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REVIEW

Adoptive cell transfer therapy for hepatocellular carcinoma

Renyu Zhang*, Zhao Zhang*, Zekun Liu, Ding Wei, Xiaodong Wu, Huijie Bian (✉), Zhinan Chen (✉)

Department of Cell Biology, National Translational Science Center for Molecular Medicine, State Key Laboratory of Cancer Biology, Fourth Military Medical University, Xi'an 710032, China

Abstract **Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. This malignancy is associated with poor prognosis and high mortality. Novel approaches for prolonging the overall survival of patients with advanced HCC are urgently needed. The antitumor activities of adoptive cell transfer therapy (ACT), such as strategies based on tumor-infiltrating lymphocytes and cytokine-induced killer cells, are more effective than those of traditional strategies. Currently, chimeric antigen receptor T-cell (CAR-T) immunotherapy has achieved numerous breakthroughs in the treatment of hematological malignancies, including relapsed or refractory lymphoblastic leukemia and refractory large B-cell lymphoma. Nevertheless, this approach only provides a modest benefit in the treatment of solid tumors. The clinical results of CAR-T immunotherapy for HCC that could be obtained at present are limited. Some published studies have demonstrated that CAR-T could inhibit tumor growth and cause severe side effects. In this review, we summarized the current application of ACT, the challenges encountered by CAR-T technology in HCC treatment, and some possible strategies for the future direction of immunotherapeutic research.**

Keywords adoptive cell transfer therapy; hepatocellular carcinoma; T cell; chimeric antigen receptor; immunotherapy

Hepatocellular carcinoma is a major health challenge

In recent years, cancer has become the leading cause of death and a major public health problem worldwide as a result of its rising morbidity and mortality rates. The latest cancer statistics show that 1 735 350 newly diagnosed cancer cases and 609 640 cancer deaths will occur in the United States in 2018 [1]. The number of newly diagnosed cancer cases and cancer-related deaths in China will be several times higher than that in the United States given the former's massive and aging population. Lung cancer, liver cancer, and gastric cancer are the leading causes of cancer death in China [2].

Liver cancer is characterized by high malignancy degree and mortality rates, rapid progression, recurrence, and metastasis; hepatocellular carcinoma (HCC) accounts for the majority of liver cancer cases [3]. Currently, surgery is the most effective method for the treatment of HCC [4,5]. The recurrence rate of HCC remains high after tumor resection, and the five-year recurrence rate of patients with HCC is as high as 70% [6]. Moreover, only 20% to 30% of patients with HCC have the opportunity to adopt surgical treatment because most cases of this malignancy are accompanied by liver cirrhosis or are diagnosed at an advanced stage [7,8]. Conventional treatment regimens (e.g., chemotherapy, embolization, and radiotherapy) usually fail to considerably prolong the median overall survival of patients with advanced HCC [9]. The Food and Drug Administration (FDA) has approved several drugs that target HCC. These drugs, which are currently applied in clinical practice, can only exert a weak therapeutic effect. For example, sorafenib could only prolong the overall survival of patients with HCC for 2 months to 3 months and has a nonoptimistic clinical effect [10]. Lenvatinib is another targeted drug used as the first-line treatment for advanced HCC. Although phase III clinical trial data have shown that lenvatinib can prolong the overall survival of patients, it only increased overall survival by 1.3 months relative to that in the sorafenib group [11]. Regorafenib, a second-line drug for the treatment of advanced HCC, has been approved by the FDA for patients with HCC progressing to sorafenib treatment. The results of clinical trials showed that the median overall survival of patients with HCC in the regorafenib group was extended from 7.8 months to 10.6

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Correspondence: Huijie Bian, hjbian@fmmu.edu.cn;
Zhinan Chen, znchen@fmmu.edu.cn

*These authors contributed equally to this work.

months relative to that in the placebo group [12]. Cabozantinib, a multitargeted small molecule inhibitor, and ramucirumab, an antagonistic anti-VEGFR2 monoclonal antibody, are second-line drugs applied for the treatment of advanced HCC and have been shown to improve the prognosis of patients [13]. Our team has developed [^{131}I]-labeled metuximab, a radioimmunoconjugate that is combined with radiofrequency ablation for the treatment of HCC; our treatment method extended the median time to overall tumor recurrence by 7 months [14]. However, the median overall survival of patients with advanced HCC is still less than 1 year after treatment with current methods. Thus, new treatment strategies are urgently needed.

The liver is the largest solid organ in the human body. It can accept the dual blood supply of the portal vein and the hepatic artery. In contrast to classical lymphoid organs, such as the thymus and spleen, the liver consists of cells with nonimmunological primary functions but that still play a key immune role. In addition to the vast majority of parenchymal cells, the liver includes nonparenchymal cells, such as liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and lymphocytes. The lymphocyte population localizes not only in the portal tract but is also distributed throughout the parenchyma. T cells, B cells, natural killer (NK) cells, and natural killer T (NKT) cells collectively constitute the lymphoid repertoire; the composition of the lymphoid repertoire enables the liver to participate in the immune regulation of the body [15]. Moreover, after the blood from the portal vein and the hepatic artery is transferred into the hepatic sinus, the deceleration of the blood flow rate enables antigens from the gut and systemic immunity to come into contact with the immune component in the liver to effectively regulate the immune response [16]. The dysregulation of the tightly regulated immune networks in the liver often results in the development of liver diseases, including chronic infections, autoimmune diseases, and tumors. Therefore, immunological methods for the treatment of HCC may provide improved therapeutic results. For example, the response rates of patients with advanced HCC to sorafenib and regofenib treatment were only 2%–3% and 7%, respectively, whereas the objective response rates of patients with advanced HCC treated with nivolumab, a monoclonal antibody that disrupts programmed cell death protein-1 (PD-1) immune checkpoint signaling, were 15%–20% [17]. Clinical trial results indicate that other antibody drugs, such as pembrolizumab and durvalumab, have acceptable safety and clinical efficacy in the treatment of patients with HCC [13,18]. Although the clinical efficacy of immunological methods for the treatment of HCC is currently unsatisfactory, the immunotherapy strategy is one of the inevitable choices for the future treatment of HCC.

Adoptive cell transfer therapy shows advantages in cancer therapy

Cancer immunotherapy was considered as the first major breakthrough in 2013 by the *Science* magazine. Adoptive cell transfer therapy (ACT) is the most representative cancer immunotherapy method. In 1985, Rosenberg *et al.* initially demonstrated that the transfusion of autologous lymphokine-activated killer cells (LAK) could mediate objective cancer regression [19]. Three years later, another type of ACT based on tumor infiltrating lymphocytes (TILs) induced distinct tumor responses in patients with metastatic melanoma [20]. The application of a lymphodepletion regimen, such as nonmyeloablative cyclophosphamide and fludarabine, prior to TIL reinfusion greatly improved the efficiency of antitumor therapy [21,22]. The advent of ACT therapies that are based on cytokine-induced killer (CIK) cells and chimeric antigen receptor T cells (CAR-Ts) could prevent cancer recurrence and progression. Clinical trials on the applicability of TIL and CIK for the treatment of HCC are summarized in Table 1 [23–30].

ACT shows unique advantages over traditional treatment strategies. The basic principle of ACT is to improve the quantity and quality of cells that restrain the growth and survival of tumor cells to break the immune tolerance of tumor cells. Lymphocytes, the major effective cells of ACT, are extracted from patients, modified or amplified *in vitro*, and then transferred to patients [31]. In contrast to antibodies or other targeted drugs, ACT is a living treatment that can be activated and proliferate *in vivo* with intensely durable antitumor effects [21]. ACT is considered as a highly individualized cancer therapy given that most effector cells are harvested autogenously from patients. Notably, because expanded or gene-modified effective cells can recognize and target tumor antigens, ACT is more specific than chemotherapy [32]. Circulating tumor cells and small residual lesions after operation can be effectively targeted and removed through ACT *in vivo*. Hence, ACT is not limited by clinical stage.

Application of TILs in HCC treatment

The TIL population represents an important cluster of antitumor effector cells that can be isolated from surgical tumor specimens. Given that T-cell receptors (TCRs) that are expressed on T cells that were isolated from TILs recognize diverse antigens, the antitumor effects of TILs are more intense than those of therapies targeting single antigens or mutations. Many previous studies have shown that TILs in HCC are rare but may have considerable implications for tumor recurrence and patient prognosis [33]. Patients with HCC and prominent lymphocyte infiltration who underwent surgical resection demonstrated

Table 1 Summary of clinical trials on the applicability of TIL and CIK as HCC treatment strategies

Cells used for ACT	Patients (*n*) total	Patients (*n*) treated with ACT	Overall survival (ACT vs. Control or ACT alone)	Recurrence rate (ACT vs. Control or ACT alone)	Study reference
Tumor-infiltrating lymphocytes	15	15	100% (14 months)	20% (14 months)	[23]
Tumor-infiltrating lymphocytes	150	76	68% vs. 62% (5 years)	59% vs. 77% (4.4 years)	[24]
Cytokine-induced killer cells	132	66	42.4% vs. 24.2% (3 years)	–	[25]
Cytokine-induced killer cells	127	41/43	37.9%/38.1% vs. 36.9% (5 years)	73%/72% vs. 76% (5 years)	[26]
Cytokine-induced killer cells	230	115	–	39.5% vs. 49.1% (4.4 years)	[27]
Cytokine-induced killer cells	85	45	100% vs. 100% (18 months)	15.6% vs. 40.0% (18 months)	[28]
Cytokine-induced killer cells	83	42	–	7.14% vs. 23.1% (1 year)	[29]
Cytokine-induced killer cells	146	72	62.4% vs. 18.8% (2 years)	–	[30]

ACT, adoptive cell transfer therapy.

reduced recurrence rates by 38.6% and increased five-year survival rates by 34.9% relative to those without substantial lymphocyte infiltration [34]. In a single randomized clinical trial, 150 patients with HCC showed improved recurrence-free survival after hepatic resection following adoptive immunotherapy using autologous lymphocytes activated with IL-2 *in vitro* [24]. However, TILs are difficult to isolate from the tumor tissues of patients with HCC and to amplify *in vitro*. In addition, only a few patients with HCC can tolerate lymphocyte deletion, which is essential for distinct antitumor effect, before TIL infusion [35].

Application of CIK in HCC

CIK cells are heterogeneous immune cell populations that are generated *ex vivo* through the expansion of PBMCs. The sequential addition of recombinant human IFNγ, anti-CD3 monoclonal antibody, and recombinant human IL2 is essential for the acquisition of CIK cells through cell culture. After 14–21 days of incubation, CIK cells can be harvested and transfused into the patient to exert antitumor functions. Supplemented cytokines and antibodies not only improve the ability of PBMC to activate and proliferate *in vitro* but also increase the cytolytic activity of CIK cells against tumor cells. CIK cells consist of NKT cells ($CD3^+CD56^+$), cytotoxic T cells ($CD3^+CD56^-$), and NK cells ($CD3^-CD56^+$), which are capable of recognizing and killing tumor cells directly [36]. Furthermore, activated cells exert a cytolytic effect against a variety of tumor cells independently of TCR-MHC class I interaction [37]. A large retrospective clinical study of 410 patients with HCC showed that the overall survival rates of the CIK adjuvant treatment group were higher than that of the surgery-alone group and revealed that patients with large lesions in the CIK group displayed drastically increased overall survival rates than those in the surgery-alone group [38].

Schmeel *et al.* summarized clinical results from 24 phase I and 21 phase II clinical trials of CIK treatment for different cancer diseases. Their results indicated that the overall survival of patients with HCC who underwent CIK cell treatment was prolonged by more than 40% compared with that of the control group [39]. However, a perfect prognostic system and additional guidance on large-scale clinical trials are still needed to be established to help clinicians determine whether patients with HCC can benefit from adjuvant CIK cell immunotherapy [40].

CAR-T immunotherapy, a special kind of ACT

CAR-T immunotherapy is a special type of ACT that utilizes gene engineering technology to modify T cells to perform antitumor functions [41]. The design and integration of a chimeric antigen receptor (CAR) into T cells to form CAR-T cells throughout the entirety of the immunotherapy process is the most critical technology of this treatment modality. At present, the most widely used CAR structure consists of a single-chain antibody extracellular domain that recognizes and binds specific antigens, an extracellular hinge region, a transmembrane region, and an intracellular domain that provides proliferation and activation signals. In contrast to the TCR structure on

traditional T cells, this CAR structure is independent of major histocompatibility complex (MHC) antigen presentation, avoids the restriction of MHC molecules, and solves the immune escape problem of tumors attributed to the downregulation of MHC molecule expression [42]. In addition to some universal CAR-T treatments at the research stage, the most widely used CAR-T immunotherapy is a personalized cell therapy method, and each patient must complete the reinfusion treatment with their own immune cells that have been engineered *in vitro*. Typical CAR-T immunotherapy is mainly divided into the following five steps: the separation of peripheral blood mononuclear cells from patients, the integration of CARs into T cells, the cultivation and extensive expansion of CAR-T cells *in vitro*, the transfusion of CAR-T cells, and the strict monitoring of patients after cell reinfusion. Although CAR-T treatment is very expensive, it is a promising strategy for cancer treatment given its ability to kill tumor cells accurately and mild side effects.

CAR-T immunotherapy has achieved breakthrough results in the treatment of hematological malignancies, such as chronic lymphocytic leukemia, acute lymphocytic leukemia, and multiple myeloma [43,44]. Notably, Kymriah and Yescarta, two kinds of CAR-T therapeutic products for relapsed or refractory lymphoblastic leukemia and refractory large B-cell lymphoma, respectively, were approved by the FDA in 2017 [45,46]. Clinical trials on CAR-T therapy for solid tumors, such as breast cancer, colorectal cancer, HCC, and prostate cancer, have been performed [47]. Although the clinical trial data that could be obtained are limited, preclinical studies have demonstrated that CAR-T is a promising approach for the treatment of solid tumors. For example, CAR-T cells targeting disialoganglioside GD2 demonstrated a potent and long-lasting therapeutic effect and improved survival in mice with diffuse intrinsic pontine glioma orthotopic xenografts [48]. To date, an increasing number of clinical trials have been performed to illustrate the value of CAR-T cell therapy for solid tumors. Clinical trials on the applicability of CAR-T therapy for the treatment of HCC are summarized in Table 2. Antigens involving GPC3, ErB2, GD2, EGFR, PSMA, and MUC1 are targeted in CAR-T therapy for solid tumors [47,49,50]. As is the case in hematologic malignancies, the suitable target for solid tumors must be selected to avoid side effects resulting from on-target, off tumor toxicity [32].

Although no universal standard guide for the selection of antigen targets exists, the antigen that is expressed at high levels in tumor tissues and at low levels in normal tissues must be selected in consideration of the safety of CAR-T treatment and the scarcity of tumor-specific antigens. Specifically, the appropriate target can be selected in accordance with the degree of tumor differentiation and the reinfusion method used in CAR-T treatment. For example, the expression of some tumor-associated antigens drastically increases with the malignant progression of the tumor. The selection of a highly expressed antigen target for a particular grade of tumor is more rational than the broad-spectrum selection of an antigen target for a certain tumor. At present, in many clinical trials, CAR-T cells are returned to the patient through local administration for the treatment of solid tumors to ensure maximum safety. In addition to the selection of antigen targets that are expressed at high levels in tumor tissues, antigens that are expressed in low levels in local tissues should be preferred, and those that are expressed at low levels in distant tissues may be considered as secondary choices.

Currently, although CAR-T cell immunotherapy for solid tumors has become a research hotspot, clinical studies on the treatment of HCC have not been reported, and the achievements of preclinical studies are rare. GPC3, a member of the glypican family of heparin sulfate proteoglycans, is widely chosen in registered clinical trials on the applicability of CAR-T therapy for HCC treatment [51]. Gao *et al.* presented the first report on CAR-T cell therapy for HCC treatment. Their results indicated that $GPC3^+$ HCC cells were effectively lysed by T cells expressing GPC3-targeted CAR (GPC3-CART). Notably, the lysis of $GPC3^+$ HCC cells was positively correlated with the expression levels of GPC3 in tumor cells. Furthermore, GPC3-CART showed similar inhibitory abilities *in vivo*: the potent suppression of tumorigenesis in HCC subcutaneous xenograft models and the considerable prolongation of the survival of orthotopic-xenograft mice [9].

Another phase I clinical trial on cell immunotherapy aiming to explore the safety of CAR-T hepatic artery infusions (HAI) for unresectable carcinoembryonic antigen (CEA)-positive liver metastases demonstrated that increased neutrophil/lymphocyte ratios were indicative of poor outcomes for patients with cancer following CAR-T HAI [52]. A case reported by Morgan *et al.* illustrated the most serious adverse effect that occurred after the administration of T cells transduced with a CAR that recognizes ERBB2. In this case, a patient with colon cancer and liver metastases experienced respiratory distress shortly after receiving an intravenous infusion of 1×10^{10} ERBB2-CART cells in 125 mL over 30 min; the patient died 5 days later despite undergoing prompt treatment [53]. Another study on CEA-CART in a murine model of CEA-positive liver metastases revealed that liver myeloid-derived suppressor cells could inhibit CAR-T function [54]. These data collectively indicate that although GPC3-CART cell immunotherapy showed commendable efficacy, the toxic adverse effects of immune cell infusion and the immunosuppressive microenvironment in the liver are potentially important barriers to the application of CAR-T in HCC therapy.

Table 2 Summary of clinical trials on the applicability of CAR-T therapy as a HCC treatment strategy

Clinical trial identifier	Antigen	Phase	Estimated enrollment	Infusion	Doses	Sponsor	Status
NCT02715362	GPC3	I/II	30	Transcatheter arterial infusion	$(1–10) \times 10^6$/kg	Shanghai Gene Chem Co., Ltd.	Recruiting
NCT03130712	GPC3	I/II	10	Intratumor injection	$(1–10) \times 10^6$	Shanghai Gene Chem Co., Ltd.	Recruiting
NCT03198546	GPC3	I	30	–	–	Second Affiliated Hospital of Guangzhou Medical University	Recruiting
NCT03146234	GPC3	–	20	Intravenous injection	Self-controlled dose escalation	Renji Hospital	Recruiting
NCT03349255	AFP	I	18	Intravenous infusion and intrahepatic artery infusion	–	Aeon Therapeutics (Shanghai) Co., Ltd.	Recruiting
NCT02587689	MUC1	I/II	20	–	–	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	Recruiting
NCT02959151	GPC3	I/II	20	Vascular interventional therapy or intratumor injection	$(1.25–4) \times 10^7$ /cm^3 tumor bulk	Shanghai GeneChem Co., Ltd.	Recruiting
NCT03013712	EpCAM	I/II	60	Vascular interventional mediated or endoscopy infusion	$(1–10) \times 10^6$/kg	First Affiliated Hospital of Chengdu Medical College	Recruiting
NCT02905188	GPC3	I	14	–	$1 \times 10^7/m^2$ $3 \times 10^7/m^2$ $1 \times 10^8/m^2$ $3 \times 10^8/m^2$ $1 \times 10^9/m^2$	Baylor College of Medicine	Not yet recruiting
NCT03084380	GPC3	I/II	20	Transcatheter arterial chemoembolization combined with CAR-T infusion	–	Xinqiao Hospital of Chongqing	Not yet recruiting
NCT03302403	GPC3	–	48	Intravenous injection	Self-controlled dose escalation	First Affiliated Hospital of Wenzhou Medical University	Not yet recruiting
NCT02723942	GPC3	I/II	60	–	–	Fuda Cancer Hospital, Guangzhou	Completed
NCT02395250	GPC3	I	13	–	–	Renji Hospital	Terminated

GPC3, glypican-3; AFP, α-fetoprotein; MUC1, mucin-1; EpCAM, epithelial cell adhesion molecule.

Challenges and strategies of CAR-T immunotherapy for HCC treatment

Traffic and reinfusion manners

CAR-T cells must be trafficked and infiltrate into tumor sites to exert their cytolytic effects. Intravenous injection is an effective approach for the reintroduction of CAR-T cells into patients with hematological malignancies. However, this approach is unfeasible for HCC treatment because of the relatively limited blood distribution of regional organs. Human liver tissues account for approximately 14% of the

blood supply of the whole body, and only a small faction of blood is required to nourish the growth of liver neoplasms. CAR-T cells infused through intravenous flow with blood in the vessels rarely infiltrate the HCC site and may even severely damage the lungs and other normal organs because of their low antigen specificity [53]. In addition, HCC tumors that developed from liver fibrosis and cirrhosis are highly fibrotic and difficult to penetrate physically. These features complicate the infiltration of CAR-T cells into tumor sites; similarly, patients showed weak response to other immunotherapies (e.g., checkpoint inhibitors) [55].

Numerous studies focusing on the introduction of chemokines to the CAR structure to surmount the infiltration barrier have been conducted. Adachi *et al.* transferred the IL-17 and CCL19 genes to CAR-T cells to produce 7 × 19 CAR-T cells. The survival rates of tumor-bearing DBA/2 mouse models treated with 7 × 19 CAR-T cells reached almost 100%, whereas that of models treated with conventional CAR-T treatment was only 30%. The immunofluorescence technique was used to detect the number of immune cells in tumor tissues and revealed that 7 × 19 CAR-T cells can promote the infiltration of T cells and dendritic cells into tumor tissues effectively. In addition, IL-7 is necessary for increasing the proliferation and survival of 7 × 19 CAR-T cells and for achieving a good therapeutic effect on solid tumors [56]. Runx3, a key transcription factor that promotes T-cell residency in nonlymphoid sites, has been recently identified and is a potentially essential cofactor of CAR-T cell therapy. The expression levels of Runx3 and other tissue-residency gene-expression signatures drastically increased in tissue-resident memory T cells but were low in circulating central memory T cells [57]. These studies provided new insights on the preferential infiltration of immune cells into solid tumor tissue.

Some other strategies can also be used to facilitate the infiltration of CAR-T cells into tumor tissues. Transcatheter arterial chemoembolization (TACE) is an interventional therapy that combines imaging diagnosis with clinical treatment and is the preferred method for the nonoperative treatment of liver cancer [58]. Given the adverse effects of intravenous injection on the treatment of HCC, clinicians may transfuse CAR-T cells in an interventional manner. This approach can improve the efficiency of killer cell infiltration into tumor tissues and drastically reduce the occurrence of systemic side reactions. However, the determination of cell reinfusion doses for TACE remains problematic and depends on the sizes and numbers of hepatic tumors and individual differences. Thus, identifying the most effective and safe input dose may be a difficult task that must be tackled in the future.

Tumor microenvironment and immune checkpoint inhibitors

Previous studies have demonstrated that patients with HCC and prominent lymphocyte infiltration may exhibit reduced tumor recurrence and prolonged overall survival [59,60]. In addition, Flecken *et al.* revealed that the production of interferon-γ was lower in $CD8^+$ T cells derived from TIL than in those derived from intrahepatic lymphocytes and peripheral blood mononuclear cells [61]. These findings support the existence of a suppressive microenvironment that may inhibit the cytotoxic function of CAR-T cells in HCC.

The tumor environment in HCC comprises immunosuppressive stromal cells and molecules, including myeloid-derived suppressor cells; tumor-associated macrophages; regulatory T cells; immune checkpoint molecules; and other immune-inhibitory factors, such as adenosine and indoleamine 2, 3-dioxygenase [62,63]. All of these ingredients constitute a complex network that promotes tumor growth by exhausting T cells in the HCC microenvironment.

Immune checkpoint molecules, such as PD-1 and T-cell immunoglobulin domain and mucin domain protein-3 (TIM3), are currently considered as the key factors in the HCC microenvironment. Antibodies against one or more of these molecules can restore the functions of TIL in HCC [64]. Given that the cytotoxicity of CAR-T cells is reduced by immune checkpoint molecules, the combinations of CAR-T and immune checkpoint inhibitors might represent an effective strategy for HCC treatment. Nevertheless, the disruption of PD-1 in CAR-T cells by the CRISPR/Cas9 system may enhance antitumor functions *in vivo* and *in vitro* [65]. Likewise, other immune checkpoint molecules can also be disrupted by genome editing technology, which contributes to the treatment of HCC.

Main side effects of CAR-T

CAR-T cell therapy has made great strides in the clinical treatment of hematological malignancies. However, fatal side effects have also occurred in clinical practice. In a typical case of CAR-T cell therapy, a patient with liver metastases suffered severe respiratory failure and ultimately died [53]. Neelapu *et al.* reviewed the toxic effects of CAR-T cell therapy. These toxic effects include cytokine-release syndrome (CRS), hemophagocytic lymphohistiocytosis, and CAR-T cell-related encephalopathy syndrome. The supervision of vital signs and serum biochemical indexes; imageological examination; and the preparation of first-aid medicine, such as tocilizumab, an anti-IL-6-receptor monoclonal antibody that alleviates the symptoms of CRS, are essential for patients receiving

CAR-T cell infusion [66]. Reducing adverse events and maximizing the clinical efficiency of CAR-T cells are critical because side effects cannot be completely avoided.

Conclusions

ACT therapy brings hope for the treatment of cancer. Its success is crucial for the development of translational medicine and will accelerate the transfer of the results of cancer treatment research from the bench to the bedside. CAR-T therapy, the most promising ACT treatment, will inevitably provide breakthroughs in the treatment of solid tumors in the future. Considering the excellent antitumor effects of CAR-T, the heterogeneity of tumors, and the expensiveness of currently available treatments, the research and development of universal and individualized CAR-T therapeutic products are the future direction of CAR-T therapy. Preparing CAR-T cells targeting tumor-associated antigens that are expressed at high levels in tumor tissues and at low levels in other tissues is a viable option given the difficulties in screening tumor-specific antigens. The transformation of CAR-T cells that target tumor-associated antigens to maximize the avoidance of adverse reactions will be a future research hotspot. At the same time, given the heterogeneity of tumors, research on the preparation of CAR-T cells by using neoantigens (epitope-specific antigens produced by tumor cell mutations) for individualized tumor immunotherapy should not be stagnant. A comprehensive and feasible clinical solution with minimal adverse reactions must be developed to prolong the median overall survival of patients with advanced HCC. The optimal cell dose, rational infusion method, and appropriate treatment schedule are problems that require further exploration. Given the complexity of tumor immune mechanisms, CAR-T immunotherapy combined with other therapeutic methods may represent promising strategies for HCC. In addition, the establishment of immunologist-led, clinician-assisted multicenter tumor immunotherapy teams will greatly promote technological innovation and improve the clinical efficacy of immunotherapies for HCC in the future.

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Compliance with ethics guidelines

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