Future of anti-PD-1/PD-L1 applications: Combinations with other therapeutic regimens

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Abstract

Programmed cell death 1 (PD-1)/programmed cell death 1 ligand (PD-L1) blockade has shown promising effects in cancer immunotherapy. Removing the so-called "brakes" on T cell immune responses by blocking the PD-1/PD-L1 check point should boost anti-tumor immunity and provide durable tumor regression for cancer patients. However, 30%–60% of patients show no response to PD-1/PD-L1 blockade. Thus, it is urgent to explore the underlying resistance mechanisms to improve sensitivity to anti-PD-1/PD-L1 therapy. We propose that the mechanisms promoting resistance mainly include T cell exclusion or exhaustion at the tumor site, immunosuppressive factors in the tumor microenvironment (TME), and a range of tumor-intrinsic factors. This review highlights the power of studying the cellular and molecular mechanisms of resistance to improve the rational design of combination therapeutic strategies that can be translated to the clinic. Here, we briefly discuss the development of PD-1/PD-L1 blockade agents and focus on the current issues and future prospects for potential combinatorial therapeutic strategies that include anti-PD-1/PD-L1 therapy, based upon the available preclinical and clinical data.

Keywords: Cancer immunotherapy; combination therapy; PD-1; PD-L1; resistance; tumor microenvironment

Introduction

The programmed cell death 1 (PD-1) pathway has become an attractive therapeutic target in numerous cancers (1). PD-1 was first characterized as a marker of apoptosis, and is now known as an important immune checkpoint that regulates T cell function. PD-1 is widely expressed on tumor-infiltrating lymphocytes (TILs), particularly on exhausted T cells (2). Its ligand, programmed death 1 ligand (PD-L1), is mainly expressed on cancer and immune cells, such as dendritic cells (DCs), macrophages, myeloid-derived suppressor cells (MDSCs), and B cells (3).

Mechanistic studies have shown that PD-1/PD-L1 signaling can inhibit the activation of T cells and thus suppress their proliferation, migration, and effector function by delivering a co-inhibitory signal (4-6). It has been reported that CD8+ T cells often become exhausted when entering a suppressive tumor microenvironment (TME) (7), and exhausted CD8+ T cells are characterized by overexpression of multiple inhibitory receptors, such as PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin mucin-3 (TIM-3), and lymphocyte activation gene 3 (LAG-3), as well as impaired effector cytokine production and cytolytic activity, leading

to the failure of cancer elimination (8). Antibody-mediated blockade of these inhibitory receptors and their ligands have yielded breakthrough immunotherapies for numerous cancers (9).

Inhibitors targeting PD-1 and PD-L1 have shown impressive anti-tumor effects and clinical benefits in a subset of patients (10). Food and Drug Administration (FDA) has approved various anti-PD-1/PD-L1 monoclonal antibodies (mAbs) for the treatment of metastatic melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), bladder cancer, recurrent or metastatic head and neck cancer, refractory classical Hodgkin lymphoma, microsatellite instability (MSI)-high colorectal cancer (CRC), and metastatic Merkel cell carcinoma. Despite these unprecedented successes, most patients experience intrinsic resistance, and even responding patients can develop acquired resistance to anti-PD-1 therapy (11). Numerous studies are currently underway to systematically elucidate the mechanisms underlying this resistance. Here, we summarize the major resistance mechanisms: 1) T cell exhaustion at the tumor site; 2) exclusion of pre-existing T cells or TILs; 3) the immunosuppressive TME formed by suppressive cells and inhibitory molecules and 4) tumor-intrinsic factors, such as oncogenic signaling pathways, PD-L1 expression levels, tumor immunogenicity, expression of major histocompatibility complex class I (MHC-I) molecules and immunosuppressive metabolites. Therefore, exploring effective combinatorial approaches to recover sensitivity in PD-1 blockade-resistant patients is attracting research directions. Herein, we briefly review the development of PD-1 pathway, discuss the current limitations of anti-PD-1/PD-L1 therapies, and then focus on potential combinatorial strategies, aiming to guide future combination trials that might yield better outcomes for cancer patients.

Discovery of PD-1 and its ligands

In 1992, Ishida *et al.* isolated the cDNA of *PD-1* and identified PD-1 as a marker of classical programmed cell death (apoptosis) (12). Subsequent mechanistic studies identified PD-1 as a negative regulator of T cell immune responses, which also played a role in preventing autoimmune diseases (13). In 2000, B7 homolog 1 (B7-H1, also called PD-L1 or CD274), a member of the B7 family of costimulatory molecules, was confirmed as a specific ligand of PD-1 (14). Similar to PD-1-deficient mice, PD-

L1-deficient mice exhibited immune activation and autoimmune diseases (14). PD-L1 was shown to deliver inhibitory signals by binding to CD80 on activated T cells (15). In addition to PD-L1, programmed death ligand 2 (PD-L2) was also demonstrated to be a second ligand of PD-1 that is expressed by DCs, macrophages, and some cancer cells. Interaction of PD-L2 with PD-1 also mediated potent inhibitory signals to hinder the proliferation and function of T effector cells (16). PD-L2 was also reported to be involved in inducing respiratory tolerance through interaction with repulsive guidance molecule family member b (RGMb), which is highly expressed on lung macrophages (17). Collectively, interaction of PD-L1 with PD-1 suppresses the initial activation and effector function of T cells, and blocking PD-1/PD-L1 pathway would enhance T cell immune responses.

Interaction of PD-1 with PD-L1 in the TME

In 2002, Dong *et al.* first reported the role of PD-1 pathway in promoting T cell apoptosis in the TME (18). Subsequent studies demonstrated that interaction of PD-1 with PD-L1 attenuated T cell migration, proliferation, and cytotoxic capacity (4-6) (*Figure 1*). Interestingly, a recent study revealed a novel role of PD-1 in cancer progression; PD-1-positive tumor-associated macrophages (TAMs) could attenuate tumor immunity by inhibiting phagocytosis (19). The major mechanism by which PD-1/PD-L1 limited the host immune response was through upregulation of their expression in the TME, and elevated PD-1 expression was detected on TILs, particularly tumor antigen-specific T cells, which was presumably induced by chronic antigenic stimulation (2). PD-L1 can be highly expressed by tumor cells and tumor-associated antigen-presenting cells (APCs) (3), and the levels of PD-L1 expressed by tumor cells can be regulated by various mechanisms (*Figure 1*). One of these mechanisms may involve inflammatory cytokines, especially interferon-γ (IFN-γ) (20). Another mechanism is aberrant expression of tumor-intrinsic signaling pathways during carcinogenesis, including activation of epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), and/or PI3K-Akt signaling pathways, and upregulation of signal transducer and activator of transcription 3 (STAT3) or hypoxia induced factor 1 (HIF-1) (21,22). PD-L1-positive cells not only inhibited T cell survival and function but also improved the suppressive activity of DCs (23) and T
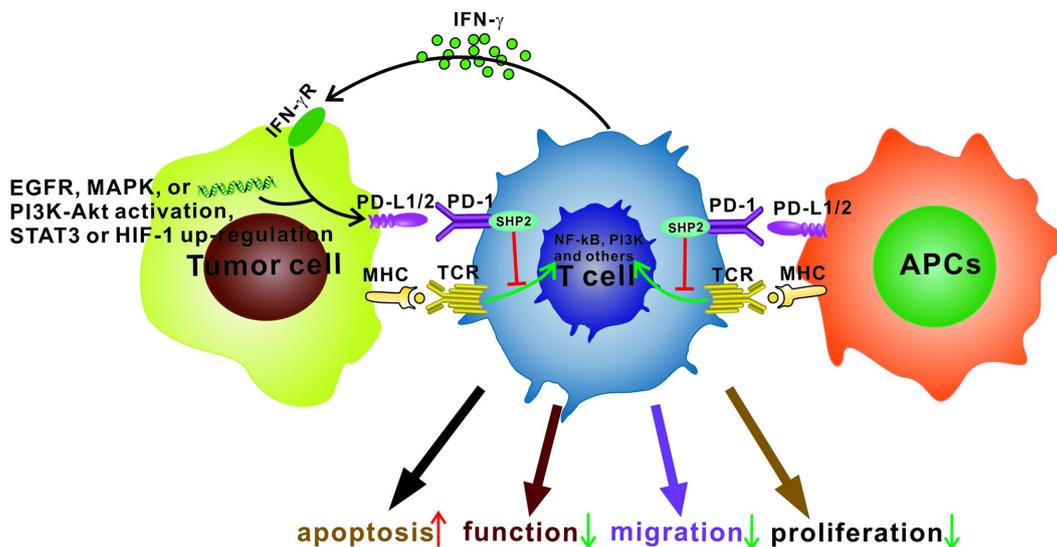
Figure 1 The effects of programmed cell death 1 (PD-1)–programmed death 1/2 ligand (PD-L1/2) interaction on T cells. PD-L1/2 is expressed by tumor cells and antigen-presenting cells (APCs). In tumor cells, PD-L1 expression may be induced by interaction between IFN-γ and IFN-γ receptor (IFN-γ R), activation of epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), and/or PI3K-Akt signaling pathways, and upregulation of signal transducer and activator of transcription 3 (STAT3) or hypoxia induced factor 1 (HIF-1). Then, interaction of PD-1 with PD-L1 can activate Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) and inhibit activation of T cell receptor (TCR)/major histocompatibility complex (MHC)-triggered NF-κB, PI3K, or other signaling pathways, leading to increased apoptosis and impaired T cell function, migration, and proliferation.

regulatory cells (Tregs) (24). Additionally, PD-L1 delivered an anti-apoptotic signal to tumor cells through its interaction with PD-1 and protected tumor cells from attack by cytotoxic T lymphocytes (CTLs) (25). PD-1/PD-L1 blockade has been shown to recover the survival and function of T cells and increase their tumor migration, significantly reinvigorating their anti-tumor immunity (26,27). In summary, PD-1/PD-L1 pathway could be used as a therapeutic target based on its immunosuppressive role in the TME, and thus modulating PD-1/PD-L1 pathway could remodel the immunosuppressive microenvironment.

Anti-PD-1/PD-L1 agents

Anti-PD-1/PD-L1 mAbs augment T-cell anti-tumor response by blocking PD-1/PD-L1 interaction. FDA has approved the anti-PD-1 mAb nivolumab for advanced melanoma, squamous cell carcinoma, metastatic NSCLC, advanced kidney cancer, Hodgkin lymphoma, and urothelial carcinoma. Pembrolizumab, another anti-PD-1 mAb, was approved by FDA for the treatment of unresectable or metastatic melanoma, advanced NSCLC, and MSI-high CRC. In 2016, the anti-PD-L1 mAb MPDL3280A was approved by FDA for the treatment of

advanced or metastatic urothelial bladder cancer (UBC). Clinical trials of another PD-L1 mAb, BMS-936559, have also been conducted for various advanced cancers (28). The immune-related adverse events (irAEs) of these anti-PD-1/PD-L1 mAbs were mostly consistent, including fatigue, asthenia, pyrexia, myalgia, arthralgia, rash, pruritus, nausea, vomiting, colitis, liver and kidney toxicity, pneumonia, dyspnea, anemia, injection-related reactions, and endocrine disorders.

Limitations of anti-PD-1/PD-L1 therapy

Despite these encouraging clinical results, anti-PD-1/PD-L1 agents are not always effective. The majority of patients did not benefit from anti-PD-1/PD-L1 therapy (primary resistance), some responders relapsed after a period of response (acquired resistance) (11), and some patients had to stop treatment because of the development of irAEs. Here, we summarize the known mechanisms promoting resistance to anti-PD-1/PD-L1 therapy (*Figure 2*). First, emerging evidence shows that decreased therapeutic efficacy can be caused by T cell exhaustion. Upregulation of alternative immune checkpoints or a lack of effector and memory epigenetic profile resulted in T cell re-exhaustion
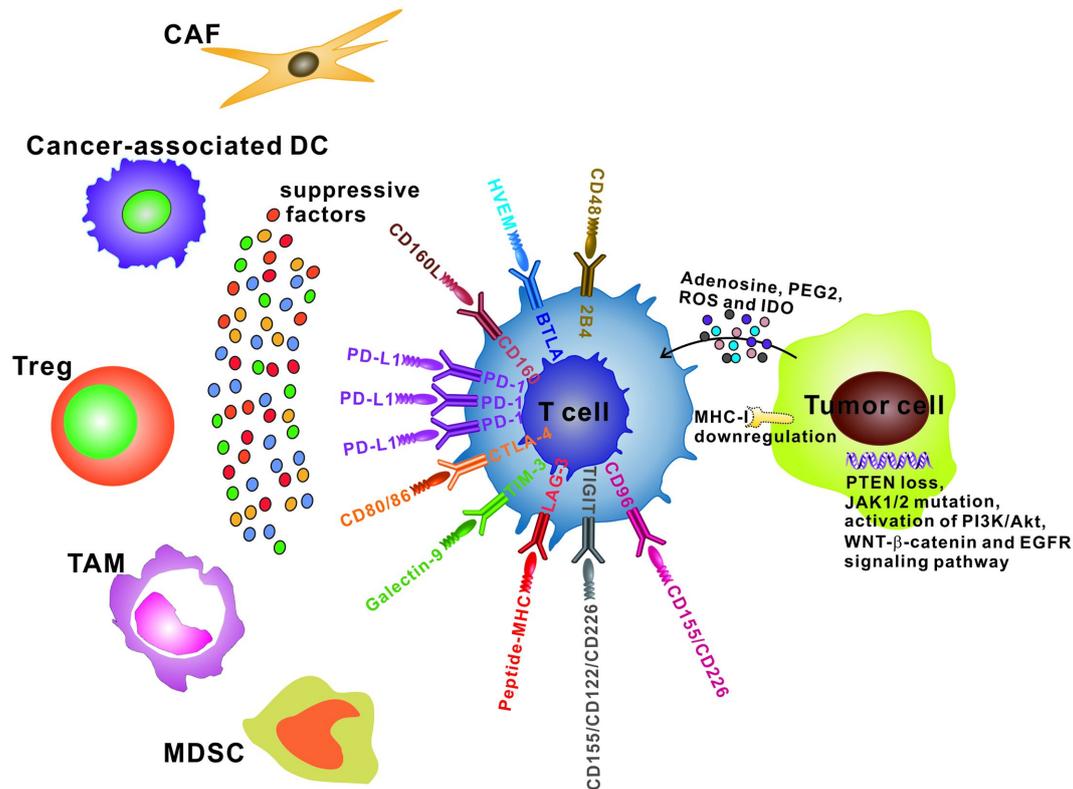
Figure 2 Mechanisms of resistance to programmed cell death 1 (PD-1)/programmed death 1 ligand (PD-L1) blockade. PD-1 expression above the threshold may result in severe exhaustion of T cells, which cannot be rescued by anti-PD-1 monoclonal antibodies (mAbs). Upregulation of alternative immune checkpoints, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin mucin-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), CD96, CD160, B- and T-lymphocyte attenuator (BTLA), and 2B4, accelerated T cell exhaustion by binding to their ligands, thus decreasing the therapeutic efficacy of anti-PD-1/PD-L1 therapy. Suppressive factors, such as interleukin 6 (IL-6), interleukin 10 (IL-10), TGF-β, vascular endothelial growth factor (VEGF), and reactive oxygen species (ROS) produced by cancer-associated fibroblasts (CAFs), cancer-associated dendritic cells (DCs), T regulatory cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) can limit the anti-tumor effect of anti-PD-1/PD-L1 therapy by mediating immune suppression. Tumor cell-intrinsic PTEN loss, JAK1/2 mutation, and activation of PI3K/Akt, WNT-β-catenin, and epidermal growth factor receptor (EGFR) signaling pathways are closely associated with resistance to anti-PD-1/PD-L1 therapy through alteration of the tumor microenvironment (TME). Downregulation of major histocompatibility complex class I (MHC-I) molecules impaired the antigen presentation process and limited the antigen-specific T cell response. Various tumor metabolites, such as adenosine, PEG2, reactive oxygen species (ROS), and indoleamine 2,3-dioxygenase (IDO), could reduce the T cell anti-tumor response and increase resistance to anti-PD-1/PD-L1 agents.

after PD-1 blockade, limiting the durability of reinvigoration (29,30). Ngiow *et al.* analyzed the expression of PD-1 by intratumor CD8+ T cells in anti-PD-1 sensitive and resistant tumors and identified a threshold for PD-1 expression, below which, release of adaptive immune resistance could be achieved with anti-PD-1 mAb, whereas T cells with expressing PD-1 levels above the threshold were in severe exhausted status and failed to be rescued by anti-PD-1 therapy (31). As a second mechanism, exclusion of pre-existing T cells or inadequate T cell trafficking to

the tumor site was identified as a key factor contributing to resistance to PD-1/PD-L1 blockade. It was shown that sensitivity to anti-PD-1/PD-L1 therapy required efficient trafficking of pre-existing CD8+ T cells or effector T cells (Teffs) to the TME (32). Third, many suppressive factors were shown to accumulate in the TME, including Tregs, MDSCs, TAMs, immature DCs, and cytokines, such as interleukin 6 (IL-6) (33), interleukin 10 (IL-10) (34), and vascular endothelial growth factor A (VEGF-A) (35), which affected the clinical efficacy of PD-1/PD-L1 blockade. In

addition, many tumor-intrinsic factors were also extensively associated with the failure of anti-PD-1/PD-L1 therapy, including upregulation of oncogenic signaling pathways (36), expression levels of PD-L1 (37), tumor immunogenicity (3), expression of MHC-I (38), and tumor suppressive metabolites, such as indoleamine 2,3-dioxygenase (IDO) (39) and adenosine (40). Recently, a novel explanation for therapeutic resistance to anti-PD-1/PD-L1 therapy was proposed, in which, PD-1+ TAMs capture anti-PD-1 mAbs on T cell surface through specific Fc/Fcγ receptor interactions, and blocking Fcγ receptor before anti-PD-1 mAb administration prolonged the binding of anti-PD-1 mAb to CD8+ TILs and improved the tumoricidal effect in mice (41). Taken together, combinatorial therapies targeting the PD-1/PD-L1 pathway and the mechanisms of resistance provide a rationale for sensitizing the resistant patients.

Potential combinatorial strategies with PD-1/PD-L1 blockade

Based on the limited therapeutic effect of single anti-PD-1/PD-L1 therapy, it is urgent to explore effective combinatorial approaches to overcome anti-PD-1/PD-L1 therapy resistance and provide insights into clinical applications. Potential therapeutic combinations with PD-1/PD-L1 blockade are shown in *Figure 3*. In this section, the potential therapeutic regimes synergizing with PD-1/PD-L1 blockade are discussed.

Chemotherapy

Some chemotherapeutic agents treat cancers in part by enhancing anti-tumor immunity in a subset of patients by eliminating Tregs and enhancing CD8+ T cell responses (42). However, in some cancer patients, chemotherapeutic agent-induced anti-tumor immunity prompted PD-L1 expression to foster an immunosuppressive TME (43). Activation of PD-1/PD-L1 pathway was reported to confer tumor cell chemoresistance associated with increased metastasis (44). Preclinical and clinical studies have confirmed that chemotherapy combined with anti-PD-1 mAb synergistically facilitated anti-tumor immune response (45) and improved survival in a subset of patients (46). In 2017, the American Society of Clinical Oncology (ASCO) reported that the disease control rate of anti-PD-1 mAb combined with chemotherapy for colon and gastric cancer was up to 100% (47) and 92% (48), respectively.

The details of how chemotherapy, including the agents, dose and timing, should be used in combination with anti-PD-1/PD-L1 mAbs warrant further exploration in patients with different types of cancer.

Radiation therapy (RT)

High-dose ionizing radiation has been reported to eliminate tumor cells partially depending on anti-tumor immunity, particularly T cell immune response. Radiation functions by upregulating MHC-I expression and tumor-associated antigen expression, thus enhancing T cell migration, activating DCs, and diminishing the accumulation of Tregs (49). However, RT rarely resulted in tumor regression at sites distant to the irradiated field, an immune-mediated response termed the "abscopal effect" (50). Preclinical studies showed that RT-induced PD-L1 expression might compromise the anti-tumor effect and lead to local relapse after RT (51). However, the combination of anti-PD-1/PD-L1 mAbs with RT was reported to augment the abscopal effect and promote T cell anti-tumor immunity in several tumor-bearing mouse models (51,52). Encouraging clinical outcomes have been observed in patients with metastatic melanoma treated with RT and anti-PD-1 therapy (53). A new additional target of this combinatorial therapy is the GAS6/AXL pathway, and targeting this pathway resulted in greater anti-tumor immune responses after RT in some cancers (54). Based on these findings, RT could be used as an adjuvant approach for anti-PD-1/PD-L1 therapy. However, to translate such combinatorial therapies into the clinic, further preclinical and clinical studies are required to detect the ionizing radiation-induced immunogenic factors and optimize the agents, dose, schedule, location and opportunity.

Targeting T cell exhaustion and exclusion

Adoptive T cell transfusion (ACT)
Accumulating evidence has shown that exclusion of effector T cells leads to the failure of cancer regression in most patients, and ACT was considered as a personalized therapy that involved administration to the host of highly tumor-reactive T cells (55). Various ACT therapies, such as chimeric antigen receptor (CAR)-T cell and T cell receptor (TCR)-T cell transfusion, have shown great clinical benefit in hematological and solid tumors (56). However, many patients do not response because of the limited factors, such as inadequate T cell trafficking, high expression levels of inhibitory receptors, tumor metabolism and the
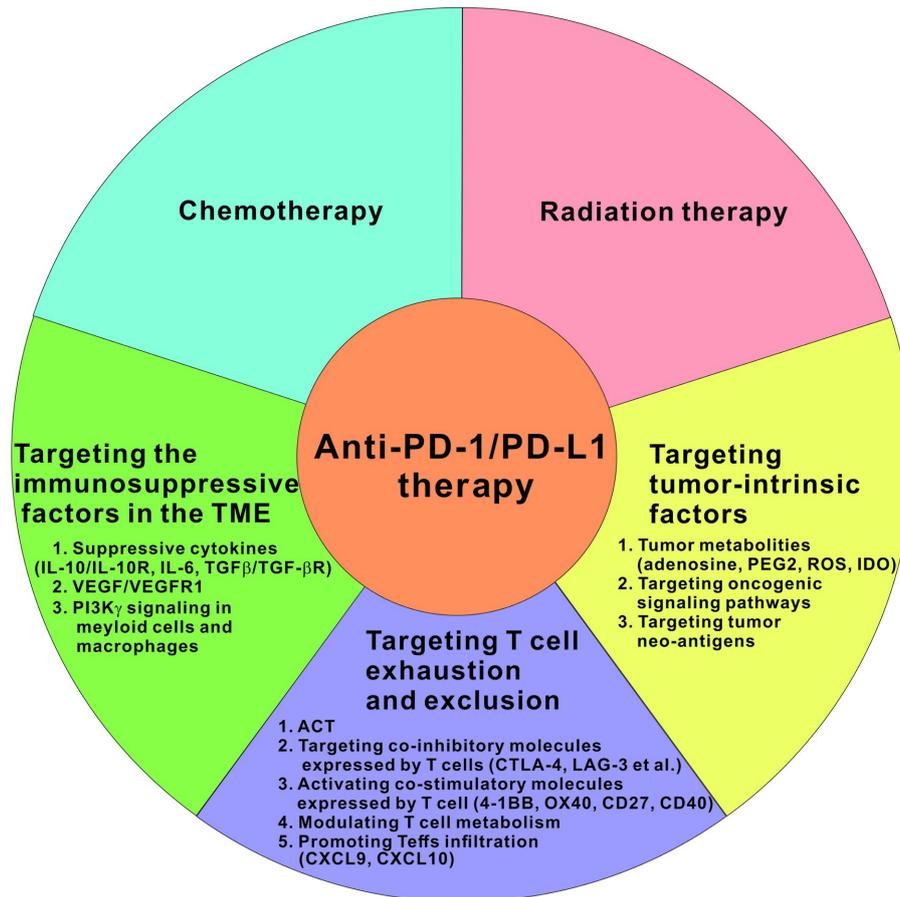
Figure 3 Potential therapeutic combinations with anti-programmed cell death 1 (anti-PD-1)/programmed cell death 1 ligand (PD-L1) therapy. Anti-PD-1/PD-L1 therapy combined with various strategies targeting the main mechanisms of resistance, including T cell exclusion or exhaustion at the tumor site, multiple immunosuppressive factors in the tumor microenvironment (TME), and a range of tumor-intrinsic factors, which become attractive research directions and may yield synergistic or additive clinical outcomes.

immunosuppressive TME (57). PD-1 was found to be upregulated on the transferred TILs, leading to their dysfunction. However, co-administration of adoptive T cells with an anti-PD-1 mAb reinvigorated exhausted TILs and strengthened anti-tumor immunity in lung tumor-bearing mice (57). A recent study showed that PD-1/PD-L1-induced resistance compromises the efficacy of CAR-T cell therapies in solid tumors, and administration of anti-PD-1 mAb, cell-intrinsic PD-1 shRNA blockade, or a PD-1 dominant-negative receptor restored the effector function of CD28 CAR-T cells (58). These findings suggest PD-1/PD-L1 blockade as a potential strategy to enhance the potency of ACT therapies. Several clinical studies of combinations of TCR-T cell or CAR-T cell transfusion with anti-PD-1/PD-L1 mAbs are ongoing (NCT02858310, NCT02775292, NCT03030001, NCT02873390, NCT02862028, and NCT02926833).

Targeting co-inhibitory molecules on T cells

Apart from PD-1, other checkpoint molecules, such as CTLA-4 (59), TIM-3 (60), LAG-3 (61), and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) (62), were also upregulated by exhausted T cells in some cancer patients. Co-expression of these inhibitory receptors probably aggravated the severity of T cell exhaustion, and combined blockade of these inhibitory molecules is an inspiring strategy for cancer therapy. It has been reported that CTLA-4 blockade not only enhanced the effector function of T cells but also eliminated Tregs and MDSCs in the TME (63,64). Clinical trials of combinatorial treatments with anti-CTLA-4 and anti-PD-1 agents have demonstrated promising clinical results in patients with advanced metastatic tumors, particularly melanoma (65). FDA approved this combinatorial therapy for the treatment of BRAF V600 wild-type unresectable or

metastatic melanoma. However, superimposed toxicity, abnormal response patterns, and other serious adverse events were observed. Thus, sequential treatment with anti-PD-1/PD-L1 mAbs followed by anti-CTLA-4 mAbs needs to be carefully considered.

Combinatorial targeting of PD-1 and the TIM-3 (60), LAG-3 (61) and TIGIT (62) was shown to synergistically recover exhausted T cells and enhance anti-tumor effects in some types of cancer. A new triple therapy of anti-TIM-3, anti-PD-1, and radiation for human glioblastoma showed 100% overall survival (OS) (66). The safety and efficacy of dual PD-1 and TIM-3 blockade along with cancer vaccines in patients with advanced melanoma have been tested in a phase I clinical trial (67). In addition, several novel co-inhibitory receptors involved in CD8+ T cell exhaustion have been identified, such as CD96 (68), 2B4 (CD244) (69), CD160 (70), and B- and T-lymphocyte attenuator (BTLA) (71), and blocking them could partially restore T cell function and/or expansion and might enhance anti-tumor immunity (69-71). Collectively, combinatorial targeting PD-1/PD-L1 and alternative checkpoints on T cells have great potential for T-cell based immunotherapy, and several clinical trials are ongoing (NCT02817633, NCT02608268, NCT02658981, NCT01968109, NCT02061761, NCT03005782, NCT02676869, NCT02966548, and NCT02460224).

Activating co-stimulatory molecules on T cells
4-1BB (CD137, TNFRSF9), OX40, CD27 and CD40 are members of tumor necrosis factor receptor (TNFR) superfamily, which provide strong costimulatory signals for augmenting and diversifying T-cell responses (72). Tumor-reactive CD8+ T cells frequently co-express PD-1 and costimulatory molecules of the TNFR family (73), offering a repertoire of potential targets for T cell-based therapy.

4-1BB has been identified as a potent mitigator of exhausted T cells, and administration of an anti-4-1BB agonist significantly enhanced CAR-T-cell efficacy in solid tumor settings (74). Preclinical studies have shown that 4-1BB activation and PD-1 blockade synergistically promoted T-cell anti-tumor responses in a mouse model of poorly immunogenic tumors (75). In addition, a recent study revealed that co-treatment with anti-4-1BB agonist, anti-PD-L1 mAb, and a tumor vaccine produced more powerful anti-tumor capacity by reprogramming suppressive and stimulatory signals in ovarian cancer (76). Clinical trials of this combinatorial therapy for the treatment of advanced solid tumors are ongoing

(NCT02179918, NCT02652455, NCT02845323, and NCT02554812). Unfortunately, severe liver toxicity has become the biggest obstacle for the clinical application of anti-4-1BB agonists.

Both OX40 and CD27 costimulatory signals could reinvigorate exhausted CD8+ T cells. Anti-PD-1/OX40 mAb treatment was shown to increase CD4+ T and CD8+ T cells and decrease Tregs and MDSCs (77). Either OX40 or CD27 agonistic mAb combined with anti-PD-L1 mAb synergistically enforced CD8+ T cell proliferation and effector cytokine generation in mice (78). The ongoing clinical trials are as follows: combination of third generation CAR-T cells, iC9-GD2-CD28-OX40 (iC9-GD2) T cells, with anti-PD-1 mAb in relapsed or refractory neuroblastoma (NCT01822652); and combination of agonist CD27 mAb and anti-PD-1 mAb in advanced refractory solid tumors (NCT02335918).

It has been reported that CD40 activation rescued antiviral CD8+ T cells from PD-1-mediated exhaustion (79). Combination of agonistic CD40 mAb with chemotherapy completely reversed the resistance to anti-PD-1 mAb in pancreatic tumors (80), and a clinical trial of nivolumab in combination with GM. CD40L vaccine in adenocarcinoma of the lung is ongoing (NCT02466568). However, the appropriate dose, schedule, route of administration, and formulation for these combinatorial therapies need to be determined for treating different cancer types.

Modulating T cell metabolism
It has been reported that a hostile TME can restrain T-cell immune response by altering cellular metabolism, such as impaired glycolysis (81,82), fatty acid metabolic dysregulation (83,84), aberrant cholesterol esterification (85), disruption of mitochondrial function, and the generation of high levels of reactive oxygen species (ROS) (86). Hypoxia-mediated glycolysis was shown to interfere with the differentiation and function of T cells by modulating the expression of co-inhibitory receptors, including PD-1, CTLA-4, and LAG-3 (87). However, anti-PD-1/PD-L1 mAbs could partially restore T cell glycolysis and function (81). One mechanism by which PD-1 inhibits T-effector cell differentiation is through the enhancement of fatty acid-oxidation (FAO) of endogenous lipids (83). Free linoleic acid has been reported to cause T cell loss by ROS-mediated depletion and promote liver carcinogenesis (83). Additionally, limiting cholesterol esterification in T cells through inhibition of acetyl-coenzyme A

acetyltransferase 1 (ACAT1), which is a key cholesterol esterification enzyme, enhanced the proliferation and effector function of CD8+ T cells, and combination treatment with ACAT1 inhibitors and anti-PD-1 mAb demonstrated remarkable tumor regression in mice with melanoma (85). In-depth investigations of the association between PD-1 expression and metabolism in dysfunctional T cells are needed to guide anti-PD-1 therapy.

Promoting T-effector cell infiltration

Chemokines play a role as chemoattractants of different leukocyte subsets and act as a double-edged sword in tumor development. For example, Th1-type chemokines C-X-C Motif Chemokine Ligand 9 (CXCL9) and C-X-C Motif Chemokine Ligand 10 (CXCL10) can increase effector T cell trafficking. It has been verified that upregulation of CXCL9 and CXCL10 improved the therapeutic efficacy of anti-PD-1 mAb (88). However, it also has been recently reported that adenosine signaling in the TME suppressed CXCL9 and CXCL10 production and T-cell infiltration in metastatic lung cancer, and this suppression could be partially reversed by adenosine receptor antagonist (89). Therefore, targeting Th1-type chemokines might offer an effective approach to combinatorial therapy with anti-PD-1/PD-L1 mAb, particularly in tumors with poor T-cell infiltration. In addition, the immunosuppressive stromal cell derived factor-1 (SDF1α)/CXCR4 axis was found to be upregulated in sorafenib-treated advanced hepatocellular carcinoma, which enhanced the expression of PD-L1 and the recruitment of Tregs and M2-type macrophages (90,91). Blockade of SDF1α/CXCR4 axis had no significant effect on CTL infiltration and function but altered the suppressive state of the TME. Dual blockade of PD-1 and CXCR4 boosted CD8+ TIL responses and prevented the polarization toward an immunosuppressive TME in mice (91), providing a rationale for combinatorial blockade of PD-1 and SDF1/CXCR4 axis in clinical studies.

Targeting immunosuppressive factors in the TME

The TME consists of cancer cells, inflammatory cells, stromal cells, and cytokines. These components form a complicated immunosuppressive network that limits T-cell activation and induces T-cell dysfunction (*Figure 2*). The immunosuppressive nature of the TME has emerged as a critical regulator of anti-tumor immune responses, and it is increasingly being recognized as a major barrier to the effectiveness of cancer immunotherapy. Some tumors

resistant to anti-PD-1/PD-L1 therapy have been shown to simultaneously utilize multiple immunosuppressive pathways in the TME, and these pathways are described below.

Suppressive cytokines

IL-10 is produced by multiple cells in the TME, and its receptor, IL-10R, was found to be upregulated on activated CD8+ T cells in some cancer patients (92). IL-10/IL-10R interaction was shown to directly limit CD8+ T cell proliferation and survival (93). PD-1 blockade augmented IL-10R expression on tumor antigen-specific CD8+ T cells, and dual PD-1 and IL-10 blockade significantly enhanced the response of antigen-specific CD8+ T cells *in vitro* (34), offering a rationale for blocking both IL-10 and PD-1 to strengthen T cell anti-tumor activity. In some cancer patients, IL-6 levels were also elevated and closely related to an aggressive cancer phenotype (94). Cancer-associated fibroblast (CAF)-derived IL-6 has been reported to recruit MDSCs and impair TIL function by upregulating inhibitory immune checkpoints, and IL-6 blockade reversed anti-PD-L1 resistance in a hepatocellular carcinoma tumor model (95). Another suppressive cytokine in the TME, transforming growth factor-β (TGF-β), was shown to directly inhibit CD8+ T cell response, promote Treg development, limit DC maturation, and upregulate PD-1 expression on T cells (95-97). It has been recently shown that TGF-inhibition reversed PD-1-induced immune tolerance and reinforced T cell responses (98). Thus, combinatorial administration of TGF-β/TGF-β receptor inhibitors and anti-PD-1/PD-L1 mAbs could be considered as a potential therapy to enhance T cell anti-tumor immunity in cancer immunotherapy.

VEGF-A/VEGF receptor 1 (VEGFR1)

VEGF-A produced by tumor cells and associated immune cells was identified to limit DCs maturation, enhance Tregs and MDSCs accumulation, and upregulate PD-1 expression by stimulating VEGFR1 (35). Anti-VEGF antibody was approved by the FDA as the first antiangiogenic agent several years ago. A clinical study demonstrated that increased levels of PD-L1 were associated with shorter survival in patients with metastatic RCC receiving VEGF-targeted agents (99). Anti-angiogenic agents targeting VEGF-A-VEGFR1 pathway have been reported to dramatically enhance the efficacy of anti-PD-1/PD-L1 therapy in a mouse model of colorectal cancer (100), suggesting that combined targeting VEGF-A-

VEGFR and PD-1/PD-L1 pathway might be a potential strategy for VEGF-expressing tumors. However, potent VEGF inhibition might increase tumor hypoxia, which would decrease the therapeutic effect. Therefore, further studies are needed to determine the proper dose titration when combining anti-VEGF therapy with anti-PD-1/PD-L1 therapy.

PI3-kinase γ (PI3Kγ) signaling in myeloid cells and macrophages
Recently, macrophage PI3Kγ was identified as a molecular switch that controlled immune suppression during inflammation and cancer (101), and high PI3Kγ expression increased the suppressive activity of macrophages and infiltrating myeloid cells, which mediated resistance to anti-PD-1/PD-L1 therapy in mouse models of cancer (101,102). Conversely, it was shown that selective inactivation of PI3Kγ could reshape the TME and promote cytotoxic-T-cell-mediated tumor regression, thus improving the therapeutic effect of anti-PD-1/PD-L1 therapy (101,102). These results provide evidence for a combinatorial approach using PI3Kγ inhibitors to overcome resistance to anti-PD-1/PD-L1 therapy in patients with accumulated suppressive myeloid cells or macrophages.

Targeting tumor-intrinsic factors

In addition to T cell autonomous factors and the suppressive TME, tumor-intrinsic factors, such as abnormal tumor metabolism, elevated oncogenic pathways, and loss of tumor antigens, might be potential therapeutic targets in combination with PD-1/PD-L1 blockade.

Targeting immunosuppressive tumor metabolites
Some tumors that are resistant to anti-PD-1 therapy have been shown to utilize multiple tumor metabolites involved in immunosuppression. For example, to survive in a hypoxic environment, cancer cells have been shown to alter purine metabolism, and this alteration also increased CD73 expression by lymphocytes, endothelial, and epithelial cells (103). High levels of CD73 induced the production of extracellular adenosines, which further increased PD-1 expression and Treg accumulation by binding to A2A adenosine receptor, thus promoting resistance to anti-PD-1 therapy (104). Dual CD73 and PD-1 blockade demonstrated synergistic anti-tumor activity in murine tumor models (40). Tumor cell-derived prostaglandin E2 (PGE2) and its synthesizing enzyme, cyclooxygenase-2

(COX-2), serve as both pro-inflammatory mediators and potent immune suppressors of anti-tumor immunity (105,106). Single PD-1 blockade enhanced the production of PGE2 and pro-tumor inflammatory cytokines, attenuating its therapeutic effect, while combinatorial inhibition of PD-1 and PGE2 significantly augmented the function and survival of CTLs during chronic viral infection (107). In addition, dual COX-2 inhibition and PD-1 blockade synergistically improved T cell anti-tumor immunity in mouse model of melanoma (106). Upregulation of IDO has also been detected in many tumors and has been shown to render experimental mouse models of melanoma resistant to anti-PD-1 therapy (39). Targeting IDO-mediated immunosuppression has demonstrated promising synergistic effects when combined with anti-PD-1/PD-L1 mAbs (108). In addition, high levels of ROS, a kind of short-lived, chemically highly reactive and oxygen-containing small molecules, have been reported to suppress the T cell immune responses. ROS could be produced by cancer cells and multiple immunosuppressive cells in the TME (86). PD-1 was shown to alter the lipid metabolism of antigen-specific T cells, which might further promote ROS production and impair their anti-tumor capacity (83,84). These results suggest that targeting the immunosuppressive metabolites of tumor cells might be an effective adjunct therapy to improve the therapeutic effect of PD-1/PD-L1 blockade.

Targeting oncogenic signaling pathways
Many tumor-intrinsic oncogenic signalings are being increasingly recognized as contributors to immune suppression, which affects the efficacy of anti-PD-1/PD-L1 therapy. It has been reported that PTEN loss and PI3K-Akt pathway activation not only directly boost tumor cell growth but also suppress anti-tumor leukocyte recruitment and function in multiple cancers. It was also shown that PI3K inhibition could improve anti-PD-1 mAb activity in murine models (109,110). Stabilization of WNT-β-catenin signaling was identified as an important mechanism of T cell exclusion from cancers by inhibiting CD103+ DC recruitment, which eventually mediated resistance to anti-PD-L1 therapy (111). Activation of EGFR pathway was reported to correlate with the upregulation of PD-1, PD-L1, CTLA-4, and pro-tumor inflammatory cytokines, which inhibited the therapeutic effect of PD-1 blockade (112). In patients with melanoma, JAK1/2 loss-of-function mutations in tumor cells can mediate primary resistance to PD-1 blockade via a genetic lack of reactive PD-L1

expression (36). In addition, tumor cell downregulation of MHC-I molecules represents another common mechanism of tumor immune escape and resistance to anti-PD-1/PD-L1 therapy (38). Taken together, these findings demonstrate that tumor-specific oncogenic signals play important roles in the development of resistance to anti-PD-1/PD-L1 therapy, and targeting them might be an effective approach to enhance the therapeutic effect in some cancer patients.

Targeting mutant tumor neoantigens

Effective anti-PD-1/PD-L1 therapy requires tumor cell recognition by tumor antigen-specific T cells in the TME. This suggests that poorly immunogenic tumors should be largely resistant to anti-PD-1 therapy. However, it has been revealed that tumor neoantigen burden is closely correlated with the immunogenicity and determines the sensitivity to anti-PD-1 therapy (113). Neoantigen-enriched tumors also showed a higher accumulation of CD8+ T cells (114), and PD-1/PD-L1 blockade has been reported to potentiate neoantigen-specific T cell responses (113). MSI-H cancer, resulting from defective mismatch repair proteins (dMMR), is a type of "hot" tumor characterized by high mutation burdens, active immune TME, and high expression levels of immune checkpoints (115). In 2017, FDA approved pembrolizumab for the treatment of MSI-H/dMMR-subtype solid tumors. A recent study demonstrated that immunodominant T cell reactivities were directed against mutant neoantigens or a cancer germline antigen, rather than the canonical antigens in human papillomavirus (HPV)-associated cervical cancer

(116). Therefore, the mutant neoantigens in a cancer might predict its sensitivity to anti-PD-1/PD-L1 therapy, and these neoantigens could be targeted in novel combination approach with anti-PD-1/PD-L1 therapy.

Clinical trials of anti-PD-1/PD-L1 mAbs

Anti-PD-1/PD-L1 mAbs have shown encouraging clinical results in both solid tumors and hematological malignancies (*Table 1*), and clinical trials of some combinatorial therapies are shown in *Table 2*. These results remind us the great potential of anti-PD-1/PD-L1 mAbs in treating patients.

Conclusions

PD-1/PD-L1 blockade represents an attractive therapeutic strategy in cancer immunotherapy. However, primary and adaptive resistance to anti-PD-1/PD-L1 therapy has been reported to occur in 30%–60% of cancer patients. Therefore, a comprehensive understanding of the resistance mechanisms and how to overcome them are critical for improving clinical responses. Combined therapies of PD-1/PD-L1 blockade with adjunctive strategies, such as chemotherapy, RT, ACT, and other immune checkpoint inhibitors, have shown promising success by improving the probability, duration, and potency of clinical activity. However, the combinations discussed here require additional research to identify the safety, efficacy and optimal dosage and timing. Additionally, as the microenvironment where immune cells

Table 1 Clinical trials of anti-PD-1/PD-L1 agents in cancer patients

Antibody	Phase	Tumor type	Clinical response rate (%)	Reference
Nivolumab (anti-PD-1)	I	Relapsed or refractory Hodgkin's lymphoma	87.0	(117)
	III	Untreated melanoma without BRAF mutation	40.0	(118)
Pembrolizumab (anti-PD-1)	II	MSI-high CRC	57.0	(115)
	III	Advanced NSCLC	44.8	(119)
Pidilizumab (anti-PD-1)	I	Hematologic malignancies	29.0	(120)
	II	Diffuse large B cell lymphoma	51.0	(121)
MPDL3280A (anti-PD-L1)	I	Metastatic bladder cancer UBC	26.2	(122)
	I	Melanoma, NSCLC, RCC, gastrointestinal cancer	36.0 (overall)	(3)
	I	Metastatic RCC	15.0	(123)
MPDL3280A (anti-PD-L1)	II	Cisplatin-ineligible, locally advanced and metastatic urothelial carcinoma	23.0	(124)

PD-1, programmed cell death 1; PD-L1, programmed death 1 ligand; MSI, microsatellite instability; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; UBC, urothelial bladder cancer; RCC, renal cell carcinoma.

Table 2 Clinical trials of combination therapies including anti-PD-1/PD-L1 agents in cancer patients

Combination	Phase	Tumor type	Clinical response rate (%)	Reference
Nivolumab plus ipilimumab	II	Advanced melanoma	63.8	(125)
	III	Unresectable melanoma	57.6	(126)
Nivolumab plus chemotherapy	I	Advanced NSCLC	54.2	(127)
	II	Advanced and metastatic urothelial carcinoma	19.6	(46)
Pembrolizumab plus chemotherapy	II	Advanced colorectal cancer	53.3	(47)
	II	Advanced gastric cancer	60.0	(48)
Nivolumab plus RT	–	Metastatic melanoma	46.7	(128)

PD-1, programmed cell death 1; PD-L1, programmed death 1 ligand; RT, radiation therapy; NSCLC, non-small cell lung cancer.

interact with cancer cells is complicated and dynamic, improved understanding of the mechanisms limiting anti-PD-1/PD-L1 therapy is needed to broaden its clinical applicability in the future.

Footnote

Conflicts of Interest: The authors have no conflicts of interests to declare.

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