CAR-T Immunotherapy to Beat Solid Tumors: From Challenges to Improvements

Chentao Li[1, †,*], Ziming Liu[2, †], Yue Zhou[3, †],

[1]Zhejiang University-University of Edinburgh Institute (ZJU-UoE Institute), Zhejiang University School of Medicine, Zhejiang University, Haining, China, chentao.20@intl.zju.edu.cn

[2]School of Medical Instruments, Shanghai University of Medicine & Health Sciences, Shanghai, China, liuziming@shanghai.email.cn

[3]St Bees Dongguan, Dongguan, China, zy425368@gmail.com

[†] These authors contributed equally

*Corresponding author: chentao.20@intl.zju.edu.cn

Keywords: Chimeric Antigen Receptor T cell (CAR-T), Tumor Microenvironment (TME), Solid Tumor, Antigenic Specificity, Antigen Heterogeneity, Immunosuppressive State.

Abstract: Chimeric antigen receptor T (CAR-T) cell immunotherapy shows potential for clinical application in solid tumor treatment, although a section of difficulties must be overcome. Compared with conventional antitumor therapies, the advantages of CAR-T cell treatment include high specificity, great killing power, and long-term effectiveness. But various difficulties in treating solid tumors by CAR-T immunotherapy include intracellular signaling of CARs, immune escape due to antigenic heterogeneity of malignant tumors, physical or cytokine barriers that prevent CAR-T cell entry or limit their persistence, tumor microenvironment of other immunosuppressive molecules, and side effects. This paper describes CAR-T immunotherapy's mechanisms, development, and applications and discusses the status, difficulties, solutions, and future directions of treating solid tumors by CAR-T immunotherapy.

1. Introduction

Chimeric antigen receptor T cell (CAR-T) immunotherapy is an anticipated anti-tumor strategy. In contrast to conventional anti-tumor therapies, CAR-T immunotherapy has high specificity, rapid onset of action, a high remission rate, and long-lasting efficacy [1]. Recurrent refractory B-cell malignancies treated by CD19-CAR-T cells have shown durable remissions [2]. Subsequently, attempts have been made to switch targets to achieve clearance of other tumors and combine other strategies to improve efficacy. The CD4+ CAR-T cells continue to exhibit a cytotoxic profile as well as continued functional activation and proliferation after ten years in patients, although the durability of CAR-T is a challenge [3]. Considering the potential of curing solid tumors through CAR-T therapy, many studies and even clinical trials have tried to apply CAR-T cells in treating solid tumors.

However, indifference to the remarkable efficacy demonstrated in hematological tumors, CAR-T cell therapy does not demonstrate critical efficacy with various difficulties in solid tumor treatment, including intracellular signaling of CARs, immune escape due to antigenic heterogeneity of malignant tumors, physical or cytokine barriers that prevent CAR-T cell entry or limit their persistence, and tumor microenvironment (TME) of other immunosuppressive molecules [1, 4]. It also carries a serious risk of adverse effects that have led to death and the discontinuation of many clinical trials. Thus, in current therapies for solid tumors, CAR-T immunotherapy must overcome extra obstacles. This present paper describes CAR-T immunotherapy's mechanisms, development, and applications and discusses the status, difficulties, solutions, and the potential development direction of solid tumor treatment with CAR-T immunotherapy in the future.

2. Mechanisms and Development

CAR-T cells are T cells genetically altered to express CAR and target cells with a specific tumor-associated antigen (TAA). CARs include extracellular antigen-binding regions (single-chain fragment variable, scFv), hinge regions (facilitating the binding of antigen receptors to tumor antigens), transmembrane spacers (used to anchor the CAR), T-cell activation structural domains - CD3 ζ (delivering the first T-cell activation signal), and intracellular co-stimulatory structural domains - CD28/4-1BB (serving as an additional cue to stimulate the T-cell response). To successfully activate T lymphocytes, the intracellular domain often includes one or more co-stimulatory domains. Increasing or decreasing the length of the hinge area allows the optimal distance between their target cells and CAR-T cells to avoid the action of the phosphatase that attenuates CAR signaling during antigen-antibody binding. When T cells localize to cells expressing non-self or neoantigens, the T cells form lytic synapses with the target cells and kill them by releasing their lytic toxins.

As technology evolves, CAR-T is constantly evolving to address the problems encountered in the clinic. The first generation of CAR solely contains the CD3ζ monoclonal antigen domain. The tyrosine activation motif on the CD3 chain or FcRI was utilized for activating T cells. It has already been illustrated that the first-generation CAR-T cells failed to multiply and proliferate in vivo, limiting its anti-tumor effects and eventually leading to apoptosis [5]. The second generation adds extra costimulatory domains to the intracellular domain, 4-1BB or CD28, which simulates the natural co-stimulatory properties of T cells and CARs to increase T cell persistence and proliferation. In general, 4-1BB transmits a longer activation signal and CD28 transmits a stronger activation signal. The main structure of the third generation is essentially the same as the second generation but carries both CD28 and 4-1BB domains. Due to the different characteristics of CD28 and 4-1BB, the third-

generation CAR-T cells should be more active and formidable to kill the target cells theoretically. However, they did not show significantly increased killing activity [6]. The fourth-generation was mainly designed to achieve precision therapy and safety. Long-term survival of CAR-T cells is beneficial for preventing tumor recurrence; however, CAR-T cells also have the potential to attack normal cells. Therefore, the addition of suicide genes or controllable suicide genes to the structure of CARs can achieve a controllable survival time frame for CAR-T cells in vivo [7]. Besides that, the fifth generation (Universal CAR-T, UCAR-T) is developing. Another way of putting it is that the fourth generation CAR-T cells are devised to release the products of modified genes into tumor tissue when CARs bind to targeted antigens. Local translocation of immunomodulatory molecules such as pro-inflammatory, cytokines and enzymes transform the T cell plasma immunosuppressive tumor microenvironment into an immune tolerant tumor microenvironment. The cytokine IL-12 is often overexpressed in CAR-T cells, a powerful drug that boosts IFNy, granzyme B, and perforin production from T cells and uses NK cells to eliminate tumor cells that CAR doesn't identify. Compared to conventional CARs, IL-12 overexpressing CAR-T cells have shown enhanced antitumor and expansion effects in preclinical models, particularly in solid tumor models. The fifth generation of CARs was designed to simultaneously activate the triple signaling of TCR, coactivation domain CD28, and cytokines as this is a prerequisite for T cells to be activated when binding antigens. The researchers demonstrated that in hematologic and solid tumor models, fifth-generation CARs were genetically modified to improve T-cell appreciation, survival, and anti-tumor effects compared to second-generation CARs.

3. CAR-T for Solid Tumor

3.1 Target Tumor Antigen Specificity

The selection of TAA is the first step toward CAR-T immunotherapy, as CAR-T cells only attack cells with targeted antigens. The ideal TAA is expressed on all tumor cell surfaces, but not (or at a very low level) on normal ones. CD19 is mainly TAA for B-cell malignancies and achieves a prospective efficiency. So far, no TAA for solid tumors has been ideal. Mutant epidermal growth factor receptor variant 3 (EGFRvIII) - CAR T immunotherapy for glioblastoma shows promising results in animal models [8]. Clinical trials (NCT02209376) show that EGFRvIII-CAR-T is viable and safe [9]. In neuroblastoma, GD2-CAR-T cell immunotherapy showed remarkable therapeutic effects [10]. Recent studies have shown that targeting "peptide-centric" chimeric antigen receptors (PC-CAR) can stimulate immune responses. It was tested in mice and showed complete regression of tumors [11]. In hepatocellular carcinoma, CAR-T cell immunotherapy targeting GPC3 had also shown a certain degree of therapeutic effect [12]. HET2-CAR-T cells also achieved therapeutic effects in phase I

clinical trials in HER2-positive advanced biliary tract cancer and pancreatic cancer [13]. All six pancreatic cancer patients in the phase I clinical trials using MSLN-CAR-T cells demonstrated distinct antitumor capabilities [14]. B7-H3 was identified as an abnormally highly expressed antigen in many malignancies, like pancreatic ductal adenocarcinoma. CAR-T cells loaded with B7-H3 antibody successfully inhibited the growth of pancreatic ductal adenocarcinoma and other solid tumors. One set of experiments led to a 100% survival rate of tumor-bearing mice, and no detectable side effects were found [15] (Fig.1).

3.2 Target to Tumor Antigen Heterogeneity

The primary definition of all proposed solid tumor TAAs is antigen heterogeneity-the differential expression of antigens on tumor cells. Furthermore, removal of highly antigenic epitopes after immunoediting may result in tumor escape, as in the case of CD19-negative leukemia escape variants. One way to address this issue is to target multiple tumor antigens simultaneously to provide better killing power. For example, dual-CAR-T (targeting both CD19 and CD20) [16] (Fig.1) and tri-CAR-T (targeting CD19, CD20, and CD22) [17] have been studied in B-cell leukemia. Novel BCMA-OR-CD38 Tan CAR T cells have shown obvious efficacy in clinical settings in multiple myeloma [18]. It is unclear which is better for dual-CAR-T cells, two CAR-T cell lines expressing different CARs, or a more complicated design. Another solution is to make CAR-T cells secrete more killing factors. The extent that which cytokines released after CAR engagement induces indirect target cells killing and recruiting other immune cells is a critical question [4]. CAR T cells release IL-12 upon activation by their CAR to attract innate immune cells to kill tumor cells (Fig.1). However, clinical trials of inducible IL-12 gene in tumor-infiltrating lymphocytes for treating metastatic melanoma resulted in serious toxicity [19].

3.3 Traffic to Tumor and Infiltrate into Tumors

The transport and infiltration of CAR-T somatic cells into malignant tumors is the basis for complete treatment. Most of the difficulties encountered in trafficking are caused by chemokine/receptor mismatch, e.g., CAR-T cells cannot effectively reach tumor sites when tumor CXCR3 and CCR5 ligands are poorly expressed. This problem could be avoided by creating CAR-T cells expressing more paired chemokine protein kinases. It is demonstrated that CCR2b has the ability to enhance CAR-T somatic cells intertumoral migration and tumor eradication when CARs with CCR2b are injected into tumors with the production of amounts of CCL2. Tumor lysing viruses combined with chemokines have also been proved to be an effective way to recruit CAR-T cells trafficking to tumor locations [20].

3.4 Immunosuppressive State of TME in Solid Tumors

The immunosuppressive TME includes immunosuppressive cells, e.g., regulatory T cells (Tregs), and suppressor molecules, e.g., TGF-β, capable of suppressing CAR-T cells [1]. Immune cells or molecules implicated in immunosuppression can be eliminated or blocked to boost the anticancer capability of CAR T cells [1]. Suppressing these immune suppressors is a topic of active research to raise the survival and remaining time of CAT-T cells. By inhibiting the recruitment of MDSCs, Olaparib may facilitate the reintroduction of CAR-T cells, resulting in stronger tumor cell killing [21]. In addition, simultaneous depletion of GR1+ cells has been illustrated that to enhance the efficacy of CEA-CAR T cells [22]. In ovarian cancer, CARs induce TAMs to produce nitric oxide, thereby enhancing tumor lysis [23].

Cytokines derived from malignant tumors are soluble molecular structures, which will endanger the actual effect of cancer immunotherapy in patients with solid tumors. TGF-β is a key soluble regulator in TME. It is a tumor-inhibitory cytokine that plays a critical function in reducing the antitumor response. because it significantly impairs CD8+ T cell cytotoxicity by downregulating granzyme expression and promoting Treg maturation [24]. Thus, by boosting the quantity, infiltration, and durability of adoptively transplanted T cells, hydration of TGF- according to antigen or small molecule water promotes CD8 T somatic cell viability and antitumor response. CAR-T cells are also damaged by several variables, including the redox microenvironment and lactate in the TME. CAR-T cells that co-express catalase can prevent the reduction of anti-tumor activity resulting from oxidative stress, and enhance the antioxidant capacity by metabolizing H2O2, enabling T cells to be more resistant against ROS in TME.Anti-PD-1 therapy could be improved by inhibiting lactate dehydrogenase A in cancer cells. Recent studies have confirmed that anti-TGF-β CAR-T therapy is safe, feasible, and effective for treating metastatic debulking resistant prostate cancer solid tumors [25].

3.5 Immune Checkpoint Ligand Inhibition

Checkpoint inhibitors are one of the most extensively utilized and effective approaches to prevent T cell exhaustion, which are antibodies that block either an IR or its ligand. T cell proliferation, activation, and effector function can be inhibited by five immunological checkpoints: ctLA-4, PD-1, LAG-3, Tim-3, and TIGIT, and maintain immune homeostasis in vivo. Specifically, PD-L1 acts as a ligand for the PD-1 receptor, inhibiting CAR-T cell activation via PD-1 binding. It has been established that the PD-1/PD-L1 pathway acts as a possible marker in a variety of cancers. The reciprocal effects of PD-1 on T cells and PD-L1 on tumor cells compromise the antitumor activity of T cells. According to a previous study, suppressing or deleting the PD-1 gene in CAR-T cells, as well as the fusion of immune checkpoint blockers with CAR-T somatic cells can significantly improve CAR-T immune cell responses and anti-tumor effects [26]. Significantly, such modifications may enhance safety,

Because the scFv released by CAR-T cells is limited to the tumor, and it prevents CAR-T cells from being inhibited by PD-1, potentially avoiding toxicity. [1].

3.6 CAR-T Promotion

Iterations of CAR-T are also dedicated to improving the killing power and stability of CAR-T, etc. Targeted knock-in or overexpression of the PRODH2 gene enhanced the anticancer result of CAR-T cells in a variety of animal models of different solid tumors, including B-cell leukemia and breast cancer [27]. The task of CAR-T somatic cells was boosted by introducing the gene encoding the immunostimulatory protein neutrophil-activating protein (NAP) from H. pylori, a promising tool to arm CAR-T cells. When NAP is released from CAR-T cells, this brings about a pro-inflammatory environment that counteracts the immunosuppressive TME to improve the function of CAR-T cells. In addition, NAP is capable of recruiting other types of immune cells that can attack tumor cells not recognized by CAR-T cells [28]. In contrast, effector T cells have limited persistence and poor expansion capacity and are prone to depletion. Stem memory T cells are perfect candidates due to their long lifespan, high self-renewal capacity, and differentiation ability among a variety of T cell populations [29].

3.7 Inhibit Side Effects

Inflammatory cytokine release syndrome (CRS, also called a cytokine storm, CS), the most common side effect, happens when many cytokines are released into the environment by the immune cells. CAR-T-related cerebrovascular disease syndrome (CRES) is also one of the most ordinary adverse reactions of CAR-T immunotherapy. Their specific manifestation is the massive cytokines released during immune responses, leading to organ damage and brain swelling, and ultimately life-threatening. As for the mechanism of CRS, a potential explanation is that Granzyme B secreted by CAR-T cells activates GSDME in target cells to mediate target cell focal death. Pyroptosis of target cells stimulates macrophages to release inflammatory cytokines like IL-6 [30]. Inhibition of CAR-T-induced cell scorching may be a solution to address the side effects. CRES often occurs in association with CRS, but the pathogenesis of CRES is different from that of CRS and the mechanism of CRES development remains unclear. One mechanism of CRES development may be the activation of independent monocytes triggered by IL-1, which then produces the expression of various cytokines, then activating T cells and macrophages and leading to increased systemic inflammation. Another possible mechanism is the disruption of the blood-brain barrier (BBB). Once the BBB is disrupted, T cells (including CAR-T cells) migrate into the brain parenchyma, resulting in higher levels of cytokines and proteins in the cerebrospinal fluid, leading to inflammation and toxicity in the central nervous system. Endothelial activation can exacerbate systemic inflammation and BBB destruction.

In addition, CAR-T cells have the potential to damage CNS directly. Researchers have detected CAR-T cells in the cerebrospinal fluid, indicating CAR-T cells may penetrate the CNS and they also found that the number of CAR-T cells in patients with neurotoxicity is apparently higher than in patients without neurotoxicity [31]. Previous studies in the CD19-CAR-T study found that blocking IL-1 and IL-6 allowed for safer CAR-T cell immunotherapy, which was as well confirmed in the other anti-aging research via uPAR-CAR-T [32, 33].

However, limiting CAR-T cell function by a switch is full of promise in circumventing side effects. Recent studies have demonstrated a transgenic expression system regulated by resveratrol, which regulates the expression (ON) and suppression (OFF) of CAR in T cells by resveratrol, leading to controlled CAR-T cell immunotherapy and improved safety of tumor immunotherapy [34]. Another study controlled the viability of CAR-T somatic cells in solid tumors by using focused ultrasound, which could lessen the off-target effect and reduce the harm of CAR-T to all normal structures [35] (Fig.2).

4. Challenges and Potential Improvements of CAR-T

CAR-T immunotherapy has been clinically efficient in curing leukemia, but the efficacy in solid tumors has not been satisfactory, and related problems and solutions have been proposed, from the low infiltration and low killing of tumors by the existing CAR-T itself, to the immune escape of tumor cells, and the TME inhibition on CAR-T cells. This paper explores the latest challenges affecting CAR-T cells for solid tumors and some potential improvements and solutions and points out the maturation and development potential of CAR-T at present.

With the "popularity" of CAR-T, immunotherapies such as CAR-NK and CAR-M have also been developed. A similar issue arises with CAR-M, which has shown highly significant therapeutic efficacy in targeting HER2 solid tumors [36], but macrophages are also unable to expand in vitro. In addition, macrophages play a protective role in innate immunity by phagocytosing exogenous substances, but this makes it more difficult to administer CAR to macrophages. At the technical level, CAR is achieved by transfecting cells with lentiviral vectors. If macrophages are transfected with a lentiviral system, they are easily phagocytosed by macrophages, so transfection can easily fail or have very low transfection efficiency. Tumor-infiltrating lymphocyte (TIL) therapy is greatly effective in the treatment of solid tumors, but in most cases, TIL cannot be applied: some patients lack tumor specimens or have few TIL in tumors and metastases; it is difficult to obtain fresh tumor tissue and isolate and expand TIL; the returned TIL cells are functionally impaired and often do not effectively recognize tumor cells in vivo; the strong immunosuppressive micro The strong immunosuppressive microenvironment in the tumor reduces the killing ability of the transfused cells.

Regarding the clinical application and development design of CAR-T immunotherapies, the time and resources consumed by CAR-T are enormous. In vivo modification of CAR-T is likely to be the future direction of development. Injection of micro lipid nanoparticles encapsulated with mRNA to achieve CAR-T treatment in mice suffering from heart failure successfully repaired the function of the mice's heart [37]. In addition to the direct killing of tumor cells by CAR-T, CAR-T cells release exosomes that express CAR on their surface. They express high levels of cytotoxic molecules, which significantly inhibit tumor growth and have a good safety profile. Out-of-body and pre-clinical in-body model descriptions of CAR exosomes do not express PD1 and that recombinant PD-L1 treatment does not diminish the antitumor effects of CAR exosomes [38]. In preclinical in vivo models of cytokine release syndrome, CAR exosomes were relatively safer to administer compared to CAR-T treatment. This provides a new idea for CAR-T iterations, where exosome engineering might improve efficacy or silence cytokine storm-related gene pathways to slow downside effects.

Future assembled CAR-T therapies may include: in vivo injection of nanovectors carrying transfected plasmids for in vivo production of CAR-T engineered cells; ideal or diversified targets for CAR-T immunotherapy to achieve optimal treatment; CAR-T engineered cells regulated by switches or negative feedback lines, and CAR-T cells functionally stable in the tumor microenvironment. We believe that more and more clinical trial data will soon emerge, and CAR-T therapies will increasingly show their potential strength in the field of solid tumors.

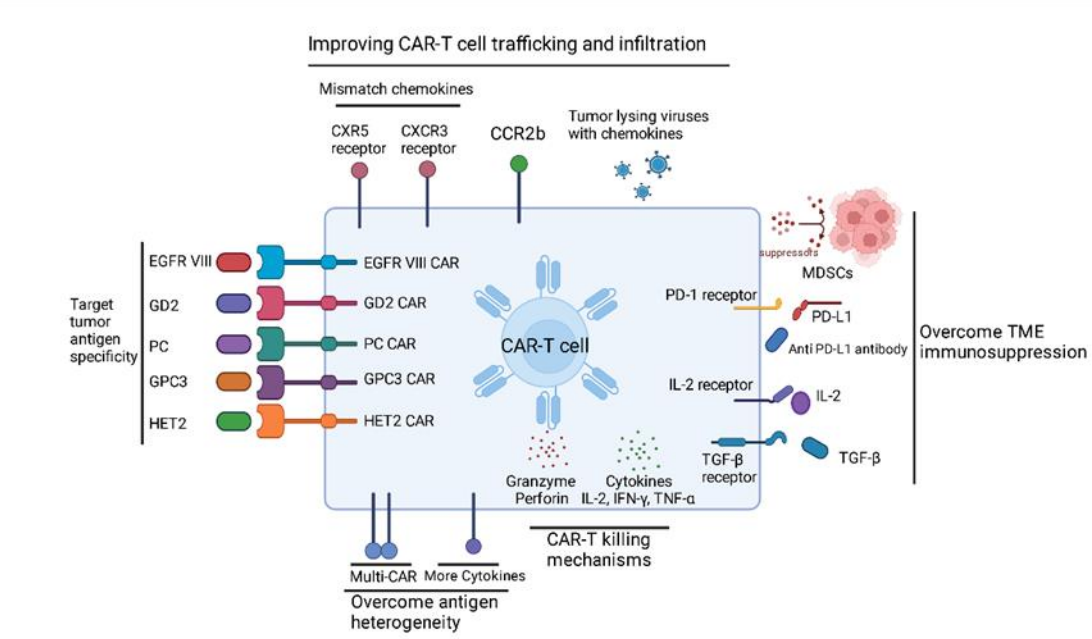

Figure 1. CAR-T cell mechanism and improvement. 1) CAR-T mechanisms: CAR-T cells eliminate target cells by releasing granzyme-perforin or other cytokines. 2) CAR-T targets tumor cell surface-specific antigen: EGFRvIII-CAR T cells for glioblastoma. GD2-CAR-T cells and PC-CAR-T cells

for neuroblastoma. GPC3-CAR-T cells for liver cancer. HET2-CAR-T cells for biliary tract cancer and pancreatic cancer. 3) Overcome antigen heterogeneity: multiple CARs or release more killing factors. 4) Enhancing CAR-T cell trafficking and infiltration: introduce CCR2b into CARs and inject these CARs with the production of CCL2. Use tumor lytic viruses with chemokines to recruit CAR-T cells. 5) Reduce tumor microenvironment suppression: Block tumor-suppressive factors and cells from acting on CAR-T cells. Use checkpoint inhibitors to resist CAR-T fatigue（Created with BioRender.com).

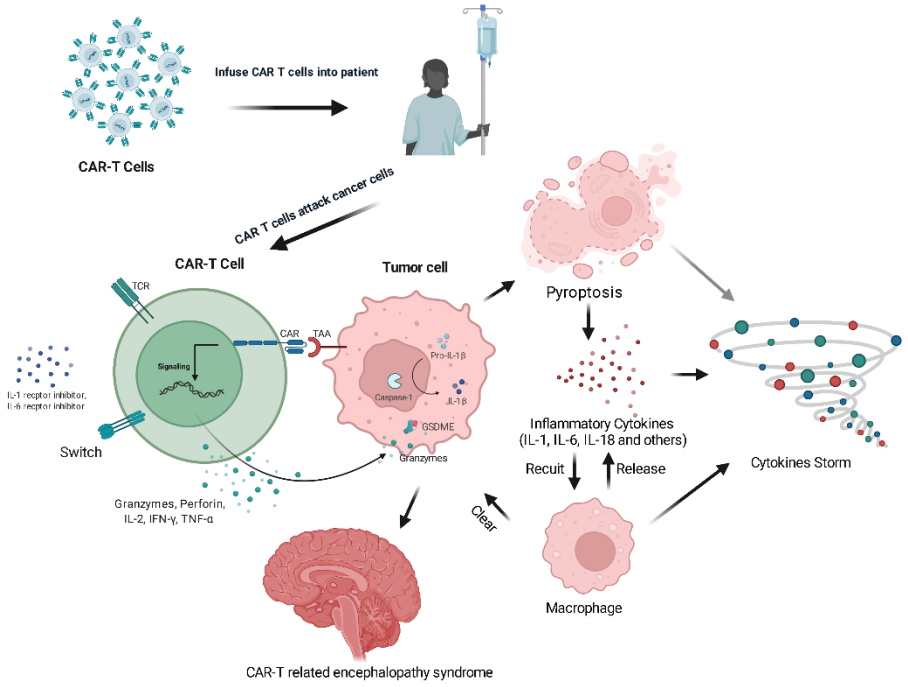

Figure 2. Side effects and suppression strategies of CAR-T therapy. After CAR-T cells injection, CAR-T cells are activated by target cell surface antigens and work properly to kill target cells. GSDME in tumor cells is activated by granzyme B and initiates a cell scorching pathway, allowing the release of inflammatory cytokines to recruit phagocytes. The inflammatory immune response of macrophages is triggered, and inflammatory cytokines are released. The accumulation of inflammatory cytokines leads to a cytokine storm. Also, CAR-T therapy leads to CAR-T-associated encephalopathy syndrome, but the mechanism is not clear. Adding a switch to modulate the function of CAR-T and using IL-1 receptor, IL-6 receptor inhibitor helps to prevent the occurrence of the cytokine storm（Created with BioRender.com).

5. Conclusion

CAR-T immunotherapy is a new anti-tumor strategy. Compared with traditional anti-tumor therapy, CAR-T immunotherapy has high specificity and a high response rate. However, the application of

CAR-T cells to the treatment of solid tumors is still a challenge, such as immune escape, adverse reactions, and other issues. The application of CAR-T immunotherapy in solid tumor treatment is full of promise and difficulties. A great variety of studies have focused on improving CAR-T cell efficacy in TME. As research progresses and clinical trials are conducted, CAR-T therapies may eventually defeat solid tumors.

References

1. Zhang ZZ, Wang T, Wang XF, Zhang YQ, Song SX, Ma CQ: **Improving the ability of CAR-T cells to hit solid tumors: Challenges and strategies**. *Pharmacological research* 2022, **175**:106036.
2. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, Bagg A, Marcucci KT, Shen A, Gonzalez V *et al*: **Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia**. *Science translational medicine* 2015, **7**(303):303ra139.
3. Melenhorst JJ, Chen GM, Wang M, Porter DL, Chen C, Collins MA, Gao P, Bandyopadhyay S, Sun H, Zhao Z *et al*: **Decade-long leukaemia remissions with persistence of CD4(+) CAR T cells**. *Nature* 2022, **602**(7897):503-509.
4. Newick K, O'Brien S, Moon E, Albelda SM: **CAR T Cell Therapy for Solid Tumors**. *Annual review of medicine* 2017, **68**:139-152.
5. Srivastava S, Riddell SR: **Engineering CAR-T cells: Design concepts**. *Trends in immunology* 2015, **36**(8):494-502.
6. Ramello MC, Benzaïd I, Kuenzi BM, Lienlaf-Moreno M, Kandell WM, Santiago DN, Pabón-Saldaña M, Darville L, Fang B, Rix U *et al*: **An immunoproteomic approach to characterize the CAR interactome and signalosome**. *Science signaling* 2019, **12**(568).
7. Chmielewski M, Abken H: **TRUCKs: the fourth generation of CARs**. *Expert opinion on biological therapy* 2015, **15**(8):1145-1154.
8. Johnson LA, Scholler J, Ohkuri T, Kosaka A, Patel PR, McGettigan SE, Nace AK, Dentchev T, Thekkat P, Loew A *et al*: **Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma**. *Science translational medicine* 2015, **7**(275):275ra222.
9. O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, Martinez-Lage M, Brem S, Maloney E, Shen A *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**. *Science translational medicine* 2017, **9**(399).
10. Tumino N, Weber G, Besi F, Del Bufalo F, Bertaina V, Paci P, Quatrini L, Antonucci L, Sinibaldi M, Quintarelli C *et al*: **Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2.CAR T-cells in patients with neuroblastoma**. *Journal of hematology & oncology* 2021, **14**(1):191.
11. Yarmarkovich M, Marshall QF, Warrington JM, Premaratne R, Farrel A, Groff D, Li W, di Marco M, Runbeck E, Truong H *et al*: **Cross-HLA targeting of intracellular oncoproteins with peptide-centric CARs**. *Nature* 2021, **599**(7885):477-484.
12. Batra SA, Rathi P, Guo L, Courtney AN, Fleurence J, Balzeau J, Shaik RS, Nguyen TP, Wu MF, Bulsara S *et al*: **Glypican-3-Specific CAR T Cells Coexpressing IL15 and IL21 Have Superior Expansion and Antitumor Activity against Hepatocellular Carcinoma**. *Cancer immunology research* 2020, **8**(3):309-320.
13. Feng K, Liu Y, Guo Y, Qiu J, Wu Z, Dai H, Yang Q, Wang Y, Han W: **Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers**. *Protein & cell* 2018, **9**(10):838-847.
14. Beatty GL, O'Hara MH, Lacey SF, Torigian DA, Nazimuddin F, Chen F, Kulikovskaya IM, Soulen MC, McGarvey M, Nelson AM *et al*: **Activity of Mesothelin-Specific Chimeric Antigen Receptor T Cells Against Pancreatic Carcinoma Metastases in a Phase 1 Trial**. *Gastroenterology* 2018, **155**(1):29-32.
15. Du H, Hirabayashi K, Ahn S, Kren NP, Montgomery SA, Wang X, Tiruthani K, Mirlekar B, Michaud D, Greene K *et al*: **Antitumor Responses in the Absence of Toxicity in Solid Tumors by Targeting B7-H3 via**

Chimeric Antigen Receptor T Cells. *Cancer cell* 2019, **35**(2):221-237.e228.
16. Tong C, Zhang Y, Liu Y, Ji X, Zhang W, Guo Y, Han X, Ti D, Dai H, Wang C *et al*: **Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma**. *Blood* 2020, **136**(14):1632-1644.
17. Fousek K, Watanabe J, Joseph SK, George A, An X, Byrd TT, Morris JS, Luong A, Martínez-Paniagua MA, Sanber K *et al*: **CAR T-cells that target acute B-lineage leukemia irrespective of CD19 expression**. *Leukemia* 2021, **35**(1):75-89.
18. Feng Y, Liu X, Li X, Zhou Y, Song Z, Zhang J, Shi B, Wang J: **Novel BCMA-OR-CD38 tandem-dual chimeric antigen receptor T cells robustly control multiple myeloma**. *Oncoimmunology* 2021, **10**(1):1959102.
19. Zhang L, Morgan RA, Beane JD, Zheng Z, Dudley ME, Kassim SH, Nahvi AV, Ngo LT, Sherry RM, Phan GQ *et al*: **Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2015, **21**(10):2278-2288.
20. Nishio N, Diaconu I, Liu H, Cerullo V, Caruana I, Hoyos V, Bouchier-Hayes L, Savoldo B, Dotti G: **Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors**. *Cancer research* 2014, **74**(18):5195-5205.
21. Sun R, Luo H, Su J, Di S, Zhou M, Shi B, Sun Y, Du G, Zhang H, Jiang H *et al*: **Olaparib Suppresses MDSC Recruitment via SDF1α/CXCR4 Axis to Improve the Anti-tumor Efficacy of CAR-T Cells on Breast Cancer in Mice**. *Molecular therapy : the journal of the American Society of Gene Therapy* 2021, **29**(1):60-74.
22. Spear P, Barber A, Rynda-Apple A, Sentman CL: **Chimeric antigen receptor T cells shape myeloid cell function within the tumor microenvironment through IFN-γ and GM-CSF**. *Journal of immunology (Baltimore, Md : 1950)* 2012, **188**(12):6389-6398.
23. Burga RA, Thorn M, Point GR, Guha P, Nguyen CT, Licata LA, DeMatteo RP, Ayala A, Joseph Espat N, Junghans RP *et al*: **Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T**. *Cancer immunology, immunotherapy : CII* 2015, **64**(7):817-829.
24. Xia AL, Wang XC, Lu YJ, Lu XJ, Sun B: **Chimeric-antigen receptor T (CAR-T) cell therapy for solid tumors: challenges and opportunities**. *Oncotarget* 2017, **8**(52):90521-90531.
25. Narayan V, Barber-Rotenberg JS, Jung IY, Lacey SF, Rech AJ, Davis MM, Hwang WT, Lal P, Carpenter EL, Maude SL *et al*: **PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial**. *Nature medicine* 2022.
26. Lind H, Gameiro SR, Jochems C, Donahue RN, Strauss J, Gulley JM, Palena C, Schlom J: **Dual targeting of TGF-β and PD-L1 via a bifunctional anti-PD-L1/TGF-βRII agent: status of preclinical and clinical advances**. *Journal for immunotherapy of cancer* 2020, **8**(1).
27. Ye L, Park JJ, Peng L, Yang Q, Chow RD, Dong MB, Lam SZ, Guo J, Tang E, Zhang Y *et al*: **A genome-scale gain-of-function CRISPR screen in CD8 T cells identifies proline metabolism as a means to enhance CAR-T therapy**. *Cell Metabolism* 2022, **34**(4):595-614.e514.
28. Jin C, Ma J, Ramachandran M, Yu D, Essand M: **CAR T cells expressing a bacterial virulence factor trigger potent bystander antitumour responses in solid cancers**. *Nature Biomedical Engineering* 2022.
29. Pietrobon V, Todd LA, Goswami A, Stefanson O, Yang Z, Marincola F: **Improving CAR T-Cell Persistence**. *International journal of molecular sciences* 2021, **22**(19).
30. Liu Y, Fang Y, Chen X, Wang Z, Liang X, Zhang T, Liu M, Zhou N, Lv J, Tang K *et al*: **Gasdermin E-mediated target cell pyroptosis by CAR T cells triggers cytokine release syndrome**. *Science immunology* 2020, **5**(43).
31. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF *et al*: **Chimeric antigen receptor T cells for sustained remissions in leukemia**. *The New England journal of medicine* 2014, **371**(16):1507-1517.
32. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M: **CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade**. *Nature medicine* 2018, **24**(6):731-738.
33. Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, Mansilla-Soto J, Boyer JA, Li X, Giavridis T

et al: **Senolytic CAR T cells reverse senescence-associated pathologies**. *Nature* 2020, **583**(7814):127-132.

34. Yang L, Yin J, Wu J, Qiao L, Zhao EM, Cai F, Ye H: **Engineering genetic devices for in vivo control of therapeutic T cell activity triggered by the dietary molecule resveratrol**. *Proceedings of the National Academy of Sciences of the United States of America* 2021, **118**(34).
35. Wu Y, Liu Y, Huang Z, Wang X, Jin Z, Li J, Limsakul P, Zhu L, Allen M, Pan Y *et al*: **Control of the activity of CAR-T cells within tumours via focused ultrasound**. *Nature Biomedical Engineering* 2021, **5**(11):1336-1347.
36. Klichinsky M, Ruella M, Shestova O, Lu XM, Best A, Zeeman M, Schmierer M, Gabrusiewicz K, Anderson NR, Petty NE *et al*: **Human chimeric antigen receptor macrophages for cancer immunotherapy**. *Nature biotechnology* 2020, **38**(8):947-953.
37. Rurik JG, Tombácz I, Yadegari A, Méndez Fernández PO, Shewale SV, Li L, Kimura T, Soliman OY, Papp TE, Tam YK *et al*: **CAR T cells produced in vivo to treat cardiac injury**. *Science (New York, NY)* 2022, **375**(6576):91-96.
38. Fu W, Lei C, Liu S, Cui Y, Wang C, Qian K, Li T, Shen Y, Fan X, Lin F *et al*: **CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity**. *Nature communications* 2019, **10**(1):4355.