



# Challenges and strategies of clinical application of CAR-T therapy in the treatment of tumors—a narrative review

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**Abstract:** Chimeric antigen receptor T-cell (CAR-T) therapy has achieved good therapeutic efficacy in the treatment of hematological malignancies. In August 2017, Novartis Kymriah (CAR-T cells targeting CD19) was approved by the FDA, indicating the real entry of CAR-T cell therapy into clinical applications and making CAR-T cell therapy the most attractive technology in the field of tumor treatment. In October 2017, the FDA approved the world's second CAR-T cell therapy—Yescarta. The launch of these products has attracted wide attention to CAR-T cell therapy. CAR-T cell therapy has achieved significant effect in the treatment of tumors, however, CAR-T therapy also faces clinical problems, such as cytokine release syndrome (CRS), poor therapeutic efficacy in solid tumors, and high rates of tumor recurrence. At present, the side effects of CAR-T therapy have attracted a large amount of attention, which has resulted in investigations into strategy establishment. With a deepening understanding of CAR-T therapy and the continuous optimization of therapeutic regimens, its toxicity and side effects have been partially controlled. This study set out to analyze the problems in the clinical application of CAR-T therapy encountered in recent years and to introduce corresponding strategies, with the aim of providing a basis of reference for clinicians and scientists in the management of CAR-T therapy in clinical practice and in the CAR-T therapy research.

**Keywords:** Chimeric antigen receptor T-cell (CAR-T); cytokine release syndrome (CRS); solid tumor treatment; tumor recurrence; strategy

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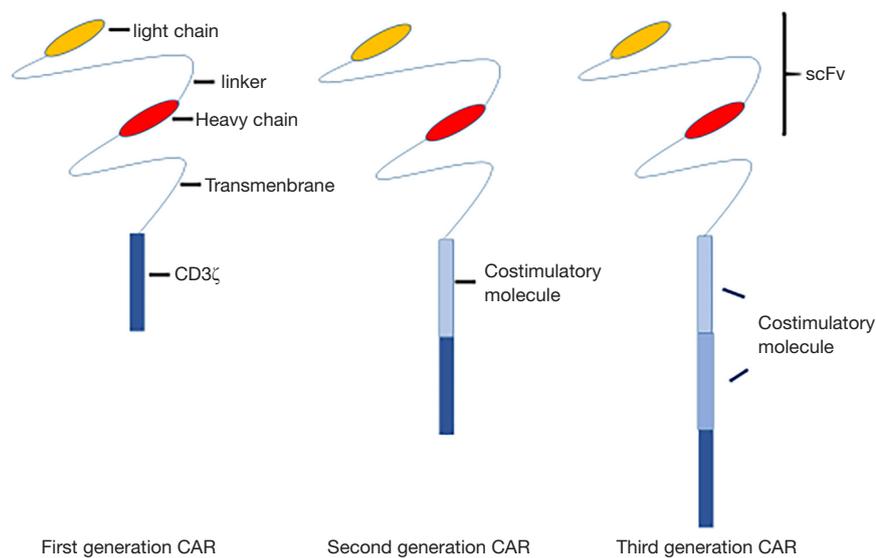
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## Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy is a new type of cell therapy that has received widespread clinical attention and has achieved impressive outcomes with hematological malignancies (1,2). The key point of CAR-T cell therapy is to make T cells express specially designed CAR through genetic modification, then proliferate *in vitro*, and then infuse into the patient. Through the CAR specifically binding to the surface antigen of targeted tumor cells, the CART-cell can accurately recognize targeted cells and then kill them to achieve the purpose

of treating tumors. The basic structure of CAR includes extracellular domain (including svFv and hinge region), transmembrane domain and intracellular domain. Among them, the scFv in the extracellular domain can specifically bind to tumor antigens, which is the “locator” for CART cells to accurately hit target cancer cells. The transmembrane domain can anchor the CAR on the T cell membrane and guide CART cells to The “retractor” of the target cell, the intracellular domain is responsible for signal activation and conduction, and is the “signal switch” that enables CART cells to exert their killing function.



**Figure 1** Chimeric antigen receptor (CAR) generations. First generation CARs were composed of the single-chain variable region (scFv) linked to intracellular CD3 zeta ( $\zeta$ ). Second generation CARs incorporated an intracellular signaling motif from a T-cell costimulatory molecule followed by CD3 $\zeta$ , while third generation CARs include more than one costimulatory molecule in addition to CD3 $\zeta$ .

So far, there are three generations of CAR (Figure 1). The US Food and Drug Administration (FDA) has approved the marketing of Kymriah (Novartis) and Yescarta as CAR-T drugs for the treatment of hematological malignancies worldwide. Despite CAR-T therapy achieving good outcomes in hematological malignancies, its clinical application still brings multiple problems, such as cytokine release syndrome (CRS), poor efficacy in solid tumors, and a high rate of recurrence. Many problems need to be solved before the use of CAR-T therapy in clinical practice can be a mainstream treatment. As the problems continue to emerge, the corresponding solution strategy seems to be more important. It seems that there are relatively few articles summarizing this, so in this paper, we mainly elucidate the existing problems of CAR-T therapy in clinical practice and propose relevant solutions and strategies.

We present the following article in accordance with the Narrative Review reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-4502>).

## CRS

### Overview of CRS

A certain degree of cytokine release is considered as a sign of effective CAR-T therapy; however, the excessive release

of cytokines can lead to CRS. Currently, CRS is the most common complication associated with CAR-T therapy (3). Defined as the excessive cascade release of cytokines, including IFN $\gamma$ , IL6, IL1, and IL2RA, CRS sees CAR-T cells massively proliferate after contact with tumor antigen-bearing cells, producing lethality against tumor cells (4). The consequential “cytokine storm” causes a syndrome leading to successive related activities that inflict damage to the patient’s body (5). The clinical manifestations of CRS are diverse, and mainly include high fever, weakness, fatigue, myalgia, nausea, anorexia, tachycardia, hypotension, capillary leakage, cardiac insufficiency, renal damage, liver failure, and diffuse intravascular coagulation. In a study of 30 patients with relapsed/refractory acute lymphoblastic leukemia (r/r ALL) (1), almost all patients had CRS, 8 of whom developed severe CRS after receiving anti-CAR-T19 therapy. Although some patients who experience the above-mentioned serious toxic reactions need to be monitored and admitted to the intensive care unit, with the condition even proving fatal for some, in most patients, the symptoms of toxic reactions are controllable. In addition, accurate assessment and timely treatment can attenuate the adverse consequences of immune cell therapy. We would mainly introduce the classification and treatment of CRS and cell-related encephalopathy syndrome (CRES).

**Table 1** CRS classification criteria

Institution	Grade 1	Grade 2	Grade 3	Grade 4
CHOP/PENN	Mild symptoms (supportive care only)	Moderate symptoms with some signs of organ dysfunction (need for hospital admission for some IV antibiotics and monitoring)	Moderate to severe symptoms with increased organ dysfunction (interventions for hypotension, coagulopathy & hypoxia)	Life threatening complications requiring aggressive interventions (high dose vasoactives, mechanical respiratory support)
MSKCC	Mild symptoms (observation and supportive care)	Hypotension requiring vasoactive <24 h or hypoxia requiring supplemental O <sub>2</sub> <40%	Hypotension requiring vasoactive >24 h. Hypoxia requiring supplemental O <sub>2</sub> >40%	Life threatening: hypotension refractory to vasoactives. Hypoxia necessitating mechanical respiratory support
Lee Criteria	Mild flu-like symptoms (supportive care only)	Moderate symptoms requiring intervention: hypotension responsive to fluids or low dose vasoactive, hypoxia responsive to supplemental O <sub>2</sub> , mild organ dysfunction	Increasing symptomatology: hypotension necessitating multiple vasoactives, hypoxia necessitating increasing O <sub>2</sub> supplementation (>40%), grade 4 transaminitis	Life-threatening symptoms (mechanical respiratory support, grade 4 organ toxicity)
CARTOX	Temperature ≥38 °C. Grade 1 organ toxicity	Hypotension responsive to fluids or low dose vasoactives. Hypoxia requiring supplemental O <sub>2</sub> <40%. Grade 2 organ toxicity	Hypotension requiring multiple vasoactives. Hypoxia requiring >40% O <sub>2</sub> . Grade 3 organ toxicity or Grade 4 transaminitis	Life threatening hypotension. Hypoxia requiring mechanical respiratory support. Grade 4 organ dysfunction
ASBMT	Temperature ≥38 °C	Temperature ≥38 °C with hypotension and/or hypoxia (not requiring additional interventions other than supplemental O <sub>2</sub> by NC)	Temperature ≥38 °C with hypotension requiring 1 vasoactive (± vasopressin) or hypoxia requiring high flow or other non-positive pressure intervention	Temperature ≥38 °C with hypotension requiring multiple vasoactives or hypoxia requiring positive pressure

CRS, cytokine release syndrome.

### Classification of CRS

CRS typically occurs within the first week after CAR-T therapy, reaching its peak within 1 to 2 weeks. Consensus guidelines for scoring CRS, which have not been widely adopted, have been established by multiple groups based on different variables (*Table 1*) (6-8). To a certain degree, these scoring models can unify institutions and homogeneous groups.

### Treatment of CRS

A strong positive correlation has been reported between the severity of CRS after CAR-T therapy and the levels of CAR-T cells in blood and serum level of IL-6 (9). For patients with moderate to severe CRS, tocilizumab

or chimeric anti-IL-6 mAb cetuximab has become the first-choice treatment (10). The timely administration of tocilizumab is recommended for Grade 3 CRS or elderly patients with Grade 2 CRS combined with other diseases, and ultimately aims to avert life-threatening Grade 4 and 5 CRS. In most patients, CRS rapidly degrades after the administration of tocilizumab. Therefore, when the US FDA approved Kymriah, tocilizumab was simultaneously approved to treat CAR-T-induced CRS. Moreover, the application of tocilizumab appears unlikely to affect CAR-T therapy and its clinical efficacy (11), and as a result, the use of tocilizumab is more common than that of cetuximab.

Both CRS and cell immunotherapy-related hemophilic lymphocyte hyperplasia (HLH) can effectively be controlled by glucocorticoids via the suppression of inflammatory

responses. Data from several clinical trials of CAR-T therapy have shown that glucocorticoids do not decrease the efficacy of CAR-T therapy when used to treat CRS (12). In a Chinese study on the treatment of acute B lymphocytic leukemia (B-ALL) patients, the use of glucocorticoids could significantly reduce severe symptoms of CRS, while eight patients experienced B-ALL recurrence (13). In another study of 30 B-ALL patients who received CAR-T19 (CTL019) therapy, 2 out of 9 patients who received glucocorticoids suffered subsequent recurrence (14). According to the above studies, glucocorticoid exerts inhibitory effects on CAR-T therapy. Therefore, glucocorticoid therapy should only be considered when treatment with anti-IL-6 fails to control the symptoms of CRS.

Therapeutic plasma exchange (TPE) has not been used as the main therapeutic approach for CRS. A small amount of literature has reported that TPE can assist with the rapid clearance of cytokines in plasma and is helpful in treating patients with severe CRS. A 23-year-old patient with relapsed and refractory B-ALL was reported to exhibit responses typical of CRS, including fever, muscle soreness, and elevated inflammatory cytokines, after the infusion of CAR-T cells. Despite treatment with anti-allergic agents, antipyretic agents, glucocorticoid, and tocilizumab, the patient's CRS symptoms were not effectively controlled and his condition rapidly deteriorated. In the intensive care unit, the patient was treated with 3,000 mL plasma exchange daily. The patient's CRS-associated symptoms were gradually alleviated, and he was eventually discharged (15). Due to the potential severe complications associated with TPE, it must be performed by experienced medical staff. In cases of CRS and ineffective treatment as well as deteriorated condition, TPE is an alternative solution for CRS after CAR-T therapy (16).

The symptoms of CRS following CAR-T cell therapy are generally diverse and complicated. Medical staff should therefore strive to fully understand and accurately evaluate the unique adverse reactions caused by immunotherapy which tocilizumab and hormones are the two most important agents.

### **CAR-T therapy in solid tumors**

CAR-T therapy has achieved significant success in treating certain human hematological malignancies, including leukemia and lymphoma (17-21). Although CAR-T therapy was first applied in the treatment of solid tumors, the progress of CAR-T therapy for this purpose has been

relatively slow and faces great challenges (22-26).

### *Causes of poor efficacy of CAR-T in solid tumors*

#### **Heterogeneity of solid tumors**

The membrane protein CD19 on the surface of B-cell leukemia cells is similarly found in hematological malignancies, and is also widely expressed in almost all B-cell leukemia; therefore, CD19-specific CAR-T therapy yields excellent efficacy (1,27-29). However, the heterogeneity of solid tumors stems from their extremely complicated composition from many tumor cells derived from multiple clones. The expression of target antigens on the surface of tumor cells is not completely consistent, making it difficult for a single type of CAR-T cells to kill all tumor cells, which causes difficulty in selecting tumor-specific antigens and also limiting the specificity of CAR-T cells and their extensive application in solid tumors.

#### **Off-target effects**

When targets exist in both tumor and non-tumor tissues, CAR-T cells can clear both tumor cells and normal cells. For instance, CAR-T cells targeting CD19 protein are very effective in identifying and destroying B-cell lymphoma and leukemia cells but can also simultaneously destroy all normal CD19+ B cells in the patient; such events are referred to as off-target effects.

#### **The difficulty faced by CAR-T cells in homing and colonization in tumor tissue**

To reach the tumor cell site, CAR-T cells have to cross the extracellular matrix, and also face unfavorable conditions posed by the immunosuppressive microenvironment. When the CAR-T cells finally reach the tumor cells, they are already reduced in number and activity and possess significantly diminished tumor-killing efficacy. Therefore, by improving their homing ability, the tumor-killing ability of CAR-T cells can be enhanced.

#### **Tumor microenvironment (TME) of solid tumors**

The TME is predominantly in a state of hypoxia and nutritional starvation, which inhibits T cell proliferation and cytokine production. The efficacy of CAR-T cells, such as prostaglandin E2 (PGE2) and adenosine, may also decrease tumor-derived soluble factors and cytokines. In addition, various immunosuppressive cells, including Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and suppressive immune molecules, such as PD-

L1 and PD-1, also exist in the TME, causing the functions of CAR-T cells to be suppressed upon entering the tumor mass (30). These immunosuppressive mechanisms are the main causes of the formation of the TME and ultimately contribute to the poor therapeutic efficacy of CAR-T cells in solid tumors.

### *Solutions and strategies for CAR-T therapy in solid tumors*

#### **Selection of specific targets**

The identification and screening of new antigen targets for various solid tumors and the construction of corresponding CAR-T cells are the critical prerequisites for the clinical application of CAR-T in the treatment of solid tumors. Glypican-3 (GPC3) is highly expressed on the surface of liver cancer cells, but its expression is restricted on normal cells (31), making it a promising target for CAR-T therapy in liver cancer. GPC3 CAR-T cells have been shown to exhibit good activation and killing effects on GPC3-positive liver cancer cells but exert almost no specific killing effects on liver cancer cells without GPC3 expression (32). Meanwhile, mucin 1 (MUC1) is a transmembrane protein. Wilkie *et al.* used MUC1 CAR-T cells to target breast cancer cells with MUC1 overexpression (33), and it was confirmed that the growth of xenograft can be significantly inhibited by MUC1-CAR-T cells in breast cancer mouse models.

#### **Promotion of the homing and aggregation of CAR-T cells in tumor tissues**

A recent study found that Runx3 protein can drive T cells from the lymphatic tissue to reaccumulate in the peripheral and tumor tissues (34). The promotion of Runx3 gene expression has been validated to significantly promote the accumulation of T cells in tumor tissues and to inhibit tumor growth in a melanoma mouse model. The homing and aggregation of CAR-T cells in tumor tissues also depend on the recognition and binding of chemokine receptors (CCRs) on the surface of T cells with corresponding chemokines on the surface of tumor cells. The combination of CCRs and corresponding chemokines might facilitate a greater proportion of T cells to reach the solid tumor. A study by Ahmed *et al.* showed that in patients with HER2-positive relapsed and refractory sarcoma, 4 out of the 17 evaluable patients treated with HER2-CAR-T therapy were stable within 12 weeks to 14 months. Three patients underwent tumor resection, with one of them exhibiting more than 90% necrosis. The median overall

survival (OS) for all 19 patients who received infusion was 10.3 months (35).

#### **Dual CAR system**

One of the main problems of CAR-T therapy is the identification of an ideal antigen, because most antigens in tumor cells are also present in normal cells of the human body. As a result, in the process of attacking tumor cells, activated T cells can also inflict damage on normal tissues. To this end, two CARs exist on engineered T cells, and T cell activation depends on the binding of these two CARs to their specific antigens. That is to say, dual tumor antigen binding with CAR-T cells is required to initiate immune response (36). Therefore, the dual CAR-T strategy renders control over the affinity of the immune response and the prevention of immune-related adverse events. Caruso *et al.* attempted to generate CAR-T cells from monoclonal antibodies with different affinities, thereby distinguishing malignant tumors from normal cells based on the different densities of EGFR expression (37). In a study on liver cancer in mice, Chen *et al.* found that dual-targeting CAR-T cells show significantly higher ability in regard to cytokine secretion, proliferation, and anti-apoptosis *in vitro* (38).

#### **Modification of CAR-T cells**

The precise activity and safety of modified T cells is directly determined by the antigen specificity of the CAR structure. However, the heterogeneity of tumor antigens can lead to ineffective immune surveillance in solid tumors, resulting in refractory and recurrent tumors. Therefore, the expressed specific antigen should be able to clearly distinguish tumor tissues from normal tissues. However, all the above requirements can be effectively overcome through the intrinsic or extrinsic modification of CAR-T cells.

CRISPR/Cas9-mediated genome editing techniques are widely used. Nuclear transfection of CRISPR-Cas9 ribonucleoprotein can destroy PD-1 in CAR-T cells, leading to better tumor controlling effects of anti-CAR-T19 cells *in vitro* and in subcutaneous xenograft mouse models, and increasing the viability of CAR-T cells (39). CRISPR/Cas9 technology might also shed novel light on the future application of CAR-T treatment.

#### **CAR-T cell transport**

The effective transport of CAR-T cells from peripheral circulation to the tumor site also determines the effectiveness of CAR-T therapy in solid tumors. We believe that the direct regional delivery of CAR-T cells

into metastatic organs or spaces would definitely enhance their safety and effectiveness. In addition, a combination of regional CAR-T delivery with an immunosuppressive method can further improve the therapeutic performance of CAR-T in solid tumors. In the mouse model of peritoneal metastasis of colorectal cancer established by Katz *et al.*, the direct peritoneal perfusion of CAR-T cells was shown to be more effective in achieving tumor control than systemic infusion in mice with peritoneal metastasis (40). Other local injections, such as hepatic arterial intubation in liver cancer and intracranial injection in glioblastomas (41) can directly deliver CAR-T cells into the tumor tissue, thereby enhancing the direct tumor killing effect of CAR-T cells.

## Tumor recurrence after CAR-T therapy

### *Causes of tumor recurrence after CAR-T therapy*

Although CAR-T therapy has demonstrated adequate efficacy in treating tumors, especially hematological malignancies, recurrence is still a problem. Long-term follow-up data shows that patients can still suffer from tumor recurrence even after complete remission (42). In the study, which investigated CD19CAR-T cells in leukemia treatment, most patients could be relieved after treatment, but some patients relapsed after a period of CD19CAR-T treatment, which was mainly caused by the loss of CD19 antigen in some leukemia cells, making recognition for CD19CAR-T cells impossible, thereby resulting in immune escape. Another underlying mechanism of tumor recurrence is the expression of immunosuppressive molecules, including PD-L1, IDO, and adenosine receptors. The combination of PD-L1 from leukemia cells and PD-1 from CAR-T cells, for instance, decreases the function of CAR-T cells (43).

### *Therapeutic strategies for tumor recurrence after CAR-T therapy*

#### Multi-target therapy

The most common cause of tumor recurrence after CAR-T therapy is the loss of CD19, which leads to the proliferation of target-negative cells, causing ineffective CAR-T therapy. Apart from CD19, common surface markers of B-ALL cells include CD20, CD22, and CD123, and by simultaneously targeting multiple targets, the therapeutic effects of CAR-T therapy can be improved. CD19 and CD123 are designed to target leukemia cells, which offers an effective strategy

for the treatment and prevention of CD19-negative recurrence (44). However, therapy-associated toxicity calls for special attention and further validation. At present, the effectiveness of multi-target therapy in tumor recurrence is controversial. The therapeutic efficacy and the safety of the treatment need to be verified by subsequent clinical trials.

#### Blocking immunosuppressive molecules

The relatively common combination of immunosuppressive molecules PD-L1 and PD-1 can significantly restrict the effectiveness of immunotherapy, thereby impeding the killing function of CAR-T cells *in vivo*. The combined application of anti-PD-1 monoclonal antibody and CAR-T19 is still under exploration. A study concerning relapsed/refractory B-cell lymphoma showed CAR-T19 therapy to be ineffective in some patients, who were subsequently treated with PD-1 inhibitor pembrolizumab. As a result, PD-1-deficient CAR-T19 cells can enhance CAR-T19 cell-mediated tumor killing *in vitro*.

Despite continuous breakthroughs in CAR-T therapy, various problems relating to its underlying technology and clinical application still need to be urgently resolved, including the various side effects represented by CRS and unsatisfactory therapeutic efficacy in solid tumors. The continuous development of cell engineering and site-specific genome editing will strengthen the effectiveness and safety of CAR-T therapy and popularize its clinical application in the treatment of various types of tumor. Although CAR-T cell therapy is still difficult to popularize in clinical applications currently, CAR-T cell therapy is rapidly becoming an effective treatment for many different types of tumors. Through this, hope will be brought to more patients.

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