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Immune checkpoint inhibition in ovarian cancer

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Abstract

Recent studies have shown that tumor cells acquire escape mechanisms to evade host immunity in the tumor microenvironment. Two key immune checkpoint pathways mediated by immunosuppressive co-signaling, the first via programmed cell death 1 (PD-1) and PD-1 ligand 1 (PD-1/PD-L1) and the second via CTLA-4 and B7 (CTLA-4/B7), have been previously described. Several clinical trials have revealed an outstanding anti-tumor efficacy of immune checkpoint inhibitors (anti-CTLA-4 antibody, anti-PD-1 antibody and/or anti-PD-L1 antibody) in patients with various types of solid malignancies, including non-small cell lung cancer, melanoma, renal cell cancer and ovarian cancer. In this review, we examine pre-clinical studies that described the local immune status and immune checkpoint signals in ovarian cancer, highlight recent clinical trials that evaluated immune checkpoint inhibitors against ovarian cancer and discuss the clinical issues regarding immune checkpoint inhibitors.

Keywords: biomarker, CTLA-4, immunotherapy, PD-1, PD-L1

Introduction

Ovarian cancer is the principal cause of mortality from gynecological malignancies. At the time of initial diagnosis, more than half of ovarian cancer patients have advanced-stage disease. Furthermore, over 70% of advanced ovarian cancer patients experience relapse after cytoreductive surgery and the combination of paclitaxel and carboplatin as a first-line therapy. There is a limited number of effective second-line chemotherapies, such as pegylated liposomal doxorubicin (PLD) or gemcitabine, thus necessitating the development of new treatment strategies (1, 2). Recent large clinical trials demonstrated the efficacy of targeted molecular drugs for patients with advanced ovarian cancer. An anti-angiogenic drug (bevacizumab, an antibody that blocks vascular endothelial growth factor A) (3–5) and an inhibitor of the enzyme poly-(ADP ribose) polymerase inhibitor (PARPi, olaparib) (6) improved the outcomes of ovarian cancer patients. However, in other clinical trials, tyrosine kinase inhibitors (TKIs) and other targeted molecular therapeutics were largely ineffective (7, 8).

Over the last two decades, remarkable advancements have been made in the research of immunosuppression in the cancer microenvironment with regard to the interaction between immune cells and tumor cells. Burnet postulated the concept of immunological surveillance in 1960s and suggested that developing tumor cells are monitored and eliminated by the host's immune system (9). Unfortunately, it is abundantly clear that tumor cells proliferate and expand in an uncontrolled manner despite immunological surveillance. One mechanism of tumor evasion from immunosurveillance is described as the cancer immunoediting theory (10).

When the TCR of a T cell binds with its specific ligand, for example, on a tumor cell, TCR signaling can be modified by co-signaling pathways (called immune checkpoints) that either enhance (co-stimulate) or suppress (co-inhibit) the signal. Two important suppressive immune checkpoints have been described: firstly, signaling via programmed cell death 1 (PD-1) and one of its ligands (PD-L1; the PD-1/PD-L1 pathway) and, secondly, signaling via CTLA-4 and its ligands B7-1 or B7-2 (the CTLA-4/B7 pathway) (11, 12). Several clinical trials of immune checkpoint inhibitors/blockers, such as anti-CTLA-4 antibody, anti-PD-1 antibody and/or anti-PD-L1 antibody, have shown dramatic anti-tumor effects in patients in some solid tumors (Fig. 1) (13–16), including ovarian cancer (17–22).

Here we provide an overview of the pre-clinical studies that evaluated the local immune status and immune checkpoint signals in ovarian cancer. Moreover, we highlight recent clinical trials using immune checkpoint inhibitors (here, blocking co-inhibition) against ovarian cancer, with an emphasis on a clinical trial conducted in our department. We close with a discussion of the clinical issues regarding the use of immune checkpoint inhibitors.

REVIEW

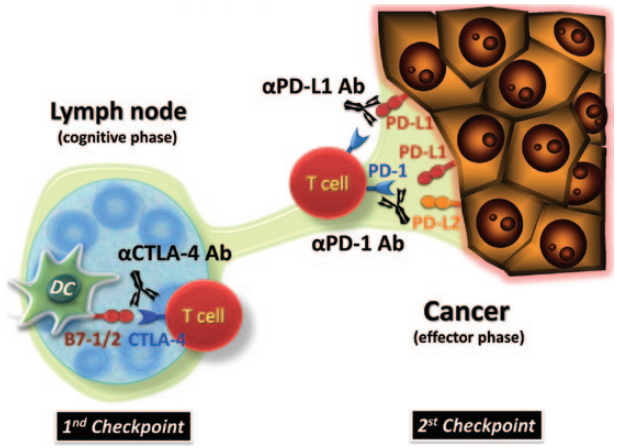

Fig. 1. Immune checkpoint inhibitors in cancer. Anti-CTLA-4 antibody (αCTLA-4 Ab) blocks CTLA-4 binding with B7-1 or B7-2 and inhibits antigen-specific T-cell recognition in the lymph nodes (cognitive phase). PD-1 inhibitors, anti-PD-1 antibody (αPD-1 Ab) and anti-PD-L1 antibody (αPD-L1 Ab) block signaling through PD-1 and the PD-1 ligands (PD-L1 and PD-L2) and block antigen-specific T-cell activation within the tumor microenvironment (effector phase).

The clinical significance of the local immune status in ovarian cancer

The initial evidence for the infiltration of tumor-antigen-specific T cells into the ovarian cancer microenvironment was reported in 1991 (23). Since that report, numerous studies have investigated the impact of T-cell infiltration on patient survival. Notably, researchers demonstrated the prognostic significance of $CD3^+$ tumor-infiltrating T cells (24) and the ratio of $CD8^+$ T cells versus $FOXP3^+$ T_{reg} cells in ovarian cancer. Our previous study also showed that intratumoral $CD8^+$ T cells were significantly associated with progression-free survival (PFS) and overall survival (25). Two recent large-scale studies further highlighted the predictive value of tumor-infiltrating T cells in ovarian cancer (26, 27).

In a meta-analysis published in 2012, 10 clinical studies comprising 1815 patients with ovarian cancer were analyzed (26). This study showed that intra-epithelial tumor-infiltrating T cells were a robust predictor of outcome in ovarian cancer despite substantial between-study heterogeneity. Another noteworthy study was The Cancer Genome Atlas (TCGA) project that analyzed 489 high-grade serous ovarian adenocarcinomas (27). The adenocarcinomas were divided into four subtypes on the basis of their gene content in microarray RNA analysis: immunoreactive, differentiated, proliferative and mesenchymal. The immunoreactive subtype was characterized by chemokine-related genes. The differentiated subtype was associated with genes suggestive of a mature stage of development. The proliferative subtype was defined by proliferative markers. The mesenchymal subtype was characterized by genes suggestive of increased stromal components.

In a follow-up study conducted in 2013, 879 publicly available expression profiles of high-grade serous ovarian carcinoma samples were analyzed for prognostic relevance (28). The results showed that, among the four ovarian cancer signatures identified in the TCGA project, the immunoreactive subtype (n = 280, 32%) was superior to other subtypes as a survival predictor. These studies demonstrated that the local immune response to tumor invasion in ovarian cancer was strongly associated with survival outcome. This gives rise to the next question: what are the determinants of the local immunological activity?

In recent reports, ovarian cancer cells acquired potential escape mechanisms to evade host immunity via several immunosuppressive factors, including a loss of MHC expression (29) and an up-regulation of immunosuppressive factors, such as TGF-β (30), indoleamine 2,3-dioxygenase (IDO) (31) and cyclooxygenases (COX-1 and COX-2) (32). Furthermore, these cells demonstrated enhanced immune checkpoint signals via B7/CTLA-4 and PD-1/PD-L1 in the tumor microenvironment. Taken together, the discovery of these immunosuppressive mechanisms highlights the need to develop new treatment strategies for ovarian cancer.

The B7/CTLA-4 signal

The B7/CTLA-4 pathway involves the B7 molecules, B7-1 (CD80) and B7-2 (CD86), and CTLA-4 (CD152) expressed on antigen-presenting cells and T cells, respectively (33). This pathway suppresses TCR signaling. CTLA-4 was identified in 1987 as the first co-inhibitory molecule (34). This molecule negatively regulates T-cell activation and diminishes the immune response by competing with CD28 (a co-stimulatory molecule) on activated T cells for binding to B7 molecules (35).

In vivo administration of antibodies to CTLA-4 resulted in rejection of tumors in mice (36). CTLA-4 is constitutively expressed on T_{reg} cells. In fact, the therapeutic effects of anti-CTLA-4 antibodies, notably human IgG1 and mouse IgG2, are likely mediated by the inhibition of T_{reg} cell activity (37). Moreover, CTLA-4 knockout mice died by 2 months of age due to systemic autoimmune diseases, suggesting that CTLA-4 inhibition could lead to autoimmune disorders in humans (33). Based on these basic research studies, clinical trials of two anti-CTLA-4 antibodies, ipilimumab and tremelimumab, were initiated in 1999 (38–40). These trials included a small population of patients with ovarian cancer.

B7/CTLA-4 signal inhibitors

Ipilimumab

Ipilimumab is a fully human anti-CTLA-4 mAb. In a pivotal, randomized, double-blind Phase III study conducted in 2010, 676 patients with unresectable Stage III or IV melanoma were randomly assigned to receive ipilimumab plus gp100 melanoma–peptide vaccine, ipilimumab alone, or gp100 peptide vaccine alone. Ipilimumab, regardless of the presence or absence of the gp100 peptide vaccine, improved overall survival, with a 1-year survival rate of 46% for ipilimumab alone versus 25% for gp100 alone (41). This study garnered extensive attention because ipilimumab appeared to be the first new and highly efficacious treatment for advanced melanoma in over a decade. Consequently, ipilimumab was approved in March 2011 for the treatment of unresectable or metastatic melanoma in the USA, followed by more than 40 other countries. In Japan, ipilimumab was approved as an orphan drug for treating malignant melanoma in March 2013. In subsequent studies,

the combination of ipilimumab and nivolumab, an anti-PD-1 immune checkpoint inhibitor, worked synergistically to provide distinctively better clinical activity than either drug used alone.

Ipilimumab can, however, cause serious and severe immune-mediated adverse reactions. The most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis, neuropathy and endocrinopathy. Pneumonitis and other clinically significant immune-mediated adverse reactions were reported at lower frequencies. The US Food and Drug Administration-approved product labeling includes a box warning regarding the severe and fatal immune-mediated adverse reactions.

In a Phase I clinical trial reported in 2003, ipilimumab was administered to seven patients with metastatic melanoma and two patients with ovarian carcinoma (42). These patients received a single 3-mg kg^{-1} dose of ipilimumab. One of the two patients with ovarian carcinoma showed a substantial reduction in the levels of the ovarian tumor marker CA-125 beginning 2 months after the treatment. The other patient had a rapid rise in CA-125 levels before treatment but achieved a plateau 1 month after the infusion, which was accompanied by a reduction in ascites and pain. Unfortunately, the patient's condition rapidly deteriorated after this period.

In a subsequent Phase I repeat-dose study of ipilimumab (3mg kg^{-1}) reported in 2008, nine Stage IV ovarian carcinoma patients pretreated with GM-CSF (GVAX®) were analyzed (42). Of these, three achieved stable disease (SD) and one had a partial response (PR) lasting for 36 or more months. Two cases of Grade 3 inflammatory toxicities that involved the gastrointestinal tract were observed. In both cases, significant diarrhea developed. Treatment with oral corticosteroids accomplished rapid improvement of symptoms in one patient, whereas the other case gradually resolved over several months. The latter patient also had Sweet's syndrome, characterized by a marked peripheral neutrophilia and rash. Biopsy of the pelvic disease showed extensive hemorrhagic tumor necrosis. Seven other patients developed minor inflammatory toxicities.

In light of the positive clinical benefits demonstrated in these studies, despite the small sample sizes, there is an ongoing Phase II trial in patients with recurrent platinum-sensitive ovarian cancer (43). Ipilimumab should be monitored closely for severe or serious immune-mediated adverse events, including pneumonitis and endocrinopathy.

Tremelimumab

Tremelimumab is a fully human monoclonal IgG2 antibody against CTLA-4. In a Phase III trial in patients with advanced melanoma, tremelimumab failed to confer a statistically significant survival advantage over standard-of-care chemotherapy (44). It is important to note that the combination of tremelimumab with other anti-tumor agents failed to improve the treatment outcomes for advanced melanoma. Currently, there is an ongoing Phase I study to evaluate the combination of tremelimumab and a PD-1 inhibitor for patients with ovarian or cervical cancer (Table 1) (45).

PD-1/PD-L1 ligand signaling

PD-1 (CD279) is an immunosuppressive co-inhibitory molecule that belongs to the CD28 family of receptors on T cells. PD-1 was discovered by Ishida *et al.* in 1992, who described it as an induced molecule on T cells undergoing apoptosis (46). Additional studies detected PD-1 expression on mature hematopoietic cells such as T and B cells as well as monocytes following activation (47).

The cognate ligands for PD-1 are the B7-family molecules, PD-L1 (CD274, B7-H1) and PD-L2 (CD273, B7-H2). PD-L1 is expressed in human tonsils, placental syncytiotrophoblasts, monocytes and lungs, where it plays a role in immune tolerance. PD-L2 is mainly expressed on dendritic cells (DCs) under normal physiological conditions (12). The PD-1/PD-L1/L2 signaling pathway has been shown to control excessive autoimmune and inflammatory responses. This pathway plays a key role in immune homeostasis, together with the B7-1/2/CTLA-4 signaling pathway described above (48). The CTLA-4 signaling pathway is primarily involved in the process of antigen presentation in lymph nodes (the 'cognitive' phase), whereas the key role of the PD-1 signaling pathway is to suppressimmune response to target (cancer) cells in peripheral tissue (the 'effector' phase; Fig. 1).

PD-1/PD-L1 signaling in pre-clinical cancer models

Several pre-clinical reports demonstrated enhanced T-cell responses and anti-tumor activity by inhibition of PD-1/PD-L1 signaling. Iwai *et al.* were the first to show that PD-L1-overexpressing tumor cells (a P815 mastocytoma cell line) suppressed the cytotoxic activity of $CD8^+$ T cells in a mouse model (49). Furthermore, tumor eradication was accelerated by the inhibition of PD-1 signaling with anti-PD-L1 or the injection of tumor cells into mice that had a knockout of *Pdcd1* (the gene for PD-1) (49–52).

Some researchers reported that tumor-associated immune cells also used PD-1 signaling to regulate anti-tumor T-cell responses (12, 25,48, 53–56). Tumor-associated myeloid DCs expressed PD-L1 and suppressed T-cell function, whereas PD-1 signal blockade with anti-PD-L1 antibody enhanced T-cell activation via antigen presentation by DCs (53). Plasmacytoid DCs in the tumor-draining lymph nodes produced the immunosuppressive molecule IDO that enhanced the function of T_{reg} cells via PD-1 signaling. Inhibition of PD-1 signaling abrogated the suppressive function of T_{reg} cells (54).

PD-1/PD-L1 signaling in clinical studies of cancer

Besides early clinical studies, many researchers reported that the expression of PD-L1 on human cancer cells and tumor-infiltrating lymphocytes (TILs) was significantly correlated with poor prognosis in several types of solid tumor such as urothelial, gastrointestinal, lung and breast cancers as well as melanoma (25, 53–63).

We previously reported that PD-L1 expression was associated with a poor prognosis in ovarian cancer (27, 64). Other researchers identified the immunosuppressive function of NY-ESO-1-specific $CD8^+$ T cells regulated by PD-1 and lymphocyte-activation gene 3 (LAG-3) (65), $PD\text{-}1^+$ DCs (66) and $PD\text{-}1^+$ myeloid-derived suppressor cells in ovarian cancer (67). Based on the positive findings from these early reports and the proof of concept that PD-1 signaling blockade is effective against cancer, many clinical trials

Table 1. Clinical trials evaluating checkpoint inhibitors in ovarian cancer

Target	Antibody	Trade/code name	IgG subclass	Phase	RR for ovarian cancer (no. of responders/total)	Mean PFS (months) [95% CI]	Mean OS (months) [95% CI]	Trial identifier (Reference)	Company
CTLA-4	Ipilimumab	Yervoy®, MDX-010	Human IgG1	I II	11% (1PR/9)	—	—	NCT01611558 (42)	Bristol–Meyers Squibb
	Tremelimumab	Ticilimumab, CP-675,206	Human IgG2	I	—	—	—	NCT01975831 (45)	MedImmune/ AstraZeneca
PD-1	Nivolumab	Opdivo®, BMS-936558, MDX1106	Human IgG4	II	15% (2CR, 1PR/20)3[a] mg kg^{-1} cohort 20% (2CR/10)	3.5 [1.7–3.9]	20.0 [7.0– not reached]	UMIN000005714 (17)	Bristol–Meyers Squibb/Ono
	Pembrolizumab	Keytruda® MK-3475, lambrolizumab	Humanized IgG4	I	11.5% (1CR, 2PR/26)	—	—	NCT02054806 (19)	Merck
	Pidilizumab	CT-011	Humanized IgG1k	I	—	—	—	NCT01386502 (–)	Cure Tech
	AMP-224	—	PD-L2 IgG2a fusion protein	I	—	—	—	NCT01352884 (–)	Amplimmune/ GlaxoSmith Klein
	MEDI0680	AMP-514	Human IgG4k	I	—	—	—	NCT02013804 (–)	Amplimmune/ GlaxoSmith Klein
PD-L1	MS-936559	MDX1105	Human IgG4	I	5.9% (1PR/17)	—	—	NCT00729664 (18)	Bristol–Meyers Squibb
	Atezolizumab	MPDL3280A	Human IgG1k	I	—	—	—	NCT02174172 (–)	Roche/Genentech
	Durvalumab	MEDI4736	Human IgG1k	I	—	—	—	NCT01693562 (–)	MedImmune/ AstraZeneca
	Avelumab	MSB0010718C	Human IgG1	I	10.7% (8PR/75)	—	—	NCT01772004 (20)	Merck Serono/ Pfizer

CI, confidence interval; CR, complete response; NCT, National Clinical Trial; OS, overall survival; PFS, progression-free survival; PR, partial response; RR, response rate. [a]3 mg kg^{-1} cohort of UMIN000005714.

were initiated to evaluate PD-1 inhibitors (anti-PD-1 or anti-PD-L1 antibodies) for the treatment of human cancers (Fig. 2), including our first clinical trial for ovarian cancer (Table 1) (17).

Clinical applications of PD-1 inhibitors in cancer

In 2010, the first Phase I clinical trial of an anti-PD-1 antibody, nivolumab (BMS-936558 or MDX1106, a fully human IgG4 mAb) at doses of 0.1 to 10.0 mg kg^{-1} was reported in 39 patients with treatment-refractory solid tumors such as advanced melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), prostate cancer and colorectal cancer (CRC). The response rate (RR) was 7.7%, including one durable complete response (CR) in CRC and two PRs in melanoma and RCC. Nivolumab was considered to be well tolerated because only one serious adverse event (AE) of inflammatory colitis was observed (68).

In 2012, a Phase I study of nivolumab was reported in a total of 296 patients with melanoma, NSCLC or RCC. The RRs were 18%, 28% and 27% for melanoma, NSCLC and RCC, respectively. The most frequent AEs were rash, diarrhea and itching; AEs that occurred in ≥1% of subjects at Grade 3 or 4 were diarrhea, hepatic dysfunction and pneumonia (13, 69). This study represented a major milestone in pharmaceutical development of PD-1 inhibitors, and these results were reported in important publications that contributed to the ongoing, rapid progress of PD-1 inhibitors. To date, at least 200 such clinical studies have been launched using nine types of antibody—anti-PD-1 antibodies nivolumab, pembrolizumab, pidilizumab, AMP-224 and MEDI0680 and anti-PD-L1 antibodies BMS-936559, atezolizumab, durvalumab and avelumab—in at least 20 types of cancer, including both solid and hematologic tumors; the total number of subjects worldwide is more than 20,000 (70).

Clinical applications of PD-1 inhibitors in ovarian cancer

Anti-PD-1 antibodies

Nivolumab. On the basis of our clinical studies of cancer immune escape in ovarian cancer mentioned above (17), we conducted the first principal investigator-initiated, Phase II, two-cohort (1 or 3 mg kg^{-1}, n = 10 each), clinical trial of nivolumab in patients with platinum-resistant recurrent ovarian cancer. This clinical trial was conducted in collaboration with Professor Tasuku Honjo. A total of 20 patients were treated with nivolumab every 2 weeks for 1 year. The antitumor effect in patients was assessed every 8 weeks, and patients with disease progression were taken off this trial. The primary end-point was the best overall response for each patient as assessed by Response Evaluation Criteria in Solid Tumors (RECIST).

Of the total 20 patients, the best overall RR across the two cohorts was 15% (2 CRs and 1 PR), and the disease control rate (DCR) was 45% (9 of 20 patients). In the 1-mg kg^{-1} cohort, one patient experienced a PR and four had SD. The objective RR (ORR) was 10%, and the DCR was 50%. In the 3-mg kg^{-1} cohort, two patients had a CR and two had SD. The ORR was 20%, and the DCR was 40%. The median overall survival was prolonged to 20.0 months, while the median PFS was 3.5 months for the two cohorts. In our ongoing follow-up study after completing the initial 1-year nivolumab treatment, two patients with CR survived without any disease progression for over 1 year in the absence of any adjuvant anti-tumor treatment (70).

The most common treatment-related AEs were fever, rash, arthralgia, fatigue, elevated aspartate transaminase or alanine transaminase, hypothyroidism and lymphocytopenia. Grade 3/4 treatment-related AEs occurred in 8 of the 20 patients. The frequency and severity of treatment-related AEs were similar between the two cohorts. The most frequently

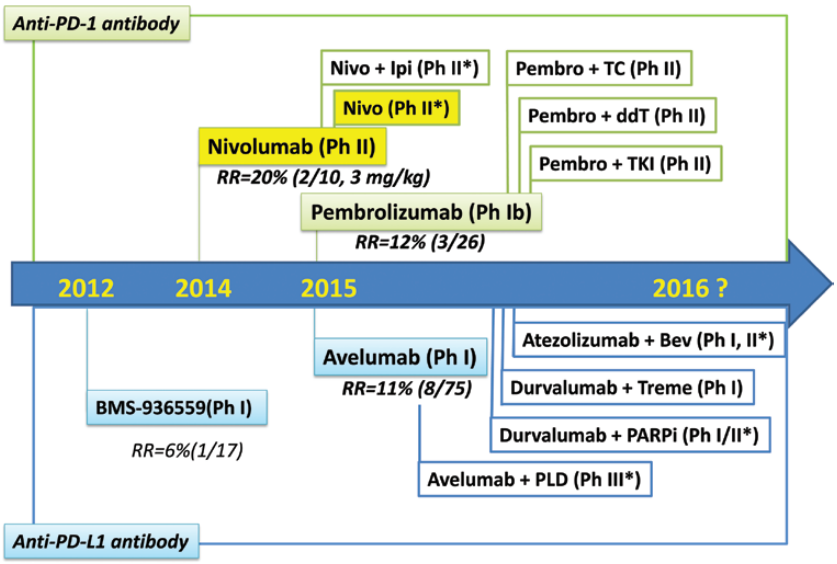

Fig. 2. The evolution of PD-1 inhibitors for ovarian cancer. Bev, bevacizumab; ddT, dose dense paclitaxel; Ipi, ipilimumab; Nivo, nivolumab; PARPi, PARP inhibitor; Pembro, pembrolizumab; Ph, Phase; PLD, pegylated liposomal doxorubicin; RR, response rate; TC, paclitaxel + carboplatin; TKI, tyrosine kinase inhibitor; Treme, tremelimumab. *Randomized clinical trial.

observed AEs were those related to thyroid function: hypothyroidism, thyroiditis, hyperthyroidism, high thyroid-stimulating hormone and low thyroid-stimulating hormone. Treatment-related serious AEs were observed in two patients. One patient in the 1-mg kg^{-1} cohort had two Grade 3 AEs of disorientation and gait disorder after developing a fever that lasted longer than 1 month. Another patient in the 3-mg kg^{-1} cohort had Grade 3 fever and deep vein thrombosis. Later, this patient was evaluated as a CR (17). As a result of the positive data from the early Phase II clinical trial, a follow-up multicenter, open-label, randomized clinical trial was initiated for platinum-resistant ovarian cancer (71).

Pembrolizumab. Pembrolizumab (MK-3475, lambrolizumab) is a humanized IgG4κ mAb against PD-1 with similar anti-tumor properties as nivolumab for several solid tumors, including melanoma and NSCLC (15, 30). The interim results recently became available from a multi-cohort, single-arm, Phase Ib clinical trial of pembrolizumab (KEYNOTE-028) for PD-L1+ solid tumors including 26 patients with recurrent ovarian cancers (19). Patients received pembrolizumab monotherapy at a dose of 10 mg kg^{-1} intravenously every 2 weeks and were treated for 24 months or until progression or intolerable toxicity. The ORR was 11.5% overall, and a DCR was observed in 34.6% of patients. Of the 26 patients in the study, 1 patient (3.8%) reported a Grade 3 AE, which was an increase in the transaminase levels. There were no discontinuations of pembrolizumab due to treatment-related AEs and no treatment-related deaths.

Anti-PD-L1 antibodies

BMS-936559. BMS-936559 is a fully human anti-PD-L1 IgG4 mAb that binds PD-L1 and inhibits PD-1 signaling. The first Phase I trial of BMS-936559 for solid tumors included 17 ovarian cancer patients (18). In that trial, ovarian cancer patients at the 10 mg kg^{-1} dose achieved objective responses; 1 patient with a PR and RR at 6.9%. The most common AEs included fatigue, infusion reactions, diarrhea, arthralgia, pruritis, rash, nausea and headache.

Avelumab. Avelumab (MSB0010718C) is a fully human anti-PD-L1 IgG1 mAb and has a native F_c receptor for antibody-dependent cell-mediated cytotoxicity. Under the JAVELIN clinical trial program, an expansive international clinical trial program exploring the use of PD-L1 inhibition with avelumab to treat metastatic and locally advanced solid tumors, a Phase Ib open-label study was conducted to investigate the tolerability, safety and efficacy of this agent in 75 patients with previously treated, recurrent or refractory ovarian cancer (20). Patients were given intravenous infusions of 10 mg kg^{-1} avelumab every 2 weeks. Anti-tumor effects were evaluated according to the RECIST version 1.1 and according to immune-related response criteria (irRC). The status of tumor PD-L1 expression was not an eligibility criterion.

Analyses of patients using the RECIST criteria (median follow-up at approximately 5 months) revealed no CRs, 8 patients (10.7%) with PRs and 33 patients (44.0%) with SD as well as an ORR of 10.7% and a DCR of 54.7%. Target tumor shrinkage of 30% or greater was observed in 11 patients (14.7%). In addition to the eight patients with PRs based on the RECIST criteria, two patients were evaluated as PRs based on irRC. Two of these positive responders had clear-cell histology. Subgroup analyses revealed that higher ORRs were associated, albeit non-significantly, with a lower tumor burden (sum longest diameter no greater than the median value of 58 mm), fewer prior therapies (≤1) and platinum-sensitive recurrence (>12 month PFS). These data were too premature to draw conclusions because of the short follow-up period.

Fifty-two patients (69.3%) developed treatment-related AEs. The most commonly reported AEs at incidences ≥5% are listed by decreasing frequencies as follows: fatigue, chills, nausea, diarrhea, infusion-related reactions, rash, vomiting, constipation and hypothyroidism. Grade 3 or higher treatment-related AEs were documented in 8% of patients, which is consistent with other antibody drugs in the same class. AEs led to premature treatment discontinuation at a rate of 12%. No treatment-related deaths were reported. Currently, there are several ongoing clinical trials investigating anti-PD-1 pidilizumab (CT-011) and anti-PD-L1 antibodies such as atezolizumab or durvalumab for solid tumors including ovarian cancers (Table 1).

Clinical issues related to PD-1 inhibitors for ovarian cancer

Accumulating data from clinical trials for ovarian cancer revealed that the response rates of immune checkpoint inhibitors were relatively low (5.9–20.0%; Table 1). There are several unresolved issues about PD-1 inhibitors that warrant further investigation (Fig. 3). Four of these will be discussed below: (i) the predictive biomarkers for anti-tumor responses, AEs and trial termination; (ii) the best combination therapy candidates; (iii) the major AEs; and (iv) assessing the 'value' of anticancer treatments.

Exploration of biomarkers

Several previous reports described the expression of PD-L1 on tumor cells. On one hand, PD-L1 expression was correlated with anti-tumor responses in several clinical trials of PD-1 inhibitors for melanoma, NSCLC and renal cancer (13, 14, 72, 73). On the other hand, PD-1 inhibition was not predictive of anti-tumor responses in more recent clinical trials of nivolumab in a Phase III study in squamous cell lung cancer (74) and a Phase III nivolumab plus ipilimumab combination therapy in melanoma (75). These conflicting results call into question the predictive validity of PD-L1 expression within the tumor. To understand and resolve the discrepancies reported in prior studies, additional validation studies of gene expression analysis methods and rigorous analytical quality control procedures are required.

In some clinical trials, the number of infiltrating T cells and the proportion of T cells positive for PD-L1 and/or PD-1 expression were correlated with therapeutic responses (72, 73, 76). However, in ovarian cancer, our Phase II nivolumab trial and Phase Ib avelumab trial showed no correlation between PD-L1 expression and anti-tumor response. The

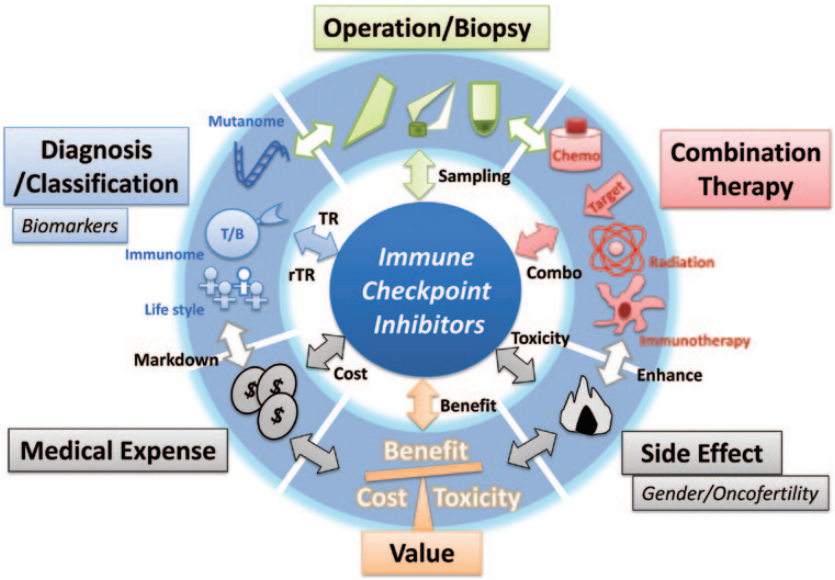

Fig. 3. How immune checkpoint inhibitors relate to other clinical issues in ovarian cancer. chemo, chemotherapy; mutanome, mutational analysis; rTR, reverse translational research; T/B, immunomonitoring; target, molecularly targeted therapy; TR, translational research.

disparate findings in these studies were likely linked to several unresolved issues including the small sample size, methodological inconsistency and differences in timing of the tissue sampling (17, 20).

Several studies sought to identify novel predictive biomarkers for the efficacy of immune checkpoint inhibitors as antitumor therapeutics on the basis of the relationship between cancer cell biology and host immune responses. Based on the analysis of the genome database of TCGA, the frequency of somatic gene mutations was highest in melanoma, followed by lung squamous cell carcinoma, lung adenocarcinoma, bladder cancer and stomach cancer (77).

These findings suggest that immune checkpoint inhibitors should be more effective in cancer types with high frequencies of somatic gene mutations (78). In fact, whole-genome mutational analysis (the mutanome) revealed durable clinical efficacy of ipilimumab for melanoma (79) and pembrolizumab for NSCLC were significantly correlated with each other (80). The therapeutic efficacy was higher in CRC patients with a DNA mismatch repair (MMR) deletion. CRC patients with normal MMR had a response rate of 0%, whereas CRC patients with MMR deletion had a response rate of 62% with a disease control rate of 92% (81). Some recent studies indicated that lifestyle choices such as the history of smoking and food (related to the resident enteric microbiota) affected anti-tumor responses to immune checkpoint inhibitors (80, 82, 83).

In ovarian cancer, mutations of breast cancer-related genes 1/2 (BRCA1/2) that have integral functions in DNA homologous recombination repair were closely correlated with TILs and the overexpression of PD-L1 on tumor cells (84). Thus, BRCA-mutated ovarian cancer may be a good candidate for PD-1 inhibitors.

Combination therapies

Several clinical trials for combination therapies of PD-1 inhibitors with chemotherapies, targeted molecular drugs for solid tumors, focal radiation therapy and the other cancer immunotherapies such as cancer-specific vaccines or immune modulators are currently ongoing (78, 85–87). In particular, a combination of nivolumab and ipilimumab for the treatment of melanoma increased PFS compared with either agent alone (88), and similar combination therapies are now being investigated in ovarian cancer (89).

Next, double-checkpoint blockade in which PD-1 inhibitors are combined with immune modulators such as anti-LAG-3 antibody, anti-OX40 agonistic antibody and anti-4-1BB agonistic antibody are currently under investigation for solid tumors (86). In addition, the combination of immune checkpoint inhibitors with conventional chemotherapies (paclitaxel and carboplatin, dose-dense paclitaxel and PLD), with PARPi molecules (olaparib or cediranib) or with a multi-TKI are being investigated for ovarian cancer (Table 2) (86, 87). The recent pre-clinical studies of ovarian cancer showed the perspective of the combined drugs with PD-1 inhibitors and anti-CD137 or anti-glucocorticoid-induced TNFR-related protein (90, 91).

Management of adverse immunological effects

On the basis of the accumulated AEs data from clinical trials using PD-1 inhibitors, severe immune-related AEs (irAEs) were milder in patients treated with PD-1 inhibitors versus anti-CTLA-4 antibodies (92). Furthermore, about 10% of patients developed Grade 3/4 irAEs, with the most commonly reported irAEs being dermatitis, colitis and thyroiditis. Life-threatening, irAEs included pneumonitis, colitis with gastrointestinal perforation, type 1 diabetes, sepsis risk after corticosteroid therapy, encephalopathy and Guillain–Barré syndrome, myelitis and myocarditis and nephritis (92, 93). The median time to onset of irAEs differed depending on the type of toxicity but can be roughly classified: either early (<2 months), featuring skin, gastrointestinal and hepatic AEs or late (>2 months), featuring pulmonary, endocrine and renal AEs (92–94).

To date, meaningful quantitative assessment of the irAE profiles for ovarian cancer trials using PD-1 inhibitors is

Table 2. Combination therapy using checkpoint inhibitors in ovarian cancer

Combination	Treatment setting	Line of therapy	Phase	Trial identifier
aCTLA-4 + PARPi	Tremelimumab + olaparib	2L+	I/II	NCT02571725
aCTLA-4 + PARPi	Tremelimumab tremelimumab + olaparib	2L	I/II	NCT02485990
aCTLA-4 + aPD-1	Nivolumab versus nivolumab + ipilimumab	2–4L	II[a]	NCT02498600
aPD-1 + TC	Pembrolizumab + paclitaxel + carboplatin	1L	II	NCT02520154
aPD-1 + ddT	Pembrolizumab + dose-dense paclitaxel	2L+	II	NCT02440425
aPD-1 + TKI	ACP-196 (TKI) versus pembrolizumab + ACP-196	2–4L	II[a]	NCT02537444
aPD-L1 + PARPi	Durvalumab + olaparib vs durvalumab + cediranib	Any	I/II[a]	NCT02484404
aPD-L1 + aCTLA-4	Durvalumab + tremelimumab	Any	I	NCT02261220
aPD-L1 + Bev	Atezolizumab + bevacizumab	2L-	II[a]	NCT02659384
aPD-L1 + TLRa + PLD	Durvalumab + motolimod + PLD	2–3L	I/II	NCT02431559
aPD-L1 + PLD	Avelumab versus avelumab + PLD versus PLD	2–4L	III[a]	NCT02580058

aCTLA4, anti-CTLA-4; Bev, bevacizumab; ddT, dose-dense paclitaxel; L, line (regimen of chemotherapy); NCT, National Clinical Trial; PARPi, PARP inhibitor; PLD, pegylated liposomal doxorubicin; TC, paclitaxel + carboplatin; TKI, tyrosine kinase inhibitor; TLRa, agonist of Toll-like receptor 8. [a]Randomized clinical trial.

precluded by the small sample sizes of the trials (17–20) Therefore, future clinical studies with careful monitoring of irAEs according to the guidelines and management algorithms described above should be conducted (92, 93).

Lastly, we, as gynecologists, would like to understand the gender differences in the frequency and severity of side effects as well as the infertility risks for younger men or women treated with PD-1 inhibitors. Currently, very little is known about the influence of PD-1 inhibitors on human fertility or pregnancy (Fig. 3) (71).

The value of immune checkpoint inhibitors in ovarian cancer

Recent drug discovery of anticancer treatments has been required to evaluate the value—referring to their benefit/cost ratio (95, 96). In order to determine the value of immune checkpoint inhibitors in ovarian cancer, there is an urgent need to establish patient selection methods that are based on predictive biomarkers of anti-tumor responses and irAEs. This is particularly important in ovarian cancer because it is not associated with a high response rate. Typically, anticancer treatments are considered to be excellent if they are associated with low medical costs, low toxicity and high 'benefits' (anti-tumor response). In addition, it is important to select treatment strategies that are tailored for each individual patient, particularly their medical history and lifestyle (Fig. 3).

Conclusions

The early phase of clinical trials of immune checkpoint inhibitors for ovarian cancer have shown the manageable safety profile (17–20) and have demonstrated a dramatic durable anti-tumor response in a certain population of the patients with a 1-year schedule of nivolumab treatment, following no adjuvant anti-tumor treatment (89). Therefore, not only to explore the predictive biomarkers of responders and to find a good combination therapy but also to conduct a clinical trial to decide the maximum anti-tumor effect with a minimum treatment period of immune checkpoint inhibitors may be a next turning point to enhance the value of immune checkpoint inhibitors for ovarian cancer.

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