CAR T-Cell Therapy in Solid Tumors: Current Review and Future Perspectives

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Abstract. Chimeric antigen receptor (CAR) T-cell therapy is a new type of targeted approach for tumors in clinical practice. At present, this technology is mainly used in hematological malignancies, while its application in solid tumors is limited, where histopathological characteristics might impede CAR-T cell infiltration and trafficking. To further expand the feasibility of CAR-T cell therapy, potential solutions have been put forward, such as enhancing CAR-T cell functioning with chemokine receptors, applying immune checkpoint inhibitors in combination, etc. In this review, we will focus on complexity of solid tumor microenvironment, adaptability of CAR-T cells, mechanisms of immune escape, and the ability of CAR-T cells to infiltrate tumor cells.

Keywords: CAR-T, cell therapy, solid tumor, hematological malignancy, genetic engineering.

1. Introduction

CAR T-cell therapy is an emerging approach for cancer treatment. It involves reinjection of *ex vivo* expanded T cells that have been genetically engineered to kill cancer cells. In other words, CAR-T cells are cells that fuse synthetic antigen receptors with T cell receptors. Therefore, CAR-T cells have high specificity and killing activity, which recognizes specific antigens on the surface of cancer cells and subsequently kills them through various killing mechanisms. With the continuous progress of genetic engineering and cell therapy technology, CAR T-cell therapy has become a promising alternative approach in the field of cancer treatment.

In hematologic malignancies, CAR T-cell therapy has been a great success in clinic. For instance, Kymriah and Yescarta have been approved by the FDA to treat B-cell malignancies, which greatly improves patients' survival. However, the efficacy of CAR-T cells in the treatment of solid tumors is limited in comparison to its promising effects in hematologic malignancies, which is possibly due to the special microenvironment and complexity of solid tumors. Therefore, development of CAR T-cell products for solid tumor treatment is an important direction for CAR T-cell investigation.

2. Definition, Characteristics, and Development of CAR-T Cell Therapy

CAR-T therapy is a new type of tumor immunotherapy that has shown promising effects in the treatment of tumors [1, 2]. Specifically, they are artificially modified T cells that can fight cancer cells [3]. CAR-T cells have a chimeric antigen receptor (CAR) to recognize specific antigens, which consists of antigen-binding structural domains, transmembrane structural domains, intracellular signaling structural domains and hinges [34-38]. When CAR-T cells interact with target antigens, the target antigens independent of the major histocompatibility complex (MHC) for mediation. CAR works by enabling T cells to directly recognize specific antigens on the surface of cancer cells and trigger cytotoxic immune responses by activating endogenous signal transduction pathways [3]. Considering its unique structure and massive production, CAR-T cells have the following characteristics: 1) They are able to identify and kill cancer cells that express specific antigens in a highly specific manner [4]; 2) A lack of co-stimulatory signaling domains may negatively affect the maturation of CAR T cells [3, 5]; 3) An antigen is initially an effective target, but due to genetic mutations, it might be lost or lose expression by cancer cells, rendering the treatment ineffective, as is often in the case of CD19 [6]; 4) They can be activated and amplified in vitro to increase quantity and potency [7]; 5) Once injected into the patient, they can survive and remain active for a long time [8]; 6) Lymphocyte-depleting chemotherapy increases the therapeutic effect by removing immune

cells from the patient's body and providing more space and nutritional conditions for the infused CAR-T cells to grow and multiply [1]; 7) For certain types of cancer, CAR T-cell therapy has shown the potential to cure patients [4, 9, 10]; 8) CAR-T cell therapy can cause serious side effects, including cytokine release syndrome (CRS) and neurotoxicity [11].

The development of the CAR-T cell has come a long way, starting in 1988 when James S. Huston et al. produced a single-chain Fv analogue in *E. coli* and successfully constructed the Single-chain Fragment Variable (scFv) [12] . In 1993, Zelig Eshhar's team constructed the first generation of CAR-T cells, which consisted of variable regions of exogenous monoclonal antibodies attached to the T-cell stimulating molecule CD3ζ. Although these cells were highly specific and lethal, they lacked tolerance and selectivity [13]. In order to enhance the function of CAR-T cells, in 2002 Michel Sadelain's team added a co-stimulatory molecule to the first generation of CAR-T cells, such as CD28, 4-1BB, etc., to form the second generation of Car T cells [14]. These cells show better tolerance and selectivity in killing tumor cells and can generate a durable memory response. The third generation of CAR-T cells were generated by Carl H. June's team around 2009 and contain two co-stimulatory molecules in their structure, such as CD28 and 4-1BB [15]. The third generation better improves cell proliferation and survival, but still suffers from unstable therapeutic effects and toxic side effects. In 2013 Hinrich Abken constructed the fourth generation of CAR-T cells, introducing a new concept named TRUCK, which is “T cells redirected for antigen-unrestricted cytokine-initiated killing”. When the CAR receptor is activated, nuclear factors NFAT is activated and migrates to the nucleus, where it transcribes a variety of genes, including IL-7/15/18/12 and other cytokines [16]. The fourth generation CART cells could produce multiple cytokines, enhance anti-tumor effects, and have a wider range of indications and better safety profile. However, it is still at the laboratory stage of research, and further validation of its efficacy as well as safety is needed.

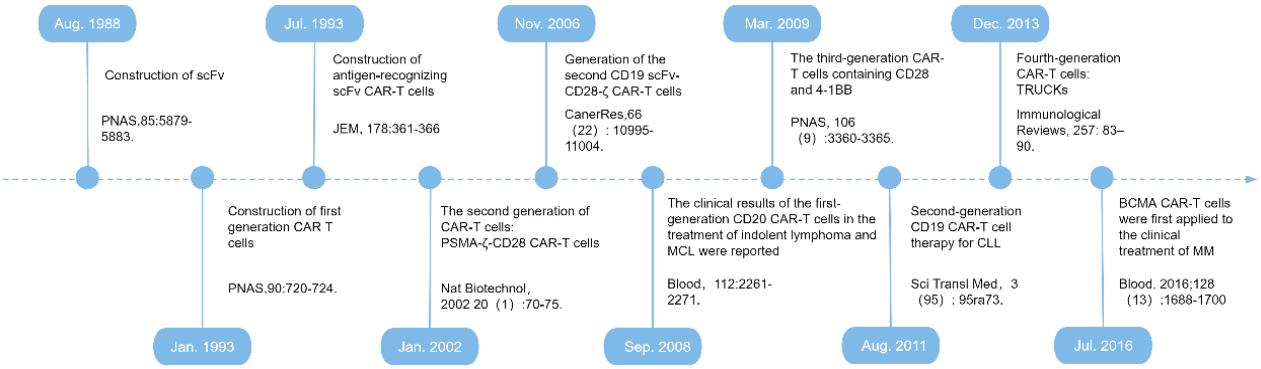


Figure 1. Timeline of CAR T-cell therapy.

3. Better CAR-T Effects in Hematological Malignancies than Solid Tumors

Solid tumor and hematological malignancies are two different types of cancer with distinct biological and clinical features. Solid tumors are usually formed by abnormal proliferation of malignant cells in solid tissues, such as cancers of the lung, liver, stomach, intestine, and breast. Hematologic tumors, by contrast, result from the proliferation of malignant clones of white blood cells, lymphocytes or plasma cells in the hematopoietic system, including leukemia and lymphoma.

The effect of CART cells on hematological malignancies has been demonstrated in several clinical trials, and in 2014, researchers conducted a study of CD19 CART cell therapy on more ALL patients in the *New England Journal of Medicine*. The objective response rate of CD19 CAR-T treatment can reach 90%. For 30 patients who failed conventional treatment, such as chemotherapy or bone marrow transplantation, 27 patients showed obvious tumor regression. And in 19 patients, the tumor remained in a state of retreat for a considerable period of time; At 6 months, 67% of patients were still in progression-free survival, with an overall survival rate of 78% at 6 months[4]. So, CAR T-cell therapy is promising for leukemia patients who have failed conventional treatments, such as traditional

chemotherapy, radiotherapy or targeted drugs. For lymphoma, in three typical clinical trials, ZMA-1, TRANSCEND, and JULIET, an objective response rate of 70 to 80 percent was achieved after CD19 CART cell therapy had failed conventional therapy. complete remission rate still accounted for half of the remission rate [17], This ratio was a considerable improvement over the efficacy of chemotherapy, targeted drugs, and even immunotherapy drugs such as PD-1/PD-L1. A study published in 2017 reported that CD19 CART cell therapy in 19 patients with relapsed or refractory B-cell lymphoma (B-NHL), which showed a complete response rate of 53% and an overall survival rate of 47% with a follow-up period of 29 months [18].

In contrast, CAR T cells were less effective against solid tumors. Eleven patients with EGFR-positive relapsed/refractory NSCLC were treated with EGFR-targeted CAR T cells. Two of the patients achieved partial responses and five were stable for two to eight months. In addition, CAR EGFR gene was detected in tumor infiltrating T cells from four biopsy patients, suggesting that EGFR-targeted CAR T cell therapy is safe and feasible in treating patients with this type of advanced relapsed/refractory NSCLC [19]. GPC3 CAR-T cell therapy was applied in the treatment of liver cancer. Among 13 patients, 2 patients had a partial response to GPC3-CART cell therapy, and their survival after treatment was more than 1-year. However, for most patients, after receiving GPC3 CAR-T treatment, the outcome is still progressive disease with a high probability, and there are 7 patients whose survival time is less than 6 months. After receiving CAR-T cell therapy, the 1-year overall survival rate of these patients with advanced liver cancer was still relatively low, and the objective remission rate and disease control rate were not high [20]. According to the meta-analysis in the field of CAR-T cell therapy for solid tumors in 2019, the effective rate of CART cell therapy for solid tumors is about 10%, which was much less than that of hematological malignancies [21].

In conclusion, CAR-T cell therapy has certain effects on solid tumors, but the effect is not as good as that of blood cancer. There are many factors leading to the poor efficacy of CART cell therapy for solid tumors, which will be illustrated in the following paragraphs.

4. Potential Mechanisms of Less CAR-T Treatment Effects in Solid Tumors

4.1. The complexity of solid tumor microenvironment

The tumor microenvironment contains a variety of immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and Tregs. Ligands for multiple immune checkpoints LAG-3, TIM-3, and TIGIT, as well as PD-L1. And immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)-β. Arginine and glutamate, which are essential for Tcell function, are depleted in TME, thereby reducing the efficacy of T cells [22, 23]. Hypoxia, insufficient nutrients, enrichment of harmful metabolites, increased concentrations of electrolytes such as potassium ions, and the presence of reactive oxygen species (ROS) in the TME are all key obstacles that restrict the proliferation of T cells and inhibit T cell immune responses [24-29].

4.2. The adaptability of CAR-T cells is weak

When T cells are chronically exposed to certain antigens such as PD-1, LAG-3, TIM3, and TIGIT, or are controlled by transcription factors such as (TOX), the expression of epigenetic programs [30-34]. T cell exhaustion may occur. These include decreased T cell function, altered imbalances in cell cycle regulation by surface markers, and apoptosis [35]. Furthermore, even when T cells are outside the TME, the ability of CAR T cells to produce cytokines, expand, and kill is affected when repeatedly exposed to tumor cells.

4.3. The ability of solid tumors to recognize antigens from CAR-T cells

Antigen targets are differentially expressed in solid tumors, and some tumor antigens are also present in normal tissues although the expression level is low, while the ideal CAR T cell targets should be uniformly and specifically expressed in high amounts in tumors, but not in normal tissues.

New tumor target antigens have specificities in different patients, and new tumor antigens are often located in cells as intracellular proteins. It is difficult to reach CAR-T cells [36].

4.4. Immune escape mechanisms and targeted de-tumoral effects

Antigen escape is common in CAR-T cell therapy. When a single antigen is used as a target, a single tumor antigen becomes resistant to the CAR domain, resulting in the loss of CAR-T cells to target the tumor. Targeted detumorization effect means that there are also some normal cells expressed antigens expressed in cancer cells, and CAR-T cells against these antigens may "kill by mistake", leading to side effects.

4.5. The ability of solid tumors to infiltrate and movement CAR-T cells

In hematologic tumors, CAR-T cells migrate well because the target tumor and CAR Tcells share a common hematopoietic origin. In contrast, in solid tumors, CAR T cells are not attracted to the target tumor [37]. CAR-T cells generally do not express some chemokine receptors, but these chemokines secreted by tumor cells such as CXCL1 are important for cell migration, It has been shown that CART cells that express the CXCR2 receptor migrate more than CAR-T cells that do not. Thus, the lack of this chemokine receptor hinders CAR-T cell trafficking [2]. The second point is that tumor stroma refers to the connective tissue and cells surrounding the tumor, including blood vessels, immune cells, fibroblasts, tumor-associated macrophages, myeloid-derived suppressor cells, T cells, etc., which secrete components into the ECM that hinder the penetration of immune cells. Tumor stroma can form very dense networks that protect tumors from immune attack and other therapeutic approaches. The tumor Extracellular Matrix is filled with tumor fibroblasts and myeloids, and these physical limitations limit direct contact between CART cells and tumor cells [38].

5. Improving Strategies for CAR T-cell Therapy in Solid Tumors

5.1. Enhancing specific antigen recognition

To avoid immune escape caused by tumor heterogeneous antigen expression, the design of CAR structure often focuses on targeting cancer stem cells or tumor initiating cells. In addition, multi-antigen targeting is also an effective strategy, because some tumor cells express multiple antigens, and targeting multiple antigen targets can increase the therapeutic effect of CAR-T cells [39-41].

For antigens expressed at high levels, off-target effects can be reduced by reducing scFv affinity, such as by reducing the scFv affinity of HER2-CAR or EGFR-CAR. 58\143\144 The risk of off-target can be reduced by using tandem CAR or loop CAR to assemble two SCFVS, both parent and target antigens. "It is also possible to target two or more antigens in the same cartcell, with CD19 / CD22 and CD19 / CD20 bis-specific cars currently only being tested in clinical trials in leukemia or lymphoma [42-45]." In solid tumors, ligands that bind multiple antigens have been designed in the antigen-binding domain of CARS; a successful example is the T1E peptide that binds to the EGFR receptor family [46]. 40 The antigen-binding domain of the universal car targets multiple antigen-recognition domains. Such as 1) avidin-CARs/biotin-labeled scFvs, (2) CD16-CAR/mAbs, (3) anti-fluorescein isothiocyanate (FITC)-CARs/FITC-labeled scFvs, (4) coiled-coil CARs (SUPRA CARs), (5) anti-PNE-CARs/PNE-scFvs, and (6) NKG2D-CARs/ULBP2-mAbs [47-53]. bystander T cells were activated by design to recognize tumor cells [54]. 166 Restrict CAR activity to the tumorsite to reduce tumor off-target, such as using synNotch to restrict CAR to the tumorsite [55] (172). In addition, two different cars can be expressed on the surface of T cells, and CAR T cells can be activated only when each CAR binds simultaneously to the corresponding target antigen, thereby restricting CAR-T cells to sites where both antigens are present [56, 57]. Successful cases include (1) PSMA - and PSCA + or (2) MUC1 - and HER2-positive tumor cells [56, 57]. Inhibitory CAR-T cells can also be designed to combine tumor-specific antigens with normal tissue antigens, such as CD19 and PSMA, to reduce tumor off-target. The most effective way to prevent off-target is a safety switch to ablate the CAR T-cell if it becomes overactivated or causes damage to the organism. These include

four approaches that rely on prodrug activation, dimeric drugs, mAb targeting, and inhibition that exploit the intrinsic vulnerability of T cells [35, 58-62].

5.2. Addressing CAR T-cell infiltration and mobility difficulties

The homing ability of tumor-specific T cells to the tumor site can be enhanced by transgenic expression of the chemokine receptor [63] and local delivery of oncolytic adenoviruses encoding RANTES and IL-15 [64], resulting in enhanced antitumor activity. In addition, targeting the extracellular matrix (ECM) can improve tumor invasion and antitumor activity in vivo due to the barrier effect of tumor stroma. For example, heparanase expression in CAR T cells targets heparin sulfate proteoglycans (HSPGs), a highly negatively charged polysaccharide molecule present in the extracellular matrix and on the cell surface [38].

5.3. Reversing the immunosuppressive tumor microenvironment

For immune checkpoints in the tumor microenvironment, gene-modified approaches can be used to inhibit the action of immune checkpoints in CAR-T cells, such as expressing PD-1/CD28 switch or truncating PD-1 receptor (major negative receptor [65, 66]. And CRISPR-Cas9 gene-editing technology to delete PD-1 [67-69]. Silencing CTLA-4 or FAS on the surface of tumor-specific T cells or CAR T cells can also improve the effector function of CAR-T cells. Suppressive cytokines present in the tumor microenvironment can inhibit the function of T cells or cause T cells to not express the corresponding receptors. This inhibitory effect can be abolished using DNRs or CSRS, thereby restoring the growth and differentiation ability of T cells. For example, DNR-TGF-beta receptor dnr and IL-4/IL-2, IL-4/IL-7 and IL-4/IL-21 CSRs are used for CAR T-cell therapy. The use of arginine and regulation of glutamine metabolism, the addition of genetic modification of enzymes critical for arginine resynthesis, and the secretion of catalase to protect T cells from ROS to improve T cell metabolism, the small molecule drug metformin has also been explored to reduce hypoxia in the tumor microenvironment [22, 70-72]. Targeting non-malignant cells in tumor stroma that promote tumor growth, metastasis and immunosuppression, CAFs can be targeted with fibroblast-activating protein (FAP) -CAR T cells, TAMs with CD123 or folate receptor β (FRβ) car T cells, CSF1R-CARS with CSF1R-positive TAMs, and NKG2D-Cars Combination therapy with NK cells and GD2-CAR T cells targets MDSCs, as well as therapies targeting tumor vascular endothelial cells, including specific cars for the EDB/EIIIB splice variants of VEGF-R2, PSMA, or fibronectin. [73-76].

5.4. Improving adaptability of T cells outside the tumor microenvironment

The improved potency of CAR T cells was facilitated by incorporating additional signals, such as the truncated cytoplasmic domain of IL-2Rβ and the STAT3-binding YXXQ motif into CD28. To provide the signal required for cell proliferation of CAR-T cells 3[77], to bind the Toll/IL-1 receptor domain to the CAR[78], to integrate the 41BB signaling domain into the CAR[79], and to promote the proliferation of CAR-T cell surface expression of T cells with other ligands of the TNF superfamily such as CD40L [80] promotes T cell activation in these ways. In addition to this, T cells were activated by transgene expression of cytokines or constitutively active cytokine receptors. Transgene expression of common cytokines including IL-2, IL-7, IL-15, IL-21, IL-12, and IL-23 activates JAK/STAT signaling in T cells and enhances the antitumor activity of CAR T cells [81-83]. Alternatively, the nuclear factor of activated T cells (NFAT) promoter can be engineered to control cytokine expression and limit systemic side effects. In clinical studies, the NFAT promoter was found to be insufficient to effectively restrict cytokine expression [84]. Cytokine genes are placed in an internal ribosome entry site (IRES) structure to limit cytokine secretion [85]. Programs currently in clinical trials include the use of IL-12-modified CAR T cells and IL-15-expressing CAR T cells, including the use of an iC9 safety switch to control potential side effects[86]. Molecules that negatively regulate T-cell activation can be screened for roles in vitro and in vivo by means of different techniques, such as short-hairpin RNA approaches and CRISPR-Cas9 gene editing. At present, the negative conditioning factors such as phosphatase pp2r2d, TCEB2, SOCS1, CBLB,

RASA2 and REGNASE1 have been screened to limit the activation of T cells, and the anti-tumor activity can be improved by knocking down negative regulators[87-89] .Transcription factor networks are essential for T cell plasticity. For example, c-Myb promotes T Cell formation [90], TOX and nuclear receptor transcription factors NR4A1, NR4A2 and NR4A3, inhibit T cell differentiation and function [30-32, 91, 92] Overexpression of c-Jun in CAR-T cells increases their functional capacity and reduces terminal T cell differentiation[93]. Thus, modulation of transcription factors may increase T-cell potency.

6. Conclusions and Prospects

CAR T-cell therapy is an emerging treatment for solid tumors, and has made some progress in clinical practice. In the future, the development direction of CAR T cells in the treatment of solid tumors may include the following aspects:

1. Develop a wider range of targeted antigens: At present, the targeted antigens of CAR-T cells for the treatment of solid tumors mainly focus on a few antigens such as CD19. Considering antigen loss that results from genetic mutations, a broader range of targeted antigens should be developed to cover more types of solid tumors.

2. Build a more appropriate CAR structure: The CAR structure of CAR T cells is critical for their therapeutic efficacy. In the future, further optimization of the CAR structure is needed to improve the antigen recognition and killing ability of CAR T cells.

3. Provide more effective treatment options: There is still some uncertainty regarding the treatment options for CAR T cells in solid tumors. Further development of more effective treatment options is needed in the future to improve the treatment effect and reduce the treatment risk.

Development of better monitoring and prediction methods: The therapeutic efficacy and safety of CAR-T cells in solid tumors need to be evaluated by monitoring and prediction methods. In the future, more comprehensive monitoring and prediction methods need to be developed to improve the accuracy and individualization of treatment.

Overall, the future direction of CAR T cell therapy in solid tumors is diversified and individualized. With the continuous progress of technology and the continuous improvement of treatment strategies, CAR-T cells will become a more effective and safe treatment for solid tumors, bringing better survival and quality of life for patients.

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