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Combinatorial therapy of immune checkpoint and cancer pathways provides a novel perspective on ovarian cancer treatment (Review)

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Abstract. An increasing number of studies have reported that immunotherapy serves a significant role in ovarian cancer treatment. In recent years, blockade of checkpoint pathways, including programmed death-ligand 1 (PD-L1)/programmed death-1 and cytotoxic T-lymphocyte-associated protein 4, has demonstrated significant clinical and preclinical benefits in the treatment of ovarian cancer. Additionally, tumor-associated angiogenesis and homologous recombination deficiency frequently occurs in patients with high-grade ovarian cancer, which makes cancer cells more susceptible to targeted therapies, including therapies targeting poly (ADP-ribose) polymerase inhibitor, and anti-angiogenic approaches. Additionally, targeted therapy has been associated with elevated PD-L1 expression in tumor cells, increased T-cell infiltration in tumors and dendritic cell stimulation. This synergistic effect provides the rationale for the joint application of targeted therapy and immunotherapy. Checkpoint blockades are able to elicit durable antitumor immune reactions and complement the transient antitumor effect of targeted therapies. The current review discusses the underlying mechanism of these therapies and novel developments in combined therapy for the treatment of ovarian cancer.

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1. Introduction

Ovarian cancer is one of the most lethal gynecological tumor types with 22,440 new cases and 14,080 associated mortalities reported in 2017 in the USA (1). Surgery is the optimal treatment for early-stage ovarian cancer, whereas platinum-based chemotherapy followed by debulking surgery is the standard therapy for advanced ovarian cancer (2). Although developments in surgery and chemotherapy have enhanced clinical outcomes, improvement of the 5-year survival rate of <30% is required (3). In recent decades, as the concepts of synthetic lethality and immune escape have emerged (4,5), several targeted drugs that differentiate from conditional chemotherapy have been applied to ovarian cancer treatment. Ovarian cancer cells, particularly high-grade serious ovarian cancer (HGSOC) cells, exhibit a high mutation rate of BRCA1 DNA repair-associated (BRCA1)/BRCA2, which is responsible for error-free repair of DNA double-strand breaks (DSB), leading to a homologous recombination defect (HRD) (6). Olaparib, the first inhibitor of the enzyme poly (ADP-ribose) polymerase (PARP) to be approved by the USA Food and Drug Administration, was demonstrated to be effective in BRCA mutation-positive ovarian cancer and was shown to kill HRD cells via tumor-selective synthetic lethality (7). Bevacizumab, an anti-angiogenic agent against vascular endothelial growth factor (VEGF), was reported to successfully improve progression-free survival (PFS) time among patients with recurrent ovarian cancer. However, no significant change in the overall survival (OS) time was identified (8). Another class of drugs that attract attention in cancer treatment are the immunotherapeutic agents, particularly drugs that inhibit the programmed death-ligand 1 (PD-L1)/programmed death-1 (PD-1) or B7/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways. Clinical studies have suggested that checkpoint blockades can stimulate an immune response against tumors (9). Targeted and checkpoint therapies are associated with the action of the immune system against the

tumor, as presented in Fig. 1. Based on their complementary and non-overlapping immune responses, the current review outlines novel combined therapies for the treatment of ovarian cancer (10).

2. Role of the PD-1/PD-L1 pathway in ovarian cancer

PD-1 is a member of the cluster of differentiation (CD)28 family and is expressed on the surface of activated T cells, B cells, dendritic cells and macrophages. PD-L1, a ligand of PD-1, is a member of the B7 family and is highly expressed in a broad range of malignant tumor types. It has been demonstrated that the interaction between PD-1 and PD-L1 negatively regulates T-cell proliferation, tumor killing and cytokine secretion, and enhances the number of regulatory T cells (Tregs), resulting in the maintenance of self-tolerance, as well as cancer progression (11-14). In a mouse ovarian model, compared with ovarian cells overexpressing PD-L1, a PD-L1-deleted ovarian cell line exhibited a higher number of cytotoxic lymphocytes and an improved prognosis (15). Several studies have demonstrated that ovarian cancer is an immunogenic tumor. It was shown that patients with increased intraepithelial $CD8^+$ T-cell infiltration and a higher $CD8^+/CD4^+$ T-cell ratio exhibited improved OS, with a median survival time of 55 months, compared with that in patients with lower frequencies of T cells (26 months) (16). Poor OS and PFS in patients are associated with high expression levels of PD-L1 in tumor cells (17). Small interfering RNA has been utilized to downregulate PD-L1 expression in dendritic cells, which significantly increases the proportion of memory-like T cells in mouse bone, as well as tumor specific $CD8^+$ T cells, demonstrating that the PD-1/PD-L1 pathway influences the immune system directly (18).

In 2010, the first phase I clinical trial of the anti-PD-1 antibody nivolumab (a fully human immunoglobulin G4 monoclonal antibody), with a dose ranging from 0.3 to 10 mg/kg, was administered to 39 patients with solid tumors, including advanced melanoma, non-small cell lung cancer, renal cell carcinoma (RCC), prostate cancer and colorectal cancer (19). The response rate was 7.7%, and nivolumab demonstrated good tolerance, as only one serious adverse event of inflammatory colitis occurred in a patient with melanoma who received five doses at 1 mg/kg. In 2012, a phase Ib study of anti-PD-L1 antibody in patients with advanced solid tumors demonstrated clinical benefit against ovarian cancer (20). Among 17 patients with ovarian cancer, 16 patients receiving 10 mg/kg exhibited a partial response, of the 16 patients, one patient exhibited a partial response, and 3 patients demonstrated stable disease for ≥24 weeks. On the basis of the aforementioned clinical data, the first clinical trial to explore the effects of nivolumab against ovarian cancer was conducted by Hamanishi *et al* (21). Among 20 patients with platinum-resistant ovarian cancer, 2 patients exhibited a durable complete response (in the 3 mg/kg cohort) and 1 patient exhibited a partial response (in the 1 mg/kg cohort). The disease control rate in all 20 patients was 45% and the median PFS time was 3.5 months. However, drug-associated treatment-emergent adverse events (TEAEs) occurred in 19 of the 20 patients (95%). Among them, 8 patients (40%) experienced adverse events of grade 3 or 4, including hypothyroidism, lymphocytopenia, fever, arrhythmia, arthralgia, and increased alanine aminotransferase and aspartate aminotransferase levels. In an ongoing phase Ib study of avelumab, an anti-PD-L1 antibody, data from 23 patients with recurrent or refractory ovarian cancer were analyzed (22). A total of 4 patients experienced a partial response, 2 patients exhibited >30% tumor remission and the median PFS was 11.9 weeks. TEAEs occurred in 18 patients (78.3%) and 2 patients (8.7%) experienced grade ≥3 drug-associated TEAEs, including increased lipase and creatine kinase levels, and autoimmune myositis, which led to treatment discontinuation. No patient experienced serious drug-associated TEAEs. The most commonly reported drug-associated TEAEs included fatigue, nausea and diarrhea. In one previous case report, a patient with radiation- and chemotherapy-resistant HGSOC demonstrated a notable complete response to the anti-PD1 immune checkpoint inhibitor pembrolizumab (23).

PD-1/PD-L1 pathway inhibitors are commonly used for patients with advanced ovarian cancer, or for patients where chemotherapy or radiotherapy failed to demonstrate an effect. However, a major limitation of immunotherapy is the extent of disease burden, which keeps the antitumor efficacy in check (24). Therefore, a combination of agents targeting the PD-1/PD-L1 pathway and other cancer pathways, may offer a promising novel therapy against ovarian cancer in the future.

3. Role of the CTLA-4 pathway in ovarian cancer

In the process of tumor killing, B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells bind to CD28 on T cells and serve a crucial role in the activation of the T cells, When the two molecules bind together, it evokes the proliferation of the T cells (24). CTLA-4 is a CD28 homolog with stronger binding affinity to B7 and is expressed predominantly on T cells, including Tregs (25). In contrast to initiating immune activation, CTLA-4 binding to B7 produces a negative signal that suppresses the immune system (26). The proportion of CD28:B7 binding compared with CTLA-4:B7 binding regulates whether the T cells undergo activation or suppression (27). The use of CTLA-4 inhibitors to reverse T-cell suppression is a promising therapy to promote the activation of immune cells against tumors. As demonstrated in an *in vivo* experiment, CTLA-4 inhibitors exhibit a capacity to improve the effect of chemotherapy and reverse the tumor suppressive environment in mice (28). In 2003, a CTLA-4-blocking antibody, ipilimumab, was administered to 7 patients with melanoma and 2 patients with ovarian carcinoma who had previously accepted therapeutic vaccine (29). Of the 2 patients with ovarian carcinoma, 1 patient exhibited a 43% reduction in the ovarian tumor marker cancer antigen (CA)-125 in the blood, beginning 2 months after treatment. However, this effect was not sustained. The other patient demonstrated a rapid increase in CA-125 levels upon treatment, but achieved a plateau 1 month after the infusion.

In order to acquire more information regarding the toxicity and antitumor effects of the CTLA-4 antibody ipilimumab, 9 patients with stage IV ovarian carcinoma were recruited (30). Each patient had received the same therapeutic vaccine and the same dose of ipilimumab. The data demonstrated that 3 patients achieved stable disease

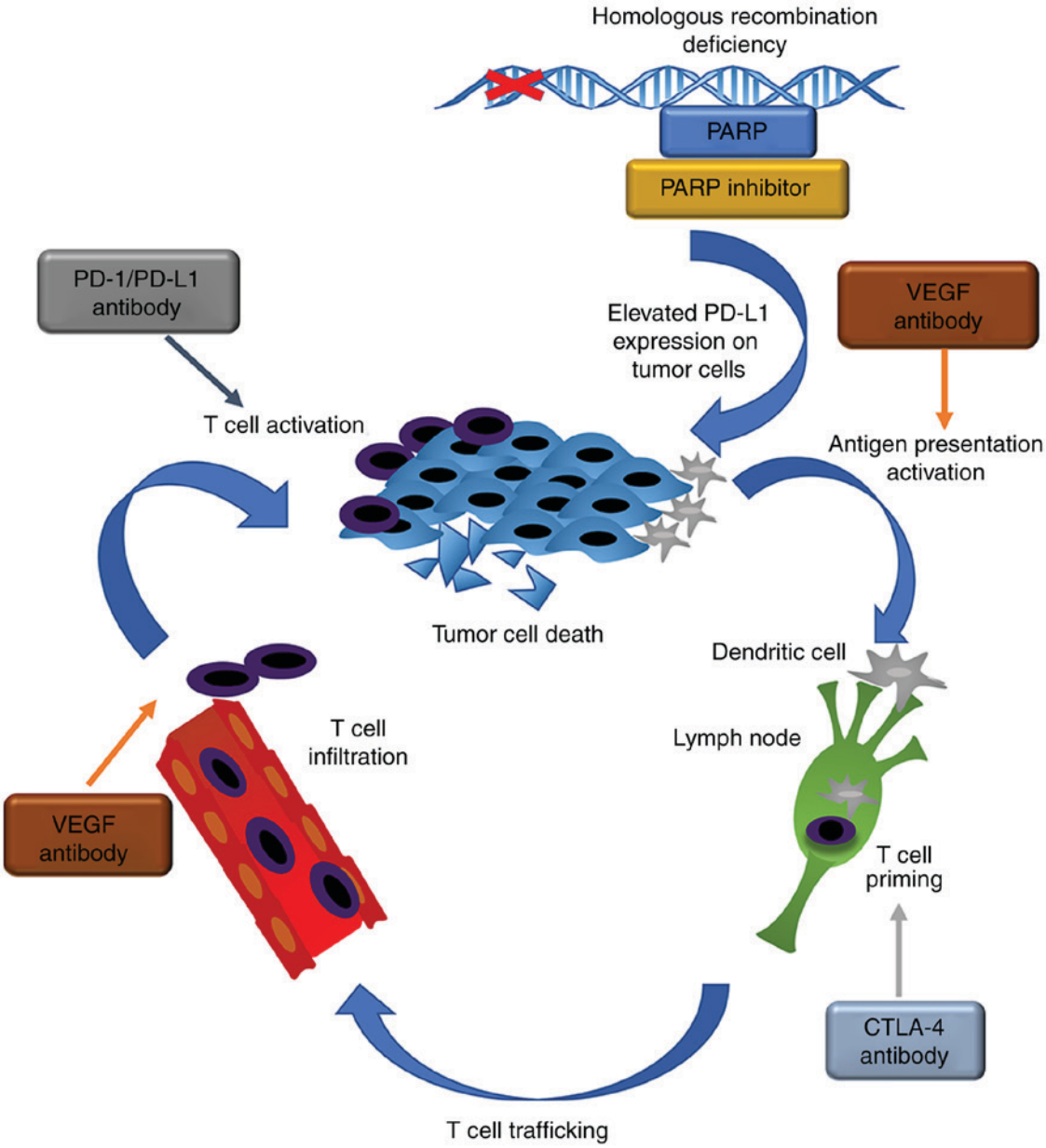

Figure 1. Mechanism of targeted therapy and immune therapy. Tumor antigens are captured and processed by dendritic cells, and then dendritic cells transfer to tumor-draining lymph nodes, where they present tumor-associated antigens to T cells and stimulate them. Activated effector T cells traffic from the lymph nodes to vessels and infiltrate into the tumor bed, where they kill cancer cells. Checkpoint therapies (anti-CTLA4 or anti-PD-1/PD-L1) induce activation and priming of T cells in the lymph node and tumor site, resulting in tumor-killing. Tumors with homologous recombination deficiency elicit higher levels of tumor antigen expression and increase PD-L1/PD-1 expression on the surface of tumor-infiltrated immune cells. PARP inhibitors enhance the PD-L1 expression on the surface of tumor cells. VEGF inhibitors complement T-cell checkpoint therapies by improving the maturation and activity of dendritic cells, as well as T-cell infiltration into tumors. PARP, poly (ADP-ribose) polymerase; VEGF, vascular endothelial growth factor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

for 2, 4 or >6 months. Among the patients, 1 patient who was treated with an initial dose of ipilimumab achieved a significant drop in CA-125 level. Although the level was not maintained, a second infusion led to a more rapid decrease in CA-125 level. Furthermore, hepatic metastasis regressed gradually and CA-125 was sustained at a low level when an additional nine infusions of ipilimumab were administered over almost 4 years. The occurrence of a grade 1 rash was the only adverse event in this patient, whereas grade 3 diarrhea was observed in 2 other patients (22.2%) and 1 patient (11.1%) developed Sweet's syndrome accompanied with tumor progression.

Due to the involvement of different ligands and functions, combinatorial targeting of PD-1/PD-L1 and CTLA-4 exhibited synergistic antitumor activity in a mouse model of colon adenocarcinoma (31). In a clinical trial, accumulating evidence revealed that patients with combined therapy demonstrated a higher overall response rate (ORR) and longer PFS time. However, the number of TEAEs that occurred following combined therapy increased significantly compared with that following single therapy (32,33). Therefore, there is a requirement to identify agents that exhibit a synergistic effect with checkpoint pathway inhibitors, but do not increase the number or severity of TEAEs.

4. Role of PARP inhibitors in ovarian cancer

PARP inhibitors have been widely studied in HGSOCs with either germline or somatic mutations of BRCA1/BRCA2. Clinical trial data have suggested that olaparib, 400 mg twice daily, can exhibit a significant antitumor effect in patients who are confirmed to be either platinum-sensitive or platinum-resistant (34,35). Compared with those patients who received a placebo and experienced a median PFS time of 5.5 months, the patients undergoing olaparib treatment experienced a significantly increased PFS time, with a median of 19.1 months. The most common adverse events were anemia, abdominal pain and intestinal obstruction. Certain patients with HGSOC undergoing olaparib maintenance therapy attained a long-term (LT) response that lasted >2 years. Germline or somatic BRCA1/BRCA2 mutations were correlated with LT response (36). However, even for patients with wild-type BRCA, the median PFS time improved moderately compared with that in the placebo group (7.4 vs. 5.5 months) (37). In another study, niraparib, a selective inhibitor of PARP-1/2, was administered to patients with platinum-sensitive, recurrent ovarian cancer by maintenance therapy (38). Those receiving niraparib exhibited a longer PFS time compared with those receiving placebo, regardless of the presence or absence of BRCA mutations or the HRD status. However, there remains a substantial number of patients with mutations that resist this agent (39).

With advancements in preclinical and clinical trials, the mechanisms involved in PARP inhibitor resistance have been partly uncovered (40). The most common mechanism of resistance is non-homologous end-joining (NHEJ), which competes with homologous recombination (HR) in the process of DSB repair (41). MicroRNA-622 (miR-622) serves a pivotal role in the modulation of the competing association between HR and NHEJ. As demonstrated in an *in vitro* experiment, miR-622 mitigates the effect of PARP inhibition and platinum in BRCA1-mutant cells (42). Furthermore, data from The Cancer Genome Atlas suggests that overexpression of miR-622 is associated with PFS and OS among patients with BRCA1 mutation and BRCA1 hypermethylation (42). In certain cases, BRCA-2 mutant cells can undergo a secondary mutation and restore the capacity of the BRCA-2 functional protein that repairs DNA damage in PARP, which results in tolerance to PARP inhibitors (43).

Although significant clinical benefits have been achieved using PARP inhibitor therapy for BRCA1/BRCA2-mutated ovarian cancer, associated resistance remains the main challenge to overcome. Therefore, the combination of PARP inhibitors with other agents may be a useful strategy to overcome this problem.

5. Role of anti-angiogenesis agents in ovarian cancer

Angiogenesis has been confirmed as an effective target for therapy in several tumor types, including ovarian cancer, renal cell carcinoma and pancreatic cancer (44). The VEGF family and their cognate receptors, the angiopoietin family of ligands, are the main elements that contribute to tumor angiogenesis (45,46). VEGF serves a critical role in the progression of ovarian cancer, ascites formation and the metastasis of tumor cells (47). Increasing evidence indicates that ovarian cancer cells exhibit high expression of VEGF and VEGF receptor (VEGFR), which are associated with ovarian cancer development and progression (48). Bevacizumab, a humanized recombinant anti-VEGF monoclonal antibody has been applied in the treatment of recurrent ovarian cancer. Clinical data revealed no significant difference in the median PFS time of patients treated by chemotherapy compared with the median PFS time of patients treated with a combination of chemotherapy and bevacizumab followed by maintenance chemotherapy (10.3 vs. 11.2 months) (8,49). However, the bevacizumab-treatment group exhibited a prolonged median PFS time of 14.1 months. The benefit is greater with respect to patients at high risk for disease progression. Patients with platinum-sensitive and -resistant cancer exhibited improved PFS and ORR times when treated with bevacizumab therapy (50,51). Cediranib, an oral inhibitor of VEGFR1-3 and c-Kit7, when combined with chemotherapy and followed by a maintenance therapy, yielded a significant improvement in PFS time among women with recurrent, platinum-sensitive ovarian cancer. The predominant cause for discontinuation of the therapy was an increase in dose-associated toxicity (52).

Although the target of anti-angiogenic agents is genetically stable, drug tolerance cases have been observed in pre-clinical and clinical settings. The most common drug resistance mechanisms are associated with the evasion of anti-angiogenic agents, as well as pre-existing indifference to anti-angiogenic agents (44,53). In addition, the immune situation appears to be associated with the anti-angiogenic effect. Groups with immune gene upregulation have been demonstrated to repress angiogenesis-associated gene expression and exhibit an improved OS time (54).

6. Combinatorial therapy of PARP inhibitor and checkpoint inhibitor in ovarian cancer treatment

An increasing number of studies have indicated that targeted therapies are able to stimulate the immune response of the host. Therefore, acknowledging the immunological effect of targeted drugs, as well as the crosslink between immunotherapy and targeted therapy, may provide improved combined therapy for patients with cancer (55). Ovarian cancer with BRCA1/2 mutation has been identified as a rational candidate for immunotherapy and targeted therapy (56). In HGSOC, tumors harboring HR-deficient/BRCA1/2 mutations demonstrated a higher neoantigen load and increased numbers of $CD3^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TILs) (57). Elevated levels of PD-1 and PD-L1 expression on tumor-infiltrating immune cells was also observed compared with that in HR-proficient tumors, which indicated that BRCA1/2-mutated HGSOCs may be more sensitive to PD-1/PD-L1 inhibitors compared with HR-proficient HGSOCs. It has been suggested that increased expression levels of PD-1/CTLA-4 attenuates cytotoxic T lymphocytes. Cisplatin treatment coupled with dual PD-L1 antibody and CTLA-4 antibody therapy substantially augmented antitumor immunity in BRCA1-deficient mice, producing a systemic immune response (57). This response consisted of enhanced dendritic cell activation and reduced number of suppressive forkhead box $P3^+$ Tregs, and was augmented in the activation of tumor-infiltrating cytotoxic

Table I. Ongoing clinical trials with combined targeted therapy and checkpoint therapy in ovarian cancer.

Clinical trial ID	Tumor type(s)	Targeted therapy	Immunotherapy	Phase	Status
NCT02657889	Ovarian cancer, breast cancer	Niraparib (PARP inhibitor)	Pembrolizumab (anti-PD-1)	I, II	Active, not recruiting
NCT02484404	Ovarian cancer, triple-negative breast cancer, lung cancer, prostate cancer, colorectal cancer	Olaparib (PARP inhibitor), cediranib (VEGFR-tyrosine kinase inhibitor)	Durvalumab (anti-PD-L1)	I, II	Recruiting
NCT02734004	Ovarian cancer, breast cancer, small cell lung cancer, gastric cancer	Olaparib (PARP inhibitor)	Durvalumab (anti-PD-L1)	I, II	Active, not recruiting
NCT03363867	Ovarian cancer	Bevacizumab (antibody against VEGF) Cobimetinib (MEK inhibitor)	Atezolizumab (anti-PD-L1)	II	Not yet recruiting
NCT02571725	Ovarian cancer	Olaparib (PARP inhibitor)	Tremelimumab (anti-CTLA-4)	I, II	Recruiting
NCT02953457	Ovarian cancer	Olaparib (PARP inhibitor)	Durvalumab (anti-PD-L1), tremelimumab (anti-CTLA-4)	I, II	Recruiting

PARP, poly (ADP-ribose) polymerase; VEGFR, vascular endothelial growth factor receptor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

$CD8^+$ and $CD4^+$ T cells. A previous study demonstrated that melanoma patients with somatic mutations and higher levels of tumor neoantigen are more susceptible to anti-CTLA-4 treatment (58). Higuchi *et al* (59) identified that a combination of CTLA-4 antibody and PARP inhibitors can enhance T-cell function and increase the amount of novel lymphocyte clones, resulting in a lasting and improved anti-ovarian cancer effect. In addition, increased levels of interferon-γ (IFN-γ) induced by combined therapy, amplified the therapeutic benefit. Another checkpoint inhibitor, anti-PD-1 antibody, did not demonstrate any significant effect on survival when co-administrated with PARP inhibitor. This unexpected result may be due to a lack of activation of lymphocytes, rather than the reversal of T-cell suppression. An *in vivo* study revealed that incorporation with PD-L1 blockade can sensitize patients to PARP inhibitor therapy (60). Furthermore, in contrast to each treatment alone, no increase in serious complications occurred. Jiao *et al* (60) identified that when the breast cancer MDA-MB-231 cell line was exposed to PARP inhibitor, the surface expression level of PD-L1 was upregulated, as expected. PARP inhibitors led to an increased level of PD-L1 expression through deactivation of glycogen synthase kinase 3β (GSK3β), and elevated PD-L1 expression attenuated T-cell function and weakened the effect of PARP inhibitors against cancer.

A BR5-AKT (BRCA1-deficient) tumor model exhibited a high level of Akt activation that inhibited GSK3β (61), which may have masked the function of PARP inhibitor. Therefore, a suitable ovarian cancer model is critical to identify the synergistic effect of PD-L1 blockade and PARP inhibitor. In a case report, a 38-year-old woman with platinum-refractory advanced ovarian adenosquamous carcinoma, who failed to respond to prior multiline chemotherapies and anti-angiogenic agents, received significant clinical benefit from a combination of targeted therapy (olaparib and pazopanib) and PD-1 inhibitors (pembrolizumab and nivolumab). The patient survived for >15 months and took nivolumab as maintenance therapy (62). A phase I study to evaluate the safety and efficacy of the combination of PARP inhibitor and anti-CTLA-4 antibody was conducted in women with BRCA mutation-associated recurrent ovarian cancer (63). No dose-limiting toxicities were identified and grade 1/2 toxicities were almost consistent with prior studies that used immune checkpoint inhibitors. Furthermore, all patients who received the combined therapy demonstrated a decrease in tumor size and CA-125 levels.

Based on the aforementioned discussion, the combination of PARP inhibitor and PD-L1 blockade can enhance the antitumor effect without an increase in serious complications. This supports the power of combined therapy by maximizing treatment response while minimizing toxicity.

7. Combination of anti-angiogenic and checkpoint agents in ovarian cancer

Combinational therapy of bevacizumab and cisplatin was applied to a xenograft ovarian cancer model, and the antitumor efficacy of cisplatin was demonstrated to be amplified (64). In addition to possessing proangiogenic properties, VEGF-A serves a critical role in the immunosuppressive

tumor environment, as it is associated with an inhibition of dendritic cell maturation, an accumulation of myeloid-derived suppressor cells (MDSCs) and an induction of Tregs (65,66). Furthermore, PD-1 and CTLA-4 have been verified to be expressed by these cells in the tumor microenvironment (67).

Previous studies have observed that VEGF enhances the expression of PD-1 expression on intratumoral $CD8^+$ T cells, which could be downregulated via VEGF antibody (68,69). Combined application of anti-PD-1 and VEGF-A blockade produces a stronger and synergistic antitumor effect in tumors with high expression of VEGF, compared with sole therapy (70). In patients with metastatic RCC who received VEGF-targeting agents, an inverse correlation was identified between high PD-L1 expression level and poor survival time (71). In addition, VEGF can upregulate other inhibitory receptors, including T-cell immunoglobulin and mucin domain 3, CTLA-4 and lymphocyte-activation gene 3, in a dose-dependent manner (68). CTLA-4 is highly associated with tumor angiogenesis. Following treatment with ipilimumab, patients with high serum levels of VEGF experience a poor prognosis (72).

The tumor vascular structure also inhibits the expression of adhesion molecules, including E-selectin, intracellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1), which limits the transmigration of TILs (73). Administering ipilimumab in combination with bevacizumab was revealed to increase T-cell infiltration and the expression levels of ICAM1 and VCAM1 in patients with advanced melanoma, in addition to enhancing T-cell accumulation, furthermore, specific cytokines that are critical to tumor inhibition, including C-C-C motif chemokine 10, tumor necrosis factor-α (TNF-α) and interleukin 1-α, were significantly increased following combined ipilimumab and bevacizumab therapy (74). Certain subgroups of patients with combined therapy even developed increased levels of antibody against Gal-1, which is known to promote tumor growth and progression, patients with elevated antibodies to Gal-1 exhibit favorable overall survival and improved clinical outcomes (74,75).

In a phase I trial, high serum levels of angiopoietin-2 (ANGPT2) contributed to tumor angiogenesis correlated with a poor survival and reduced response in patients who underwent immunotherapy (76). ANGPT2 was revealed to induce PD-L1 expression in tumor-associated macrophages and weaken the efficiency of immunotherapy. Furthermore, ipilimumab plus bevacizumab treatment downregulated ANGPT2 expression in sera and in tumors (77). The close association between angiogenesis ability and checkpoint protein expression suggests that the combination of anti-angiogenic and checkpoint treatment may complement each other's limitations and provide novel treatment strategies for ovarian cancer. In 2017, Lee *et al* (78) initiated a clinical trial to evaluate the antitumor effect of the PD-1 inhibitor durvalumab in combination with endothelial growth factor receptor 1-3 inhibitor cediranib or PARP inhibitor olaparib in women with cancer. A total of 9 patients with ovarian cancer were recruited to the durvalumab plus cediranib arm, in addition to 14 patients with gynecological or breast cancer types. The data suggested that 6 patients with ovarian cancer achieved a partial response for >5 to >8 months and a 50% ORR, which is higher compared with that recorded for PD-L1 pathway inhibition (ORR range, 10-20%) (21) or cediranib treatment alone (23% ORR) (79). However, treatment with durvalumab plus cediranib was associated with a higher frequency of TEAEs. A total of 7 patients suffered from grade 2-4 adverse events and 1 patient experienced grade 3 pulmonary hypertension and eventually succumbed.

In the context of the immune microenvironment, a combined treatment of VEGF blockade and checkpoint blockade is a double-edged sword, as it may increase the tumor-killing effect and the associated adverse events. Therefore, the safety of combined therapy in ovarian cancer should be confirmed in larger clinical trials.

8. Conclusions and perspectives

The current review provided a perspective regarding the co-administration of targeted therapy and immunotherapy in ovarian cancer treatment. Targeted therapy is capable of initiating T-cell activation and infiltration, which serves a central role in immunotherapy. High PD-L1 expression is closely associated with a poor prognosis in targeted therapy. PARP inhibitor has been proven to modulate the immune response against an ovarian cancer model with BRCA1 mutation (80). In particular, the amount of peritoneal $CD8^+$ T cells, NK cells, and their production of IFN-γ and TNF-α increases significantly, whereas the percentage of immunosuppressive MDSCs and Tregs decreases following PARP inhibitor therapy. These results conclude that a synergic effect of immune checkpoint blockade in combination with PARP inhibitor is promising. Potent anti-angiogenic treatment also can promote PD-L1 expression and hypoxia, which disables antigen-presenting cells and weakens the activation of T cells. By contrast, elevated serum levels of CTLA-4 have been reported to promote a higher risk of invasive and metastatic potential, a poor response to anti-VEGF therapy, a poor prognosis and a shorter overall survival time (81).

There are clear advantages of combined administration, including the generation of a regime where targeted therapy improves positive immune activation that could further accelerate checkpoint blockade. One additional consideration is whether adding checkpoint inhibition may reverse the immunosuppressive condition of targeted therapy. In certain *in vivo* experiments with BRCA1 mutation, only the concurrent administration of CTLA-4 antibody and PAPP inhibitor elicited dual antitumor effectiveness, and PD-1 antibody failed to demonstrate the same effect as CTLA-4 antibody (59). Combination therapy with two agents attracts research into the potential mechanisms involved. Testing various combinations of targeted treatment with immunotherapy has been performed in numerous clinical trials. The main challenge of combinational therapy is the potential of dual toxicity. Furthermore, the biological and immune system in a mouse model does not completely mimic human cancer. Therefore, there is a lack of evidence on the response to many different combined therapies (82). Understanding immune-associated mechanisms of the targeted pathways may benefit the development of safer combined therapies. Additionally, there are a number of ongoing clinical trials that are evaluating the effect of combined targeted therapy and immunotherapy in ovarian cancer (Table I).

In conclusion, the potential adverse effects of combination therapy and the choice of targeted therapy that may be most effective with a checkpoint antibody requires consideration when applying in a clinical setting.

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Authors' contributions

GZ and ZZ analyzed and interpreted the review. GZ collected the references and wrote the review and CL revised the article. The authors Hb, GC and RC contribute to the acquisition of data and ZZ designed the review. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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