

Updating CAR-T Cell Immunotherapy for Liquid and Solid Tumor

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Abstract: According to the GLOBOCAN 2020 report, there is still a 50% mortality rate observed in cancer patients despite the availability of various innovative cancer therapies. This highlights the need for specific therapies that can effectively kill cancer cells without harming normal cells. One such therapy is cancer immunotherapy, which comprises immune checkpoint blockade, adoptive cellular therapies, cancer vaccines, and CAR-T cell therapy. CAR-T cell therapy entails modifying T-cells from a cancer patient in a lab so that they attack cancer cells only. This therapy has been successful in treating some lymphomas and myelomas, and is being explored for solid tumors as well. However, there are certain limitations to its efficacy for solid tumors due to their heterogeneity. This review covers the most recent advancements and enhancements in CAR-T therapies for lymphomas and solid tumors, as well as methods to get around their drawbacks.

Keywords: CAR-T cell therapy; Immunotherapy; Cancer; lymphoma; Solid tumor.

Introduction

GLOBOCAN 2020 has estimated around 19.3 million incidences of cancer with 10.0 million deaths by cancer^[1,2]. Few examples of immunotherapies that can be used to treat cancer are immune checkpoint blockade, adoptive cellular therapy such as CAR-T and TCR-T cell therapy based on the introduction of tumor-fighting immune cells into the body and cancer vaccines^[3]. Furthermore, a combination of surgical resection, systemic chemotherapy, and local radiation therapy may be used as a treatment for various malignancies^[4,5]. The systemic toxicity and undesirable side effects brought on by the therapeutic process are the primary causes of this failure^[6]. Thereinto, Cancer Immunotherapy is an innovative technology which activates immune system and kills only cancer cells without harming normal cell of cancer patients^[7]. A variety of substances, including lymphokines, vaccines, in vitro-stimulated effector cells that are part of the immune system, or antibodies, are used in immunotherapy approaches to complement or activate the immune system^[8].

There are various immunotherapy techniques, including passive and aggressive approaches. Delivery of substances like mAbs, lymphocytes, or cytokines that boost the body's natural anti-tumor response are all included as types of passive immunotherapy^[9,10]. Active or aggressive immunotherapy activates the immune system through vaccination, non-specific immunomodulation, or the targeting of certain antigen receptors to destroy tumor cells^[11]. This review overviews the latest update on CAR-T cell therapy and its significance in developing precise therapies for the cancer in near future.

About Car-T Cell Therapy

Chimeric antigen receptor T-cell (CAR-T cell) therapy is a form of immunotherapy that entails genetically altering a patient's T-cells to more effectively target and eradicate cancer cells. It functions by removing T-cells from a patient's blood, altering them genetically to express a chimeric antigen receptor (CAR) that recognizes and targets cancer cells, and then reintroducing the altered cells into the patient's body. PD-1 protein, expressed on T-cells' surface helps to regulate the immune response. Some cancer cells are able to manipulate the immune system by secreting the protein PD-L1, which binds to PD-1 and effectively "turns off" T-cells, enabling the cancer cells to avoid detection by the immune system. Immunotherapy known as PD-1 inhibitors function by preventing the interaction between PD-1 and PD-L1, which keeps T-cells activated and improves their ability to combat cancer cells.

Antibodies such as pembrolizumab that target the programmed cell death protein 1 pathway (PD-1/PD-L1) and ipilimumab that targets cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) have shown promising results as immune checkpoint blockades in treating a number of cancers^[12]. As a novel T-cell therapy, lymphocytes created from peripheral blood mononuclear cells (PBMCs) that artificially express a desired TCR or chimeric antigen receptor (CAR) have been developed and used in clinical settings. T-cells that have been transduced with TCRs that are specific to an antigen are used in TCR-T therapy^[13].

Using gene-modified T-cells, CAR-T cells, or tumor-infiltrating lymphocytes (TIL), adoptive cell therapy (ACT) enables the immune system to recognise tumour cells and act as an effector against them^[14]. CAR-T cell treatment was groundbreaking as it led to effective and long-lasting therapeutic outcomes^[15]. Chimeric antigen receptor (CAR) is a recombinant receptor which makes immune cells to focus on and recognize antigens on surface of tumor cells^[16]. T-cells from patients (autologous) or donors (allogeneic) are altered to express a CAR to produce CAR-T cells^[1]. Autologous CAR-T cells are produced using several production

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techniques, all of which adhere to the same fundamental principles. The patient's white blood cells (WBCs) are first separated and cleaned using leukapheresis. The T-cells are subsequently activated, infected with the CAR transgene, multiplied to treatment cell quantities, prepared, and administered. Following quality assurance testing and a prophylactic lymphodepleting chemotherapy

regimen, the product is introduced into the patient^[17] (Fig.1). γ -retroviral or lentiviral transduction methods are majorly used to introduce CARs into T-cells. However, other methods have also been used, such as the Sleeping Beauty technique and electroporation of naked plasmid DNA or mRNA^[18]. MHC-unrestricted surface structures are recognized by CARs^[19].

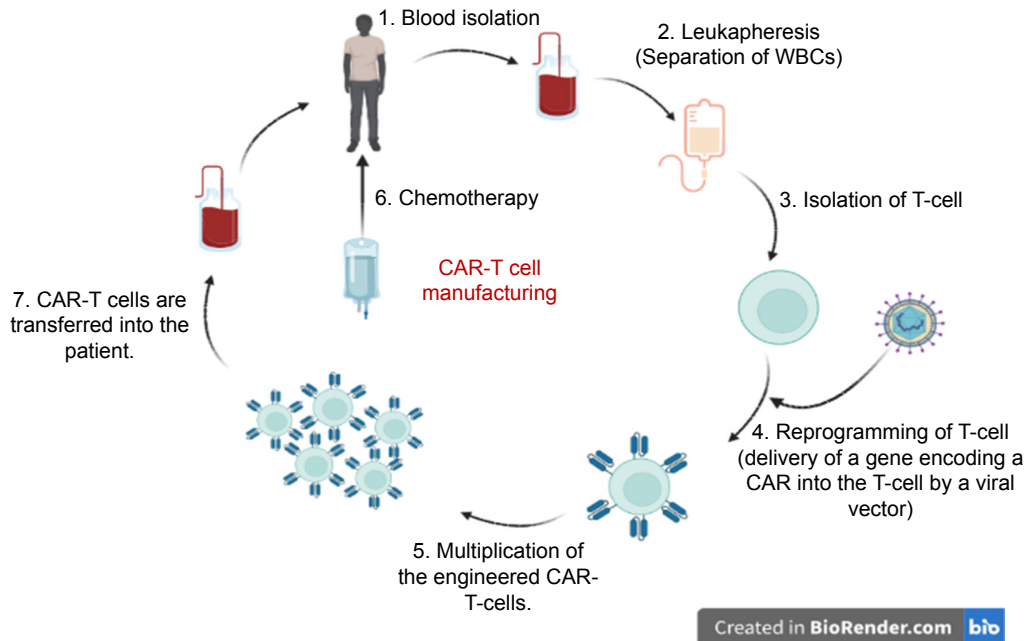


Fig. 1. CAR-T cell manufacturing.

CARs that target the B lymphocyte antigen CD19 have had extraordinary success in genetically rerouting patient T-cells in many therapy-refractory hematologic disorders^[20]. This kind of adoptive cell immunotherapy has revolutionized the management of the lymphomas of B-cell and acute lymphoblastic leukemia that originates from precursors of the B-lymphocytes^[21]. Therefore, CAR-T cell therapy could be one of the first commercially viable examples of customized synthetic biology for cancer treatment^[22]. Within a year of the initial authorization of CAR-T cell drug in August 2017 for acute lymphoblastic leukemia (ALL), there have been two more FDA approvals for anti-CD19 CARs^[23]. Currently, six CAR-T cell products were approved for treating R/R B-cell malignancies by the US FDA which target CD19 antigen and B-cell maturation antigen (BCMA): tisagenlecleucel, axicabtageneclisoleucel, brexucabtageneautoleucel, lisocabtageneumaleucel, idecabtagenevicleucel, and ciltacabtageneautoleucel^[24]. Till December 2019, 284 CAR-T cell clinical trials were still in progress worldwide, with majority of them taking place in the United States (130) and China (124). The majority of these trials were focused on treating hematological malignancies such as acute lymphocytic leukemia (ALL), non-hodgkin lymphoma (NHL), and multiple myeloma, and they primarily targeted CD19 as well as CD20, CD22, and BCMA. At least 24 nations currently employ CAR-T cell therapy^[25].

Car T Cells Design

CAR-T cells can target any cancer cell surface antigen that can be attacked by a mAb, such as proteins, carbohydrates, and glycol-

ipids. Hence, they can react to more diverse targets^[26]. The fundamental idea behind CAR design is to connect an intracellular signaling module with CD3 to an extracellular ligand recognition domain (scFv) in order to activate T-cells on antigen binding for mimicking co-stimulation provided during the recognition of T-cell receptors by antigen presenting cells (APC) which is necessary for total physiologic T-cell activation^[27]. The four primary parts of CARs are target antigen-binding domain, hinge region, transmembrane domain and intracellular signaling domain^[15] (Fig. 2).

Antigen binding domain

The receptor's ectoderm contains antigens that are recognized by the antigen-binding domain. Target antigen interacts with this domain, which is constantly exposed to the outside. This domain is obtained from the variable region of mAbs^[28]. Instead of the target antigen, single chain variable fragment (scFv) present on extracellular side determines the CAR's capacity to bind antigens^[29]. scFvs found in CARs target antigens present on cancer cell surface, activating T-cells without the need for the MHC. CAR's antigen binding affinity must be strong enough to recognize and identify tumor-associated antigens, thereby initiating CAR signalling and, as a result, T-cell activation. However, it must not be too high to cause activation-induced T-cell death or toxicities^[30].

Hinge region (spacer)

Compared to the other receptor domains, hinge area is minuscule and typically lies between the T-cell's outer membrane and anti-

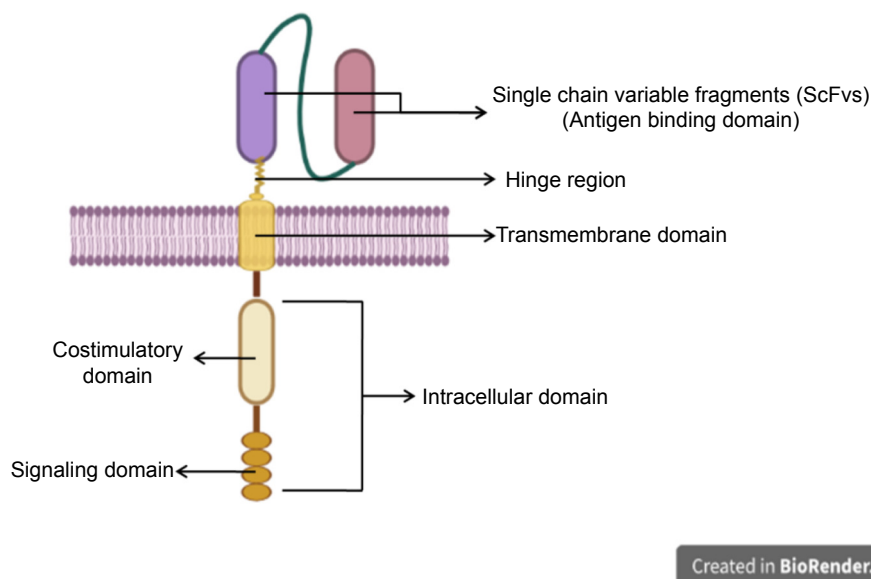


Fig. 2. Design of Chimeric Antigen Receptor.

gen recognition domain^[28]. It often provides stability for effective CAR expression and activity as well as versatility for accessing the targeted antigen. The ideal spacer length for a specific CAR is also affected by the position where the targeted epitope is located. Long hinge regions give the CAR more flexibility and improve access to complex glycosylated antigens or membrane-proximal epitopes. On the other hand, CARs with shorter hinge region are better at binding epitopes that are far away from membrane^[29]. The hinge regions are typically built using amino acid sequences derived from CD8, CD28, IgG1, or IgG4. But, IgG-derived spacers have the potential to interact with Fc receptors, which could lead to CAR-T cell degradation and subsequently reduced consistency in vivo. The solution to this issue is to either select a different spacer region or to further modify the existing spacer region in light of structural or functional issues^[15].

Transmembrane domain

The intracellular signaling domain and hinge region are sandwiched by CAR's transmembrane domain. The CD3, CD4, CD8, and occasionally CD28 molecules are the main sources of the transmembrane domain^[28]. It secures the binding of CAR to the T-cell membrane. CAR transmembrane domains have an impact on the level of CAR expression, stability, and potential for signaling or synapse formation, as well as their capacity to dimerize with endogenous signaling molecules^[15].

Intracellular domain

Binding of an antigen to the antigen recognition domain (extracellular domain) results in assembly and clustering of CAR receptors, generating activation signal that is transferred to the intracellular T-cell signaling domain, which further sends the signal inside the cell. Like other T-cells, CD-3 ζ 's cytoplasmic domain is utilized as the primary intracellular signaling domain, and the activation of CAR-T cells also requires co-stimulatory molecules or domains^[28]. The most common costimulatory endodomains are CD28, CD27, and 4-1BB. Higher effector function and self-limiting growth of CARs based on CD28 may be appropri-

ate for temporarily treating disorders with quick tumor eradication and temporary CAR persistence. On the other hand, CARs based on 4-1BB could be employed to treat conditions where prolonged T-cell persistence is necessary for full responses^[29].

Different generations of the CAR model have been created, based on the wide range of biomarker selection and structural complexity (Fig.3).

First generation CARs: Developed by Israeli immunologists Zelig Eshhar and Gideon Gross^[25]. The TCR's CD3 domain (T-cell activating chain) is linked to the scFv of the B-cell receptor or antibodies, to generate activating receptor molecules that are non-MHC-restricted^[1]. These CARs were phased out due to insufficient durability, antitumor efficacy, and signaling capacity^[6].

Second generation CARs: Designed by Fenney et. al.^[31]. These contained a co-stimulatory domain, like CD28 or 4-1BB (CD137), combined with the CD3 intracellular domain that mediates potent anti-tumor activity in patients who diagnosed with B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. In pre-clinical models, they improve T-cell persistence, cytokine release, and anti-tumor efficacy^[6]. These CARs are foundation of the recently approved CAR T-cell therapy in the form of "living drugs"^[21].

Third generation CARs: OX40 (CD134), CD28, 4-1BB (CD137), CD27, DAP10, or other sequences of co-stimulatory signals were combined with CD3 to enhance the response of third-generation CARs by enhancing cytokine production, T-cell proliferation, and killing^[8].

Fourth generation CARs: Designed by Chmielewski and colleagues^[32]. These CARs called TRUCKs (T-cells redirected for universal cytokine-mediated killing), include, among other things, extra costimulatory ligands or transgenes for the release of cytokines (such as IL-12)^[21].

Fifth generation CARs: Developed by Kagoya et. al.^[33]. These CARs are made up of cytokine receptors that have had their intracellular domains truncated (for example, the IL-2R chain fragment), and have a motif for the attachment of transcription factors like STAT-3/5. As a result, the secreted signal stimulates CAR-T

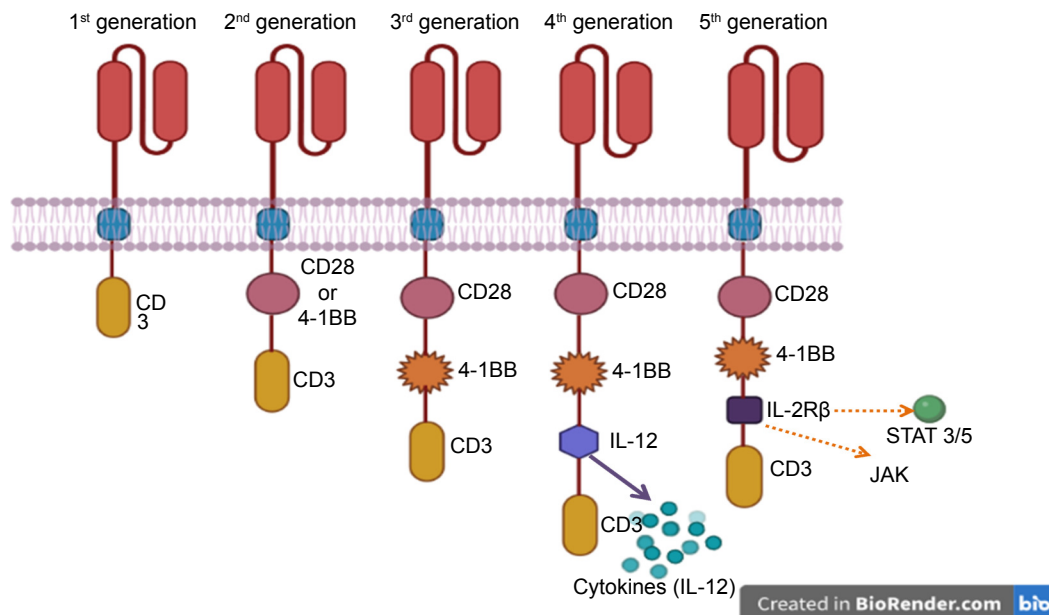


Fig. 3. Five generations of Chimeric antigen receptor (CAR).

cells to carry out their functions and produce memory T-cells while also reviving and activating the immune system^[6]. This receptor is activated by antigens in a manner that simultaneously engages the TCR (via the CD3 domains), co-stimulatory (CD28 domain), and cytokine (JAK-STAT3/5) signaling pathways, which provide all three synergistic signals necessary to promote complete T-cell activation and proliferation^[34].

Difficulties in Making Car for Solid Tumor

The difficulties posed by solid tumors are not yet sufficiently addressed by CAR-T cells such as high antigen heterogeneity in these tumors, physical barriers and highly immunosuppressive tumor microenvironments (TME) which have cellular, molecular, and metabolic characteristics that eventually cause T-cell exhaustion and dysfunction^[35]. When CAR-T cell therapy is used, the expression of the surface antigen is reduced or altered in cancer cells. As a result, CAR-T cell therapy fails to recognize them, and tumor cells can proliferate uncontrollably. Focusing on inhibitors of T-cell activation, such as Diacylglycerol (DAG) kinase, mutated tumor-specific antigens, such as epidermal growth factor receptor variant 3 (EGFRvIII) and interleukin-13 receptor alpha 2 (IL13R2), and creating CAR-T cells devoid of these enzymes may help CAR-T cell therapy for solid tumors be more effective^[36]. The abnormal vascularity, mismatch between chemokines and receptors, and fibrosis brought on by cancer-associated fibroblasts (CAFs) are just a few of the barriers that tumors present to T-cell trafficking. CAR-T cells that have been transduced to express chemokine receptors that improve targeting to the tumor include GD2 CAR designed to express CCR2b127, the CD30 CAR designed to express with CCR4128, and the CX3CL1 gradient to trigger CAR trafficking^[37]. A secure and efficient technique like regional injection in the local area, such as delivery of TAG72-CAR-T cells intraperitoneally to treat ovarian cancer and intracranial infusions of interleukin-13 receptor alpha 2 (IL13Rα2)-CAR-T cells to treat recurrent multifocal

glioblastoma, can be used to elevate the quantity and concentration of CAR-T cells^[38]. Adenosine suppresses the immune system by interacting with the prostaglandin 2 (PGE2)/EP2/protein kinase A (PKA) signaling pathway, which activates PKA and prevents TCR activation. A tiny peptide known as the "regulatory subunit I anchoring disruptor" (RIAD), reduces PKA's negative effects on TCR activation. When immune checkpoint molecules like programmed cell death ligand 1(PD-L1) are overexpressed, such tumors frequently exhibit T-cell exhaustion. CAR-T cells that express anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibodies as well as CAR-T cells that were engineered to lack PD-1 are currently being tested in clinical trials^[39].

Recent Advancements in Car-T Cell Therapy of Lymphomas and Solid Tumors

As a result of their effectiveness in treating hematological malignancies, genetic alteration and immune cell reprogramming are fast expanding fields with the aim of expanding therapeutic indications, lowering toxicity and relapse, and improving accessibility of the CAR-T cell therapy^[40].

CAR T-cell therapy in lymphoma

CAR-T cell therapy has become more well-known recently as an exciting new therapeutic alternative for patients with leukemias and lymphomas that are CD19-positive. Axicabtagenelecleucel and tisagenlecleucel, two CD19 CAR-T medicines, were approved in the US and subsequently throughout the world to treat aggressive, large B-cell non-Hodgkin's lymphoma and r/r diffuse large B-Cell lymphoma based on the outcomes of phase II trials in r/r NHL. The US Food and Drug Administration(FDA) most recently approved the CD19 CAR-T product brexucabtagene autoleucel for r/r Mantle cell lymphomas^[41]. Tisagenlecleucel, initially known as CTL019 was created as an anti-CD19 CAR and contained a CD137 signaling domain, also called tumor necrosis factor receptor superfamily member 9 (TNFRSF9), 4-1BB.

Apoptosis prevention and T-cell proliferation/survival are both influenced by CD137, which may all contribute to the protection provided by CD137-expressing CARs in circulation. Axicabtageneicel, in contrast, possesses a CD28 co-stimulatory domain. 4-1BB CAR-T cells are known to develop into central memory T-cells, on the other hand, CD28 CAR-T cells are known to generate a large significant portion of the effector memory cells^[42].

Lisocabtagene-Maraleucel (Liso-cel) is a CAR-T cell construct generated from a specified ratio of CD4 and CD8 cells. Despite the fact that long-term outcomes are unknown, preliminary data suggests that Liso-cel is an encouraging CAR-T design for individuals with recurrent NHL. A multicenter phase II Liso-cel experiment is already recruiting people based on these findings. Using CAR-T cells earlier in the course of the disease, possibly even before an autologous stem cell transplant, may produce better outcomes. Patients are currently being enrolled in TRANSFORM (NCT03575351), a large multi-center study that examines the efficacy and safety of autologous stem cell transplant (ASCT) with Liso-cel at first relapse and may offer solutions to these problems^[43].

Antigens other than CD19 may be targeted for lymphoma therapy for two main causes. First, there is no CD19 expression in Hodgkin lymphoma, T-cell lymphomas, or even in some B-cell NHLs. Second, the use of anti-CD19 CAR-T cells to treat CD19⁺ lymphomas may not be effective if all lymphoma cells do not express CD19 before treatment or if this expression is lost during treatment due to the persistence and quick expansion of resistant clones. These reasons have led to the development of CARs that target different lymphoma-associated antigens, such as CD20, CD22, Ig k-light chain, and CD30^[44].

The truncated epidermal growth factor receptor (EGFRt) or CD20 can be targeted with the cognate mAb, and suicide switches like inducible caspase 9 (iCASP9) or herpes simplex virus-derived thymidine kinase (HSV-TK) can be activated to remove CAR-T cells shortly after giving an inducer drug. Unfortunately, these traditional safety switches can only be activated

once to have an anticancer effect. Reversible inhibition by the tyrosine kinase inhibitor dasatinib is a more sophisticated strategy currently being used^[45]. Numerous clinical trials are examining the combination of approved CAR-T therapies with other additive or synergistic substances that may improve the anticancer activity of CAR-T cells, lessen T-cell exhaustion, or lessen toxicity. Checkpoint inhibitors, immunomodulators, and Bruton tyrosine kinase (BTK) inhibitors are among the CAR-T products currently undergoing phase 1/2 trials^[46]. Currently approved CAR-T products for CD-19 Lymphomas have been shown in Table 1^[47-49].

CAR T-cell therapy in Solid tumors

Target antigens present on solid tumors are typically heterogeneous, varying not only between primary and metastatic tumors but even within a single tumor^[50]. Also, the suitable antigens on tumor cells are frequently shared with the healthy cells which make possible toxicity a serious concern. In contrast to CD19 targeting lymphoma vs. healthy B-cells, there is no healthy tissue that can be sacrificed without harm in the case of solid tumors. However, some rare tumor-specific antigens do exist^[51].

Solid tumors' oncofetal antigens make excellent targets for CAR therapy since they are expressed primarily by tumor cells, which make them extremely selective. This strategy is demonstrated by CAR-T cells that target the mutant epidermal growth factor receptor (EGFR). Only malignant tumor cells express EGFR variant3 or EGFRvIII (mostly glioblastomas)^[52]. The anticancer efficacy and safety of 2 distinct anti-EGFRvIII CAR designs are being evaluated in ongoing trials. For patients with HPV-16⁺ malignancies, a TCR study focusing on an A2-restricted epitope generated from the high-risk HPV-16 serotype E6 oncoprotein has been further initiated^[53].

Tumor-associated glycoprotein 72 (TAG72) is a CAR-T cell therapy target in ovarian cancer. MUC16-CAR-T cells, Her2-CAR-T cells, and Meso-CAR-T cells are also being used to treat ovarian cancer. It has been discovered that a novel target for CAR-T cell therapy is carbonic anhydrase IX (CA-IX), which is expressed in a number of renal malignancies. Bi-specific CART

Table 1. Currently approved CAR-T products for CD-19- Lymphomas.

Product Name	Manufacturer	Brand Name	Target Antigen	Targeted Disease	Patient Population	FDA Approval
Tisagenlecleucel	Novartis	Kymriah	CD19	R/R B-cell acute lymphoblastic leukemia (ALL)	Children and adults with R/R B-cell ALL	August 2017
	Novartis			R/R B-cell non-hodgkin lymphoma (NHL)	Adults with R/R B-cell NHL	May 2018
Axicabtageneicel	Kite pharma	Yescarta	CD19	R/R B-cell non-hodgkin lymphoma (NHL)	Adults with R/R B-cell NHL	October 2017
	Kite pharma			R/R Follicular lymphoma	Adults with R/R B-cell follicular lymphoma	March 2021
Brexucabtagene autoleucel	Kite pharma	Tecartus	CD19	R/R Mantle cell lymphoma (MCL)	Adults with R/R B-cell MCL	July 2020
	Kite pharma			R/R B-cell acute lymphoblastic leukemia (ALL)	Adults with R/R B-cell ALL	October 2021
Lisocabtagene maraleucel	Juno therapeutics	Breyanzi	CD19	R/R B-cell non-hodgkin lymphoma (NHL)	Adults with R/R B-cell NHL	February 2021
Idecabtagene vicleucel	BMS and bluebird bio	Abecma	BCMA	R/R Multiple myeloma	Adults with R/R multiple myeloma	March 2021
Ciltacabtagene autoleucel	Janssen Pharmaceuticals	Carvykti	BCMA	R/R Multiple myeloma	Adults with R/R multiple myeloma	February 2022

cells, such as Trop2/PD-L1 CAR-T cells, can effectively inhibit the growth of gastric cancer than Trop2-specific CAR-T cells. Several target antigens, including CD133, CD24, PSCA, CEA, MUC-1, mesothelin, FAP, and Her-2, were identified in preclinical and clinical trials for pancreatic cancer CAR-T cell therapy^[54]. Among many cytokines released by tumors, transforming growth factor- β (TGF β) is essential for the maturation of suppressive regulatory T-cells (Tregs) and the upkeep of T-cell homeostasis. To prevent TGF- β enriched TME from applying immunosuppressive stress on CAR-T cells, researchers are investigating methods

to block the signal by developing a dominant-negative receptor or to benefit from this signal by linking the extracellular domain of the TGF- β receptor to an intracellular stimulatory domain. Using gene editing tools like CRISPR/Cas9 and transcription activator-like effector nucleases (TALENs) from xanthomonas bacteria for editing the precise target gene loci is another approach being researched to increase the effectiveness of CAR-T cell therapy^[55]. List of completed CAR-T immunotherapy studies in solid tumors is shown in [Table2](#)^[56,57].

Table 2. list of completed CAR-T immunotherapy studies in solid tumors.

Cancer Type	Clinical Trial Number	Status	Brief Title	Cell Target
Brain Cancers (Glioblastoma)	NCT01454596	Completed recruiting/ phase I	CAR-T cell receptor immunotherapy that targets EGFRvIII for patients having Malignant Gliomas expressing EGFRvIII	EGFRvIII
	NCT01109095	Completed recruiting/ phase I	Cytomegalovirus (CMV)-specific cytotoxic T-cells that express CAR targeting HER2 in patients having Glioblastoma multiform (GBM)	HER2
Hepatocellular Carcinoma	NCT02723942	Completed/phase 1/2	CAR-T cell immunotherapy for Hepatocellular carcinoma that targets GPC3	GPC3
Pancreatic Cancer	NCT02465983	Completed/phase 1	Pilot study of autologous T-cells in patients having Metastatic pancreatic cancer	Mesothelin
Breast Cancer	NCT02547961	Completed/phase 1/2	Chimeric antigen receptor-modified T- cells for patients having Breast cancer	HER-2
Sarcoma, Osteosarcoma, Neuroblastoma, Melanoma Metastatic cancer,	NCT02107963	Completed/phase 1	A phase I Trial of T-cells that express an anti-GD2 CAR in children and young adults having GD2 ⁺ solid tumors	GD2
Metastatic Melanoma, Renal Cancer	NCT01218867	Completed/phase 1/2	CAR-T Cell receptor immunotherapy that target VEGFR2 for patients having Metastatic cancer	VEGFR2

Limitations of the Cart-Cell Therapy

CAR-T resistance linked to antigen escape, antigen-positive relapses linked to poor persistence of CAR-T cell, inability to timely inject a CAR-T cell product into patients, non-responsiveness or incomplete responses, and toxicities are some of the challenges connected to CAR-T cell therapy^[58]. The two most frequently noted adverse reactions of CAR-T cell therapy in hematologic cancer studies were neurotoxicity and cytokine release syndrome^[59]. Furthermore, because of T-cell exhaustion, the infused T-cells may cease to perform their effector functions^[60].

CAR-T cell Toxicity

Neurotoxicity and cytokine release syndrome (CRS) are two side effects of CAR-T cells. CAR-T cell activation and overexpansion, as well as increased production of cytokines by CAR-T cells as well as other immune cells, such as IL-6, IL-10, interferon-gamma (IFN- γ), tumour necrosis factor-alpha (TNF- α), and granulocyte-macrophage colony-stimulating factor, all contribute to CRS^[61]. Initial symptoms of the cytokine release syndrome are noninfectious flu-like symptoms, but they can progress to potentially lethal capillary leakage with hypoxia and hypotension^[62]. Headache, aphasia, delirium, cerebral hemorrhage, seizures, and even death are some neurological symptoms of neurotoxicity. According to autopsy results that demonstrate disrupted endothelial function and blood-brain barrier disruption, the activation of endothelial cells may facilitate the development of neurotoxicity^[63].

Relapse

Relapses come in two flavors, antigen-positive and antigen-negative. Typically, antigen-positive relapse is accompanied by short CAR-T cell persistence and low potency. The potency and persistence of CAR-T cells can be affected by CAR construct components like costimulatory domains and scFv. When tumor cells lose or have their antigens altered, they exhibit an additional relapse pattern known as antigen-negative relapse, making them resistant to CAR-T cell recognition. Gene mutation, lineage switching, immune selection, selective splicing, trogocytosis, and antigen escape are among the potential causes^[64]. In antigen escape, patients experience a relapse of their disease with a phenotypically similar phenotype but without the surface expression of a CD19 molecule that can bind the anti-CD19 antibodies built into the CARs. Lineage switch is a relapse of a genetically similar but phenotypically distinct malignancy, most frequently acute myeloid leukemia (AML), in a patient^[65].

Tumor microenvironment

Numerous factors limit the efficacy of CAR-T cells in the tumour microenvironment. Transforming growth factor (TGF)- β stimulates stromal cells to create a thick, fibrogenic tumour microenvironment (TME), which starts the production of extracellular matrix (ECM) proteins. ECM blocks T-cell trafficking and motility and irregular vasculature of solid tumors result in tissue

hypoxia blocking T-cell extravasation into the TME^[16]. The effector capabilities of cytotoxic T-cells within tumors are decreased by immunosuppressive cells such as T regulatory cells, tumor associated monocytes (TAMs), tumor associated neutrophils (TANs), and myeloid-derived suppressor cells (MDSCs) along with inhibitory cytokines and factors like IL-10, TGF- β , prostaglandin E2 (PGE2), arginase, cyclooxygenase 1 (COX1), galectin-9, and indoleamine 2,3-dioxygenase (IDO)^[66]. Majority of the tumor stroma is made up of cancer-associated fibroblasts (CAFs), which are also in charge of encouraging the buildup of extracellular matrix (ECM), which physically inhibits immune cell infiltration^[67]. In addition to serving as a physical barrier to stop immune cells from entering the tumor, they also naturally produce growth factors (VEGF and PDGF) that promote growth, angiogenesis, invasion, and metastasis of the tumor^[68].

Tumor heterogeneity

Due to the heterogeneity of the CAR target antigen in solid tumors, CAR-Ts are unable to effectively detect cancer cells and are unable to produce an appropriate CAR-T mediated anti-tumor response to cancer cells that express that specific target antigen. Such ineffective antitumor responses may cause the treatment to be unsuccessful and cause the tumor to grow more^[69].

Poor CAR-T infiltration rate

In solid tumors, a number of tumor-applied mechanisms result in a decrease in the secretion of a number of vasculature-related factors, which prevents CAR-Ts from penetrating the vascular endothelium and reaching the tumor tissue. In accordance with additional studies, a number of solid tumor types are resistant to T-cell infiltration at the tumor site when the WNT/ β -catenin signaling pathway is active (including metastatic melanoma and colorectal cancer)^[69]. Successful immune cell trafficking requires the appropriate chemokine receptors on the T-cells and the coordinated expression of chemokines secreted by the tumor. Similarly, how T-cells and tumor endothelium express adhesion receptors and ligands in a coordinated manner drives the infiltration process. Sadly, tumors frequently suppress the expression of chemoattractant molecules, allowing them to evade immune surveillance^[67].

Ways to Overcome the Limitations

There are various ways which are being used and further being investigated in order to overcome the limitations associated with CAR-T cell therapy and to improve its efficacy in patients.

Removing Toxicity

A less toxic antigen for CAR-T cells or dual-targeted CARs that increase the tumor selectivity of CAR-T cells may be effective in reducing toxicity. Local (intratumoral) administration of CAR-T cells that are specifically directed towards cancer stem cells (CSCs) can also reduce toxicity. Additionally, adding suicide genes to CAR-T cells as a "safety switch" in the event of unmanageable adverse responses may restrict on-target, off-tumor toxicities^[70]. By including surface proteins like RQR8/CD20 in the CAR design, a target is created for their removal by antibodies like rituximab. With this method, the bulk of CAR-T cells disappear in a matter of hours. Improved control over effector responses and tumor target sensing is made possible by tetracycline-inducible systems or AND/NOT Boolean logic gates (Syn-Notch

receptors in CAR-T cells and iCAR) in CAR-T cells^[71]. Early administration of tocilizumab can reduce the risk of serious cytokine release syndrome (CRS) and end-organ dysfunctions without affecting the growth, persistence, and response times of CAR-T cells^[72].

Relapse

Co-expressing a CD28-based CAR with a 4-1BB ligand increased therapeutic efficacy, reconciling the tumoricidal function provided by CD28 co-stimulation with the improved T-cell persistence provided by 4-1BB engagement^[73]. T-cells that co-express two distinct chimeric receptors specific for two different tumor associated antigens (TAAs) or CAR-T cells with tandem bispecific targeting domains (Tandem CAR or TanCAR) can cytotoxically attack tumor cells that are concurrently expressing either antigen or both antigens. UniCAR-T cells can launch cytotoxic responses against escaping tumor cells when new tumor antigen-specific targeting modules are introduced, as opposed to those that are alternatively spliced, have expression loss, or are expressed at low levels^[74]. Multi-targeted CAR-T cells may be a significant way to reduce tumor immune escape given the variety of antigenic phenotypes present in solid tumors^[75]. Increasing antigen expression on target cells is another approach. By reducing cleavage of surface-expressed B-cell maturation antigen (BCMA), small-molecule γ -secretase inhibitors enhance B-cell maturation antigen (BCMA; also known as TNFRSF17) expression on myeloma cells^[76].

Overcoming the immunosuppressive tumor microenvironment

A concept-based tactic called "switch receptors" changes an inhibitory signal into a stimulatory one. The anti-TGF- β CAR, which is intended to function as a switch receptor, is composed of an anti-TGF- β scFv and intracellular signaling domains obtained from the T-cell co-stimulatory molecules used in traditional CAR. ShRNA or the CRISPR-Cas9 gene-editing system can also be used to reasonably interfere with immune checkpoint molecule expression on CAR-T cells^[77]. Epidermal growth factor receptor class III variant (EGFRvIII)-targeting CAR-T cell has the ability of significant intratumor regulatory T-cells (Treg cell) infiltration following CAR-T cell infusion. An EGFR-bispecific T-cell engager (BiTE) was created which is produced by EGFRvIII-targeted CAR-T cells and directs regular T-cells and Tregs to exert cytotoxic effect against the tumor^[76]. Fibroblast activation protein (FAP) is a protein that can be targeted by CAR-T cells in order to lessen the amount of CAFs in the microenvironment^[67]. Cancer associated fibroblasts produce the chemokine CXCL12 that keeps T-cells away from tumor tissue. T-cells can enter the tumor bed more easily and effectively if the CXCL12 and CXCR4 interaction is prevented. In contrast, when modified CAR-T cells and CXCR4/CXCL12 inhibitors like AMD3100 and NOXA12 were used to co-target the tumor, the anticancer activity was more potent and CAR-T cell accumulation in the tumor microenvironment was greater than with adoptive T-cell therapy alone^[68].

Overcoming tumor heterogeneity

Anti-EGFR BiTEs and universal CARs can be used to target multiple antigens, including avidin-linked CARs coupled with biotinylated antibodies, CARs containing scFvs that recognise a fluorescein isothiocyanate fluorophore combined to TAA-binding molecules, CARs that contain FcRys as the antigen-binding domain, and SUPRA (split, universal, and programmable) CARs

in which leucine zipper motifs are used to match CARs (zipCAR) with free scFvs (zipFv)^[78]. Diverse capabilities of physiological immune receptors like NKG2D (natural killer group 2 member D) of binding ligands can be used as a replacement for the traditional antibody-based CAR limitation. A CAR having NKG2D as targeting moiety recognizes a number of ligands that are induced by stress and are expressed inside the TME of cancers and has the potential to eradicate an extensive array of cancers while simultaneously modifying the tumor and its supporting structure. A second ligand-based CAR approach is based on the ErbB receptor family, which has at least one member expressed in 88% of solid tumors^[74].

Improving CAR-T infiltration rate

Recent studies profiled the cytokine/chemokine profiles of various tumors in order to better pinpoint the tumors that CAR-T should target. One example that is noteworthy is the generation of anti-CD70 CAR-T cells which express high levels of IL-8R and are more effective at recruiting to the tumor site than CD70-CAR alone, considering that CD70 + gliomas produce high levels of interleukin IL-8. Similar to this, CAR-T cells that target mesothelin and concurrently express the CCL2 receptor CCR2b can effectively eradicate lung tumors that secrete high amounts of CCL2. Oncolytic viruses, which only infect tumor cells, are the basis of another tactic^[67]. Local administration of CAR-T cells in tumor sites has favored CAR-T cell trafficking and tumor penetration^[79].

Combination therapies to enhance CAR-T cell efficacy in solid tumors

CAR-T cell-secreted PD-1 blocking antibodies can bind to PD-1 in a competitive manner, enhancing CAR-T cell growth and cytotoxicity^[80]. CRISPR-Cas9-mediated disruption of the PD-1 locus in CAR-T cells has been shown to improve therapeutic efficacy in vitro and in vivo. Several clinical trials are currently underway to investigate these methods^[81]. Another strategy is to genetically modify CAR-T cells to express PD1 dominant-negative receptors (DNR) or PD1:CD28 switch receptors (by switching the transmembrane and intracellular domains of PD1 with CD28), which disrupt PD1 inhibitory signaling and are thus tolerant to PDL1 upregulation^[68]. CAR-T therapy can be combined with chemotherapy and radiotherapy to improve the outcomes in solid tumors. Chemotherapy has an immunomodulatory effect when used sparingly; it promotes dendritic cell activation and tumor antigen presentation to CAR-T cells, blocks immunosuppressive cells and hence increases CAR-T cell persistence, and sensitizes tumor cells to CAR-T cell activity by encouraging granzyme B penetration into tumor cells. Radiotherapy can explicitly induce apoptosis which promote tumor antigen presentation and dendritic cell maturation and activation. Damage-associated molecular patterns (DAMPs) and INF- γ are released following radiation, causing CAR-T cells to migrate and infiltrate the tumor more effectively^[80]. Some of the CAR-T cell combination therapies are listed in Table 3^[82,83].

Table 3. List of some CAR-T cell combination therapies^[82,83].

Drug Class	Combination Strategy	CAR-T cell Target Antigen
PD-1 inhibitor	Pembrolizumab	GD-2 CD-19 Mesothelin EGFRvIII CD-19 CD-19 B-cell Ag
	Atezolizumab Durvalumab Nivolumab	
	Ipilimumab	IL13Ra2
Immunomodulators	Lenalidomide	BCMA CS1 CD20 CD19 CD133 HER-2 EGFRvIII CD19/CD20
	Decitabine	
GM-CSF inhibitor	Lenzilumab	CD19
TGF-beta inhibitor	Galunisertib	CD133 HER2 ROR1
	SD-208	
Vaccine	Imovax Rabies VZV PCV13	B-cell Ag GD2 CD19
Oncolytic virus (OV)	Binary OAV	HER2
Chemotherapy	Fludarabine Cyclophosphamide ALLO-647 (anti-CD52)	CD19/BCMA
mAb	Rituximab	CD19

Conclusion

In CAR-T cell therapy, T-cells are fused with CAR to instruct the immune system to target tumor cells and perform an effector function against tumors. Despite the fact that this therapy has been very effective in treating cancer patients, there are some drawbacks to its therapeutic efficacies, including relapse, tumor heterogeneity and associated toxicities. Numerous clinical trials are looking at the interaction of approved CAR-T therapies with additional additive or synergistic drugs that may improve CAR-T cell anticancer activity, reduce T-cell exhaustion, or reduce toxicity. CAR-T efficacy can be improved in patients in a number of ways, including using multi-specific CAR-T-cells to lower the toxicity, antigen escape, and tumor heterogeneity, using an automated system (CliniMACS Prodigy System) to speed up production, etc. For improving the specificity and efficacy of CAR-T cell therapy, many more approaches are being used and further researched. Given how new this type of immunotherapy is, there is still much to learn about its efficacy.

Abbreviations

ACT, Adoptive Cell Therapy; ALL, Acute Lymphoblastic Leukemia; AML, Acute Myeloid Leukaemia; APC, Antigen Presenting Cell; ASCT, Autologous Stem Cell Transplant; BCMA, B-cell Maturation Antigen; BiTE, Bispecific T cell Engager; BTK, Bruton Tyrosine Kinase; CAF, Cancer Associated Fibroblasts; CAF, Cancer-Associated Fibroblast; CA-IX, Carbonic Anhydrase IX; CAR, Chimeric Antigen Receptor; CEA, Carcinoembryonic Antigen; CMV, Cytomegalovirus; COX1, Cyclooxygenase 1; CRS, Cytokine Release Syndrome; CSC, Cancer Stem Cell; CTLA 4, Cytotoxic T-Lymphocyte Associated Antigen 4; DAG, Diacylglycerol; ECM, Extracellular Matrix; EGFRt, Truncated Epidermal Growth Factor Receptor; EGFRvIII, Epidermal Growth Factor Receptor Variant 3; FAP, Fibroblast Activation Protein; FDA, Food and Drug Administration; HPSE, Heparanase; HSPG, Heparan Sulphate Proteoglyca; HSV-TK, Herpes Simplex Virus-Derived Thymidine Kinase; iCASP9, Inducible Caspase 9; IDO, Indoleamine 2, 3-Dioxygenase; IFN, Interferon; Ig, Immunoglobulin; IL, Interleukin; IL13R α 2 - Interleukin-13 Receptor Alpha 2; JAK - Janus Kinase; mAb, Monoclonal Antibody; MDSC, Myeloid-Derived Suppressor Cell; MHC, Major Histocompatibility Complex; NHL, Hodgkin Lymphoma; NK, Natural Killer; NKG2D, Natural Killer Group 2 Member D; NKT, Natural Killer T-cell; PD-L1, Programmed Cell Death Ligand 1; PGE2, Prostaglandin E2; RIAD, Regulatory Subunit I Anchoring Disruptor; scFv, Single-Chain Variable Fragment; STAT, Signal Transducers and Activators of Transcription; SUPRA, Split, Universal and Programmable; TAA, Tumor Associated Antigen; TAG72, Tumor-Associated Glycoprotein 72; TALEN, Transcription Activator-Like Effector Nuclease; TAM, Tumor Associated Monocytes; TAN, Tumor Associated Neutrophils; TCR, T-cell Receptor; TGF β , Transforming Growth Factor β ; TIL, Tumor-Infiltrating Lymphocytes; TME, Tumor Microenvironment; TNF, Tumor Necrosis Factor; TNFRSF9, Tumor Necrosis Factor Receptor Superfamily Member 9; Treg, Regulatory T cells; TRUCK, T-cells Redirected for Universal Cytokine-Mediated Killing; UniCAR-T, Universal CAR-T; WBC, White Blood Cells.

Conflict of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

PDP contributed to the consensus concept and design, PDP and AS were responsible for data acquisition, and AS was responsible for manuscript drafting. All authors approved the final version of the manuscript.

References

- [1] Mohanty R, Chowdhury CR, Arega S, et al. CAR T cell therapy: A new era for cancer treatment (Review). *Oncol Rep*, 2019, 42(6): 2183-2195.
- [2] Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer*, 2021, 149(4): 778-789.
- [3] Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol*, 2020, 20(11): 651-668.
- [4] Mao JJ. Introduction. *Cancer J*, 2019, 25(5): 305-306.
- [5] Ahles TA, Root JC. Cognitive Effects of Cancer and Cancer Treatments. *Annu Rev Clin Psychol*, 2018, 14: 425-451.
- [6] Akkin S, Varan G, Bilensoy E. A Review on Cancer Immunotherapy and Applications of Nanotechnology to Chemioimmunotherapy of Different Cancers. *Molecules*, 2021, 26(11): 3382.
- [7] Mehrabadi AZ, Ranjbar R, Farzanehpour M, et al. Therapeutic potential of CAR T cell in malignancies: A scoping review. *Biomed Pharmacother*, 2022, 146: 112512.
- [8] Schuster M, Nechansky A, Kircheis R. Cancer immunotherapy. *Biotechnol J*, 2006, 1(2): 138-147.
- [9] Miliotou AN, Papadopoulou LC. CAR T-cell Therapy: A New Era in Cancer Immunotherapy. *Curr Pharm Biotechnol*, 2018, 19(1): 5-18.
- [10] Taefehshok S, Parhizkar A, Hayati S, et al. Cancer immunotherapy: Challenges and limitations. *Pathol Res Pract*, 2022, 229: 153723.
- [11] Zhang H, Chen J. Current status and future directions of cancer immunotherapy. *J Cancer*, 2018, 9(10): 1773-1781.
- [12] Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol*, 2015, 33(17): 1974-1982.
- [13] Igarashi Y, Sasada T. Cancer Vaccines: Toward the Next Breakthrough in Cancer Immunotherapy. *J Immunol Res*, 2020, 2020: 5825401.
- [14] Rohaan MW, Wilgenhof S, Haanen JBAG. Adoptive cellular therapies: the current landscape. *Virchows Arch*, 2019, 474(4): 449-461.
- [15] Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*, 2021, 11(4): 69.
- [16] Kankeu Fonkoua LA, Sirpilla O, Sakemura R, et al. CAR T cell therapy and the tumor microenvironment: Current challenges and opportunities. *Mol Ther Oncolytics*, 2022, 25: 69-77.
- [17] Vormittag P, Gunn R, Ghorashian S, et al. A guide to manufacturing CAR T cell therapies. *Curr Opin Biotechnol*, 2018, 53: 164-181.
- [18] Turtle CJ. Chimeric antigen receptor modified T cell therapy for B

- cell malignancies. *Int J Hematol*, 2014, 99(2): 132-140.
- [19] Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat Rev Immunol*, 2012, 12(4): 269-281.
 - [20] Almásbak H, Aarvak T, Vemuri MC. CAR T Cell Therapy: A Game Changer in Cancer Treatment. *J Immunol Res*, 2016, 2016: 5474602.
 - [21] Subklewe M0, von Bergwelt-Baildon M, Humpe A. Chimeric Antigen Receptor T Cells: A Race to Revolutionize Cancer Therapy. *Transfus Med Hemoth*, 2019, 46(1): 15-24.
 - [22] Feins S, Kong W, Williams EF, et al. An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. *Am J Hematol*, 2019, 94(S1): S3-S9.
 - [23] Sermer D, Brentjens R. CAR T-cell therapy: Full speed ahead. *Hematol Oncol*, 2019, 37 Suppl 1: 95-100.
 - [24] Zhang X, Zhu L, Zhang H, et al. CAR-T Cell Therapy in Hematological Malignancies: Current Opportunities and Challenges. *Front Immunol*, 2022, 13: 927153.
 - [25] Styczyński J. A brief history of CAR-T cells: from laboratory to the bedside. *Acta Haematologica Polonica*, 2020, 51(1): 2-5.
 - [26] Brentjens RJ, Curran KJ. Novel cellular therapies for leukemia: CAR-modified T cells targeted to the CD19 antigen. *Hematology Am Soc Hematol Educ Program*, 2012, 2012: 143-151.
 - [27] Srivastava S, Riddell SR. Engineering CAR-T cells: Design concepts. *Trends Immunol*, 2015, 6(8): 494-502.
 - [28] Ahmad U, Khan Z, Ualiyeva D, et al. Chimeric antigen receptor T cell structure, its manufacturing, and related toxicities; a comprehensive review. *Adv Cancer Bio-Metastasis*, 2022, 4: 100035.
 - [29] Guedan S, Calderon H, Posey AD Jr, et al. Engineering and Design of Chimeric Antigen Receptors. *Mol Ther Methods Clin Dev*, 2018, 12: 145-156.
 - [30] Zhang C, Liu J, Zhong JF, et al. Engineering CAR-T cells. *Biomark Res*, 2017, 5: 22.
 - [31] Finney HM, Lawson AD, Bebbington CR, et al. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol*, 1998, 161(6): 2791-2797.
 - [32] Chmielewski M, Kopecky C, Hombach AA, et al. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res*, 2011, 71(17): 5697-5706.
 - [33] Kagoya Y, Tanaka S, Guo T, et al. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat Med*, 2018, 24(3): 352-359.
 - [34] Tokarew N, Ogonek J, Endres S, et al. Teaching an old dog new tricks: next-generation CAR T cells. *Br J Cancer*, 2019, 120(1): 26-37.
 - [35] Hou AJ, Chen LC, Chen YY. Navigating CAR-T cells through the solid-tumour microenvironment. *Nat Rev Drug Discov*, 2021, 20(7): 531-550.
 - [36] Jindal V, Arora E, Gupta S. Challenges and prospects of chimeric antigen receptor T cell therapy in solid tumors. *Med Oncol*, 2018, 35(6): 87.
 - [37] Ramakrishna S, Barsan V, Mackall C. Prospects and challenges for use of CAR T cell therapies in solid tumors. *Expert Opin Biol Ther*, 2020, 20(5): 503-516.
 - [38] Miao L, Zhang Z, Ren Z, et al. Obstacles and Coping Strategies of CAR-T Cell Immunotherapy in Solid Tumors. *Front Immunol*, 2021, 12: 687822.
 - [39] Dana H, Chalbatani GM, Jalali SA, et al. CAR-T cells: Early successes in blood cancer and challenges in solid tumors. *Acta Pharm Sin B*, 2021, 11(5): 1129-1147.
 - [40] Wehrli M, Maus MV. New CARs on and off the road: challenges and new developments in CAR-T cell therapy. *Curr Opin Pharmacol*, 2021, 59: 116-126.
 - [41] Ghilardi G, Braendstrup P, Chong EA, et al. CAR-T TREK through the lymphoma universe, to boldly go where no other therapy has gone before. *Br J Haematol*, 2021, 193(3): 449-465.
 - [42] Ahmad A, Uddin S, Steinhoff M. CAR-T Cell Therapies: An Overview of Clinical Studies Supporting Their Approved Use against Acute Lymphoblastic Leukemia and Large B-Cell Lymphomas. *Int J Mol Sci*, 2020, 21(11): 3906.
 - [43] Kallam A, Vose JM. Recent Advances in CAR-T Cell Therapy for Non-Hodgkin Lymphoma. *Clin Lymphoma Myeloma Leuk*, 2019, 19(12): 751-757.
 - [44] Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. *Nat Rev Clin Oncol*, 2018, 15(1): 31-46.
 - [45] Mestermann K, Giavridis T, Weber J, et al. The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. *Sci Transl Med*, 2019, 11(499): eaau5907.
 - [46] Qualls D, Salles G. Optimizing CAR T cell therapy in lymphoma. *Hematol Oncol*, 2021, 39 Suppl 1: 104-112.
 - [47] Gill S, Brudno JN. CAR T-Cell Therapy in Hematologic Malignancies: Clinical Role, Toxicity, and Unanswered Questions. *Am Soc Clin Oncol Educ Book*, 2021, 41: 1-20.
 - [48] Boettcher M, Joehner A, Li Z, et al. Development of CAR T Cell Therapy in Children-A Comprehensive Overview. *J Clin Med*, 2022, 11(8): 2158.
 - [49] Bourré L, Zhong B. The Progress of Precision Medicine in Immunology - Crown Bioscience. Available online: <https://blog.crownbio.com/the-progress-of-precision-medicine-in-immunology> [Last access on 2022].
 - [50] Yong CSM, Dardalhon V, Devaud C, et al. CAR T-cell therapy of solid tumors. *Immunol Cell Biol*, 2017, 95(4): 356-363.
 - [51] Titov A, Valiullina A, Zmievskaya E, et al. Advancing CAR T-Cell Therapy for Solid Tumors: Lessons Learned from Lymphoma Treatment. *Cancers (Basel)*, 2020, 12(1): 125.
 - [52] Newick K, O'Brien S, Moon E, et al. CAR T Cell Therapy for Solid Tumors. *Annu Rev Med*, 2017, 68: 139-152.
 - [53] Klebanoff CA, Rosenberg SA, Restifo NP. Prospects for gene-engineered T cell immunotherapy for solid cancers. *Nat Med*, 2016, 22(1): 26-36.
 - [54] Marofi F, Motavalli R, Safonov VA, et al. CAR T cells in solid tumors: challenges and opportunities. *Stem Cell Res Ther*, 2021, 12(1): 81.
 - [55] Baybutt TR, Flickinger JC Jr, Caparosa EM, et al. Advances in Chimeric Antigen Receptor T-Cell Therapies for Solid Tumors. *Clin Pharmacol Ther*, 2019, 105(1): 71-78.
 - [56] Filley AC, Henriquez M, Dey M. CART Immunotherapy: Development, Success, and Translation to Malignant Gliomas and Other Solid Tumors. *Front Oncol*, 2018, 8: 453.
 - [57] Patel U, Abernathy J, Savani BN, et al. CAR T cell therapy in solid tumors: A review of current clinical trials. *EJHaem*, 2021, 3(Suppl 1): 24-31.
 - [58] Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol*, 2019, 16(6): 372-385.
 - [59] Grigor EJM, Fergusson D, Kekre N, et al. Risks and Benefits of Chimeric Antigen Receptor T-Cell (CAR-T) Therapy in Cancer: A Systematic Review and Meta-Analysis. *Transfus Med Rev*, 2019, 33(2): 98-110.
 - [60] Abrantes R, Duarte HO, Gomes C, et al. CAR-Ts: new perspectives in cancer therapy. *FEBS Lett*, 2022, 596(4): 403-416.
 - [61] Akhoundi M, Mohammadi M, Sahraei SS, et al. CAR T cell therapy as a promising approach in cancer immunotherapy: challenges and opportunities. *Cell Oncol (Dordr)*, 2021, 44(3): 495-523.

- [62] June CH, Sadelain M. Chimeric Antigen Receptor Therapy. *N Engl J Med*, 2018, 379(1): 64-73.
- [63] Chen L, Chen F, Niu H, et al. Chimeric Antigen Receptor (CAR)-T Cell Immunotherapy Against Thoracic Malignancies: Challenges and Opportunities. *Front Immunol*, 2022, 13: 871661.
- [64] Xu X, Huang S, Xiao X, et al. Challenges and Clinical Strategies of CAR T-Cell Therapy for Acute Lymphoblastic Leukemia: Overview and Developments. *Front Immunol*, 2021, 11: 569117.
- [65] Majzner RG, Mackall CL. Tumor Antigen Escape from CAR T-cell Therapy. *Cancer Discov*, 2018, 8(10): 1219-1226.
- [66] Masoumi J, Jafarzadeh A, Abdolalizadeh J, et al. Cancer stem cell-targeted chimeric antigen receptor (CAR)-T cell therapy: Challenges and prospects. *Acta Pharm Sin B*, 2021, 11(7): 1721-1739.
- [67] Boccalatte F, Mina R, Aroldi A, et al. Advances and Hurdles in CAR T Cell Immune Therapy for Solid Tumors. *Cancers (Basel)*, 2022, 14(20): 5108.
- [68] Marofi F, Achmad H, Bokov D, et al. Hurdles to breakthrough in CAR T cell therapy of solid tumors. *Stem Cell Res Ther*, 2022, 13(1): 140.
- [69] Safarzadeh Kozani P, Safarzadeh Kozani P, Ahmadi Najafabadi M, et al. Recent Advances in Solid Tumor CAR-T Cell Therapy: Driving Tumor Cells From Hero to Zero? *Front Immunol*, 2022, 13: 795164.
- [70] Cui X, Liu R, Duan L, et al. CAR-T therapy: Prospects in targeting cancer stem cells. *J Cell Mol Med*, 2021, 25(21): 9891-9904.
- [71] Knochenhahn HM, Smith AS, Dwyer CJ, et al. CAR T Cells in Solid Tumors: Blueprints for Building Effective Therapies. *Front Immunol*, 2018, 9: 1740.
- [72] Schubert ML, Schmitt M, Wang L, et al. Side-effect management of chimeric antigen receptor (CAR) T-cell therapy. *Ann Oncol*, 2021, 32(1): 34-48.
- [73] Springuel L, Lonez C, Alexandre B, et al. Chimeric Antigen Receptor-T Cells for Targeting Solid Tumors: Current Challenges and Existing Strategies. *BioDrugs*, 2019, 33(5): 515-537.
- [74] Safarzadeh Kozani P, Safarzadeh Kozani P, Rahbarizadeh F, et al. Strategies for Dodging the Obstacles in CAR T Cell Therapy. *Front Oncol*, 2021, 11: 627549.
- [75] Zhang ZZ, Wang T, Wang XF, et al. Improving the ability of CAR-T cells to hit solid tumors: Challenges and strategies. *Pharmacol Res*, 2022, 175: 106036.
- [76] Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer*, 2021, 21(3): 145-161.
- [77] Adachi K, Tamada K. Paving the road to make chimeric antigen receptor-T-cell therapy effective against solid tumors. *Cancer Sci*, 2022, 113(12): 4020-4029.
- [78] Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol*, 2020, 17(3): 147-167.
- [79] Drougkas K, Karampinos K, Karavolias I, et al. Comprehensive clinical evaluation of CAR-T cell immunotherapy for solid tumors: a path moving forward or a dead end? *J Cancer Res Clin Oncol*, 2023, 149(6): 2709-2734.
- [80] Maalej KM, Merhi M, Inchakalody VP, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. *Mol Cancer*, 2023, 22(1): 20.
- [81] Umut Ö, Gottschlich A, Endres S, et al. CAR T cell therapy in solid tumors: a short review. *Memo*, 2021, 14(2): 143-149.
- [82] Al-Haideri M, Tondok SB, Safa SH, et al. CAR-T cell combination therapy: the next revolution in cancer treatment. *Cancer Cell Int*, 2022, 22(1): 365.
- [83] Stock S, Kluever AK, Endres S, et al. Enhanced Chimeric Antigen Receptor T Cell Therapy through Co-Application of Synergistic Combination Partners. *Biomedicines*, 2022, 10(2): 307.