

Progress and Prospects of CAR-T Therapy for the Treatment of Solid Tumors

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Abstract. CAR-T therapy utilizes cytokines, cytotoxic particles, and ligand receptors in order to eradicate malignant cells. The objective may be accomplished by either directly inducing tumor cell death or indirectly augmenting the immune response against these cells. Recent days, in treating hematological malignancies, CAR-T therapy demonstrated high efficiency and multiple successful clinical applications. However, the available literature reveals a scarcity of research regarding the impact of CAR-T therapy on solid tumors although they account for about 90% of the cancer cases in the adult population. Solid tumors present challenges to CAR-T therapy because of trafficking or infiltration issues, tumor antigen heterogeneity, and an immunosuppressive microenvironment (TME). This review aims to assess these obstacles and explore various techniques and innovative approaches developed to address these limitations. Moreover, in this review, we review the structure and mechanism of CAR-T and further discuss the potential future applications of CAR-T therapy in treating solid malignancies.

Keywords: CAR-T cell therapy, solid tumor treatment, immunotherapy.

1. Introduction

Cancer has emerged as a very lethal disease over the course of human history. There are two distinct categories of tumors: solid and liquid. Solid tumors however, account for 90% of cancer cases in adult populations [1]. Numerous conventional therapeutic approaches, including chemotherapy, radiation, and surgical interventions, have been used to address the formidable challenge of cancer. Nevertheless, all of them had restricted outcomes. In recent years, there has been a shift in the scientific emphasis towards the immune system. Researchers have found various ways to augment the immune system to combat and eradicate cancer. This particular kind of medical intervention is often referred to as immunotherapies.

One of the immunotherapy that has displayed promising results against cancer is CAR-T cell therapy. In this method, clinicians collect the T cells derived from the patient and engineered it to eradicate cancer [2]. These autologous T cells are transfected and express specific receptors that can bind to the proteins on tumor cell membrane [2]. This interaction activates killing mechanisms of CAR-T cells such as triggering ligand receptors, and secreting cytokines and cytotoxic molecules. Current CAR-T therapy limitations for both hematologic and solid malignancies are the emergence of grafts versus host disease, neurotoxicity, and CRS. There are also on-target off-tumor effects, trafficking or infiltration issues, and antigen escape.

Currently, CAR-T cell therapy has demonstrated spectacular results for hematologic malignancies treatment like treating leukemia, lymphoma, and multiple myeloma. Therefore, researchers are beginning to concentrate on the treatment for solid tumors. This review examines CAR-T therapy's current advances and limitations for treating solid cancers. Additionally, it explores recent preclinical endeavors undertaken by researchers to address these problems and provides an outlook on the potential future developments of CAR-T therapy for solid tumors.

2. The structure of CAR

2.1. Antigen binding domain

This domain is responsible for recognizing specific antigen of CARs. This region comprises a combination of V_H and V_L chains of mAbs, which composes scFv [3]. The ScFvs bind to receptors expressed on tumor cells. The outcome of this interaction leads to T cell activation that is not reliant on the MHC.

The features of ScFv, including affinity, avidity, aggregation tendency, and the position of its antigen epitope, are important parameters that may impact the function of CAR. The modulation of affinity and avidity in ScFv enhances the ability to selectively recognize target cells with a dense expression of specific ligands to alleviate on-target off-tumor effects [4]. Meanwhile, the assembly of scFv is accountable for tonic signaling–antigen-independent signaling – and may arise from diminished folding stabilities within the V_H and V_L domains [4]. Finally, the placement of epitopes may influence the functional responses of CAR-T cells. For example, CARs that use scFv to target MSLN molecule regions that are membrane-proximal exhibited enhancement in cytotoxicity and cytokine production in contrast to CARs targeting membrane-distal epitopes [4]. This phenomenon was attributed to the inflexible configuration of the membrane-proximal area, which enhanced signal transduction [4].

2.2. Hinge region

This region is considered as the bridge between the antigen-binding domain and the transmembrane protein [5]. The incorporation of this region in CAR provides flexibility to the scFv, which improves the ability of CARs to recognize target membrane receptors that are sterically inaccessible [5]. It also contributes to the length that facilitates binding between the antigen-binding domain and target epitope. Selected hinge regions for CAR manufacture have significant influences on the functionalities of CAR because of its length and composition, which can affect CAR expression, flexibility, signaling, and epitope recognition [5].

Hinge lengths can significantly influence cytolytic activity and signaling capabilities of CAR-engineered cells [5]. Hence, it is essential to have an adequate intercellular distance between antigen-binding domain and transmembrane protein to facilitate the establishment of immunological synapses. Theoretically, the ideal hinge length is contingent upon the target epitope location and its steric hindrance [5]. Long hinges provide more flexibility, enhancing accessibility to epitopes located in close proximity to the membrane [5]. Meanwhile, shorter hinges demonstrate greater efficacy in binding epitopes that are remote to the membrane [5]. In practical applications, the appropriate length of the hinge is often found by empirical methods and customized for each pair of antigen-binding domains.

The constituents of hinges are often sourced from the immunoglobulin G (IgG) family or the co-receptor of T lymphocytes (CD4/CD8). The four subclasses of immunoglobulin G (IgG), including IgG1, IgG2, IgG3, and IgG4, exhibit variations in their constant region [6]. These variants have discernible effector functions in relation to their capacity to stimulate cells expressing $Fc\gamma R$, including macrophages, neutrophils, dendritic cells, and NK cells. The aforementioned activation gives rise to antibody-dependent cell-mediated cytotoxicity, a process that potentially leads to CAR-T cells exhaustion [6].

Therefore, it is crucial to take into account the length and composition of hinges during the engineering process of CAR-T cells.

2.3. Transmembrane domain

Transmembrane domains are integral components of CAR structures, which function to anchor the extracellular CAR domain firmly to the T cell's membrane. The structure and composition of this domain is responsible for membrane stability via hydrophobic alpha-helix structure and expression levels of CARs. Transmembrane domains are also found to manage key reactions such as CAR

assembly, activation, and clustering, which positively increases the efficacy of CAR-related therapies [7]. Moreover, it is capable of exerting influence on cytokine secretion [7].

The transmembrane region often consists of hydrophobic amino acid residues identical to the proteins originating from the neighboring hinge or intracellular signaling domains [7]. Most of these domains originate from CD3 ζ , CD4, CD8 α , or CD28, each exhibiting unique features when presented to CAR-T cells [7]. For instance, CD3 ζ -derived transmembrane domains facilitate the integration of endogenous T-cell receptors (TCR) with CAR [7]. This leads to an increased activation and decreased complex stability of T cells. Meanwhile, the presence of CD8 α or CD28-derived transmembrane domains may contribute to the enhancement of membrane stability [7].

2.4. Intracellular signaling domain

The intracellular signaling domain is also known as the endodomain of CAR. This particular domain is responsible for transmitting activation signals to T cells upon the binding of an antigen to the antigen binding domain.

Costimulatory signals are crucial for the maintenance and propagation of signals that initiate a cascade of reactions that are capable of inducing the direct or indirect elimination of cancerous cells; It is found to dictate the T cell differentiation and metabolic pathways, as well as programmed cell deaths [7]. The major signaling domain is derived from CD3 ζ in most FDA-approved CAR-T cells. The single structure of CD3 ζ -chain in the endodomain is a typical characteristic for first generation CAR-T cells. Second, third, and fourth generations of CAR-T cells include costimulatory domains (assimilated adjacent to CD3 ζ) that are derived from CD28 and 4-1BB [7]. Other costimulatory signals including CD27, ICOS, OX40 and CD40, have also shown effectiveness in preclinical studies [7].

3. Development of CAR-T cells

3.1. First Generation

The first generation of CARs is characterized by a solitary structural component, the CD3 ζ - chain or Fc ϵ RI γ , inside the intracellular signaling region [8]. A limitation to first generation CAR-T cells is its inability to produce sufficient amounts of interleukin (IL) -2 [8]. Therefore, an additional administration of exogenous IL-2 is required before the usage of CAR-T therapy [8].

3.2. Second Generation

In second generations of CARs, T cell activation is achieved through dual signaling. TCR triggers the first signal through the recognition of antigenic peptides and MHC complexes on the surface of target cells [8]. The second signal refers to the costimulatory signal generated by co-stimulatory molecules [8]. The aforementioned signal induces the synthesis of IL-2 to facilitate the full activation of T cells and inhibit programmed cell death [8]. Moreover, additional costimulatory protein receptors in the intracellular signaling domain such as CD28 and CD137 improves proliferation, cytotoxicity, sustain response, and prolong CAR-T cell lifespan in vivo [8].

3.3. Third Generation

Several signaling domains were integrated into the third generation of CARs to enhance their potency, cytokine production, and lethal potential. These scFv CD20-CD28-CD137-CD3 ζ -CAR-T and HER2-CAR-T cells have shown notable effectiveness in combating lymphoma and colon cancer [8]. Nevertheless, the observed results did not demonstrate a substantial improvement in outcomes compared to second-generation chimeric antigen receptor (CAR) therapies.

3.4. Fourth Generation

The fourth iteration of CARs was designed by incorporating supplementary IL-12 into the foundational framework of the second-generation CAR structure [8]. These cellular entities are often regarded as TRUCKs. TRUCKs demonstrated its ability to augment T-cell activation and facilitate the recruitment of functional immune cells to eradicate malignant cells [8].

4. The Major Killing Mechanisms of CAR-T Therapy

There are three major killing mechanisms of CAR-T therapy that contribute to the effective eradication of cancer cells.

4.1. Perforin and Granzymes

Perforin and granzymes are cytotoxic chemicals that are stored in the granules of cytotoxic effector T cells [9]. The exocytosis of these cytotoxic molecules are the fast-acting killing mechanisms of CAR-T therapy.

The effective and accurate elimination of cancer cells relies on the attachment between the cytotoxic granules and the microtubules of the effector cell [9]. The granules migrate toward the interface and fuse with the membrane after the establishment of the immunological synapse [9]. Subsequently, the vesicles containing cytolytic cargo are released into the synaptic cleft [9]. Within this space, Perforin forms pores on the membrane of tumor cells to facilitate the entry of granzymes [9]. Upon entry into the cytoplasm of the designated cell, the granzymes activate apoptosis by processes that include both caspase-dependent and caspase-independent pathways, accomplished by cleaving specific substrates [9].

Research has shown that human T lymphocytes, equipped with a CAR, can effectively eradicate tumor cells via a mechanism that is not reliant on MHC molecules or Fas signaling. Hence, the degranulation process involving Perforin and granzymes is widely regarded as the primary mechanism of CAR-T cells inducing targeted cell death.

4.2. Ligand expression

The induction of cellular death in target cells may occur via the expression of membrane-bound ligands belonging to the TNF family [9]. Apoptosis is initiated upon the interaction of these ligands with their corresponding receptors [9]. This is the slow-acting killing mechanism of CAR-T therapy.

The Fas ligand (FasL) from the Fas and FasL axis is a well-acknowledged TNF family ligand expressed on CAR-T cells [9]. The engagement between FasL and the Fas receptor (CD95/Apo-1), initiates the activation of caspase 8 and pro-caspase 8, leading to the assembly of the DISC [9]. Following this, the activation of caspase 8 is responsible for the enzymatic transformation of pro-caspase 3 into its fully developed state, known as mature caspase 3 [9]. Subsequently, the mature form of caspase 3 effectively induces programmed cell death by enzymatically cleaving more than 500 substrates, proficiently executing the apoptotic program [9]. The Fas/FasL system regulates immune homeostasis, and its dysfunction has been associated with immunological and inflammatory mechanisms that enhance chronic inflammation in the tumor microenvironment [9]. Thus, it is essential to maintain the function of the Fas and FasL axis to increase the anti-tumor functionalities of CAR-T cells.

4.3. Cytokine secretion

Cytokines have been shown to have a substantial influence on mediating tumor lysis. Moreover, it has the capacity to modify the tumor microenvironment, hence bestowing resistance onto CAR-T cells against immunosuppression instigated by Tregs, and MDSCs [9]. Preclinical investigations demonstrated enhanced anti-tumor abilities of CAR-T cells by the secretion of IL-12 and IL-18.

The induction of IL-12 results in enhanced anti-cancer effects. Mechanisms include a variety of events that contribute to the augmentation of cytolytic activity in T cells, the mobilization and

stimulation of the innate immune system, and the modification of immunological suppressor cells associated with the stroma [10].

In the meantime, the release of IL-18 has the potential to augment the innate immune response against tumors, modulate the TME, and stimulate the activation of lymphocytes that have infiltrated the tumor [10]. Other cytokines that are secreted by CAR-T cells include IL-6, INF- γ , IL-2, and IL-10.

Table 1. FDA Approved CAR-T Cell Products

CAR-T Cell Product and Date of FDA Approval	Target Antigen	Potential diseases
Abecma [®] (idecabtagene vicleucel) 2021	BCMA	Refractory MM.
Breyanzi [®] (lisocabtagene maraleucel) 2021	CD19	Follicular lymphoma grade 3B, DLBCL, high-grade BCL, and primary mediastinal BCL
Carvykti [™] (ciltacabtagene autoleucel) 2022	BCMA	Relapsed/refractory MM patients
Kymriah [™] (tisagenlecleucel) 2017	CD19	Adult DLBCL and ALL
Tecartus [™] (brexucabtagene autoleucel) 2020	CD19	Mantle cell lymphoma patients B-cell precursor ALL
Yescarta [™] (axicabtagene ciloleucel) 2017	CD19	DLBCL PMBCL HGBL

5. Current FDA approved clinical trials and outcomes

The FDA approved six CAR-T cell products (Table 1). All products are approved to treat hematological malignancies. Four medications authorized by the FDA are designed to specifically target CD19, whilst the other two products are intended to target BCMA. CD19 is a transmembrane glycoprotein with type-1 characteristics. It is classified under the immunoglobulin superfamily and is known for its widespread expression on B cells. Hence, these FDA-approved CD-19 CAR-T cell products effectively target B-cell malignancies. The BCMA is a member of the TNF family and has substantial expression on fully developed B-lymphocytes and plasma cells [11]. This characteristic renders BCMA to be a potentially effective therapeutic target for the treatment of myeloma cancer [11]. Yet, these approved CAR-T cell products are limited to certain potential diseases; Further investigation is required for cancer types beyond B cell malignancies and myeloma.

6. Comparison with traditional therapy

Three conventional methods are often used for the treatment of cancer. Each of these conventional medicines has shown efficacy in clinical studies. However, in order to achieve optimal eradication of cancer cells, they are often used in conjunction with one another. Meanwhile, CAR-T Therapy has the potential to mitigate or significantly alleviate the adverse effects associated with other therapies, therefore positioning it as a promising avenue for cancer treatment.

6.1. Surgery

The primary goal of surgical interventions is to remove tumor tissue and areas containing malignant cells, such as the lymph nodes [13]. Three distinct surgical procedures are capable of effectively eliminating malignant tissues inside the human body. Laser surgery is a surgical procedure that utilizes focused beams of light to eradicate areas affected by cancerous tumor cells [13]. Meanwhile, electrosurgery utilizes the potentials of electric currents, whereas cryosurgery entails the deliberate administration of reduced temperatures to facilitate the freezing of cancerous cells [13].

In contrast to CAR-T therapy, surgical intervention provides a more physically direct modality for the eradication of cancerous cells. However, it is crucial to acknowledge that although surgical procedures might palliate pain and address symptomatic concerns related to cancer, it should be emphasized that surgery alone does not constitute a curative approach to cancer therapy. Surgery is often used as a supplementary technique in combination with chemotherapy or radiation therapy to considerably improve the efficacy of cancer treatment. On the contrary, CAR-T therapy is a treatment mechanism that may be used either alone or in combination with other therapeutic strategies. Furthermore, CAR-T treatment is a less invasive alternative to surgical interventions, so effectively reducing the probability of complications and related risks that may occur during surgical procedures. Furthermore, this therapeutic strategy routinely exhibits a notable degree of efficacy in the treatment of cancer.

6.2. Chemotherapy

Chemotherapy is a therapeutic approach that utilizes potent chemical agents such as Zalcitidine and cladribine to target and eradicate rapidly dividing cells inside the human body, focusing on malignant cells [14]. For cancer, the main objectives of chemotherapy are to inhibit and reduce the growth of tumors and to promote tumor regression, hence reducing symptoms associated with cancer [14]. However, chemotherapy is linked with a plethora of unfavorable outcomes. Chemotherapy has the capacity to induce damage to non-cancerous cells, resulting in the emergence of enduring symptoms like nausea, baldness, fatigue, and anorexia [14]. Considering the regular administration of chemotherapy, the accompanying side effects may last for a prolonged period of time.

In contrast, CAR-T treatment can selectively target antigens that are unique to cancer cells, resulting in a significant reduction in the elimination of non-malignant cells. Although the issue of off-target toxicity remains, the detrimental effects on healthy tissues are significantly mitigated compared to those caused by chemotherapy.

6.3. Radiation therapy

Radiation therapy, often known as radiotherapy, is a cancer treatment approach that utilizes high-intensity focused energy beams to eliminate malignant cells [15]. The delivery of radiation therapy can also be conducted by internal or external methods on the patient's body. The aforementioned approach demonstrates a high level of precision, as it has the ability to selectively target cancer cells while safeguarding adjacent healthy tissues from the deleterious effects of elevated radiation levels [15].

However, radiation therapy elicits biological harm by causing genetic damage inside cells. Genetic material plays a crucial role in regulating cellular development and division mechanisms; it is conceivable that non-cancerous cells may also experience damage in addition to malignant cells [15]. Yet, healthy cells have a far greater ability to undergo self-repair than cancer cells.

CAR-T treatment has the ability to alleviate this limitation since it bypasses the need for radiation in its therapeutic methodology. During the CAR-T treatment process, genetic material is directly introduced into T cells to facilitate chimeric CAR expression. As a result, healthy cells' genetic material stays unchanged, eliminating the need for them to engage in repair mechanisms.

7. Current Progress of CAR-T Therapy for solid tumors

Currently, none of the CAR-T therapies for solid tumors are approved by the FDA. However, multiple target antigens have been identified and investigated for CAR-T cell therapies to treat solid malignancies. These target antigens can either enhance anti-tumor activity or are therapeutic targets and biomarkers for different types of cancer (Table 2).

8. The Major Challenges of CAR-T Therapy in solid tumors

8.1. Tumor antigen heterogeneity

Antigen heterogeneity significantly hinders CAR-T therapy functionalities. TAAs are the most useful targets for CAR engineering. However, the heterogeneous presentation of TAA across distinctive tumor cells makes it challenging to recognize and target tumor cells, hence diminishing the efficacy of CAR-T treatment [17]. Additionally, diverse tumor sites exhibit varying degrees of antigen expression, impairing CAR T cells' functionalities as antigen diversity makes it difficult to identify and target cell-specific antigens, especially those with lower expression levels [17].

8.2. Trafficking and infiltration into tumor tissue

Table 2. Target Antigens for Solid Tumors

Target Antigen	Malignancies	Effects
EGFRvIII	NSCLC	Stimulates angiogenesis, survival, and invasion tumor cells Provides resistance against radiation and chemotherapy
	Advanced biliary tract cancer	
	Colorectal cancer Glioblastoma (GB)	
IL3R α 2	Recurrent or refractory malignant glioma	Augmented specificity Can enhance trafficking to tumor regions
	Glioblastoma	
	Breast cancer	
Mesothelin	Lung cancer	Rise in cytokines and immune cells Augmented specificity Regression of tumors
	Malignant pleural disease	
	Mesothelioma	
HER2	Breast cancer	Enhance cell proliferation and tumorigenesis Overexpression on multiple cancer types, making predictive biomarker
	Gastric cancer	
	Ductal cancer Pancreatic cancer	
PSMA	NSCLC	Enhancing specificity of CAR T-cells Improve antigen recognition
	GB	
PSMA	Prostate Cancer	Enhancing specificity of CAR T-cells Improve antigen recognition
Mucin-1	Pancreatic cancer	Enhanced resistance against immunosuppressive cytokines
GD2	Neuroblastomas	Increases proinflammatory cytokines and chemokines in TME
	Melanomas	
NKG2D	Retinoblastoma	Essential regulator of effector immune cells
	Multiple Myeloma	
	Ovarian cancer	
CLDN18.2	Osteosarcoma	Allows effective invasion into tumor sites Assist with partial or total tumor eradication
	Gastric cancer	
CEA	Gastroesophageal cancer	Address on-target off tumor toxicity Reduces toxicity
	CEA-positive malignancies	
EpCAM	Breast cancer	Tumor suppressive protein: Cell adhesion, differentiation, proliferation and migration
	Pancreatic cancer	
GPC3	Gastric cancer	Important for differentiating, multiplying, and migration of cells
	Lung squamous cell carcinoma	
B7H3	Neuroblastoma	Immune checkpoint molecule that enhances tumor immune evasion
	Ovarian Cancer	
	PDAC Pediatric malignancies	

In order for CAR-T cells to effectively engage with the target proteins present on the membrane of tumor cells, they must undergo trafficking or infiltration processes to reach the tumor locations. These processes are crucial for CAR-T therapy to function optimally and efficiently. The trafficking of CAR-T cells to solid tumor sites is significantly constrained compared to hematological malignancies due to an immunosuppressive milieu [18].

Impediment in trafficking and infiltration into tumor tissue is primarily caused by a variety of mechanisms that diminish the production of vascular-related components [17]. An example of this phenomenon is the upregulation of endothelin B receptors in cancerous tissues, resulting in the downregulation of ICAM-1 expression. Consequently, this inhibits the egress of CAR-T cells from the bloodstream [17].

Solid tumors also secrete chemokines such as CXCL 1, CXCL 12, and CXCL 5 to prevent T cells from trafficking to tumor sites [18]. As T cells have a low expression of corresponding chemokine receptors, it is difficult for infiltration, largely inhibiting the ability of immuno-cytotoxicity functions of CAR T cells. Moreover, the dense fibrotic matrix in solid tumors further hinders the infiltration of CAR T cells.

8.3. Immunosuppressive tumor microenvironment (TME)

The immunosuppressive TME is another challenge of CAR-T treatment in solid tumors. There are three major factors in the immunosuppressive environment that affect the anti-tumor abilities of T cells:

8.3.1 Immune suppressor cells

Multiple types of cells that infiltrate into the solid tumor microenvironment can facilitate tumor development, angiogenesis, and metastasis. These include Tregs, MDSCs, and M2 TAMs [17]. These immunosuppressive cells promote the development and multiplication of tumors by generating growth factors, local cytokines, and chemokines inside solid tumors. These include VEGF, TGF- β , IL-4, and IL-10 [17].

8.3.2 Tumor-derived cytokines

Tumor-derived cytokines are secreted by the immunosuppressive cells. TGF- β is an inhibitory tumor cytokine which can alleviate antitumor response [18]. It has the potential to downregulate CD8⁺ effector T cell functions while upregulating maturation of T_{reg} [18]. Meanwhile, VEGF is a proinflammatory cytokine which is responsible for angiogenesis. Furthermore, the anti-inflammatory properties exhibited by cytokines IL-4 and IL-10 contribute to the pre-existing immunosuppressive TME inside solid tumors [18].

8.3.3 Checkpoint inhibitory ligands

Overexpression of inhibitory immune-checkpoint ligands in solid tumors suppresses the immune system. For example, PD-L1 binds to the PD-1 receptor and inhibits CAR-T cells from becoming active [18]. Other immune checkpoint proteins like CD80 and CD86, found in some solid tumors, can interact with CTLA-4 and CD28 receptors on T cells to hinder anti-tumor activity [18].

9. Current strategies and new approaches of CAR-T therapy

Presently, researchers have used many methods and innovative approaches in preclinical and clinical investigations to tackle the numerous challenges associated with CAR-T therapy for solid tumors. These methodologies can improve the functions of CAR-T therapy in distinct ways.

9.1. Tumor-associated antigen (TAA) heterogeneity

Two novel engineering strategies have been investigated in pre-clinical trials and models as potential solutions to address the issue of antigen heterogeneity in solid tumors.

One potential strategy involves creating multitarget CAR-T cells. This type of CAR-T cell can mitigate tumor antigen evasion by effectively recognizing and targeting several antigens.

A noteworthy dual targeting strategy for solid tumors involves the implementation of BiTE-secreting CAR-T cells, which augments the therapeutic capabilities of CAR-T therapy [19]. ScFvs are the compositions that make up BiTEs. One of the components is specific to CD3, while the other component is particular to a TAA [19]. A bendable linker connects these parts, which makes it possible for a T-cell to attach itself to a tumor cell. Furthermore, BiTE-secreting CAR-T cells mitigated difficulties of antigen variations and antigen escape [19]. For example, in vivo and in vitro research outcomes provide evidence that the Nb-CAR.BiTE- $\gamma\delta$ T substantially eliminates solid tumors that express PD-L1 or HLA-G [19]. The PD-L1/CD3 ϵ Nb-BiTE, upon administration, can guide Nb-CAR- $\gamma\delta$ T cells and recruit un-transduced bystander T cells towards tumor cells that exhibit PD-L1 expression [19]. Hence, this particular technique demonstrates notable efficacy in treating lung cancer, breast cancer, B cell malignancies, and melanoma.

9.2. Trafficking and infiltration into tumor tissue

Local infusion is a strategy in which CAR-T cells are directly delivered to the tumor sites or cranial cavity [20]. This overcomes this limitation of CAR-T therapy as it disregards or shortens the process of infiltration or trafficking. Moreover, this approach avoids the toxicity of system injection and off-target effects while allowing activated CAR-T cells to function appropriately within the TME [20]. Currently, efficacy is found through intracranial infusion of CAR-T cells in breast cancer and brain metastases, and intraperitoneal delivery in ovarian cancer [20]. However, these strategies are not effective when encountering extensive tumor metastasis.

Another strategy is to directly optimize CAR-T cells to express favorably matched CCRs [20]. This leads to enhanced interaction with the chemokine ligands that are produced by tumor cells. In their study, Moon et al. used lentiviral vectors as a means to generate anti-mesothelin CAR-T cells that expressed CCR2b, a receptor known to interact with CCL2 that is released by cancer cells [20]. The outcome of the study demonstrated enhanced infiltration of T cells in the mesothelioma xenograft model, which was followed by an increase in antitumor activity [20].

Moreover, the enhancement of infiltration capability in solid tumors may be achieved by the disruption of physical barriers. One potential strategy for attaining this goal involves the genetic alteration of CAR T cells to recognize antigens linked to stromal cells selectively or to generate enzymes that can degrade the extracellular matrix [20].

9.3. Immunosuppressive tumor microenvironment (TME)

Several investigations have been undertaken to address the immunosuppressive effects caused by the TME in the context of CAR-T cell therapy.

The first therapeutic approach involves modifying CAR-T cells to induce pro-inflammatory cytokines synthesis [21]. Numerous research has been conducted to augment the functioning of CAR-T cells by genetic modifications that result in increased production of pro-inflammatory cytokines, such as IL-12, IL-15, and IL-18. These modified CAR-T cells are often called armored CAR T cells. This methodology is often regarded as a more secure method for regulating the nearby microenvironment compared to the adverse effects caused by the systemic administration of stimulating molecules. The efficacy of engineered IL-12 has been validated in promoting enhanced proliferation, survival, and cytotoxicity abilities while conferring resistance to apoptosis and functional inhibition mediated by PD-L1 [21]. In leukemia, it was shown that the use of IL-15-expressing anti-CD19 CAR-T cells resulted in a longer lifespan within the system and induced remission [20]. The process is also believed to be associated with the development of a specific group of memory cells. In addition, it has been shown that IL-15 may effectively enhance the functionality of GD2 CAR-T cells, leading to enhanced antitumor efficacy in a neuroblastoma metastasis model [20]. Moreover, previous studies demonstrated enhanced proliferation and infiltration capabilities of

IL-18-expressing CAR-T cells while also facilitating the recruitment of immune cells to regulate the TME [20].

Other preclinical research approaches target immune suppressor cells in the TME. Most research focuses on the redirection or repolarization of these cells to achieve a hostile state [21]. For instance, Sun et al conducted coadministration of Laparib in conjunction with CAR T cells. Laripa is a poly (ADP-ribose) polymerase inhibitor that kills tumor cells through its involvement in the defect repair pathways of DNA [21]. The simultaneous administration of treatment resulted in an augmentation of the anti-tumor effects by inhibiting the movement of MDSC along the SDF1a/CXCR4 axis in mouse models of breast cancer [21]. The co-administration of CAR-T cell treatment with folate-targeted Toll-like receptor agonists resulted in the repolarization of MDSCs and TAMs, inducing a shift in their functional phenotype from antagonistic to hostile [21]. Furthermore, it facilitated the collection and activation of CAR-T and native T cells. Implementing this approach decreased the immunosuppressive TME seen in solid tumors, promoting and augmenting the efficacy of CAR-T cells.

10. Summary

CAR-T therapy has undeniably transformed the landscape of cancer treatment for solid and hematological malignancies. Despite the persisting obstacles associated with tumor antigen heterogeneity, trafficking or infiltration, and the immunosuppressive TME in solid tumors, researchers have developed or are developing many innovative approaches to surmount these problems in the foreseeable future. The increasing number of clinical and preclinical studies underscores the growing awareness and urgency surrounding the development of remedies for solid tumors. Further research is necessary to develop FDA-approved treatments for solid tumors. Further studies would also prioritize the creation of novel CAR-T therapies tailored with newly discovered antigens of different types of solid malignancies. Moreover, subsequent investigations would also seek to minimize adverse effects associated with the therapeutic treatments that address the challenges of CAR T therapy with solid tumors.

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