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Review

Chimeric antigen receptor-T cell therapy for solid tumors require new clinical regimens

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Abstract

Introduction: Chimeric antigen receptor modified T cell (CAR-T) therapy has achieved encouraging breakthroughs in the treatment of hematological malignancies. Nevertheless, this success has not yet been extrapolated to solid tumors. This review focuses on new clinical regimens that could improve the therapeutic efficacy of CAR-T in solid tumors.

Areas covered: Herein, the authors reviewed recent clinical trials using CAR-T therapies for the treatment of solid tumors. Specifically, this review covered the following areas: (1) the current status of CAR-T cells in the treatment of solid tumors; (2) the major factors constraining the efficacy of CAR-T cells in solid tumors; and (3) opinions regarding the future of CAR-T as a treatment for solid tumors.

Expert commentary: While some recent studies have shown promising results, the ultimate success of CAR-T therapies in solid tumor patients will require the following improvements to clinical regimens: (1) local delivery of CAR-T cells; (2) combination of CAR-T cells with chemotherapeutic drugs to treat metastatic tumors; (3) combination of CAR-T with immune checkpoint inhibitors; (4) combination therapy using CAR-T cells targeting two different antigens; and (5) the use of CAR-T as a strategy to prevent tumor recurrence and metastasis after radical resection.

Keywords: chemotherapy; clinical regimen; CAR-T; radical resection; solid tumor

1. Introduction

Chimeric antigen receptor (CAR) is an artificial T cell receptor that simulates the physiological function of the native T cell receptor (TCR). The CAR is constructed by fusing the antigen-binding domain of an antibody with the activation and co-stimulation signaling moiety of T cells. The CAR-modified T cell (CAR-T) can be activated in an MHC-independent manner upon antigen recognition by the CAR [1]. According to the composition of their intracellular signaling domain, the CARs are grouped into three generations. The first generation includes CARs containing a single signaling unit derived from the CD3ζ chain or FcεRIγ. Second-generation CARs contain an additional costimulatory component, such as CD28, 4-1BB or OX40. Third-generation CARs contain a combination of costimulatory components [2].

CAR-T strategies targeting the CD19 antigen have made major breakthroughs in treating patients with advanced B-cell leukemias and lymphomas. A clinical trial of CTL019, an anti-CD19 CAR-T product, showed that complete remission (CR) was achieved in 27 of 30 (90%) patients with acute lymphoblastic leukemia (ALL) [3]. Another anti-CD19 product (KTE-C19) showed a CR rate of 57% (total of 7 patients) in a phase I trial and a CR rate of 47% (total of 51 patients) in a phase II trial for non-Hodgkin lymphoma (NHL) [4,5]. In Jan 2015, the European Commission (EC) designated KTE-C19 (Kite Pharma) as an orphan medicinal product for the treatment of diffuse large B-cell lymphoma (DLBCL). Currently, three anti-CD19 CARs (CTL019, JCAR015 and KTE-C19) have been designated “breakthrough therapies” by the United States Food and Drug Administration. In July 2017, CTL019 was approved by FDA for the treatment of children and young adults (ages 3-25) with relapsed or refractory B-cell ALL. Several CARs against CD20 [6], CD22 [7], etc. have also shown promise in the treatment of hematologic malignancies. With regards to the treatment of hematologic malignancies using CAR-T, malignant cells in the circulatory system are easily reached by CAR-T cells delivered intravenously. The current successes of CAR-T in the treatment of hematologic malignancies should be credited mainly to the existence of lineage-restricted surface antigens, e.g., CD19, as

well as the ease of delivery of CAR-T cells to tumor sites [2].

In contrast to hematologic malignancies, the clinical efficacy of CAR-T cells in solid tumors is less impressive. Previous reviews have mainly focused on the optimization of CAR-T in order to enhance accumulation of the cells in tumor tissues and immune response to cancer cells. Optimization strategies have included arming CAR-T with chemokine or signaling receptors, optimizing costimulatory molecules, and engineering CAR-T to secrete enzymes or cytokines [8]. In this review, we will summarize the state of CAR-T cell therapies with a focus on solid tumors. We will discuss (1) the current status of CAR-T cells in the treatment of solid tumors; (2) the major factors constraining the efficacy of CAR-T cells in solid tumors; and (3) our opinions regarding the future of CAR-T in the treatment of solid tumors.

2. Therapeutic efficacy of CAR-T for solid tumors

The overall status of CAR-T cell therapies for the treatment of solid tumors in clinical trials is shown in Table 1. CAR-T cells targeting α-folate receptor (FRα), carbonic anhydrase IX (CAIX), HER2 and mesothelin did not generate an obvious clinical response in patients with ovarian cancer [9], metastatic renal cell carcinoma [10,11], colon cancer [12] and malignant pleural mesothelioma, respectively [13]. However, in 2007, the results of a clinical trial showed that CAR-T cells targeting L1-CAM had a therapeutic effect in patients with metastatic neuroblastoma [14]. One of 5 patients achieved partial remission (PR). In 2011, CAR-T cells targeting GD2 showed a more promising therapeutic effect in patients with neuroblastoma [15]. In this trial, 3 of 19 patients achieved complete remission (CR), and 2 patients were alive with disease.

In 2015, CAR-T cells targeting human epidermal growth factor receptor 2 (HER2) also had a therapeutic effect in patients with sarcoma [17]. Of 17 evaluable patients, 4 had stable disease for 12 weeks to 14 months. Tumors were resected from 3 of these patients, and 1 tumor showed 90% necrosis. The median overall survival of all 19 infused patients was 10.3 months. In 2016, Junghans *et al*. reported a clinical trial

using CAR-T cells targeting prostate-specific membrane antigen (PSMA) to treat patients with advanced prostate cancer. Of 5 patients received PSMA-specific CAR-T therapy, 2 patients achieved PR, one patient achieved minor response [21]. In the completed phase I/II clinical trial (NCT01218867), 24 patients received VEGFR2-specific CAR-T therapy. Of these, 1 patient achieved PR, 1 patient achieved SD, 22 patients with PD. In 2017, a 52-year-old female patient with advanced cholangiocarcinoma was treated with a CAR-T cocktail immunotherapy composed of successive infusions of CAR-T cells targeting epidermal growth factor receptor (EGFR) and CD133 [23]. The patient finally achieved an 8.5-month PR with the EGFR-CAR-T therapy and a 4.5-month PR with the CD133-CAR-T treatment.

CAR-T cells were infused through intravenous (i.v.) injection in all clinical trials described above. In recent years, some scientists have attempted to treat patients with solid tumors through local delivery of CAR-T cells. In 2015, Brown *et al*. used locally delivered CAR-T cells to treat patients with solid tumors for the first time [18]; 3 patients with recurrent glioblastoma (GBM) were treated with CAR-T cells targeting IL13Ralpha2. Patients received intracranial delivery of the CAR-T cells into their resection cavity. Transient anti-glioma responses were observed in 2 of the 3 patients [18]. In 2016, Brown *et al*. reported another exciting result: a patient with recurrent multifocal glioblastoma who received multiple infusions of CAR-T cells targeting IL13Ralpha2 into the resection cavity achieved CR [20]. The therapeutic effects of locally infused CAR-T cells targeting carcinoembryonic antigen (CEA) [19], mucin 1 (MUC1) [22] and tumor-associated glycoprotein (TAG)-72 [24] were tested in patients with liver metastases from adenocarcinoma, metastatic seminal vesicle cancer and metastatic colorectal cancer, respectively. Although antitumor immune responses were observed in some patients, an overall clinical response was not obvious (Table 1).

3. Main reasons for the limited therapeutic efficacy of CAR-T cells in solid tumors

Although the therapeutic efficacy of CAR-T cells was promising in some patients with neuroblastoma, glioblastoma and prostate cancer, current clinical trials demonstrate that the overall therapeutic efficacy of CAR-T cells is limited in patients with solid tumors (Table 1). The difference in the clinical responses between patients with solid tumors and those with hematological tumors, especially leukemia, is very obvious [1]. There are at least three reasons for this great difference. The first reason is that the dense tumor extracellular matrix is a significant obstacle in the homing of CAR-T cells to the interior of solid tumors. The second reason is that the functions of CAR-T cells (infiltration, expansion, survival, etc.) are suppressed by the extremely hostile microenvironment. Normally, the microenvironment is composed of immunosuppressive cells, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and macrophages, and immunosuppressive molecules, including TGF-β and PD-L1. The third reason is that the cancer cells and the target antigens present on solid tumors are generally heterogeneous [30]. How can we improve the therapeutic efficacy of CAR-T cells for the treatment of solid tumors? In the following sections, we will discuss future strategies to augment the effects of CAR-T therapies for the treatment of solid tumors.

4. Expert commentary & five-year view

4.1 Local delivery of CAR-T cells

Zuccolotto *et al*. developed PSMA-specific CAR-engineered T cells and investigated their therapeutic efficacy in a mouse model of prostate cancer [31]. Their results showed that the PSMA-specific CAR-T cells did not display therapeutic activity when they were administered systemically to mice bearing s.c. tumors. In a previous report using TF (tissue factor)-specific CAR-T for lung cancer therapy in a mouse model, we showed that the growth of s.c. xenografts were inhibited significantly by CAR-T cells

delivered by intratumoral injection but not by i.v. injection [32]. In a phase I clinical trial of α-folate receptor (FR)-specific CAR-T cells against ovarian cancer carried out by Kershaw *et al*., no reduction in tumor burden was seen in 14 patients treated with CAR-T cells [9]. Additionally, the CAR-T cells did not specifically localize to tumor sites. Therefore, the poor therapeutic efficacy of systemically administered CAR-T cells is likely due to the poor capacity of infused CAR-T cells to reach the tumor site.

It is difficult for CAR-T cells infused via i.v. injection to overcome the immunosuppressive microenvironment in order to home to the interior of a solid tumor [1]. In addition, CAR-T cells can disperse throughout the body through the circulatory system, which may cause off-target toxicity due to the expression of target antigens in normal tissue [33]. These are two main obstacles facing the use of CAR-T cells in solid tumor therapy. Based on the results of the clinical trials described above (Table 1), local delivery of CAR-T cells may be promising. Local delivery can simultaneously enhance the penetrance of CAR-T cells into tumors and, to a certain extent, avoid off-target effects of CAR-T cells on normal tissue.

For cancers presenting near the surface of the body (such as melanoma or head and neck cancers), CAR-T cells can be injected directly into the tumor. For patients receiving surgical treatment, CAR-T cells can be infused into the cavity left by the resected tumor [18,20]. For patients with large tumor lesions, CAR-T cells can be delivered intratumorally through interventional treatment [22]. For patients with liver metastases, CAR-T cells can be delivered through hepatic artery infusions [22,24]. Based on current clinical results, local delivery is a promising strategy for the delivery of CAR-T therapies to solid tumors. However, its therapeutic efficacy needs to be further studied in larger clinical trials.

However, local delivery requires more complicated equipment and operational processes. This may increase the risk of immediate or acute device-related adverse events, including occlusion, malfunction, or infection. To achieve the best therapeutic efficacy, it is imperative that the CAR-T cells are able to traffic to distant sites of

infiltrative and/or multifocal disease. It is not yet evident whether local delivery strategies will be able to target infiltrative and/or multifocal disease. This needs to be further investigated.

4.2 Combination of CAR-T cells with chemotherapeutic drugs to treat metastatic tumors

Immunosuppressive factors in the tumor microenvironment, including immunosuppressive cells and cytokines, significantly impede the efficacy of immunotherapeutic approaches. The mechanisms by which these factors suppress the immune system have been well defined in previous reviews [34-36]. Elimination or inhibition of these immunosuppressive factors will significantly promote an antitumor immune response and enhance the response to CAR-T therapy. Treatment with chemotherapeutic agents may be a promising strategy to remodel the immunosuppressive tumor microenvironment and facilitate CAR-T therapy. First, extensive evidence has demonstrated that some chemotherapeutic agents, such as doxorubicin, sunitinib, sorafenib and gemcitabine, can eliminate or inhibit immunosuppressive factors and promote an antitumor immune response. Second, treatment with approved chemotherapeutic agents is convenient for clinical application. The immune modulating effects of these drugs will be discussed in the following sections.

4.2.1 Doxorubicin

Doxorubicin is an antineoplastic drug broadly used for the treatment of hematological malignancies, soft tissue sarcomas, and several other types of carcinomas [37]. This drug induces an "immunogenic type" of tumor cell death leading to the stimulation of dendritic cell antigen-presenting function [38]. Doxorubicin administration has also been reported to eliminate MDSCs by promoting cleavage of caspase-3, triggering an

apoptotic response. In addition, doxorubicin impedes the suppressive activity of residual MDSCs by impairing both the production of reactive oxygen species (ROS) and the expression of arginase-1 and indoleamine 2,3-dioxygenase (IDO) by residual MDSCs. By impairing the immune suppressive function of MDSCs, doxorubicin enhances the proliferation and infiltration of NK cells and tumor-specific $CD4^+$ and $CD8^+$ T cells [39,40]. It also increases the permeability of tumor cells to granzyme B produced by cytotoxic T lymphocytes [41]. The combination of doxorubicin and T lymphocytes has been shown to enhance the therapeutic efficacy of adoptive T cell transfer in a mouse model [40,42].

4.2.2 Sunitinib

Sunitinib is a receptor tyrosine kinase inhibitor that is currently being used with significant clinical effect in the treatment of metastatic renal cell carcinoma (mRCC). Sunitinib inhibits signaling through the vascular endothelial growth factor receptors (VEGFRs) as well as through platelet-derived growth factor receptor, stem cell factor receptor (c-kit), Flt3, and colony-stimulating factor (CSF)-1 receptor [43]. The c-kit ligand is required for MDSC accumulation and Treg development [44]. Sunitinib could reverse MDSC-mediated immune suppression and modulate the tumor microenvironment by (1) reducing the quantity and function of MDSCs and Tregs [45,46]; (2) decreasing the expression of the negative costimulatory molecules CTLA4 and PD-1 in both CD4 and CD8 T cells and PD-L1 in MDSCs and plasmacytoid dendritic cells [44]; (3) reprogramming tumor-associated macrophages toward classically activated or "M1" polarization [47]; and (4) increasing the type-1 T cell immune response [45,46]. These findings provide a rationale for combining sunitinib with immunotherapy for the treatment of solid tumors.

Combined treatment with sunitinib and an agonistic antibody against glucocorticoid-induced TNFR related protein (GITR) elicited a remarkably synergistic antitumor response in a model of mRCC [47]. Sunitinib was also shown to

enhance the efficacy of vaccines [48-50], agonistic CD40-antibody [51] and ALT-803 (IL-15/IL-15 receptor alpha complex) [52] therapy in mouse models of advanced melanoma and cervical cancer, respectively. Strategies combining sunitinib and immunotherapy were also tested in clinical trials. In a phase II clinical trial [53], 23 patients with mRCC were treated with sunitinib combined with rIL-21. Of these, 7 reached PR (30%) and 14 reached SD (61%). In a pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with sunitinib for mRCC, 1 of 8 patients reached CR, 1 patient reached PR, and 3 patients reached SD [54]. These studies indicated that sunitinib could synergistically enhance the therapeutic efficacy of immune-based therapies for some solid tumors.

4.2.3 Sorafenib

Sorafenib is another multikinase inhibitor that targets the Raf/MEK/ERK pathway as well as receptor tyrosine kinases, including VEGFR-2 and -3, PDGFR-β, Flt-3, and c-kit [55,56]. In December 2005, sorafenib was approved by the FDA for the treatment of patients with mRCC. Busse *et al*. reported that the frequency of Treg cells in peripheral blood was significantly decreased in sorafenib-treated patients with mRCC [57]. Sorafenib has differential impacts on subsets of T cells: it selectively increases the activation of effector T cells while blocking Treg function in patients with hepatocellular carcinoma (HCC) [58] and in a mouse model of HCC [59]. A study where sorafenib was combined with adoptive T cell therapy for the treatment of an E.G7/OT-1 mouse model showed that sorafenib can enhance the therapeutic efficacy of adoptive T cell therapy by improving the tumor microenvironment [60]. Although associated mechanisms need to be further investigated, these results indicate that sorafenib represents a potential targeted agent that is suitable in combination with immunotherapeutic approaches to treat cancer patients.

4.2.4 Gemcitabine

Gemcitabine is a chemotherapeutic used to treat a number of types of cancer, including lung cancer, mesothelioma, and pancreatic cancer [61-63]. It is a nucleoside analog and works by blocking the creation of new DNA, resulting in cell death [64]. Several previous reports demonstrated that gemcitabine was able to dramatically and specifically reduce the number of immunosuppressive cells, including MDSCs and Tregs, by inducing apoptosis in these cells. Additionally, gemcitabine was able to increase the antitumor activity of $CD8^+$ T cells and NK cells in mice bearing large tumors [61,63,65]. Furthermore, combining gemcitabine with immunotherapy, IFN-β or WT1-specific T cells markedly enhanced treatment efficacy in a mouse model [61,62].

In addition to the chemotherapeutic drugs described above, 5-fluorouracil (5-FU) [66], docetaxel [67], cabozantinib [32,68,69], dacarbazine, temozolomide and cisplatin [70] have also been studied for their ability to improve the tumor immune microenvironment and enhance the response to immunotherapy. In conclusion, based on the cytotoxic activity and immune modulating functions of these chemotherapeutic drugs, treatments combining these drugs with CAR-T cells is a promising strategy for the treatment of solid tumors.

When considering the combined application of CAR-T cells and chemotherapeutic agents, three important problems should be addressed. First, the mechanism of action of the drug should be clarified, including the signaling pathway by which the drug carries out its immune-modulating functions. Some drugs inhibit the activity of immunosuppressive cells while also inhibiting the function of immune effector cells. However, other drugs inhibit the activity of immunosuppressive cells while promoting the function of immune effector cells. Second, the dose at which the drug has a positive effect on immune regulation should be confirmed. Third, the timing of treatment with each therapy should be optimized. In general, combination therapy schedules should be designed based on the mechanisms of the drugs. Considering the potential negative effects of these drugs on CAR-T cells and the results of recent preclinical studies, it seems that the optimal regimen would be one where

chemotherapy is followed by CAR-T treatment at an interval of one to two days [40,60].

4.3 Combination of CAR-T with immune checkpoint inhibitors

Accumulating evidence demonstrates that tumors can escape immune surveillance by stimulating immune inhibitory receptors on T cells, including T cell immunoglobulin and mucin domain-3 (TIM-3), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and programmed death-1 (PD-1) [71]. The majority of solid tumors often up-regulate immune checkpoint ligands, leading to the inhibition of CAR-T cells through the stimulation of immune inhibitory receptors [72]. Antibodies that block CTLA-4 (ipilimumab, tremelimumab), PD-1 (nivolumab, pembrolizumab, pidilizumab) and PD-L1 (MDX-1105, MPDL3280A) have recently been approved by the FDA for use in certain solid tumors [73].

Preclinical studies by John, *et al*. and Liu, *et al*. have demonstrated that inhibition of CAR-T and PD-1 is highly synergistic, leading to long-term survival without any signs of pathology in mouse models [74,75]. Clinical trials have further evaluated the efficacy of combined CAR-T and PD-1 inhibitor therapies. Heczey *et al*. revealed that a PD-1 checkpoint inhibitor could augment CAR-T cell efficacy and persistence in patients with neuroblastoma [27]. Therefore, combination treatment with immune checkpoint inhibitors can improve the therapeutic efficacy of CAR-T cells for solid tumors.

To study combined therapies, a self-antigen mouse model was used to evaluate the therapeutic activity of CAR-Ts combined with an anti-PD-1 antibody in Her-2^{+} tumors. CAR-T cells were infused on days 7 and 8 after tumor implantation, and the PD-1 blocking antibody was injected on days 7, 11, and 15 [76]. In a separate study evaluating the combined treatment of anti-hPSMA CAR-T cells and anti-PD-1 antibody on a mouse model of prostate cancer, the anti-PD-1 antibody was

administered to mice 3 hours before the i.v. infusion of CAR-T cells and every other day thereafter (between days 10 and 20 of tumor growth) [77]. In a clinical study evaluating the efficacy of CAR-T cells in combination with a PD-1 inhibitor in $GD2^+$ neuroblastoma, the PD-1 inhibitor (pembrolizumab) was given 1 day before and 21 days after infusion of CAR-T cells [27]. Therefore, based on results of preclinical and clinical studies as well as the mechanisms of immune checkpoint inhibitors, it is reasonable that immune checkpoint inhibitors should be administered slightly before or during CAR-T cell treatment.

4.4 Combination therapy with CAR-T cells targeting two antigens

Antigenic heterogeneity is a main limitation for the therapeutic efficacy of CAR-T in the treatment of cancers, including solid tumors. Cancer cells escape immune recognition by employing a number of antigen-evasion strategies, including antigen mutation and downregulation or deletion of target antigens [78]. Infusion of CAR-T cells targeting a single tumor-associated antigen may lead to target antigen modulation under this selective pressure, with subsequent tumor immune escape. This could explain why targeting a single antigen using CAR-T cells allows an initial robust antitumor response, followed by a relapse due to the outgrowth of antigen-null tumor cells [79]. This phenomenon has been reported as a cause of failure in both preclinical and clinical studies using adoptively transferred CAR-T cells to treat heterogeneous tumors [80]. The probability of immune escape by spontaneous mutation and selective expansion of antigen-null tumor cells decreases with each additional antigen that can be recognized by the CAR-T cells. Therefore, a potential prophylaxis against immune escape is to generate CAR-T cells capable of recognizing multiple antigens. Currently, combinational targeting of two tumor-associated antigens is an important strategy aiming to offset the immune escape of heterogeneous cancer cells.

Three multiple receptor configurations have been adopted to achieve bispecific signal

computation: (1) combination therapy with two CAR-T cell lines, each targeting a different antigen [81]; (2) co-expression of two different CARs in one T cell [80]; and (3) engineering dual-antigen recognition capability into a single CAR molecule (TanCAR) [78,79,82]. Anurathapan, *et al*. studied the impact of co-administration of CAR-T cells targeting two distinct antigens (MUC 1 and PSCA) in a mouse pancreatic tumor model. The combination therapy showed superior antitumor effects compared with single-antigen CAR-T monotherapy [81]. In one of our clinical trials (clinicaltrials.gov, NCT02903810), patients with lymphoma received sequential infusions of CAR-T cells targeting CD19 and CD22 (data not published). Combination therapy with two CAR-T cell lines has the following advantages. First, expressing a single CAR in one T cell can guarantee transduction efficiency, expression efficiency and antitumor activity of each CAR as well as the proliferation efficiency of CAR-T cells. Second, the combination of two CAR-T cells using existing CARs is convenient, efficient and inexpensive.

Hegde *et al*. developed biCAR T cells coexpressing HER2.CD28ζ and IL-13Rα2.CD28ζ and targeting two glioma-restricted antigens, HER2 and IL-13Rα2 for the treatment of mice with glioma xenografts [80]. biCAR T cells were generated by tandem retroviral transduction in order to express the two CARs in a single T cell. Near-complete tumor cell targeting can be achieved using the bispecific combinational approach. Furthermore, treatment with the biCAR T cells could offset antigen escape and achieve better tumor control, conferring a survival advantage to the treated animals. A potential disadvantage of the tandem retroviral transductions needed to generate biCAR T cells is that two retroviral transductions could possibly compromise the proliferation potential and antitumor activity these cells. In addition, this transduction strategy may also result in different transduction efficiencies of the two CARs, further compromising the antitumor activity.

In 2013, Grada, *et al*. constructed a novel bispecific chimeric antigen receptor by engineering dual-antigen recognition capability into a single CAR molecule, named TanCAR [82]. For the first TanCAR, the anti-CD19 scFv was linked to anti-HER2

scFv by a 3× G4S linker and then sequentially linked to a short hinge, the CD28 transmembrane and signaling domains, and the signaling domain of the CD3ζ-chain. In follow-up studies, Zah *et al*. and Schneider *et al*. developed TanCARs targeting both CD19 and CD20 and used these TanCAR T cells to treat advanced B-cell malignancies in mice [78,79]. The TanCAR T cells could effectively prevent antigen escape and showed good therapeutic efficacy in mouse models. Compared with the first two strategies, the strategy of engineering dual-antigen recognition capability into a single CAR molecule significantly reduces the costs of CAR-T cell production. However, based on current study results, the design of the TanCAR molecule is challenging and does not simply involve linking two scFv to each other. The design must be based on the configuration of the two antigens and their scFvs. Otherwise, the TanCARs cannot exert their antitumor effects [82].

4.5 CAR-T as a strategy for preventing tumor recurrence and metastasis after radical resection

Recurrence and metastasis after radical resection are the main reasons why some tumors cannot be cured [83]. Tumor metastasis is a very complex process, including the dissociation of tumor cells from the primary locus, invasion of the surrounding tissue, entrance into and extravasation from the circulation, and growth in distant organs. One of the important steps in tumor metastasis is where cancer cells reach the distant organs through the blood [84]. It is well known that one of the main reasons that CAR-Ts have achieved success in the treatment of hematologic malignancies is that cancer cells are easily recognized by CAR-T cells infused into the circulation. Our previous study showed that TF-specific CAR-T cells could significantly suppress metastasis of TF-positive cancer cells in a pulmonary metastasis mouse model established by i.v. injection [32]. Therefore, if CAR-T cells were intravenously infused into patients either before or after radical tumor resection, the metastatic cancer cells could be killed by circulating CAR-T cells during the metastatic process.

In conclusion, although current clinical responses to CAR-T cells in solid tumors were less than impressive, CAR-Ts remain a promising treatment strategy for solid tumors. Further improvement of the therapeutic efficacy of CAR-T therapy for solid tumors requires new clinical regimens, including new delivery strategies, combination chemotherapy approaches, immune checkpoint inhibitors and radical resection. These new clinical regimens or therapeutic strategies need to be further verified in both the laboratory and the clinic.

Key issues

- Current results of strategies using CAR-T cells to treat solid tumors are not very satisfactory. New clinical regimens may be one of the strategies can be used to improve the therapeutic efficacy of CAR-T cells for solid tumors.
- The poor homing ability of CAR-T cells to the interior of solid tumor is a main obstacle in solid tumor treatment. The most direct way to solve this problem is delivering CAR-T cells intratumorally or locally.
- Some chemotherapeutic drugs can modulate the tumor microenvironment and promotes antitumor immunity. It is a promising strategy that combining these drugs with CAR-T cells to treat solid tumors.
- Combining with immune checkpoint inhibitor may be another strategy to improve the therapeutic efficacy of CAR-T cells for solid tumors.
- Antigenic heterogeneity is a main limitation for the therapeutic efficacy of CAR-T in the treatment of cancers, including solid tumors. Combinational targeting of two tumor-associated antigens is an important strategy aiming to offset the immune escape of heterogeneous cancer cells.
- It is one of the important steps of tumor metastasis that cancer cells move to distant organs through the blood. CAR-T cells may be an effective strategy to prevent tumor recurrence and metastasis after radical resection.

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Declaration of Interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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Reference annotations

* Of interest

** Of considerable interest

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Table **1** Clinical performance of CAR-T cells for solid tumors.

Publication Year	Antigen	CAR Generation	Co-stimulatory Domains	Cancer	Delivery Route	Patient No.	Clinical Response	Reference
2006	FRα	First	no	Ovarian cancer	i.v.	14	14 PD	[9]
2007	L1-CAM	First	no	Metastatic neuroblastoma	i.v.	6	1 PR, 5 PD	[14]
2010	HER2	Third	CD28, 4-1BB	Colon cancer	i.v.	1	Died of CAR-T related toxicity	[12]
2011	GD2	First	no	Neuroblastoma	i.v.	19	3 CR, 7 NED, 5 PD, 1 PR, 1 SD, 2 tumor necrosis	[15,16]
2013	mesothelin	Second	4-1BB	Malignant pleural mesothelioma	i.v.	3	1 DPD, 2 NR	[13]
2015	HER2	Second	CD-28	Sarcoma	i.v.	19	4 SD, 13 PD	[17]
2016	CAIX	First	no	Metastatic renal cell carcinoma	i.v.	12	No clinical response	[11]
2015	IL13Ralpha2	First	no	Recurrent glioblastoma	Local delivery	3	transient antiglioma responses	[18]
2015	CEA	Second	CD-28	Adenocarcinoma liver metastases	Local delivery	6	1 SD, 5 DPD	[19]
2016	IL13Ralpha2	Second	4-1BB	Recurrent glioblastoma	Local delivery	1	CR	[20]
2016	PSMA	First	no	Prostate cancer	i.v.	5	2 PR, 1 minor response	[21]
2016	MUC1	Third	CD28, 4-1BB	Metastatic seminal vesicle cancer	Local delivery	1	Positive cytokine response, tumor necrosis	[22]
2016	VEGFR2	Undisclosed	Undisclosed	Metastatic melanoma and renal cancer	i.v.	24	1 PR, 1 SD, 22 PD	NCT01218867
2017	EGFR & CD133	Second	4-1BB	Cholangiocarcinoma	i.v.	1	PR for 13 months	[23]
2017	TAG-72	First	no	Metastatic colorectal cancer	i.v. & Local delivery	16	NOR	[24]
2017	HER2	Second	CD28	Progressive Glioblastoma	i.v.	17	1 PR, 7 SD, 8 PD	[25]
2017	HER2	Second	4-1BB	Advanced biliary tract cancer, pancreatic cancer	i.v.	11	1 PR, 5 SD, 5 PD	[26]
2017	GD2	First	no	Neuroblastoma	i.v.	11	2 CR, 3 AWD, 5 DOD	[27]
2017	CEA	Second	CD28	Metastatic Colorectal Cancer	i.v.	10	8 PD, 2 SD	[28]

2017	CEACAM5	First	no	Metastatic CEACAM5+ cancers, including Colon, Stomach, Rectum, Pancreas, Caecum, Oesophagus, Gastro-oesophageal junction and Pseudomyxoma peritonei cancers.	i.v.	14	7 SD, 7PD	[29]

CR, complete remission; PR, partial remission; PD, progressive disease; NED, no evidence of disease; SD, stable disease; NOR, no objective response; DPD, died of progressive disease; AWD, alive with disease.