# Current status and future prospects of the strategy of combining CAR-T with PD-1 blockade for antitumor therapy (Review)

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**Abstract.** The immune system serves an important role in controlling and eradicating malignant cells. Immunotherapy for treating tumors has received much attention in recent years due to its marked effect. There are two approaches which currently lead this field: Chimeric antigen receptor-modified T-cell immunotherapy (CAR-T) and programmed cell death protein-1 blockade (PD-1 blockade). CAR-T has emerged as a promising regimen for the treatment of a range of types of cancer, including chronic lymphoid leukemia and neuroblastoma, with studies of long term remission in certain patients. PD-1 blockade has been reported to exert marked clinical responses in patients against a range of types of solid cancer, including advanced melanoma, non-small-cell lung cancer and renal cell carcinoma, in addition to hematological malignancies. While increasing the power of the immune system to fight cancer has been a long-standing goal in oncology, a number of studies have demonstrated the synergistic antitumor effects of combination therapies under the umbrella of immunotherapy. The present review focused on a novel combination approach involving CAR-T and PD-1 blockade. The present reviews aimed to discuss the following four aspects of such an approach: i) Current monotherapy status; ii) rationale for the combination of CAR-T and PD-1 blockade; iii) current status of the combination of CAR-T and PD-1 blockade; and iv) conclusions and future perspectives.

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**Contents**



## 1. Introduction

Chimeric antigen receptor (CAR) T cells, an example of adoptive cellular immunotherapy, and is a potentially curative therapy for a multitude of cancer types (1). CARs are engineered fusion proteins that generally consist of an extracellular single-chain variable fragment (scFv) of an antibody for target recognition, the transmembrane domain that is fused with co-stimulation signaling domains, such as cluster of differentiation (CD) 28 or 4-1BB, and a CD3ζ signaling domain to provide T-cell activation signals (2-4). Additionally, antigen recognition by CARs occurs in a major histocompatibility complex (MHC)-independent manner, in order to overcome the tumor's immune escape by downregulation of MHC molecules on the cell surface (5,6).

Targeted immunotherapy using chimeric antigen receptor (CAR) molecules to redirect the specificity of cytotoxic T-cells has emerged as a promising strategy for the treatment of a broad range of malignancies (7,8). However, despite encouraging outcomes, accumulating evidence has demonstrated that the immunosuppressive microenvironment induced by tumors and host regulatory cells may limit the full potential of adoptive T-cell immunotherapy (9). Tumors may evade immune surveillance by stimulating immune inhibitory receptors, including hepatitis A virus cellular receptor 2 (TIM-3), cytotoxic T-lymphocyte protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1), on T-cells (10). An example regulatory pathway includes PD-1/programmed cell death protein ligand 1 (PD-L1), which acts as a negative feedback loop to switch off adaptive immunity following the initial immune response (11). The CAR-T and PD-1 blockade techniques have achieved notable results in the therapy numerous types of cancers (12,13). However, a number of clinical trials have

demonstrated that the efficacy of CAR-T and PD-1 blockade therapy remains limited (14-16). Due to these reported issues, the present review aimed to discuss the status of combination therapies using a combination of CAR-T and PD-1 blockade.

## 2. Current monotherapy status

CAR-T technology, a promising immunotherapy tool, utilizes artificial T-cell surface receptors that stimulate the physiological functions of the native T-cell receptor (TCR) (16). The CAR is composed of an extracellular antigen recognition domain, a spacer, a transmembrane domain and an intracellular T-cell activation domain (5,17). Through the use of genetic modification techniques, effector T-cells may be induced to exhibit improved properties with regard to targeting, killing activity and durability, compared with conventional immunotherapies. CARs combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity and avidity, independent of major histocompatibility complex restriction (18,19). Additionally, compared with other T-cell immunotherapy strategies, CAR-T-cells may overcome the local immunosuppressive tumor microenvironment and break down host immune tolerance to tumor cells. CAR-T therapies have generated encouraging results for treating malignant tumors in clinical trials, including cluster of differentiation (CD) 20 for the treatment of non-Hodgkin's lymphoma (20), GD2 for neuroblastoma (21), and CD19 for chronic lymphocytic leukemia (22). However, the application of such therapies to solid tumors has been less encouraging due to a number of factors, including the difficulty in identifying unique tumor-associated antigens, inefficient homing of CAR-T-cells to tumor locations, low persistence of CAR-T-cells following infusion and their functional impairment in the immunosuppressive microenvironment of solid tumors (23,24). Meanwhile, numerous additional potential risks and challenges must be addressed, including the potential for off-target effects, insertion mutations, immune evasion, tumor lysis syndromes and B-cell aplasia.

Tumors are associated with the immune system, and may evade immune surveillance by stimulating immune inhibitory receptors (25). TIM-3, CTLA-4 and PD-1 are all inhibitory receptors with sustained expression in T-cells which may be involved in tumor immune evasion (26). The PD-1/PD-L1 axis, a potential barrier to adoptive T-cell immunotherapeutic strategies, is rapidly emerging as a clinically important immune inhibitory pathway (27). PD-1 is an inhibitory receptor expressed by activated T-cells, activated B cells, natural killer cells and myeloid cells (28). PD-1 inhibits T-cell activation when engaged by its ligands PD-L1 or PD-L2, which are expressed on tumor cells and stromal cells (29). The interaction of PD-L1 with PD-1 may provide an inhibitory signal to induce apoptosis and to suppress the activation or proliferation of T-cells, meaning that immune-checkpoint inhibitors may block the inhibitory signal of T-cells to prevent T-cell anergy (30-32). Previously, checkpoint inhibitor therapies, including PD-1 blockade (Fig. 1), which promote T-cell responses by preventing T-cell exhaustion and anergy, have been reported to exert marked antitumor responses in patients with renal clear cell carcinoma (ccRCC) (33), non-small-cell lung cancer (34), advanced melanoma (35), urothelial carcinoma (36) and other solid tumors (37,38), in addition to lymphoid malignancies (39). Similar to other cancer therapies, toxicity remains a concern. Toxicity associated with PD-1 blockade is typically immune-associated, and may include pneumonitis, colitis, hepatitis, hypophysitis and thyroiditis (34,40).

## 3. Rationale for the combination of CAR-T and PD-1 blockade

A prominent example of a clinically successful CAR-T therapy is the treatment of hematological malignancies using a second-generation CD19-specific CAR, which has demonstrated antitumor activity in clinical trials (41,42). However, the application of CAR-T-cells in the treatment of solid tumors is associated with a number of challenges; one important obstacle is the immunosuppressive effects of tumors (43). The early success of checkpoint inhibitors in enhancing T-cell immunity presented the possibility that these reagents may be used to enhance the antitumor activity of genetically-modified T-cells (44). The most successful cases reported for CAR-T have involved hematological lymphoid malignancies (45), whereas blockade of the PD-1/PD-L1 pathway has demonstrated signs of efficacy against solid tumors (46,47). It was hypothesized that CAR-T in combination with PD-1 blockade may be a promising immunotherapeutic strategy for tumors, which may enhance the antitumor efficacy and extend the scope of treatment.

An improved understanding of the mechanisms of action of CAR-T may aid the design of novel CAR-T-based combination therapies. CAR is an artificial T-cell surface receptor which stimulates the physiological functions of the native TCR (17). Common elements of all CARs include a single-chain antibody for antigen recognition on the surface of tumor cells, and a membrane domain and intracellular signaling domains borrowed from the CD3ζ chain and costimulatory receptors, including CD28, CD137 and CD27, to supply a costimulatory signal, which appears to be important for expansion and persistence *in vivo* (48,49).

Additionally, the mechanisms of action for PD-1 blockade merit further investigation. PD-1 is an inhibitory receptor expressed by activated T-cells, activated B cells, natural killer cells and myeloid cells (29). Engagement of the PD-1/PD-L1 pathway results in the phosphorylation of tyrosine-based motifs in the cytoplasmic tail of the PD-1 inhibitory receptor, which promotes the recruitment of tyrosine-protein phosphatase non-receptor type 11 (SHP-1), leading to dephosphorylation of phosphatidylinositol 3-kinase (PI3K). The resulting inhibition of PI3K generates downstream activation of RAC-α serine/threonine protein kinase, decreasing T-cell activation, proliferation and survival (50) (Fig. 2).

In order to further examine the combination strategy, it is necessary to understand in detail the mechanism of action of the combination approach, which is not completely clear at present. However, certain insights may be obtained from previous studies. For instance, John *et al* (51) observed a significant decrease in the percentage of $Grl^{+}CD11b^{+}$ myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment of mice treated with the combination therapy. L-MDSC

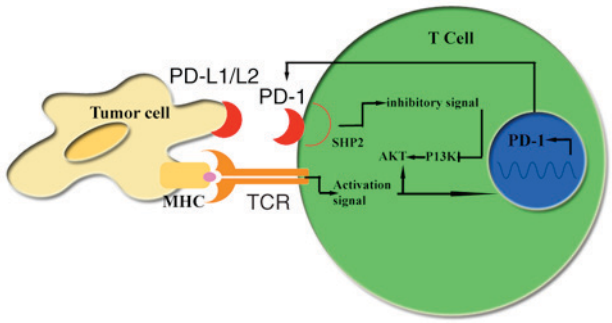


Figure 1. Mechanism of action of PD-1 blockade. PD-1, programmed cell death protein 1; PD-L, PD-2 ligand; MHC, major histocompatibility complex; TCR, T-cell receptor; AKT, RAC-α serine/threonine protein kinase; PI3K, phosphatidylinositol 3-kinase; SHP2, tyrosine-protein phosphatase non-receptor type 11.

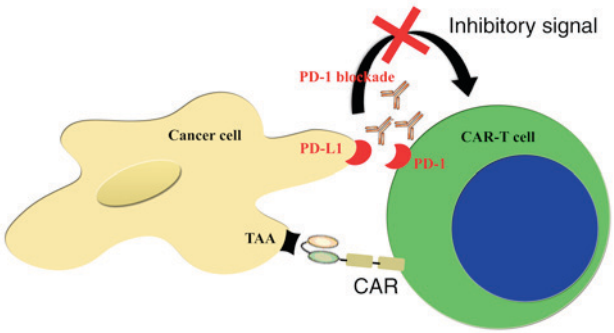


Figure 2. Simplified mechanism of action of the combination of CAR-T and PD-1 blockade. CAR-T, chimeric antigen receptor-modified T-cell immunotherapy; PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; TAA, tumor-associated antigen.

may circumvent the effects of PD-L1 blockade by exploiting alternative suppressive pathways, including indolamine 2,3-dioxygenase (52), arginase or inducible NO synthase (53). In addition, CAR-T proliferation in the presence of L-MDSC was rescued by SHP-1 and SHP-2 inhibition, which prevented PD-1 signaling within CAR-T (54). The results of ongoing and future studies may facilitate the understanding of the mechanism of action for this combination modality in order to improve patient prognosis.

## 4. Current status of the combination of CAR-T and PD-1 blockade

Immunotherapy frequently utilizes combination approaches to increase efficacy. There are two individual approaches which are currently leading this field: CAR-T and PD-1 blockade (46). Given the promising results from CAR-T and PD-1 blockade monotherapies, it is of importance to investigate whether a combined immunotherapeutic approach involving blockade of the PD-1 pathway may enhance the function of genetically-modified T-cells expressing a CAR, leading to enhanced tumor eradication. Studies into the combination strategy have primarily focused on PD-1 blockade using monoclonal antibody (mAb) and genetic approaches.

An area of interest is CAR-T therapy in combination with PD-1 blockade using mAbs. A study from an Australian group provided promising results; their work demonstrated for the first time that PD-1 blockade was able to enhance the efficacy of CAR-T-cell therapy against established solid tumors *in vitro* and *in vivo* (51). The researchers generated primary mouse T-cells expressing an anti-receptor tyrosine-protein kinase erbB-2 (Her-2) CAR containing an extracellular scFv-anti-Her-2 human mAb region, fused to a transmembrane, intracellular costimulatory CD28 domain and intracellular TCR-ζ domain. The study examined whether administration of anti-PD-1 monoclonal antibodies was able increase the therapeutic activity of CAR-T-cells against two different Her-2$^+$PD-L1$^+$ tumors. Preclinical evidence for the synergistic combination of adoptive T-cell therapy with T-cells expressing CARs and anti-PD-1 mAbs was reported (51). A similar result obtained in a study from Moon *et al* (55) indicated that the addition of a blocking PD-L1 antibody to an *ex vivo* CAR tumor infiltrating lymphocyte killing assay was able to restore the defect in tumor cell killing, suggesting that the PD-1 pathway serves a role in maintaining the dysfunction of exhausted CAR-T-cells. An additional similar result reported by Burga *et al* (56) on the combination of CAR-T and anti-PD-L1 antibodies supported the potential clinical merit of neutralizing L-MDSC in order to allow for optimal antitumor efficacy. The researchers demonstrated that CAR-T therapy in combination with PD-1 blockade through mAbs may be highly synergistic.

A second area of interest is CAR-T therapy in combination with PD-1 blockade through genetic approaches. The results of a previous preclinical trial indicated that anti-carbonic anhydrase IX (CAIX) CAR-T-cells secreting anti-PD-L1 antibodies were able to diminish T-cell exhaustion *in vitro* and further decrease tumor growth in an orthotopic mouse model of human renal cell carcinoma (RCC). Suarez *et al* (57) developed a novel CAR therapy for CAIX+RCC that was able to block T-cell exhaustion. The group engineered a bicistronic lentiviral vector to express the anti-CAIX scFv bound to CD28 and CD3ζ signaling domains in one cassette, and anti-PD-L1 immunoglobulin G1 (IgG1) or IgG4 in a second expression cassette subsequent to an internal ribosome entry site site, thus engineering human anti-CAIX-targeted CAR-T-cells that secreted human anti-PD-L1 antibodies at the tumor site. Compared with the anti-CAIX CAR-T-cells alone in a humanized mouse model of ccRCC, tumor growth was decreased 5-fold and tumor weight was decreased by 50-80% (57). The results of a preclinical trial performed by Liu *et al* (43) demonstrated that, while PD-1 blockade augmented the antitumor efficacy of CAR-T-cells, the use of CAR-T-cells expressing PD1CD28 was superior in controlling tumor burden. In order to address this possibility, the researchers used anti-PD1 antibodies in combination with CAR-T-cells, followed by a genetic approach described by others, in which T-cells were transduced with a CAR and a chimeric switch-receptor containing the extracellular domain of PD1 fused to the transmembrane and cytoplasmic domain of the costimulatory molecule CD28. When the PD1 portion of this switch-receptor engages its ligand, PD-L1, it transmits an activating signal via the CD28 cytoplasmic domain instead of the inhibitory signal generally transduced by the PD1 cytoplasmic domain. The aforementioned previous study tested the effect of this PD1CD28 supplement on human CAR-T-cells targeting

aggressive models of human solid tumors expressing relevant tumor antigens. Treatment of mice bearing large, established solid tumors with PD1CD28 CAR-T-cells led to a significant regression in tumor volume due to enhanced CAR-T-cell infiltration, decreased susceptibility to tumor-induced hypofunction and attenuation of insulin receptor expression, compared with treatment with CAR-T-cells alone or PD-1 antibodies (43). The group demonstrated that CAR-T therapy in combination with PD-1 blockade through genetic approaches may be synergistic.

Combination therapy with CAR-T and PD-1 blockade has been further evaluated in clinical trials. Gargett *et al* (58) demonstrated that PD-1-targeted combination therapy approaches may be useful for augmenting CAR-T-cell efficacy and persistence in patients. The phase 1 CARPETS trial (registration no. ACTRN12613000198729) utilized GD2-iCAR consisting of CD3ζ, CD28 and OX40 signaling domains coupled to a 14g2a scFv and an inducible caspase-9 suicide gene, with PD-1 blocked using pembrolizumab. In a protocol amendment for the GRAIN trial of GD2-specific CAR-T-cells in neuroblastoma patients, concurrent treatment with anti-PD-1 mAb was used (58). The researchers applied their understanding of the *in vitro* results to an analysis of peripheral blood samples derived from patients enrolled in the ongoing CARPETS clinical trial. During the investigations, it was observed that PD-1 blockade restored CAR-T-cell cytokine production and promoted GD2-iCAR T-cell survival and the killing of $GD2^+PD\text{-}L1^+$ tumor cells (58). However, the limited number of patients enrolled means that the results that were presented were descriptive and may not be used to form definitive conclusions until more patients are enrolled in the study.

Preclinical studies have illustrated the synergistic efficacy of the combination of CAR-T and PD-1 blockade. By contrast, fewer clinical trials have been performed to evaluate the effects of the combination approach; therefore, whether CAR-T in combination with PD-1 blockade is a rational strategy for clinical trials requires further elucidation. However, it may be hypothesized that if translated to the clinic, PD-1 blockade and CAR-T may be an efficacious treatment, since preclinical evidence supports the synergistic combination of CAR-T and PD-1 blockade.

## 5. Conclusions and future perspectives

The immune system serves an important role in controlling and eradicating malignant cells. Based on the rapid development of immunotherapy, combination therapy using CAR-T and PD-1 blockade has become a novel research area. Preclinical studies have demonstrated that CAR-T and PD-1 blockade are synergistic, leading to long-term survival without causing any signs of pathology *in vivo*. Moon *et al* (55) reported that the combination strategy was able to slow tumor growth, although it did not result in regression or cure. Despite recent progress, the field remains at the preclinical phase. However, previous data have suggested that combination therapy may enhance therapeutic efficacy and broaden the range of anti-tumor treatments (43,51,55-57,59). It may be hypothesized that the combination strategy may be a rational approach for future clinical trials, although further research is required. Prior to wide adoption of the CAR-T and PD-1 blockade combination in clinical practice, a number of challenges must be addressed, including low response rates, toxicity, relatively short response duration, inability to achieve curative effects, and lack of effective and specific tumor-associated antigen targets. Trial-and-error approaches may be used to optimize the strategy in order to provide more rational principles for future clinical practice. At present, further research is required to improve the efficacy and decrease the toxicity of the combination treatment.

Future strategies may improve the efficacy of the combination therapy of CAR-T and PD-1 blockade. Immunotherapy for cancer is primarily dependent on T-cells, particularly $CD8^+$ CTL and $CD4^+$ T-helper cells (10). The ability to identify important T-cell characteristics and systematically optimize CAR-T-cell preparation has the potential to markedly improve the efficacy of adoptive T-cell therapy. A previous study demonstrated that CAR-T-cells are enriched in the central memory (TCM) phenotype and that TCM-derived CAR-T-cells are functionally superior to those generated using bulk $CD8^+$ T-cells (60). Methods to increase the persistence of CAR-T-cells to promote treatment efficacy include using allogeneic virus-specific T-cells and a combination of $CD8^+$ TCM cells and $CD4^+$ T-cells (61,62). Strategies to increase the efficacy of CAR-T-cells through the modification of CAR constructs, including the use of 3rd generation and 4th generation armored constructs, are being evaluated (63). An additional approach is to infuse patients with polyspecific CAR-T-cells that target multiple cell surface proteins to prevent immune evasion.

The toxicity of the combined therapy requires further investigation. Due to previous studies of toxicity in certain CAR-T-cell (64), anti-PD-1 (34) and combination CAR-T and PD-1 blockade trials (51,56,57), future studies are required to further optimize the dose and timing regimens of CAR-T-cells with PD-1 blockade in self-antigen mouse models prior to phase I clinical trials. However, identifying an ideal dose of CAR-T-cells to use in combination with PD-1 blockade is difficult as the *in vivo* expansion of the cells is variable, potentially resulting in inconsistent responses and unpredictable toxicity. Novel methods to increase therapeutic safety are being evaluated and include the introduction of a suicide gene via herpes simplex virus thymidine kinase or inducible caspase-9, in addition to the use of targetable cell-surface proteins, including truncated epidermal growth factor receptor or CD20 (65,66). Sadelain *et al* (5) reported the cotransfection of two different CARs that recognize two different tumor surface antigens, one providing TCR-like signals and the other co-stimulation. The need for simultaneous recognition of two antigens may provide increased specificity and safety.

The combination therapy of CAR-T and PD-1 blockade may be promising for patients with cancer as the research continues and the techniques improve. The combination strategy requires optimization through repeated preclinical and clinical trials in order to minimize toxicity and maximize treatment efficacy for patients with malignancies. The results of ongoing and future studies may facilitate understanding of the differential use of these treatments as a single or a combined modality that improves patient prognosis. It may be hypothesized that immunotherapies will be

increasingly applied in the clinic due to the rapid development of cellular immunology and molecular biology, and that an era of novel immunotherapies for malignancy may be approaching.

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