulating factor; HLH, hemophagocytic lymphohistiocytosis; ICAHT, immune effector cell–associated hematotoxicity; TPO, thrombopoietin.

② The possibility for spontaneous hematological recovery must be carefully assessed. As the 3-6 months after CAR-T cell infusion are still within the reasonable timeframe for potential spontaneous hematological recovery, it is necessary to consider the possibility of spontaneous hematological recovery and infection during this period and carefully assess the individual benefits and risks of allogeneic hematopoietic stem cell transplantation.

Table 15. Short-Term Interventions for Cytopenia[35]

	Timing	Method	Precautions	Explanation
Packed red blood cells (PRBC)/ platelet infusion	According to the guidelines of the health care institution, based on the patient risk profile	• According to the guidelines of the health care institution • When using packed red blood cells, consider using only one unit each time to avoid iron overload	Perform irradiation of the blood products before their use, starting at 7 days before leukapheresis and continuing until at least 90 days after CAR-T cell infusion.	• As fludarabine is used, blood products may undergo irradiation to prevent the development of TA-GvHD
G-CSF	**Prophylactic G-CSF:** Start use on Day +2 for patients with a high-risk profile for ICAHT based on the CAR-HEMATOTOX score	• For patients with a high-risk profile for ICAHT, consider early administration of G-CSF (starting at Day +2) as prophylaxis • Dose: 5 μg/kg once daily	G-CSF may not be necessary for patients with a low risk profile for ICAHT	• Reduce the risk of neutropenia with fever (does not increase the risk of severe or Grade 3 and above CRS or ICANS) • Does not produce adverse effects on CAR-T cell expansion or treatment outcomes • Patients with intermittent neutrophil recovery often respond rapidly to G-CSF stimulation, while aplastic patients are often unresponsive to G-CSF
	Therapeutic G-CSF: Patients with severe neutropenia (ANC <500/μL), with or without infectious complications, must receive G-CSF immediately	• Recommended for severe neutropenia with or without infectious complications • Dose: 5 μg/kg once daily. If there is no treatment response, consider increasing the dose		
Antibacterial prophylaxis	• Use not recommended for patients with a low-risk profile for ICAHT • Patients with anticipated long-term neutropenia (e.g., ANC <100/μL for over 7 days) may be administered antibiotics as antibacterial prophylaxis • Infection prophylaxis may be considered for patients with a high-risk profile for ICAHT once ANC <500/μL • Use of fluoroquinolones as prophylactic antibiotics is not recommended for patients with a low-risk profile	• According to the guidelines of the health care institution (e.g., levofloxacin or ciprofloxacin)	It is necessary to be alert for colonization by multi-drug resistant (MDR) pathogens	• Consider local bacterial epidemiology. A high local prevalence of multi-drug resistant Gram negative bacteria may affect the use of prophylactic antibiotics • A recent study has found that overuse of prophylactic antibiotics may diminish the diversity of intestinal flora, which in turn affects immunoregulatory function, leading to a worse prognosis

	Timing	Method	Precautions	Explanation
Antiviral agents	All patients	• Starting with lymphodepletion and continuing until 1 year after CAR-T cell infusion and/or until $CD4^+$ cell count >0.2 x 10^9/L • Use valaciclovir 500 mg twice daily or acyclovir 800 mg twice daily		
Antipneumocystis agents	All patients	• Starting with lymphodepletion and continuing until 1 year after CAR-T cell infusion and/or until $CD4^+$ cell count >0.2 x 10^9/L • Use co-trimoxazole 480 mg once daily or 960 mg three times weekly	In case of a co-trimoxazole allergy, use of inhaled pentamidine 300 mg once monthly, dapsone 100 mg once daily, or atovaquone 1,500 mg once daily may be considered	• Implemented in according to the guidelines of the health care institution • The start may be postponed in case of prolonged myelosuppression
Systemic Primary Antifungal Prophylaxis	Infection prophylaxis may be considered for patients with severe neutropenia (ANC <500/μL), a high-risk profile for ICAHT based on the CAR-HEMATOTOX score, and (or) prolonged neutropenia	• Mold-active prophylaxis must be maintained for 1-3 months (depending on the duration of neutropenia and use of steroids) • Oral posaconazole 300 mg daily or intravenous micafungin 50 mg daily		Infection prophylaxis is recommended for patients in the following three situations: • Prior allo-HCT • Experiencing invasive aspergillosis • Prior corticosteroid therapy (use of high doses or >72 hours)

Adapted from: Table 3, Rejeski K, et al. *Blood* 2023;142:865-877.
allo-HCT, allogeneic hematopoietic cell transplantation; ANC, absolute neutrophil count; CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; G-CSF, granulocyte colony stimulating factor; ICANS, Immune effector cell-associated neurotoxicity syndrome; ICAHT, immune effector cell-associated hematotoxicity; MDR, multi-drug resistant; PRBC, packed red blood cells; TA-GvHD, transfusion associated with graft-versus-host disease.

3.2 Late Complications

3.2.1 Infection and Prophylaxis

Opportunistic infections are one of the common long-term complications. According to the EBMT/EHA recommendations, patients receiving CAR-T cell therapy should receive prophylactic interventions for infection before immune reconstitution (see **Table 15**).

Risk factors for infection include:[8]

- Before CAR-T cell therapy, patients exposed to >4 lines of therapy
- Prior autologous/allogeneic hematopoietic cell transplantation

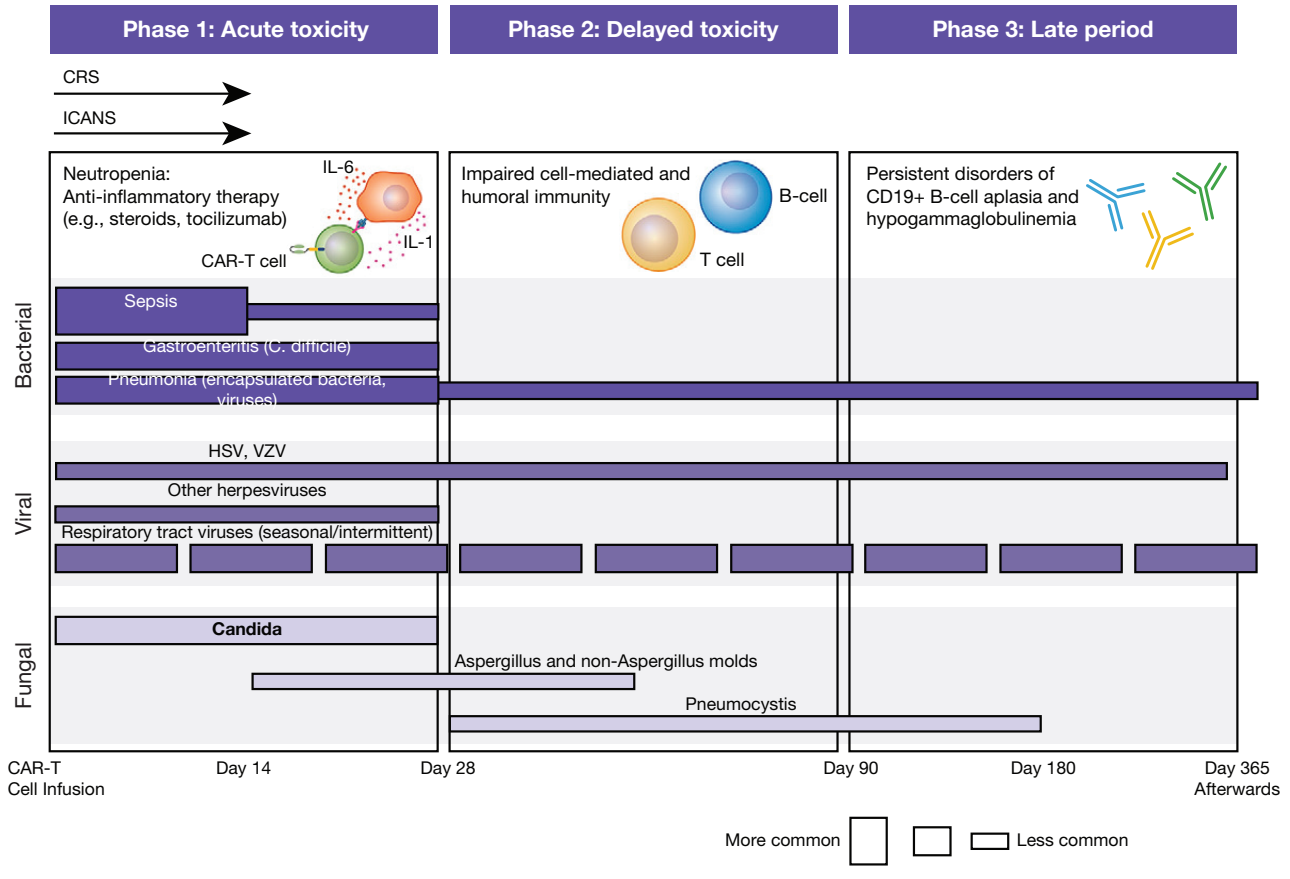
- Prior bridging therapy
- High-dose CAR-T cell infusion
- Occurrence of ≥Grade 3CRS or Grade ≥2 ICANS
- Prior use of steroids/tocilizumab to treat CRS or ICANS
- Neutropenia
- Prolonged CD4+ T lymphopenia
- Prolonged B-cell aplasia and hypogammaglobulinemia

As **Figure 10** shows, early infections occurring within the 30 days after CAR-T cell therapy are typically bacteria-induced or respiratory tract infections, and invasive fungal infections are uncommon. [35] After 30 days have elapsed since treatment, viral infections are in the majority; for patients receiving prophylactic valaciclovir, reactivation of herpes simplex virus (HSV) and varicella-zoster virus (VZV) is uncommon. Reactivation of CMV, EBV, adenovirus, human herpes virus type 6 (HHV6), BK virus, and John Cunningham virus is uncommon. Consequently, it is unnecessary to perform excessive routine monitoring unless the patient has received autologous bone marrow transplantation or prolonged high-dose steroids.

There is current evidence that CAR-T cell production may still proceed* despite latent infection with hepatitis B virus (HBV), hepatitis C virus, and human immunodeficiency virus (HIV), and that treatment is safe provided that the viral load is undetectable before leukapheresis and lymphodepletion. In addition, after CAR-T cell therapy, at least 6 months of entecavir/tenofovir or equivalent medications should be given to patients with an HBV infection, including those with positive HBV surface antigen or anti-HBc antibody (particularly if both HBV surface antigen and HBV DNA are positive) as long-term prophylaxis. [8]

* Different CAR-T cell products have their respective raw material collection guidelines and should be handled according to these stipulations

Figure 10. Possible Infections at Each Phase After CAR-T Cell Therapy[36]



Adapted from: Figure 4, Hill JA, Seo SK. *Blood* 2020;136:925-935.
CAR-T, chimeric antigen receptor T; CRS, cytokine release syndrome; HSV, herpes simplex virus; ICANS, immune effector cell-associated neurotoxicity syndrome; IL, interleukin; VZV, varicella-zoster virus.

3.2.2 Vaccination

In case of incomplete immune reconstitution or continued immunosuppression, vaccines (including COVID-19 vaccines) may exhibit diminished efficacy, but the current consensus remains that vaccination is still able to reduce infection rates and improve clinical outcomes. Please refer to **Table 16** for the EBMT/EHA recommendations for vaccination before/after CAR-T cell therapy; these recommendations apply to both adult and pediatric patients. [8,37]

Table 16. Vaccination Recommendations Before and After CAR-T Cell Therapy[8]

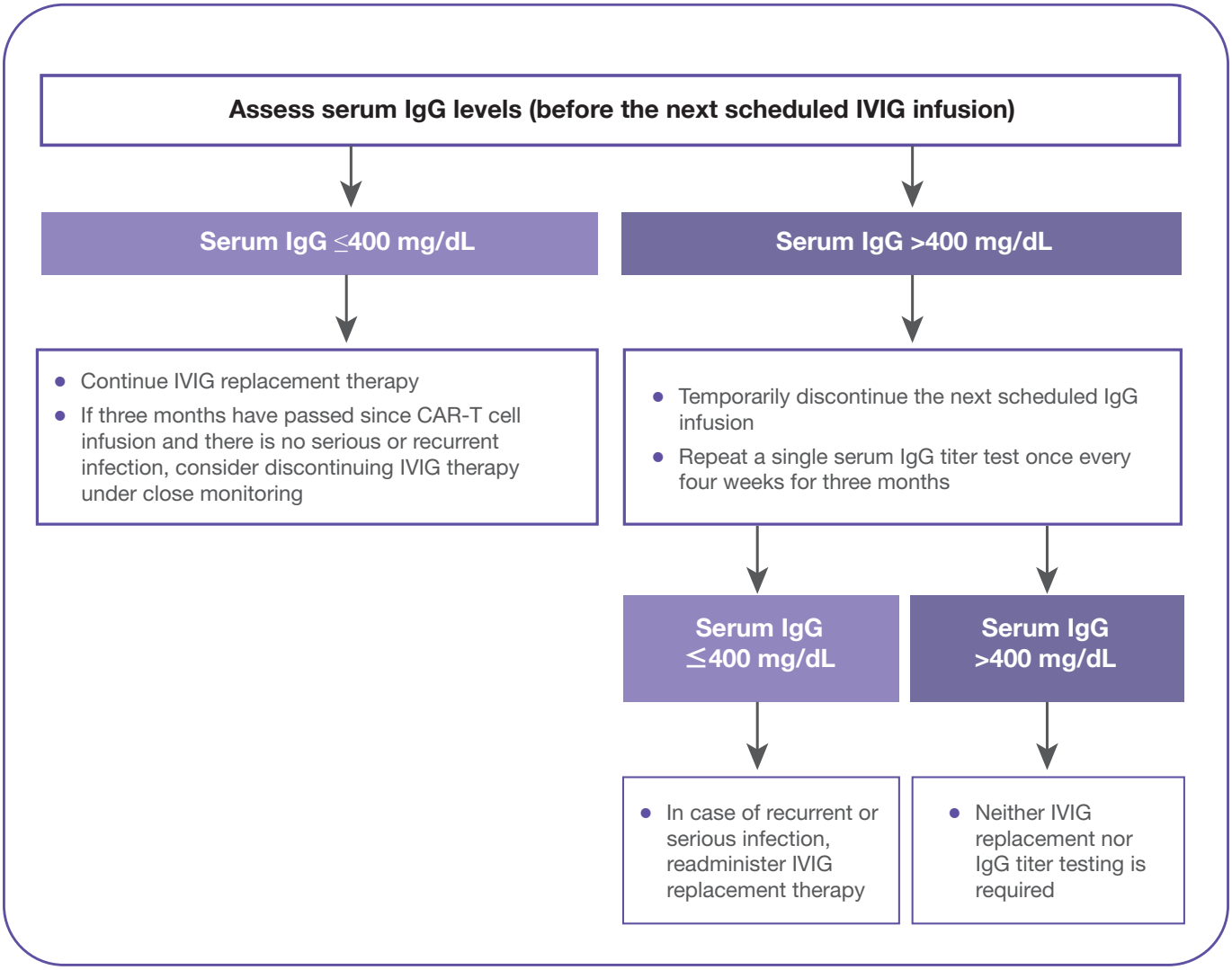
	EBMT/EHA Recommendations		Explanation
	Pre CAR-T Cell Therapy	Post CAR-T Cell Therapy	
Influenza vaccine	• Preferably vaccinate 2 weeks before lymphodepletion • There is a lower chance of generating immune serological responses in B-cell aplasia	Over 3 months after CAR-T cell therapy, patients should be vaccinated regardless of recovery of immunological reconstitution	• The response to vaccination may be diminished in cases of incomplete immune system recovery or ongoing immunosuppression • Current consensus: Vaccination may still be beneficial in reducing infection rates and improving clinical outcomes. Boosters may be considered after B-cell recovery
SARS-CoV-2 (COVID-19) Vaccines	• Preferably vaccinate before receiving CAR-T cell therapy • There is a lower chance of generating immune serological responses in B-cell aplasia	Over 3 months after CAR-T cell infusion	• Evidence of vaccine response after CAR-T cell therapy is limited, and preliminary study reports have shown incomplete immune serological responses • SARS-CoV-2 (COVID-19) vaccine-induced protection is heavily reliant on T-cell mediated immunity, so B-cell aplasia is not a contraindication; however, no T-cell threshold has been defined. Post-vaccination monitoring is recommended
Inactivated vaccines		Over 6 months after CAR-T cell therapy and over 2 months after immunoglobulin replacement therapy	Contraindications include: • Concurrent immunosuppressive or cytotoxic therapy
Attenuated live and non-live adjuvant vaccines		1 year after CAR-T cell therapy and complete immune reconstitution (absolute $CD4^+$ T-cell count >0.2 x 10^9/L, CD19 or $CD20^+$ B-cell count >0.2 x 10^9/L, and no concurrent immunosuppressive or cytotoxic therapy)	Contraindications include: • Within 2 years after receiving allo-HCT • Within 8 months of completed immunoglobulin replacement therapy

Adapted from: Table 13, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
allo-HCT, allogeneic hematopoietic cell transplantation; CAR-T, chimeric antigen receptor T; EBMT, European Society for Blood and Marrow Transplantation; EHA, European Hematology Association; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

3.2.3 B-Cell Aplasia and Hypogammaglobulinemia

CAR-T cell therapy displays high specificity for its target, so off-target toxicity is seldom observed. Conversely, it is more common to observe toxicities associated with CAR-T cells attacking normal cells bearing the target (on-target/off-tumor toxicity). B-cell aplasia almost always occurs after successful CD19 CAR-T cell therapy. [37] In the ELIANA clinical study, 83% of pediatric B-ALL patients still had B-cell aplasia within 6 months; in the ZUMA-1 clinical study, 25% of the CAR-T cell therapy responders still had B-cell aplasia within 12 months. [8] 43% of pediatric patients receiving tisagenlecleucel were observed to have hypogammaglobulinemia, but the actual incidence may be higher

Figure 11. Timing for Administering Immunoglobulin After CAR-T Cell Therapy[38]



Adapted from: Figure 3, Hill JA, et al. *Blood Rev* 2019;38:100596.
CAR-T, chimeric antigen receptor T; IgG, immunoglobulin G; IVIG, intravenous immunoglobulin.

still because a subset of the patients had already undergone empiric immunoglobulin replacement before hypogammaglobulinemia was confirmed. [37]

During the period of functional CAR-T cells persistence, pediatric patients often receive empiric immunoglobulin replacement to reduce the risk of acute infectious complications, but no conclusion has been reached as to whether adult patients require such treatment. [37] Serious hypogammaglobulinemia may increase the risk of respiratory tract and other bacteria-induced infections (e.g., Streptococcus pneumoniae and Haemophilus influenza type b). Before establishing data on the association between hypogammaglobulinemia and infection, it is recommended to closely monitor serum IgG levels after CAR-T cell therapy and administer intravenous immunoglobulin (IVIG) at appropriate times to maintain IgG >400 mg/dL. Please refer to **Figure 11** for the timing of administration. [37]

3.3 Long-term Follow-up after CAR-T Cell Therapy

Long-term follow-up after CAR-T cell therapy requires collaboration by a multidisciplinary team (including the CAR-T cell therapy physicians, specialists, nurse practitioners, and case managers) to manage the post-treatment disease status and late effects. Persistent cytopenia, hypogammaglobulinemia, and infections are very common; neurological complications and pulmonary toxicity increase the mortality risk; secondary malignancies have a lengthy latency period but tend to be rare. Further long-term follow-up information from clinical research and epidemiology are needed for precise estimation of the incidence of secondary malignancies. The U.S. Food and Drug Administration requires at least 15 years of long-term follow-up for tisagenlecleucel and axicabtagene ciloleucel, and the post-market registra-

tion study conducted by the Center for International Blood and Marrow Transplant Research (CIBMTR) will consider post-treatment incidence of malignancy as an important endpoint for assessment. Please refer to **Table 17** and **Table 18** for the EBMT/EHA recommendations on long-term follow-up frequencies and assessment items. [8]

Long-term follow-up after CAR-T cell therapy is mainly considered from three levels:

1. **Disease status**
   Currently international clinical treatment guidelines have not reached a conclusion regarding follow-up frequency for disease status, but studies have shown that bone marrow minimal residual disease (MRD) and PET imaging studies 28 days after CAR-T cell infusion can predict the relapse after CAR-T cell therapy in ALL[39] and DLBCL[40] patients. Therefore, follow-up of disease status at 1 month after CAR-T cell therapy, every 3 months in Year 1, and every 6 months in Year 2 may be considered.
2. **Presence of CAR-T cells in the body**
   Anti-CD19 CAR antibodies may be used for follow-up of CAR-T cell counts, but the majority of hospitals do not have access to this test; alternatively, B-cell aplasia may also be used for functional assessment of CD19 CAR-T cells. B-cell recovery is defined as the time from disease resolution until $CD19^+$ cells occupy ≥1% or the earliest time when the cells achieve ≥3% among lymphocytes. [41]
3. **Follow-up of long-term post-treatment side effects**
   Follow-up items include: cytopenia, hypogammaglobulinemia, recovery from neurological or psychiatric side effects, infection/cardiotoxicity/secondary neoplasm incidence, pregnancy status, and other immunological responses. [42]

Table 17. EBMT/EHA Recommended Minimum Frequency of Attendance at CAR-T Cell Therapy Centers for Patients in Remission for Monitoring[8]

Period	Visiting Frequency	Items to be Monitored
• Post-treatment Day 100 to Year 1 • 1-2 years • 2-15 years	• Monthly • Every 6 months • Annually	• Disease status – Remission, minimal residual disease (MRD), relapse, death • 'Subsequent treatments – including allogeneic transplantation, other immune effector cell (IEC) therapies or advanced therapy medicinal products (ATMPs) • Immunological status – including immune effector cell markers, immunoglobulins, CAR-T cell persistence • New cancers and secondary bone marrow diseases • Autoimmunity and new autoimmune diseases • Endocrine, reproductive, and bone health, including growth and developmental status • Nervous system status (recovery from ICANS) • Psychological status and quality of life • Cardiovascular disease, including metabolic syndrome and other risk factors • Respiratory function • Gastrointestinal and hepatic health • Vaccination status • Please refer to the recommendations proposed by Dr. Majhail and colleagues in 2012 for long-term follow-up of patients with subsequent allogeneic transplantation, cytotoxic therapy, and/or other IEC therapies.[43]

Adapted from: Table 14, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
ATMPs, advanced therapy medicinal products; CAR-T, chimeric antigen receptor T; EBMT, European Society for Blood and Marrow Transplantation; EHA, European Hematology Association; ICANS, immune effector cell-associated neurotoxicity syndrome; IEC, immune effector cell; MRD, minimal residual disease.

Table 18. EBMT/EHA Recommendations for Tests During Long-Term Follow-up[8]

Test	Purpose	Frequency	Comments
Complete blood count, biochemistry panel	Standard follow-up	At every visit	
Viral infections (peripheral blood PCR, nasopharyngeal aspirate)	Viral reactivation/ infection	As clinically indicated	
Quantitative immunoglobulins ± serum protein electrophoresis	Immune reconstitution	At every visit	
Peripheral blood immunophenotyping—CD 3/4/8/16+56/19	Immune reconstitution	At every other visit	No longer required after normalization
CAR-T cell monitoring where commercial kits are available for routine monitoring of anti-CD19 CAR-T cell therapy	CAR-T cell persistence	At every visit	No longer required when undetectable for two consecutive tests
Endocrine function and other standard late effects testing depending on patients' age	Standard follow-up	Annually or as clinically indicated	

Adapted from: Table 15, Hayden PJ, et al. *Ann Oncol* 2022;33:259-275.
CAR-T, chimeric antigen receptor T; EBMT, European Society for Blood and Marrow Transplantation; EHA, European Hematology Association; PCR, polymerase chain reaction.

Disease Status Follow-up

Although CD19 CAR-T cell therapy has achieved exceptionally complete remission rates in treating patients with relapsed and refractory B-ALL and B-cell lymphoma, ultimately 40-60% will elapse. [44] Please refer to **Table 19** for factors associated with remission durability after CAR-T cell therapy. Antigen escape is a mechanism of relapse after CAR-T cell therapy that occurs when tumor cells no longer express the target antigen. Currently, it is known that antigen escape occurs in 20-28% of B-cell lymphoma patients who have received CAR-T cell therapy; this occurs in 16-68% of B-ALL patients. [45]

Table 19. Factors Associated with Remission Durability After CAR-T Cell Therapy[45]

Depth of Treatment Response	• Patients with a greater initial depth of remission are more likely to maintain long-term treatment responses; however, relapse may still occur even after achieving deep MRD(-) remission
Disease Type	• B-cell lymphoma patients are less likely to achieve complete remission, but it is more durable once achieved • Patients with B-ALL or MM are more likely to achieve complete remission, but it is less durable
Tumor Burden and Location	• Patients with a lower tumor burden before infusion are more likely to achieve deep treatment responses • Extramedullary disease will reduce response rates
Lymphodepleting Chemotherapy (LDC)	• Patients who receive lymphodepleting chemotherapy have better treatment responses • The most-effective lymphodepleting chemotherapy regimen and dosing strategy remain unknown, but fludarabine in combination with cyclophosphamide is the most commonly used regimen
CAR-T Cell Levels	• Higher peak blood CAR-T cell levels are associated with an initial response and remission durability

Adapted from: Box 1, Cappell KM, Kochenderfer JN. *Nat Rev Clin Oncol* 2023;20:359-371.
B-ALL, B-cell acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T; MM, multiple myeloma; MRD, minimal residual disease.

04

CAR-T Cell Therapy in Solid Tumors

Chapter 4

CAR-T Cell Therapy in Solid Tumors

4.1 Overview of CAR-T Cell Therapy in Solid Tumors

CAR-T cell therapy is primarily used in hematological cancers and has demonstrated excellent clinical efficacy for the indications mentioned in the preceding sections. However, as of December 2023, the applications of CAR-T cell therapy in the treatment of solid tumors are still in the early stages of research and development, and have faced certain challenges.

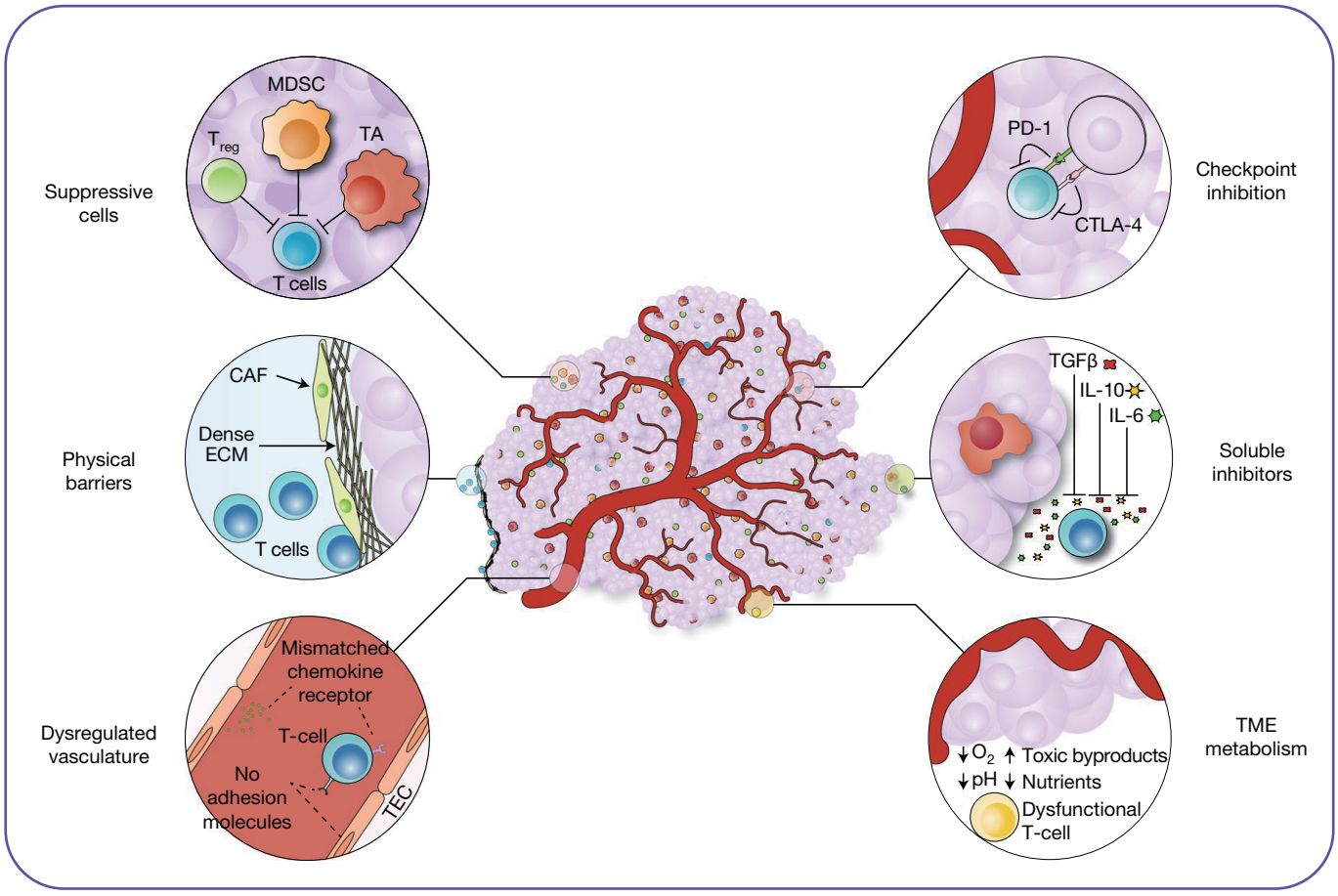
Currently claudin 18.2 CAR-T cells have entered early clinical studies, where it has achieved an overall response rate (ORR) of 49% in gastrointestinal tumors. Cancers of the stomach or gastroesophageal junction are even more sensitive, with ORR reaching 57%. These show good efficacy and controlled, tolerable toxicity. [46] Besides standard overexpressed proteins or novel mutant antigens, non-protein antigens such as lipids and carbohydrates have not been investigated in depth and may provide additional options for targets in CAR-T cell therapy. Among these categories of non-protein antigens, ganglioside GD2 has obtained favorable initial results in the treatment of neuroblastoma in early clinical studies. Approximately two-thirds of subjects were able to achieve a tumor response, including complete disappearance of the tumor in one-third, and study results revealed a long tail effect in the progression-free survival curve. [47]

The use of CAR-T cell therapy in solid tumors has encountered two main challenges, the lack of tumor-specific antigens and complex tumor microenvironments (as shown in **Figure 12**). [48,49] This section will discuss these two challenges and possible responses.

4.2 Lack of Unique Tumor-Specific Antigens and the Resulting On-Target/Off-Tumor Toxicity

Ideal tumor antigens are tumor-specific antigens (TSA) rather than tumor-associated antigens (TAA). In reality, however, TSAs are extremely rare, so in most cases only TAAs may be used in the design of CAR-T cells. Currently, many TAAs have already been identified, providing a sound foundation for testing CAR-T cells in patients with solid tumors.

Figure 12. Immunosuppressive Mechanisms in the Tumor Microenvironment[49]



Adapted from: Figure 6, Labanieh L, et al. *Nat Biomed Eng* 2018;2:377-391.
CAF, cancer-associated fibroblast; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ECM, extracellular matrix; IL, interleukin; MDSC, myeloid-derived suppressor cell; PD-1, programmed cell death protein 1; TAM, tumor-associated macrophage; TEC, tumor endothelial cell; TGFβ, transforming growth factor-β; TME, tumor microenvironment; Treg, regulatory T cell.

Because tumor-associated antigens are insufficiently specific, they may also be expressed at low levels by normal cells. Such situations will cause CAR-T cells to produce on-target/off-tumor toxicity, inflicting damage on normal cells bearing the target antigen. In previous phase I clinical studies for CAR-T cell therapy, a subset of patients with solid tumors who receive CAR-T cell therapy may develop severe toxicity,[50] which causes organ failure and in turn leads to the risk of death. [51]

When developing CAR-T cell therapy for solid tumors, how to resolve or at least reduce off-target toxicity is of utmost importance, guiding the development of a safe and effective treatment strategy. Several approaches are under development to solve this challenge.

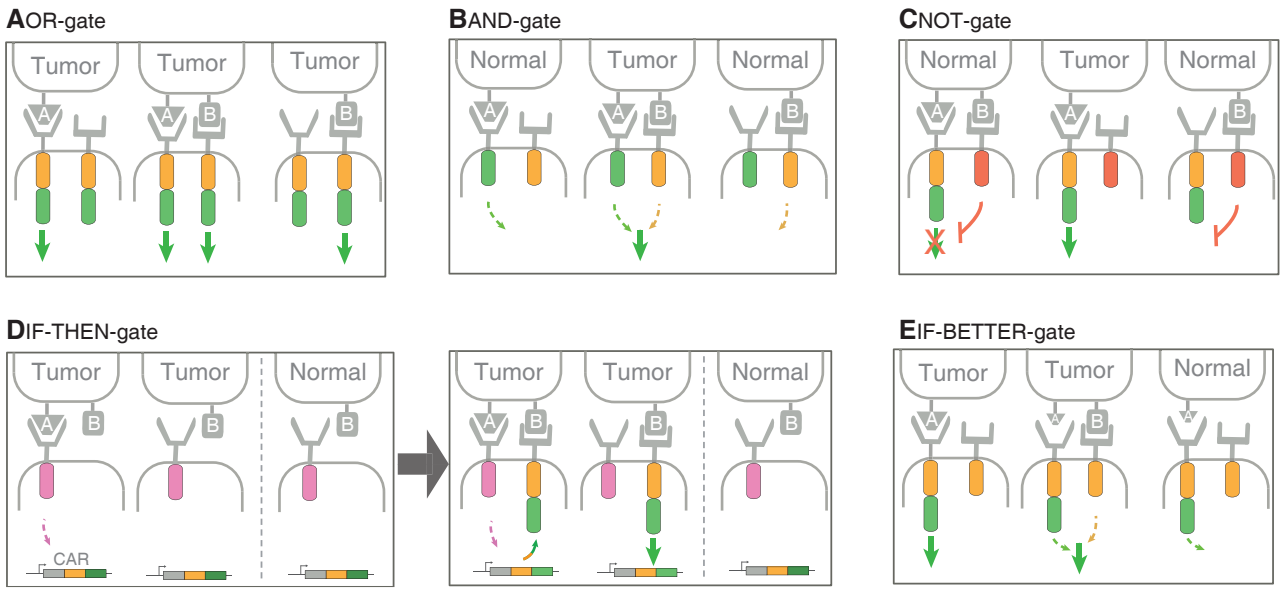
1. Establish CAR-T cell therapy system with greater specificity, including systems with externally controlled T-cell function or survival, which may take the form of a drug-off or drug-on mode.
   - ① Drug-off mode: CAR-T cells are on by default. In GD2-CART01 studies, an inducible caspase 9 acts as a suicide switch, so that drug administration allows GD2-CART01 cells to be eliminated by CAR-T cells when they develop hazardous toxicity. In clinical studies, there were in fact patients with severe neurotoxicity for whom administration of rimiducid activated the suicide gene in GD2-CART01 cells. This rapidly eliminated most of the GD2-CART01 cells within 4 hours, thus safeguarding patient safety. [47]
   - ② Drug-on mode: CAR-T cells are off by default and require the administration of specific small molecule substances to stabilize the CAR structure and activate the CAR-T cells' attack mode. 52,53 In general, this approach regulates CAR-T cell activity through altering small-molecule drug levels.
2. Using modifications to single-chain variable fragment (scFv) affinity or CAR structure so that the CAR-T cells exhibit cytotoxicity toward tumor cells with high antigen expression but reduced or absent cytotoxicity toward normal cells with low antigen expression. This may aid in reducing the risk of off-target toxicity, and this enhancement greatly boosts the effectiveness of HER2 CAR-T cell therapy. [54]
3. Many protein-based logic-gating strategies (OR gate, AND gate, NOT gate, IF-THEN gate, IF-BETTER gate, etc.) have been investigated in in vitro models. They use the presence and degree of expression of two or more antigens to further confine CAR-T cell activity and toxicity to the tumor site. Please refer to **Figure 13** for their mechanisms of action. [55]
4. Administer CAR-T cells locally (e.g., intrathecal, intraventricular, intraperitoneal, intratumor). These methods can concentrate antitumor activity within the tumor environment and may prevent systemic off-target toxicity. Such methods have already been realized in early clinical studies.

4.3 Immunosuppressive Tumor Microenvironment

The microenvironment of solid tumors often has abundant fibrous tissue, varying cell types, and complex cell-cell interactions. This makes it difficult for CAR-T cells to move effectively and penetrate deep into the tumor, so they are unable to attack all cancer cells. Therefore, enabling CAR-T cells to efficiently traverse the fibrous structures surrounding tumor tissue and overcome the immunosuppressive microenvironment is a major dilemma in the use of CAR-T cell therapy for treating solid tumors. [56]

The area around a tumor forms a highly immunosuppressive microenvironment. For example, the tumor locally produces vascular endothelial growth factor (VEGF), inducing the formation of malformed blood vessels, inhibiting lymphocyte adhesion, promoting tissue hypoxia, and restricting the oxidative metabolism of infiltrating immune effector cells. Furthermore, the tumor attracts myeloid-derived suppressor cells (MDSC), which secrete immunosuppressive factors that inhibit immune effector cells. [57] At the same time, expression of chemokines by tumor-associated macrophages (TAMs) and the tumor itself, and high expression of immunosuppressive factors and inhibitory immune checkpoints by regulatory T cells, as well as cytokines with immunosuppressive actions and exosomes expressing the programmed death-ligand, are all important contributors in forming the highly immunosuppressive environment around the tumor, which helps tumor cells successfully evade identification or attacks by immune effector cells.

Figure 13. Protein-Based Logic Gating Strategies for Restricting CAR-T Cells[55]



A, OR-gate, T cells coexpress fully functional CARs targeting distinct tumor antigens A and B.

B, AND-gate, T cells coexpress a CAR specific for antigen A and a CCR specific for antigen B. CAR T cells are fully activated when the CAR and the CCR simultaneously engage with antigens A and B coexpressed on the tumor but not on normal cells.

C, NOT-gate, T cells coexpress a fully functional CAR specific for antigen A and an inhibitory CAR (iCAR) specific for antigen B. T cells are fully activated when the CAR engages with antigen A expressed exclusively on tumor cells. iCAR engagement with antigen B expressed on normal cells reversibly inhibits CAR T cells.

D, IF-THEN-gate, T cells coexpress synthetic Notch (SynNotch) receptor specific for antigen A. Engagement of SynNotch receptor with antigen A (left) induces transient expression of fully functional CAR specific for antigen B (right) in the tumor environment. The decay of CAR expression (spatiotemporal regulation) in circulating T cells should allow for the protection of normal cells expressing the tumor-associated antigen B. The gray arrow between implies time.

E, IF-BETTER-gate, T cells coexpress a fully functional CAR specific for antigen A and a CCR specific for antigen B. CAR T cells are fully activated if the CAR engages with antigen A expressed at high levels on tumor cells. If tumors express antigen A at low levels (small-size antigen A, middle tumor cell), full T cell activation requires CCR engagement with antigen B on the same tumor cells. Antigen A can be expressed alone, not with antigen B, at low levels in normal tissues.

Adapted from: Figure 3, Hamieh M, et al. *Cancer Discov* 2023;13:829-843.
CAR-T, chimeric antigen receptor T; CCR, chimeric costimulatory receptor.

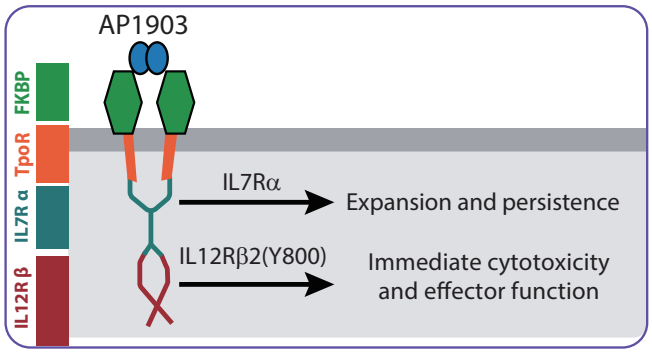
To address tumor microenvironments and the challenge of enhancing CAR-T cell therapy performance, scientists are seeking to improve CAR-T cell efficacy in solid tumors through synthetic biology approaches and development,[48] as in the following examples:

1. **Have CAR-T cells express specific tumor chemokines at the same time**
   Examples include CCR5, CXCR3, and CXCR4. It is hoped that more CAR-T cells will be able to enter the interior of the tumor by using these tumor chemokines.
2. **Have CAR-T cells secrete specific cytokines**
   Examples include IL-2, IL-7, IL-15, and IL-18. It is hoped that this will modify the tumor microenvironment. This method shows potential for development.
3. **Dominant Negative Receptor (DNR)**
   These modified receptors bind ligands with immunosuppressive actions but do not induce the transduction of downstream inhibitory signals by immune effector cells, thus consuming immunosuppressive ligands such as TGF-β and VEGF in the tumor microenvironment. In an animal study on pancreatic cancer, TGF-β DNR-equipped anti-PSMA CAR-T cells were demonstrated to enhance anti-tumor effects.
4. **Cell-Intrinsic Checkpoint Inhibition**
   Similar to the previous mechanism, this allows CAR-T cells to overcome PD-1 immunosuppression through the use of PD-1 DNR. A preclinical study and Phase 1 trial showed that PD-1 DNR-carrying anti-CD19 CAR-T cell therapy is safe and effective.
5. **Switch Receptors (SR)**
   Intracellular modification of the CAR-T cells causes receptors that originally bind immunosuppressive ligands to bind immunostimulatory signals instead, achieving the effect of reversing extracellular inhibitory signals into intracellular stimulatory signals.For example, linking 4-1BB to the TGF-β receptor causes CAR-T cells to generate intracellular 4-1BB immunostimulatory signaling after re-

ceiving extracellular TGF-β signaling. This method has been implemented in a melanoma model and was demonstrated to increase proliferation and tumor clearance by T cells.

6 **iTurbo Platform**
This is a transduction platform that can use small molecule drugs (such as AP1903 in **Figure 14**) to initiate cytokine signaling (such as IL-7 receptor and IL-12 receptor) and control JAK/STAT signal transduction and its strength, thus effectively prolonging T-cell persistence.[58]

Figure 14. The iTurbo Platform for AP1903[58]

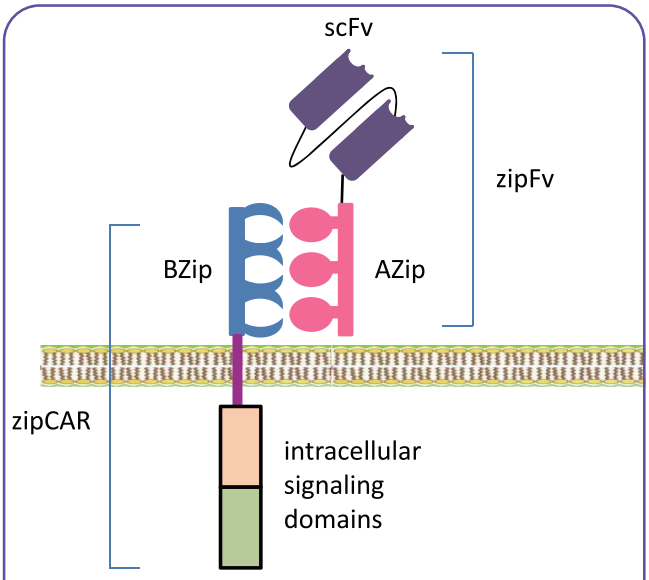


Adapted from: Figure 3, Lin RJ, et al. Cancer Immunol Res 2022;10:1069-1083.
IL, interleukin.

7 **Universal CARs**
"universal" CARs (such as zipCAR in **Figure 15**) may pair with different tumor-associated antigen targeting domains (such as zipFv in **Figure 15**) or even bispecific T-cell engagers (BiTEs) for flexible switching of CAR-T cell specificity in tumor toxicity with an exogenous means of persistently activating CAR-T cells.[59] There are many variations of this method; for example, using avidin CARs to bind specific ligands can increase CAR-T cell specificity and activity.

8 **Using anti-FAP CAR-T cells to eradicate fibroblasts**
Fibroblasts express the antigen fibroblast activation protein (FAP), so FAP CAR-T cells are used in the first line of treatment, followed by second-line mesothelin CAR-T cell therapy. This method can effectively treat pancreatic cancer in mice, and has currently achieved significant results in animal studies. [60]

Figure 15. Universal CAR Systems Can Flexibly Regulate CAR Cell Activity and Specificity[59]



A zipCAR has a cognate leucine zipper (BZip) that can bind to the AZip. Through the binding between A- and B-leucine zippers, any extracellular signal linked to the AZip can activate the T cells. The affinity between the A- and B-leucine zippers can be adjusted so that the signaling strength and activity can be dialed up or down as desired.

Adapted from: Figure 3, Zhao J, et al. J Hematol Oncol 2018;11:132.

CAR, chimeric antigen receptor; scFv, single-chain variable fragment; zipCAR, a CAR system consisting of a universal receptor with leucine zipper adaptor; zipFv, a separate scFv with leucine zipper adaptor.

9 **Delivering multiple courses of tumor antigen stimulation during CAR-T cell therapy is expected to extend CAR-T cell persistence and increase CAR-T cell quantity**
For example, CARVac (a lipid nanoparticle-based RNA vaccine) serves to amplify the stimulation of claudin 6 CAR-T cells, which has achieved good preliminary results in early clinical studies.

4.4 Conclusion

Currently, researchers are dedicated to making continuous improvements to CAR-T cell therapy, particularly in the domain of solid tumor therapy. This includes modifications to CAR-T cell design or routes of administration, reduction of off-target toxicity, or use of synthetic biology approaches. These innovative receptor designs and cell engineering techniques allow CAR-T cell therapy to conquer the complex tumor microenvironment, and aid in the development of combined treatment regimens that incorporate other treatment strategies. We believe that solid tumors do not pose an impenetrable barrier to CAR-T cell therapy. Through continuous development of the CAR-T cell therapy domain, new research findings and therapeutic advances provide the opportunity to bring revolutionary changes to clinical tumor treatment strategies.

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English	Abbreviation	Chinese	Page
European Society for Blood and Marrow Transplantation	EBMT	歐洲血液與骨髓移植學會	5–7, 15, 18–20, 33, 42, 44, 46, 47
Event-free survival	EFS	無事件存活期	8
Extramedullary disease	EMD	骨髓外疾病	8, 15, 47
Follicular lymphoma	FL	濾泡性淋巴瘤	5, 20
Glucose-6-phosphate dehydrogenase	G6PD	葡萄糖-6-磷酸鹽去氫酶	23
Graft-versus-host disease	GvHD	移植物抗宿主疾病	5, 10, 19, 20, 41, 42
Granulocyte colony-stimulating factor	G-CSF	白血球生長激素	33, 37, 40–42
Hemophagocytic lymphohistiocytosis	HLH	噬血症候群	29–34, 37–40
Hyperferritinemia		高血鐵蛋白血症	30–32
Hypofibrinogenemia		低纖維蛋白原血症	31, 32, 39
Hypogammaglobulinemia		免疫球蛋白低下	43–46
Immune effector cell-associated encephalopathy	ICE	免疫效應細胞相關腦病變	27–30
Immune effector cell-associated hematotoxicity	ICAHT	免疫效應細胞相關的血球毒性	33–37, 40–42
Immune effector cell-associated HLH-like syndrome	IEC-HS		31–34, 37–39
Immune effector cell-associated neurotoxicity syndrome	ICANS	免疫效應細胞相關神經毒性症候群	4, 5, 19, 20, 25–31, 39, 41–43, 46
Immune reconstitution inflammatory syndrome		免疫重建發炎症候群	40
Immunoglobulin G	IgG	免疫球蛋白G	37, 45
Intravenous immunoglobulin	IVIG	靜脈注射免疫球蛋白	45
Lactate dehydrogenase	LDH	乳酸脫氫酶	9, 19, 20, 31, 34, 39
Large B-cell lymphoma	LBCL	大B細胞淋巴瘤	7, 35
Leukapheresis		白血球分離術	6, 7, 9, 10, 14, 41, 43
Leukemia-free survival	LFS	無白血病存活期	8
Lumbar puncture	LP	腰椎穿刺術	20, 30
Lymphodepleting chemotherapy	LDC	淋巴細胞清除性化療	5, 9, 14–16, 18–20, 23, 34–36, 47
Macrophage activation syndrome	MAS	巨噬細胞活化症候群	31, 32, 34, 37–39
Mantle cell lymphoma	MCL	被套細胞淋巴瘤	7, 35
Minimal residual disease	MRD	微量殘存疾病	15, 46, 47

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Authors (ordered by ascending number of strokes in the Chinese names):
Yu-Wen Wang, Chiao-En Wu, Yi-Rong Lee, Si-Huei Lee, Shu-Wei Chou, Chien-Ting Lin, San-Chi Chen, Zhao-Neng Zheng, Sheng-Hsuan Chien

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Address:
Rm. 2805, 28F., No. 66, Sec. 1, Zhongxiao W. Rd., Zhongzheng Dist., Taipei City; Rm. 203D, 2F., Taita Jing-fu Building, No. 1, Changde St., Taipei City; Rm. 204, 2F., Taita Jing-fu Building, No. 1, Changde St., Taipei City; Linkou Changgung Memorial Hospital, No. 5, Fuxing St. Guishan Dist., Taoyuan City (Clinic 13, 5F., Pathology Building)
Tel:
02-23819661, 02-23751091, 02-23753867, 0905-911-325

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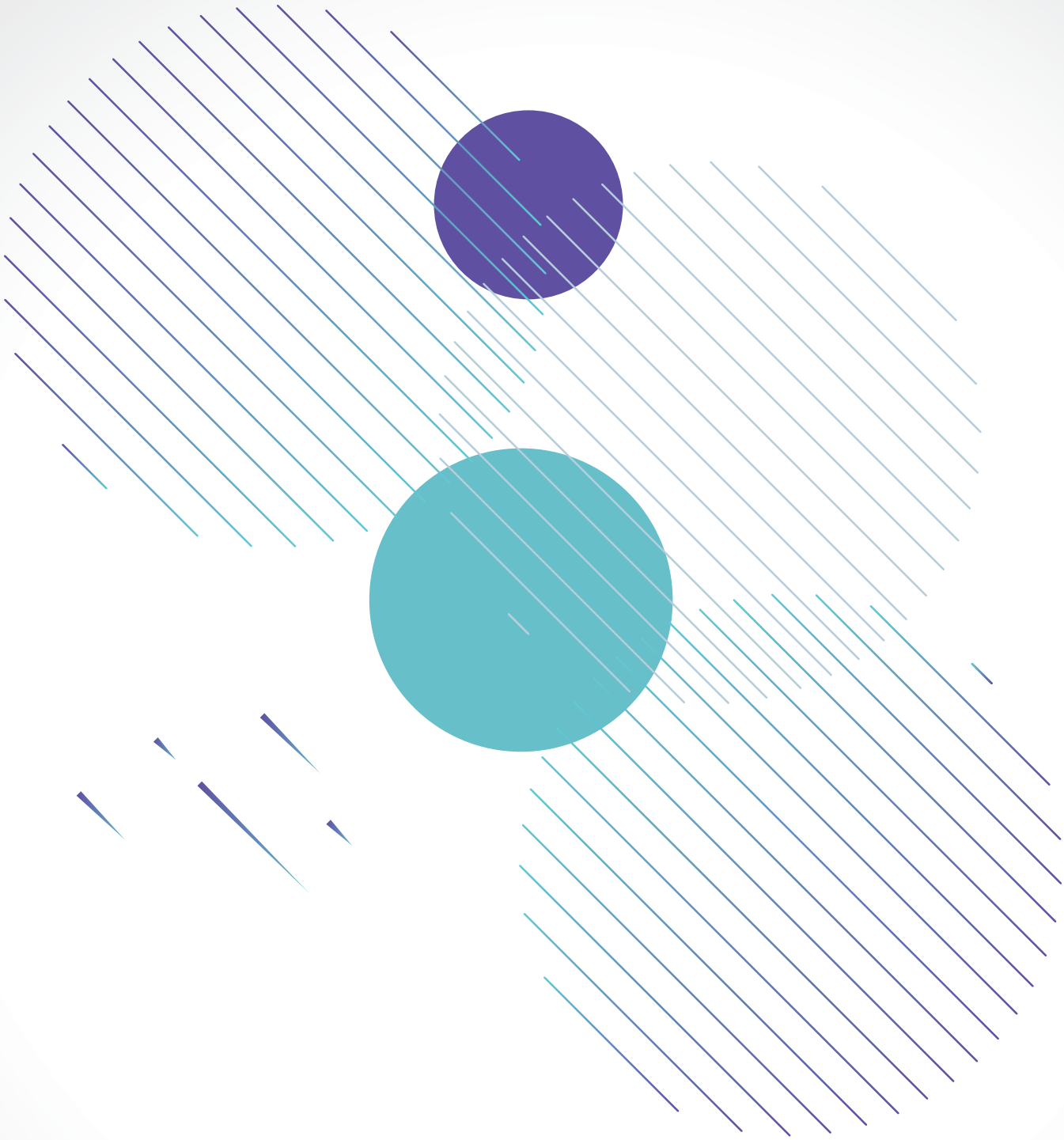

Editorial Team
(ordered by ascending number of strokes in the Chinese names):
Dr. Yu-Wen Wang, Dr. Chiao-En Wu, Nurse Yi-Rong Lee,
Dr. Si-Huei Lee, Dr. Shu-Wei Chou, Dr. Chien-Ting Lin,
Dr. San-Chi Chen, Dr. Zhao-Neng Zheng, Dr. Sheng-Hsuan Chien

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