

Car-T Therapy for Hepatocellular Carcinoma: Recent Advanced Progress

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Abstract. Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer. Human health is at risk due to the fact that only 10% survive for five years. HCC is treated with surgery, radiotherapy, and chemotherapy as the main methods. However, traditional treatment methods have certain side effects, surgical treatment requires postoperative intensive care, radiotherapy and chemotherapy can damage normal liver tissue, lead to fatigue and nausea and other symptoms, affecting the quality of life of patients. The treatment of hepatocellular carcinoma has been greatly benefited from the development of Chimeric antigen receptor T (CAR-T) cell therapy, which is considered one of the most innovative immunotherapies. Compared to traditional therapies and other immunotherapies, it is highly targeted and can survive and kill tumor cells for a long time. CAR-T cell therapy has become the focus of modern basic medical research and clinical trials. This article reviews the advantages, disadvantages, prospects and challenges of CAR-T cell therapy for liver HCC.

Keywords: CAR-T cell; Hepatocellular carcinoma; Traditional therapy.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer worldwide and the third largest cause of cancer-related deaths worldwide. Malignant cells in the liver grow uncontrolled and are characterized by this. The most common symptoms of HCC are abdominal pain or discomfort, weight reduction, irritability, vomiting, fatigue, yellowing of the skin and eyes (jaundice), and abdominal swelling.

Asia and Africa are the areas where HCC is most common worldwide, with Mongolia having the highest incidence at 93.7 cases per 100 000 people, while China has the highest number of HCC cases due to its large population, with an incidence of 18.3 cases per 100 000 people, and the morbidity and mortality are increasing year by year. HCC develops and progresses due to a number of risk factors, which include hepatitis B virus (HBV) infections, fatty liver disease, alcohol-related cirrhosis, smoking, obesity, diabetes, iron overload, and various dietary exposures. Currently, due to unclear causative factors. There are no effective therapeutic strategies for HCC in the clinic [1].

Traditional treatments for HCC include surgical resection, radiotherapy, and chemotherapy. Surgical resection refers to the treatment of HCC by removing tumor tissue and is an early primary method of HCC treatment. However, surgical treatment usually requires invasive procedures, which may require postoperative intensive care, ventilator and blood transfusion. For radiotherapy, hepatocellular carcinoma is less sensitive, so the therapeutic effect of radiotherapy is limited. In addition, normal liver tissue may be damaged by radiotherapy, leading to side effects and complications, including fatigue, nausea, skin inflammation, radiation hepatitis, liver function damage and gastrointestinal reactions [2].

Chemotherapy involves the use of drugs to kill or stop the growth of cancer cells. It is frequently utilized in advanced patients or as an adjunct therapy prior to or following surgery. Despite chemotherapy being an important treatment for advanced hepatocellular carcinoma, its effectiveness is still insufficient and patients' prognoses are poor.

CAR-T-cell therapy is an innovative form of immunotherapy that entails collecting a patient's immune cells (typically T cells), by genetically modifying and expanding them in vitro and then reinserting them back into the patient, the immune system's ability to attack tumors can be improved. CAR-T cell therapy is well targeted, durable, and highly effective. It has many advantages over

traditional therapies and solves the shortcomings of traditional therapies, such as overcoming immune escape and overcoming chemotherapy resistance. Recently, researchers discovered that CAR-T cell therapy has important therapeutic significance for those patients suffering from the HCC. The targets of CAR-T cell therapy for HCC include GPC3 (Glypicans), CD133 (Prominin-1), CD147 (EMMPRIN), EGFR (Epidermal Growth Factor Receptor), HER2 (Human Epidermal Growth Factor Receptor), etc. Among them, GPC3 and CD133 were highly expressed in HCC tissues [3].

Glypicans is a class of proteoglycans attached to the cell surface via a glycosyl phosphoinositol (GPI) anchor. In 1997, According to Hsu et al., GPC3 mRNA levels in the majority of HCC were significantly elevated compared to those in normal and non-malignant liver lesions. Clinical evidence suggests that CAR T Cell therapy that targets GPC3 can effectively decrease tumor growth in advanced patients. Out of the 18 patients, 1 was labeled with PR (Partial Response) and 10 with SD (Stable Disease), with 6 of them developing SD more than 6 months before progression. No DLT (Dose-limiting toxicities) was observed [4].

CD133, a glycoprotein with one chain and five diffusing domains, is deposited in the bulging regions of the cell's plasma membrane. These rings are divided into two large glycosylated extracellular rings and two small intracellular rings. Hanren Dai et al. conducted a clinical trial on 21 HCC patients and found that CD133-directed CAR-T cell therapy showed some efficacy in patients with advanced hepatocellular carcinoma. The study revealed that patients with advanced hepatocellular carcinoma had an average survival of 12 months and an average progression-free survival of 6.8 months, which are encouraging findings [4].

Currently, the treatment of HCC with CAR-T cell therapy appears to be highly promising. We explore existing challenges and possible solutions, as well as potential tactics to improve CAR-T cell efficacy in HCC patients, in light of the application status of these cells in this disease.

2. Traditional Therapies

Surgical and non-surgical procedures are the mainstays of traditional HCC treatment. The surgical treatment of HCC includes resection, liver transplantation and radiofrequency ablation. Non-surgical treatments for HCC include radiotherapy, chemotherapy and immunotherapy.

2.1. Surgical treatment

Surgical resection of HCC is the treatment of HCC by surgically removing the tumor tissue in the liver. However, surgical resection will cause bleeding infection, bile leakage, liver dysfunction, etc. The incidence of infection after surgery ranges from 3.9% to 6.8%. The 90-day mortality rate increased by 1.3% for patients who underwent reoperation compared to those who did not [5].

Liver transplantation is the treatment of choice for patients with cirrhosis, decompensated disease, acute liver failure, and hepatocellular carcinoma. The survival rates for liver transplantation at 1-5 years were 69%, 56%, and 44%, as reported [6]. However, liver transplantation has many complications. Numerous studies have shown that common complications of liver transplant surgery include biliary complications, of which bile leakage and bile duct stricture are the most common.

2.2. Non-surgical treatment

2.2.1. Radiation therapy

Radiotherapy for HCC is a non-invasive local treatment that causes both direct and indirect DNA double-strand breaks through ionizing radiation [7]. The advantage of radiotherapy for HCC is that with the continuous progress of radiotherapy technology, modern radiotherapy technology can provide higher radiation doses while reducing radiation damage to normal tissues. However, radiotherapy also has many disadvantages, for example, toxic side effects, high cost, unsuitable for combination with other treatments, etc [8]. Some studies have suggested that radiotherapy may affect the tumor microenvironment (TME), inhibiting the effect of immune checkpoint suppression in irradiated areas. First, radiation can raise the expression of Class I (MHC I) proteins that are

associated with radiation on the tumor surface, which could lead to cytotoxic T cells recognizing tumor-associated antigens (TAAs). Second, radiotherapy can attract macrophages by releasing hazardous associated molecular pattern (DAMPs) immune molecules on the cell surface. Radiation therapy may enhance immunogenic cell death by causing immune cells and macrophages to send phagocytosis signals by moving calprotectin from the endoplasmic reticulum to the cell surface. Additionally, by increasing the release of particular chemokines like CXCL16, CXCL9, and CXCL10 as well as the induction of interferon gamma signaling, radiotherapy can increase the expression of E-selectin and intercellular adhesion molecule (ICAM-1) in human endothelial cells and promote the recruitment and penetration of CD4+ and CD8+ T cells. Modified angiogenesis of tumors [9].

2.2.2. Chemotherapy

Chemotherapy is a treatment that uses chemicals to treat cancer. It kills cancer cells by inhibiting the growth and division of cancer cells. Commonly used drugs include Sorafenib, Docetaxel, Cisplatin, Capecitabine, etc. These drugs can be administered by hepatic arterial infusion (HAIC). The median overall survival for patients with hepatocellular carcinoma treated with sorafenib in a clinical trial was 4.9 months, the median survival time was 2.0 months, the objective response rate was 10 percent, and the disease control rate was 35.0% [10]. Its therapeutic effect is limited, it does not clear the tumor, and it is less effective for patients with large tumors or vascular invasion. The disadvantages of chemotherapy include side effects and limited therapeutic effectiveness. Side effects include nausea, vomiting, diarrhea, damage to normal liver tissue, etc. Chemotherapy may also have a suppressive effect on the immune system, leading to decreased immune function. This may affect the treatment outcome of patients with HCC. Therefore, chemotherapy is not an ideal treatment for HCC.

3. Innovative therapy Car-T cell

A promising therapeutic approach that can enhance prognosis and reduce treatment-related harm is immunotherapy. By genetically engineering their own T cells to express a particular chimeric antigen receptor (CAR), patients undergoing car-t cell therapy can enhance their immune system's capacity to identify and eliminate cancerous cells. As depicted in **Fig 1**. The artificial protein complex CAR is composed of four domains: the transmembrane domain, the hinge domain, the endogenous signal transduction domain, and the exogenous antigen recognition domain. Tumor-associated antigen recognition is accomplished by use of exogenous antigen recognition domains, which are often formed from single-chain variable fragments (ScFv) [11].

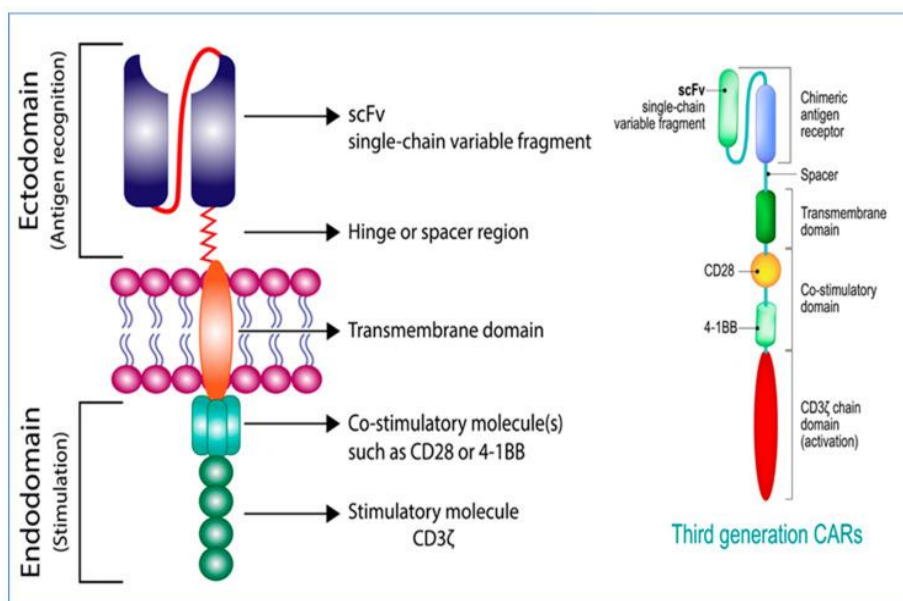


Fig 1. The structure of Car-T cells [12].

After introducing the CAR into the patient's own T cells by genetic engineering technology, the outer part of the cell membrane of CAR-T cells features an antibody against tumor antigens, which guides CAR-T cells to specifically recognize tumor cells expressing relevant antigens. CAR T cells' binding to target cells triggers the activation of the CD3 chain inside the cell membrane, which initiates the division and proliferation of CAR T cells. As reported by Li, Dan et al., the CAR-T cells can recognize and bind to the specific surface antigen GPC3 of HCC cells. The researchers prepared GPC3-targeted CAR-T cells by engineering the C-lobe and N-lobe of GPC3 using antibodies with high-affinity hYP7 and HN3, respectively. CAR-T cells eliminate GPC3-positive HCC cells not only by inducing perforin, granase mediated apoptosis, but also by binding GPC3 antigen to inhibit the Wnt/ β -catenin signaling pathway in tumor cells [13].

3.1. Design of Car-T Cells

CAR-T cell therapy is now in its fourth generation. As shown in **Fig 2**, first-generation CAR-T cells are the earliest designs that contain a single antigen-recognition construct, usually a single-chain antibody (scFv), linked to a T-cell activation signaling domain, such as CD3 ζ [14]. By incorporating a costimulatory signaling domain, like CD28 or 4-1BB, second-generation CAR-T cells have improved over the first generation. This design could provide additional costimulatory signals that enhance CAR-T cell viability, proliferation, and antitumor effects. Third generation CAR-T cells are further improved on the second generation and usually contain two costimulatory signaling domains, such as CD28 and 4-1BB. This design is intended to further enhance the activity and persistence of CAR-T cells and improve adaptation to the complex tumor environment [15].

Finally, fourth-generation CAR-T cells (TRUCKs) possess a costimulatory region and a CAR-induced interleukin-12 (IL-12) gene. In this context, the interaction of the CAR-T cell with the antigen activates T-cell signaling and releases the proinflammatory interleukin-12 to recruit natural killer cells and macrophages that attack antigen-negative cells [15].

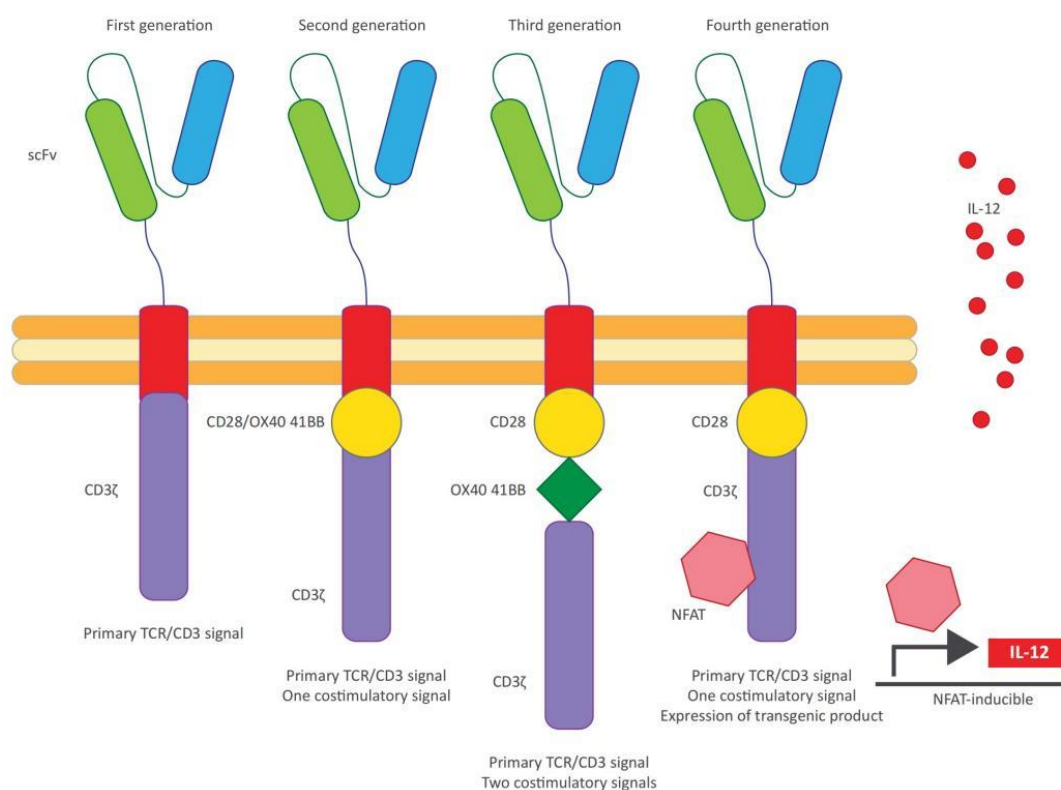


Fig 2. Design of Car-T cells [15].

Every generation of CAR-T cells has generally been engineered to increase the cells' capacity for activation and proliferation as well as their capacity to kill tumor cells. The development of CAR-T cell design is expected to continue as technology advances.

3.2. The Target of CAR-T Therapy for HCC

Certain antigen molecules that are widely expressed on the surface of cancer cells have been investigated in order to create CAR-T therapy for solid tumors. These include glypican-3 (GPC3) and CD133, which are overexpressed in most HCC tissues but have minimal expression in healthy adult tissues and are linked to a poor prognosis in HCC patients. These targets make them attractive candidates for HCC CAR-T cell treatment.

3.2.1. Glypican-3

GPC3 is a fetal antigen highly expressed in hepatocellular carcinoma (HCC), which promotes Wnt-dependent cell proliferation. GPC3-targeted CAR-T cells caused tumor regression by reducing active β -catenin levels and Wnt signaling in HCC cells. Specifically, GPC3-targeted CAR-T cells were found to eliminate GPC3-positive HCC cells by inducing apoptosis mediated by perforin and granzyme. In addition, these CAR-T cells can be continuously expanded in the tumor microenvironment and the spleen, and they can be selected by antigen induction to form a multifunctional CAR-T cell subset. These CAR-T cells persisted in the mice for up to 7 weeks, the tumors began to shrink in the first week, the CR ratio of the mouse tumors was 13/15 by the end of the third week, until the seventh week, nearly 30% of the tumors were less than 500 cubic millimeters in size, eliminating xenograft or orthotopic liver tumors, and the survival of the mice was significantly extended [16].

3.2.2. Prominin-1

Prominin-1 is a surface marker, also known as CD133, which is a highly conserved five-transmembrane glycoprotein. CD133 is expressed in a variety of tissues and cell types, including stem cells, neural stem cells, and tumor stem cells. In tumors, CD133 is considered to be a tumor stem cell marker whose expression is associated with tumor proliferation, invasion, and drug resistance [17].

CD133-directed CAR-T cell therapy has shown some efficacy in patients with advanced hepatocellular carcinoma. Chaopin Yang et al. used the Sleeping Beauty transposon system to construct CD133-specific CAR-T cells that secrete PD-1 blocking single chain antibody (scFv) from microcyclic carriers. The antitumor efficacy of CD133 CAR-T cells was analyzed by *in vitro* and *in vivo* experiments. Studies have shown that late-stage (stage II and III) CD133 expression in male patients is significantly associated with poorer progression-free survival (PFS) ($P=0.0057$) and overall survival (OS) ($P=0.015$), making CD133 a reasonable target for immunotherapy in patients with advanced HCC [18].

The reason for the surprising therapeutic effect may stem from the marker's own functioning. CD133 is currently the most mature surface marker on endothelial precursor cells, and it has increased circulation in the peripheral blood of patients with highly vascularized hepatocellular carcinoma and is involved in angiogenesis. Therefore, CD133-directed CAR-T cell therapy may not only disrupt the nutrient supply of tumors but also improve the infiltration of small molecule drugs and other therapies at the tumor site.

4. Advantages

So far, a lot of research has shown that, Compared to conventional therapy, car-T cell therapy for HCC offers numerous benefits. Its targeting is more precise. Car-T therapy has stronger targeting, can precisely detect and destroy tumor cells, and lessens damage to normal tissues. It does this by converting T cells into CAR-T cells through genetic engineering technology. Long-term survival and tumor cell killing are two of CAR-T cells' many capabilities. Moreover, CAR-T cell therapy can be designed and prepared individually according to the specific conditions of patients. CAR-T cells can be engineered by gene editing technology to improve their antitumor efficacy and survival. More importantly, HCC is often resistant to traditional chemotherapy drugs, but CAR-T cell therapy can overcome this problem. By recognizing and attacking specific antigens of tumor cells, CAR-T cells are not affected by the drug resistance mechanism of tumor cells [19].

5. Limitations and Challenges

Despite the promising treatment, CAR-T cell therapy for HCC faces many difficulties and obstacles in clinical application, including cytokine release syndrome, neurotoxic syndrome immune, escape tolerance, limited tolerance to the tumor microenvironment, high recurrence rate, high cost of treatment.

5.1. Cytokine release syndrome (CRS)

Cytokine release syndrome, is a serious side effect of CAR-T cell therapy. CAR-T cells interact with tumor cells which would further release large amounts of cytokines such as interleukin-6 (IL-6), necrosis fact- α (TNF- α), and so on. The release of these cytokines leads to an inflammatory response that triggers CRS. Symptoms of CRS include hyperthermia, chills, low blood pressure, dyspnea, tachycardia, and pulmonary edema [20].

5.2. Expensive treatment

Beyond the improvements to CAR-T therapy itself, the journey towards universal accessibility and cost-effectiveness presents critical challenges that must be addressed to fully realize its potential. The production of CAR-T cells is highly personalized, involving the genetic modification of a patient's own T cells, which leads to the high costs of treatments like Kymriah and Yescarta. These treatments can cost over \$400,000 per infusion, not including additional hospitalization expenses for potential side effects [21].

5.3. Tumor microenvironment

The tumor microenvironment plays an important role in the therapeutic effect of CAR-T cells. The first aspect to consider is immunosuppressive cells in the tumor microenvironment inhibit the function of CAR-T cells such as regulatory T cells (Tregs). Tregs can inhibit the activity of CAR-T cells through cell contact and cytokine release, and the expressed cytokines such as IL-10 and TGF- β , inhibitory receptors PD-1 and CTLA-4 interact with CAR-T cells, thereby inhibiting the proliferation and function of CAR-T cells [22]. Moreover, hypoxic and acidic environments in the tumor microenvironment also affect the function of CAR-T cells. Hypoxic environment can reduce the activity and proliferation capacity of CAR-T cells, thus reducing their anti-tumor effect. In a low-oxygen environment, tumor cells release lactic acid, resulting in acidification of the tumor microenvironment. The expression of immunosuppressive substances including TGF β , IL-10, and IL-6 is encouraged by this acidic environment. Furthermore, tumor cells decrease antigen downregulation and surface mutation to avoid being recognized and attacked by CAR-T cells, which reduces the therapeutic efficacy of CAR-T cells. For example, in B-cell malignancies, about 50% of recurrences are related to the loss of CD19, while in lymphomas, about 30% of recurrences are due to CD19 expression levels below the antigen density threshold of commercial CAR-T cells [22]. Another difficulty is determining the exact location of CAR-T cells within the tumor microenvironment. The therapeutic impact of CAR-T cells is limited because of the tumor tissue's complicated structure and uneven blood vessel density, which prevents the cells from entering the tissue efficiently.

6. Improvement Strategy

With the progress of scientific research, the preparation methods and sources of CAR-T cell have been relatively changed, making CAR-T therapy more convenient.

6.1. Cytokine release syndrome (CRS)

The process of preparing CAR-T cells from autologous T cells is complicated and time-consuming. In addition, patients often experienced radiotherapy, chemotherapy and other treatments before

choosing CAR-T cell therapy, which made the number of autologous T cells insufficient and their vitality decreased. Under the combined influence of the above factors, the failure rate of autologous CAR-T cells preparation is high and the price is expensive, which limits the wide application of CAR-T cell immunotherapy [23]. To solve this problem, the researchers designed a Universal Car-T cell.

General-purpose CAR-T cells use allogeneic CAR-T cells from a healthy donor, while traditional CAR-T cells are prepared using the patient's own T lymphocytes, which means that general-purpose CAR-T cells can be prepared in advance and stored for use by multiple patients, without the need to customize CAR-T cells for each patient. The preparation process of general-purpose CAR-T cells is relatively simple and can be mass-produced to improve the quality and availability of CAR-T cell products [24].

6.2. Combination of drugs

Preclinical studies have shown that the efficacy of CAR-T in the treatment of solid tumors is limited, mainly due to the existence of immunosuppressive tumor microenvironment, so the need for combined therapy. Studies have found that CAR-T cell therapy and immune checkpoint inhibitor (ICIs) therapy can well overcome the immunosuppression of tumor microenvironment. In PD-L1-positive mice models of HCC, GPC3-targeted CAR-T cells shown reduced cytotoxic effects; in contrast, GPC3-targeted CAR-T cells that were carrying PD-1 blockers demonstrated noticeably more tumor inhibition. Consequently, it makes sense to combine ICIs with CAR-T treatment [22]. The other is the combination of CAR-T cells and multi-target tyrosine kinase inhibitors (sorafenib), which can better enhance the tumor killing effect of CAR-T cells. In HCC patients, sorafenib has been shown to have an average blood concentration of 400mg twice daily (FDA) recommended dose between 3-10 mg /L (about 5-16 mm). Comparing monotherapy and combination therapy, XiuQi Wu and colleagues discovered that the tumor volume in mice was 1326.1 cubic mm and 557.1 cubic mm, respectively. Based on these findings, they came to the conclusion that sorafenib could improve the anti-tumor activity of CAR T cells in both humans and mice by controlling macrophages or encouraging apoptosis [25].

7. Conclusion

With a high incidence, HCC is the third most common cancer-related cause of death globally. Traditional treatments for HCC include chemotherapy, surgical removal and surgical treatment. The advantage of these traditional treatments is that they can effectively reduce the size of the tumor and control the growth and spread of the tumor. However, these methods also have some disadvantages. Chemotherapy can cause serious side effects such as nausea, vomiting, and suppression of the immune system. Surgical resection may result in postoperative complications and liver insufficiency. In addition, these traditional therapies have a limited therapeutic effect on HCC, which can easily lead to tumor recurrence and metastasis. Further research and development of more effective treatment strategies are needed to target recurrence and metastasis of HCC. In particular, using GPC-3, CD133, and CD147 as the target therapy, CAR-T cell therapy has demonstrated excellent therapeutic impact and few adverse effects. Emerging tumor immunity, as represented by CAR-T cell therapy, is projected to become one of the most successful treatment approaches for HCC patients. CAR-T Cell is expected to become an important immunotherapy for the cure of HCC.

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