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# *CAR-T Cell Immunotherapy Combined with Immune Checkpoint Blockade Reverses T Cell Exhaustion and Enhances Solid Tumor Immunotherapy*

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***Abstract.*** Chimeric antigen receptor T-cell (CAR-T) therapy has achieved remarkable results in hematological malignancies, but its effectiveness in treating solid tumors is still lacking, with T-cell exhaustion being a key bottleneck. This article analyzes the bottlenecks in cell therapy, examines the advantages and limitations of immune checkpoint inhibitors (ICI), and explores the synergistic mechanism of combination therapy. Specifically, ICI reverses T-cell exhaustion through checkpoint inhibition mechanisms to restore partial function, while reshaping the tumor microenvironment (TME) to transform "cold tumors" into "hot tumors" and activate endogenous anti-tumor T cells. The results indicate that combination therapy has potential for synergistic anti-tumor effects in theoretical mechanisms and has shown certain efficacy in clinical studies of some solid tumors, effectively overcoming key bottlenecks in solid tumor treatment. This review suggests future research and challenges to promote the strategy from clinical exploration to widespread application, providing a solid theoretical basis for in depth exploration and clinical practice of solid tumor immunotherapy.

***Keywords:*** CAR-T, T-cell depletion, Immune checkpoint inhibitors, Solid tumors, Combination therapy

## 1. Introduction

Immunotherapy for malignant tumors represents one of the most remarkable breakthroughs in contemporary biomedical research. Specifically, by using genetic engineering, the CAR-T therapy can modify a patient's own T cells to efficiently recognize and kill tumor cells. This approach has achieved revolutionary success in treating B-cell lymphoma. However, this remarkable efficacy has not been effectively replicated in the field of solid tumors, and there are various factors contributing to this dilemma. Among all the obstacles, T-cell functional fatigue is the most critical mechanism. Under the combined effects of persistent antigen stimulation and immunosuppressive signals, CAR-T cells infiltrating the tumor site gradually lose their potent killing ability and overexpress a series of co-inhibitory molecules, severely impairing the effector function of CAR-T cells and ultimately preventing them from effectively eliminating tumor cells.

Meanwhile, ICI can restore the anti-tumor function of endogenous T cells and induce a durable clinical response by blocking key signaling pathways such as PD1/PD-L1 [1]. However, ICI therapy

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also has its limitations, with limited response rates for monotherapy and its efficacy largely dependent on the pre-existing and exhausted functional T cells in the patient's tumor microenvironment.

Based on the aforementioned scientific background, a treatment strategy has emerged: combining CAR-T cells, which can specifically target tumors, with immune checkpoint inhibitors, which can relieve microenvironment inhibition and reverse T cell exhaustion. Exploring the mechanism, efficacy, and challenges of the combination therapy in reversing T cell exhaustion and treating solid tumors holds significant theoretical and clinical value in breaking through the current bottleneck of solid tumor immunotherapy. This review will systematically elaborate on the mechanism of action and the latest clinical translational progress of the combination therapy, and thoroughly analyzing the present obstacles and prospects of growth, providing new ideas for the advancement of global cancer treatment.

## 2. Bottleneck of CAR-T cell immunotherapy

### 2.1. CAR-T cell therapy

Hematological malignancies primarily encompass various types of leukemia, multiple myeloma and so on. In 2025, there were over 1,500 global CAR-T trials, with the proportion of solid tumor trials rising to 40%, representing an approximate growth rate of 30%. Notably, the number of therapy trials for solid tumors increased by 27.3%, and the number of specific targets increased by 10% [2]. The data indicate that the therapy is widely used in tumor treatment. Most cases of hematological malignancies are alleviated by targeting CD19 during the preparation of the cell. However, a small number of patients still experience relapse. To address the issue of single target downregulation or loss, multi-target CAR-T cell therapies such as CD19/CD22 have been developed, marking a new breakthrough in the treatment of acute B-lymphoma [3]. Nevertheless, in the treatment of solid tumors, T-cell exhaustion is considered one of the core obstacles. During prolonged exposure to high loads of tumor antigens, CAR-T cells gradually lose their proliferative capacity and cytotoxicity, and overexpress a series of co-inhibitory molecules. Studies have shown that the tumor microenvironment (TME) accelerates T-cell exhaustion through upregulation of immune checkpoint molecules and an acidic environment, leading to severe impairment of the function of the cell and ultimately preventing effective tumor cell clearance.

### 2.2. T cell depletion mechanism and CAR-T specificity

A chronic antigen-rich environment causes T cell fatigue, leading to a gradual loss of effector function and the expression of a series of inhibitory receptors, which are difficult to reverse. T cell exhaustion has the following characteristics: firstly, loss of effector function: this loss is gradual, starting with the loss of the ability to secrete IL-2 and proliferate, followed by a decrease in the ability to secrete tumor necrosis factor, and ultimately, even the secretion of the core effector molecules, gamma interferon (IFN-γ) and granzyme B, is severely impaired [4]. Secondly, sustained high expression of inhibitory receptors: this is the most significant molecular marker of exhaustion. Exhausted T cells simultaneously express multiple inhibitory receptors, such as PD-1, TIM-3, LAG-3, etc., collectively inhibiting T cell activity. Thirdly, epigenetic remodeling: the chromatin openness and DNA methylation patterns of exhausted T cells undergo fundamental changes, forming a unique and stable "epigenetic imprint". Even if the cells are temporarily released from inhibition, they tend to revert to an exhausted state rather than fully recovering to functional T cells. Lastly, metabolic

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dysregulation: the metabolic changes in exhausted T cells are mainly due to the inhibition of mitochondrial respiration and aerobic glycolysis [5], resulting in an inability to effectively produce energy. Recent studies have found that exhaustion is not completely irreversible. Epigenetic intervention (such as DNMT inhibitors) and metabolic reprogramming (such as inhibition of CD38 enzyme activity) can restore T cell fatigue to some extent.

CAR-T cells exhibit high affinity for tumor antigens. When they are brought into contact with solid tumors, which contain these antigens, they undergo stronger stimulation than natural T cells, thereby accelerating their exhaustion process. The characteristics of CAR-T cell exhaustion (Figure 1) include reduced cytokine secretion and proliferative capacity, high expression of immune checkpoint molecules, and changes in transcriptional characteristics (such as levels of genes like NR4A, TOX, and NFAT). These changes affect their function and activity, thereby impacting immune efficiency. The chronic signaling of the key transcription factor NFAT induces the expression of exhaustion master regulators such as NR4A and TOX. Mitochondrial dysfunction serves as an "endogenous trigger," which is a key internal factor initiating exhaustion. IDH2 has been identified as a key molecule regulating mitochondrial adaptability and functional persistence. IDH2 blocks the differentiation of memory-type CAR-T cells, thereby enhancing exhaustion and reducing the persistent efficacy of these cells. The above are all proven mechanisms of CAR-T cell exhaustion. According to these mechanisms, scientists have used gene editing to alleviate or interrupt the cell exhaustion. For example, in preclinical studies, engineered T cells that express anti-PD-1 scFv-fused to the cell or secrete IL-12 have demonstrated the ability to reverse exhaustion, thereby exploring the key mechanisms of CAR-T cell exhaustion [6].

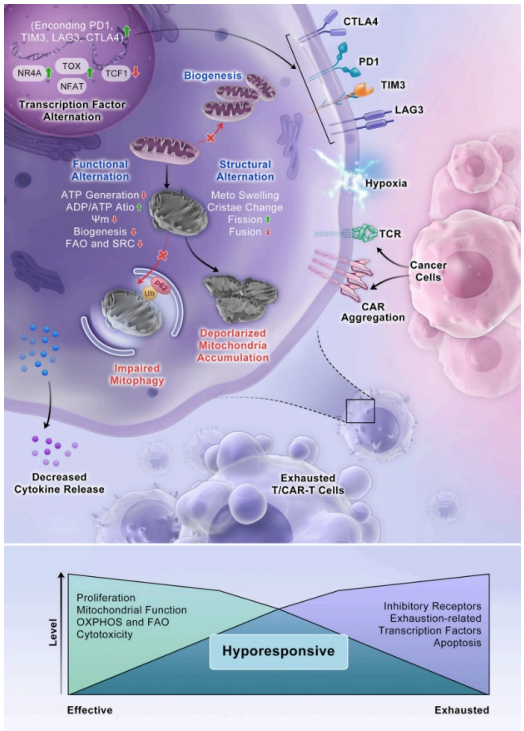



Figure 1. CAR-T cell exhaustion mechanism [7]

## 2.3. Tumor microenvironment

TME encompasses both tumor cells and a large number of nontumor stromal cells and non-cellular components (Figure 2). There are three major stromal cells in the tumor microenvironment [8] that

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promote tumor growth and metastasis. The TME is filled with inhibitory cells and molecules (such as TGF-β, PGE2, IL-4, IL-10), collectively forming an immunosuppressive microenvironment. In addition to these components, the harsh environment characterized by hypoxia, acidity (low pH), and high pressure in the TME is also an important factor promoting tumor growth, metastasis, and diffusion. Therefore, the TME and tissue structure of solid tumors hinder the efficacy of CAR-T cells.

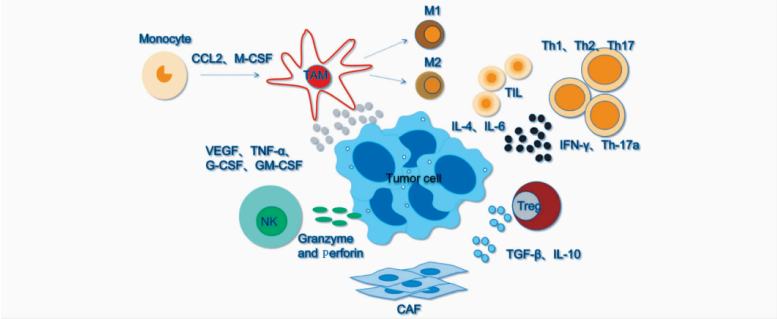



Figure 2. Tumor microenvironment [9]

## 3. Advantages and limitations of immune checkpoint inhibitors

ICI, as a form of immunotherapy, has brought revolutionary advancements to the treatment of solid tumors. Immune checkpoint inhibitors can remove the brakes and activate endogenous anti-tumor immunity. Exhausted T cells simultaneously express multiple inhibitory receptors, which provide a clear target for ICI. By blocking these overly active inhibitory pathways, ICI allows T cells to regain some of their functions. However, immune checkpoint inhibitors have certain limitations. The response rate of ICI in cold tumors is usually less than 20%. The role of ICI is to "remove the brakes on endogenous T cells," which relies on pre-existing tumor-infiltrating lymphocytes whose functions are suppressed [10]. CAR-T cells, through adoptive transfer, swiftly replenish the tumor microenvironment with specific killer T cells, solving the problem of T cell deficiency and transforming "cold tumors" into "hot tumors." Existing data show that after CAR-T transforms "cold" tumors into "hot" ones, the response rate increases to over 40%. Therefore, this offers fresh thoughts on the integration of therapy.

## 4. Synergistic mechanism of combination therapy

### 4.1. Immune checkpoint inhibitors reverse T cell fatigue

In the tumor microenvironment, CAR-T cells also face chronic antigen stimulation, leading to their high expression of immune checkpoint molecules. Signaling pathways like Akt/Ras are suppressed by the PD-1/PD-L1 pathway, which directly inhibits the proliferation of T and B cells. Therefore, the high expression of PD-L1 on CAR-T cells can weaken the body's antitumor immune response [11]. Blocking the PD-1/PD-L1 pathway can reverse CAR-T cell exhaustion, prolong their survival in vivo, restore their ability to proliferate, kill, and secrete cytokines, reduce the expression of exhaustion markers, and enhance the anti-tumor immune response of CAR-T cells. Such immune checkpoint inhibitors have achieved significant efficacy in the treatment of various cancers, including melanoma, non-small cell lung cancer, and renal cell carcinoma, demonstrating their good effectiveness and safety. However, as tumors evolve, other immunosuppressive mechanisms may emerge, leading to the failure of PD-1 inhibitors. Recent studies have found that Tim-3 (T cell

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immunoglobulin mucin molecule-3) is highly expressed in tumor-infiltrating lymphocytes, and its upregulation is associated with T cell exhaustion. In models where resistance to PD-1 inhibitors occurs after treatment, T cells express Tim-3 in large quantities. Therefore, blocking Tim-3 may restore T cell partial function and overcome PD-1 resistance.

In various human tumor models, LAG-3 is often co-expressed with PD-1 on T cells, which is associated with T cell exhaustion. Notably, in specific cancers such as non-small cell lung cancer, LAG-3 is primarily expressed on Tregs rather than effector T cells. Clinical studies (BMS-986016combined with Nivolumab) have shown that the combination of LAG-3 monoclonal antibody (Relatlimab) and immune checkpoint inhibitors can improve the treatment outcome for melanoma patients who have developed resistance to PD-1 inhibitors [12]. In addition, research has also demonstrated that the combination of Tim-3/LAG-3 overcomes compensatory mechanisms and significantly enhances efficacy in NSCLC models, providing more ideas for addressing ICI resistance.

### 4.2. Reshaping the tumor microenvironment, transitioning from "cold" to "hot"

Recruit endogenous immune cells to transform the tumor microenvironment from an immunosuppressive "cold tumor" (lacking T cell infiltration) to an immunoinflammatory "hot tumor", creating an overall environment conducive to attacking the tumor. Immune checkpoint inhibitors have a low response rate in "cold tumors", ICI's effectiveness can be improved by creating an inflammatory environment with CAR-T cells using pre-existing T cells.

In the tumor microenvironment, Treg cells and various cytokines can strongly suppress immune responses, thereby facilitating tumor immune escape [13]. The function of CAR-T cells can be affected and even depleted by the secretion of the cytokine INF-γ, creating a hot tumor environment for ICIs to exert their effects. Macrophages in the TME are commonly referred to as tumor-associated macrophages (TAMs). Activated macrophages mainly include M1 and M2 macrophages, and TAMs can differentiate into either of these subtypes. Specifically, M2 macrophages promote tumor growth, angiogenesis, and tissue repair. The cytokines may contribute to the polarization of M2 macrophages towards M1 macrophages, which play a proinflammatory and anti-tumor role, therefore M1 macrophages can enhance the efficacy of ICIs.

Research has found that in neuroblastoma models, GD2-specific T cells expressing IL-7 and CCR2b exhibit stronger tumor infiltration capabilities and antitumor effects [14]. These two key signaling molecules can recruit more T cells and dendritic cells to the tumor area, not only creating an immune "hotspot" within the tumor, but also significantly enhancing the antitumor effect of CAR-T cells.

CAR-T cells recognize and kill tumor cells in the tumor microenvironment (TME), triggering immunogenic cell death and releasing a large amount of tumor associated antigens and neoantigens. This is equivalent to sounding an "alarm" for the body's natural immune system, recruiting and activating the host's own antigen presenting cells and endogenous T cells. This process transforms an immunosuppressive "cold" tumor into a "hot" tumor with a large infiltration of immune cells. With the assistance of immune checkpoint inhibitors (ICIs), these recruited endogenous T cells can be further activated to attack solid tumors together with CAR-T cells. However, combination therapy also carries toxicity risks, it may also trigger autoimmune diseases, necessitating further optimization of drug administration strategies and dosages.

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## 5. Clinical research

Numerous scientists have conducted trials on GPC3 CAR-T cell therapy and anti-PD-1 antibody to treat GPC3-positive hepatocellular carcinoma sequentially. With a 70.8% disease control rate and a boost in CD3+ cells, the data demonstrated that the combination treatment's safety is manageable. Furthermore, signs of tumor shrinkage were observed in some patients, indicating promising clinical outcomes and providing preliminary evidence for subsequent research. In addition, research has demonstrated that CD8+ T cell viability may be activated in individuals with advanced liver cancer by using CAR-T and nivolumab, thereby enhancing the immune response to tumors in patients [15].

Multiple studies have explored the combination therapy of mesothelin-targeted CAR-T with pembrolizumab or nivolumab for pancreatic cancer. Early evidence suggests that combination therapy can help promote the growth and survival of CAR-T cells and enhance the infiltration of T cells, each has been associated with improved patient survival and demonstrations of efficacy in clinical settings. Currently, hundreds of clinical trials of the combination therapy are underway globally, including integrating PD-1scFv secretion units into the c-Met CAR structure, allowing CAR-T to locally secrete PD-1 blocking antibodies while recognizing c-Met antigens, thereby releasing the inhibition of CAR-T and endogenous immune cells. The results demonstrate that PD-1scFv-c-MetCAR-T, constructed by combining CAR-T therapy with immune checkpoint inhibitor therapy, exhibits good antitumor activity in many experiments [16].

In the research progress of CAR-T therapy targeting EGFRvIII for glioblastoma, despite the limited efficacy of early single-drug CAR-T and the presence of antigen escape, attempts to combine it with PD-1 inhibitors have shown significant success in restoring T cell exhaustion. Song et al. [17] confirmed that ICIs combined with EGFRvIII-CART cells can significantly enhance anti-tumor effects and address the challenges posed by "off-target" effects. Further research has shown that neoadjuvant therapy can significantly improve patient survival, with an overall response rate exceeding 30%, effectively overcoming the challenges posed by antigen escape.

## 6. Conclusion

CAR-T therapy combined with ICIs has become a core strategy to reverse T-cell exhaustion and break through the bottleneck of solid tumor treatment, through the synergistic mechanism of "functional remodeling + signal release". However, there are still urgent challenges to be addressed in this field, as T-cell exhaustion is a complex network of mechanisms mediated by multiple inhibitory receptors, epigenetic regulation, and metabolic disorders. This combined therapy has not yet achieved long lasting efficacy and overcome the compensatory mechanisms of this network. In addition, the heterogeneity of solid tumor antigens makes it difficult to replicate the efficacy of combined therapy, and combined treatment increases the risk of adverse reactions while enhancing immune responses. Therefore, in the future, multi-target CAR-T structures should be developed to address antigen heterogeneity; epigenetic intervention and metabolic regulation should be integrated to fundamentally reverse exhaustion; and novel combination modes should be explored, such as developing CAR-T+ICI+ nanomedicine delivery systems. Future research should focus on these aspects, ultimately dismantling the barriers of solid tumors and enabling combined therapy to make a leap from "effective" to "universal", making more outstanding contributions to human health.

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