


Review article

Chimeric antigen receptor T-cell therapy: challenges and opportunities in lung cancer

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the paradigm in hematological malignancies treatment, driving an ever-expanding number of basic research and clinical trials of genetically engineering T cells to treat solid tumors. CAR T-cell therapies based on the antibodies targeting Mesothelin, CEA, EGFR, EGFR, MUC1, DLL3, and emerging novel targets provide promising efficacy for lung cancer patients. However, clinical application of CAR T-cell therapy against lung cancer remains limited on account of physical and immune barriers, antigen escape and heterogeneity, on-target off-tumor toxicity, and many other reasons. Understanding the evolution of CAR structure and the generalizable requirements for manufacturing CAR T cells as well as the interplay between lung tumor immunology and CAR T cells will improve clinical translation of this therapeutic modality in lung cancer. In this review, we systematically summarize the latest advances in CAR T-cell therapy in lung cancer, focusing on the CAR structure, target antigens, challenges, and corresponding new strategies.

Statement of Significance: Application of CAR T-cell therapy in lung cancer holds both promise and challenge. Here, we summarize the advances of CAR T-cell therapy in lung cancer treatment, providing a comprehensive insight and some inspiration for researchers and clinicians.

KEYWORDS: lung cancer; CAR T-cell therapy; CAR structure; challenges; new strategies

INTRODUCTION

Lung cancer is the second most common cancer and primary reason for cancer-related mortality all over the world, with 2.2 million new diagnoses and 1.8 million deaths in 2020 [1]. There are two main types of lung cancers: ~85% of all diagnosed lung cancers are classified as non-small cell lung cancer (NSCLC), with its most copious types being adenocarcinoma and squamous cell carcinoma, and ~15% are categorized as small cell lung cancer (SCLC; [2]). Despite the progress in surgical techniques, radiotherapy, chemotherapy, small molecular targeted therapy, and antibody immunotherapy, the 5-year survival rates remain only 4–17% [3]. Therefore, new treatments are urgently needed for lung cancer patients to decrease mortality and improve their quality of life.

Chimeric antigen receptor (CAR) T-cell therapy has gained fresh prominence as a promising novel strategy

for antitumor treatment, especially in hematologic malignancies. CAR T strategy aims to isolate T cells from peripheral blood of patients or other donors and genetically engineer T cells with CAR structure to equip them with the capability of recognizing specific antigens on the tumor cell surface. After infusion back into patients, these super T cells recognize and eliminate the cancer cells that expresses specific target antigens. The molecular structure of CARs comprises four main components: an antigen-binding domain, an extracellular hinge, a transmembrane domain, and an intracellular domain. The antigen-binding domains of CARs are generally single-chain variable fragments (scFv) targeting tumor-associated antigens (TAA), thus conferring major histocompatibility complex (MHC)-independent T cell activation [4]. According to previous reports, MHC-I was absent or downregulated in 25–94% of NSCLC [5]. Thus, CAR T-cell therapy can overcome the limitation of T-cell receptor (TCR) in these patients.

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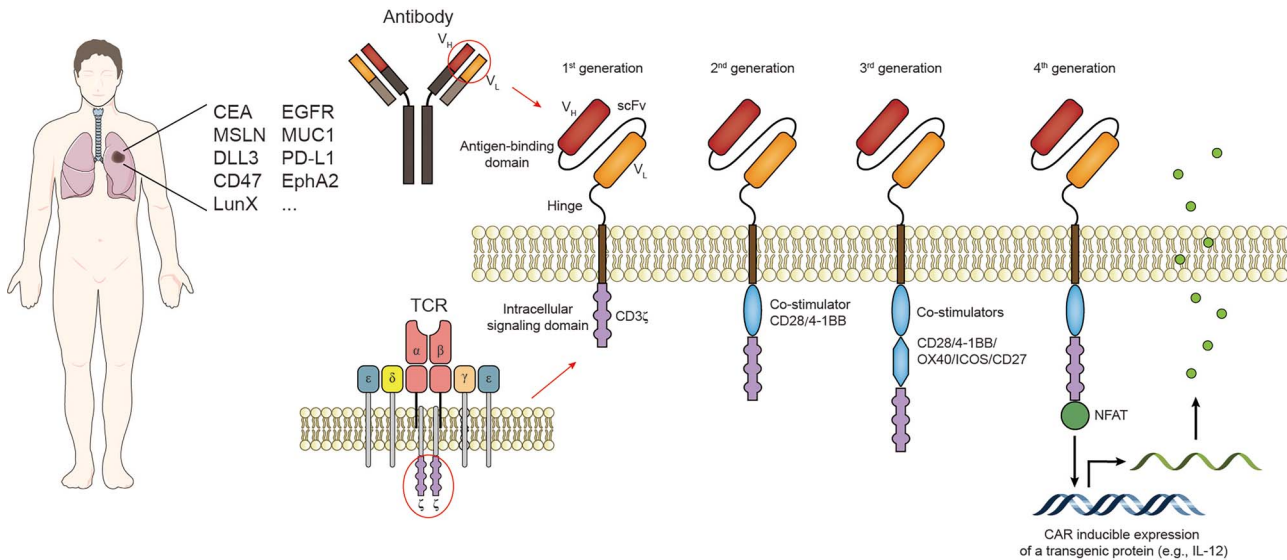


Figure 1. Blueprint of the evolution of CAR structures and the potential targets for CAR T therapy. CARs are consisted of extracellular antigen-binding domain, short hinge, transmembrane region, and intracellular signaling domain. Generally, antigen-binding domain is a scFv molecule derived from a monoclonal antibody or a VHH molecule derived from a nanobody. Intracellular signaling domain often comprises CD3 ζ intracellular signaling domain and CD28/4-1BB, costimulatory molecules.

Since 2017, seven CAR T-cell products have been approved for treating pre-B-cell acute lymphoblastic leukemia, specific types of large B-cell lymphoma, refractory/relapsed mantle cell lymphoma, or multiple myeloma. Although limited to targeting CD19 or B-cell maturation antigen (BCMA) for hematologic malignancies, these successes have already inspired a growing exploration of CAR T-cell therapy in solid tumors. This review concentrates on the advances in CAR T-cell therapy for lung cancers. We summarize the investigated antigens and generated CAR T cells both in preclinical and clinical studies, discuss main challenges in the practical application of CAR T-cell therapy for lung cancer treatment, and introduce the updated strategies to address these obstacles.

EVOLUTION OF THE CAR STRUCTURE

The CAR structure has mainly undergone at least four generations of evolution in design to augment CAR T-cell expansion, tumoricidal activity, cytokine secretion, and long-term persistence (Fig. 1). The first-generation CAR is designed with only CD3 ζ as the intracellular signaling domain. Owing to the lack of robust stimulation and activation, these CAR T cells are prone to anergy and rapid death *in vivo* [6]. For the natural T-cell activation process, in addition to the first signal mediated by TCR binding to the MHC-peptide complex and delivered via CD3, T cells also require a co-stimulatory signal such as CD28-B7 interaction to fully activate themselves. Consequently, the second-generation CAR incorporates a co-stimulatory domain (e.g., CD28 and 4-1BB co-stimulatory domain) [7]. On this basis, the third-generation CAR was equipped with two tandem co-stimulatory domains to achieve more potent tumoricidal efficacy [8]. However, some researchers considered that multiple co-stimulatory elements might result in

a low threshold for T-cell stimulation, possibly inducing an excessive release of cytokines. The fourth-generation CARs are equipped with universal cytokine-mediated killing, so they are also termed TRUCKs. These CARs are modified with activated T-cell-responsive elements for the CAR-induced protein expression, especially some interleukins such as interleukin (IL)-12 and IL18 [9–14].

Currently, more novel CAR design strategies are emerging continuously. Some CARs modified with the IL2 receptor β fragment can induce JAK-STAT3/5 signal transduction, resulting in better persistence of CAR T cells [15]. In addition, universal CAR T cells become a highly sought-after research topic in recent years. In terms of universal CARs, adaptor CARs have the capacity to bind to the Fc portion of IgG, certain types of tags or bispecific antibodies, relying on exogenous soluble adaptors to regulate CAR T-cell activation [16]. During universal CAR T-cell generation, TRAC, CD52, and B2M genes of non-autologous T cells were inactivated by gene editing techniques including CRISPR-Cas9, zinc finger nuclease (ZFN), and transcription activator-like effector nuclease (TALEN; [17–20]). These CD52, MHC-I, and TCR-negative T cells did not cause strong graft-versus-host responses when injected into the recipients, thus removing the requirement of autologous derived T cells and offering a lot of advantages and prospects to manufacture “off-the-shelf” CAR T-cell products for large-scale clinical applications [21]. The universal CAR T cells obtained by the above strategies have also been referred to by some scholars as the fifth generation of CAR T [22].

TARGET ANTIGENS FOR CAR

With the advancement of CAR T-cell therapy, various attempts to cure lung cancer have continually emerged, yet choosing the optimal target remains a considerable

challenge. An ideal target should be expressed with high coverage and specificity on lung cancer cells and not easily lost.

The lung TAAs investigated in current clinical trials are mainly carcinoembryonic antigen (CEA), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), mesothelin (MSLN), disialoganglioside (GD2), receptor tyrosine kinase-like orphan receptor 1 (ROR1), mucin 1 (MUC1), glypican-3 (GPC3), delta-like ligand 3 (DLL3), and PD-L1. Table 1 summarizes the current clinical trials of CAR T-cell therapy for lung cancer treatment. The characteristics and recent progress of the primary targets are detailed below.

CEA

CEA is a glycoprotein expressed in the colon during fetal development and is usually no longer produced in normal tissues after birth. However, in some cancers like colorectal cancer and certain lung cancers, tumor cells upregulate CEA expression levels [23]. Although no evidence shows the relevance of serum CEA level and overall survival in SCLC, it can be used as a prognostic marker for NSCLC patients [24]. Preclinical studies have demonstrated that serum CEA concentration in advanced NSCLC patients has a strong correlation with tumor brain metastasis [25]. Thus, CEA can be used as a biomarker for prediction and prognosis as well as a promising target antigen of NSCLC.

Two Phase I clinical trials of CAR T-cell therapy targeting CEA-positive tumors containing lung carcinoma are ongoing (NCT02349724, NCT04348643). Preliminary results of anti-CEA CAR T cells targeting metastatic cancers exhibited well tolerability even in a high dose of $1 \times 10^8/\text{CAR}^+/\text{kg}$ cells and demonstrated effectiveness in 70% of the treated patients (NCT02349724; [26]).

EGFR

Also known as ErbB-1 or HER-1, EGFR is a transmembrane receptor tyrosine kinase that supports cell growth and division. It is an important member of the ErbBs family and is the expression product of proto-oncogene *c-ErbB1*. The extracellular domain of EGFR consists of four subdomains and the ligand can interact with the pocket formed by domain I, II, and III. Upon binding with the ligand, EGFR polymerizes from a monomer to a homodimer or heterodimer and activate downstream signaling related to cell proliferation, motility, and differentiation [27]. When EGFR is mutated to an overexpressed status, the epithelial cells will grow and divide abnormally, eventually leading to tumorigenesis and progression [28]. Given that over 60% of NSCLCs express EGFR and EGFR-activating mutations are estimated to be present in 15–20% of NSCLC patients, EGFR has already been a potent therapeutic target for lung cancer [29, 30]. Although a variety of tinibs remain the first-line agents for lung cancer treatment, EGFR-targeting antibodies such as Cetuximab, Necitumumab, and Amivantamab are approved and widely being used, especially for NSCLC with EGFR exon 20 insertion mutations. These antibodies are able to compete with the ligand for the

extracellular binding site of EGFR, in particular the third domain, thus hindering its dimerization [31–34].

A Phase I clinical trial of EGFR-targeting CAR T-cell therapy to treat patients with EGFR-positive (> 50% expression) relapsed/refractory NSCLC has achieved initial success (NCT01869166). Some patients showed shrinkage of EGFR-positive tumors on pathology biopsy with acceptable mild skin toxicity and dyspnea after receiving dose-escalating CAR T-cell infusions. Statistically, among 11 volunteer patients, 2 patients showed partial response, and 5 patients maintained stable disease (SD; [35]). This result provides preliminary evidence that EGFR-targeting CAR T therapy is safe and feasible in certain relapsed/refractory NSCLC, where conventional tyrosine kinase inhibitors (TKIs) such as Gefitinib fail to work. In addition, CAR T cells targeting EGFR variant III have also shown safety and significant antitumor activity against metastatic lung cancer in vitro and in mice [36].

MSLN

MSLN is an emerging target with appealing characteristics of differentiated expressions between normal mesothelial cells and an array of solid tumors, including lung cancer [37]. Besides, preclinical and clinical studies showed that high MSLN expression is associated with tumor aggressiveness, thus leading to a poor prognosis in lung adenocarcinoma [38, 39].

Although MSLN appears to be an ideal target, a terminated Phase I/II clinical trial of MSLN-directed CAR T-cell therapies for patients with metastatic cancers, including lung cancer, did not yield satisfactory results (NCT02414269). Of the 15 enrolled participants, only one patient had SD for 3.5 months, whereas the others had progressive disease (PD). Moreover, six patients experienced severe adverse events, including one death in the group administered at the highest dose ($1 \times 10^8/\text{CAR}^+/\text{kg}$ cells). This reminds us of the possible safety risks of using MSLN as a target for CAR T cells. There is also a Phase I/II clinical trial of MSLN-directed inducible caspase 9-M28z CAR T-cell therapy for lung metastatic malignant pleural disease currently ongoing (NCT02414269).

MUC1

MUC1 is a transmembrane glycoprotein strictly expressed in the luminal side of normal epithelial cells. However, during lung cancer progression, cancerous epithelial cells lose their polarity and express MUC1 over the entire cell surface [40]. A study on 172 patients with stage IB NSCLC showed that high MUC1 expression was detected in 64% of specimens, especially in lung adenocarcinoma (86.3%; [41]). MUC1 regulates the survival and invasion of lung cancer cells [42], and downregulation of MUC1 expression inhibits NSCLC progression in human cell lines [43]. However, in a patient-derived xenograft model of NSCLC, MUC1-targeting CAR T cells displayed no efficient suppression on tumor growth, despite exhibiting synergistic antitumor effects when combined with PSCA-targeting CAR T cells [44].

Table 1. Summary of clinical trials of CAR T-cell therapy in lung cancer

Target Antigen	NCT#	Phase	Malignancies	Signaling domain	Estimated enrollment	Sponsor	Other information
CD276	NCT04864821	Early/I	CD276 positive advanced solid tumor (include lung cancer)	N/A	24	PersonGen BioTherapeutics (Suzhou) Co., Ltd.	
CEA	NCT02349724	I	CEA positive cancers (include lung cancer)	N/A	75	Southwest Hospital, China	
	NCT04348643	I/II	Relapsed or refractory CEA positive cancers (include lung cancer)	N/A	40	Chongqing Precision Biotech Co., Ltd, China	
DLL3	NCT03392064	I	Relapsed or refractory SCLC	N/A	6	Angen	
	NCT01869166	I/II	Chemotherapy refractory EGFR positive advanced solid tumors (include NSCLC)	CD28/4- IBB/CD3 ζ	60	Chinese PLA General Hospital, China	
EGFR	NCT02862028	I/II	EGFR family member positive advanced solid tumors (include lung cancer)	N/A	20	Shanghai International Medical Center	Herm CAR-T cells expressing PD-1 antibodies
	NCT03638206	I/II	Multiple malignancies (include lung cancer)	N/A	73	Shenzhen BinDebio Ltd, China	Anti-EGFR VIII CAR-T cells
GPC3	NCT04153799	I	Advanced NSCLC	N/A	11	Sun Yat-sen University, China	CXCR5-modified anti-EGFR CAR-T cells
	NCT03060796	Early/I	Advanced NSCLC	N/A	11	Second Affiliated Hospital of Guangzhou Medical University, China	CXCR5-modified anti-EGFR CAR-T cells
GPC3	NCT02876978	I	Recurrent or refractory lung squamous cell carcinoma	N/A	20	Carsgen Therapeutics, Ltd.	
	NCT03198546	I	GPC3 positive cancers (include squamous cell lung cancer)	3rd/4th generation CAR-T	30	Second Affiliated Hospital of Guangzhou Medical University, China	Anti-GPC3 and/or TGF β
HER2	NCT01935843	I/II	Chemotherapy refractory HER2 positive advanced solid tumors (include NSCLC)	CD28/4- IBB/CD3 ζ	10	Chinese PLA General Hospital, China	
	NCT02713984	I/II	HER2 positive cancers (include lung cancer)	N/A	N/A	Southwest Hospital, China	Reform CAR structure due to safety consideration
MSLN	NCT03740256	I	Advanced HER2 positive solid tumors (include lung cancer)	N/A	45	Baylor College of Medicine, USA	HER2 specific CAR-T cells in combination with intra-tumor injection of oncolytic adenovirus CA4VEC
	NCT02414269	I/II	Malignant Pleural diseases (include NSCLC metastatic to the pleura)	N/A	113	Memorial Sloan Kettering Cancer Center, USA	Completed on 2018/12/17. 1 participant got stable disease, and 14 had progressive disease
MSLN	NCT01585686	I/II	MSLN positive metastatic cancers (include lung cancer)	N/A	15	National Cancer Institute (NCI), USA	rCasp9M28 ζ T cells
	NCT04489862	Early/I	MSLN positive advanced solid tumors (include NSCLC)	N/A	10	Wuhan Union Hospital, China	MSLN-targeted CAR-T cells secreting PD-1 nanobodies
MUC1	NCT03054298	I	MSLN positive cancers (include lung adenocarcinoma)	N/A	18	University of Pennsylvania, USA	Leniviral transduced huCAR-meso cells with or without lymphodepletion
	NCT02587689	I/II	MUC1 positive advanced refractory solid tumors (include NSCLC)	N/A	20	PersonGen BioTherapeutics (Suzhou) Co., Ltd., China	
MUC1	NCT03525782	I/II	Lung neoplasm malignant and NSCLC	N/A	60	The First Affiliated Hospital of Guangdong Pharmaceutical University, China	Anti-MUC1 CAR-T cells combining PD-1 knockout
	NCT03330834	I	PD-L1 positive advanced NSCLC	CD28/4- IBB/CD3 ζ	22	Sun Yat-sen University, China	Ultimately only 1 participant who experienced severe CRS-induced interstitial pneumonia disease after the treatment
PD-L1, CD80/CD86	NCT03060343	Early/I	Relapsed or refractory NSCLC	N/A	10	Yu Fenglei	Zeushield Cytotoxic T Lymphocytes
	NCT0463148	I	PSMA expression positive cancers (include NSCLC)	N/A	35	Cellex Patient Treatment GmbH, Germany	T cells carrying universal CAR (UniCAR02-T) in combination with PSMA peptide target module (Tm/PSMA)
ROR1	NCT02706392	I	ROR1 positive advanced malignancies (include metastatic/unresectable/Stage III/IV NSCLC)	N/A	60	Fred Hutchinson Cancer Research Center, USA	
TnMUC1	NCT04025216	I	TnMUC1 positive advanced cancers (include NSCLC)	N/A	112	Tmunity Therapeutics, USA	
HER2, MSLN, Lewis-Y, PSMA, MUC1, GPC3, AXL, EGFR, B7-H3, Claudin 18.2	NCT03198052	I	Lung cancers	CD28/4-IBB/ OX-40/CD3 ζ	30	Second Affiliated Hospital of Guangzhou Medical University, China	
	NCT03356808	I/II	Lung cancers (SCLC and NSCLC)	N/A	20	Shenzhen Geno-Immune Medical Institute, China	

DLL3

DLL3 is an inhibitory Notch ligand expressed on cell surface in >80% of SCLC but only has a minimal expression in normal tissues. A study of 63 SCLC patients showed ~83% of patients were DLL3 positive, and 32% were DLL3 highly expressed (at least 50% of cancer cells were DLL3 positive; [45]). Thus, DLL3 has been identified as an appealing novel target for lung cancer immunotherapy.

Several clinical trials of DLL3-targeting immunotherapies for SCLC have been conducted, including the antibody-drug conjugate rovalpituzumab tesirine, the bispecific T-cell engager (BiTE) AMG 757, and the CAR T cells AMG 119 [46]. However, Phase II (NCT02674568) and Phase III (NCT03061812, NCT03033511) clinical researches showed disappointing results with a high occurrence of adverse events and limited survival benefits [47]. The sponsor has announced abandoning all programs of DLL3-related cancer therapeutics after the failure of the Phase III MERU trial in 2019. In previous studies, AMG 119 showed robust ablation of DLL3-positive target cells in vitro and remarkable antitumor activity in mice, but the clinical performance remains to be examined [48]. Amgen is pinning its hope on the CAR T product AMG 119 to perform better in the ongoing Phase I clinical trial of DLL3-targeting SCLC treatment (NCT03392064).

GPC3

GPC3 is a member of heparan sulfate proteoglycan family anchored to cell membrane by glycosyl-phosphatidylinositol. In general, healthy adults do not express this protein, except for the placenta [49]. However, GPC3 has been found to be abundant in lung squamous cell carcinoma (LSCC) and promoted cell proliferation and tumorigenesis through Wnt/ β -catenin signaling [50].

Studies in LSCC xenograft models showed that GPC3-redirectioned CAR T cells almost eliminated target cells with durable persistence and tumor tissue infiltration [51]. A Phase I clinical trial of GPC3 CAR T-cell therapy for LSCC, is currently underway (NCT03198546).

Other promising targets. In addition to the above antigens, there are a number of familiar or newly emerging targets that are being attempted for the construction of CAR for lung cancer treatment.

PD-L1 is recognized as one of the most comprehensively investigated immune checkpoints and its blocking antibodies such as Atezolizumab and Durvalumab have been approved for NSCLC treatment. Researchers constructed anti-PD-L1 second-generation CAR T with Atezolizumab-derived scFv, demonstrating potent antitumor effects in NSCLC xenograft model with EGFR mutation [52]. For PD-L1^{low} NSCLC tumors, local radiotherapy was used to increase PD-L1 expression and subsequently combined with CAR T-cell therapy [52].

CD47 is another immune checkpoint highly expressed on tumor cell membranes. It binds to signal-regulated protein alpha (SIRP α) on macrophages to release inhibitory signals and evade phagocytosis [53]. A third-generation CAR T targeting CD47 has recently been designed to treat lung

cancer cell metastasis. It effectively killed A549 tumor cells and attenuated the expression of genes related to cancer invasion and metastasis [54].

Erythropoietin-producing hepatocellular carcinoma A2 (EphA2) is a tyrosine kinase belonging to the Eph receptors family and is aberrantly upregulated in >90% of NSCLC while lowly expressed in most normal adult tissues [55, 56]. Silenced EphA2 protein expression or small-molecule EphA2 inhibitors can overcome acquired resistance to EGFR-TKIs in lung carcinoma [57]. Furthermore, scFv of humanized EphA2 antibody 4H5 has been used to construct a second-generation CAR T and EphA2-targeting CAR T cells showed efficient antitumor effects in A549 xenograft model. Hence, EphA2 is also regarded as a promising target for NSCLC [56].

Lung-specific X (LunX) is a member of the PLUNC protein family highly expressed on NSCLC while not expressed on most of the normal cells. Its antibody is able to restrain the cell proliferation, metastasis, and invasion of LunX-positive NSCLC cells [58]. LunX-targeting CAR T cells were also demonstrated to induce regression of tumors and prolonged survival in the metastatic A549 xenograft model [59].

Melanoma-associated antigen-A1 (MAGE-A1) is a kind of cancer/testicular antigen expressed only in testicular germ cells or in certain malignancies. MAGE-A1 was found to be highly expressed in cell lines and tumor tissues of human lung adenocarcinoma and positively correlated with malignant behavior of cancer. The second-generation CAR T containing MAGE-A1-scFv also demonstrated tumoricidal activity against lung adenocarcinoma in vitro and in NCI-H1299 mouse xenograft model [60].

In addition to these, more targets have been preliminarily demonstrated feasible for CAR T-cell therapies in lung cancer, such as CD56, CD44v6, and fibroblast activation protein (FAP; [61–63]).

CHALLENGES IN TREATING LUNG CANCERS WITH CAR T CELLS

In despite of the great success of CAR T-cell therapy in the treatment of hematological malignancies, there are numerous significant challenges in expanding its application into solid tumors including lung carcinoma. Identifying a specific target that is only highly, homogeneously and stably expressed in lung tumors rather than healthy tissue is a great difficulty. Off-tumor effects due to poor specificity and excessive activation of T cells without control can cause severe toxic side effects. Moreover, CAR T cells have to undergo layers of physical and immune barriers before exerting desired effects. Among all kinds of obstacles, several core challenges are selected for detailed discussion.

Toxicity

Adverse events accompanying CAR T-cell therapy are always a non-negligible problem. The most significant toxicities are cytokine release syndrome (CRS) and on-target off-tumor toxicity. Recently, neurotoxicity that occurred in CD19 CAR T-cell therapy has also been

demonstrated associated with the on-target off-tumor toxicity produced by brain mural cells expressing CD19 [64].

CRS usually occurs shortly after CAR T cells administrations, signaling the peak of CAR T-cell expansion. Its clinical manifestations include high fevers, hypotension, hypoxia, tachycardia, hemodynamic instability, and organ dysfunction [65]. Moreover, a certain percentage of severe CRS may worsen to hemophagocytic lymphohistiocytosis [66], even leading to fatal events [67]. The mechanism of CRS occurrence is associated with CAR T cell-induced gasdermin E-mediated target cell pyroptosis and cytokines produced by recipient macrophages [68, 69]. Cytokines involved in CRS include IL-1, IL-2, IL-6, IL-10, IL-12, IL-15, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, C-reactive protein, and macrophage inflammatory protein-1 alpha [70, 71]. Among them, IL-6 and IL-1 are considered to be critical for the symptom onset [72]. After being stimulated by granzyme B released from CAR T cells, tumor cells undergo pyroptosis and further induce macrophages to produce IL-6 and IL-1 β [69]. IL-6 signaling contributes to an array of key symptoms of CRS, including complement activation, vascular leakage, disseminated intravascular coagulation, and myocardial dysfunction [73–75]. Timely administration of IL-6 receptor antibody, tocilizumab, can effectively alleviate CRS. A recent clinical trial revealed that preemptive tocilizumab was also effective in preventing the occurrence of severe CRS without compromising the antitumor efficacy of CAR T-cell therapy [76]. In addition, IL-1 triggers inducible nitric oxide synthase (iNOS), leading to vasodilation and hypotension [68]. Blocking IL-1 signaling by anakinra, an IL-1 receptor antagonist, can mitigate CRS severity and decrease CRS-related deaths by CAR T cells, especially the fatal neurotoxicity characterized by paralysis and seizures [72]. Although a uniform CRS grading scale and management guidelines have been established, they are mainly based on the clinical experience of hematologic oncology, thus may require further adaptation in the application for lung cancer [67].

On-target off-tumor toxicity is caused by cross-targeting of CAR T cells to antigens in normal tissues. Although the demand for high antigen density for CAR T-cell therapy provides a therapeutic window to differentiate malignant and nonmalignant tissues based on different antigen density levels, during solid tumor treatment, some CAR T cells may attack normal tissues with low antigen density, resulting in cases of death [77, 78]. The powerful targeting and killing ability of CAR T cells makes it more severely toxic than traditional antibody drugs and therefore imposes higher standards on the target antigen selection.

Compared to hematologic malignant tumors, toxicity may be more prominent in the treatment of solid tumors, including lung carcinoma. One reason is that the high cell density of solid tumors places a higher demand on the effector-to-target ratio, yet higher dose of CAR T cells means a greater risk of adverse events. Another reason is that the on-target off-tumor toxicity of CAR T cells in solid tumors tends to be more intolerable than that in CD19-targeting leukemia treatment. The latter prolonged B-cell aplasia can be resolved with immunoglobulin

supplementation, whereas the former is often difficult to control and salvage [79]. Therefore, the survival benefits and toxicity of CAR T cells for lung cancer patients need to be carefully weighed.

Physical barrier and immune barrier

As with other solid tumors, two major barriers limit the efficacy of CAR T-cell therapy for lung cancer treatment: physical barrier and immune barrier. The former impedes the trafficking and homing of CAR T cells into tumor masses, and the latter is often referred to as immunosuppressive tumor microenvironment (TME).

The first obstacle encountered by injected CAR T cells is the physical barrier of trafficking and homing to the lung tumor tissue. Several studies showed that the integrins CD11a/CD18 and CD49a are required for the entry and retention of CD8⁺ T cells in the lung, respectively [80, 81]. And CXCR3 and CCR5 are related to the localization of effector or memory CD8⁺ T cells in the lung [82, 83]. Moreover, it is worth noting that cytolytic CD8⁺ T cells in the blood tend to be confined to the intravascular circulation, whereas CD8⁺ T cells emigrating into thoracic duct lymph and tissues are prominently non-cytolytic [84]. Besides, abundant fibroblastic stromal cells in TME of lung cancer also influence local angiogenesis and T-cell infiltration [85]. The tumor stroma is similar in composition to the stroma from the wound, with active angiogenesis and a large number of proliferating fibroblasts. These activated cancer-associated fibroblasts (CAFs) occupy a major part of the stromal cells with a pivotal role in secreting a complex extracellular matrix (ECM). This kind of desmoplastic reaction characterized by proliferation of CAFs and deposition of ECM may establish a more robust physical barrier for CAR T-cell infiltration [86, 87]. CAFs were demonstrated to suppress the function of BCMA CAR T cells in the treatment of multiple myeloma, and CAR T cells that dual-target CAF and BCMA successfully overcame this suppression [88]. Therefore, using relevant strategies to increase the homing of CAR T cells to the lung and infiltration into the tumor mass may lead to better therapeutic outcomes.

Upon arrival at the lung tumor, CAR T cells are inevitably confronted with the immune barrier. A large number of stromal cells undergo tumor reprogramming and collectively result in an immunosuppressive TME. A variety of inhibitory factors exist in the lung TME, such as transforming growth factor β (TGF β) and IL10 released by immature dendritic cells; ARG1 and iNOS expressed by myeloid-derived suppressor cells; COX2, PGE₂, and indolamine-2,3-dioxygenase (IDO) produced by tumor cells; PD-L1, PD-L2, and FASL expressed by cancer-associated fibroblasts, and many other substances [89, 90]. Exhausted CAR T cells also overexpress inhibitory receptors and interact with the corresponding ligands upregulated in TME [89]. These inhibitory factors can be categorized into several aspects including metabolic regulation, cytokine networks and immune checkpoints. To give some examples, IDO secreted by myeloid-derived suppressor cells and tumor cells exercised the function of converting tryptophan to kynurenine and resulted in CAR

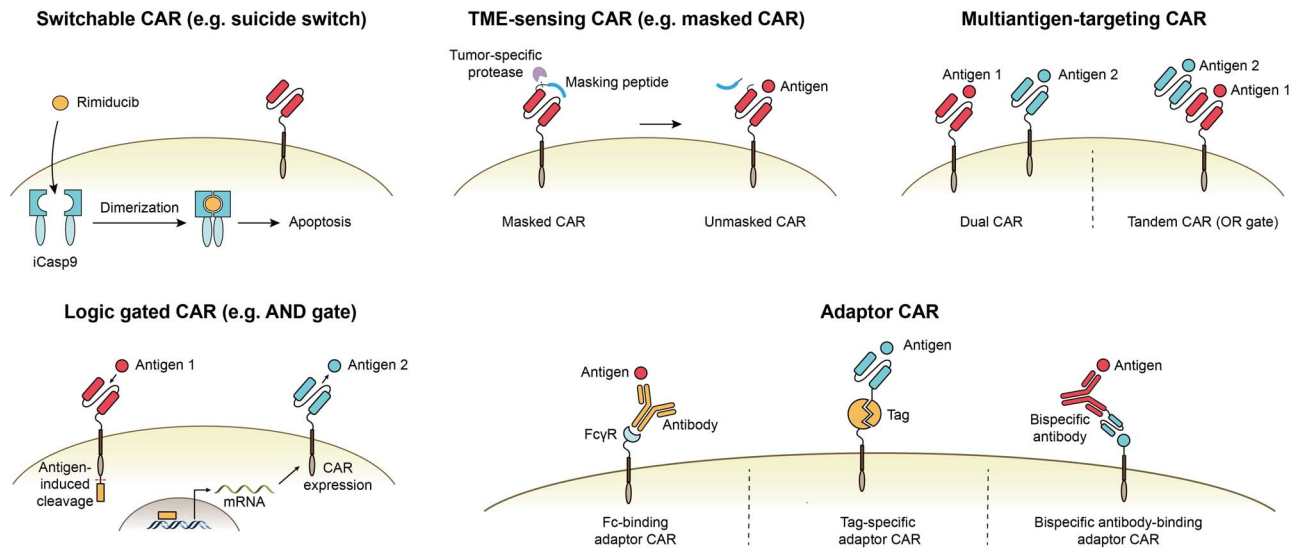


Figure 2. Mechanisms of switchable CAR, TME-sensing CAR, multiantigen-targeting CAR, logic gated CAR, and adaptor CAR.

T-cell anergy. Conversely, inhibition of IDO expression promoted the killing ability of CD19 CAR T cells against solid tumors [91]. In terms of cytokines, $TGF\beta$ is one of the most noteworthy factors in TME. $TGF\beta$ plays a complex role in tumor growth, differentiation, migration, invasion, angiogenesis, and immune modulation [92]. It also has a well-documented capacity to attenuate cytolytic activity of CAR T cells through complex mechanisms. Blockade of $TGF\beta$ signaling such as using small molecular inhibitor SD-208, $TGFBR2$ knock-out via CRISPR/Cas9, and expression of dominant-negative $TGF\beta$ receptor augmented the therapeutic effect of CAR T cells against solid tumor [93–96]. These factors collectively lead to progressive inactivation and dysfunction of T cells and further limit the persistent efficacy of CAR T-cell therapy in lung cancer.

Antigen heterogeneity

A study of tumor tissue biopsies from NSCLC patients revealed that local mutational diversity of lung tumors drove high spatial heterogeneity of intratumoral immune microenvironment [97]. The expression of PD-L1 and some other immunosuppressive molecules showed significant discordance or heterogeneity throughout NSCLC patients' tumors [97, 98]. After struggling with CAR T cells, antigen-deficient tumor cells and antigen-low expressing variants that escape recognition by CAR may be retained under immune pressure, thereby giving rise to tumor relapse and hindering clinical success.

STRATEGIES TO OVERCOME THE OBSTACLES

Switchable and locally sensitive CAR T cells

To address the toxicity problem, in addition to commonly used anti-IL6 antibodies to alleviate CRS, tuning CAR T-cell activity *in vivo* has become a research hotspot [99]. Switch-based control may be a reliable strategy to control

CAR T-cell toxicity within acceptable limits. In this strategy, activity of CAR T cells is controlled by exogenous switch molecules. “Suicide switch” or “off switch” is used to suppress CAR T-cell activation at the onset of severe toxicity, and “on switch” can activate the cytotoxicity of T cells when needed [100–103]. The research on bifunctional switches that can effectively control and balance the anti-tumor activity and toxicity of CAR T cells has also been brought to the fore [104, 105]. The most commonly used “suicide switch” is the inducible caspase-9 (iCasp9) system. Under normal conditions, iCasp9 does not affect CAR T-cell activity as a monomer, but after the addition of exogenous small molecules such as Rimiducid, iCasp9 undergoes dimerization, thus leading CAR T cells towards apoptosis [100]. Currently, CAR T cells with the iCasp9 system have been practised in clinical studies for lung cancer treatment (NCT022414269).

In the regulatory system based on local sensitivity, CAR T cells are activated when they come into contact with specific physiological features of TME, thereby enabling site-specific identification and activation. For example, the masked CARs (mCARs) contain a masking peptide that blocks the extracellular domain of the CAR. When exposed to proteases secreted locally in TME, the linking peptide between inhibitory mCARs and CAR structure is hydrolytically cleaved by protease, releasing intact CARs for recognition and bind of target antigens [106] (Fig. 2).

Empowering CAR T cells to migrate into tumor sites and reshape TME

How to promote the migration of CAR T cells into tumor sites is a crucial problem in their extensive application from hematologic malignancies to solid tumors. Genetically modifying CAR T cells with specific chemokine receptor expression for better migration into tumor sites is an ideal strategy. CAR T cells expressing CCR2b, CCR4, CXCR2, or CXCR5 have been shown to enhance tumor localization and eradication [107–110]. Of particular

interest was that chemokine CXCL13, the ligand of CXCR5, was found highly expressed on tumor lesions of NSCLC patients [111]. The latest research indicated that equipping EGFR-targeted CAR T cells with an additional CXCR5 gene sequence following CD3 ζ augmented its oriented migration and tumoricidal activity [110]. In addition, to overcome the physical barriers formed by dense extracellular matrix, redirecting CAR T cells to recognize FAP or secrete matrix-degrading enzymes for better infiltration may be a feasible strategy.

To counteract the inhibition of TME, cell-extrinsic and cell-intrinsic combination therapy strategies have been investigated. In terms of simple co-administration, besides CAR T cells combined with immune checkpoint inhibitors (ICIs), which are currently under investigation in clinical trials, several combinational strategies are emerging in recent years. For instance, the antitumor efficacy can be promoted by combining CAR T cells with avasimibe to intervene lipid metabolism, with STING agonist to activate immune response, and with PAK4 inhibitor to normalize the tumor vascular microenvironment [112–114].

In respect of cell-intrinsic combination therapy, a case in point is the TRUCK T cells. IL12 released by TRUCK T cells into TME augments CAR T-cell activation, modulates immunological TME and vascular microenvironment, and recruits innate immune cells such as NK cells and macrophages to eliminate tumors regardless of target antigen expression [9]. In addition to IL12, an array of cytokines secreted by CAR T cells including IL7, IL9, IL15, and IL18 can also enhance the therapeutic effect on solid tumors [115–119]. Besides, CAR T cells expressing other transgenic products such as ICIs, BiTE, and ineffective receptor for inhibitory cytokines also display resistance to immunosuppressive TME [96, 120–123].

Antigen optimization and combinatorial TAA targeting

Heterogeneous manner of antigen expression on tumor cells and a certain level of TAA expression on normal tissues make it tough to find an ideal target for CAR T cells to treat lung cancer. Besides, a large proportion of patients become resistant to CAR T-cell therapy due to antigen escape after a period of treatment [77]. To overcome the heterogeneity and downregulation of tumor antigen, the following strategies have been developed. The first option is to target multiple antigens. When the expression of one target is low or insufficiently specific, the recognition of other antigens can help CAR T cells achieve tumor recognition and killing to a greater extent. Concrete approaches that can be taken include multiple CAR T combinations, dual CAR constructs, bivalent tandem CARs and other CAR structures designed with “AND/OR/NOT” logic gate. For example, PSCA-targeting CAR T cells in combination with MUC1-targeting cells were shown to synergistically eliminate NSCLC tumors on a patient-derived xenograft model [44]. Tandem CAR T cells simultaneously targeting CD70 and B7-H3 also exhibited significantly enhanced antitumor activity compared to single-target CAR T cells in a xenograft model of human lung cancer NCI-H460 [124]. The second method is to increase TAA expression on solid tumors. A representative and innovative attempt is

the combination of oncolytic virotherapy and CAR T cells. Through infection and genetic modification of tumors, the oncolytic virus was able to deliver a CAR targetable TAA to solid tumors, which enhanced CAR T-cell recruitment and antitumor immunity [125]. The third strategy is to construct adaptor CARs. As aforementioned, adaptor CARs make it possible to artificially transform the antigen recognized by CAR T cells by means of Fc-binding, tag-specific, and bispecific antibody-binding adaptors [16]. They have been successfully applied in simultaneous or sequential multi-antigen targeting to overcome antigen escape and heterogeneity. Finally, in addition to targeting antigens on the tumor cell surface, CARs targeting the TME provide a new perspective to overcome tumor escape [126]. Taking CAF as an example, it was reported that CAF in the TME influenced the tumor heterogeneity of lung cancer [127, 128]. Treatment targeting CAF may be able to reverse resistance to CAR T cells [88] (Fig. 2).

CONCLUSIONS

In recent years, CAR T-cell therapy has undoubtedly been an increasingly popular hotspot in biopharmaceuticals. Although it has shown promising achievements in the treatment of hematologic tumors, it is still far from the actual application for solid tumors, including lung cancer. The selection of targets, toxicities after T-cell infusion, immunosuppressive TME, tumor heterogeneity, antigen escape, T-cell dysfunction, and many other issues are yet to be addressed. From the future perspective, universal CAR T overcomes the dependence on autologous T cells and offers the possibility of more efficient and economical preparation of CAR T. Switchable CARs and masked CARs enable control of CAR T activity, limiting toxicity to an acceptable level. Combining other therapies including cytokines, immune checkpoint inhibitors, and oncolytic viruses overcomes the physical and immune barriers of TME. Besides, the use of Boolean logic gates and various modifications of CAR structure are expected to address tumor heterogeneity and antigen escape. In summary, despite all kinds of obstacles, a number of intelligent engineering approaches and encouraging preclinical and clinical study results provide us with more confidence in the application of CAR T-cell therapy for lung cancer treatment.

AUTHORS' CONTRIBUTIONS

D.J. and X.Z. designed and proofread the concept. C.X. and X.Z. wrote the paper. All authors approved the final paper.

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DATA AVAILABILITY STATEMENT

The data in this study are openly available.

CONFLICT OF INTEREST STATEMENT

D.J. and X.Z. hold the position of Editorial Board Member and Assistant Editor for Antibody Therapeutics respectively and are blinded from reviewing or making decisions on the manuscript. The authors declared no other conflicts of interest in this manuscript.

ETHICS AND CONSENT STATEMENT

The consent is not required.

ANIMAL RESEARCH STATEMENT

This is not applicable.

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