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Clinical status, drug resistance, and countermeasures of CAR-T cell cancer immunotherapy

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Abstract. The field of adoptive immune cell therapy, known as CAR-T cells (chimeric antigen receptor-modified T-cells), has advanced significantly in recent years. However, the drug resistance of CAR-T therapy limits the further development of this technology, and a large proportion of tumor patients have drug resistance or cancer recurrence. Immune evasion due to drug resistance may be caused by CAR-T cell dysfunction, the effects of the tumor microenvironment, or drug-resistant tumor cells. The clinical status of CAR-T is reviewed here, along with the intrinsic mechanisms by which tumor cells resist CAR-T therapy and potential development options to address CAR-T resistance..

Keywords: Car-T, Resistance, Hematological Tumor, Solid Tumor, AML.

1. Introduction

Cancer is known as an 'incurable disease' due to its inability to be completely cured, limited treatment options, and high cost of treatment. Therefore, research on cancer and methods and drugs for treating it have been the object of unremitting efforts of oncologists and clinicians for decades. Traditional cancer treatments include radiation therapy, chemotherapy, and surgery for solid tumors [1]. Traditional cancer treatment methods have serious side effects, and it is difficult to completely cure cancer. Compared with traditional therapy, CAR-T therapy has relatively fewer side effects, and CAR-T cells can survive for a long time and continue to remove cancer cells in patients [2]. CAR-T cell technology has been developed for several generations and has made great progress. The first-generation CAR-T technology's single-chain Fragment variable (scFv) integrates only the CD3ζ signaling domain, which can trigger tumor-specific cytotoxicity. The latest CAR-T technology is designed to simultaneously activate TCR, co-stimulatory domain CD28, and cytokine triple signaling; because this is a prerequisite for T cells to be activated when they bind antigen [2, 3]. Although this technology has many clinical advantages, the drug resistance generated during CAR-T therapy hinders the further development of its clinical application [4]. Here, this paper will introduce the clinical status, drug resistance, and countermeasures of CAR-T therapy in tumor immunotherapy.

2. Clinical status

As emerging cancer immunotherapy, CAR-T research mainly focuses on hematological tumors and solid

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tumors, especially in hematological tumors, it has made great breakthroughs [2].

Clinical trials have demonstrated that CD19-targeting CAR-T cells could successfully treat B-cell malignancies in hematological cancers [5] and have promising treatment results for relapsed and refractory B lymphoblastic leukemia (B-ALL) [5]. A study revealed that the overall number of patients was improved when 16 patients with relapsed and refractor B-ALL received autologous T cells that expressed the CD19 antigen-specific 19-28z chimeric antigen receptor (CAR). The rate of full recovery was 88% [6]. The clinical treatment mechanism and efficacy of some rapidly developing CAR-T technologies are shown in TABLE 1.

Table 1. Clinical treatment mechanism and efficacy of CAR-T technology.

Tumor Type	Therapeutic Mechanism	Clinical Efficacy
Acute lymphoblastic leukemia	Specifically recognizes the tumor antigen CD19 molecule, and enhances the antitumor effect through the co-stimulatory signals of CD28 and CD137 molecules [7, 8].	Long survival time of CAR-T cells
Non-Hodgkin lymphoma	Specifically recognizes the tumor antigen CD20 molecule, and takes advantage of the co-stimulatory signals of CD28 and CD137 to enhance the anti-tumor impact [9, 10].	High clinical remission rate and long survival time of CAR-T cells
Myeloid leukemia	It specifically recognizes the LeY tumor antigen and enhances the anti-tumor effect through the costimulatory signal generated by the CD28 molecule [11].	Long survival time of CAR-T cells
Glioma	Specifically recognizes the tumor antigen EGFRvIII and enhances the antitumor effect through the co-stimulatory signal of ICOS [12].	Significant inhibitory effect on tumors

In a clinical study by Li SQ et al., 10 patients with relapsed or refractory ALL were treated with anti-CD19 CAR-modified T-cells containing CD28 or CD137. The first 5 patients received CD28-CAR treatment and the other 5 patients received CD137-CAR treatment (All 10 patients were response-evaluable). Three patients had complete remission following CD28-CAR-T therapy, while one extramedullary patient experienced partial remission. Three patients in the CD137-CAR therapy group experienced complete remission. The clinical remission rate based on experiments is above 60 %. In addition, this study highlights that no serious adverse events were observed in the 10 treated patients [8]. Ying, ZT et al. reported that the co-stimulatory signals of CD28 and CD137, among other molecules, increased the antitumor activity of T cells. Additionally, the effectiveness and side effects of these two signal molecules in the treatment of non-Hodgkin's lymphoma were studied. The comparison shows that CD137 has more clinical advantages in reducing side effects than CD28 [10]. Patients with R/R EBV-negative, CD30+ HL, or T-cell anaplastic large cell lymphoma recently participated in a clinical trial using a second-generation targeted CD30-CAR-T(NHL). Before receiving a CAR-T infusion, patients in clinical trials did not undergo any conditioning therapy. At the 6-week follow-up, 1 patient had a CR, 1 had a very good PR, and 4 had an SD [13]. The research of autologous LeY antigen-specific CAR-T therapy in acute myeloid leukemia (AML) preliminarily demonstrated that CAR-T cells can infiltrate established disease sites in the treatment of AML using LeY antigen-specific CAR-T cells. Subsequent serial PCR examination of LeY-transgenic peripheral blood and bone marrow showed that the infused CAR-T cells persisted in patients for up to ten months [11].

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The use of CAR-T technology to treat solid tumors is currently mainly concentrated in the clinical trial stage [14]. In recent years of CAR-T therapy research, researchers carried out a series of therapy targeting solid tumor targets, including CEA, HER-2, GD2, EGFR, GPC3, and other tumor targets [15]. Related antigens, the use of second or third-generation CAR-T, the introduction of co-stimulatory signals CD28 and CD137, etc., greatly enhance the proliferation, survival, and killing ability of CAR-T cells [8]. The solid tumor-related CAR-T therapeutic targets currently under study are shown 1in TABLE 2 [15-19].

Table 2. Solid tumor-related CAR-T therapeutic targets currently under investigation.

Target	Tumor Type
EGFR	Head and neck tumors, malignant gliomas
CEA	Colorectal, ovarian, stomach, liver, lung, breast, and pancreatic cancers
CD133	Liver, brain, pancreatic, ovarian, breast, and colorectal cancers
HER-2	Advanced osteosarcoma, glioblastoma multiforme, HER-2 positive tumors
GPC3	hepatocellular carcinoma, lung cancer
EphA2	malignant glioma
Mesothelin **(**MSLN**)**	Metastatic pancreatic tumor, malignant mesothelioma, epithelial ovarian cancer, epithelial malignant pleural mesothelioma, ovarian tumor, breast cancer, pancreatic cancer, endometrial cancer
PSCA	Pancreatic cancer

Compared with hematological tumors, due to the higher complexity of the tissue structure and immune characteristics of solid tumors, the selection of appropriate targets is essential [14]. When treating solid tumors, certain antigens expressed on the surface of tumor cells, such as EGFR, HER-2, and MSLN may have a substantial impact. The skin, digestive system, kidney, and other normal tissues all have high levels of EGFR expression, but many epithelial malignancies, including lung, pancreatic, colon, and breast cancers, have substantially higher levels of EGFR expression [20]. Although there are currently targeted drugs against EGFR, because EGFR is prone to mutation, the efficacy in some patients is short-lived. Therefore, CAR-T cell therapy targeting mutant EGFR may become a new means of treating solid tumors. Variant III mutations of epidermal growth factor receptor (EGFRvIII) result from deletion of part of the extracellular domain. Some research teams used second-generation CAR-T cells as a carrier backbone, which was based on the efficacy of mouse scFv CARs in glioblastoma xenograft models. In a mouse model receiving normal human skin transplants, the main CAR candidate's specificity for EGFR-expressing keratinocytes in vitro and in vivo was evaluated. In allogeneic subcutaneous and orthotopic models of human EGFRvIII (+) glioblastoma, tumor development was also inhibited by EGFRvIII-targeted CAR-T cells [21]. HER-2 is also a transmembrane glycoprotein of the EGFR family and is widely overexpressed in breast cancer, ovarian cancer, osteosarcoma, and glioma. In patients with advanced biliary tract cancer and pancreatic cancer, clinical research confirmed the efficacy, safety, and activity of CAR-T therapy targeting HER2 [16, 22]. MSLN is also a tumor-specific antigen on the surface of cell membranes, with low expression in normal tissues, but high expressions in various malignant tumors, such as malignant glioma, ovarian cancer, pancreatic cancer, and NSCLC. A research team has verified that MSLN-specific CAR-T cells have a significant anti-tumor effect in a mouse model, and the combination with CD137 and CD28 can improve the secretion of specific CAR-T cells and enhance their tumor-killing ability [18, 23]. Researchers discovered that while CAR-T cells targeting MSLN might considerably slow tumor development, they could not entirely eradicate malignancies. The reason may be due to some inhibitory proteins, such as PD-1, which can inhibit T cell function. Therefore, combined use with immune checkpoint inhibitors such as PD-1 can enhance the activity of MSLN-specific CAR-T cells [24].

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3. Drug Resistance, and Countermeasures

However, even if the efficacy of CAR-T therapy is significant, there are still a considerable number of patients with primary or secondary resistance to it. The tumor microenvironment, which includes immunosuppressive cells, and immunosuppressive cytokines, as well as tumor cells themselves, may all be important for the resistance mechanism of CAR-T. CAR-T cell issues, such as insufficient proliferation, insufficient persistence, defective/exhausted effector functions, and tumor cells themselves may also have an impact [25].

Among the numerous drug resistance mechanisms, target antigen loss is one of the most extensively studied, which can lead to cancer cells becoming resistant or evading CAR-T therapy [25]. The recurrence of target antigen-negative clones may be due to selective growth of pre-existing antigen-negative subclones by immune editing, or it may be due to the acquired loss of target antigens originally expressed by tumor cells. The mechanisms of antigen loss that lead to drug resistance include the following aspects. Due to the existence of mutation or alternative splicing, the target antigen whose structure has been changed cannot be recognized; the existence of tumor heterogeneity will make the antigen-negative cells in tumor cells unable to be cleared by CAR-T cells, Thus, drug resistance occurs; the lineage switch of tumor cells may lead to changes in their phenotypic markers; under some special conditions, tumor cells may acquire the carrier CAR, causing the CAR to mask the tumor cell's epitope, making the CAR-T cells cannot recognize tumor cells resulting in resistance [25, 26]. It should be noted that the incidence of antigen loss varies among patients with different histology, and in B-ALL, CD19 loss accounts for approximately 7-25% of patients who relapse on CAR-T therapy; a BCMA-CAR In the T-treated multiple myeloma (MM) study, 1 of 12 patients (8%) had BCMA loss at relapse [27].

At present, there are many targeted ideas to circumvent the drug resistance of CAR-T cell therapy. First, antigen-negative tumor cell relapse can be prevented by targeting multiple antigen-targeted CAR-T cells. This is accomplished by administering a patient with a combination of CAR-T cells with distinct but different specificities or CAR-T cells with numerous specificities, which can be given either sequentially or concurrently [25, 28]. In a clinical study, CD22 CAR-T treatment was given to 21 B-ALL patients, 17 of whom had previously undergone CD19-targeted immunotherapy. All five CD19dim or CD19-negative B-ALL patients in this research experienced full remission following CD22 CAR-T treatment, with a median remission length of six months [28]. Similarly, a study reported that 3 patients with CD19 CAR-resistant DLBCL achieved complete remission after receiving CD22 CAR-T treatment [29]. Another idea is to reduce the loss of target cell surface antigens. Since the efficiency is closely related to the density of target antigens on tumor cells, increasing the expression of target antigens on tumor cells is extremely important for CAR-T therapy [25]. There is also an idea to deal with drug resistance by relying on the non-antigen-dependent helper killing function. CAR-T cells inhibit tumor cell proliferation by secreting immune-stimulating cytokines or expressing co-stimulatory ligands and other non-antigen-dependent ways. CAR-T cells can increase their anti-tumor activity by secreting IL12 in an autocrine way. IL12 can also change the tumor microenvironment, which enables CAR-T cells to overcome immunological suppression by regulatory T cells [30]. CAR-T cells capable of constitutively expressing the co-stimulatory ligand CD40L can produce cytotoxic effects by direct binding of CD40L to CD40-positive tumor cells and, similarly, to antigen-presenting cells (APCs). CD40L-CD40 binding can activate para tumor APCs and increase the expression of co-stimulatory molecules such as CD40, CD86, and MHC-II. According to the aforementioned mechanism, if CAR-T cells express CD40L, they can encourage the patient's immune system to produce endogenous anti-tumor T cells that will recognize tumor cells and kill them through their cytotoxic effect, thereby reducing the immune response brought on by the risk of a single antigen eluding detection [31].

The immune checkpoint inhibitor pathway (CAR-T and PD-L1) and the inhibitory ligands PD-L1 and PD-L2, which can be produced by tumor cells or their surroundings, mean that the ligand and its interaction will have an impact on the activation of T cells. The receptor binds and blocks it so that tumor cells can achieve immune escape [32]. Regarding the mechanism of resistance caused by the loss of non-antigen targets, there are currently three research directions. Tumor cells lack CD58 expression, which prevents co-stimulatory factors from being delivered to CAR-T cells, which impairs the cytotoxic

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function of some T cells; even if CAR can recognize antigen targets, tumor cells can well express inhibitory ligands. The CAR-T cell cytotoxicity is successfully suppressed; the intrinsic apoptotic mechanism of tumor cells is damaged due to mutations and other factors, and tumor cells acquire endogenous drug resistance, making CAR-T cells unable to pass through the cells after recognizing tumor cells. Toxic effects lyse tumor cells [33, 34].

There are currently only a few methods for overcoming PD-L1-mediated CAR-T cell treatment resistance, primarily the combination of CAR-T with anti-PD1/PD-L1 antibodies, as well as anti-CAR-T secretory CAR-T cells. adjuvant therapy, etc. In a clinical study, 15 patients with DLBCL received PD-L1 monoclonal antibody durvalumab, and the treatment response rate could reach 50 %, of which 5 patients developed cytokine release syndrome, 1 patient presented with symptoms of neurotoxicity [35]. A research team has developed "armored" CAR-T cells that can secrete CAR-T-blocking scFv through paracrine and autocrine means. After experiments with mice, it was discovered that these CAR-T cells were at least as effective against tumor cells that already expressed PD-L1 as the combination of CAR-T cells plus anti-CAR-T monoclonal antibodies. This shows a relatively good development prospect for the follow-up related research [33, 36].

4. Conclusion

At present, in the clinical treatment of hematological cancers, CAR-T therapy has demonstrated great effectiveness, and certain mature patented medications subject to government regulation have been developed. Some blood cancer patients have been cured and are in good condition after recovery. However, in combating solid tumors, the lack of specific tumor antigens limits the application of this therapy. The majority clinical studies that are now being conducted worldwide are in the early stages of development in terms of clinical research.

In the field of CAR-T, the most important drug resistance mechanism is the lack of target antigens and strategies to overcome this include CAR-T cells that specifically bind to multiple antigen targets. Other mechanisms of CAR-T therapy resistance include inhibitory ligand expression, lack of costimulatory ligands, and tumor cell-intrinsic resistance. Although the coping methods have not yet met the full needs, relevant studies have supported the correctness of the solution idea of resistance.

With the development of biochemistry and oncology and the continuous optimization of CAR-T therapy, how determining the resistance mechanism of individual tumors in patients to select the best product or combination to give personalized CAR-T therapy will become increasingly significant.

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