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Review Article

CAR-T cell therapy: breakthroughs, challenges and emerging horizons in cancer treatment

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ABSTRACT

Chimeric Antigen Receptor (CAR)-T cell therapy has recently emerged as a breakthrough technology, offering a targeted approach in the treatment of cancers. It is a form of cancer immunotherapy which involves genetic modification of autogenic or allogenic T cells to express a chimeric receptor that can target a specific tumor antigen on the malignant cells. The receptors are chimeric because both antigen-binding and T cell activating functions are integrated into a single receptor. The greatest potential of CAR-T cell therapy lies in its power to use the patient's immune system to fight cancer besides its durability. It also overcomes certain limitations such as limited effectiveness in resistant cancers, lack of precision in blood cancers etc. associated with the traditional cancer therapies like chemotherapy and radiation. CAR-T cell therapy has proven significantly efficacious in clinical trials for the treatment of patients with relapsed or refractory haematological malignancies and to a lesser extent in solid tumors too. A few of these CAR- T cells therapies have finally been approved by the FDA after decades of pre-clinical developments. CAR-T cell therapy though causes long term remissions in some cancer patients, yet few patients either relapse after the therapy or suffer severe toxic and adverse effects, leaving innovation space for further research. This review discusses the structure of CAR-T cells, principle of CAR-T cell therapy and its clinical applications, efficacy, safety, challenges and future directions for its use in cancer patients.

Keywords: Chimeric antigen receptor, T cells, CAR-T cell, Relapsed or refractory leukaemia, Solid tumors, Cytokine release syndrome, Immune effector cell-associated neurotoxicity syndrome, Immunotherapy, Genetic engineering

INTRODUCTION

There exist different types of immunotherapies having varied degrees of efficacy in treating malignancies. To name a few, these are immune checkpoint inhibitors, monoclonal antibodies, immune system modulators, treatment vaccines or onco-vaccines etc.[1-4] A promising new therapy coming up in cancer treatment is chimeric antigen receptor (CAR)-T cell therapy, which is a novel immunotherapeutic methodology to kill cancer cells with the help of genetically engineered autologous or allogenic T cells.[5] The immunotherapy in cancer treatment primarily is either active or passive based on whether it specifically targets tumor cells (via the immune system) or augment the power of the immune system to target cancerous cells respectively. The CAR - modified T cells function as an active therapy wherein the genetically engineered CAR-T cells, having supra-physiological activities, associate with the tumor antigens resulting in both immediate and sustained anti-cancer effects. The tumor antigens targeted by CAR-T cell therapy in clinical trials, so far, are mainly CD19, CD22, CD30, CD33, CD123, FLT3 (FMS like tyrosine kinase 3), BCMA(B- cell maturation antigen), PSCA (prostate stem cell antigen), PSMA (prostate specific maturation antigen), PLAP (placental alkaline phosphatase antigen), EGFR (epidermal growth factor receptor), EGFRvIII (epidermal growth factor receptor variant III), HER2 (human epidermal growth factor receptor 2), MSLN (mesothelin), etc in different haematological and solid cancers.[6-8] The list is further expanding, day by day, by targeting new tumor antigens using CAR-T cell therapy in lab experiments. The thrust

and benefits of CAR-T cell therapy, however, have mainly been on lymphomas and leukemia as compared to solid malignancies due to restricted T cell trafficking, antigen heterogeneity, hostile tumor microenvironment etc in the latter.[9]

The CAR-T cells in contrast to conventional effector T cells can detect antigens independent of MHC presentation, thus expanding the range of its clinical applications. This also makes it more versatile in targeting the malignant cells which may otherwise escape the traditional immune response.[10] The CAR-T cells, however, are restricted in identifying surface expressed antigens only and on encountering the targeted surface antigen gets activated, proliferate and turn cytotoxic to kill tumor cells. The cytotoxic effects of CAR-T cell therapy are mediated through several mechanisms such as perforin granazyme pathway, FAS and FAS ligand pathway leading to cell death, release of cytokines etc.[11-14]

CHIMERIC ANTIGEN RECEPTOR (CAR) STRUCTURE

The different CAR-T cell therapies evaluated in clinical trials though differ in their distinct features that may influence their effectiveness, yet they all have common elements. The success of CAR-T cells lies in their unique structure, which basically is an integration of the specificity of antibodies with the potent cytotoxic power of the T cells.[15,16] CAR contains four distinct regions - an extracellular antigen recognition domain, an extracellular hinge region, trans-membrane domain and an intracellular signalling/activation domain (Figure1).[17] The intracellular domain is known to activate the T cell when the antigen binds to the extracellular recognition domain.[18] All the four domains of CAR play a significant role in identifying cancerous cells and T cell activation.

The antigen recognition domain is usually derived from the variable regions of a monoclonal antibody, combined into a single-chain variable fragment (scFv). The latter (scFv) is a chimeric protein composed of the light (VL) and heavy (VH) chains of immunoglobulins, linked by a short peptide which acts as a linker (Figure 1).[19] The linker contains hydrophilic residues, glycine and serine, for flexibility as well as glutamate and lysine for enhanced solubility to recognize wide range of tumor specific antigens across different cancers.[19,20] The VL and VH regions are pre-chosen for their ability to bind the target antigens such as CD19, CD22, CD30 etc. with high precision and minimum binding to normal cells. It is the chosen regions which determine how effectively the receptor identifies or binds to the tumor antigen on the cell surface.[21] The recombinant single-domain antibodies (sdAb), such as VHH (camelid heavy- chain variable domain) and VNAR (variable domain of new antigen receptor), have also been used as antigen recognition domains in CARs because of their high transduction efficiency in T cell.[21-23]

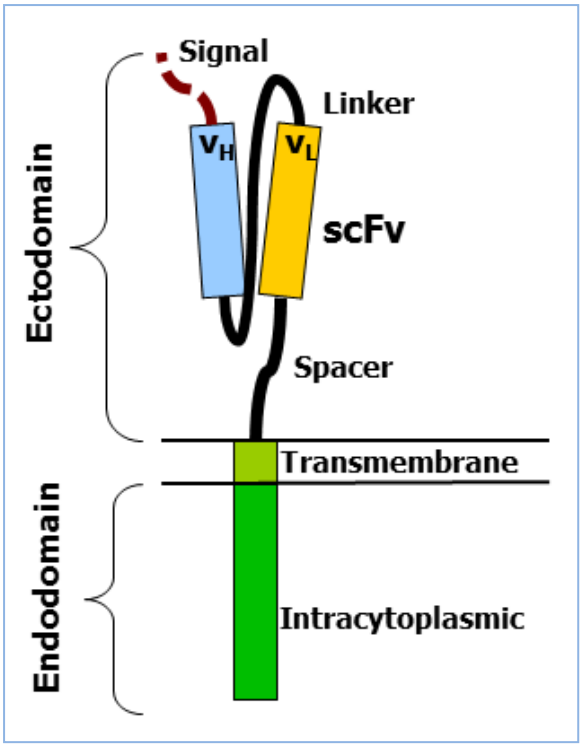

Figure 1: Different components of a chimeric antigen receptor (CAR).[110]

The hinge region, or spacer, is tiny as compared to other domains. It acts as a bridge between the antigen recognition domain and the trans-membrane domain. The spacer's ideal length depends on the position of the epitope on the antigen. It increases the flexibility of the scFv, minimizes spatial constraints between the antigen head and CAR, and improves the recognition of target antigen epitopes.[24] Hinge sequences are usually derived from membrane-proximal regions of other immune molecules such as IgG, CD8, and CD28.[25]

The trans-membrane domain is made up of a hydrophobic alpha helix that spans across the membrane. It couples the extracellular hinge and antigen recognition domains to the intracellular signalling domain. The trans-membrane domain is mostly derived from CD3-ζ, CD4, CD8, and at times from CD28 molecules. It secures the CAR to the plasma membrane for receptor stability through its hydrophobic alpha-helix structure.[25]

The intracellular T cell signalling domain is the endodomain of CAR (Figure 1). The binding of the antigen to the CAR extracellular domain causes clustering of the CAR receptors. This, consequently, initiates an activation signal that gets transmitted through the cytoplasmic T cell signaling domain into the cell. It is the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) present in the cytoplasmic domain of CD3- ζ which causes normal T cell activation. Therefore, the CD3- cytoplasmic domain, similar to normal T cells, is used as the primary CAR signaling endodomain component in CAR- T cells. Besides this, other co-

stimulatory molecules like CD27, CD28 or 4-1BB, CD28-OX40, CD- 134, CD-137 etc which are known to increase activation in the normal T cells are also used along with the primary signaling domain in CAR-T cells (Figure 2). The co-stimulatory domains provide additional benefits like enhanced T cell proliferation, in vivo persistence, cytokines release etc.[26-29]

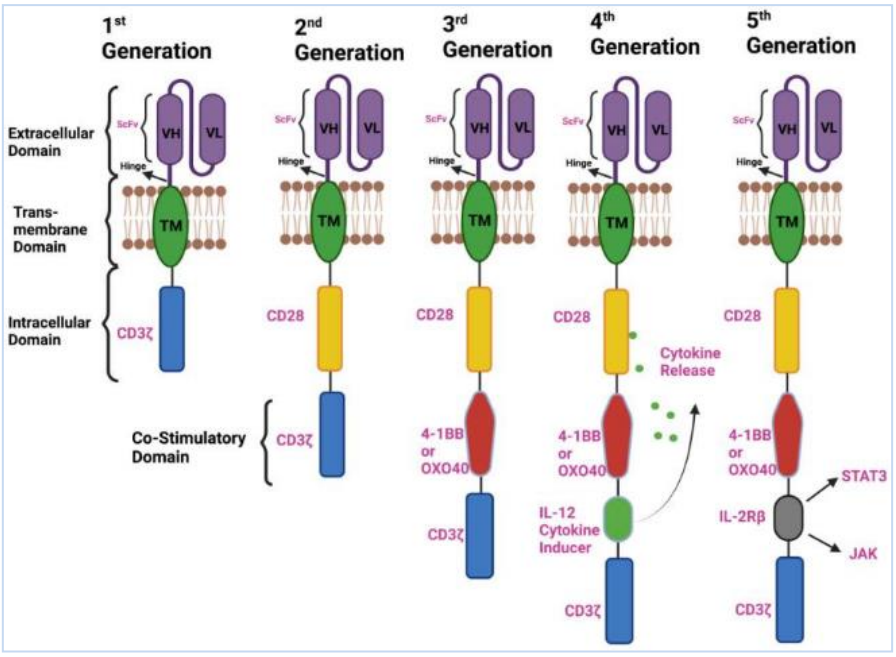

Figure 2: Different generations of chimeric antigen receptors (CARs).[7]

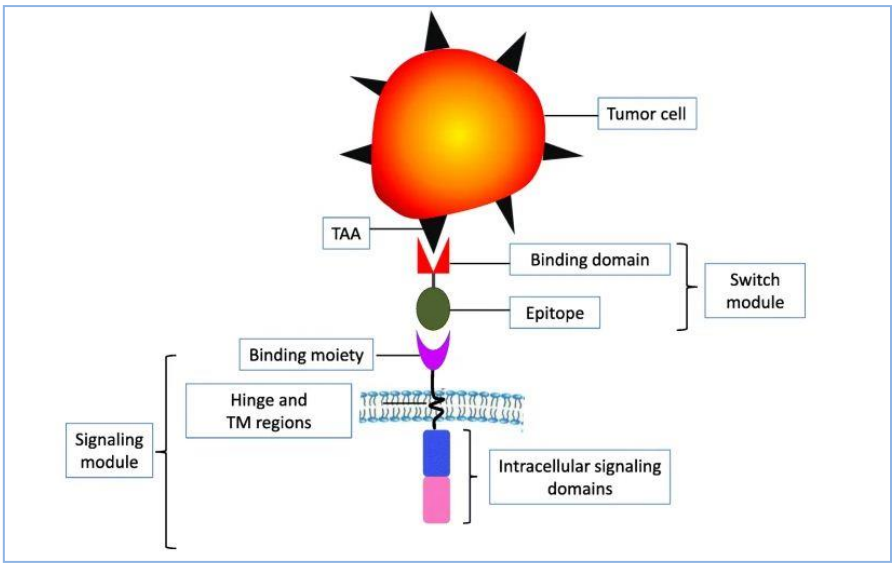

Figure 3: Diagrammatic representation of a UniCAR.[7]

DIFFERENT GENERATIONS OF CARs

The intracellular signaling domain also defines the type of "generation" of CAR.[30] First generation CARs have CD3-domain only whereas addition of a co-stimulatory domain turn the first-generation CARs into second generation (Figure 2). The addition of multiple co- stimulatory domains, to further boost up the T cell activity, results in the formation of robust third generation CARs which have enhanced effector functions and in vivo persistence than the second-generation CARs. The third generation CARs demonstrate increase phosphorylation of their downstream targets than second-generation CARs leading to enhanced intracellular signalling. The third generation CARs may however, elevate the infection risk because of enhanced B-cell aplasia, an increased capacity for

immune-related adverse effects and acute occurrences of cytokine storms.[19,31] Their favourable characteristics justify their potential selection as successors to the first and second generations CARs despite their adverse effects.

Fourth-generation CARs, which are also known as armored CARs or "TRUCKs" (T cells Redirected for Universal Cytokine Killing) contain a genetically engineered cytokine-induced domain IL-12 (cytokine inducer), to secrete pro-inflammatory cytokine such as IL-12, upon activation.[32] TUCKS enhance cytokines secretion, overcome tumor immune-suppression, enhanced persistence and overall survival for sustained immune response in the tumor microenvironment. TRUCKs enable the use of CAR-T cell therapy to target varied spectrum of malignancies, particularly the solid cancers.

Fifth generation CARs also known as next generation CARs consist of truncated intracellular cytoplasmic IL-2 beta receptor (IL-2Rβ) chain domain in addition to the CD28 or 4-1BB co-stimulatory domains in earlier CARs (Figure 2). The truncated intracellular cytoplasmic IL-2 beta chain receptor domain has a binding site for STAT3/5 transcription factors allowing antigen dependent activation of JAK/STAT pathway when the targeted antigen binds the fifth generation CAR. The added signaling complexity increases T cell survival, proliferation, ability to better differentiate between normal and cancerous tissues, and decreases the risk of off-target toxicity including adverse effects like cytokine release syndrome (CRS).[33,34]

The recent development is the generation of universal CARs (UniCARs), for broader applications in various tumor types, that can act as "off-the-shelf" therapeutic agents for wider and large scale clinical use. These are programmable universal CARs having modular approach in CAR-T cell therapy which like conventional CARs have no fixed antigen recognition domain. The antigen recognition domain in UniCAR is separated from the signaling domain so that it can be interchanged or switched without the need of re-engineering the CAR-T cells (Figure 3).[35] These are named UniCAR since the antigen binding module can be added or removed outside the body, making it highly universal, programmable and reversible.

The signaling module binds to a specific epitope on the switching module and is connected to intracellular signaling domains through hinge and trans-membrane (TM) regions. The switching module primarily has a switching molecule with a tumor associated antigen (TAA)-binding domain and a switching epitope specifically recognized by the signaling module (Figure 3). The switching module is thus, a bi-specific fusion molecule that can bind both the signaling domain and the targeted tumor associated antigen (TAA). This consequently provides an opportunity to simultaneously target multiple antigens in case of heterogeneous solid tumors.[36] A variety of switchable CARs have been engineered with different switchable modular designs such as biotin binding immune receptor CAR (BBIR CAR), split, universal, and programmable CAR (SUPRA CAR) etc.[35]

MECHANISM OF CAR-T CELL THERAPY

The patient's own immune cells to eliminate the tumors have remained the main focus of adoptive immunotherapy research since 1960s. The research in this direction resulted in the production of genetically engineered first CART constructs in 1993 by an immunologist Zelig Eshar from Israel.[37] The process of generating CAR-T cells involves several key steps such as T cell collection, gene modification, expansion, and infusion which ultimately eliminate cancerous cells.

The autogenic or allogenic T cells are harvested via leukapharesis and are genetically modified to express CARs that can specifically bind to antigens on cancer cells, such as CD19 in B-cell malignancies (Figure 4). The genetic modification is done using either viral and non-viral gene delivery or other gene editing methods such as CRISPR/Cas9, TALENS ((Transcription Activator-Like Effector Nucleases), ZFNs (Zinc Finger Nucleases), RNAi (RNA interference), base editing, prime editing etc to produce T cells having phenotype of CAR-T cells.[15,16] The most commonly employed technique for modification, however, has been the genetic transduction with the retroviral vectors encoding CAR constructs or the CAR gene is integrated using Crispr/Cas9 system.[38] The modified T cells are further expanded ex vivo using cytokine, interleukin 2 (IL2) and anti-CD3 or antiCD3/CD28 antibodies that stimulate their proliferation. These CAR-T cells are infused into the patients who have been pre- subjected to lympho-depletion chemotherapy. The infused CAR-T cells on encountering the target antigen gets activated, proliferate, and release cytotoxic substances to kill the cancerous cells.[39]

THERAPEUTIC APPLICATIONS OF CAR-T CELLS

The CAR-T cells have provided a new option for cancer treatment, particularly where only few treatments options existed earlier. The first clinical use of CAR-T cells was initiated in the year 2005 in Rotterdam, Netherland, for treating metastatic renal cell carcinoma with a parallel trial at the National Cancer Institute (NCI), USA, for metastatic ovarian cancer which was however, unsuccessful. The true clinical breakthrough using anti-CD19 CAR-T came in the years 2009 and 2011 to treat a patient of refractory follicular lymphoma (FL) at National Cancer Institute (NCI) and patients with chronic lymphocytic leukemia (CLL) and B cell acute lymphoblasic leukemia (B-ALL) at University of Pennsylvania (Penn) respectively.[40,41] The success of CAR-T therapy at NCI and Penn, USA was followed by many early clinical trials of CAR-T therapy across US on chemo refractory patients with B cell

malignancies. These trials demonstrated remarkable response to the therapy which later on resulted in the approval of anti-CD19 CAR-T cells therapies for B cell malignancies in US and Europe. These are tisagenlecleucel, Axicabtagene ciloleucel, lisocabtagene maraleucel etc (Figure 5).[42-44]

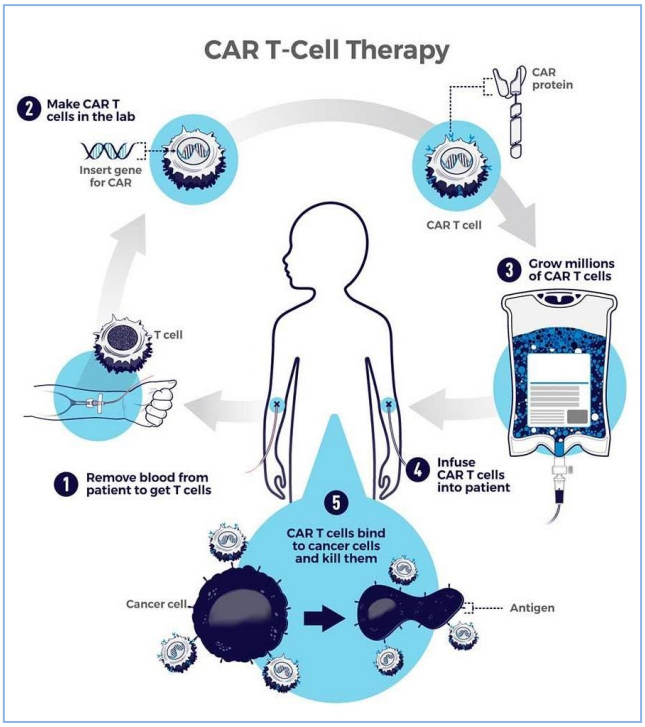

Figure 4: Mechanism of CAR- T cell therapy- a T cell-based immunotherapy involves genetically engineered T cell expressing the chimeric receptor, which binds tumor specific antigen on cancerous cell to destroy it. Patient's WBCs are separated from the blood through leukapheresis and T cells are isolated (1) which are genetically engineered using different techniques to express a chimeric antigen receptor (CAR) protein on the T cell surface, resulting in the generation of CAR-T cells (2). These CAR -T cells are further expanded in millions in vitro (3) and are intravenously infused back to the patient (4) to bind the tumor antigen and consequently killing the tumor cells.[39]

Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

Figure 5: CAR-T cell therapies approved by the FDA.[39]

Hematological malignancies

The tisagenlecleucel (tisa-cell, brand name Kymriah®) was the first US FDA approved CAR-T cell treatment in the year 2017 to be used in patients up to 25 years of age with refractory or relapsed B-cell precursor acute lymphoblastic leukemia (BCP-ALL).[42] It uses 4-1BB co-stimulatory construct and a retroviral gene transfer. FDA also issued guidelines for use of CAR-T in relapsed or refractory large B- cell lymphoma adult patients including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B –cell lymphoma and DLBCL transformed from follicular lymphoma (FL).[45] Axicabtagene ciloleucel (axicell, brand name Yescarta®)

was the second US FDA approved CAR-T cell therapy for treatment of adult patients with relapsed or refractory large B-cell lymphoma after 2 or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.[46,47] The axicabtagene ciloleucel uses the CD-28 co-stimulatory domain and a lentiviral gene transfer.

Lisocabtagene maraleucel (liso-cel, brand name Breyanzi®) is another autologous, CD19-directed CAR- T cell therapy used in patients with relapsed or refractory large B-cell lymphomas.[48] It consists of 4-1BB co-stimulatory signal and a lentiviral gene transfer. Tisa-cel and liso-cel have similar co-stimulatory domains but both products differ by hinges which are CD8a and CD28 respectively.[49] Out of the 256 patients included in the efficacy-evaluable set for lisocabtagene maraleuce, an objective response was achieved by 186 (73%, 95% CI 66·8–78·0) patients and a complete response by 136 (53%, 46·8–59·4) in a multicentre US trial for patients with relapsed or refractory large B–cell lymphoma.[50]

Brexucabtagene autoleucel (brand name Tecartus®) is an autologous customized CAR -T cell based treatment having CD-28 co-stimulatory construct targeted against CD 19 in patients of relapsed or refractory B cell precursor acute lymphoblastic leukemia (r/r BCP- ALL) and mantle cell lymphoma (MCL) in adults.[51,52] The relapsed or refractory MCL patients had previously been treated with anthracycline- or bendamustine-containing chemotherapy, an anti-CD20 antibody, and a Bruton tyrosine kinase inhibitor. The efficacy of this product was tested in a single-arm multicentre trial and participants were given a single infusion of brexucabtagene autoleucel after completion of lymphodepleting chemotherapy.[53] Tecartus® in case of all, demonstrated deep molecular remission (97%) with undetectable minimal residual disease (MRD).[54]

Tisagenlecleucel (Kymriah®) for r/r B-cell acute lymphoblastic leukemia (B-ALL), Brexucabtagen autoleucel (Tecartus®) for patients with r/r mantle cell lymphoma (MCL) and follicular lymphoma (FL) have demonstrated an unprecedentedly high rates of tumor responses and durable disease remissions in patients relapsed or refractory (r/r) after two systemic treatment lines.[55] Axicabtagene ciloleucel (Yescarta®) in the clinical trial led to an objective response rate (ORR) as high as 82%, with a complete response rate (CRR) of 54%, in patients with r/r DLBCL, transformed FL and primary mediastinal B-cell lymphoma. However, complete response rates between 70% and 94% across distinct trials using CAR-T cell therapy have been observed in patients with B-ALL.[56]

In a phase I trial involving 16 non-Hodgkin lymphoma (NHL) patients, the concurrent infusion of second-generation (2G) anti-CD19 CAR-T cells having the CD28 domain and third-generation(3G) anti-CD9 CAR-T cells containing both CD28 and 4-1BB domains showed that the third-generation CAR-T cells had superior expansion and persistence. This, in turn, resulted in remarkable clinical outcomes, including sustained complete responses (CRs) in patients with relapsed or refractory(r/r) NHL, a classification of heterogeneous malignant neoplasms of the lymphoid tissue. This difference was most remarkable in patients with a low disease burden whose normal CD19+ B cells are depleted by prior lympho-depletion therapy.[57]

The CAR-T cell therapy, utilizing the benefits of both molecular antibody-like target specificity and the cytotoxicity of T cells, has also emerged as a promising treatment for multiple myeloma targeting B–cell maturation antigen (BCMA). BCMA is encoded by TNFRSF17 gene and regulates B-cell survival, proliferation, and maturation.[58,59] Idecabtagene vicleucel (brand name Abecma®), also known as Ide-cel and bb2121, a 2G CAR-T-cell therapy targeting the BCMA has recently (2021) been approved by the FDA to be used in patients of RRMM. It consists of a murine anti-BCMA single-chain variable fragment (scFV), a 4-1BB co-stimulatory domain, a CD3 signaling domain, a CD8α hinge, and a transmembrane domain.

A dose of bb2121, in a phase 2 clinical trial on 128 patients, varying from 150–450×10^6 CAR-T cells was given to the patients with RRMM who previously had three lines of therapies including immunomodulatory (IMiD) and proteasome inhibitors (PIs). The 94 patients out of 128, on follow up after 11.3 months, showed clinical responses with a better response on a higher dose of CAR-T cells as patients receiving the highest dose of 450×10^6 cells achieved an 81.5% ORR and a 35.2% CR.[60]

Ciltacabtagene Autoleucel (brand name Karvykti®), is another FDA approved (year 2022) second generation CAR-T cell immune-therapy for RRMM and consists of a chimmeric construct with two OBAMA-binding scFv domains, a trans-membrane part, a CD3 signalling domain along with 4-1BB co-stimulatory domain. The promising efficacy of cita-cel was demonstrated in open–label clinical trials including both phases 1b/2 on RRMM patients who had previously received at least three lines of therapies. The 97 study participants received a single infusion of cilta-cel and nearly all of them (98%) showed good clinical response and 78% of the participants had a stringent complete response. Overall responses stayed for a median of 22 months.[7,61,62]

Solid tumors

CAR-T cells therapy has shown remarkable efficacy in hematological malignancies, with approved treatment for specific leukaemias, lymphomas, and more recently myeloma showing consistently high response rates as described in the previous section. It is moving ahead with a rapid pace, targeting new and multiple antigens at one time. The efforts too are underway using CAR-T cells to target solid tumors like breast, kidney, liver, brain cancers etc., but the results so far have not been clinically encouraging and consistent besides numerous examples of

toxicity as well. The ongoing studies on CAR -T cells in solid tumors mainly focus on assessing safety concerns and have, so far, reported only early-stage research outcomes. It is yet to overcome certain challenges, in solid tumors, such as physical barriers, immunosuppressive tumor microenvironment (TME), specificity and safety.[63]

Various trials have been conducted and many are still in progress to explore the potential of CAR- T cell therapy for solid tumors including pancreatic cancer, colorectal cancer, renal carcinoma, hepatic cancers, breast carcinoma, sarcoma, neuroblastoma, and others. These trials have targeted surface proteins and integrins, such as heparan sulfate-proteoglycan Glypican3(GPC3) and CD147, a type I transmembrane glycoprotein in hepatocellular carcinoma (HCC); classic tumor marker carcinoembryonic antigen (CEA), transmembrane 4 L six family member 1 (TM4SF1) and epithelial cell adhesion molecule (EpCAM) for colorectal adenocarcinoma; PSCA in patients with PSCA+ metastatic castration-resistant prostate cancer (mCRPC); sphingolipid namely disialoganglioside GD2 for neuroblastoma and osteosarcoma, fibroblast activation protein (FAP) for malignant pleural mesothelioma; human epidermal growth factor receptor 2 (HER2) for HER2-positive sarcoma; interleukin-13 receptor α (IL-13Rα), HER2, and epidermal growth factor variant III(EGFRvIII) for glioma; MSLN, HER2 and a cell surface receptor tyrosine kinase c-Met (mesenchymal-epithelial transition factor) in breast cancer; carcinoembryonic antigen-related cell adhesion molecule (CEACAM7) for pancreatic ductal adenocarcinoma(PDAC); receptor tyrosine kinase targets AXL and ROR2 in renal cell carcinoma; EGFR, HER2, MSLN, MUC1, CEA, ROR1, and PD-L1 in lung cancer etc.[64-76]

Thus, a range of targets, a few of which are even common in different solid malignancies, have been evaluated for CAR- T cells therapy as described previously. A few targets for CAR-T therapy in phase 1 trials in solid cancers seem to be better as compared to others due to the antigen's higher specificity and lower on target, off-tumor toxic effects. However, the trial outcomes of CAR-T cell therapy in solid tumors have generally been unsatisfactory, inconsistent and currently low on feasibility of translation to phase 2/3 trials mainly due to their safety and specificity concerns.

CHALLENGES IN TREATMENT OF SOLID TUMORS BY CAR-T CELL THERAPY

Solid tumors exhibit large tumor heterogeneity, as compared to homogenous nature of blood cancers. The solid cancer type not only can be molecularly different in different patients suffering from a particular cancer but also between the primary and metastatic stage of the same cancer. These tumors may even lack a targetable antigen or may not have sufficient antigens for CAR-T cells to function effectively.[77] Solid tumors release certain chemokines like CXCL5 and CXCR2 in their surroundings which inhibit the migration of T cells to the targeted cancer.[7] It also forms the fibrotic extra cellular matrix constituted by the tumor microenvironment (TME) with the recruitment of myeloid cells and fibroblasts which further prevents the infiltration of T cells to the solid tumor.[6]

The TME, in fact, presents a variety of molecular and cellular factors that make CAR-T cells ineffective. Elements such as nutrient deprivation, oxidative stress, acidic pH, and low oxygen levels compromise the CAR-T cell efficacy.[79] Additionally, the release of immunosuppressive cytokines like TGF-β and IL-10; the presence of immune-suppressive cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages and neutrophils (TAMs and TANs); and checkpoint inhibitors like PD-L1 further impair CAR- T cells' ability to initiate a strong anti-tumor response.[80,81] PD-L1 (programmed cell death ligand-1) is a checkpoint protein on tumor cells that, when bound to PD-1(programmed cell death protein-1) on T cells, prevents T cells from attacking tumor cells. Further, CAR-T cells due to their own immunogenicity and potential toxicity, may also affect the surrounding immune environment in solid tumors. The CAR- T cells in solid tumors can also attack normal tissues, including the liver, lungs, brain, and heart, because the targeted antigens for CAR-T cells are generally present on these vital organs as well.[82]

STRATEGIES TO OVERCOME CHALLENGES IN CAR- T CELLS THERAPY OF SOLID TUMORS

The different strategies have recently been developed to override the hurdles of CAR-T therapy to increase its efficacy by means of regulating physical barriers and guiding CAR- T cells to tumors; addressing the immunosuppressive tumor microenvironment; and enhancing CAR- T cell specificity and safety in solid tumors (Figure 6).

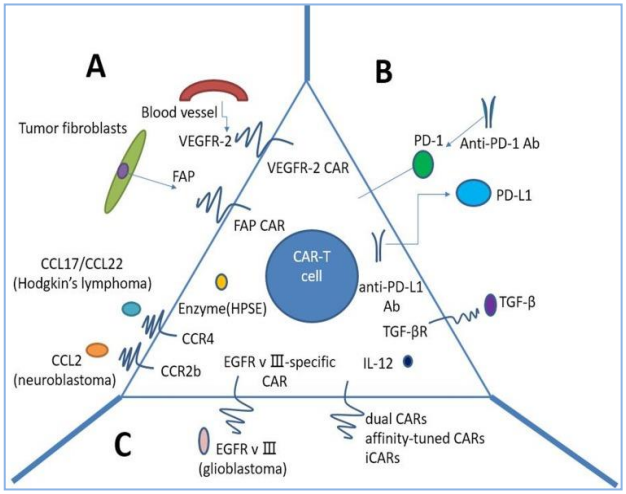

Figure 6: Methodologies to improve CAR-T cells for treatment of solid tumors:(A) Penetrating physical barriers and homing to the tumors; (B) B Immunosuppressive tumor microenvironment and; (C) Specificity and safety of CAR-T cells.[82]

Regulating physical barriers

Targeting tumor stroma

Studies have demonstrated that the use of fibroblast activation protein (FAP)- targeted CAR- T cells (FAP-CAR- T) in solid tumors like mesothelioma, lung and pancreatic cancers could reduce the number of tumor-associated fibroblasts which consequently inhibits tumor growth.[83,84]

Secreting enzymes

The CAR- T cells expressing the enzyme heparanase (HPSE) have been shown to break down heparan sulfate proteoglycans, a key component of the extracellular matrix (ECM) which results in overcoming the physical barriers and increase T cell infiltration and antitumor activity.[85]

Expressing chemokine receptors

The genetically altered CAR-T cells expressing chemokine receptors that match the tumor's chemokines, like CCR4-bearing CAR-T cells in Hodgkin's lymphoma and CCR2b CAR-T cells in mesothelioma and neuroblastoma xenografts enable a large number of T cells to migrate to the tumor mass.[86, 87]

Addressing the immunosuppressive tumor microenvironment

CAR-T cells secreting anti-PD-L1 antibodies and PD-1 blockade

The CAR- T cells secreting anti-PD-L1 antibodies instead of anti-PD-L1 mAbs along with CAR-T cell therapy have been investigated to mitigate the immunosuppressive TME.[88] Such CAR-T cells releasing anti-PD-L1 antibodies leads to the recruitment of human NK cells to the tumor site; and a consequential decrease in tumor growth in vivo in a humanized mouse model with renal cell carcinoma. The studies have also shown that using anti-human PD-1 antibodies along with HER CAR-T cells remarkably improves tumor regression by CAR- T cell therapy besides decreasing significantly the number of immune suppressive cells such as myeloid derived suppressor cells (MDSCs) in TME in mouse model.[89]

Blocking IL-10/TGF-β receptors

The blockade of binding of transforming growth factor β to TGF-β receptors enhances $CD8^{+}$T-cell mediated anti-tumor immune responses as well as increase in the efficacy of adoptive CAR-T in solid tumors in animal models.[90]

Armored CARs and TRUCKs

The use of armored-CARs or TRUCKS secreting pro-inflammatory cytokines such as IL-12 in tumor microenvironment results in enhanced antitumor efficacy with increased survival, prolonged persistence of T cells, and higher systemic IFNγ in mice with human ovarian cancer xenografts.[91] CRISPR/Cas9 has also been used to help CAR-T cells secrete specific cytokines by knocking in cytokines such as IL-15, IL-23 and IL-18 which enhance the anti-tumor activity and long term persistence of CAR-T cells.[92,93]

Enhancing CAR T cell specificity and safety

EGFRvIII-specific CARs

The CAR-T cells specific to tumor antigen EGFR variant III (EGFRvIII) fully restricted to human cancers mainly glioblastoma increase the efficacy and decrease the toxicity in animal model.[94]

Dual CARs

The dual CAR technology employs two modified CARs, one CAR contains the CD3ζ signaling domain to activate T cell function, while the second CAR provides co-stimulation via CD28 and/or CD137 which helps in identifying the tumor cells from normal cells and works only when both are present. This helps in preventing "on-target, off- tumor" toxicity effects on normal cells. For example, two CARs having specificity for two different antigens i.e. mesothelin and α- folate receptor (FRα), concurrently expressed in the majority of epithelial ovarian cancers, were genetically engineered to co-express signal 1(anti-meso scFV-CD3ζ) and signal 2 (Anti-FRa scFv-CD28) in trans.[95,96] The trans signaling CAR-T cells exhibited increased cytokine secretion on coming across tumor cells co- expressing both the antigens but not against the normal cells expressing only low amount of mesothelin which is also a tumor associated antigen (TAA). Moreover, the dual trans- signaling CAR-T cells are better placed to second generation CAR-T cells (co-expressing both the antigens in cis) due to their lack of ability not to target normal cells expressing low amount of TAA.[95,96]

Affinity-tuned CARs

A recent strategy involving affinity-tuned CAR-T cells demonstrates that these cells can differentiate between tumor cells and normal cells expressing the same antigen at lower levels. This is achieved by adjusting the CAR's affinity, while still preserving strong antitumor activity in vivo. Adjusting the sensitivity of CAR through scFv affinity provides an alternative to expand the use of targets that are over-expressed on solid tumors for CAR-T cell therapy. To specify, the development of synthetic CARs capable of adjusting T-cell activity based on EGFR expression in which a CAR with reduced affinity, allowed T cells to differentiate between malignant and non-malignant cells in glioblastoma (GBM).[97]

iCARs

One of the alternatives to reduce off-target effects is the use of inhibitory CARs (iCARs) which are designed to recognize antigens present on normal cells but absent on tumor cells. iCARs initiate negative signaling upon binding to normal cells that prevents CAR-T cells from attacking healthy tissue and thus, sparing normal cells from CAR-T cell-mediated damage. The feasibility of using iCARs that leverage natural T cell inhibition, through PD-1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), to protect normal tissue from off-target effects in preclinical mouse models have been illustrated by a recent study.[98] Studies using CTLA-4 or PD-1–based iCARs showed that their use could selectively restrict cytokine secretion, cytotoxicity, and proliferation induced via endogenous T cell receptor or an activating chimeric receptor in primary human T cells.[99]

TOXICITIES ASSOCIATED WITH CAR-T CELL THERAPY

The CAR-T cell therapy has shown promising clinical benefits especially in hematological malignancies like B-cell lymphoma and leukemia. It is steadily emerging as a revolutionary treatment for cancers. However, at times it is linked to side effects and toxicities that can be lethal such as "on-target, off-tumor" effects, cytokine release syndrome (CRS), neurological toxicities, also known as CAR-related encephalopathy syndrome (CRES) etc.

"On-target, Off-tumor" effect

The "On-target, Off-tumor" effect happens when the modified T cells target not only the cancerous cells displaying a particular antigen but also healthy cells that carry the same or similar antigens, causing unwanted damage. For example, B-cell aplasia is a frequent side effect event in CAR-T cell trials targeting B-cell malignancies because both the CD1 and CD20 antigens are found on transformed malignant as well as healthy B-cells. As a result, both the normal and the cancerous B-cells are, therefore, killed by CD19 specific or CD20 specific CAR-T cells leading to B-cell aplasia.[100] However, in most trials, B-cell aplasia correlates with clinical benefit. The B-cell aplasia can consequently be successfully treated with gamma globulin infusions as a replacement therapy, though this approach can be expensive, particularly for long-term treatments. Furthermore, CD20 CAR-T cells have been reported to damage normal tissues near lesions site due to low-level expression of CD20 on non-B-cell tissues, causing symptoms such as dyspnea and respiratory distress.[101]

The studies to minimize the off-target effects by enhancing CAR-T cell specificity and safety using more refined and specific CARs like dual CARs, iCARs, affinity tuned CARs, EGFRvIII-specific CARs etc. have demonstrated lesser degrees of "on- target off- target" effects which have already been discussed in the preceding sections.[94-99] The mitigation of the "on-target, off-tumor" effect is one of the key focuses of research in CAR-T therapy.

Cytokine release syndrome

Cytokine release syndrome (CRS) has so far been the most frequently observed adverse drug reaction. CRS, an inflammatory condition occurs when immune cells, specifically T cells, release excessive amounts of cytokines such as interleukins (IL6), interferons (IF-γ), and tumor necrosis factors (TNF-α) into the bloodstream. CRS in case of CAR- T cell therapy is generated when the modified T cells are activated on encountering their target antigen on cancer cells.[102]

The immune activation in CAR-T cell therapy is essential for effective killing of tumors cells, but excessive cytokine production often results in extensive inflammation and tissue damage. The severity of CRS depends on many factors, including the patient's underlying health condition, the tumor burden, and the specific CAR-T therapy being used etc. The overproduction of cytokines may lead to organ dysfunction including respiratory failure, kidney injury, and cardiovascular complications. The severe form of CRS can become fatal, if not treated well in time.[102]

The clinical trial of the CAR-T therapy tisagenlecleucel (Kymriah®), which targets CD19 for the treatment of relapsed or refractory B-cell ALL caused CRS in 70% of patients, with approximately 20% experiencing severe life-threatening symptoms.[103] The trials for axicabtagene ciloleucel (Yescarta®), another CD19-targeting CAR-T therapy used for DLBCL also resulted in CRS affecting up to 94% of patients, with 13% suffering from severe forms.[104] The risk of CRS is not confined to CD19-targeting therapies. The patients undergoing CAR-T cells treatment directed against other antigens, such as BCMA for the treatment of multiple myeloma and mesothelin in solid tumors also developed CRS. It indicates that it is not limited to CD-19 only thus, highlighting the systemic nature of the immune activation caused by CAR T cell therapy.[7,82]

The use of Tocilizumab, an anti-IL-6 receptor therapy; corticosteroids like dexamethasone and methylprednisolone as immunosuppressive agents; early detection of CRS to prevent its further progression to severe form; risk stratification and personalized treatment plans for patients who are more vulnerable to CRS; CAR-T cells engineering and modifications like use of "suicidal" switches and "tunable" CARs, where selective destruction and strength of the immune response can be modulated; and gradual dosing regimens by infusing CAR-T cells in a staggered or lower initial dose are some of the main modalities to reduce the severity of CRS.[105,106] These modalities prevent the sudden and overwhelming activation of the immune system.

Neurological toxicity

Neurological toxicity, commonly referred to as CAR-T cell related encephalopathy syndrome (CRES) or immune effector cell-associated neurotoxicity syndrome (ICANS) creates a significant challenge in the use of CAR-T cell therapy. The symptoms can vary from mild cognitive impairments, aphasia, motor weakness to life-threatening cerebral edema and seizures. The exact cause of neurological toxicity is still not fully understood. It is however, clear that a combination of systemic inflammation, cytokine release, and blood-brain barrier (BBB) disruption plays a role in development of encephalopathy and other neurological symptoms.

There are instances where the death has occurred in CD19 CAR clinical trials because of neurotoxicity due to cerebral edema and surprisingly no fatality due to cerebral edema was also reported at the same time in another clinical trials using CD19 CAR. Additionally, several other clinical trials reported reversible neurotoxicity symptoms such as confusion, delirium, expressive aphasia, encephalopathy, and seizures.[107] The postulated patho-physiological mechanisms include cytokine diffusion and/or translocation of activated CAR-T cells across the blood–brain barrier as CD19 CAR-T cells have been found in cerebrospinal fluid (CSF) in few patients.[108]

The management of CRES is directed by the severity of symptoms based on the CARTOX-10 (CAR-T cell therapy-associated toxicity 10-point neurological assessment), intracranial pressure, and the presence of seizures or motor weakness.[109] The treatment modality for CRES is similar to that for CRS to ameliorate ICANS effects without compromising the efficacy of CAR-T cells. It involves supportive care, corticosteroids, and IL-6 antagonists along with continuous neurological consultation and evaluation after the administration of CAR-T cell therapy.

CONCLUSION

Chimeric Antigen Receptor (CAR)-T cell therapy is a revolutionary breakthrough in the field of cancer immunotherapy which offers a powerful and personalized approach to treat cancers. It involved many years of biomedical research to produce the first CAR construct and subsequent evolution in its development to strengthen the effectiveness of this methodology to be used for cancer treatment. The different clinical trials have demonstrated that CAR-T cell therapy is effective in treating cancers, particularly certain blood cancers. A few of these treatments like Kymriah®, Yescarta®, Breyanzi®, Tecartus®) Abecma® and Karvykti® have already been approved by the FDA for use in treating different types of B-cell malignancies. Certain others are still undergoing clinical trials for different tumors including multiple myeloma, CNS tumors, hepatocellular carcinoma, lung cancer etc, and a few are awaiting FDA approval. However, the success rate of CAR-T cell therapy with solid tumors remains relatively unsatisfactory due to factors like antigen heterogeneity, tumor micro environment, on target-off tumor effects etc. The clinical trials of CAR-T cell therapy though inclusively look promising, but several challenges still persist including toxicities and suboptimal responses in some patients.

New strategies like use of next generations CARs including dual CARs, iCARs, TRUCKs and UniCARs, CRISPR Cas9 technology to knock in cytokine genes in CAR T cells etc are being developed to overcome these limitations which may result in better efficacy, in vivo persistence, low toxicity and avoidance of antigen escape. Other challenges like cost effectiveness and quality assurance, with combined co-operation between industry partners and academic institutions, also need to be addressed thoroughly to make the CAR-T cells therapy easily available for cancer patients. In the near future, one can hope that it will transform the treatment picture for cancer patients and their well-being.

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REFERENCES

1. Shiravand Y, Khodadadi F, Kashani SMA, Hosseini-Fard SR, Hosseini S, Sadeghirad H, et al. Immune Checkpoint Inhibitors in Cancer Therapy. Curr Oncol. 2022; 29(5):3044-60.
2. Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. Antibodies. 2020;9(3):34.
3. Goswami A, Goyal S, Khurana P, Singh K, Deb B, Kulkarni A Small molecule innate immune modulator in cancer therapy. Front. Immunol. 2024;15:1395655
4. Park R, Eshrat F, Al-Jumayli M, Saeed A, Saeed A. Immuno-Oncotherapeutic Approaches in Advanced Hepatocellular Carcinoma. Vaccines. 2020; 8(3):447.
5. Sharma R, Suravarjhula L, Banerjee M, Kumar G, & Kumar, N. Chimeric antigen receptor T-cell therapy in cancer: A critical review. Current Drug Research Reviews Formerly. Current Drug Abuse Rev. 2023;15(3):241-61.
6. Patel U, Abernathy J, Savani BN, Oluwole O Sengsayadeth S, Bhagirathbhai D. CAR T cell therapy in solid tumors: A Review of Current Clinical Trials. 2021;3(1):24-31.
7. Mishra AK, Gupta A, Dagar G. CAR-T-Cell Therapy in Multiple Myeloma: B-cell maturation antigen (bcma) and beyond. Vaccines (Basel). 2023;11(11):1721.
8. Haslauer T, Greil R, Zaborsky N, Geisberger R. CAR T-Cell Therapy in Hematological Malignancies. Int J Mol Sci. 2021;22(16):8996.
9. Dana H, Chalbatani GM, Jalali S , Mirzaei HR, Grupp SA , Suarez ER , Rapôso C, Webster TJ. CAR-T cells: Early successes in blood cancer and challenges in solid tumors. Acta Pharmaceutica Sinica B. 2021;11(5):1129-47.
10. Benmebarek MR, Karches CH, Cadilha BL, Lesch S, Endres S, Kobold S. Killing Mechanisms of Chimeric Antigen Receptor (CAR) T Cells. Int J Molec Sci. 2019;20(6):1283.
11. Tschumi BO, Dumauthioz N, Marti B, Zhang L, Schneider P, Mach JP, Donda A. CART cells are prone to Fas-and

DR5-mediated cell death. J Immunother of Cancer. 2018; 6(1):71.
12. Huan T, Chen D, Liu G, Zhang H, Wang X, Wu Z, et al. Activation-induced cell death in CAR-T cell therapy. Human Cell. 2022;35(2):441-7.
13. Rodriguez-Garcia A, Palazon A, Noguera-Ortega E, Powell Jr DJ, Guedan S. CAR-T cells hit the tumor microenvironment: strategies to overcome tumor escape. Frontiers in Immunol. 2020;11:1109.
14. Scarfò I, Maus M V. Current approaches to increase CAR T cell potency in solid tumors: targeting the tumor microenvironment. J Immuno Cancer. 2017;5:1-8.
15. Zhang C, Liu J, Zhong JF et al. Engineering CAR-T cells. Biomark Res. 2017;5:22.
16. Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, et al. Recent advances in CAR-T cell engineering. J Hematol & Oncol.2020;13:1-19.
17. Ahmad U, Khan Z, Ualiyeva D, Amissah OB, Noor Z, Khan A, et al. Chimeric antigen receptor T cell structure, its manufacturing, and related toxicities; A comprehensive review. Advances in Cancer Biology-Metastasis. 2022;4:100035.
18. Sievers NM, Dörrie J, Schaft N. CARs: beyond T cells and T cell-derived signaling domains. Int J Mol Sci. 2020;21(10):3525.
19. Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. Biomark Res. 2017;5:22.
20. Dotti G, Gottschalk S, Savoldo B, Brenner MK. Design and development of therapies using chimeric antigen receptor-expressing T cells. Immunol Rev. 2014;257(1):107-26.
21. Baldo BA. Chimeric Fusion Proteins Used for Therapy: Indications, Mechanisms, and Safety. Drug Saf. 2015;38:455–79.
22. Li C, Tang Z, Hu Z, Wang Y, Yang X, Mo F, & Lu X. Natural single-domain antibody-nanobody: a novel concept in the antibody field. J Biomed Nanotechnol. 2018;14(1):1-19.
23. Zhu L, Yang X, Zhon D, Xie S, Shi W, Li Y, et al. Single-domain antibody-based TCR-like CAR-T: a potential cancer therapy. J Immunol Res. 2020;20(1):2454907.
24. Kennewick KT, Yamaguchi Y, Gibson J, Gerdts EA, Jeang B, Tilakawardane D, Murad JP, Chang WC, Wright SL, Thiel MS, Forman SJ. Non-signaling extracellular spacer regulates tumor antigen selectivity of CAR T cells. Molecular Therapy Oncol. 2024;32(2).
25. Fujiwara K, Tsunei A, Kusabuka H, Ogaki E, Tachibana M, Okada N. Hinge and Transmembrane Domains of Chimeric Antigen Receptor Regulate Receptor Expression and Signaling Threshold. Cells. 2020;9(5):1182. Published 2020 May 9. doi:10.3390/cells9051182
26. Mazinani M, Rahbarizadeh F. CAR-T cell potency: from structural elements to vector backbone components. Biomark Res. 2022;10(1):70. Published 2022 Sep 19. doi:10.1186/s40364-022-00417-w
27. Jayaraman J, Mellody MP, Hou AJ, Desai RP, Fung AW, Pham AHT, et al .CAR-T design: Elements and their synergistic function. EBioMedicine.2020; 58:102931
28. Smirnov S, Mateikovich P, Samochernykh K, Shlyakhto E. Recent advances on CAR-T signaling pave the way for prolonged persistence and new modalities in clinic. Frontiers in Immunology. 2024;15:1335-424.
29. Harrison AJ, Du X, Scheidt BV, Kershaw MH, Slaney CY. Enhancing co-stimulation of CAR T cells to improve treatment outcomes in solid cancers. Immuno ther Adv. 2021;1(1):16.
30. Subklewe M, Bergwelt-Baildon MV, Humpe A. Chimeric Antigen Receptor T Cells: A Race to Revolutionize Cancer Therapy. Transfus Med Hemother. 2019;46(1):15–24.
31. Styczyński J. A brief history of CAR-T cells: from laboratory to the bedside. Acta Haematologica Polonica. 2020;51(1):2-5.
32. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. Expert opinion on biological therapy. 2015; 15(8):1145-1154.
33. Chen YJ, Abila B, Mostafa Kamel Y. CAR-T: what is next. Cancers. 2023;15(3):663.
34. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang CH, Saso K, Butler MO, Minden MD, Hirano N. A novel chimeric antigen receptor containing a JAK-STAT signalling domain mediates superior antitumor effects. Nat Med. 2018;24(3):352–9.
35. Zhao J, Lin Q, Song Y, Liu D. Universal CARs, universal T cells, and universal CAR T cells. J Hematology & Oncol .2018;11:1-9.
36. Bachmann M. The UniCAR system: a modular CAR T cell approach to improve the safety of CAR T cells. Immunology letters. 2019;211:13-22.
37. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA. 1989;86(24):10024-8.
38. Goel R. CRISPR/Cas9-mediated genome editing: from basic research to gene therapy. Int J Res Med Sci. 2024;12(6):2200.
39. CAR T Cells: Engineering Patients' Immune Cells to Treat Their Cancers. Source: National Cancer Institute, USA. Available at: https://www.cancer. gov/about-cancer/treatment/research/car-t-cells. Accessed on 3 June 2024.
40. Cappell KM, Sherry RM, Yang JC, Goff SL, Vanasse DA, McIntyre L, Rosenberg SA, Kochenderfer JN. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. Journal of Clinical Oncology. 2020;38(32):3805-15.
41. Chavez JC, Yassine F, Sandoval-Sus J, Kharfan-Dabaja MA. Anti-CD19 chimeric antigen receptor T-cell therapy in B-cell lymphomas: current status and future directions. Int J Hematol Oncol. 2021;10(2):33.
42. Lu P, Hill HA, Navsaria L J, Wang ML. CAR-T and other adoptive cell therapies for B cell malignancies. Journal of the National Cancer Center. 2021;1(3):88-96.
43. Bouchkouj N, Kasamon YL, de Claro RA, George B, Lin X, Lee S, et al. FDA approval summary: axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma. Clinical Cancer Research. 2019;25(6):1702-8.
44. Elmacken M, Peredo-Pinto, Wang C, Xu Z, Tegenge M, Jaigirdar AA, et al. FDA Approval Summary: Lisocabtagene Maraleucel for Second-Line Treatment of Large B-Cell Lymphoma. Clinical Cancer Research .2024; 30(11):2309-16.
45. O'Leary MC, Lu X, Huang Y, Lin X, Mahmood I, Przepiorka D. Pazdur R. FDA approval summary: tisagenlecleucel for treatment of patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia. Clinical Cancer Research, 2019; 25(4):1142-6.
46. Jain MD, Bachmeier CA, Phuoc VH, Chavez JC Axicabtagene ciloleucel (KTE-C19), an anti-CD19 CAR T therapy for the treatment of relapsed/refractory aggressive B-cell non-Hodgkin's lymphoma. Therapeutics and Clinical Risk Management.2018;14:1007–17.
47. Viardot A, Wais V, Sala E, Koerper S. Chimeric antigen receptor (CAR) T-cell therapy as a treatment option for patients with B-cell lymphomas: perspectives on the therapeutic potential of Axicabtagene ciloleucel. Cancer Management and Research. 2019;2:2393-404.

48. Hirayama A, Chou C, Maloney DG, Marcondes MQ, Turtle CJ. A Phase Ib Open-Label Study Evaluating the Safety and Efficacy of NKTR-255 in Combination with CD19-Directed CAR-T Cell Therapy in Patients with Relapsed/Refractory (R/R) Large B-Cell Lymphoma (LBCL). Blood. 2022;140(1):12747-8.
49. Ho M, Zanwar S, Paludo J. Chimeric antigen receptor T-cell therapy in hematologic malignancies: Successes, challenges, and opportunities. European Journal of Haematology. 2024 Feb;112(2):197-210.
50. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. The Lancet. 2020;396(10254):839-52.
51. Anderson MK, Torosyan A, Halford Z. Brexucabtagene Autoleucel: A novel chimeric antigen receptor t-cell therapy for the treatment of mantle cell lymphoma. Ann Pharmaco.2022;56(5):609-19.
52. CD19-Directed CAR T Improves OS in B-Cell Acute Lymphoblastic Leukemia. Available at: https://www.cancernetwork.com/view/cd19-direct-car-t-improves-os-in-b-cell-acute-lymphoblastic-leukemia. Accessed on 3 June 2024.
53. Bouchkouj N, Lin X, Wang X, Przepiorka D, Xu Z, Purohit-Sheth,T, Theoret M. FDA approval summary: brexucabtagene autoleucel for treatment of adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia. The Oncol. 2022;27(10):892-9.
54. Benjamin Holmes DVM. CAR T-cell therapy indications grow significantly in 2021. Targ Ther Oncol. 2021;10(18):10.
55. Wittibschlager V, Bacher U, Seipel K, Porret N, Wiedemann G, Haslebacher C, et al. CAR T-Cell persistence correlates with improved outcome in patients with B-Cell Lymphoma. Int J Mole Sci. 2023;24(6):5688.
56. Blüm P, Kayser S. Chimeric Antigen Receptor (CAR) T-Cell therapy in hematologic malignancies: clinical implications and limitations. Cancers. 2024;16(8):1599.
57. Ramos CA, Rouce R, Robertson C S, Reyna A, Narala N, Vyas G, et al. In vivo fate and activity of second-versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. Molecular Ther. 2018; 26(12): 2727-37.
58. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. Leukemia. 2020;34(4):985-1005.
59. Abramson HN. B-cell maturation antigen (BCMA) as a target for new drug development in relapsed and/or refractory multiple myeloma. Int J Mol Sci.2020;21(15):5192.
60. Abecma (Idecabtagene Vicleucel) First-in-Class BCMA-Directed CAR T-Cell Therapy Approved for Relapsed or Refractory Multiple Myeloma. Available at: https://www.ahdbonline.com/issues/2021/december-2021-twelfth-annual-payers-guide/abecma-idecabtagene-vicleucel-first-in-class-bcma-directed-car-t-cell-therapy. Accessed on 3 June 2024.
61. Martin T, Usmani SZ, Berdeja JG, Jakubowiak A, Agha M, Cohen AD, et al. Updated results from CARTITUDE-1: phase 1b/2Study of ciltacabtagene autoleucel, a b-cell maturation antigen-directed chimeric antigen receptor T cell therapy, in patients with relapsed/refractory multiple myeloma. Blood. 2021;138:549.
62. FDA approves ciltacabtagene autoleucel for relapsed or refractory multiple myeloma. Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-ciltacabtagene-autoleucel-relapsed-or-refractory-multiple-myeloma. Accessed on 3 June 2024.
63. Lionel A, Kankeu F. Olivia S, Reona S, Elizabeth LS, Saad SK. CAR T cell therapy and the tumor microenvironment: Current challenges and opportunities. Molecular Ther Oncol. 2022;25:69-77.
64. Du G, Dou C, Sun P, Wang S, Liu J, Ma L. Regulatory T cells and immune escape in HCC: understanding the tumor microenvironment and advancing CAR-T cell therapy. Front Imm. 2024;15:1431211.
65. Liu YG, Jiang ST, Zhang JW, Zheng H, Zhang L, Zhao HT, et al. Role of extracellular vesicle-associated proteins in the progression, diagnosis, and treatment of hepatocellular carcinoma. Cell & Bioscience. 2024;14(1):113.
66. Wilczyński J, Paradowska E, Wilczyński M. High-grade serous ovarian cancer—a risk factor puzzle and screening fugitive. Biomedicines. 2024;12(1);229.
67. Dorff TB, Blanchard MS., Adkins LN, et al. PSCA-CAR T cell therapy in metastatic castration-resistant prostate cancer: a phase 1 trial. Nat Med. 2024;30:1636-44.
68. Yu T, Jiang W, Wang Y, Zhou Y, Jiao J, Wu M. Chimeric antigen receptor T cells in the treatment of osteosarcoma. Intern J Oncol. 2024; 64(4):1-23.
69. Bughda R, Dimou P, D'Souza RR, Klampatsa A. Fibroblast activation protein (FAP)-targeted CAR-T cells: launching an attack on tumor stroma. Immun Targets and Therapy. 2021;3: 313-23.
70. Budi HS, Ahmad FN, Achmad H, Ansari MJ, Mikhailova M., Suksatan W et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor (CAR) for tumor immunotherapy; recent progress. Stem Cell Research & Therapy. 2022;13(1):40.
71. Yoon WS, Chung DS. Advanced t and natural killer cell therapy for glioblastoma. J Kor Neurosurg Soc. 2023;66(4):356-81.
72. Xie Y, Hu Y, Zhou N, Yao C, Wu L, Liu L, Chen F. CAR T-cell therapy for triple-negative breast cancer: Where we are. Cancer letters. 2020;491:121-31.
73. Ho-Yen CM, Jones JL, Kermorgant, S. The clinical and functional significance of c-Met in breast cancer: a review. Breast Cancer Res. 2015;17:1-11.
74. Xie H. The progress of optional targets and approaches to enhance efficacy of CAR-T for pancreatic ductal adenocarcinoma. In Third International Conference on Biological Engineering and Medical Science. 2024;12924:367-76.
75. Ma HY, Das J, Prendergast C, De Jong D, Braumuller B, Paily J, et al. Advances in CAR T cell therapy for non-small cell lung cancer. Curr Issues in Mol Bio. 2023;45(11):9019-38.
76. Khorasani AB, Sanaei MJ, Pourbagheri-Sigaroodi A, Ghaffari SH, Bashash D. CAR T cell therapy in solid tumors; with an extensive focus on obstacles and strategies to overcome the challenges. Int Immunopharmacol.2021;101:108260.
77. Stern LA, Jonsson VD, Priceman SJ. CAR T cell therapy progress and challenges for solid tumors. Tumor Microenvironment. 2020;4:297-326.
78. Foeng J, Comerford I, McColl SR. Harnessing the chemokine system to home CAR-T cells into solid tumors. Cell Rep Med. 2022;3(3):100543.
79. Li W, Pan X, Chen L, Cui H, Mo S, Pan, et al. Cell metabolism-based optimization strategy of CAR-T cell function in cancer therapy. Front Immunol. 2023;14:1186383.

80. Liu Z, Zhou Z, Dang Q et al. Immunosuppression in tumor immune microenvironment and its optimization from CAR-T cell therapy. Theranostics. 2022;12(14):6273-90.
81. Liu M, Wang X, Li W, Yu X, Flores-Villanueva P, Xu-Monette ZY, et al. Targeting PD-L1 in non-small cell lung cancer using CAR T cells. Oncogenesis. 2020;9(8):72.
82. Xia AL, Wang XC, Lu YJ, Lu XJ, Sun B. Chimeric-antigen receptor T (CAR-T) cell therapy for solid tumors: challenges and opportunities. Oncotarget. 2017;8(52):90521.
83. Wang L, Zhang L, Zhang Z, Wu P, Zhang Y, Chen X. Advances in targeting tumor microenvironment for immunotherapy. Front. Immunol. 2024;15:1472772.
84. Rodriguez-Garcia A, Palazon A, Noguera-Ortega E, Powell Jr DJ, Guedan S. CAR-T cells hit the tumor microenvironment: strategies to overcome tumor escape. Front Immunol. 2020;11:1109.
85. Vlodavsky I, Kayal Y, Hilwi M., Soboh S, Sanderson RD, Ilan N. Heparanase—A single protein with multiple enzymatic and nonenzymatic functions. Prot Res. 2023;1(3):6.
86. Yong CS, Dardalhon V, Devaud C, Taylor N, Darcy PK, Kershaw MH. CAR T-cell therapy of solid tumors. Immunol and Cell Biol. 2017;95(4): 356-63.
87. Wang Y, Wang J, Yang X, Yang J, Lu P, Zhao L, et al. Chemokine receptor CCR2b enhanced anti-tumor function of chimeric antigen receptor T cells targeting mesothelin in a non-small-cell lung carcinoma model. Front Immunol. 2021;12:628-906.
88. Najafi S, Mortezaee K. Modifying CAR-T cells with anti-checkpoints in cancer immunotherapy: A focus on anti PD-1/PD-L1 antibodies. Life Sciences. 2023;1:223-87.
89. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest. 2016;126(8):3130-44.
90. Liu G, Rui W, Zhao X, Lin X. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. Cell Molecul Immunol. 2021;18(5):1085-95.
91. Li X, Chen T, Li X, Zhang H, Li Y, Zhang S, Luo S, Zheng T. Therapeutic targets of armored chimeric antigen receptor T cells navigating the tumor microenvironment. Experimental Hematol Oncol. 2024;13(1):96
92. Wei W, Chen ZN, Wang K. CRISPR/Cas9: A powerful strategy to improve CAR-T cell persistence. International Journal of Molecular Sciences. 2023;24(15):12317.
93. Andrea AE, Chiron A, Mallah S, Bessoles S, Sarrabayrouse G, Hacein-Bey-Abina S. Advances in CAR-T cell genetic engineering strategies to overcome hurdles in solid tumors treatment. Frontiers in Immunology. 2022;13:830292.
94. Sterner RC, Sterner RM. EGFRVIII and EGFR targeted chimeric antigen receptor T cell therapy in glioblastoma. Front Oncol. 2024;14.
95. Terlikowska KM, Dobrzycka B, Terlikowski SJ. Chimeric antigen receptor design and efficacy in ovarian cancer treatment. Int J Mol Sci. 2021;22(7):3495.
96. Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH, et al. Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer Immunol Res. 2013;1(1):43-53.
97. Comoli P, Chabannon C, Koehl U, Lanza F, Urbano-Ispizua A, Hudecek M, et al. Development of adaptive immune effector therapies in solid tumors. Annals of Oncology. 2019;30(11):1740-50.
98. Karnik I, Her Z, Neo SH, Liu WN, Chen Q. Emerging preclinical applications of humanized mouse models in the discovery and validation of novel Immunotherapeutics and their mechanisms of action for improved cancer treatment. Pharmaceutics. 2023;15(6):1600.
99. Fedorov VD, Themeli M, Sadelain M. PD-1–and CTLA-4–based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Science Translat Med. 2013;5(2):172.
100. Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. Nature Rev Clin Oncol. 2013;10(5):267-76.
101. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO molecular medicine. 2017;9(9):1183-97.
102. Giavridis T, Stegen SJ, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nature Med. 2018;24(6):731-8.
103. Yoo JW. Management of adverse events in young adults and children with acute B-cell lymphoblastic leukemia receiving anti-CD19 chimeric antigen receptor (CAR) T-cell therapy. Blood research. 2023;58(1):20-8.
104. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. New England J Med. 2017;377(26):2531-44.
105. Jain MD, Smith M, Shah NN. How I treat refractory CRS and ICANS after CAR T-cell therapy. Blood, The J Am Soc Hematol. 2023;141(20):2430-42.
106. Moghanloo E, Mollanoori H, Talebi M, Pashangzadeh S, Faraji F, Hadjilooei F, et al. Remote controlling of CAR-T cells and toxicity management: Molecular switches and next generation CARs. Transl Oncol. 2021;14(6):101070.
107. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med. 2017;9(9):1183-97.
108. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood, J Am Society Hematol. 2014;124(2):188-95.
109. Gonzalez Castro LN, Dietrich J. Evaluation and management of chimeric antigen receptor (CAR) T-cell-associated neurotoxicity. Neuro-Oncol Practice. 2021;8(3):259-65.
110. CAR T Cell. Available at: https://en.wikipedia.org/wiki/CAR_T_cell. Accessed on 4 July 2024.
111. Liu D, Zhao J, Song Y. Engineering switchable and programmable universal CARs for CAR T therapy. J Hematol & Oncol. 2019;12:1-9.

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