

# A combination of PD-1/PD-L1 inhibitors: The prospect of overcoming the weakness of tumor immunotherapy (Review)

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**Abstract.** Programmed cell death protein-1 (PD-1)/programmed death protein ligand-1 (PD-L1) inhibitors for treatment of a various types of cancers have revolutionized cancer immunotherapy. However, PD-1/PD-L1 inhibitors are associated with a low response rate and are only effective on a small number of patients with cancer. Development of an anti-PD-1/PD-L1 sensitizer for improving response rate and effectiveness of immunotherapy is a challenge. The present study reviews the synergistic effects of PD-1/PD-L1 inhibitor with oncolytic virus, tumor vaccine, molecular targeted drugs, immunotherapy, chemotherapy, radiotherapy, intestinal flora

and traditional Chinese medicine, to provide information for development of effective combination therapies.

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**Abbreviations:** CTLA-4, cytotoxic T-lymphocyte antigen-4; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; IDO, indoleamine 2, 3-dioxygenase; IFN- $\gamma$ , interferon- $\gamma$ ; LAG3, lymphocyte activation gene-3; ICIs, immune checkpoint inhibitors; IR, irradiation; MDSCs, myeloid-derived suppressor cells; MSI, microsatellite instability; NSCLC, non-small cell lung cancer; OVs, oncolytic virus; ORR, objective response rate; PR, partially responsive; PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1; PFS, progression-free survival; RT, radiotherapy; SD, stable disease; SBRT, stereotactic body radiotherapy; TIL, tumor infiltrating lymphocyte; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TMB, tumor mutation burden; US FDA, United States Food and Drug Administration; VEGF, vascular endothelial growth factor

**Key words:** oncolytic virus, cancer vaccine, molecular targeted therapy, immunotherapy, intestinal flora, Traditional Chinese Medicine

## 1. Introduction

*Advances in immunotherapy have revolutionized cancer therapy.* Programmed death receptor-1 [programmed cell death protein 1 (PD-1)] and programmed death protein ligand-1 [programmed death-ligand 1 (PD-L1)] inhibitors, have improved tumor therapy in cancer immunotherapy (1). The combination of PD-1 and PD-L1 inhibits the activity of T cells and act as the 'brake' of immunity, thereby preventing effector immune cells from killing cancer cells (2). Common PD-1/PD-L1 inhibitors in clinical used include Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab and Avelumab (3). PD-1/PD-L1 inhibitors block PD-1/PD-L1 pathway to restore normal immune function of T cells. Effector T cells play a role in recognizing and killing tumors (4). Various PD-1/PD-L1 inhibitors have been approved by the United States Food and Drug Administration (US FDA) for the treatment of various tumors (5). PD-1/PD-L1 inhibitors are characterized by high efficacy and fewer adverse events (6). However, PD-1/PD-L1

inhibitors are associated with low response rate when used as a monotherapy with few patients meeting the treatment conditions and the high cost of treatment (7). In addition, immune regulatory signaling pathways are complex, so even those patients who are initially sensitive to PD-1/PD-L1 therapy may develop resistance or relapse. Therefore, there is need to develop approaches to improve sensitivity of PD-1/PD-L1 inhibitors (8).

Tumor-related gene deletions and mutations are implicated in anti-PD-1/PD-L1 resistance (9). For example, Janus kinase (JAK)1, JAK2 and  $\beta$ 2 microglobulin mutations cause antigen presentation barriers, which induce CD8-infiltrated T cells to lose major histocompatibility complex (MHC) I and reduce sensitivity to IFN- $\gamma$  (10). Mutation or activation of epidermal growth factor receptor (EGFR), T cell immunoglobulin mucin 3 (Tim-3), lymphocyte activation gene-3 (LAG-3), T cell Ig and ITIM domain (TIGIT) and other T cell depletion-related protein receptors results in a gradual loss of T cell proliferative potential and effector function, thus inducing drug resistance against PD-1 inhibitors (11-13). Immune checkpoint inhibitors (ICIs) inhibit checkpoints of the immune system rather than directly enhancing immune function. A single ICI is not effective in activating immune response. Therefore, there is a requirement to explore novel alternative strategies and personalized immunotherapy strategies through a combination of PD-1/PD-L1 inhibitors with small molecular targets, chemotherapy and radiotherapy to improve sensitivity to activated anti-tumor immune response and the response rate of patients and solve the bottleneck of drug resistance (14). The present study summarizes previous studies on the anti-tumor effects of PD-1/PD-L1 inhibitors combination therapy to provide information for clinical and basic research (Fig. 1).

## 2. Combination of oncolytic virus (OVs) with PD-1/PD-L1 inhibitors

Tumor virus therapy induces immunogenic death on target cells and induces immune response by releasing pathogen-associated molecular pattern and damage-associated molecular pattern (15,16). As a result, tumor virus therapy improves the sensitivity of tumor cells to immunotherapy thus improving therapeutic effect. OVs mediates clearance of cancer cells or killing of cancer cells by targeting the tumor vascular system and inducing immunity (17). *Talimogene laherparepvec*, a herpes simplex virus expressing granulocyte-macrophage colony stimulating factor, was the first US FDA approved oncolytic therapy (18). Local intratumoral injection of the virus into tumors improves the overall survival rate of patients (19). A previous study (7) reported on the treatment of 21 patients with advanced melanoma with *Talimogene laherparepvec* combined with Pembrolizumab. The study report that therapy was well tolerated, with fatigue, fevers and chills as the common adverse events. The therapy showed no dose-dependent toxic reaction and an objective response rate (ORR) of 62%. Patients who responded to the combination therapy showed an increase number of CD8+T cells (7). Vaccinia virus is a highly immunogenic oncolytic immunotherapy vector (20,21). Previous studies report that vaccinia virus attracts effector T cells in mouse

model of colorectal cancer and ovarian cancer (22,23). A combination of vaccinia virus with PD-L1 inhibitor enhances the infiltration of effector CD4+ and CD8+T cells and increases granzyme B, ICOS, perforin and IFN- $\gamma$ , thus improving the survival rate (23).

PD-1/PD-L1 drug resistance is a main challenge, therefore, studies are required to explore novel approaches to improve immunogenicity of tumors and overcome resistance to immunotherapy (8). Rotavirus vaccine has immunostimulatory and anti-tumor effects (24). Administration of rotavirus in tumors overcomes drug resistance against PD-L1 inhibitors and has a synergistic effect with PD-L1 inhibitors. Heat- and UV-inactivated rotaviruses have no oncolytic activity but offer a synergistic effect with immune checkpoint-targeted antibodies through upregulation of the double-stranded RNA receptor retinoic acid-induced gene 1 (25). Rotaviruses have been used clinically and can be used for clinical sensitization of anti-PD-1/PD-L1 therapy (25) (Table I).

## 3. Combination of cancer vaccine with PD-1/PD-L1 inhibitors

Tumor vaccine enhances immunogenicity and activates the immune system of the patient, thus controlling or eliminating tumors (26). DNA vaccine, a universal and personalized cancer treatment containing multiple new antigen coding sequences, is ideal for new antigen vaccination (27). DNA vaccine induces Cytotoxicity of CD8 T cells. A single dose of DNA vaccine combined with anti-PD-1 treatment significantly delays tumor growth in tumor-bearing mice inoculated with MC38 colon cancer cell line and some of the tumors are cleared completely, with a cure rate of 25%, and this indicates that tumor vaccine works synergistically with immune checkpoint blocking therapy (28).

Lmdd-MPFG vaccine promotes expression of PD-L1 in HCC cells but re-sensitizes tumor local T cell to respond to anti-PD-1 immunotherapy (29). Lmdd-MPFG vaccine activates NF- $\kappa$ B pathway and autophagy pathway in tumor-associated macrophages (TAMS). In addition, it converts M2 TAMS to M1 and induces the expression of antineoplastic factors, thus restoring T cell response to PD-1 inhibitors (29). Lmdd-MPFG vaccine acts synergistically with PD-1 inhibitors in treatment of liver cancer (29).

The tumor vaccine OVA @ Mn-DAP with nano-scale coordination polymer as a carrier, prepared from Mn<sup>2+</sup> ions, Nod1 agonist and DAP as organic ligands, promotes maturation of dendritic cells and cross-presentation of antigens. Further, it prevents occurrence of B16-OVA tumors and works synergistically with PD-1 inhibitors to inhibit tumor growth (30).

Nivolumab combined with a multi-peptide vaccine (gp100, MART-1 and NY-ESO-1 with Montanide ISA 51 VG) was investigated as adjuvant therapy in resected stage IIIC and IV melanoma patients (31). The study findings showed that the treatment strategy was well tolerated. Common adverse events observed included fatigue, rash/pruritus, nausea/diarrhea, arthralgias and endocrinopathies. Although related grade 3 events occurred in 4 out of 33 patients, they were manageable. Notably, the combination therapy significantly increases CD8+ T-cell levels and decreases PD-1 expressing T-cells. In

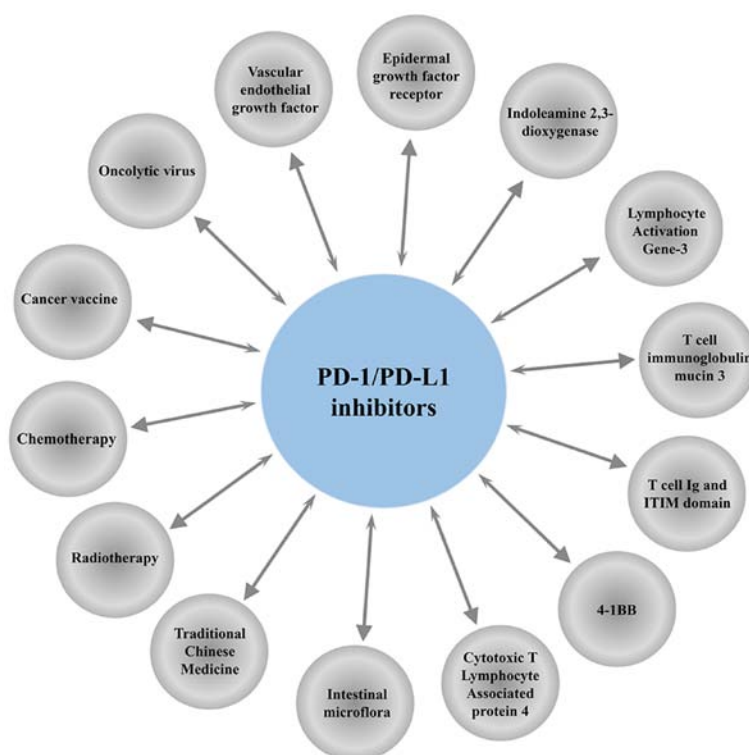


Figure 1. Schematic diagram of combined treatment regimen for PD-1/PD-L1 inhibitors. PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

addition, significant increases in CD25+ regulatory T cells (Tregs)/CTLA4+/CD4+ T-cell populations are observed with anti-PD-1 therapy (31). These findings imply that synergistic activity of nivolumab and anti-PD-1 therapy is mediated through CTLA-4 and/or Tregs (32).

A previous phase I study (33) evaluated a vaccine that targets  $\leq 20$  predicted personal tumor neoantigens in patients with previously untreated high-risk melanoma following surgical resection. In that study, vaccine-induced polyfunctional CD4+ and CD8+ T cells targeted 58 (60%) and 15 (16%) of the 97 unique neoantigens used across patients, respectively. These T cells discriminated mutated antigens from wild-type antigens and recognized autologous tumors. Out of the six vaccinated patients, four showed no recurrence at 25 months following vaccination, whereas two showed recurrent disease and were subsequently treated with anti-PD-1 (pembrolizumab) therapy achieving complete tumor regression, with the expansion of a repertoire of neoantigen-specific T cells (33).

A previous study (34) explored the synergistic effect of a vaccine targeting HER2 $\Delta 16$  on anti-PD-1 therapy in enhancing antitumor immunity in a model of advanced HER2+ breast cancer. HER2 $\Delta 16$  is a critical oncogenic pathway and spontaneous tumors driven by HER2 $\Delta 16$  are reflective of clinically advanced immunosuppressive HER2+ breast cancer. Endogenous HER2 $\Delta 16$ + breast cancers show no response to anti-PD-1 as a single agent. Treatment with anti-PD-1 is not effective in increasing systemic anti-HER2 T-cell responses. However, combination of anti-PD-1 with Ad-HER2 $\Delta 16$ -KI significantly increases survival rate, with ~30% of mice exhibiting complete tumor regression and long-term tumor-free survival. These findings show that vaccinated mice are characterized by a high IFN- $\gamma$  gene signature score. In addition,

the results show that HER2 $\Delta 16$  vaccination induces systemic adaptive immune responses and increases HER2-specific CD8 T cells that infiltrate into tumors. Therefore, addition of anti-PD-1 effectively induces HER2-specific T cells in TME (34) (Table II).

#### 4. Combination of molecular targeting drugs with PD-1/PD-L1 inhibitors

*Combined application of vascular endothelial growth factor (VEGF) and PD-1/PD-L1 inhibitors.* VEGF is an angiogenic factor that regulates the growth and survival of vascular endothelial cells, thereby causing immunosuppression (35) (Fig. 2). VEGF inhibitors are used to prevent angiogenesis and to promote differentiation of immune cells. Co-blocking of PD-1 and VEGF enhances efficacy of PD-1 inhibition (36). A clinical trial showed that the combination of VEGF and PD-1 inhibitors is effective in cancer treatment (NCT01472081) (37). Another study reported that PD-L1 inhibitors combined with VEGF receptor 2 (R2) small molecule inhibitors significantly downregulated the expression levels of PD-1 and PD-L1, and inhibited tumor growth by increasing tumor infiltrating lymphocytes (TILs) and decreasing Tregs and myeloid-derived suppressor cells (MDSCs) (38).

Bevacizumab was the first antiangiogenic drug and vascular modulator used for clinical treatment of solid tumors (39). Bevacizumab binds to vascular endothelial growth factor A, blocks interaction of its receptor VEGFR-1/VEGFR-2, induces tumor vascular degradation and inhibits tumor growth. Bevacizumab confers immunomodulatory effects by inhibiting VEGF and promoting DC maturation (40). In addition, it reverses immunosuppression by increasing T cell

Table I. Combination therapy of oncolytic viruses with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Ribas <i>et al.</i> , 2017	Talimogene laherparepvec + Pembrolizumab	ORR CD8+ T cells	62% Increased	(7)
Liu <i>et al.</i> , 2017	Vaccinia virus + Anti-PD-L1	Tumor burden Survival rate Granzyme B, Perforin, IFN- $\gamma$ , ICOS, Effector CD4+ and CD8+T cells	Reduced Improved Increased	(23)
Shekarian <i>et al.</i> , 2019	Rotavirus vaccine + Anti-PD-L1	Tumor size Percent survival	Reduced Improved	(25)

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

Table II. Combination of cancer vaccines with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Tondini <i>et al.</i> , 2019	DNA vaccine + Anti-PD-1	Tumor growth Cure rate	Delayed 25%	(28)
Xu <i>et al.</i> , 2020	Anti-PD-1 + Lmdd-MPFG vaccine	Percent survival Tumor volume TAMS PD-L1	Prolonged Retardation Converted M2 TAMS to M1 Promoted	(29)
Zhao <i>et al.</i> , 2019	OVA@Mn-DAP vaccine + Anti-PD-1	Tumor-infiltrating lymphocytes Tumor size Percent survival	Increased Inhibited Prolonged	(30)
Gibney <i>et al.</i> , 2015	Nivolumab + A multi-peptide vaccine	CD8+/CD25+Treg/ CTLA4+/CD4+ T-cells PD-1	Increased Decreased	(31)
Crosby <i>et al.</i> , 2020	Ad-HER2D16-KI + Anti-PD-1 vs. Anti-PD-1	Survival IFN- $\gamma$	Prolonged Increased	(34)

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

infiltration. Furthermore, it enhances anti-tumor activity of PD-L1 antibody Atezolizumab (41). Phase III randomized controlled trials showed that Atezolizumab combined with chemotherapy and Bevacizumab improves progression-free survival (PFS) and overall survival (OS) of patients with metastatic NSCLC. Moreover, Bevacizumab monoclonal antibody increases sensitivity of Atezolizumab therapy (42) (Table III).

*Combined application of EGFR and PD-1/PD-L1 inhibitors.* EGFR is a transmembrane tyrosine kinase receptor, implicated in tumor cell proliferation, invasion and metastatic angiogenesis (43) (Fig. 2). EGFR tyrosine kinase inhibitor (EGFR-TKI) inhibits EGFR, reduces T cell apoptosis and increases production of IFN- $\gamma$  (44). However, most patients develop acquired drug resistance following EGFR-TKIs treatment (45). Activation of

EGFR pathway during tumorigenesis induces tumor immune escape mediated by PD-L1 (46). A previous study has explored the combination of PD-1/PD-L1 inhibitors and EGFR-TKIs for clinical use. Efficacy of PD-1/PD-L1 inhibitors combined with EGFR-TKIs in treatment of advanced EGFR mutant NSCLC has not yet been fully explored. Advanced patients with NSCLC and acquired tolerance to first or second generation of EGFR-TKIs should be treated with third generation of EGFR-TKIs before PD-1/PD-L1 inhibitors in case of a T790M mutation (47). In EGFR-TKIs-resistant EGFR mutant NSCLC, positive expression rate of PD-L1 in T790M negative patients was higher compared with that in T790M-positive patients (48). T790M negative patients were more sensitive to anti-PD-1 therapy after EGFR-TKIs treatment (48). Another clinical study reports that patients with advanced NSCLC and EGFR mutations show immune responses to PD-1/PD-L1

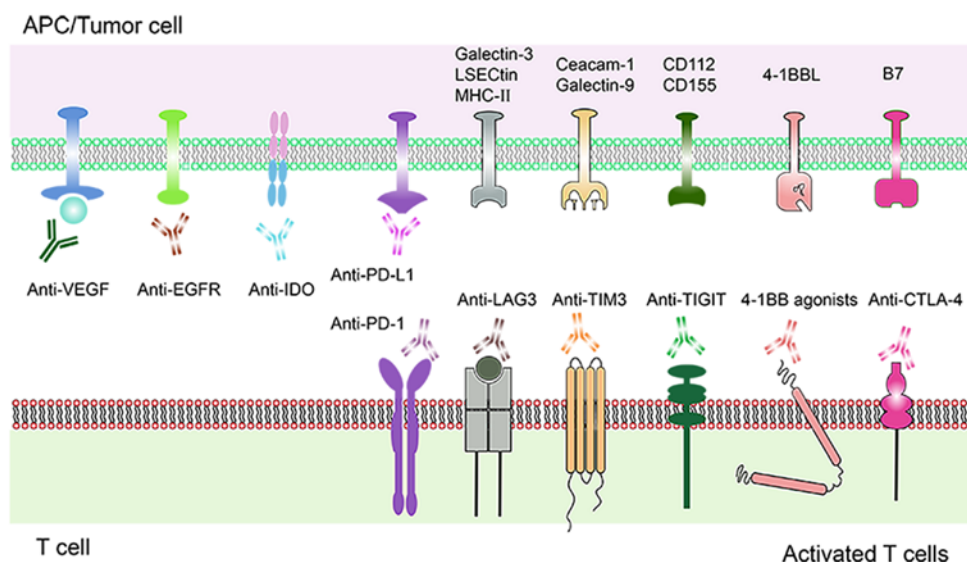


Figure 2. Schematic diagram of a combination therapy comprising molecular targeting drugs and PD-1/PD-L1 inhibitors. Current and emerging molecular targeting drugs. Various molecular targets expressed on T cells and tumor cells are shown. Immune molecular targets such as PD-1, LAG-3, TIM-3, TIGIT, 4-1BB, CTLA-4, IDO bound to their respective specific antibodies, triggering a positive signal to T cells response. Inhibition of VEGF and EGFR mediated angiogenesis. PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1; LAG3, lymphocyte activation gene-3; TIM-3, T cell immunoglobulin mucin 3; TIGIT, T cell immunoreceptor with Ig and ITIM domains; CTLA-4, cytotoxic T-lymphocyte antigen-4; IDO, indoleamine 2,3-dioxygenase; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor.

inhibitors following EGFR-TKIs pretreatment and chemotherapy (49).

However, a clinical study reports that EGFR inhibitors do not improve sensitivity to PD-1/PD-L1 inhibitors. Phase I/II clinical trials (NCT02039674; keynote-021) explored effect Erlotinib or Gefitinib combined with Pembrolizumab for treatment of advanced NSCLC patients with EGFR sensitive mutations. The results showed that combination of these drugs could not improve efficacy and showed no synergistic effect with Pembrolizumab in killing tumor cells (50) (Table III).

**Combined application of indoleamine 2,3-dioxygenase (IDO) and PD-1/PD-L1 inhibitors.** IDO is a rate-limiting enzyme that breaks down tryptophan, reduces the number and activity of CD8T cells and is implicated in immunosuppression (Fig. 2). Increase in IDO activity is associated with poor clinical efficacy of PD-1 inhibitors (51). A clinical trial on immunotherapy combined with IDO inhibitors showed high efficacy. A combination therapy of Bms-986205, a potent oral IDO1 inhibitor and Nivolumab resulted in grade 1-2 toxicities with the exception of 3 cases of grade 3 hepatitis, rash and hypophosphatemia (52). Phase II clinical trials of the effect of Indoximod, an IDO inhibitor on melanoma (NCT02073123) (53), pancreatic cancer (NCT02077881) (54) and castrated prostate cancer (NCT01560923) (55) are underway with promising results. The ORR of melanoma patients treated with Indoximod combined with Ipilimumab, Nivolumab or Pembrolizumab was 52% (56). *Epacadostat*, an oral drug targeting IDO pathway is in phase I/II clinical trials (NCT 02327078, NCT 02178722) for treatment of multiple malignant tumors. Preliminary results show that ORR for melanoma is 75 and 4% for colorectal cancer. A combination with Pembrolizumab is relatively safe, however 3% of patients stopped treatment due to adverse events (57) (Table III).

**Combined application of LAG3 and PD-1/PD-L1 inhibitors.** LAG3 serves a protective role in autoimmunity through direct inhibition of T-helper (Th) cell response by MHCII. LAG3 has a negative regulatory effect on T cells. Continuous exposure of antigens in tumor microenvironment leads to sustained expression of LAG3 (58) (Fig. 2). LAG3 and PD-1 have synergistic effects, and a previous study has recently explored combined immunotherapy for LAG3 and PD-1 (12).

A combination of anti-LAG3 and PD-1 inhibitors yielded a 100% tumor clearance in an EG7 lymphoma model, whereas tumor clearance rate in mice treated with PD-1 inhibitors alone was 50% (59). Targeted inhibition of LAG3 and PD-1 showed significant tumor regression in B16-F10 recurrent melanoma model (60).

These findings show that LAG3 and PD-1 acts synergistically. Bispecific LAG3/PD1 antibodies are being developed to improve efficacy of PD-1 inhibitor monotherapy by inhibiting both LAG3 and PD-1 (61). BMS-986016 was the first anti-LAG3mAb to be developed. The first phase of I/IIa trial has been launched to evaluate efficacy of LAG3 inhibitors combined with Nivolumab in treatment of advanced malignant tumors (NCT01968109) (62). Merck conducted a phase I clinical trial of anti-LAG3 monoclonal antibody (MK-4280) to evaluate safety and tolerance of the drug (63). MK-4280 combined with PD-1 blocker (Pembrolizumab) is currently under clinical trial of 70 patients with metastatic solid tumors (NCT02720068) (58) (Table III).

**Combined application of Tim-3 and PD-1/PD-L1 inhibitors.** Overexpression of Tim-3 is positively associated with poor prognosis of lung, gastric, prostate and cervical cancer (64). Interaction between Tim-3 on effector T cells and Galectin-9 on tumor cells induces T cell apoptosis and suppresses immune response (Fig. 2). Blocking Tim-3 enhances T cell proliferation and immune function (65). Tim-3 is highly expressed in

Table III. Combination of molecular targeting drugs with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Zhao <i>et al.</i> , 2019	PD-L1 inhibitors + VEGFR2 small molecule inhibitors (apatinib)	TILs TAMs, MDSCs, TGF- $\beta$ , Tumor growth Survival	Increased Hindered Decreased Prolonged	(38)
Reck <i>et al.</i> , 2019	Anti-PD-L1+ Bevacizuma + Chemotherapy vs. Bevacizuma + Chemotherapy	PFS OS	10.2 months vs. 6.9 months 13.3 months vs. 9.4 months	(42)
Haratani <i>et al.</i> , 2017	PD-1/PD-L1 inhibitors+ EGFR-TKIs	ORR	T790M-negative patients (24%) vs. T790M-positive patients (13%)	(48)
Yang <i>et al.</i> , 2019	Pembrolizumab + Erlotinib vs. Pembrolizumab+ Gefitinib	ORR PFS	41.7% vs. 14.3% 19.5 months vs. 1.4 months	(50)
Siu <i>et al.</i> , 2017	IDO1 inhibitor (BMS-986205) + Nivolumab vs. BMS-986205	Safety	All treatment-related adverse events were grade 1/2 except three grade 3 toxicities	(52)
Zakharia <i>et al.</i> , 2016	IDO inhibitor (Indoximod) + Ipilimumab, Nivolumab or Pembrolizumab	ORR	52%	(56)
Hamid <i>et al.</i> , 2017	IDO inhibitor (Epacadostat) + Pembrolizumab	ORR	75% of melanoma and 4% of colorectal cancer	(57)
Huang <i>et al.</i> , 2015	Anti-LAG3 + Anti-PD-1 vs. Anti-PD-1	Tumor clearance	100% vs. 50%	(59)
Goding <i>et al.</i> , 2013	Anti-PD-L1 + anti-LAG-3 antibodies	Tumor area	Reduced	(60)
Sakuishi <i>et al.</i> , 2010	Co-blocking Tim-3 and PD-1 pathways	Tumor Size	Reduced	(67)
Friedlaender <i>et al.</i> , 2019	Co-blocking Tim-3 and PD-1 pathways	An ongoing phase I trials	Anti-tumor study of TIM3 and PD-L1 inhibitors is under way (NCT03099109; NCT02608268)	(71)
Davar <i>et al.</i> , 2018	Anti-Tim-3(TSR-022)+ anti-PD-1(TSR-042)	PR  SD	1 case of 11 evaluable patients with 100 mg dose vs. 3 cases of 20 evaluable patients with 300 mg dose 3 cases of 11 evaluable patients with 100 mg dose vs. 8 cases of 20 evaluable patients with 300 mg dose	(72)
Chauvin <i>et al.</i> , 2015	Anti-TIGIT+ anti-PD-1 vs. anti-TIGIT vs. anti-PD-1	NY-ESO-1-specific CD8+ T cell	Anti-TIGIT+ anti-PD-1>anti-TIGIT/anti-PD-1	(74)
Johnston <i>et al.</i> , 2014	Anti-TIGIT + anti-PD-L1 vs. anti-TIGIT vs. anti-PD-L1	Tumor volume  Percent survival	Anti-TIGIT+ anti-PD-L1 <anti-TIGIT/anti-PD-L1 Anti-TIGIT+ anti-PD-L1 >anti-TIGIT/anti-PD-L1	(76)
Morales-Kastresana <i>et al.</i> , 2013	Combination of anti-4-1BB, anti-OX40 and anti-PD-L1	Survival Tumor-infiltrating lymphocytes	Extended Increased	(80)
Tolcher <i>et al.</i> , 2017	4-1BB (Utomilumab) + Pembrolizumab	Safety  Activated memory/ effector CD8+ T cells	Treatment-emergent adverse events were mostly grades 1-2 Increased	(83)



Table III. Continued

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Postow <i>et al</i> , 2015	Nivolumab + Ipilimumab vs. Ipilimumab	ORR The median reduction in tumor volume	61% vs. 11% 68.1% vs. 5.5%	(87)
Larkin <i>et al</i> , 2015	Nivolumab + Ipilimumab vs. Ipilimumab vs. Nivolumab	PFS Safety	11.5 months vs. 2.9 months vs. 6.9 months, Grade 3 or 4 adverse events: 55.0% vs. 27.3% vs. 16.3%	(88)
Omuro <i>et al</i> , 2018	Nivolumab + Ipilimumab vs. Ipilimumab vs. Nivolumab	Tolerance Safety	80% vs. 70% vs. 90%, Fatigue: 55% vs. 80% vs. 30% Diarrhea: 30% vs. 70% vs. 10%	(89)

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

CD8 positive tumor infiltrating lymphocytes in solid tumor mice (66). Tim-3 (+) PD-1 (+) TILs is a severe failure phenotype, which does not proliferate to produce IL-2, TNF and IFN- $\gamma$  (67). A previous study reported that blocking Tim-3 and PD-1 pathways effectively controls tumor growth through synergistic activity (67). A combination of Tim-3 inhibitor and PD-1 inhibitor in mice with lung cancer upregulates expression of TILs (68). Administration of PD-1 inhibitors only results in drug resistance promoting tumor progression. Co-administration with Tim-3 inhibitor restores anti-tumor effect and increases survival time (69,70). These findings imply that Tim-3 inhibitor may increase IFN- $\gamma$  levels and increase T cell proliferation (13). Co-administration of Tim-3 and PD-1 shows synergistic effect on anti-tumor cells. An anti-tumor study on combination of Tim-3 and PD-L1 inhibitors is underway (NCT03099109 and NCT02608268) (71). Currently, only Phase I results have been reported (72) on Tim-3 antibodies (TSR-022) and PD-1 inhibitors (TSR-042) combination therapy. A total of 39 patients with NSCLC who were treated with PD-1 inhibitors were further treated with TSR-042, at a fixed dose of 500 mg combined with TSR-022 100 mg (14 cases)/3 weeks and 300 mg (25 cases)/3 weeks. Of the 11 patients who received a dose of 100 mg TSR-022, 1 case was partially responsive (PR) and 3 cases were stable disease (SD). For 20 patients who received a dose of 300 mg TSR-022, 3 cases were PR and 8 cases were SD. All reactions occurred in PD-L1 positive patients. Only 12 PD-L1 positive patients were analyzed, 4 were PR and 6 were SD (the 2 other patients were not specifically identified). The current dose was well tolerated. The disease control rate was 55% and the disease control rate was 83% in PD-L1 positive subgroups (72) (Table III).

**Combined application of TIGIT and PD-1/PD-L1 inhibitors.** TIGIT is a member of the immunoglobulin superfamily which is exclusively expressed in lymphocytes. When it binds to its ligand CD155, TIGIT inhibits T cell proliferation and IFN- $\gamma$  production. Therefore, activation of TIGIT pathway induces tumor immune escape (73) (Fig. 2). Co-blocking of PD-1/PD-L1 and TIGIT pathways restores the function of failed CD8+T cells. In patients with melanoma, co-blocking

of TIGIT and PD-1 increases the proliferation of CD8 TILs, cytogenesis and degranulation (74). In a mouse CT26 tumor model, co-inhibition of TIGIT and PD-L1 enhances CTL functions and restores CD8+T functions (75). Combination therapy induces tumor regression and tumor antigen-specific protective memory (76). TIGIT synergized with other co-suppressor molecules PD-1 and Tim-3 to inhibit effector T cell response and promote T cell dysfunction. Therefore, inhibiting TIGIT with PD-1 or Tim-3 may promote anti-tumor immunity and induce tumor regression (77). Phase I clinical trials are underway to evaluate the safety and efficacy of anti-TIGIT monoclonal antibodies (OMP-31M32; NCT 03119428) (78) (Table III).

**Combined application of 4-1BB (CD137) agonists and PD-1/PD-L1 inhibitors.** 4-1BB (CD137) is an inducible costimulatory receptor. On binding to its ligand (4-1BBL), it triggers the proliferation and activation of immune cells (79) (Fig. 2). A combination of PD-1 inhibitors and 4-1BB agonists has a strong synergistic effect. The combination also exerts significant effects on mice cancer models with poor immunogenicity (80). Utomilumab (PF-05082566) is a human monoclonal antibody that stimulates 4-1BB (81). As an accelerator of the immune system, Utomilumab has been investigated in clinical research (82). A study has shown that the level of activated memory/effector CD8+T cells in peripheral blood increases following treatment with a combination of Utomilumab (0.45-5.0 mg/kg) and Pembrolizumab (2 mg/kg). The combination is safe and well tolerated, consistent with the expected side effects of Pembrolizumab alone (83). Urelumab (BMS-663513) was the first anti-4-1BB drug to enter clinical trials. Studies show that a combination use of Urelumab and Nivolumab is well tolerated. The overall response rate of metastatic melanoma was 47% (84) (Table III).

**Combined application of cytotoxic T lymphocyte associated protein 4 (CTLA-4) and PD-1/PD-L1 inhibitors.** Ipilimumab (Anti-CTLA-4) is an immunomodulatory monoclonal antibody that targets cell surface antigen CTLA-4 as an ICL (85) (Fig. 2). The use of CTLA-4 and PD-1 inhibitors, either as

singly or as combinations, has been approved by US FDA for the treatment of metastatic melanoma (86).

In a phase II clinical study, the objective response rate of patients with advanced melanoma who received Nivolumab + Ipilimumab was significantly higher than that of patients who received Ipilimumab + placebo (61 vs. 11%). In the Nivolumab + Ipilimumab group, 22% of the patients showed complete response (87). A clinical study has shown that Nivolumab combined with Ipilimumab yields a PFS of 11.5 months, whereas the PFS of Ipilimumab or Nivolumab alone was 2.9 and 6.9 months, respectively. The probability of treatment-related grade 3 or 4 adverse events in Nivolumab group, Ipilimumab group and combination group was 16.3, 27.3 and 55.0%, respectively (88). Another clinical study has explored the safety and tolerance of Nivolumab with or without Ipilimumab in the treatment of recurrent glioblastoma. It has been reported that the tolerance of Nivolumab 3 mg/kg group exceeds that of Nivolumab 1 mg/kg + Ipilimumab 3 mg/kg and Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg subgroups (90 vs. 70 vs. 80%, respectively). Fatigue and diarrhea were the most common treatment-related adverse events associated with the aforementioned drugs (30 vs. 80 vs. 55%; 10 vs. 70 vs. 30%, respectively) and no other side effects were observed. Tolerance to the combination was negatively influenced by the dose of Ipilimumab (89) (Table III).

## 5. Combination of chemotherapy with PD-1/PD-L1 inhibitors

Chemotherapy usually kills cancer cells by targeting their DNA synthesis and replication (90). It also promotes the presentation of tumor antigens following cancer cell death, activates tumor specific T cells, facilitates DCs maturation, stimulates type I interferon response and eliminates bone marrow-derived immunosuppressive cells (91). Appropriate combination of chemotherapeutic drugs and PD-1/PD-L1 inhibitors can enhance the efficacy of PD-1 blockers and produce a more sustained anti-tumor response, especially in tumors with poor immunogenicity and sensitivity to chemotherapy. A study has shown that Pembrolizumab combined with pemetrexed/carboplatin enhances improves symptoms of metastatic non-squamous NSCLC and has been approved by US FDA (92). Application of Pembrolizumab in combination with pemetrexed and platinum increases the PFS of metastatic NSCLC (93). For untreated patients with metastatic squamous NSCLC, the PFS and OS of Pembrolizumab combined treatment group versus the placebo group were 6.4 months vs. 4.8 months and 15.9 months vs. 11.3 months, respectively. The risk of death decreased by 36% and the risk of disease progression or death reduced by 44% in the Pembrolizumab combined treatment group (94) (Table IV).

## 6. Combination of radiotherapy with PD-1/PD-L1 inhibitors

Radiotherapy (RT) has profound immunological effects. Basic research studies have demonstrated that RT can improve the efficacy of PD-1 inhibitors (95). Cancer cells can be killed by radiation. RT activates the immune system by triggering the release of tumor antigens (96). Basic research and clinical trials have revealed that RT synergizes with immunotherapy when

applied together (97,98). A study has shown that PD-1 inhibitors combined with RT can activate CTLs and reduce immunosuppressive cells (99). A combination of RT and PD-1/PD-L1 inhibitors significantly improves the survival rate and reduces the tumor volume in mice (100). Compared with the control group, co-treatment of RT and PD-1 significantly increased the expression of PD-L1, CD8+T cells and interferon- $\gamma$  in tumor cells (101). Clinical studies have provided evidence that anti-PD-1 therapy can significantly improve the control rate and OS rate of patients with melanoma brain metastasis who received stereotactic radiotherapy (102). Clinical trials of the efficacy of Nivolumab combined with RT in the treatment of NSCLC (NCT02768558) and glioblastoma (NCT02617589) are under way (103).

Immunotherapy amplifies the rare systemic effects of radiotherapy, while radiotherapy renders immune-excluded tumors quickly responsive to immunotherapy (104). MDSCs have been implicated in development of radioresistance. Accumulation of MDSC in the tumor microenvironment promotes tumor relapse by directly affecting tumor cell survival and indirectly affecting local T cell suppression (105). A combination of irradiation (IR) and anti-PD-L1 therapy enhanced the activation of CD8+ T cells and inhibition of TUBO tumor growth. CD8+ T cells induce the apoptosis of MDSCs through TNF- $\alpha$  following combination therapy (95). PD-L1 is upregulated in the tumor microenvironment following IR. IR-induced increases in tumor-infiltrating lymphocytes (TILs) and upregulation of PD-L1 could provide an opportunity for PD-L1 blockade (106). The combination of IR and anti-PD-L1 treatment optimizes the tumor immune microenvironment and results in tumor regression (95). Local radiotherapy significantly adds to the systemic efficacy of immunotherapy. Combining single-site stereotactic body radiotherapy (SBRT) with pembrolizumab improves response rates in metastatic NSCLC. PD-L1-negative patients benefited from SBRT (107). This study also suggested that the way to improve the effect of immunotherapy was to treat with local radiotherapy to synergize the local and systemic effects of both modalities. Immunotherapy increased the local effect of radiotherapy in all treated sites. Radiotherapy suppresses the tumor burden allowing immunotherapy better to eliminate micro-metastatic disease (108). In addition, a study demonstrated that higher single doses of RT from 12-18 Gy blunt the efficacy of anti-tumor immunity. They also reduce IFN- $\beta$  production and abrogate DC-mediated CD8+T-cell priming, suggesting that RT doses below 12 Gy may be more immunogenic (109). Another study confirmed that a single dose of 15 Gy irradiation results in higher tumor immune cell infiltration than a fractionated (3 Gy x 5) schedule (110). The differences in the above results might be due to differences in the genetic backgrounds of mice, immune competence and immunogenicity of models and radiosensitivity of cell lines (111) (Table IV).

## 7. Combination of intestinal microflora with PD-1/PD-L1 inhibitors

Intestinal microflora influences the effects of immunotherapy in cancers. A previous study reported that oral *Bifidobacterium* can significantly decrease the growth rate of melanoma, promote the maturation of dendritic cells and



Table IV. Combination of chemotherapy or radiotherapy with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Langer <i>et al</i> , 2016	Pembrolizumab + Chemotherapy vs. Chemotherapy	ORR The incidence of grade 3 or worse treatment-related adverse events	55% vs. 29% 39% vs. 26%	(92)
Gandhi <i>et al</i> , 2018	Pembrolizumab + Chemotherapy vs. Placebo + Chemotherapy	Rate of Overall survival at 12 months PFS	69.2% vs. 49.4% 8.8 months vs. 4.9 months	(93)
Paz-Ares <i>et al</i> , 2018	Pembrolizumab + Chemotherapy vs. Placebo + Chemotherapy	PFS OS	6.4 months vs. 4.8 months 15.9 months vs. 11.3 months	(94)
Deng <i>et al</i> , 2014	Irradiation (IR) + Anti-PD-L1 vs. Anti-PD-L1 vs. IR	Tumor volume  The percentage of MDSCs in the total CD45+ cell population	25.59±10.26 mm vs. 587.3±169.1 mm vs. 402.8±76.73 mm 0.38±0.16% vs. 7.33±2.22% vs. 4.78±2.49%	(95)
Sharabi <i>et al</i> , 2015	XRT + Anti-PD-1	Tumor volume T-cell infiltration	Inhibited Increased	(99)
Dovedi <i>et al</i> , 2014	RT + PD-1/PD-L1 blocking	Tumor volume Percent survival	Inhibited Improved	(101)
Ahmed <i>et al</i> , 2016	Stereotactic radiation + Anti-PD-1	local lesions control rates at 6 and 12 months OS rates at 6 and 12 months	91 and 85% 78 and 55%	(102)

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

production of IFN- $\gamma$  and enhance the anti-tumor effect of PD-1 inhibitors (112). The abnormal composition of intestinal flora may affect the response of patients to cancer immunotherapy (113). Transplantation of fecal bacteria improved the anti-tumor effect of PD-1 inhibitors (114). A study has shown that the clinical response of PD-1 inhibitors is dependent on the relative abundance of *Akkermansia muciniphila*. Oral supplementation of *Akkermansia muciniphila* restores the efficacy of PD-1 inhibitors in an IL-12-dependent manner (115). In another study, intestinal microflora regulated the response of anti-PD-1 immunotherapy to melanoma patients (116).

Patients with abundant beneficial intestinal bacteria (*Ruminococcaceae*/*Faecalibacterium*) have improved antigen presentation, effector T cells function in peripheral and tumor microenvironment and strong anti-tumor immune response (117). By contrast, the intestinal harmful bacteria (*Bacteroidales*) weakened antigen presentation and impaired anti-tumor immune response (118,119). Response to PD-1 inhibitors is influenced by the composition of intestinal flora, but not to oral flora (120). Other studies suggest that patients with melanoma responsive to Nivolumab were rich in *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, *B. longum*, *C. aerofaciens* and *E. faecium*. Patients who responded well to Pembrolizumab were rich in intestinal *Dorea formicogenerans* (121,122) (Table V).

## 8. Combination of Traditional Chinese Medicine with PD-1/PD-L1 inhibitors

Diosgenin is a natural steroidal saponin (123). A combination of diosgenin with PD-1 inhibitor suppresses tumor growth, increased T cell infiltration and IFN- $\gamma$  expression in tumor tissues. Diosgenin stimulates the immune cells thereby improving the response rate and therapeutic effect of PD-1 inhibitors (124). Diosgenin treatment downregulates intestinal *Bacteroidetes* but upregulated *Clostridiales*, *Lactobacillus* and *Sutterella* (124).

Icariin possesses a variety of pharmacological and biological activities. Icariin is now under clinical trial for the treatment of PD-L1 positive advanced liver cancer (NCT03236649) and advanced breast cancer (NCT01278810). Pre-clinal studies have shown that Icariin can effectively reduce the tumor load of B16F10 melanoma and MC38 colorectal cancer in mice and its therapeutic effect is T cell-dependent. It increased CD8 T cell infiltration and the number of effector memory T cells. A combination of PD-1 inhibitor and Icariin significantly suppressed tumor growth (125).

*Rhus verniciflua* Stokes (RVS) has been shown to contain a large number of bioactive phytochemicals, including alkaloids, polyphenols and flavonoids, which block the interaction between PD-1/PD-L1 and CTLA-4/CD80. Thus, RVS might be used as an immune checkpoint blocker (126).

Table V. Combination of intestinal microflora with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Sivan, 2015	<i>Bifidobacterium</i> + Anti-PD-L1	Tumor volume	Reduced	(112)
		IFN- $\gamma$ , DCs	Increased	
Routy <i>et al.</i> , 2018	<i>A. muciniphila</i> + Anti-PD-1 vs. Anti-PD-1	PR	69% vs. 31%	(115)
		SD	58% vs. 42%	
		PD	34% vs. 66%	
		Tumor size	<i>A. muciniphila</i> + Anti-PD-1 < Anti-PD-1	
Frankel <i>et al.</i> , 2017	Ipilimumab + Nivolumab vs. Pembrolizumab	RECIST response	67% vs. 23%	(121)
		SD	8% vs. 23%	
Matson, 2018	Fecal material from three responder patient donors + Anti-PD-L1(R) vs. Fecal material from three non-responder patient donors + Anti-PD-L1(NR)	IFN- $\gamma$ , Tumor-infiltrating specific CD8+ T cells	R>NR	(122)
		Tumor volume	R<NR	

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

*Ganoderma lucidum* reduces the proportion of PD-1 positive cells in B lymphocytes. It can, therefore, be used to develop a new type of immunomodulator for the prevention and treatment of cancer (127). The combination of *Ganoderma lucidum* and paclitaxel inhibits the expression of immune checkpoints (PD-1 and Tim-3) and restored TILs. The combination regulates the development of 4T1-breast cancer in mice (128) (Table VI).

## 9. Conclusion and future perspectives

The anti-tumor response rate of PD-1 inhibitors is low. Patients sensitive to PD-1/PD-L1 inhibitors develop drug resistance, tumor recurrence and disease progression and the mortality rate of patients with advanced tumor stages is high. A study has reported that patients with melanoma sensitive to anti-PD-L1 antibody treatment show increased levels of interferon- $\gamma$  and related genes in blood prior to treatment (129). Anti-PD-1 therapy downregulates expression of IFN receptor-related genes and MHC I and upregulates inhibitory receptors on the surface of T cells (10). Furthermore, the inhibitory receptors inhibit the cytotoxic activity of T cells and these effects can be attributed to drug resistance against ICIs. Several basic and clinical studies are exploring effective combination and sequence of PD-1/PD-L1 inhibitors and other anti-tumor therapies to induce tumor cell immunogenicity and improve effectiveness of anti-tumor effect of PD-1/PD-L1 inhibitors. These advances will provide effective therapies for patients who are unresponsive to current treatment regimens. However, development of these combination therapies possesses several challenges.

Development of effective antineoplastic therapy should consider medical costs and adverse reactions for each treatment. Therefore, it is necessary to determine predictive biomarkers for individualized therapy, so as to predict efficacy

and adverse reactions of PD-1/PD-L1 inhibitors. At present, some patients are not sensitive to PD-1/PD-L1 inhibitors. Lack of biomarkers for predicting response rate limits the effectiveness of clinical treatment strategies, thus there is need to screen novel biomarkers for predicting immunotherapy responses in patients.

In order to increase the proportion of patients benefiting from PD-1/PD-L1 inhibitors, studies should explore potential predictive biomarkers for anti-tumor treatment. PD-L1 expression is a potential biomarker for predicting effectiveness of PD-1/PD-L1 immunotherapy on patients with cancer thus identifying patients who may benefit from immunotherapy. Expression of PD-L1 is associated with several TILs and activated tumor antigen-specific T cells induces expression of PD-L1 (130,131). However, expression of PD-L1 in tumor tissues is heterogeneous and changes with tumor treatment (132,133). Several staining antibodies are used in immunohistochemical methods (IHC) to detect PD-L1 expression and the staining techniques (manual and automated) vary (134-136). Currently, effectiveness of PD-L1 detection as an anti-tumor immune response index is still controversial. The association between the expression of PD-1 or PD-L1 at the tumor site and disease outcome varies in patients with different tumors (137-139). Therefore, it is difficult to achieve consistent results with PD-L1 detection, hindering application of anti-PD-1/PD-L1 therapy as precision medicine.

TIL in tumor tissue demonstrates the presence of immune response by the body (140). TIL positive + PD-L1 positive group show improved PD-1/PD-L1 inhibitor immune response compared with TIL negative + PD-L1 positive group. This implies that the number of TIL can predict efficacy of PD-1/PD-L1 inhibitors (141). TIL mainly infiltrates into tumor nests, tumor stroma and tumor invasive margins of tumor tissues and different parts have different associations

Table VI. Combination of Traditional Chinese Medicine with PD-1/PD-L1 inhibitors.

Author(s) (year)	Interventions	Primary end point(s)	Results	(Refs.)
Dong <i>et al</i> , 2018	Diosgenin + anti-PD-1 vs. diosgenin vs. anti-PD-1	Mean tumor weigh	1,980.00±861.22 mg vs. 3,203.33±641.43 mg vs. 2,530.00±584.04 mg	(124)
Hao <i>et al</i> , 2019	Icariin + anti-PD-1 + anti-CTLA-4 vs. anti-PD-1 + anti-CTLA-4	Average inhibition rates	65% vs. 34.2%	(125)
Li <i>et al</i> , 2019	Rhusverniciflua Stokes	The IC50 of blocking PD-1/PD-L1 interaction	26.22 µg/ml	(126)
Wang <i>et al</i> , 2019	Ganoderma lucidum	PD-1	Decreased	(127)
Su <i>et al</i> , 2018	Ganoderma lucidum + Paclitaxel	Tumor weight Tumor infiltration lymphocytes PD-1, Tim-3	Decreased Increased Inhibited	(128)

PD-1, programmed cell death protein-1; PD-L1, programmed death protein ligand-1.

with therapeutic effects (142). Therefore, it is necessary to further determine the association between the quantity and quality of TIL and other infiltrating immune cells and tumor immune response. In addition, local radiotherapy is effective in inducing inflammation, which may benefit patients without sustained immune response (99). Radiotherapy should not be used in patients with significant tumor infiltration, as this may impair the ongoing immune response (143,144). Effects of different therapies on immune response should be considered when designing combination therapies with chemotherapy and targeted therapy.

Mismatch repair (MMR) is a set of susceptibility genes isolated from hereditary non-polyposis colorectal cancer. Mutations in these gene leads to loss of mismatch repair function, resulting in microsatellite instability (MSI) which is prone to tumors (145). Microsatellite instability high (MSI-H) attracts tumor-infiltrating lymphocytes (TILs) and upregulates PD-L1 expression in tumor epithelial cells (146). MMR deficiency (MMR-D) type solid tumors have more tumor neoantigens to enhance anti-tumor immune response and show an improved response to PD-1 monoclonal antibody, thereby improving immune suppression and restoring anti-tumor immunity (147). MMR-D is a predictor for anti-PD-D efficacy. However, MMR-D only occurs in a small number of patients. Further pre-clinical and clinical research should be performed before clinical application.

The therapeutic effect of PD-1 inhibitors is high in patients with a high mutation load of tumor mutation burden (TMB). Tumor cells with high TMB expression have higher levels of neoantigens, which stimulate a stronger anti-tumor immune response (148). TMB and PD-L1 have similar predictive function. However, TMB is not associated with PD-L1 expression. TMB is an important and independent predictive biomarker, which can predict the effectiveness of ICIs (149).

Further studies should explore ways to alleviate side effects of immunotherapy. Resistance of malignant tumors against PD-1/PD-L1 inhibitors can be overcome by use of combination therapy of PD-1/PD-L1 inhibitors (150).

Notably, a combination of anti-PD-1/PD-L1 therapy is more effective compared with use of anti-PD-1/PD-L1 inhibitors alone. However, combination therapy is associated increased side effects (88). A study revealed that patients younger than 65 years old benefit more from nivolumab plus ipilimumab treatment than patients older than 65 years old. Therefore, combination therapies with ICIs should be carefully chosen for patients >65 years of age (151). Goals for treatment of patients with advanced cancer is usually palliative, prolonging survival, controlling symptoms and improving quality of life. Therefore, studies should explore combination therapies with ICIs and fully understand the toxic effects of immunotherapy, chemotherapy and radiotherapy to make sound treatment decision. Side effects such as immune disorders caused by ICIs are called immune-related adverse events. Common adverse reactions include diarrhea, fatigue, itching, rash, nausea and loss of appetite. Severe adverse reactions include severe diarrhea, colitis, myocarditis and cardiac insufficiency, liver dysfunction, pneumonia and glomerulonephritis (88,152,153). Serious side effects may require discontinuation of treatment, although patients may have an immune response thereafter. Intravenous corticosteroids or immunosuppressive drugs should be given if necessary. Some treatment-related autoimmune responses, such as rashes, are associated with improved prognosis (154). This implies that occurrence of adverse reactions is manifested by activation of immune system and represents action of PD-1/PD-L1 inhibitor, which eliminates tumors. There is an overlap between autoimmune reaction and anti-tumor immune reaction. Further studies should be performed to explore adverse drug reactions associated with immunotherapy.

Clinical application of molecular targeted drugs is associated with challenges such as acquired drug resistance and side effects which need to be minimized. Several studies are exploring the development of molecular targeted drugs with higher efficiency and fewer side effects (155,156). Studies exploring sequence, dosage and safety of PD-1 inhibitors and EGFR should be performed (157,158). Development of combination therapies will improve efficacy and reduce side effects

of molecular targeted drugs. A number of clinical studies on combination therapies between chemotherapy and radiotherapy with immunotherapy are underway. An open-label, randomized phase 3 study showed that pembrolizumab+chemotherapy significantly improved OS in the total population. The data support the use of pembrolizumab+platinum +5-FU as new first-line standards of care for recurrent/metastatic head and neck squamous cell carcinoma (NCT0235803) (159). In the KEYNOTE-189 and KEYNOTE-407 studies (phase III), PFS and OS were significantly longer in patients treated with pembrolizumab and chemotherapy compared with those in patients treated with chemotherapy alone (93,94). Anti-PD-1 therapy enhances the efficacy of radiotherapy in metastatic gastric cancer treatment by increasing the CD8<sup>+</sup> T cell/effecter regulatory T cell ratio in TILs (160). Another study showed that patients with metastatic NSCLC treated with nivolumab or pembrolizumab+radiotherapy did not have increased grade 3/4 immune-related adverse events (161). The combination of chemotherapy and radiotherapy with PD-1/PD-L1 inhibitors induces lasting immune response in treatment of tumors when other treatment strategies fail.

Despite a significant number of basic and ongoing clinical trials aimed at improving effectiveness of combination therapies, intestinal flora combined with PD-1/PD-L1 inhibitors is a novel approach for cancer treatment. However, differences between basic and clinical trial results occur due to high variability of bacteria in intestinal tract and the effects produced by bacteria in the laboratory. Less diverse bacteria used in basic trials may not fully represent the complicated environment in the intestinal tract. Therefore, further studies should explore the mechanism of intestinal flora, side effects, optimal dosage and species for human use for development of effective combination therapies.

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### Authors' contributions

All authors contributed to the content development of this article. XK conceived and designed the study. PL, CL and YG reviewed literature and collated appropriate information. XK, YY, YP, FW, ZB and XD wrote the manuscript. HS generated figures and tables. JM reviewed and edited the manuscript. All authors approved the final manuscript

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### Competing interests

Authors declare that they have no competing interests.

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