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Optimizing CAR-T cell therapy in solid tumors through multi-target and vaccine strategies

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Abstract. Chimeric antigen receptor (CAR) T cells can be used to treat B-cell malignancies and represent a novel approach in cancer immune cell therapy. Although the therapy has shown significant clinical success in treating B-cell malignancies, its efficacy in solid tumors is limited by challenges such as tumor antigen heterogeneity, antigen loss, and immunosuppressive tumor microenvironment. This review understands the shortcomings of CAR-T cell therapy and proposes several optimization strategies to face different dilemmas in treating tumors. Targeting fibroblast activation protein (FAP) to suppress the TME; RNA vaccines that can deliver specific antigens to lymph nodes to activate and expand CAR-T cells to enhance their immune response against solid tumors; and multi-targeting design to target multiple antigens at the same time to effectively mitigate tumor escape due to antigen loss. In addition, this review enumerates the strategies of combining therapies with chemotherapy, radiotherapy, immune checkpoint inhibitors, and co-stimulatory molecules to improve the durability and targeting precision of CAR-T cells and reduce side effects. Thus, this review demonstrates a series of optimized uses of CAR-T cell therapies and improves the efficacy of CAR-T cells in solid tumors. This has important clinical implications for advancing cancer immunotherapy and driving the development of immune cancer in the future.

Keywords: CAR-T Cell Therapy, Cancer, Antigen, Immunosuppression, Microenvironment.

1. Introduction

Cancer is mainly public health problem in the worldwide [1]. A multitude of causes, including the aging of the world population and environmental changes, are projected to contribute to the expected continued rise in cancer incidence in the ensuing decades. It is anticipated that the number of new cancer cases annually would surpass 20 million by 2025 [1]. Instead of being a singular illness, cancer is the umbrella term for a wide range of conditions that are all characterized by the unchecked growth of altered cells that arise as a result of natural selection [2]. Because of the aberrant cells' unchecked proliferation, cancer can spread to any area of the body.

The nature of cancer is that they will lose their original growth inhibition mechanism when the increasing of cells surpasses the normal range of controlling, infiltrate nearby tissues, and proceed to move to other organs via the lymphatic or circulatory systems. Malignant tumors are the result of cancer's unchecked growth and capacity to spread, which transforms the disease from a localized to a systemic one. The name "malignant" refers to these tumors because they not only disregard the body's

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warnings and keep growing, but they also invade and kill nearby healthy tissue. Cancer eventually rises to the top of the global death toll due to its multiplication and tissue damage [3].

The burden of cancer remains a significant concern for both developed and developing economies, even though overall cancer incidence and mortality rates have been gradually declining in developed nations like the United States over the past ten years as a result of new research on tumor mechanisms, improved diagnostic techniques, and innovative therapeutic approaches [4]. Thus, cancer still poses a serious threat to human health [4]. Thus, resolving the issue of tumor-related illnesses can significantly enhance people's health and increase their average lifespan. Malignant tumors are the foundation of research cancer treatment options that cannot be disregarded. Cancer has grown to be a significant worldwide health concern.

Immunotherapy, particularly CAR-T cell therapy, has shown to be remarkably effective in treating hematologic cancers, demonstrating strong, long-lasting anti-tumor effects. However, there are still many important obstacles to overcome before immunotherapy can be used to solid tumors. First, the distinct TME of solid tumors is primarily responsible for their complexity. Strong immunosuppressive mechanisms and characteristics such as antigenic heterogeneity and antigen loss are present in this milieu, significantly reducing CAR-T cells' therapeutic impact [4]. The second reason is that the diverse antigen distribution in solid tumors suggests that certain tumor cells may still be able to evade treatment, even if CAR-T cells can identify some of the tumor cells. Apart from the unique milieu of solid tumors, CAR-T cell treatments encounter many hindrances while treating different types of cancer. On the one hand, the treatments' potentially fatal severe adverse effects restrict their applicability and safety. However, in some patients, CAR-T cells' anticancer activity is milder, making it challenging to eradicate tumors entirely [5]. Further limiting the effectiveness are antigen escape, limited CAR-T cell movement, and limited tumor invasion. The intricate interplay between the host immune system and the tumor microenvironment further impacts CAR-T cell activity [5].

Therefore, based on the ongoing research on CAR-T cell therapy, a combination of new technologies and approaches could be more effective in overcoming these challenges and developing therapeutic strategies to increase anti-tumor activity while reducing its toxicity and side effects on the body. Strategies that can be employed to optimize this novel therapeutic strategy and overcome these obstacles include multi-target design, vaccination strategies, fibroblast activating proteins (FAPs), combination therapies, and costimulatory molecules. tumor molecules in the TME that influence CAR-T cell efficacy through the study of effective cancer targets, as well as the augmentation of immune cells. reduce escape from antigen loss. In addition, RNA vaccines can enhance the detection and killing ability of CAR-modified T cells in solid malignancies by providing tumor-specific antigens. Using multi-target design and RNA vaccine technology, this review aims to optimize CAR-T cell therapy in solid tumors by addressing the challenges of immunosuppression and antigenic heterogeneity in the TME. The shortcomings of existing CAR-T therapies in solid tumors will first be explored, followed by a look at the potential application of future combination therapies in the treatment of these tumors. The implementation of these optimized techniques will be examined next.

2. Mechanisms

2.1. Characteristics and immunosuppressive environment of solid tumors

In solid cancerous growth, the organization of the TME is highly structured and plays a crucial role in the tumor's immune escape mechanisms. Solid tumors are embedded in a complex network of non-tumor cells within the TME, which facilitates immune evasion and prevents the immune system from identifying and eliminating the tumor [6, 7]. Solid tumors consist of more than merely cancerous cells. The immunosuppressive properties of the TME are the main way solid tumors avoid immune responses.

The local environment around malignant and non-cancerous cells is known as the tumor microenvironment (TME); it is primarily self-regulated by the tumor [8,9]. Blood arteries, inflammatory cells that have spread throughout the body, developing tumor cells, tumor stroma, and other pertinent tissues comprise the TME [8]. In addition, the TME harbors a wide variety of innate immune cells that

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mediate adaptive immunological responses, such as macrophages, polymorphonuclear leukocytes, and natural killer (NK) cells; moreover, T lymphocytes, dendritic cells (DCs), and infrequently B cells are found there [8]. Although the immune system uses these cells to fight cancer, the suppressive environment within the TME makes it more difficult for them to do so. Rather, it encourages the growth of cancers. This immunosuppressive environment not only reduces the effectiveness of conventional therapies but also promotes immune escape from the tumor. The TME's cells and the chemicals they release are now being utilized as targets for cancer treatment because it is now believed that they are essential to the pathophysiology of cancer [10].

The immune escape mechanism of solid tumors provides more insight into the process of immunosuppression in TME. The purpose of the human immune system is to maintain homeostasis and shield the body against outside pathogens. Usually, it accomplishes this by carefully controlling immune responses through diversity and adaptability [6]. Even though adaptive immune cells are able to recognize and eliminate cells expressing non-self-antigens, cancer cells manage to evade the immune system through a number of complex strategies and ultimately grow into full tumors. Immunological checkpoints, a mechanism that prevents the immune system from attacking healthy cells, have been overrun by tumor cells. As a result, the tumor cells can avoid being found and eliminated by T cells [11]. These systems allow tumors to persist in the host for extended periods of time and continuously elude the immune system's surveillance.

Through immunological and antigen editing, cancer cells can progressively become immune-evading [12]. Cancer cells alter their own antigens through antigen editing in the early stages of a tumor in order to evade the immune system's detection and attack. In contrast, immunoediting consists of three stages: escape, equilibration, and elimination. Most tumor cells are identified and eliminated by the immune system during the elimination stage; any cancer cells that remain will move on to the equilibrium stage. Even though the tumor cells are still present at this time, they are increasingly able to withstand more immune onslaught by downregulating the production of their antigen. Ultimately, these cells go into the escape phase, where they successfully elude immune system surveillance by up-regulating immune checkpoint inhibitory ligands or down-regulating *MHC-I* molecules. After this, they multiply swiftly to create clinically detectable tumors [12]. Immune escape mechanisms, therefore, pose a severe therapeutic challenge to cancer patients and are essential to the survival and metastasis of malignancies. Efficient immunotherapies, including CAR-T cell therapy, have the potential to successfully limit tumor growth in two ways: by rerouting tumors from the escape phase to the elimination phase and by reactivating the immune system [12]. However, immunotherapy will not stop the tumor from expanding and moving forward into the challenging escape phase. Therefore, understanding the immune escape mechanism is essential to developing more effective treatments.

2.2. Working Mechanisms of Car-t Cell Therapy

An effective novel immunotherapeutic strategy is the use of chimeric antigen receptor (CAR) T cells, which can address the problems posed by immune escape and the TME [13]. It works on the principle that T cells are transduced on chimeric antigen receptors specific to tumor-associated antigens (TAA) to produce CAR-activated T cells [13]. The extracellular antigen-binding structural domain, the transmembrane structural domain, and the intracellular signaling structural domain constitute the three structural domains of CAR. The intracellular signaling structural domain is activated when CAR-T lymphocytes attach to antigens of malignant cells. During this process, T cells multiply, and cytokines kill and protect healthy cells from cancer [14]. As a result, cytotoxic T lymphocytes (CTL) from the patient are converted into effector cells by CAR-T cells, which recognize specific cancer antigens and directly target cancer cells [14]. The main advantage of CAR-T therapy over conventional drugs is that it identifies and destroys tumor cells that evade observation by the host immune system while eliminating the need for traditional antigen presentation pathways. Cancers related to the hematologic system include multiple myeloma, diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), and, more recently, solid tumors that have shown a favorable response [14]. This

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novel cell therapy provides new advances in combating tumor immune evasion and holds promise for treating these related cancer diseases.

Although CAR-T cell immunotherapy has been approved for the treatment of patients with relapsed and refractory multiple myeloma or B-cell malignancies, in reality, it still has several pending issues and obstacles to be addressed in the face of research into therapeutic approaches for solid tumors [13,15]. One of the main problems with CAR-T cell therapy is antigen loss, which means that the cells may lose the antigens the CAR targets. Since cells are designed to recognize and attack specific antigens on the surface of cancer cells, if these antigens are eliminated or reduced, the cells' ability to identify and destroy cancer cells is compromised [14]. Therefore, the process of antigen loss or reduction and the resulting decreased capacity to target cancer cells may considerably limit the efficacy of CAR-T cells in the therapy of solid tumors that refuse to go away. Furthermore, CAR-T cell depletion and malfunction may result from extended exposure to the TME and persistent antigenic stimulation, which may further reduce the cells' capacity to mount a potent anti-tumor response.

Research addressing this issue has shown that vaccinations, when combined with CAR-T cell treatment, can successfully limit the formation of antigen-negative tumors by triggering an endogenous immune response in the patient [16]. Vaccines boost the anti-tumor coverage and the effectiveness of CAR-T cell attacks on tumors by inducing the patient's immune system to combat new tumor antigens. This "antigen spreading" phenomenon provides a fresh way to treat CAR-T patients in the face of tumor heterogeneity and antigen loss [16]. Based on data from the few successful CAR-T cell therapy trials, the most promising strategies include using pluripotent stem cells, co-targeting two or more immune evasion mechanisms, using multiple co-stimulatory domains, and using CAR ligands to target vaccines [15].

3. Optimizing the use of CAR-T cell therapy

3.1. Fibroblast activation protein (FAP)

FAP is a membrane-bound type II protein that is a member of the prolyl oligopeptidase family and the dipeptidyl peptidase (DPP) subfamily [13]. The serine protease-like enzyme fibroblast activation protein (FAP) is overexpressed by fibroblasts associated with cancer-associated fibroblasts (CAFs) in a variety of tumor types [13]. FAP is thought to be a potent therapeutic target when it comes to cancer treatment. TME cells include immune cells, the basement membrane, the tumor vasculature, and CAFs. These cells might be malignant or non-malignant. FAP is widely expressed in CAFs and contributes to immune suppression, invasion, angiogenesis, and tumor formation. TME-targeting immunotherapy is considered an essential treatment approach for TME-induced chemoresistance and tumor recurrence, two issues that conventional therapies cannot successfully handle [13]. Genetic engineering and CAR-T cell therapy have made it possible to treat hematological malignancies with T cells specifically targeted against tumor antigens [13]. It is anticipated that by eradicating FAP-positive CAFs in TME, CAR-T cells targeting FAP will reduce immunosuppression and improve anti-tumor responses in solid tumors [13].

FAP is a marker produced specifically for CAFs. They are genetically more stable in the TME compared to cancerous cells. Finding and targeting specific tumor antigens that are consistently expressed in TME is one of the major challenges for genetically modified T cell therapy [13]. FAP has been a major target for tumor immunotherapy in recent years due to its specificity as a marker for CAFs. This is because FAP is under-expressed in normal tissues, and thus targeting FAP can improve the specificity of targeted T cell therapy and minimize the impact on healthy cells, thereby improving the safety and duration of treatment. In addition, preclinical studies have shown that CAR-T cells targeting FAP can effectively remove FAP-positive tumor cells and extend the lifespan of experimental animals. For example, Schuberth et al. showed that CAR-T cells with FAP could be used for the first time in a malignant pleural mesothelioma (MPM) model [13]. Their study showed that CAR-T cells with FAP significantly prolonged the survival time of MPM mouse models and successfully eliminated FAP-

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expressing MPM cell lines. These results suggest that FAP-targeted CAR-T therapy can eliminate many cells in TME and inhibit it, thereby improving the overall antitumor efficacy.

Targeting FAP-positive stromal cells may increase the use of CAR-T cells even if FAP is only expressed in a small percentage of cancer cells. Cancer-associated fibroblasts, or CAF, encourage the formation of tumors; hence, removing FAP-positive CAF may enhance the prognosis of patients. For the first time, Kakarla et al. discovered that FAP CAR-T cells increased survival in a rat lung cancer model and effectively destroyed FAP-positive cells by producing proinflammatory factors [13]. FAP-positive cells and extended survival in a model of lung cancer in mice, and co-targeting additional antigens could increase the effectiveness even further [13].

Subsequent research has also shown that FAP CAR-T cells effectively reduce tumor development in a range of cancer types without causing appreciable damage [13]. Nevertheless, some research indicates that FAP CAR-T cells may cause osteotoxicity, therefore their use should be exercised caution [13]. Moreover, it has been discovered by researchers that utilizing CAR-T cell co-stimulatory structural domains, including CD28, can greatly enhance tumor clearance [13].

3.2. RNA Vaccine Enhanced CAR-T Cells

Although CAR-T cell therapy has shown impressive clinical results in B-cell malignancies, its effectiveness in patients with solid tumors is more limited. This is mostly because tumor targets are not unique to CAR-T cells, and TME inhibits antigen-specific T cells. In response to these problems, scientists created an RNA vaccine in the form of nanoparticles that, by transferring CAR antigens to lymph nodes, stimulates CAR-T cell activation and proliferation [16]. By using antigen-presenting cells (APCs) to exhibit certain tumor antigens, this RNA vaccine lessens systemic toxicity while assisting CAR-T cells in accurately identifying and attacking tumor targets [17].

In this context, *Claudin 6* (*CLDN6*), a tetra-transmembrane protein that is highly specific and expressed in a variety of solid tumors, has been selected as a novel target for CAR-T cell therapy. *CLDN6* is barely expressed in healthy adult tissues, thus its high expression in cancer makes it an ideal target [17]. To assess its potential, researchers designed a second-generation *CLDN6*-CAR incorporating the 4-1BB co-stimulatory structural domain in combination with a nanoparticle RNA vaccine (RNA-LPX) delivering *CLDN6* antigen to further enhance CAR-T cell efficacy [17]. In a mouse model, RNA-LPX successfully delivered *CLDN6* antigen to the surface of APCs in the spleen and lymph nodes, which activated and proliferated *CLDN6*-CAR-T cells, displaying a strong immune response, including a significant increase in *IFN-γ* secretion, as well as effective recognition and killing of *CLDN6*-positive tumors [17].

In human healthy adult tissues, the *CLDN6* gene is tightly silenced, but in a variety of solid malignancy that require medical attention, it is aberrantly activated and results in high protein level expression. This makes it an excellent target for CAR-T cell-based therapy for solid malignancies, especially when combined with the possibility of creating a strong CLDN6-guided CAR with high sensitivity, exact specificity, and targeting of this surface molecule. A tumor with uneven *CLDN6* expression is less able to produce antigen-losing variants. On the other hand, potent effector cells that release *IFN-γ* are activated CLDN6-CAR-T cells. Therefore, it is thought that their anticancer action causes the release of endogenous tumor antigens and inflammatory remodeling of the suppressive TME, which in turn promote the distribution of antigens and slow down the rapid development of antigen-losing variants [17].

This strategy demonstrates how RNA vaccines can improve CAR-T cell targeting, persistence, and ability to overcome substantial hurdles in solid tumors, hence greatly enhancing CAR-T therapy.

3.3. Multi-targeting Design

Multi-antigen-targeted CAR-T cells reduce the escape problem associated with antigen loss by attacking many antigens simultaneously. One important challenge to CAR-T treatment is antigen loss [18]. Moreover, producing a significant number of multifunctional CAR-T cells that can reliably target and eradicate tumor cells over time is the primary objective of T-cell-based cancer therapy. Furthermore, by

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adding particular genetic modifications in T cells, the persistence and preventive hypofunction of CAR-T cells can be addressed [15]. A logical solution to the issue of antigen loss following CAR-T cell therapy is to target several antigen receptors. There are four possible ways to go about doing this: Co-administration refers to the process of creating multiple cell populations that express distinct CARs and combining them or using a double cis-trans vector to express two CARs on the same cell. Co-transduction involves transforming T-cells with two CAR constructs at the same time, resulting in the formation of three CAR-T subpopulations: dual-CAR-expressing cells and single-CAR-expressing cells. Finally, co-transduction involves using a single vector to express two CARs on the same chimeric protein, also known as dual-specific or tandem CARs [18]. One of the newest multi-targeted CAR structures, the Dual-CAR structure, targets two different antigens on tumor cells. CAR-T will be more effective and safe if it targets several antigens [19]. Furthermore, it has proven to be an effective treatment for adult patients who have relapsed or are refractory to B-cell acute lymphoblastic leukemia (R/R ALL) [19]. Furthermore, compared to traditional varieties, dual CAR-T cells demonstrate substantial cytokine production, high persistence of CAR-T cells, decreased "on-target/off-tumor" toxicity, and tailored and easily managed anticancer efficacy [19].

3.4. Combination Therapy

Solid tumors may not respond well to monotherapy due to antigen escape. Therefore, advances in CAR-T cell combination therapy and other treatment modalities offer hope for more successful cancer treatment, especially for patients with solid tumors [20]. Combination therapies have greatly reduced the negative effects of the novel therapy while improving efficacy. Multiple therapeutic approaches can be used to alter TME, enhance the structure of the CAR, and establish a link between tumor cells and CAR-T cells. Combination therapeutic approaches that can be used in conjunction with CAR-T cell therapy to target multiple antigens, minimize therapeutic toxicity, circumvent tumor immune escape mechanisms, and improve the efficacy and safety of CAR-T cells include checkpoint inhibitors, cytokines, radiotherapy, chemotherapy, immunomodulators, hematopoietic stem cell transplantation (HSCT), lysogenic viruses, cancer vaccines, and metabolic inhibitors [20]. For example, combining chemotherapy and CAR-T cell therapy increases the survival of CAR-T cells in vivo. It maximizes the benefits of both therapies by reducing autoimmune and immunosuppressive cells. Past studies by researchers have shown that immunosuppressive cells are more susceptible to the effects of chemotherapy drugs than normal T cells, even though these therapies are safe for T cells. By striking the right balance, chemotherapy may enhance the immunogenicity of T cells when administered before other treatments [20]. In addition to this, chemotherapeutic agents such as cyclophosphamide, adriamycin and fluorouracil may enhance the efficacy of CAR-T cell therapy [20].

Additionally, combining radiotherapy with it improves anti-tumor effects [20]. Radiation inhibits distant cancers and induces immunity to malignancy by activating local cytotoxic T lymphocytes (CTL) [20]. Radiation therapy promotes MHC class I expression, which optimizes cytotoxic T-cell recognition by improving peptide production and antigen presentation, aiming to enhance the efficacy of local and distant dominant CTL immunotherapy. CAR-T cell therapy also enhances T-cell responses. In addition to inducing the release of pro-inflammatory cytokines such as *IL-6*, *IL-1α/β*, *IFN-α/β*, and tumor necrosis factor α (TNF-α), radiotherapy stimulates the release of DAMP and *IFN-γ*, which results in the entry of immune effector cells into the TME and enhances their activity [20].

Checkpoint signaling functions as a regulatory mechanism to balance the immune response and avoid autoimmunity by blocking excessive immunological activity. Immune checkpoint inhibitors (CPIs) are a class of highly effective and novel immunotherapies that enhance immune system performance and encourage immune cell infiltration and persistence.

Combining the therapy with anti-PD-1/PD-L1 antibodies, also known as PD-1/PD-L1 blocking antibodies, can improve efficacy. This is because *PD-1*/*PD-L1* can unlock the immune system's inhibition of tumor cells, thereby enhancing the tumor's defense against the immune system. Studies have shown that the anti-PD-1 monoclonal antibody Pembrolizumab can be combined with CAR-T cells to restore specific immune responses, including the production of *TNF-α* and *IFN-γ*, albeit with a weaker

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effect of *IL-2*. The combination therapy also improved the robustness and functionality of CAR-T cells in melanoma patients. A different study showed that the combination of anti-Her-2 CD8 + T cells and anti-PD-1 antibodies improved T cell killing and resulted in more successful tumor clearance in a mouse model of breast cancer. However, using PD-1 blockers in systemic therapy may result in reduced efficacy and increased treatment costs because the drugs may not be able to access the TME, which is the unique environment surrounding the tumor. In addition, systemic use of PD-1 blockers may result in several side effects, including hyperactivity of T-cells, which may affect multiple organs and produce problems such as kidney failure and abnormal thyroid function. Researchers have suggested using CAR-T cells to direct PD-1 blockers to the tumor site and minimize systemic side effects while improving efficacy [20].

3.5. Costimulatory molecules

CD28 and 4-1BB are two key signaling molecules that enhance cellular activity in CAR-T cell therapy [20]. It has been shown that CAR-T cells containing 4-1BB are less depleted and express fewer immunosuppressive chemicals, thus targeting tumors for longer. On the other hand, CD28 is more likely to lead to cellular depletion and the appearance of inhibitory chemicals, making the cellular function decline faster. Therefore, 4-1BB is a better choice for enhancing CAR-T therapy's efficacy than CD28 [20].

4. Conclusion and Expectation

CAR-T cell therapy has shown remarkable clinical results and can effectively treat B-cell malignancies [1, 2]. However, the efficacy of thisl therapy has been considerably lower than expected and still poses several therapeutic barriers when confronted with solid malignancies. Examples include limited targeting, immunosuppressive TME, antigenic heterogeneity, T-cell fatigue, and impaired infiltration [3, 17]. These problems make CAR-T cells less efficient at penetrating tumors and eliminating tumor cells. T cells must pass through the immunosuppressive impact of TME to enter and function inside the tumor, which is primarily responsible for the complexity of solid tumors. During this process, tumor endothelial cells emit chemokines, which T lymphocytes first recognize to infiltrate the tumor tissue [15]. Lack of T cell adhesion, mismatch issues between chemokines and their receptors, and the extracellular matrix's physical barrier impact typically make this process ineffective [17].

Multi-target CAR-T cell design has been considered as a strategy to cope with antigen loss. However, multi-target design does not fully address all the problems in solid tumors, such as immune escape or tumor heterogeneity [15, 18]. Although tumor recognition by CAR-T cells can be improved by targeting multiple antigens, this approach is also challenged by safety and manufacturing costs. Multi-targeting designs may lead to more potent cytokine release syndrome (CRS) and increase the therapeutic burden [19].

Once T cells enter the tumor, their main task is to traverse the tumor stroma, a process that requires overcoming the physical barriers provided by perivascular cells and the extracellular matrix [17]. Even if a small percentage of T cells manage to enter the tumor interior and bind to ICAM1 on tumor cells, most T cells fail to function properly due to issues with chemokine-receptor matching and adhesion, resulting in an inefficient immune response [15,17]. To address these challenges, researchers have developed several innovative strategies. First, RNA vaccines (such as RNA-LPX) deliver specific tumor antigens (e.g., *Claudin 6*) to lymph nodes, activating and expanding CAR-T cells [16]. By displaying tumor-specific antigens on antigen-presenting cells (APCs), RNA vaccines not only enhance the targeting of receptor-engineered T cells but also significantly enhance their persistence in the TME [17]. In addition, targeting FAP in CAFs has been demonstrated to be an effective therapeutic strategy for immune cancers. CAR-T cell therapies have been genetically engineered to accurately target FAP in solid tumors to reduce tumor proliferation and suppression of immunity induced by the high expression of FAP, thereby enhancing anti-tumor responses [13]. Antigen loss is resolved by multi-targeting design, which allows CAR-T cells to target and recognize numerous tumor antigens at once, decreasing the possibility of tumor escape and increasing therapeutic efficacy [18, 19]. I Additionally, immune

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checkpoint inhibitors, radiation, and chemotherapy combined with other treatments can improve the anti-tumor effectiveness of CAR-T cells. For instance, PD-1/PD-L1 inhibitors aid in regaining the immune system's capacity to combat malignancies, whereas radiation also enhances the immune response to both local and distant tumors [20]. CAR-T cell depletion can be better averted and its functional lifetime maintained by optimizing the choice of co-stimulatory molecules, using 4-1BB instead of CD28, minimizing the effect of CD28 on the body, and utilizing 4-1BB [20]. Integrating these strategies not only overcomes the significant obstacles of CAR-T therapy in solid tumor treatment but also dramatically improves its efficacy, providing a broad prospect for future clinical applications. It also provides an upward step for people to study immune cancer-related fields afterward.

Although CAR-T cell therapy has made significant progress in treating solid tumors, it is still confronted with these diseases' adverse effects. Cancer patients' lives may be at jeopardy if the issue of immune tumors is not promptly resolved. Researchers are concentrating on CAR-T cell-targeting clinical trials, cell persistence, and immunosuppression of the TME and heterogeneity as they continue their research and development. It is essential to address these problems with concentrated attention and research in order to enable a wider deployment of global CAR-T treatments. Patients with solid tumors have a greater chance of achieving a long-term remission or even a cure thanks to the development of CAR-T cell therapies, which are especially optimized by the integration of multi-target design, RNA vaccines, and other multiple therapeutic techniques. Future developments in research, innovation, and clinical validation will allow CAR-T cell therapy to assume a more significant position in cancer immunotherapy.

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