Al-Rafidain J Med Sci. 2024;6(2):21-31.
DOI: https://doi.org/10.54133/ajms.v6i2.726

Review Article

CAR-T Cell Therapy for Cancer

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Received: 13 March 2024; Revised: 13 April 2024, Accepted: 14 April 2024

Abstract

Objective: To provide a basic overview of the status of CAR-T cell therapy and foresee its future applicability in cancer treatment. ***Method***: The search engines PubMed, Google Scholar, ResearchGate and Web of Science were employed in obtaining peer-reviewed articles using the criteria outlined in the method section. ***Main points***: CAR-T cell therapy has proved a lifesaving option for hematological malignancies despite its huge cost per treatment. Clinical trials are still ongoing to improve the effectiveness of this therapy for solid tumors as well as make it more affordable and easier to set up. ***Conclusion***: CAR-T cell therapy represents a useful addition to the arsenal in the fight against cancer, particularly in lifesaving scenarios in dealing with serious hematological malignancies.

Keywords: CAR-T cells, Chimeric antigen receptor, Immune cell therapy.

علاج السرطان بالخلايا التائية المحورة

الخلاصة

الهدف: تقديم مراجعة اساسية لوضعية علاج السرطان بالخلايا التائية المحورة والتنبؤ عن امكانية تطبيقها في المستقبل. **الطريقة**: تم استخدام العديد من أدوات البحث في اعداد هذه الدراسة للحصول على المقالات المقيمة بالمعايير الموضحة في قسم الطريقة من المراجعة. النقاط الرئيسية: اثبت علاج السرطان باستخدام الخلايا التائية المحورة انه خيار منقذ للحياة بالنسبة لبعض سرطانات الدم الخبيثه على الرغم من كلفته الباهضة. ولاتزال التجارب السريرية مستمرة لتحسين فعالية هذا العلاج مع الأورام السرطانية الصلبة، فضلًا عن جعله في المتناول وأسهل في الاعداد. **الاستنتاج**: يمثل علاج السرطان باستخدام الخلايا التائية المحورة اضافة مفيدة في مكافحة هذا المرض وخاصة مع إنقاذ الحياة عند التعامل مع بعض السرطانات الدموية الخبيثة والخطرة.

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Article citation: Al-Janabi II, CAR-T Cell Therapy for Cancer. *Al-Rafidain J Med Sci*. 2024;6(2):21-31. doi: https://doi.org/10.54133/ajms.v6i2.726

INTRODUCTION

The link between immunity and cancer was first recorded around 150 years ago, when the development of a microbial skin condition, erysipelas, was seen to be protective against cancer [1]. Later in 1893, William Coley demonstrated that erysipelas was associated with a better outcome in patients with sarcoma [2]. These epidemiological associations between what we now recognize as the immune response of the body to the presence of cancer constitute one of history's early evidences of the deployment of our immune system to fight cancer. Just over a century ago, a hypothesis was formulated that the human body constantly generates cancer cells that our immune system can eliminate, thus preventing most potential tumors from taking hold [3]. Over 60 years ago, scientists conceived the cancer immunosurveillance hypothesis, which postulates that antigens from cancer cells can be recognized and targeted by the immune system to prevent the development of carcinogenesis [1]. The introduction of knockout mouse models provided the necessary means to experimentally demonstrate the relationship between the immune response and tumorigenesis. Following this, there was a surge in interest in harnessing and modifying the immune response to combat cancer. Cancer immunotherapies have now revolutionized the field of oncology by extending the lives of many patients with cancers that would have otherwise been fatal [4]. Cancer immunotherapies use cytokines, antibodies and immune checkpoint

inhibitors (ICIs) as well as immune cells to modulate the host immune response to cancer [5–8]. The latter type of treatment employs living immune cells to control carcinogenesis in a general approach called adoptive cell therapy (ACT). Rapid progress has been made in recent years in our understanding of the antitumor functions of immune cells, and ACT has emerged as a major platform for therapeutics [9]. Our immune cells can recognize and eliminate infected, damaged and cancerous cells, and a type of immune cell known as killer T cells (toxic T lymphocytes) is particularly good at that when it comes to cancer cells. These T cells, as we will refer to them in this review, can be employed in several ways, including: A) tumor-infiltrating lymphocytes (TILs), which involves harvesting the T cells from freshly resected tumor tissues followed by expansion in the laboratory and then re-infusing them into the patient [10–13]; B) engineered T cell receptor (TCR) therapy, where T cells are usually taken from the patients and engineered to have a manipulated T cell receptor that enables them to target a specific cancer antigen; C) CAR-T cell therapy, where T cells are usually taken from the patients and engineered to have a synthetic receptor, known as CAR (chimeric antigen receptor). The therapies mentioned in a and b above can only target and eliminate cancer cells that present their antigens in a certain context and are bound by the human leukocyte antigen (HLA) complex. The general name for this complex is the major histocompatibility complex (MHC), but it will be referred to as HLA for humans. The ability of CARs to bind to cancer cells despite their inside antigens not being seen by T cells, thus representing a distinct advantage of CAR-T cells over the previous immune cell therapies, However, CAR T cells can only recognize antigens that are naturally expressed on the cell surface, thus limiting their potential antigen targets. The main two components of CAR-T cell therapy, which will be the focus of this narrative review, are the T cells and the engineered chimeric antigen receptor (CAR).

METHODS

A literature search was carried out for peer-reviewed articles using PubMed, Google Scholar, ResearchGate and Web of Science, covering the period between November 2003 and October 2023. The keywords and key phrases employed in the search were “CAR-T cells," “chimeric antigen receptor,” and “immune cell therapy." The evaluation and selection of the chosen hits were carried out by the author, taking into consideration the citations of the article and the impact factor of the journal. Many hits resulted from the initial search; however, studies deemed to be insufficiently reflecting the basic narrative review intended for the present work were excluded. Publications before November 2003 were only considered if the initial reading of the article indicated that they represented a significant or historic contribution.

T cells and T cell receptors

T cells originate from progenitors in the bone marrow that migrate to the thymus, hence the name T cells, where they differentiate and proliferate [14]. Cell surface HLA molecules load fragments of antigens, derived from peptides degraded within the cell, for recognition by T cells. There are two broad classes of T cells with distinct effector mechanisms, even though both types express T cell receptors (TCRs). One class of T cells is additionally characterized by the expression of CD4 co-receptor (called $CD4^+$ T cells, where CD stands for a protein called cluster of differentiation that exists in different forms), while the other class additionally expresses CD8 co-receptor (called $CD8^+$ T cells) [14,15]. $CD4^+$ T cells recognize antigens in the context of HLA class II molecules and initiate an immune response by producing cytokines with chemotactic, pro-inflammatory and immunoprotective effects [15]. At least one subtype of $CD4^+$ T cells, $CD4^+$ $CD25^+$ T cells, functions to dampen the immune response following exposure to allergens [16]. CD8+ T cells, on the other hand, detect antigens in HLA class I molecules and direct the elimination of infected cells or cancer cells [14]. Many CAR-T cell therapies have utilized both CD4+ T cells and CD8+ T cells [17]. This review will refer to the cells utilized in manufacturing as simple T cells, regardless of their subtype. Each T cell has a specific and sensitive T cell receptor (TCR) that constantly looks out for foreign (non-self) signals that, when engaged, trigger a cascade of immune responses [18]. The T cell receptor is a surface-located complex of eight polypeptides, usually represented by their Greek alphabets (see Figure 1).

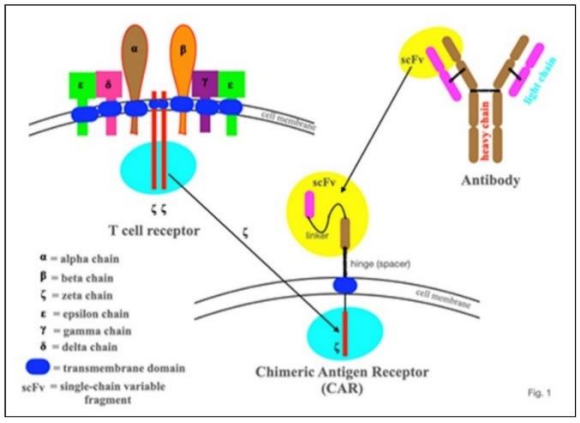

Figure 1: A schematic representation of the basic construction of a chimeric antigen receptor (CAR).

The α (alpha) and the β (beta) chains bind the foreign antigen and form the core of the receptor. This heterodimer core associates with one copy each of δ (delta) and γ (gamma) chains and two copies each of ε (epsilon) and ζ (zeta) chains to make up a total of 8 polypeptides (refer to Figure 1). A smaller population of T cells expresses a different class of T cell receptors using γ and δ chains (instead of the respectively similar α and β chains). However, the T cells expressing α β chains have been studied more extensively, and the rest of this review will use the phrase T cells when referring to α β T-cells unless

otherwise specified. A T cell requires binding of its TCR to its cognate antigen presented by the HLA molecule, and this binding results in the so-called "signal 1." Some extra stimuli are needed for the T cell to become fully activated, though. If these extra stimuli aren't present, the T cell could die or become anergized, which means it doesn't react to anything [19]. Some of these additional stimuli include activating ligands (such as CD80 and CD86) displayed on the antigen-presenting cells, which can bind to co-stimulatory molecules (such as the CD28 receptor) expressed by T cells, leading to the generation of "signal 2" [20,21]. Other additional stimuli can include cytokines secreted by the same (or neighboring) cells and are often referred to as "signal 3." When activated, through the engagement of their T cell receptor with the antigen, T cells can kill cancer cells by several mechanisms, including: A) Secretion of cytotoxic granules containing perforin and granzyme to directly kill cancer cells via apoptosis [22]. B) expression of death receptors on their surface to bind with death receptor ligands on cancer cells, resulting in their death [23–24]. C) Secretion of multiple cytokines such as IL-2 (interleukin-2), IL-6 (interleukin-6), INF-γ (interferon gamma) and GM-CSF (granulocyte-macrophage colony-stimulating factor), which recruit and activate other immune cell types like macrophages and natural killer cells (NKs) [25].

The design of CAR-T cell

CAR-T cell therapy is designed to re-direct a patient's (or donor's) T cells to specifically target and destroy cancer cells [26]. To establish this therapy, viable T cells are required, for which a chimeric antigen receptor (CAR) is engineered. There are two basic components to the CAR design connected through a transmembrane domain: "a binding part" that attaches strongly to the antigen being targeted and "a signaling part" that is responsible for signal initiation and transduction leading up to T cell activation (see figure 1) [19]. The binding part is often derived from the antigen binding fraction (often abbreviated as Fab for fragment antigen-binding) of a monoclonal antibody that has a high affinity for the antigen being targeted. The Fab part of the antibody itself arises from two genes: the variable light chain (V_L) gene and the variable heavy chain (V_H) gene [27,28]. The sequences of these two genes are often combined using a short link to create a single-chain fragment variable denoted by the symbol scFv [29,30]. The signaling part of the CAR construct is usually obtained from the intracellular domain of one of the zetas (ζ) chains. The ζ chain is responsible for the specificity signal in T cells (signal 1) and for IL-2 production, which is widely known to be a crucial promoter of T cell expansion [31,32]. The binding part of CAR is attached to the signaling part via a hinge region to provide flexibility, reach and length to the construct and improve the binding to the antigen (33–37). The binding of this chimeric receptor to its target antigen causes conformational changes in the CAR construct, leading to signaling and culminating in T cell activation.

Multiple generations of CARs

The 1st generation of CARs constructs contained the minimum components in the form of scFv, a hinge, a transmembrane domain and an intracellular zeta chain signaling domain as illustrated in Figure 2.

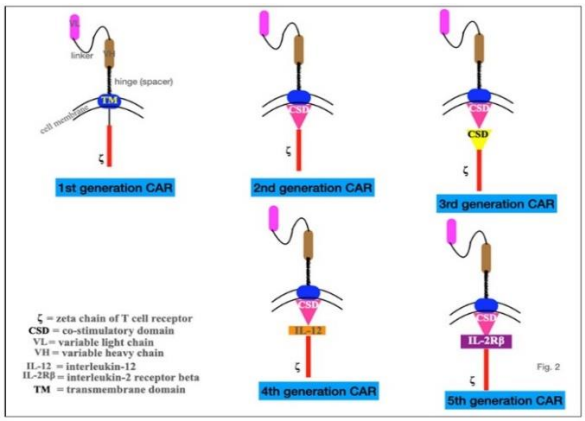

Figure 2: Five generations of chimeric antigen receptors (CARs).

However, it soon became clear that the stimulatory action of the zeta chain (ζ) alone is insufficient to elicit an adequate T cell response and avoid exhaustion and apoptosis, despite it being a common feature of all the currently licensed American and European products [37,38]. Tumor cells lack the expression of co-stimulatory ligands often required for the activation of T cells [39]. The reason for the modest T cell activation, expansion and persistence seen with the 1st generation CARs is often attributed to the absence of co-stimulatory ligands. The 2nd generation CARs were developed to include a co-stimulatory signal such as CD28, CD134 and CD137 in the intracellular portion of their receptor (CD28 and CD137 being the co-stimulatory signals employed in licensed products so far) [40–49]. These co-stimulatory molecules are incorporated in the CAR construct and can bypass the need for their respective ligands to activate the T cells and prevent anergy and apoptosis that can result from the solitary signal 1 [40,42-49]. The 2nd generation CARs have been shown to have improved potency and persistence compared to the 1st generation [44,50,51]. Third-generation CARs are usually based on the addition of further co-stimulatory signals, such as combining CD28 and CD137, to provide even stronger signal activation [44,52,53]. The additional co-stimulatory signals provided by the 3rd generation can enhance T cell activation and proliferation, leading to a more effective antitumor response. However, the inclusion of more signaling molecules in the CAR design might not always be advantageous [54]. The 4th generation of CARs was developed to allow the recognition of a broader range of cancer cells and to treat different types of malignancies. The 4th generation CAR-T cells, also called TRUCK (T cells Redirected for Antigen-Unrestricted Cytokine-Initiated Killing), were based on the 2nd generation format but contain a transgene for cytokine release [55]. When the 4th generation CAR-T cells are activated, they not only kill cancer cells but also constitutively secrete cytokines such as IL-12 or IL-

18 that recruit and activate other immune cells to eliminate cancer with potentially fewer side effects [55–57]. The 5th generation of CAR-T cells is currently emerging to address some of the limitations of previous versions and have multiple functions. One type of CAR has a truncated intracellular domain of the cytokine receptor IL-R2 (interleukin receptor 2) containing a motif for binding transcription factors such as STAT-3/5 (signal transducer and activator of transcription 3/5). The cytokine release signal not only maintains T cell activation and promotes memory T cell generation, but it also reactivates and stimulates the immune system [58]. Other 5th generation CARs contain domains that suppress immune checkpoint molecules such as PD-1 (programmed cell death-1 protein) or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) to reduce immune exhaustion and enhance antitumor activity [59].

Processing CAR-T cells

Delivering CAR-T cells to the patient is a complex process involving several stages, including the collection of the T cells, engineering them to contain the desired construct, increasing their numbers and infusing the modified resultant T cells back into the patient [26,37,60,61]. Blood is first collected from the patient's (or donor's) peripheral circulation, usually from the antecubital veins, and then it undergoes a process called leukapheresis [62,63]. This involves the removal of the white cells from the blood before it is returned to the patient. The white blood cells are then separated and sorted to isolate the desired type of T cells. These T cells are then genetically engineered to express the desired construct and activated with CD3/CD28 to render them susceptible to viral transduction [64,65]. The construct is then transduced into the T cells using a viral vector (usually a lentivirus) or through electroporation of its *in vitro*-transcribed RNA [60,66]. The construct is often encoded in the DNA and integrates into the T cell genome, imparting a permanent expression. RNA-based constructs are sometimes used to permit transient expression. The next step will be the expansion of CAR-T cells *ex vivo* over several days so that large numbers of these cells can be produced to meet clinical-grade criteria for administration. Finally, the CAR-T cells are ready to be re-infused back into the patient's bloodstream after receiving conditioning chemotherapy to help make space for the engineered T cells to grow and attack the cancer.

Selection of target antigen

As mentioned earlier, the binding part of the CAR is often made from a monoclonal antibody (mAb) with a high affinity to the target antigen of the cancer cell to be eliminated. Cancer cell antigens are proteins that can be classified into two broad categories: a) tumor-associated antigens (TAAs) and b) tumor-specific antigens (TSAs) [67]. The TAAs include antigens originating from 1) overexpressed genes, 2) differentiation antigens and 3) germline/testis antigens. The antigens derived from over-expressed genes are normal self-proteins that are minimally expressed in healthy tissues but over-expressed in cancer, such as the EGRF protein family. An interesting example of this family is the HER2/Neu protein, which is overexpressed in epithelial tumors of the breast and ovary [68,69]. The targeting of this over-expressed protein (antigen) by the mAb, trastuzumab, has revolutionized the treatment of breast cancer. Differentiation antigens represent normal proteins performing a specific function in certain tissues, as in the case of prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) expression in the prostate [70]. Cancer germline/cancer testis antigens (thereon referred to as CTAs) are a large family of TAAs expressed in different cancers but not in normal tissues, except for testis and placental tissues, for example, the MAGE (melanoma antigen) family of proteins [71,72]. The protein MAGE A3 is one of the most frequently expressed antigens in many cancers, including melanoma, head and neck cancer, and non-small cell lung carcinoma [73]. The CTAs have been particularly attractive as cancer-specific therapeutic targets. The tumor-specific antigens (TSAs) are, as the name indicates, proteins that are restricted to tumors and not found in normal cells. TSAs arise because of genetic perturbations or from the expression of viral elements within the cell and include neoantigens, oncoviral antigens and endogenous retroviral elements [67,74,75]. Neoantigens are a subset of TSAs produced as a direct result of genetic alterations caused by DNA mutations in incipient cancer cells, and in that sense, they are highly immunogenic nonself-antigens, leading to effector T cells escaping central tolerance [76]. The expression of tumor antigens is not sufficient to elicit a complete immune response, as cancer cells often employ several mechanisms to avoid being recognized and targeted by effector T cells [77]. To date, the best studied and clinically validated target antigen for CAR-T cells is CD19, a TTA expressed on the surface of B cells, making it useful for the treatment of B cell cancers and producing remarkably effective and durable clinical responses [78,79]. The display of CD19 on the surface of B cells and the consequent targeting by CAR-T cells are independent of the HLA mechanisms that normally operate to externalize foreign antigens found inside the cells. For a protein to be recognized as being foreign within the cell, it must be processed to produce a peptide, followed by the display of that peptide on the surface. Two types of molecules are responsible for presenting these peptides as a complex of peptide-HLA on the cell surface. HLA-class I molecules are tasked with presenting the complex to $CD8^+$ cells, and HLA-class II molecules are responsible for presenting the complex to $CD4^+$ cells (Figure 3) [80]. Normally, endogenous antigens (including tumor antigens) are presented through HLA-class I, and exogenous antigens are presented through HLA-class II [81]. There is an additional mechanism called "cross-presentation," whereby exogenous antigens are presented by HLA-class I molecules [82]. While HLA-class I proteins are expressed by all nucleated cells, only a fraction of cell types (usually referred to as professional antigen-presenting cells, APCs), such

as dendritic cells and macrophages, express HLA-class II proteins.

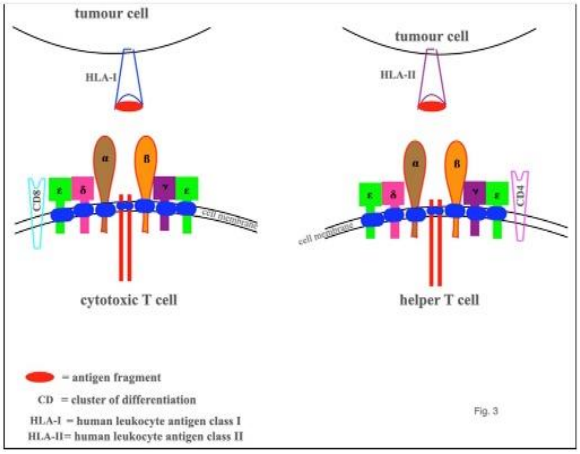

Figure 3: Cancer antigen presentations to T cells.

Although cancer cells can present self-antigens through HLA-class I, the mere existence of an antigen inside the cancer cell does not translate to a successful antigen presentation. This presentation is particularly impaired in malignant cells due to the lack of co-stimulatory signals to present the antigen coupled with the expression of high levels of inhibitory proteins such as PD-L1 (programmed cell death ligand 1 protein) [77]. Moreover, cancer cells can downregulate their HLA-class I molecules and other members of the processing machinery [83]. Although HLA-class II molecules are restricted to APCs, some types of tumors, such as melanoma, can often express these proteins [84]. There is a rich diversity of tumor antigens from intracellularly expressed proteins that CAR-T cells are unable to target, limiting the expansion of this therapy to surface antigens [85]. Despite the unprecedented clinical success of CAR-T cell therapy for hematological disorders, more potent and specific CARs are needed to treat solid tumors [86]. So far, no surface antigen with comparable characteristics to CD19 has emerged regarding solid tumors [87]. An ideal molecule for CAR should be overexpressed on the cancer cell surface of many patients, with negligible expression in normal tissues. A selection of surface antigens is given in Table 1, along with some of the solid malignancies they were described for [88].

Table 1: A selection of target antigens for CAR-T cells in some solid malignancies

Target antigen	Cancer type	Reference
CEA	GIT adenocarcinoma, CRC	[89-91]
HER2	Glioblastoma, sarcoma, BC	[92,93]
EGFR/CD133	Cholangiocarcinomas, NSCLC	[94]
IL13α2	Glioblastoma	[95]
EGFRvIII	Glioblastoma	[96]
PSMA	Prostate	[97]
GD2	Melanoma, osteocarcinoma, neuroblastoma	[98]
Mesothelin	NSCLC, BC, pancreatic cancer	[99-101]

CEA: carcinoembryonic antigen, GIT: gastrointestinal tract, CRC: colorectal cancer, HER2: human epidermal growth factor receptor 2, BC: breast cancer, EGFR: epidermal growth factor receptor, CD133: cluster of differentiation 133, IL13α2: interleukin 13 alpha 2, EGFRvIII: epidermal growth factor receptor viii, PSMA: prostate-specific membrane antigen, GD2: disialoganglioside 2, NSCLC: non-small cell lung cancer.

Status of CAR-T cell therapy

As of June 2023, six CAR-T cell cancer therapies have been approved for use in Europe and the United States of America (see Table 2) [37]. Four of these products are based on using anti-CD19 antibodies for the treatment of B cell malignancies with success due to the limited (but ubiquitous) expression of the target antigen, CD19, on the surface of B cells [102]. These four licensed products are: Tisagenleleucel (Kymriah), Axicabtagene ciloleucel (Yescarta), Lisocabtagene maraleucel (Breyanzi) and Brexucabtagene autoleucel (Tecartus). The remaining two CAR-T cell therapies, Idecabtagene vicleucel (Abecma) and Ciltacabtagene autoleucel (Carvykti), are based on using anti-BCMA antibodies (BCMA are B cell maturation antigen proteins) and are approved for the treatment of multiple myeloma [103]. The BCMA proteins are exclusively expressed in B cell lineages, including normal and malignant plasma cells [37]. In addition to the six currently available CAR-T cell therapies, several others are in clinical trials for hematological as well as solid cancers. The personalized approach of collecting T cells from the patients and then genetically modifying and expanding them is time-consuming and expensive, which severely limits its widespread use. The cost of collecting autologous CAR-T cells from the patient can be up to 0.5 million US dollars per treatment [21]. Furthermore, the T cells harvested from patients may already suffer from exhaustion and are less active than those from healthy donors. Allogenic T cells (T cells collected from other humans other than the patient and are likely to be genetically dissimilar) provide a promising alternative in seeking the development of off-the-shelf CAR-T cell therapies [110–112]. However, when administering allogenic CAR-T cells to patients, there is always a risk of graft versus host disease (referred to as GvHD), manifested as rejection by the host immune system. To mitigate this risk, the TCR genes are deleted from CAR-T cells using appropriate gene editing technology, such as CRISPR/Cas9. Additionally, the beta-2-microglobulin gene (*B2M*) is also deleted to abolish the formation of the HLA class I complex and avoid host rejection of CAR-T cells [21,113]. Nevertheless, a significant challenge with the allogenic CAR-T cell approach is that the cells do not survive for a long time within the patient, possibly due to the lack of HLA class I presentation and the consequent elimination by the NK cells [21,114]. One interesting strategy to lower the cost of producing CAR-T cells is through their transient generation *in vivo* by delivering mRNA encoding the FAP-targeting CAR in lipid nanoparticles (LNPs) [115]. Another field of research was where CAR-T cells were engineered to recognize and attack two different targets on cancer cells, thus making the therapy more useful to treat cancer by utilizing two different ways to evade the immune system. There are two main types of dual-targeted CAR-T cell therapies. I) bispecific CAR-T cells, and II) tandem CAR-T cells.

Table 2: FDA/EMC-approved CAR-T cell cancer therapies as of 30/06/2023

Product	Trade name	Based on	Indicated for	Reference
Tisagenlecleucel	KYMRIAH	CD19-directed genetically modified autologous T cells	LBCL FL B-ALL	[104]
Axicabtagene ciloleucel	YESCARTA	CD19-directed genetically modified autologous T cells	LBCL PMBCL FL	[105]
Lisocabtagene maraleucel	BREYANZI	CD19-directed genetically modified autologous T cells	LBCL PMBCL FL	[106]
Brexucabtagene autoleucel	TECARTUS	CD19-directed genetically modified autologous T cells	MCL	[107]
Idecabtagene vicleucel	ABECMA	BCMA-directed genetically modified autologous T cells	MM	[108]
Ciltacabtagene autoleucel	CARVYKTI	BCMA-directed genetically modified autologous T cells	MM	[109]

FDA: Food and Drug Administration, EMC: European Medicines Agency, BCMA: B-cell maturation antigen, CD19: cluster of differentiation 19, MM: multiple myeloma, LBCL: large B-cell lymphoma, PMBCL: primary mediastinal large B-cell lymphoma, FL: follicular lymphoma, MCL: Mantle cell lymphoma, B-ALL: B-cell acute lymphoblastic leukaemia.

Bispecific CAR-T cells are those that are engineered to express two different antigen recognition domains, allowing for the simultaneous targeting of these two antigens. The bispecific CAR-T cells can be designed to recognize two antigens on the same cancer cell type or two different cell types [61,116]. As an example, the bispecific CAR-T cells are those targeting CD19 and CD22, developed for the treatment of B-cell acute lymphoblastic leukemia [117]. The tandem CAR-T cells, on the other hand, are those that express two separate CARs, each targeting a different antigen [118]. In contrast to bispecific CAR-T cells, which use a single CAR to target two antigens, tandem constructs use two separate CARs. The tandem type can target two different cancer cell types or two different antigens on the same cancer cell type, as in the case of constructs having recognition domains for both mesothelin (a protein overexpressed in solid tumors) and folate receptor alpha, engineered for the treatment of mesothelioma [119]. Although CAR-T cell therapy has shown impressive results in the treatment of hematological malignancies, its efficacy in solid tumors (representing approximately 90% in adults and 30% in children) has been rather limited, possibly because of the lack of suitable antigens to target and the immuno-suppressive tumor microenvironment (TME) [21]. Figure 4 depicts a schematic illustration of the basis on which CAR-T therapy is designed to target cancer cells displaying suitable antigens. The lack of CAR-T cell targets exclusive to solid tumors represents a challenging obstacle. The ideal target should be solely expressed on the surface of tumor cells and not in any normal tissue. Currently, several targets for solid tumors are in various stages of clinical development, including GD2 (disialoganglioside 2), HER2 (human epidermal growth factor receptor 2), EGFRviii (epidermal growth factor receptor viii), mesothelin, claudin 18.2, IL13Ra2 (interleukin 13 receptor subunit alpha 2), CEA (carcinoembryonic antigen), PSMA (prostate-specific membrane antigen), PSCA (prostate stem cell antigen), GPC3 (glypican-3), MUC1 (mucin-1) and others [120]. A recent publication reported that a potent antitumor CAR-T cell activity against mesothelin in pre-clinical models is currently being progressed to clinical trials [121].

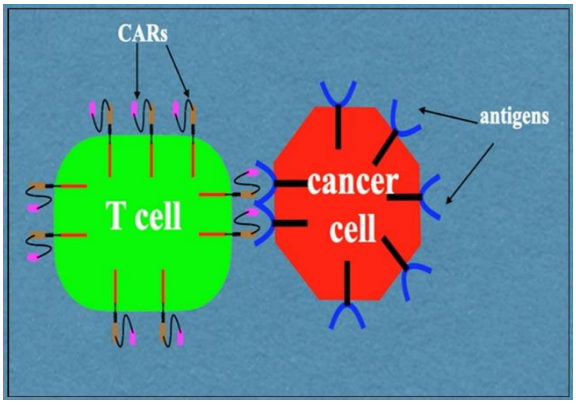

Figure 4: A simplified diagram illustrating the recognition of a CAR-T cell of antigens presented on the surface of a cancer cell as a first step to the eventual elimination of the tumor.

The tumor microenvironment is enriched with immunosuppressive cells such as Tregs (regulatory T cells), TAMs (tumor-associated macrophages) and Th2 (T helper 2) and to overcome this, the CAR-T cells are engineered to express various molecules. These accessory molecules, when expressed, provide the CAR-T cells with an armory to enhance their antitumor capabilities, and the constructs are called "armored" CARs. In addition, negative regulators of CAR-T cell function, such as PD-1, are deleted using gene editing technology to achieve enhanced anticancer activity [122,123]. To improve the effectiveness of CAR-T cells against solid tumors, other approaches focused on employing combination therapies. One such approach is to combine CAR-T cell therapy with other treatments such as oncolytic viruses, radiotherapy, chemotherapy or immune checkpoint inhibitors (ICIs) [124–126]. More recently, the use of gene editing technology such as CRISPR-Cas9 to enhance CAR-T cell functions has been proposed [127]. Eyquem et al. have used the CRISPR/Cas9 system to place the CD19-specific CAR sequence at the T cell receptor alpha constant locus to knock out the existing T cell receptor while knocking in the CD19-specific CAR to sustain T cell

function in mouse models [127]. CAR-T cells are living drugs, and their persistence in the body beyond their need can mean their continued attack of normal tissues expressing the target antigen. To avoid this, one strategy is to eliminate the CAR-T cells from the body through the incorporation of a safety switch after curing cancer. The inducible caspase 9 (iC9)/Rimiducid system has demonstrated its effectiveness in eliminating CAR-T cells both *in vitro* and in mice [128]. In addition to the "off" switches, there are also "on" switches that can be used to control CAR-T cell activity, such as the controllable CAR-T cell therapy using a Rimiducid-inducible GoCAR-T [129]. Although CAR-T cell therapy has shown great promise in treating hematological cancers, a major concern with this approach is the potential to cause life-threatening side effects. Two of the most common adverse events are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (iCANS) [130]. Despite the seriousness of these events, they can be treated and reversed if managed early enough during therapy. The management of CRS can range from supportive care to administering corticosteroids and/or Tocilizumab (Actemra, an anti-IL-6 receptor antagonist), depending on the severity of this adverse event. Low-grade neurological events are primarily managed with supportive care. High-grade neurological side effects with concurrent CRS are best managed with Tocilizumab. Neurologic events without concurrent CRS do not respond to anti-IL-6 therapy and are best treated with corticosteroids [21,130].

Conclusions

Despite its exorbitant cost, CAR-T cell therapy has made steady progress, and six such treatments have reached the market. They proved particularly effective against B-cell cancers and were lifesavers for many patients. Progressive generations of CAR-T cells have added much-needed improvements to their efficacy, range of targets and persistence in the recipient patients. Targeting solid tumors remains, for the time being, challenging due to the lack of well-defined targets and their immunosuppressive microenvironment. Nevertheless, several clinical trials are ongoing on a range of solid malignancies targeting their specific or associated antigens. The adverse events associated with CAR-T cell therapies could be serious, such as cytokine release syndrome and neurotoxicity. However, with appropriate management of these adverse events, the potential lifesaving benefits of CAR-T cell therapy can become paramount.

Epilogue

After writing this article, the American FDA, on January 19, 2024, asked manufacturers of CAR-T cell therapies to add a warning to such products regarding the risk of secondary cancers. However, a spokesperson for this agency has said that the overall benefits of these products continue to outweigh their possible risks.

Conflict of interests

No conflict of interests was declared by the author.

Funding source

The author did not receive any source of fund.

Data sharing statement

N/A

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