New Approaches in CAR-T Cell Immunotherapy for Breast Cancer

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Abstract

Despite significant advances in surgery, chemotherapy, radiotherapy, endocrine therapy, and molecular-targeted therapy, breast cancer remains the leading cause of death from malignant tumors among women. Immunotherapy has recently become a critical component of breast cancer treatment with encouraging activity and mild safety profiles. CAR-T therapy using genetically modifying T cells with chimeric antigen receptors (CAR) is the most commonly used approach to generate tumor-specific T cells. It has shown good curative effect for a variety of malignant diseases, especially for hematological malignancies. In this review, we briefly introduce the history and the present state of CAR research. Then we discuss the barriers of solid tumors for CARs application and possible strategies to improve therapeutic response with a focus on breast cancer. At last, we outlook the future directions of CAR-T therapy including managing toxicities and developing universal CAR-T cells.

Keywords

Breast cancer • Immunotherapy • T cells • Chimeric antigen receptor

17.1 Introduction

Breast cancer is the most diagnosed cancer in women. Despite significant advances in surgery, chemotherapy, radiotherapy, endocrine therapy, and now molecular-targeted therapy, breast cancer remains the leading cause of death from malignant tumors among women [1, 2]. After decades of researches and trials, it seems that manipulation and utilization of antitumor properties of the immune system have begun to show

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promise for a variety of tumors [3, 4]. Through the years, many advances have been made in the immunotherapy of breast cancer. Immunotherapy has become an important part of breast cancer treatment, along with encouraging activity and mild safety.

Immunotherapy for breast cancer involves a wide range of therapies including monoclonal antibodies (mAbs), vaccinations, immune checkpoint inhibition, and adoptive T-cell transfer immunotherapy. HER-2/neu monoclonal antibody has been successfully used in treatment for breast cancer patients. However, overexpression of HER-2/neu accounts for only 25–30% of breast cancer patients. Vaccinations induce specific antitumor immunity, but objective tumor regression is rarely observed in clinic [5]. Cytotoxic T cells play a key role in immune-mediated control of cancer [6–12]. Plenty of studies have proved that the extent of cytotoxic T cell-infiltrating tumors is a key factor in determining the natural progression of a variety of cancers [6–9, 13–15]. Over the past two decades, T-cell-based immune therapy has gained general acceptance with its curative potency for several types of malignant diseases [16]. Current T-cell-based immune therapies are generally based on two methods. The first involves the isolation of antitumor T lymphocytes from the primary tumor tissues of the patients, which is called tumor-infiltrating lymphocytes (TILs). However, due to the difficulties of TIL isolation and culture, TIL therapy is limited to a few types of tumors with high number of TIL [17]. Another way is to generate T cells with a predetermined antitumor specificity via gene therapy-based approaches. There are two gene modification strategies, including TCR gene transfer and chimeric antigen receptor (CAR) gene transfer, which are used to endow polyclonal T cells with an antigen specificity of choice. We highlight the CAR-T cell therapy in this review.

17.2 Present State of CAR-T Therapy

Genetically modifying T cells with CARs is the most common method of producing tumor-specific T cells. CARs usually consist of an extracellular ligand-binding domain of a single-chain antibody (scFv), a hinge, a transmembrane domain, a cytoplasmic signaling chain, and/or costimulatory molecules. CAR-engineered T cells combine the specificity of mAbs with the homing and killing capacity of T cells. Specifically, CAR-T cell therapy is considered to have several advantages when compared with other cellular immunotherapies. Firstly, CAR-T cells are generated using nonspecifically activated polyclonal T cells. Therefore, they overcome the difficulty of isolation and amplification of natural tumor-specific CD4+ and CD8+ T cells [18, 19]. Secondly, CAR-T cells recognize the target antigens in a MHC-independent manner. This property enables CAR-T cells to recognize target cells with reduced HLA expression or antigen processing, which are considered as an important factor in tumor immunological escape [20–22]. Thirdly, CAR-T cells can home to tumor sites actively and specifically and possess the capacity to expand and persist over a long term after tumor recognition in vivo. Therefore, CAR-T cells targeted to tumor-associated antigens (TAAs) may be more effective than mAbs in producing long-lasting tumor responses [23]. Another particular advantage of CAR-T cells is the capacity to cross the blood–brain barrier [24]. This characteristic is highly useful for treating malignant tumors that involve in or have been transferred to the central nervous system, though adverse reactions relevant to central nervous system must be considered as well.

The concept of the CAR was put forward by Gross and colleagues in 1989, who fused the antibody-binding domain Fab with the TCR signaling domain CD3ζ and named it as T body. Since then,

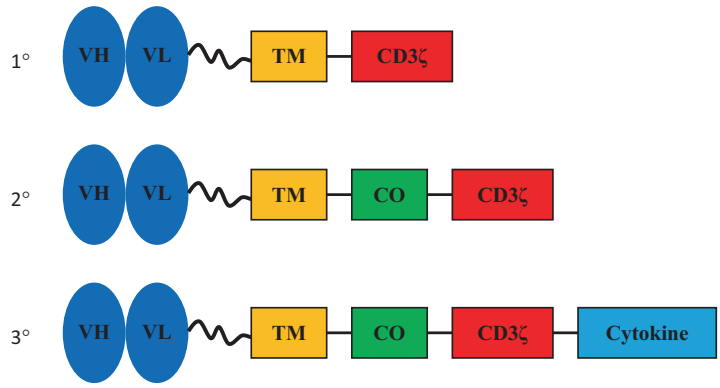

Fig. 17.1 The evolution of chimeric antigen receptors (CARs). CARs are classified into first-generation (one), second-generation (two), or third-generation (three) CARs. Abbreviation: *VH* heavy chain variable region, *VL* light chain variable region, *TM* transmembrane domain, *CO* costimulatory signaling domain

different generations of CAR-T cells have been claimed with confusing definition. In our opinion, based on the three signals required for T-cell activation, which are TCR, costimulation, and cytokine, CAR-T cells could be divided into three generations (Fig. 17.1). The first generation of CARs contained scFv and only a single signaling domain derived from CD3ζ [25]. However, the effect of the first-generation CAR trials was disappointing. Both complete T-cell activation and prevention of apoptosis required a costimulatory signal [26]. The second-generation CARs were subsequently developed, which contained two or three costimulatory signal domains of CD28 and/or 4-1BB, or other costimulatory molecules, to complete the activation signal of the CAR-T cells [27, 28]. The third-generation CARs were embedded into a cytokine cassette which endowed the CAR-T cells with a better function or survival environment. Other features such as migration, homeostatic proliferation, suppression resistance, etc. were subsequently embedded into CAR-T cells, which were described as TRUCK CAR-T cells [29, 30]. For example, the transgenic cytokine IL-12 produced by TRUCK T cells not only improves T-cell activation and modulates the immunological environment but also recruits other immune cells for the fight against those antigen-negative cancer cells that are not recognized by CAR-T cells. Other cytokines like IL-23, IL-27, and IL-15 are alternative payload for TRUCK T cells. In treatment for solid cancer, such TRUCK T cells might have an advantage to modulate the tumor environment, thus enhancing the T-cell antitumor response [31, 32].

T cells engrafted with CAR recognize a wide variety of TAAs expressed on a broad range of tumors, representing both solid and hematologic malignancies. One of the most impressive clinical results ever achieved by CAR-T cells is that polyclonal T cells express CD19-specific CARs with CD28-CD3ζ or 41BB-CD3ζ as signaling domains [24, 33–37]. Complete responses were achieved after infusion of 2nd generation CAR-T cells in patients with CD19+ hematological malignancies including NHL, acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). There are also clinical studies with 2nd generation CAR-T cells specific for the κ-light chain of human immunoglobulin or for CD30. Clinical responses including CRs have been observed [38, 39]. In contrast to B-cell malignancies, clinical experiences of CARs in treatment of T-cell or myeloid-derived malignancies are limited.

17.3 CAR-T Therapy for Breast Cancer: Problems and Solutions

CAR-based therapy for solid tumors involves the use of CARs targeting colorectal cancer [40, 41], ovarian cancer [42], prostate cancer [43], metastatic renal cell carcinoma, and so on [44].

There are also studies targeting HER-2, Lewis Y, mesothelin, folate receptor alpha (FR-α), and Muc1 for breast cancer in vitro and in animal models [28, 40, 45–55]. HER-2 expression is known to impact breast cancer recurrence and ultimately survival [56]. The use of anti-HER2 mAbs has significantly improved breast cancer prognosis. HER-2-targeted therapies are now a main component of HER-2 overexpressing breast cancer treatment [57, 58]. There are several clinical trials of CAR-T cells targeting HER-2 in progress, such as a phase I/II study of HER-2-targeted CAR-T cells in chemotherapy or HER-2 antibody inhibitor therapy for refractory HER-2-advanced breast cancer (NCT01935843) and a phase II study of anti-CD3 x anti-HER2/Neu-armed activated T cells after second-line chemotherapy in women with HER2/Neu (0, 1+ or 2+) metastatic breast cancer (NCT01022138). Moreover, clinical trials of CAR-T-cell therapy targeting other antigens for patients with breast cancer are ongoing, including a phase I study of CAR-T cells targeting cMet, which is aberrant activation in cancer and correlates with poor prognosis, in metastatic breast cancer refractory to at least one standard treatment or newly diagnosed patients with operable triple-negative breast cancer (TNBC) (NCT03060356), and a phase I study of CAR-T cells targeting mesothelin, a tumor antigen associated with TNBC, in metastatic HER2-negative breast cancer (NCT02580747). Despite the successes in treating hematological malignancies, CAR-T cells have encountered significant challenges for treatment of solid tumors [44, 59–62]. Some of the key problems are the rarity of target antigens, limited persistence of the CAR-T cells, inefficient homing of T cells to tumor sites, and less cytotoxicity in the local tumor immunosuppressive microenvironment [63]. The preclinical and clinical studies on treatment for breast cancer with CAR-T therapy are summarized in Table 17.1.

17.3.1 Target Antigen

Antigens currently targeted in clinical studies include HER2, mesothelin, CEA, carbonic anhydrase IX (CAIX), FR-α, CD171, GD2, EGFRvIII, fibroblast activation protein (FAP), and vascular endothelial growth factor receptor 2 (VEGF-R2) [64]. Like other forms of cancer immunotherapy, CARs should ideally target antigens that are expressed only on cancer cells but not on normal tissues. Besides, unlike the

Table 17.1 Preclinical and clinical studies on treatment for breast cancer with CAR-T therapy

Antigen	Gene transfer	Signaling domain	Clinical trial identifier	Phase	References
ERBB2	γ-retrovirus	CD28, 4-1BB, CD3ζ	–	–	[31]
ErbB	Retrovirus	CD28, CD3ζ	–	–	[36]
ErbB2	Retrovirus	CD28, 4-1BB, CD3ζ	–	–	[37]
ErbB2	Retrovirus	CD28, CD3ζ	–	–	[38]
Mesothelin	Lentivirus	4-1BB, CD3ζ	–	–	[51]
Lewis-Y	Retrovirus	CD28, CD3ζ	–	–	[43]
MUCI	Retrovirus	CD28, OX40, CD3ζ	–	–	[55]
FRα	Lentivirus	CD27, CD3ζ	–	–	[46]
Her-2		4-1BB, CD3ζ	NCT01935843	I/II	–
CD3 × HER2			NCT01022138	II	–
cMet	RNA electroporated	4-1BB, CD3ζ	NCT03060356	I	–
Mesothelin	Retrovirus	4-1BB, CD3ζ	NCT02580747	I	–

native TCR, the CARs containing scFv only recognize target antigens expressed on the cell surface, rather than internal antigens which are processed and rendered by the cells' MHC. Consequently, only few solid tumor antigens are available, though numerous antigens are being actively explored for CAR-T cell therapy. An alternative approach is to target antigen-MHC complex, which could make intracellular antigens available, though the generation of this kind of antibody is quite difficult. Conventional T cells only recognize single antigens, but CAR-T cells could be genetically modified to recognize multiple antigens, which should allow the recognition of unique antigen expression patterns on tumor cells. One example is the "split signal CARs," which limit full T-cell activation to tumors that express multiple antigens [43, 65, 66]. Other strategies for recognizing multiple antigens include tandem CARs, ectodomains of which are 2 scFvs [67], and so-called universal ectodomain CARs that incorporate avidin or a fluorescein isothiocyanate-specific scFv to identify tumor cells incubated with labeled monoclonal antibodies [43, 65, 68, 69]. Another possible concern is immune escape. Antigenic shift may cause tumor cells to produce new tumor antigens that may not be identified by the original CAR-T cells. Such escape variants are not rare because most of the cancer cells are genetically unstable [70]. Immune escape, previously described as a drug resistance mechanism in chemotherapy, may become a dilemma in cell-based therapies. The risk of immune escape can be reduced by targeting multiple antigens. Another solution is to target antigens that are expressed on the tumor stroma. The tumor stromal compartment supports tumor growth directly by secreting cytokines and growth factors, providing nutrients, and contributing to tumor-induced immunosuppression [71]. Moreover, tumor stroma is demonstrated to be genetically more stable by studies targeting FAP expressed on cancer-associated fibroblasts or VEGFR-2 expressed on the endothelial cells of the tumor vasculature [72–75].

17.3.2 Persistence

It is important to achieve high levels of CAR-T cells persisting in the peripheral circulation of patients, in order to ensure sufficient cells are available to penetrate into tumor sites. Early trials using the first generation of CAR-T cells targeting ovarian [76] and renal cell antigens [44] indicated that the lack of persistence might be induced by lack of patient preconditioning or anti-CAR immune responses. CARs were then added with costimulatory signals to improve persistence in vivo, particularly when administered to lymphodepleted hosts [36, 77, 78]. Another effort to improve the persistence of CAR-T cells focuses on the range of cytokines that are used to culture the T cells. IL-2 has been selected as an essential cytokine to drive the expansion of T cells in vitro. There are other cytokines including IL-15, IL-7, and IL-21 that can result in cultured T cells preferential to IL-2-expanded T cells. Studies show that IL-15 can promote the proliferation of T lymphocytes, prevent apoptosis and exhaustion [79, 80], reverse anergy [79], stimulate long-lasting antigen-experienced memory cells [81], and overcome Treg-mediated inhibition [82–85]. IL-7 plays an important role in maintaining the homeostasis of mature T cells and the maintenance of memory T cells [86]. Meanwhile, CAR-T cells can be genetically modified to produce cytokines to improve the expansion and persistence in vivo while avoiding systemic toxicity [30, 82, 84, 87]. The function of CAR-T cells may be enhanced not only by adding stimulatory signals (costimulation, cytokines/cytokine receptors) but also by blocking down regulatory signals. Antibodies that block the programmed death-1 (PD-1) receptor or the PD-L1 ligand or the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) have produced encouraging clinical results as single agents [3, 88]. Convincing evidence also demonstrates their benefit for triple-negative breast cancer [89]. The combination of these antibodies with CAR-T cells prolongs the effector function of CAR-T cells at tumor sites, which is a logical evolution of current clinical strategies.

Besides, the source and phenotype of T cells used to generate CAR-T cells will affect the latter's persistence. Selecting T cells that express naive markers such as CD62L before the genetic modification may produce CAR-T cells that possess better persistence ability than effector or more differentiated T cells [90]. Alternatively, virus-specific cytotoxic T lymphocytes (CTLs) have the potential for life-long persistence, and the CTLs contain both CD4+ and CD8+ subsets, with the latter being a critical compartment for the former's long-term persistence [91, 92]. Virus-specific CTLs also feature expression of homing/chemokines receptors commensurate with their capacity for trafficking to and residing in the designated lymphoid or non-lymphoid tissues [93]. Memory T-stem cell could differentiate into memory T cells, leading to a continuous supply of CAR-T cells. On the other hand, hematopoietic stem cells could be engineered with CARs to produce CAR-T cells in a sustained way [94].

17.3.3 Homing

Since the direct binding of tumor antigen is the primary condition of CAR to display its function, the efficient migration of CAR-T cells into tumor sites is essential to the success of the CAR-based therapeutic approach. The success of CAR-T cell therapy for B-cell malignancies is probably caused by the fact that the target B cells are readily accessible to CAR-T cells and express a variety of costimulatory receptor ligands that can promote CAR-T cell function [95]. Chemokines play an important role in the migration of lymphocytes [96], as typified by recent studies [97–99]. However, the chemokine system is complex. Therefore, it is important to develop a strategy to make use of the important homing chemokines and avoid the potential regulatory effect of other tumor-expressed chemokines, in order to achieve efficient targeting of CAR-T cells [17].

17.3.4 Tumor Microenvironment

The tumor microenvironment possesses a variety of pro-tumorigenic and immunosuppressive qualities that are consistent with supporting tumor growth and proliferation and with preventing the antitumor effects of the immune system. The tumor microenvironment comprises several factors such as immunosuppressive cytokines, regulatory modulators, and coinhibitory receptors [100]. The immunosuppressive cell populations include regulatory T cells, immature myeloid cell populations, and tumor-associated macrophages [9, 101–103]. As highly complex interactions among different components in the tumor microenvironment contribute to clinical outcomes, CAR-T cells must be armed and thrive in the environment. Genetically engineering of the CAR vector to include dominant negative TGFβ receptors to overcome the adverse effects of tumor-derived TGFβ [104], and to adopt knockdown strategies to avoid apoptosis mediated by Fas/Fas ligand [105] or the expression of survival genes such as BCL-XL [106], may protect the CAR-T cells against the tumor immunosuppressive microenvironment. Besides, transgenic expression of cytokines such as IL-15 or IL-12 can reverse the immunosuppressive tumor environment. In an alternate strategy, silencing of genes that inhibit the function of T cells in the tumor microenvironment or the transgenic expression of constitutively active signaling molecules may improve CAR-T cell function [105, 107]. Lastly, a combined treatment of agents that propagate cell-based immunotherapies and agents that circumvent antitumor mechanisms may be beneficial for CAR-T cells to overcome the tumor microenvironment.

17.4 Toxicities and Management

As the potency of CARs was enhanced, toxicity induced by this immunotherapeutic approach was unfortunately observed. The continued expansion of CAR-T cells implies that the

associated toxicities may show corresponding persistence and deterioration with time. "On target, off tumor" toxicity is currently a major concern, which results from the activation of CAR-T cells by targeting antigen within healthy tissues. This is a well-recognized phenomenon and has led to several different side effects. Prevention of on-target toxicity requires accurate selection of antigens that are more restricted in their expression. Another approach is to infuse CAR-T cells with transient expression of the CARs only. Thus, the expression level decreases with the cell division, and the transcription becomes diluted gradually [108–110]. Another well-documented clinical side effect is systemic inflammatory response syndrome (SIRS) or cytokine storm, which is driven by a variety of cytokines, including IFN-γ, TNF-α, IL-2 [33, 77], and the most important IL-6 [24]. To reduce the onset or severity of SIRS, researchers are modifying the dose escalation of T cells and have introduced the prompt use of antibodies that block the effects of IL-6. In addition, there are genetically modified T cells expressing a suicide or safety switch along with the CAR. These cells would retain their long-term expansion and expression capacity, but could be eliminated by activating the suicide genes once toxicity occurs [111–113]. Although the expression of multiple CARs in T cells is likely to increase safety [43, 65, 66], it remains to be proved whether the benefits can be summarized within heterogeneous human malignancies, as the patterns and levels of antigen expression may vary between different malignancies.

17.5 Universal CAR-T Cells

The current standard CAR-T cell therapy requires autologous adoptive cell transfer, which is expensive and time-consuming. For newborns and elder patients, it is often difficult to obtain enough T cells with good quality to generate patient-specific CAR-T cells. To make CAR-T therapy more accessible, it is highly desirable to develop an allogeneic adoptive transfer strategy, in which universal CAR-T cells derived from healthy donors can be applied to treat multiple patients circumventing the inherent variability of individualized patient. For this strategy to work, human leukocyte antigens class I (HLA-Is) on CAR-T cells need to be removed to minimize their immunogenicity, and the T-cell receptor (TCR) on allogeneic CAR-T cells needs to be eliminated to avoid graft-versus-host disease (GVHD) [114]. There have been studies to efficiently generate CAR-T cells with TCRα subunit constant (TRAC) and beta-2 microglobulin (β_2M) genes disrupted. However, these TRAC/β_2M-negative CAR-T cells need to be further tested for their efficacy and safety in clinical studies [114–116].

17.6 Combinatorial CAR-T Cell Therapy

It may be better to fight a war with a well-orchestrated army than a "single bullet," so combining CAR-T cells with other therapies offers the potential to improve antitumor effects. For example, combining blocking antibodies (CTLA-4, PD-1, and PD-L1) to the coinhibitory receptors, epigenetic modifiers that upregulate the expression of TAA [117], or targeted therapies that inhibit tumor cell growth without impairing T cells may be beneficial [118]. In the future, experimental treatment will be needed to determine how the CAR-T cell approach will be combined with other therapies for solid tumors, such as breast cancer.

17.7 Conclusions

The general concept of CAR-T cell was invented about 20 years ago. CAR-T cells are changing from being simply "promising" to being "effective" regimens for treating hematological malignancies. As we continue to improve the function of CAR-T cells in tumor microenvironment, broader application can be expected beyond hematological tumors and into solid tumors. Clinical trials comparing different genetic modi-

fication strategies will be important in the future for optimizing CAR-T cell therapy, which would be a potentially effective method to cure breast cancer disease.

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