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REVIEW ARTICLE

Chimeric Antigen Receptor T Cell Immunotherapy for Tumor: A Review of Patent Literatures

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Abstract: ***Background***: Chimeric Antigen Receptor (CAR) T cell immunotherapy, as an innovative method for tumor immunotherapy, acquires unprecedented clinical outcomes. Genetic modification not only provides T cells with the antigen-binding function but also endows T cells with better immunological functions both in solid and hematological cancer. However, the CAR T cell therapy is not perfect because of several reasons, such as tumor immune microenvironment, and autologous limiting factors of CAR T cells. Moreover, the safety of CAR T cells should be improved.

Objective: Recently many patents and publications have reported the importance of CAR T cell immunotherapy. Based on the patents about CAR T cell immunotherapy, we conclude some methods for designing the CAR which can provide useful information to readers.

Methods: This review presents recent patents and publications, summarizes some specific antigens for oncotherapy from patents and enumerate some approaches to treatment of immunosuppression and reinforcing the immune response of CAR T cells. We also sum up some strategies for improving the safety of CAR T cell immunotherapy.

Results: CAR T cell immunotherapy as a neotype cellular immunotherapy has been proved effective in oncotherapy and authorized by the FDA. Improvements in CAR designing have enhanced the functions of CAR T cells.

Conclusion: This review, summarizing antigens and approaches to overcome defects of CAR T cell immunotherapy from patents and publications, might contribute to a broad readership.

Keywords: Antigen, CAR T cells, efficiency, immune checkpoints, immune therapy, safety.

1. INTRODUCTION

According to the GLOBOCAN estimate in 2012, approximately 14.1 million cancer patients were reported, and among them, 8.2 million people died. Lung cancer leads to higher death rates among men, and, breast cancer among women [1]. Fortunately, the number of survivals have risen over the years and will keep on increasing owing to early treatment [2]. Radiotherapy cures tumor combined with some radiation sensitizers, such as Vandetanib and Sunitinib [3, 4]. Chemotherapy can also treat tumors by some cytotoxic drugs, such as doxorubicin [5]. However, normal cells are inevitably impaired for lack of specificity when tumor cells are killed. Targeting therapeutic drug has been developed as another choice for tumor therapy and has acquired an uplifting effect, but it has the limitation of drug resistance.

Therefore, a new oncotherapy method emerged. Chimeric Antigen Receptor (CAR) T cell immunotherapy has revealed tremendous potential. Conventional T Cell Receptor (TCR) needs the Major Histocompatibility Complex (MHC) to recognize an antigen. T cells can be activated and can express immune function through the MHC [6]. Simply, CAR T cells which are reconstructive T cells carry artificial domains and can be activated without MHC, which provides CAR T cells with more flexible functions.

Some solid and hematological tumors can be relieved by utilizing CAR T cell therapy technology, for example, CD19, a transmembrane glycoprotein, is highly expressed on Non-Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL) cells when B lymphocytes are deteriorated [7]. Therefore, CD19 is an appropriate antigen for oncotherapy. CD19 CAR T cells were reported as an effective treatment method in 2003 and first applied to therapy in 2007. In recent years, CD19 CAR T cell products were approved by the FDA and gradually applied to NHL, CLL and Acute Lymphoblastic Leukemia (ALL) therapy [8]. Zhang *et al*. [9] also reported that CD20 CAR T cells had a significant function in NHL therapy. On Phase I clinical trial, six of these cells were completely relieved and three were partially relieved among eleven patients. Carbonic anhydrase IX (CAIX) which is

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highly expressed on Renal Carcinoma Cells (RCC) rather than adjacent normal cells, plays the role of a biomarker in renal carcinoma. Lo *et al*. [10] reported that G36-CD28-TCRζ CAR T cells exhibited potent function in CAIX + RCC therapy.

In this review, we summarized patents, which were published at the European Patent Office (EPO) and the United States Patent and Trademark Office (USPTO), and publications to reveal some methods for CAR designing. The disagreement about patents of CAR T cell immunotherapy has never been resolved, such as the dispute among Juno, Kite and Novartis. Although CAR T cell therapy faces many challenges, it provides oncotherapy with a promising prospect.

2. SPECIFIC CARS FOR TUMOR IMMUNOTHERAPY

Universally, CARs are designed including the activation domain, costimulatory domain, transmembrane domain and antigen-binding domain. The activation domain is an intracellular domain, such as CD3. The costimulatory domain can increase the activity of the CAR T cell, like proliferation and persistence [11]. The transmembrane domain plays a role of the structural anchor [9]. The antigen-binding domain which can combine with the target antigen is an important component of CARs. It always consists of Single Chain Variable Fragments (scFvs) [11]. The characteristics of CAR T cell therapy technique include the specific combination of CARs and antigens. Currently, antigens are divided into two types, mutated antigens and self-antigens [12]. It is of great importance to design CARs which can specifically bind to the target antigen and can activate T cells to generate an immune response.

Some new target antigens are useful with oncotherapy as shown in patents (Table **1**) [13-27, 29-40].

For example, B7-H4, which is overexpressed in breast carcinoma and merely in normal tissues, is used as a tumor biomarker in women's reproductive system [28]. Hu *et al*.

Table 1. Antigens for Cancers.

Patent Numbers	Antigens	Cancers	References
AU20160243124	LHR	Ovarian cancer, prostate cancer	[13]
WO2016CN92577	Robo1	Robo1+tumor, like HCC	[14]
CN20171326932	CEA	CEA+ tumor, like colon cancer	[15]
WO2017EP63862	BCMA	Multiple myeloma, NHL	[16]
WO2016US14985	IL13Rα2	Neuroglioma	[17]
CN20171406177	MUC1	Adenocarcinoma	[18]
CN20161602458	HER2	Breast, ovarian, endometrial, cervical cancer *et al.*	[19]
CN20161327611	Mesothelin	Malignant pleural mesothelioma, pancreatic cancer	[20]
CN20161327646	CD30	Hodgkin's lymphoma, non-Hodgkin's lymphoma	[21]
CN2016102324	PSA	Prostatic cancer	[22]
CN20171406296	AFP	Liver cancer	[23]
14/830,392	CD123	Acute myeloid leukemia	[24]
14/805,236	CD33	Acute myeloid leukemia	[25]
14/994,403	EGFRIII	Glioblastoma	[26]
13/875,560	VEGFR2	Liver cancer	[27]
AU2016024312	B7-H4	Breast, ovarian, renal cancers	[29]
AU20160243128	HLA-G	HLA-G^+ tumor, like papillary thyroid carcinoma	[31]
WO2015132604	GD2	Neuroblastoma	[32]
WO2018181207	GM2	Myeloma	[33]
AU2017232431	CLEC14A	CLEC14A+ solid tumor	[34]
WO2018165913	NKG2DL	NKG2DL+tumor	[35]
US2018251568	CSPG4	Melanoma, breast cancer, head and neck cancer, mesothelioma, glioblastoma, renal cancer	[36]
WO2018145649	CD20	B lymphomas	[37]
CN108276495	CSF1R	M2 type tumor related macrophage	[38]
CN108277205	CXCR4	CXCR4+tumor	[39]
US2018104308	CD5	B lymphomas	[40]
CN2016102321	HCA153	Breast carcinoma	[44]
CN201611102044	CD19 and CD20	Malignant B lymphocytic leukemia	[45]
CN201611158116	CD19 and CD20 or CD138 or CD123	B cell lymphoma	[46]

[29] constructed anti-B7-H4 CAR T cells for breast, ovarian, and renal cancers therapy and proved that they were safe and effective. Human Leukocyte Antigen G (HLA G) which is found in tumor cells and is related to the poor prognosis of cancer patients might be considered as a promising tumor therapeutic target. Inventors introduced human anti-HLAG CAR T cells for papillary thyroid carcinoma therapy [30] [31]. Cluster determinant 30 (CD30) is a membrane protein which is highly expressed in lymphoma, particularly Hodgkin lymphoma [41]. Wang *et al*. [42] pointed out that autologous T cells which express CD 30 CARs could efficiently relieve relapsed or refractory Hodgkin lymphoma. Seven patients were relieved locally, and six patients' condition was controlled steadily among eighteen patients who suffered from Hodgkin lymphoma. Qi *et al*. [21] disclosed recombinant lentivirus vectors of CD30 CAR T cells in the patent and the efficient clinical effect of this kind of CAR T cells.

Studies have shown that different kinds of tumors express the same type of antigens. Hence one type of CARs can be applied to different types of tumors. For example, roundabout 1 (Robo1), a member of Robo immunoglobulin family, increases proliferation and microvessel formation of hepatocellular carcinoma cell. Moreover, it is overexpressed in breast cancer, colon cancer, pancreatic cancer, prostate cancer and glioma [43]. Therefore, anti-Robo1 CAR T cells are appropriate for different oncotherapy. And same is the case with anti-B7-H4, anti-LHR CAR T cells and so on. At the same time, different kinds of antigens appear in one type of tumors. Bi-specific CAR T cells have been applied to oncotherapy. For instance, inventors designed CAR T cells which expressed CD19 and CD20 or CD138 or CD123 for B-cell lymphoma therapy, and HER and HCA153 for breast cancer therapy [44-46].

Perfect CARs can only combine with tumor antigens. However, some CARs combined with "antigens" express on normal cells which cause an adverse reaction in adoptive CAR T cell therapy. Moreover, the expansion of CAR T cells is restricted by several factors, such as Tumor Microenvironment (TME) and the structure of the CAR T cell.

3. ENHANCE CAR T CELLS' EFFICIENCY

CAR T cells immunological therapy has been proved to be efficient in both hematological and solid cancers. However, this treatment is accompanied with some negative effects. CAR T cells exist transitorily and exhaust fleetly when patients adopt modified T cells. Researchers also indicate that solid tumor therapy is very difficult mainly because of the tumor microenvironment. O'Rourke *et al*. [47], from the University of Pennsylvania, discovered that tumor microenvironment is a significant drawback when they used EGFRvIII CAR T cells for glioma therapy.

3.1. Inhibit Immune Checkpoints

Some regulatory factors participate in activating immune response of T lymphocytes when T lymphocytes perform an immune function. It is worth mentioning that immune checkpoint proteins reside on T lymphocyte surfaces, such as Programmed Cell Death Protein 1 (PD-1), and Cytotoxic T Lymphocyte Antigen 4(CTLA-4) [48]. These proteins inhibit the activity of T lymphocytes and avoid immune access by combining with relative ligands. However, ligands of these proteins which also lie on tumor cell surfaces support tumor immune escape. PD-1 and CTLA-4 impede the function of T lymphocytes by the PI3K/Akt pathway. Differently, PD-1 blocks the activity of PI3K and CTLA-4 inhibits Akt directly [49].

In order to enhance the functions of CAR T cells, some measures should be taken to suppress immune checkpoints in oncotherapy (Fig. **1**). A few PD1 monoclonal antibodies which could combine with immune checkpoint proteins have been used in clinical trials [50]. Drugs for the inhibition drugs of immune checkpoint ligands may play a commendable role. In recent years, several antibodies targeting PDLI were approved by the FDA, such as atezolizumab, avelumab and durvalumab [51]. siRNA can downregulate the expression of immune checkpoint protein [52]. Therefore, enhancing the efficiency of CAR T cells is both autologous and requires external modulation (Table **2**) [53-109].

Table 2. Improvement Measurements of CAR T Cell Therapy in Patents.

Patent Numbers	Functions	Simple Description	References
CN107119021 CN106480097	Enhance CAR T cells efficiency	sgRNA or CRISPR/Cas9 knocked out the PD1 gene in CAR T cells	[53] [55]
WO2017177575		PD1 CAR T cells specifically recognized and targeted with over-expressing PDL1 tumor cells	[56]
CN106350533		Anti-PDL1 CAR T cells not only inhibited the combination of tumor cells and T cells, but also recognized tumor cells through PDL1 surface protein	[57]
CN105796597		CAR T cells carried PD-L1 and CTLA-4 antibody genes	[58]
CN107337736, CN107164410, CN107325185, CN107267555, CN107245500, CN107177632, CN107299110		One CAR with Two ScFvs (OCTS) technology	[60-66]
US2016340406		Costimulatory molecules were introduced to CARs	[71]
WO2017176289		Lenalidomide applied to increase immunological efficacy CAR T cells	[73]

Table (2) contd….

Patent Numbers	Functions	Simple Description	References
CN107058232		Inhibited SOAT1 was able to enhance lethality of CAR T cells	[74]
WO2017049166		CAR T cells with reduced Tet2 exhibited an improved efficacy in oncotherapy	[76]
CA2985156		Nucleic acid vaccinations had functions on CAR T cells activation and amplification	[77]
PH12017500596, AU2017249694 CN106916789		Improved CAR T cells contained antigen-binding domain and secretory cytokines	[78-80]
WO2017172952	Enhance CAR T cells safety	CD 19-FLAG CAR T cells were able to decrease IFN γ, IL2 and IL6	[89]
CN106636090		siRNA of humanized interleukin 6 reduced CRS	[90]
CN105640990		IL-6 receptor antagonistic drugs, like Tocilizumab, were applied to combination oncotherapy	[91]
CN106591363 CN106544321		Universal CAR T cells applied to allotransplantation	[92, 93]
WO2017177149		Some small molecular ligands assisted connections between CAR T cells and tumor cells	[96]
CA2982532 CN105087495		Double receptor enhanced CAR T cells safety	[97, 98]
US2017354724		Psoralen limited the proliferation capacity of effector cells	[99]
US2018256744		Cotinine induced T cells exhausted	[100]
CN107365798		iCasp9 suicide gene induced T cells apoptosis	[101]
CN105330750		Molecular brake rapidly stopped killing effect of CAR T cells	[102]
CN105524943		AAVS1 locus was safe and reliable for gene editing	[108]
WO2018184074		mesenchymal stem cells (MSC) relieved the side effect of CAR T cells therapy	[109]

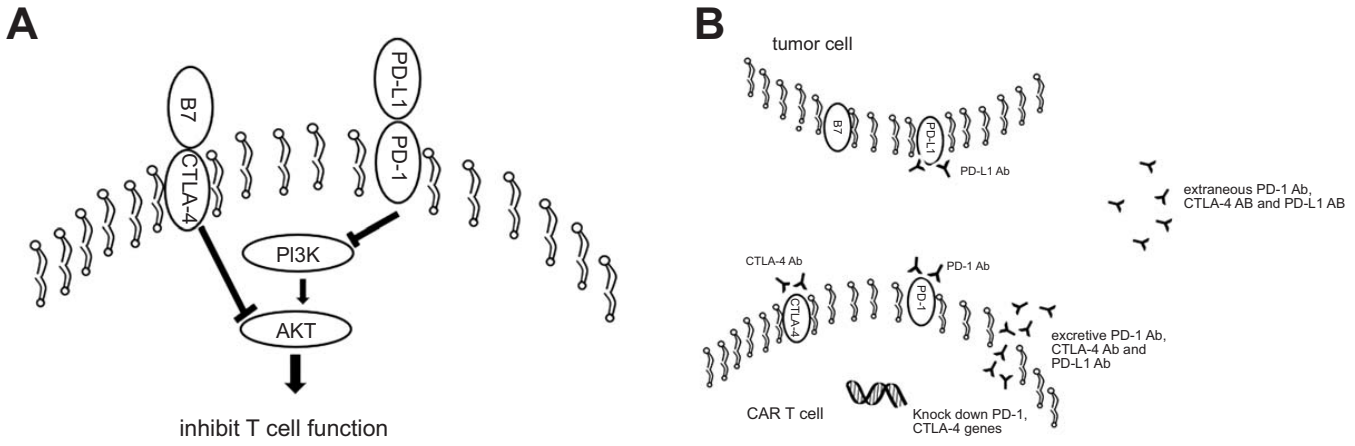

Fig. (1). PD-1 and CTLA-4 in tumor therapy. (A) PD-1 combines with PD-L1, which impedes PI3K activity. CTLA-4 combines with B7, which inhibits AKT activity. These combinations affect T cell activity. **(B)** several methods for inhibiting immune checkpoint. Antibodies bond to relevant loci that hinder the combination of receptors and ligands. Knockdown of PD-1 and CTLA-4 genes in T cells by siRNA, sgRNA and CRISPR/Cas9.

Shang *et al*. [53] enhanced the capacity and safety of CD19 CAR T cells by constructing a sgRNA for knocking out the PD1 gene in their patent. CRISPR/Cas9 is a promising gene editing technique for modifying CAR T cells. In recent patent and publications, this technology has been devoted to remold CD19 CAR T cells and enhance tumor immunity of CAR T cells [54, 55]. In brief, T lymphocytes' immunosuppression will be relieved partly when the combination of immune checkpoints and relevant ligands is restricted.

Other indispensable methods in patents which combine CAR T cells and immune checkpoints should be emphasized. Inventors provided PD1 CAR T cells which were specifically recognized and targeted with over-expressing PDL1 tumor cells in their patent, such as squamous cell carcinoma of the lung, esophagus, head and the neck [56]. At the same time,

patents have also been published on the preparation method and application of anti-PDL1 CAR T cells which not only inhibited the combination of tumor cells and T cells, but also recognized tumor cells through the PDL1 surface protein [57]. Similarly, T cells carrying PD-L1 and CTLA-4 antibody genes were applied to immunotherapy which could save the cost than the direct antibody injection [58]. Significantly, some investigators provided a new combination immunotherapy in their patents and publications. They constructed a type of CAR T cells which contained a specific antigen-binding domain and a secretion domain. On one hand, CAR T cells can maintain immune competence and attack tumor specifically. On the other hand, secretion can hinder immune escape. Suarez *et al*. [59] used a single bicistronic lentiviral vector to design a kind of CAR T cells which included carbonic anhydrase IX scFv and PD-L1 antibody secretion domain. CAIX scFv precisely targeted renal carcinoma cells, and PD-L1 antibody decreased and reversed T cell exhaustion.Moreover, one CAR with two ScFvs (OCTS) technology has been proved to be effective [60]. For instance, Qi *et al*. [61, 62]exhibited a kind of CAR T cells for prostate cancer treatment which included PSMA or PSCA and PDL1 single chain antibodies. In addition, they also constructed other therapy vectors for myeloid glioblastoma [63], lymphoblastic leukemia [64], myeloid leukemia [65], pancreatic cancer and malignant mesothelioma [66] through OCTS technology.

3.2. Increase Autologous Immunological Competencies and Lifespans of CAR T Cells

The T lymphocyte exhaustion is an undesirable factor for immune responses against tumor when the T lymphocyte exposed in tumor microenvironment. Exhausted T cells experience restricted bioactivity, such as proliferation and cytokine production, and have a high apoptosis rate [67]. Long *et al*. [68] presented that the early T cells' exhaustion was considered as a primary factor which limited anti-tumor competence of the CAR T cells, and that the structure of CARs played a dominating factor in the activation and exhaustion of the CAR T cell. The first-generation CAR T cells contained CD3, a transmembrane domain and an antigen-binding domain. They could characteristically bind to tumor but be consumed easily [69, 70]. The reason why the first-generation CAR T cells failed in oncotherapy is that an insufficient number of CARs result in low durability. Therefore, costimulatory molecules were introduced to CARs in the second and third generation CAR T cells in patents, such as CD28, 4-1BB, X40, CD70, CD83, CD80, CD86 and so on [71]. These receptors are superior for the release of cytokine and for increasing proliferation of CAR-T cells [72].

Methods which appear in patents can enhance the effect of CAR T cells and cytokines release. Lenalidomide, as a kind of immunomodulatory drugs, has been applied to increase immunological efficacy of EGFRvIII CAR T cells for glioma therapy by enhancing the cytotoxicity of CAR T cells [73]. Sterol O-Acyltransferase 1 (SOAT1) was used to change cholesterol into cholesteryl ester by the lipid synthesis metabolic pathway. A series of experiments indicated that inhibition of SOAT1 enhanced the lethality of CAR T cells [74]. The methylcytosine dioxygenases include Tet1, Tet2 and Tet3 [75]. Gregory *et al*. [76] provided that CAR T cells with reduced Tet2 exhibited an improved efficacy in oncotherapy. Some nucleic acid vaccinations have shown functions in activation and amplification of CAR T cells in the patent [77]. Some patents also provided the co-expression of CAR T cells which contain an antigen-binding domain and secretory cytokines [78, 79]. For example, inventors constructed the CD19 CAR plasmid and the IL12 secretion plasmid. They were transfected to the third generation CAR T cells. Secretion of IL12 could assist NK or T cells' activation. These cytokines included IL2, IL12, IL6, IL7, IL15 and so on [80].

In order to reveal the efficiency of CAR T cells, some methods in patents can be used to detect CAR T cell numbers and its activities. Indicator cells split and release luciferase when CAR T cells attack cytotoxic indicator cells which carry TAA and luciferase. Therefore, cytotoxicity of CAR T cells could be calculated through luciferase activity [81]. Moreover, the TaqMan real-time fluorescent quantitative PCR kit and the fluorescent probe could test CAR T cells in peripheral blood [82, 83].

Liu [84] pointed out that the quality of synapse reflected the effectiveness of CAR T cells in the patent.

4 ENHANCE CAR T CELLS' SAFETY

CAR T cell immunotherapy can remit malignancies but it causes some side effects. Recently, CD 19 CAR T cells were regarded as a promising therapy for lymphoma. However, CD 19 also occurs on the normal B lymphocyte surface. The normal B lymphocyte is damaged during this process and results in the B lymphocyte mal-development [85]. The release of cytokines not only enhances the immune competence of CAR T cells but also undesirably causes cytokines release syndrome [86]. Cytokines which can cause fever and hypotension include interferon-γ, IFN, IL2, IL6, IL8 and IL10 [87]. The neurological toxicity is another non-negligible side effect of CAR T cell therapy [88] (Table **2**).

4.1. Decrease Cytokines

Some methods are provided for reducing cytokines release in patents. CD 19-FLAG CAR T cells which added FLAG sequence to CD 19 CAR T cells were able to decrease IFN γ, IL2 and IL6. And CD 19-FLAG CAR T cells could be applied to live-cell imaging and screening [89]. Inventors expounded siRNA of humanized interleukin 6. These kinds of CAR T cells reduced CRS in ALL, pancreatic cancer, and glioma [90]. Moreover, IL-6 receptor antagonistic drugs, like Tocilizumab, were applied to combination oncotherapy [91]. Some universal CAR T cells were applied to allotransplantation [92] [93]. Some ELISA kits which monitor cytokines expediently were invented to detect cytokines secretion, such as IFN γ and IL2 [94, 95].

4.2. Reduce Off-Target

Inventors established connections between CAR T cells and tumor cells with some small molecular ligands whose receptors are overexpressed on tumor cells. [96]. John *et al*. [97] also showed us a way to use a double receptor T cell for immunotherapy to enhance CAR T cells' safety (Fig. **2**). γ-δ

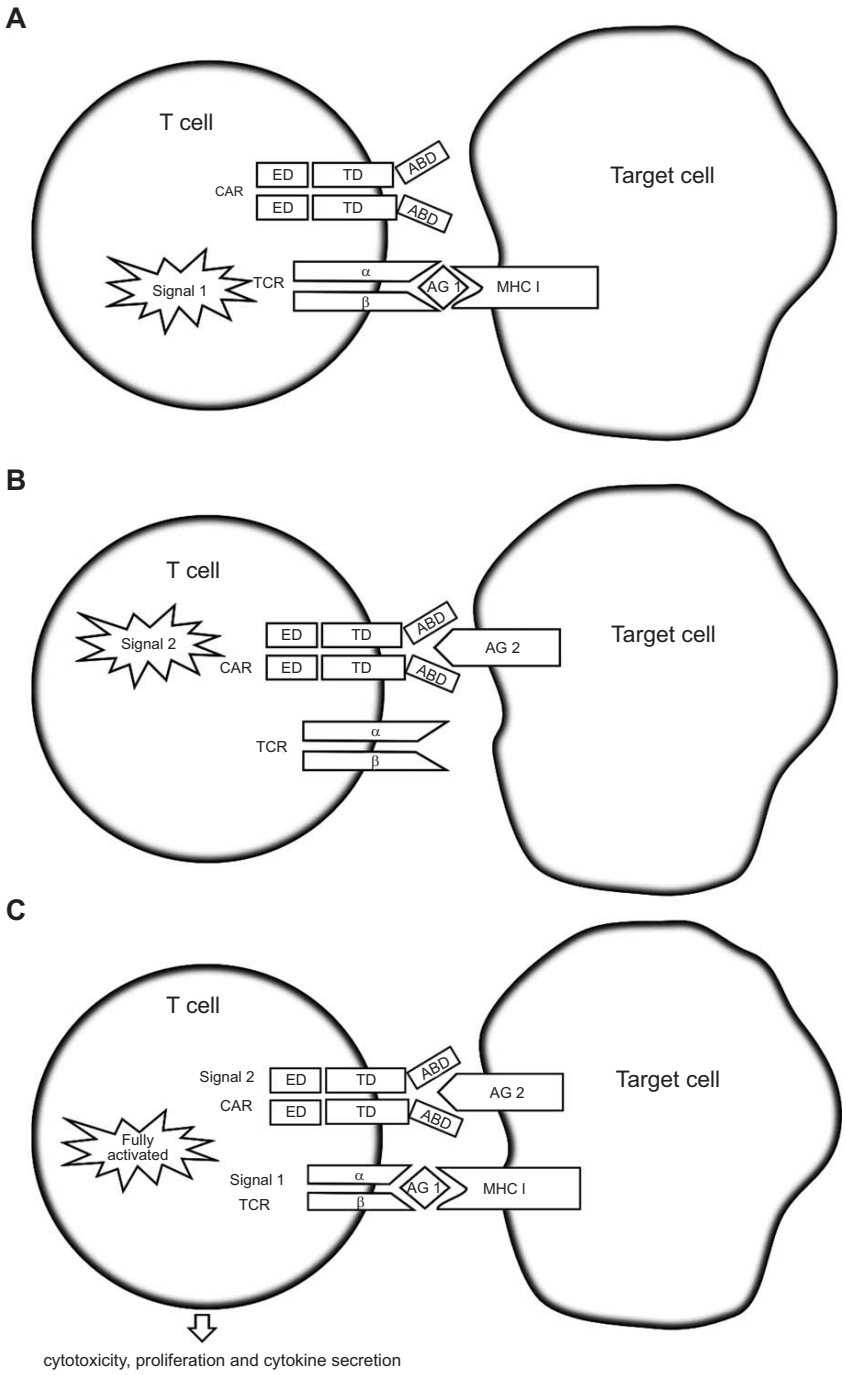

Fig. (2). Double receptor T cell for immunotherapy. (A) The TCR recognizes antigen 1 and presents signal 1 to the T cell. Antigen 1 contains some dangereous signals, such as phosphoantigen. **(B)** The CAR recognizes antigen 2 and presents signal 2 to the T cell. The CAR includes antigen binding domain, the transmembrane domain and costimulatory signal conduction domain (DAP10, CD28, CD27, 41BB, OX40, CD30, IL2-R, IL7-R, IL21-R, NKp30, NKp44 or DNAM-1(CD226)). **(C)** The T cell is fully active by signal 1 and 2, and reveals cytotoxicity, proliferation and cytokine secretion.

T cells were found to contain the TCR for signal one which was activated by red light, like phosphorylated antigens, and CAR provided signal two for γ-δ T cells when CAR was combined with the target antigen. γ-δ T cells could be completely activated by a combination with the signal one and two and play immune responses. Another similar patent provided CAR T cells with two CARs, one CAR had low affinity, while another had high affinity, and other different antigens were also recognized. CAR T cells could be activated only when two antigens are recognized [98]. These double receptor systems can partly strengthen CAR T cells' safety and avoid the off-target.

4.3. Restrict CAR T Cells' Proliferation

Several methods can limit CAR T cells proliferation *in vivo*. The patent stated that the proliferation capacity of ef-

fector cells was limited by adduct, like psoralen. The psoralen was activated by UVA radiation-induced cross-linking between the chains of genomic DNA double helix and led to cellular replicative senescence [99]. Moreover, some drugs could induce T cells exhausted, like cotinine [100]. Some suicide genes which induce T cells apoptosis were introduced into CAR T cell systems, including metabolic suicide genes [101], apoptosis genes and cell surface molecules. Inventors elaborated a molecular brake mechanism for rapidly stopping the killing effect of CAR T cells. An antigen polypeptide, as a molecular brake, was be recognized by a specific antibody and inserted into a single chain of the CAR. The single-chain antibody region of CAR changed the conformation and became inactive. CAR T cells were cleared away by Antibody Dependent Cell-Mediated Cytotoxicity (ADCC) or Complement Dependent Cytotoxicity (CDC) [102].

4.4 Other Measures for Enhancing CAR T Cells' Safety

There are other methods mentioned in patents to enhance drug safety. Traditional gene modification modes are virus vectors, such as lentivirus [103], adenovirus [104] and retrovirus [105]. For the fear of VSVG sequence pollution of lentivirus vector, some inventors published the detection method for CAR T cell products in their patents [106]. Some patents also presented electro-transfection, Piggy-Bac transposable system and CRISPR/Cas9 for CAR T cells preparation, and they confirmed that the AAVS1 locus located at the first intron of human chromosome 19 was safe and reliable for gene editing [107, 108]. These methods can avoid the negative effects of virus vectors and exogenous genetic toxicities. Moreover, Kelly *et al*. [109] applied Mesenchymal Stem Cells (MSC) to relieve the side effect of CAR T cell therapy in their patent.

5. CAR T CELL THERAPY FOR OTHER DISEASES

Besides the application in cancer therapy, CAR T cell therapy can also be used in autoimmune disease [110], HIV and transplant rejection. Several patents and publications explain that CAR T cells for HIV therapy can remit the disease [111-113]. The patent pointed out that CAR T cells were bound to BCMA polypeptide for pathogenic B cells therapy [16]. MacDonald *et al*. [114] described the therapeutic potential of A2 CAR in transplantation and utilized A2 CAR to decrease the immune response.

CONCLUSION

The concept of CAR T cells adoptive immune therapy was first proposed as an anticancer strategy in the last century. Nowadays, CAR T cell immune therapy has acquired remarkable achievements against different types of cancer, such as CLL, NHL, ALL, ovarian, prostate cancer and so on. Moerover,, CAR T cells have been shown to give significant responses to autoimmune disease, HIV and transplant rejection. According to patents and publications, the key to designing CAR is to identify specific antigens. Despite CAR T cell immune therapy has acquired remarkable clinical effect of oncotherapy, many difficult problems remain, such as T cells exhaustion, lack of activity etc. Fortunately, recent patents and publications emphasize on theseissues and challenges. In this review, we mainly summarize patents about CAR T cells. Our target is to analyze antigens to overcome challenges of CAR T cell therapy as discussed in patents.

CURRENT & FUTURE DEVELOPMENTS

The value of CAR T cell immune therapy is considerable. Some CAR T cell products have reached the market as approved by the FDA which were convenient for use in patients of cancer. Furthermore, combining CAR T cell immune therapy with radiotherapy and chemotherapy may obtain spectacular feedback. Moreover, many patents disclosed effective measurements to improve immune function and safety. It might be better to change them into products by cooperating with companies or transferring to companies, such as universal CAR T cells for allotransplantation. Nevertheless, many unsolved barriers are still there for researchers. Complex immune microenvironment affects CAR T cell immune response, but the regulatory mechanism is elusive. Hence, in-depth research works are needed to unmask the mechanism of the immune microenvironment and some measures should be taken to overcome perplexities. Besides, as a therapeutic drug for human diseases, the safety of CAR T cell products is credible.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65(2): 87-108.

[2] Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, *et al*. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016; 66(4): 271-89.

[3] Ansiaux R, Dewever J, Grégoire V, Feron O, Jordan BF, Gallez B. Decrease in tumor cell oxygen consumption after treatment with vandetanib (ZACTIMA; ZD6474) and its effect on response to radiotherapy. Radiat Res 2009; 172(5): 584-91.

[4] Zhou QP, Guo JM, Gallo P. Impact of angiogenesis inhibition by sunitinib on tumor distribution of temozolomide. Clin Cancer Res 2008; 14(5): 1540-9.

[5] Miller KD, Sweeney CJ, Sledge GW Jr. Redefining the target: Chemotherapeutics as antiangiogenics. J Clin Oncol 2001; 19(4): 1195-206.

[6] Malissen, B., P. Bongrand. Early T cell activation: Integrating biochemical, structural, and biophysical cues. Annu Rev Immunol 2015; 33: 539-61.

[7] Ramos, C.A., B. Savoldo, G. Dotti. CD19-CAR trials. Cancer J 2014; 20(2): 112-8.

[8] Sadelain, M. CD19 CAR T Cells. Cell 2017; 171(7): 1471-8.

[9] Zhang WY, Wang Y, Guo YL, Dai HR, Yang QM, Zhang YJ, *et al.* Treatment of CD20-directed Chimeric Antigen Receptor-modified T cells in patients with relapsed or refractory B-cell non-Hodgkin lymphoma: An early Phase IIa trial report. Signal Transduct Target Ther 2016; 1: 16002-9.

[10] Lo AS, Xu C, Murakami A, Marasco WA. Regression of established renal cell carcinoma in nude mice using lentivirus-transduced human T cells expressing a human anti-CAIX chimeric antigen receptor. Mol Ther Oncolytics 2014; 1: 14003-8.

[11] Sadelain, M., R. Brentjens, I. Riviere. The basic principles of chimeric antigen receptor design. Cancer Discov 2013; 3(4): 388-98.

[12] Kakarla S, Gottschalk S. CAR T cells for solid tumors: armed and ready to go? Cancer J 2014; 20(2): 151-5.

[13] Epstein, A.L, Hu, P.S, Pinski, J.K**.** CAR T-cell therapy directed to LHR for the treatment of solid tumors. AU2016243124 (**2017**).

[14] Li HS. Anti-robo1 CAR-T cell, and preparation and application thereof. WO2017181552 (**2017**).

[15] Jiang, J.T., Lu, B.F., Chen, Y.A., Chen, L.J. CEA.CAR-T (carcinoembryonic antigen.chimeric antigen receptor-T) and preparation method and application thereof. CN107056952 (**2017**).

[16] Rehm, A., Höpken, U.E., Bluhm, J., Uckert, W., Kieback, E., Marino, S. Chimeric antigen receptor and CAR-T cells that bind BCMA. WO2017211900 (**2017**).

[17] Balyasnikova, I.V., Lesniak, M.S., Gottschalk, S.M.G. CAR T-cells recognizing cancer-specific IL13Rα2. WO2016123143 (**2016**).

[18] Liu, W.B. Anti-MUC1 CAR-T cell and its preparation method and application. CN107227299 (**2017**).

[19] Zeng, X.Z., Zhang, Y. CAR-T cell as well as preparation method and application thereof. CN106047817 (**2016**).

[20] Qi, W., Yu, L., Ou, H.G. Mesothelin-targeted replication-defective recombinant lentivirus CAR-T transgenic carrier as well as establishment method and application thereof. CN105969805 (**2016**).

[21] Qi, W., Yu, L., Ou, H.G. CD 30-targeting replication-defective recombinant lentivirus CAR-T transgenic vector as well as construction method and applications thereof. CN105950663 (**2016**).

[22] Zhou, X. CAR-T cell preparation for treating prostatic cancer and preparation method thereof. CN105640991 (**2016**).

[23] Liu, W.B. Anti AFP (alpha-fetoprotein) CAR-T (chimeric antigen receptor T) cell, preparation method thereof and application of cell. CN107254447 (**2017**).

[24] Brogdon, J., Gill, S., Glass, D., Kenderian, S., Loew, A., Mannick, J.M,, Michael C., Murphy L,, Porter. D.L., Ruella, M., Wang, Yq., Wu, Q.L., Zhang, J.Q. Treatment of cancer using a CD123 chimeric antigen receptor. US 20160068601 (**2016**).

[25] Ebersbach, H.E., Huber, T., Jascur, J., Richardson, C., Singh, R., Song, H.J, Wu, Q.L, Zhang, J.Q. Treatment of cancer using a CD33 chimeric antigen receptor. US 20160096892 (**2016**).

[26] Morgan, R.A., Rosenberg, S.A. Anti-epidermal growth factor receptor variant III chimeric antigen receptors and use of same for the treatment of cancer. US 20160200819 (**2016**).

[27] Rosenberg, S.A., Chinnasamy, D. Anti-vascular endothelial growth factor receptor-2 chimeric antigen receptors and use of same for the treatment of cancer. US 20130225668 (**2013**).

[28] Leong SR, Liang WC, Wu Y, Crocker L, Cheng E, Sampath D, *et al.* An anti-B7-H4 antibody-drug conjugate for the treatment of breast cancer. Mol Pharm 2015; 12(6): 1717-29.

[29] Epstein, A.L., Hu, P.S. CAR T-cells for the treatment of B7-H4 expressing solid tumors. AU2016243126 (**2017**).

30] Loumagne L, Baudhuin J, Favier B, Montespan F, Carosella ED, Rouas-Freiss N. *In vivo* evidence that secretion of HLA-G by immunogenic tumor cells allows their evasion from immunosurveillance. Int J Cancer 2014; 135(9): 2107-17.

[31] Epstein, A.L., Hu, P.S. HLA-G as a novel target for CAR T-cell immunotherapy. CA2981166 (**2016**).

[32] Pulé, M., Anderson, J., Thomas, S. Chimeric antigen receptor. WO2015132604 (**2018**).

[33] Tamada, K., Sakoda, Y., Ishizaki, H. Chimeric antigen receptor. WO2018181207 (**2018**)..

[34] Bicknell, R., Lee, S., Mount, N., Sharpe, M. Chimeric antigen receptor. AU2017232431(**2018**).

[35] Dai, H.J., Zhang, Z., Li, X.L., Zhou, J., Zhao, X.D., Xiong, B. Specific chimeric antigen receptor targeting NKG2DL and CAR-T cell thereof and use thereof. WO2018165913(**2018**).

[36] Dotti, G., Ferrone, S. CSGP4-specific chimeric antigen receptor for cancer. US2018251568 (**2018**).

[37] Yao, Y.H., Huang, J.Q., Zhu, S.G., Zhu, W., Yao, X., Li, Z.Y., Zhang, L., Zhu, L., Ma, A.Y., Wei, Y.T., Li, Y.F., Wang, Q.X., He, J.P. Construction of chimeric antigen receptor targeting cd20 antigen and activity identification of engineered t cells thereof. WO2018145649 (**2018**).

[38] Yang, L., Zhang, P. Targeted CSF1R chimeric antigen receptor modified NK92MI cell, T cell, and preparation method and application of cells. CN108276495 (**2018**).

[39] Wang, Y.S., Wei, Y.Q., Guo, F.C., Wang, W. Chimeric antigen receptor modified lymphocyte capable of expressing CXCR4, and preparation method and applications thereof. CN108277205 (**2018**).

[40] Mamonkin, M., Brenner, M.K. CD5 chimeric antigen receptor for adoptive T cell therapy. US2018104308 (**2018**).

[41] Muta, H., E.R. Podack. CD30: from basic research to cancer therapy. Immunol Res 2013; 57(1-3): 151-8.

[42] Wang CM, Wu ZQ, Wang Y, Guo YL, Dai HR, Wang XH, *et al*. Autologous T Cells Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin Lymphoma: An Open-Label Phase I Trial. Clin Cancer Res 2017; 23(5): 1156-1166.

[43] Ao JY, Chai ZT, Zhang YY, Zhu XD, Kong LQ, Zhang N, *et al*. Robo1 promotes angiogenesis in hepatocellular carcinoma through the Rho family of guanosine triphosphatases' signaling pathway. Tumour Biol 2015; 36(11): 8413-24.

[44] Liang AB, Liu J, Sun Y, Zhang WJ, Wang JB, Li P, Tang XC, Chen CQ, Zhu YH. Multi-target combined CAR (chimeric antigen receptor)-T cell preparation and preparation method. CN106822184 (**2017**).

[45] Zhu FL, Liu XM. Transformation, multiplication culture and preservation method for T cell for expressing CD19 and CD20 antibody gene CAR (chimeric antigen receptor). CN106701827 (**2017**)**.**

[46] Zhou X. CAR-T cell preparation for treating breast cancer and preparation method thereof. CN105640990 (**2016**).

[47] O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, *et al*. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017; 9(399).

[48] Ghoneim HE, Zamora AE, Thomas PG, Youngblood BA. Cell-Intrinsic Barriers of T Cell-Based Immunotherapy. Trends Mol Med 2016; 22(12): 1000-1011.

[49] Boussiotis, V.A., P. Chatterjee, L. Li. Biochemical signaling of PD-1 on T cells and its functional implications. Cancer J 2014; 20(4): 265-71.

[50] Serganova I, Moroz E, Cohen I, Moroz M, Mane M, Zurita J, *et al*. Enhancement of PSMA-Directed CAR Adoptive Immunotherapy by PD-1/PD-L1 Blockade. Mol Ther Oncolytics 2017; 4: 41-54.

[51] Hyun TL, Ju YL, Heejin L, Sang HL, Yu JM, Hyo JP, *et al*. Molecular mechanism of PD-1/PD-L1 blockade via anti-PD-L1 antibodies atezolizumab and durvalumab. Sci Rep 2017; 7(1): 5532.

[52] Condomines M, Arnason J, Benjamin R, Gunset G, Plotkin J, Sadelain M. Tumor-Targeted Human T Cells Expressing CD28-Based Chimeric Antigen Receptors Circumvent CTLA-4 Inhibition. PLoS One 2015; 10(6): e0130518.

[53] Shang XY, Zhang LL, LI G. Preparation of PD-1 knocked-out CD19 CAR (Chimeric Antigen Receptor)-T cell. CN107119021 (**2017**).

[54] Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, *et al*. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. Scientific Reports 2017; 7(1).

[55] dai HJ, Li XL, Sun B, Zhang Z, Zhao XD, Xiong B. Method for knocking out PD-1 gene by utilizing CRISPR/Cas9 technology to construct MSLN-targeted novel CAR-T cell and application of method. CN106480097 (**2017**).

[56] Li HS. PD-1 CAR-T cell, preparation method therefor, and application thereof. LI HUASHUN. WO2017177575 (**2017**).

[57] Shi WX, Yang CX, Du Y, Fang YS. Anti-PD-L1-CAR-T, and preparation method and application thereof. CN106350533 (**2017**).

[58] Xin BC. Application of CAR-T cell carrying PD-L1 and CTLA-4 antibody genes in tumor immunology. CN105796597 (**2016**).

[59] Suarez ER, Chang de K, Sun J, Sui J, Freeman GJ, Signoretti S, *et al*. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. Oncotarget 2016; 7(23): 34341-55.

[60] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. OCTS-CAR double-targeting chimeric antigen receptor, coding gene, recombinant expression vector and establishment and application of OCTS-CAR double-targeting chimeric antigen receptor, coding gene and recombinant expression vector. CN107337736 (**2017**).

[61] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. OCTS technology-based prostatic cancer CAR-T therapy vector and construction method and application thereof. CN107164410 (**2017**).

[62] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. PSCA (prostate stem cell antigen) and PD-L1 targeted CAR based on OCTS (one CAR with two ScFvs)-CAR (chimeric antigen receptor), encoding gene and expression vector. CN107325185 (**2017**).

[63] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. OCTS (One CAR (Chimeric Antigen Receptor) with two ScFvs (Single-chain variable Fragments)) technique based CAR-T (Chimeric Antigen Receptor-T cell immunotherapy) therapeutic vector for glioblastoma and construction method and application thereof CN107267555 (**2017**).

[64] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. Lymphoblastic leukemia CAR-T (Chimeric Antigen Receptor-T Cell Immunotherapy) therapy carrier based on OCTS (One CAR with Two SeFvs) technology as well as constructing method and application of lymphoblastic leukemia CAR-T therapy carrier CN107245500 (**2017**).

[65] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. Myeloid leukemia CAR-T treatment carrier based on OCTS technology and construction method and application of myeloid leukemia CAR-T treatment carrier. CN107177632 (**2017**).

[66] Qi W, Yu L, Kang LQ, Lin GW, Yu Z. OCTS technology-based pancreatic cancer and malignant mesothelioma CAR-T therapy vector, as well as construction method and application thereof CN107299110 (**2017**).

[67] Wherry, E.J., M. Kurachi. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 2015; 15(8): 486-99.

[68] Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, *et al*. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med 2015; 21(6): 581-90.

[69] Chang, Z.L., Y.Y. Chen. CARs: Synthetic Immunoreceptors for Cancer Therapy and Beyond. Trends Mol Med 2017; 23(5): 430-450.

[70] Barrett DM, Singh N, Porter DL, Grupp SA, June CH. Chimeric antigen receptor therapy for cancer. Annu Rev Med 2014; 65: 333-47.

[71] Zhao YB, Shedlock D, June CH, Liu XJ. Enhanced antigen presenting ability of RNA CAR T cells by co-introduction of costimulatory molecules. US2016340406 (**2016**).

[72] Jensen, M.C., S.R. Riddell. Designing chimeric antigen receptors to effectively and safely target tumors. Curr Opin Immunol 2015; 33: 9-15.

[73] Natsume A, Kuramitsu S, Ohno M. Uses of lenalidomide and CAR T-cells. WO2017176289 (**2017**).

[74] Qi W, Yu Z, Kang LQ, Yu L. CAR-T cells with inhibited sterol o-acyltransferase 1 (SOAT1) as well as preparation method and application of CAR-T (Chimeric Antigen Receptor-T-cell Immunotherapy) cells. CN107058232 (**2017**).

[75] Ichiyama K, Chen T, Wang X, Yan X, Kim BS, Tanaka S, *et al*. The methylcytosine dioxygenase Tet2 promotes DNA demethylation and activation of cytokine gene expression in T cells. Immunity 2015; 42(4): 613-26.

[76] Motz G, Bushman FD, Fraietta JA, June CH, Melenhorst JJ, Nobles CL, Young RM. CAR T cell therapies with enhanced efficacy. WO2017049166 (**2017**).

[77] Sahin U, Reinhard K, Simon P, Mroz KA, Hobohm K. Enhancing the effect of CAR-engineered T cells by means of nucleic acid vaccination. CA2985156 (**2016**).

[78] Tamada K, Sakoda Y, Adachi K. CAR expression vector and car-expressing t cells. PH12017500596 (**2017**).

[79] Berdel W, Rossig C, Schliemann C, Altvater B, Kailayangiri S. Combination therapy comprising an inflammatory immunocytokine and a chimeric antigen receptor (CAR)-T cell. AU2017249694 (**2018**).

[80] Shang XY. Preparation method of CD19 CAR-T cell secreting IL-12. CN106916789 (**2017**).

[81] Sun XL, Li X. CAR-T cytotoxicity indicating vector. CN106086078 (**2016**).

[82] Zhang QX, Wang H, Du X, Chen WH. TaqMan real-time fluorescent quantitative PCR kit for detecting peripheral blood CAR (Chimeric Antigen Receptor)-T cells. CN106399550 (**2017**).

[83] Lu XA, He T, Ma L. Capture probe for detecting CAR-T cell, method for detecting cell content, and application. WO2017133222 (**2017**).

[84] Liu DF. Quality of immunological synapse predicts effectiveness of chimeric antigen receptor (CAR) T cells. US2018231523 (**2018**).

[85] Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, *et al*. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 2012; 119(12): 2709-20.

[86] Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, *et al*. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014; 6(224): 224ra25.

[87] 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 2014; 124(2): 188-95.

[88] Brudno, J.N., J.N. Kochenderfer. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 2016; 127(26): 3321-30.

[89] Wu LJ, Golubovskaya V, Lewis M, Zhou H. FLAG tagged CD19-CAR-T cells. WO2017172952 (**2017**).

[90] Qi W, Kang LQ, Yu L, Xia RH. SiRNA of humanized interleukin 6, recombination expression carrier CAR-T and construction method and application of recombination expression carrier CAR-T. CN106636090 (**2017**).

[91] Zhou X. CAR-T cell preparation for treating breast cancer and preparation method thereof. CN105640990 (**2016**).

[92] Zhang YD, Li XG, Xie HT. Preparation method of universal heterologous CAR-T cells and application. CN106591363 (**2017**).

[93] He T, Lu XA, You YN, Hu XL. Universal type CAR-T cell, and preparation method and application thereof. CN106544321 (**2017**).

[94] Dong J, Yang Y, Liu YZ, Xiong W, Cai M. Do you detect car T cell IFN paper ELISA kit of gamma release? CN205861688 (**2017**).

[95] Dong J, Yang Y, Liu YZ, Xiong W, Cai M. Do you detect car T cell IL 2 ELISA kit of release? CN205786667 (**2016**).

[96] Low PS, Chu HY, Lee YGU. Methods and compositions for CAR T cell therapy. WO2017177149 (**2017**).

[97] Anderson J, Fisher J, Pule M, Gustafsson K. T cell which expresses a gamma-delta T cell receptor (TCR) and a chimeric antigen receptor (CAR). CA2982532 (**2016**).

[98] Jiang S, Ji XL, Yun Zhang, Luo CX. Double chimeric antigen receptor (CAR) modified T lymphocyte and preparation method thereof. CN105087495 (**2015**).

[99] Stassinopoulos A, Greenman WM. Compositions and methods for improved car-t cell therapies. US2017354724 (**2017**).

[100] Choi K, Park H, Lee JE, Oh Y, Kim K, Kim S, Kim H, Chung J. Chimeric antigen receptor to which anti-cotinine antibody is linked, and use thereof. US2018256744 (**2018**).

[101] Tan Y, Zhang HH. CD19-CAR-T cell carrying iCasp9 suicide gene and use thereof. CN107365798 (**2017**).

[102] Qian QJ, Jin HJ, Li LF, Ye ZL, Xu JY, Lyu SQ, Wu HP, Wu MC. Molecular brake for rapidly stopping killing effect of CAR-T (T cell engineered with chimeric antigen receptors) and application of molecular brake. CN105330750 (**2016**).

[103] Terakura S, Yamamoto TN, Gardner RA, Turtle CJ, Jensen MC, Riddell SR. Generation of CD19-chimeric antigen receptor modified CD8+ T cells derived from virus-specific central memory T cells. Blood 2012; 119(1): 72-82.

[104] Zhang WF, Wu FL, Shao HW, Wang T, Huang XT, Li WL, *et al*. Chimeric adenoviral vector Ad5F35L containing the Ad5 natural long-shaft exhibits efficient gene transfer into human T lymphocytes. J Virol Methods 2013; 194(1-2): 52-9.

[105] John LB, Chee TM, Gilham DE, Darcy PK. Genetic modification of mouse effector and helper T lymphocytes expressing a chimeric antigen receptor. Methodgs Mol Biol 2014; 1139: 177-87.

[106] Sun XL, Li X, Xu H. Method for qualitatively detecting pollution of VSVG sequence in CAR-T product. CN106086235 (**2016**).

[107] Xiong B. Method for preparing CAR-T cell by CRISPR/Cas9. CN104894068 (**2015**).

[108] Zhang C, Zhang Y, Liu XM, Zhang YL. Method for site-specific integration of CAR gene to AAVS1 site of T cell based on double-strand micro carrier. CN105524943 (**2016**).

[109] Kelly K, Slukvin I. Method for treating a side effect of chimeric antigen receptor (CAR) T cell therapy. WO2018184074 (**2016).**

[110] Blazar B, Flynn RP, Pennell CA. Chimeric antigen receptor (CAR) T cells as therapeutic interventions for auto- and allo-immunity. US2018264038 (**2018**).

[111] Wagner T, Scharenberg AM, Rawlings DJ, Sather B, Sahni J. Ccr5 disruption of cells expressing anti-hiv chimeric antigen receptor (CAR) derived from broadly neutralizing antibodies. US2017044240 (**2017).**

[112] Zhang TC, Hu G; Gu CJ, Zhang SK, Huang Z, Tang J, Tang L. Recombinant gene construction of chimeric antigen receptor (CAR) for treating HIV (human immunodeficiency virus) infection and application of chimeric antigen receptor. CN107098969 (**2017**).

[113] Zhen A, Peterson CW, Carrillo MA, Reddy SS, Youn CS, Lam BB, *et al*. Long-term persistence and function of hematopoietic stem cell-derived chimeric antigen receptor T cells in a nonhuman primate model of HIV / AIDS. PLoS Pathog 2017; 13(12): e1006753.

[114] MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, *et al*. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. J Clin Invest 2016; 126(4): 1413-24.