Strategic combination therapies for ovarian cancer

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Abstract
Ovarian cancer remains the leading cause of gynecologic cancer-related death among women worldwide. The dismal survival rate is partially due to recurrence after standardized debulking surgery and first-line chemotherapy. In recent years, targeted therapies including anti-angiogenic agents or poly (ADP-ribose) polymerase inhibitors represent breakthroughs in the treatment for ovarian cancer. As more therapeutic agents become available supplemented by deeper understanding of ovarian cancer biology, a range of combination treatment approaches are being actively investigated to further improve the clinical outcomes of the disease. These combinations, which involve DNA-damaging agents, targeted therapies of signaling pathways and immunotherapies, simultaneously target multiple cancer pathways or hallmarks to induce additive or synergistic antitumor activities. Here we review the preclinical data and ongoing clinical trials for developing effective combination therapies in treating ovarian cancer. These emerging therapeutic modalities may reshape the treatment landscape of the disease.

Abbreviations:
ACT, adoptive cell transfer; ATR, ataxia telengiectasia and Rad3-related protein; CAR-T, chimeric antigen receptor T; CHK1, checkpoint kinase 1; CHO, Chinese hamster ovary; CI, confidence interval; CTLA-4, cytotoxic T-lymphocyte antigen-4; CXCR4, C-X-C chemokine receptor type 4; DCR, disease control rate; DOR, duration of response; EGFR, epidermal growth factor receptor; FA, Fanconi anemia; HDR, homology-directed DNA repair; HR, hazard ratio; ICL, interstrand crosslinks; IL-2, interleukin-2; INF-γ, interferon-gamma; MAPK, mitogen-activated protein kinase; MDSCs, myeloid-derived suppressor cells; MEK, mitogen-activated protein kinase kinase; ORR, objective response rate; OS, overall survival; PARP, poly (ADP-ribose) polymerase; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; PDGFR, platelet-derived growth factor receptor; PDX, patient-derived xenograft; PFS, progression-free survival; PI3K, phosphoinositide 3-kinase; PLD, pegylated liposomal doxorubicin; PR, partial response; RTKs, receptor tyrosine kinases; SD, stable disease; TAA, tumor-associated antigen (TAA); TILs, tumor infiltrating lymphocytes; TME, tumor microenvironment; Treg, regulatory T cells; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

1. Introduction

Ovarian cancer is the most lethal gynecological malignancy. The clinical outcomes of ovarian cancer patients vary at different stages and survival rates decrease rapidly from stage I to stage IV. After cytoreduction surgery, the 5-year survival rates of patients with stage I ovarian cancer can be as high as 90% (1). In contrast, the 5-year survival rates at late stages are around 20%. Due to the lack of obvious symptoms at the early stages of cancer growth, 80% of ovarian cancer patients are diagnosed at advanced stages (2).

The current mainstay first-line treatment for ovarian cancer is complete surgical cytoreduction accompanied with adjuvant platinum/taxane chemotherapy. Debulking surgery removes as much visible tumor as possible, regardless of the cancer stage. The first-line chemotherapy regimen has progressed over the past few decades to improve clinical benefits and to reduce side effects. Platinum-based chemotherapeutics inhibiting DNA synthesis were first considered as the first-line treatment. Then two-drug combination of cisplatin plus cyclophosphamide (an alkylating agent that also interferes with DNA replication) became the standard therapy because such combination was more effective than either agent alone (3). Ten years later, cisplatin-paclitaxel doublet was shown to prolong patient survival compared with the cisplatin-cyclophosphamide regimen (4), since then cyclophosphamide has been less commonly used in ovarian cancer treatment. Subsequently, multiple clinical trials established that carboplatin-paclitaxel doublet causes less toxicity than cisplatin-paclitaxel without compromising efficacy (5).

Although ovarian cancer is relatively more sensitive to chemotherapy than many other malignancies, most of the ovarian cancer patients who initially respond to chemotherapy would relapse. The optimal treatments for recurrent or chemo-resistant ovarian cancer patients remain unresolved. Over the past decade, we have witnessed a remarkable increase in the understanding of ovarian cancer tumorigenesis at the molecular and cellular levels. There is also a growing number of molecular targeted therapies. These together drive the expansion of treatment options for ovarian cancer. For examples, pharmacological inhibition of the angiogenic factor vascular endothelial growth factor (VEGF) and the DNA damage sensor protein poly (ADP-ribose) polymerase (PARP) has demonstrated impressive antitumor activities. In addition, immunotherapy has emerged as a new strategy in anti-cancer treatment, which is being actively investigated in ovarian cancer. Importantly, accumulating evidence supports the combined uses of these therapeutic agents to simultaneously block multiple cancer pathways, thereby achieving greater therapeutic effects or overcoming drug resistance. Better treatment outcomes can be achieved by combinatorial approaches in light of the underlying resistant mechanisms. Drug resistance can be intrinsic or acquired. Intrinsic resistance is the innate ability of cancer cells to remain insensitive to the initial treatment. In contrast, acquired resistance represents the evolution of cancer cells to an adaptive status upon treatment exposure. For examples, genomic aberrations of members along the same signaling cascade of the target may affect primary treatment response (6). In such case, targeting multiple nodes of the signaling axis may overcome the resistance. Alternatively, chemotherapeutic drugs and inhibitors targeting PARP, receptor tyrosine kinases (RTKs), mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) may result in the activation of compensatory pathways, allowing cancer cells to bypass the drug-induced toxicity (6, 7). The occurrence of network rewiring highlights the importance of combination

strategies against acquired drug resistance. Further, it has been suggested that immunosuppression initiated by genomic aberrations or the tumor microenvironment (TME) prevents ovarian tumors from responding to immunotherapies (8). In this review, we discuss the combinatorial treatment approaches evaluated in preclinical studies and clinical trials for ovarian cancer.

2. Combination treatment approaches that involve chemotherapy or PARP inhibitor

2.1 Synthetic lethality leveraging defective DNA damage response

Defect in cellular response to DNA damage can be exploited by synthetic lethality. A poster child is the susceptibility of BRCA-inactivated cells to PARP inhibitors (9). BRCA1 and BRCA2 are essential enzymes in homologous recombination (HR), whereas PARP involves in multiple DNA repair pathways and can often compensate for the loss of BRCA. In cancer cells without functional BRCA, PARP inhibitor causes an accumulation of DNA double-strand breaks and thereby mitotic catastrophe. Multiple drug combinations have been developed based on this cell death mechanism triggered by DNA damage. The commonly used platinum and taxane chemotherapies target cell division by inhibiting DNA replication and causing mitotic arrest, respectively (10). Under such conditions, DNA damage response is activated to maintain genomic integrity. Hence, blocking DNA damage repair through inhibiting PARP together with chemotherapy could augment antitumor effect. A preclinical study has shown that the PARP inhibitor ABT-888 (veliparib) markedly sensitized ovarian cancer cells to FdUrd, an FDA approved DNA-damaging chemotherapy (11). In addition, combination treatment of *BRCA*-deficient Chinese Hamster Ovary (CHO) cells and xenograft models with ABT-888 and carboplatin delayed tumor growth more efficiently compared with single drug treatment (12) (Table 1).

Clinically, PARP inhibitor olaparib in combination with paclitaxel and carboplatin has entered phase II trial (13) (Table 2). Platinum-sensitive and recurrent ovarian cancer patients were randomized into two treatment groups: (1) olaparib plus chemotherapy followed by olaparib maintenance monotherapy or (2) chemotherapy alone without further treatment. The median progression-free survival (PFS) in olaparib plus chemotherapy group was significantly longer than chemotherapy alone group (12.2 vs. 9.6 months). This trial also revealed the clinical benefit of the 3-drug regimen for patients with *BRCA* mutations. Among these *BRCA*-mutated patients, the 12-month progression-free rate in olaparib plus chemotherapies treatment group reached 70%, while that of chemotherapy only group was 12.5% (13). The therapeutic potential of the other chemotherapy reagents in combination with PARP inhibitors has also been investigated. In a phase I trial, ovarian cancer patients were treated with continuous or intermittent olaparib plus pegylated liposomal doxorubicin (PLD; a chemotherapy drug disrupting DNA replication) (14). The objective response rate (ORR; complete or partial response) was 50%, which was higher than previously reported in trials of single-agent olaparib (31%-41%) or doxorubicin (18%-20%). It is noteworthy that the response rates in platinum-sensitive patients and *BRCA*-mutated patients were 71% and 61% respectively (14). Consistently, another phase I study showed that 69% of *BRCA*-deficient ovarian patients responded (45% partial response and 24% complete response) to veliparib in combination with carboplatin and gemcitabine (15). These

studies together suggest that combining chemotherapy and PARP inhibitor is more effective in chemo-sensitive or *BRCA*-deficient ovarian tumors.

Examples other than chemotherapy and PARP inhibitor combination exist. Remarkably, some of these combinations might be effective in *BRCA* wild-type ovarian tumors. Carboplatin induces DNA damage not only by intrastrand DNA lesions but also by interstrand crosslinks (ICL), which can only be repaired by the Fanconi anemia (FA) pathway. HSP90 inhibitor ganetespib targets the repair of ICL, providing a mechanistic rationale of combining ganetespib and carboplatin. Ganetespib enhanced the antitumor potential of carboplatin by suppressing FA pathway-mediated repair of ICL induced by carboplatin, triggering massive chromosome fragmentation (16). The consequences of the DNA lesions were most clearly manifest in *TP53*-mutant cells because the unrepair cells proceeded through aberrant mitosis and eventually cell death (16). This synergy could be observed in *BRCA* wild-type cells. The second example is the simultaneous inhibition of ATR/CHK1 and PARP. ATR (ataxia telengiectasia and Rad3-related protein) and its downstream target CHK1 (checkpoint kinase 1) are activated in response to replication stress and stalled replication forks, thereby promoting cell cycle control and DNA repair through HR. Cells with high levels of replication stress, for example upon inhibition of PARP, have an increased reliance on the ATR/CHK1-dependent replication fork protection pathway. Accordingly, dual blockade of PARP with ATR or CHK1 resulted in increased chromosomal abnormalities and cell death *in vitro* as well as reduced tumor burden in patient-derived xenograft (17). Intriguingly, combined inhibition of PARP and ATR caused stronger antitumor effects than that of PARP and CHK1. Further, while combined blockage of PARP and ATR caused additive effects in both *BRCA*-deficient and *BRCA*-proficient ovarian cancer cells, PARP and CHK1 combination treatment was effective only in *BRCA*-deficient cells (17). It was hypothesized that ATR inhibition may affect multiple downstream targets in addition to CHK1 and therefore resulting in stronger therapeutic efficiency. The third example is combination of PARP inhibitor/chemotherapy with WEE1 kinase inhibitor. WEE1 kinase regulates G2/M cell-cycle checkpoint arrest for DNA repair before mitotic entry. *TP53*-mutated cells with DNA damage depend largely on the G2 checkpoint, creating a synthetic lethality between DNA damage and checkpoint inhibition. *In vitro*, the combination of WEE1 kinase inhibitor AZD1775 with olaparib or gemcitabine was synergistic in *TP53*-mutated ovarian cancer cells (18). Complementarily, combination therapy of AZD1775 and carboplatin demonstrated encouraging antitumor activity in a phase II clinical study with *TP53*-mutated ovarian cancer refractory or resistant to first-line platinum-based therapy (19). However, additional evidence has suggested that the combination activity is likely independent of *TP53* mutation status. Synergistic inhibition could be observed in *TP53* wild-type cells with mutations in *KRAS* or *BRAF* or *ARID1A* (20). Further, the addition of AZD1775 did not induce synergy in PARP inhibitor-sensitive cells. Noteworthy, while drugs were concurrently administered in most of the other studies, sequential treatment with PARP inhibitor and WEE1 inhibitor preserved efficacy with reduced toxicity such as weight loss and anemia which could be the results of concurrent treatment (20). Lastly, synergy of bromodomain protein BRD4 inhibitor with PARP inhibition has been demonstrated in *BRCA*-proficient ovarian cancers (21). The inhibition of BRD4, which is an epigenetic modulator regulating gene expression, suppressed the expression of WEE1 as well as the DNA damage response protein TOPBP1 leading to DNA damage and mitotic catastrophe.

2.2 Targeting the apoptosis pathway

Encouragingly, more therapeutic strategies have been under investigation to enhance the efficacy of chemotherapy or PARP inhibitor. A strategy capitalizes on the reactivation of cancer cell death pathway because resistance to cell death is a cancer hallmark. ABT-263 causes cell death by inhibiting the apoptosis suppressor proteins Bcl-2, Bcl-xL and Bcl-w. Combinational treatment of ABT-263 and PARP inhibitor BMN 673 (talazoparib) in *BRCA* wild-type ovarian cancer cell lines synergistically decreased cell viability as well as increased DNA fragmentation and apoptotic cell death (22). Mechanistically, reduction of apoptosis threshold by ABT-263 through suppressing the anti-apoptotic proteins may sensitize cells to BMN 673. Another independent preclinical study involved Bcl-2 inhibitor ABT-199, which induced synergistic effect with paclitaxel in ovarian cancer cell lines (23). Importantly, low JNK1 protein level is potentially a predictive biomarker for the synergism because of its correlation with Bcl-2 phosphorylation upon paclitaxel treatment (23).

2.3 Reactivation of p53

TP53, which encodes p53 protein, is frequently mutated in ovarian cancer patients. The drug APR-246 reactivates *TP53* mutants and triggers apoptosis via induction of caspases and other downstream proteins. Strong synergy was observed *in vitro* upon combination treatment of APR-246 with cisplatin, carboplatin or doxorubicin in primary cancer cells derived from ascitic fluid of ovarian, fallopian tube, or peritoneal cancer, suggesting the clinical benefits of combination of APR-246 with DNA-damaging agents (24). Moreover, this combination regimen may re-sensitize chemo-resistant patients to platinum agents (24). p53 controls a broad range of cellular processes, the mechanisms underlying the observed synergism remain to be fully elucidated.

3. Combinatorial therapeutic approaches that involve kinase inhibitors

The oncogenic phenotypes of cancer cells are often activated by kinases, which are enzymes that transfer phosphate groups to proteins, lipids or carbohydrate substrates to initiate downstream signaling. Importantly, many of these kinases are druggable and have attracted immense interests as cancer therapeutic targets. The kinase activity can be blocked by specific inhibitors through competitive binding to ATP-binding site or allosteric site.

3.1 Receptor tyrosine kinases

RTKs are a group of cell-surface expressed growth factor receptors that relay extracellular signals into the cell. These receptors are often found aberrantly overexpressed or activated in cancer cells.

3.1.1 VEGFR signaling

Notably, inhibition of vascular endothelial growth factor receptor (VEGFR) signaling, which activates angiogenesis, has shown efficacy in ovarian cancer. However, patients who initially display positive response to VEGF inhibition may unfortunately develop relapsed disease, driving researches on additional approaches to maximize its clinical impact. Phase III clinical trials have demonstrated that chemotherapy and bevacizumab (Avastin, VEGF-A monoclonal antibody) resulted in statistically significant better treatment outcomes in both platinum-sensitive recurrent (PFS 12.4 vs. 8.4 months; (25)) and platinum-resistant recurrent (PFS 6.7 vs. 3.4 months; (26))

ovarian cancer patients (Table 2). In addition, bevacizumab plus dose-dense paclitaxel and carboplatin as first-line adjuvant therapy for advanced stage ovarian cancer showed acceptable tolerability and efficacy after primary cytoreductive surgery (27).

Phase II trials that combined PARP inhibitor olaparib and VEGFR-1/2/3 inhibitor cediranib in platinum-sensitive, relapsed ovarian cancer patients have demonstrated extended median PFS (16.5 vs. 8.2 months) than olaparib single-agent group (28). Intriguingly, subset analysis according to *BRCA* status yielded different results. In *BRCA* wild-type group, olaparib and cediranib combination achieved significant improvement in median PFS (23.7 vs. 5.7 months) and median overall survival (OS; 37.8 vs. 23.0 months). In contrast, PFS and OS appeared to be similar between the two arms in *BRCA*-mutated patients (28). The significant improvement of PFS in *BRCA* wild-type patients leads to an interesting hypothesis that cediranib treatment may result in a condition that mimics *BRCA* deficiency. Indeed, a recent study has unveiled the underlying mechanism of olaparib and cediranib combination in *BRCA* wild-type cells. Cediranib conferred sensitivity to olaparib by down-regulating HR repair proteins BRCA1/2 and RAD51 recombinase (RAD51) via inhibition of platelet-derived growth factor receptor (PDGFR) signaling and induction of hypoxia (29) (Table 1). While phase III clinical trial of olaparib and cediranib combination treatment is currently underway, a recently published phase III trial showed a significant benefit for bevacizumab plus olaparib in terms of PFS (22.1 months vs. 16.6 months with bevacizumab only) (30). The clinical benefit was more prominent in patients with deleterious *BRCA* mutation (PFS 37.2 vs. 17.7 months) or *BRCA* wild-type tumor but with HR deficiency (HRD) score >=42 (PFS 28.1 vs. 16.6 months). These investigations together support the application of the combination regimens in HRD-positive tumor regardless of *BRCA* status.

3.1.2 ErbB family

ErbB family belongs to class I RTK and comprises four structurally-related members: epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). It is reported that ErbB family members, particularly EGFR, are overexpressed in ovarian cancer and associated with poor prognostic outcomes (31). To date, inhibition of EGFR by either monotherapy or in combination with other drugs have not been efficacious in ovarian cancer. Combining EGFR inhibitors with chemotherapy as first-line or second-line approach in recurrent ovarian cancer treatments showed modest activity (32, 33). The EGFR inhibitor erlotinib plus bevacizumab was not superior to single-agent bevacizumab in a phase II clinical trial, yet fatal gastrointestinal perforation was observed (34). Similarly, blockage of EGFR signaling by multi-target inhibitor vandetanib (targeting VEGFR-2/3, EGFR and RET; (35)) or lapatinib (dual blockage of EGFR and HER2; (36)) together with chemotherapy failed to show improved clinical activities. Further investigations are warranted to understand the underlying mechanism of the insensitivity towards EGFR inhibition and to derive rational combinational treatments accordingly. Interestingly, although combining EGFR inhibitor with chemotherapy or anti-angiogenic agent lacks sufficient antitumor activity, preliminary data has suggested dual blockage of EGFR and PARP in EGFR-overexpressing ovarian tumor xenograft (37).

Several clinical trials of inhibitors targeting the other ErbB family members were conducted, which have revealed potentially effective biomarkers for patient stratification. In a phase II clinical trial of HER2 monoclonal antibody pertuzumab combined with gemcitabine in platinum-resistant ovarian cancer, tumors with low HER3 transcript levels demonstrated better clinical benefit than gemcitabine alone (38). The effectiveness of this combination is further supported by a phase III trial later on, which evaluated pertuzumab plus chemotherapy in patients with platinum-resistant ovarian carcinoma. Consistently, patients with HER3 mRNA-low tumors had favorable PFS upon pertuzumab plus gemcitabine or paclitaxel (39). Noteworthy, the clinical activity of this combination was not recaptured in platinum-sensitive recurrent ovarian cancer (40). The predictive power of low HER3 transcript level in the responsiveness to HER2 inhibition can possibly be attributed to the heterodimeric interaction between HER2 and HER3 for signaling activation. HER3 mRNA expression is downregulated upon ligand-induced HER2-HER3 dimer activity. Therefore low HER3 mRNA level reflects HER2-HER3 activation and susceptibility to HER2 inhibition. HER2 mRNA did not correlate with the response, but the reason is unknown. Intriguingly, HER2 may however inform response to HER3 inhibition. The addition of HER3 monoclonal antibody seribantumab to paclitaxel did not improve PFS in unselected patients, but patients with low HER2 level and detectable heregulin might benefit from this combination (41). In contrast, high HER3 levels appeared to correlate with favorable response compared to patients with low HER3.

3.1.3 Multi-target RTKs

The antitumor activity of multi-target RTK inhibitors, which have shown clinical benefits in other cancer types, was also evaluated in ovarian cancer patients. Pazopanib is an inhibitor that targets VEGFR, PDGFR and c-Kit. Although pazopanib as single-agent maintenance therapy might prolong PFS (17.9 vs. 12.3 months compared with placebo) in patients with advanced ovarian cancer who have not progressed after first-line chemotherapy (42), the combination of pazopanib with paclitaxel in persistent or recurrent ovarian cancer patients was not superior to paclitaxel alone (43).

3.2 Cytosolic kinases

Non-receptor cytosolic kinases, such as PI3K and MAPK, are key mediators of signal transduction and therapeutic targets of significant interest. *KRAS* mutations can drive hyperactivation of PI3K and MAPK, and importantly, *KRAS* is found frequently mutated in ovarian cancer. Dual blockade of PI3K and MAPK pathways in *RAS*-driven tumors induced synergistic antitumor effect (44). Several phase I clinical trials that co-targeted PI3K and mitogen-activated protein kinase kinase (MEK) have been conducted and showed promising antitumor activities, especially in patients with *RAS* mutations (45, 46). In addition, Src and MAPK are coactivated in 31% of TCGA high-grade serous ovarian cancer patients. Combination of selumetinib (AZD6244; MEK inhibitor) and saracatinib (AZD0530; Src inhibitor) could overcome Src mono-inhibition mediated MEK/MAPK bypass activation, resulting in autophagy and apoptosis *in vitro* as well as decreasing tumor burden *in vivo* (47). Another rational combination strategy is MEK inhibitor GDC-0973 (cobimetinib) and dual BCL-2/XL inhibitor (ABT-263), which is built upon the observation that inhibition of MEK caused apoptotic priming, leading to increased dependency specifically on BCL-XL for cell survival (48).

Genomic deletion of *PIK3R1*, which is the coding gene of the class IA PI3K regulatory subunit p85α, has been suggested to activate both AKT and STAT3 pathways (49).

Dual inhibition of AKT and STAT3 induced synergistic antitumor activity in *PIK3R1*-loss ovarian tumors (49). Moreover, due to the prevalence of PI3K pathway activation in ovarian cancer, the efficacy of combining PI3K inhibitors and DNA damage drugs has been assessed in preclinical and clinical studies. A pan-PI3K inhibitor buparlisib (BKM120) enhances the cytotoxicity of PARP inhibitor in ovarian cancer cells (50). Repressed expression of BRCA1/2 and HRD were observed in these cells (50). Encouraging results were obtained in two phase I clinical trials, in which combination of olaparib and buparlisib or PI3Kα-specific inhibitor alpelisib (BYL719) achieved 29% and 36% partial responses in ovarian cancer, respectively (51, 52). Phase II trials are needed to further compare the efficacy of dual PI3K/PARP inhibition and PARP inhibition alone.

4. Combinational approaches in immunotherapy

Immunotherapy, which stimulates the immune system to induce a robust antitumor immune response, is an effective treatment for a number of malignancies including melanoma and lung cancer (53). Immunotherapy options studied in ovarian cancer can be broadly divided into four categories: immune checkpoint inhibitor, cancer vaccine, oncolytic virus and adoptive cell transfer (ACT). In ovarian cancer patients, the presence of tumor infiltrating lymphocytes (TILs) positively correlates with improved clinical outcomes (54). Yet, immunotherapy as monotherapy has only achieved modest benefits in ovarian cancer patients as demonstrated in early clinical trials (55). Therefore, there is no approved immunotherapy for the treatment of ovarian cancer currently. Ovarian cancer has a lower somatic mutation burden than tumor types that are responsive to immunotherapy (56), implying less neo-antigens are present to trigger the immune machinery. The highly immunosuppressive TME in ovarian cancer patients is thought to be another major factor in attenuating the antitumor response induced by immunotherapy. In view of this, immunotherapy can be combined with therapies that modulate the immunosuppressive TME to achieve maximal therapeutic benefits in ovarian cancer patients.

4.1 Immune checkpoint inhibitors

Immune checkpoint inhibitors, which include antibodies targeting programmed cell death-1 (PD-1) and its associated ligand programmed death-ligand 1 (PD-L1) as well as cytotoxic T-lymphocyte antigen-4 (CTLA-4), are currently in the forefront of immunotherapy research in cancers including ovarian cancer. Checkpoint inhibitors act by promoting the activity of T cells in the tumor through the release of inhibitory signals on T cells thereby allowing effective induction of antitumor responses. Chemotherapy and targeted therapies, including PARP inhibitors and anti-angiogenic agents, associate with immunomodulation in the TME (57-59) and have been demonstrated to complement the action of checkpoint inhibitors in inducing antitumor immunity. Combinations of checkpoint blockade with these therapeutic agents have been extensively evaluated in a number of preclinical studies and clinical trials.

Given the role of chemotherapy in increasing tumor immunogenicity, combinations of checkpoint inhibitors and chemotherapy have been explored in ovarian cancer treatment. Building on the observations that chemotherapy elevated the expression of MHC class I (which can be recognized by CD8+ T lymphocytes) and PD-L1 as well as the number of TILs, combination therapy of paclitaxel and anti-PD-L1 or anti-PD-1

antibody yielded better survival in an immunocompetent murine ID8 ovarian cancer model compared to either monotherapy alone (59) (Table 1). Another study showed that anti-PD-1 antibody provided a strong antitumor effect when combined with a chemotherapy drug trabectedin in the same model through an increase in CD8+ and CD4+ T cells and depletion of immunosuppressive regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) (60). While some of these combinational approaches have entered clinical trials at phases I to III, two recent phase III clinical trials (NCT02580058, NCT02718417) exploring the combinations of avelumab (anti-PD-L1 antibody) and chemotherapies including carboplatin/paclitaxel and PLD failed to show improvement in OS or PFS. Investigating the alterations of immune interaction upon treatment with the therapeutic agent may reveal strategy to improve efficacy. A recent study assessed the impact of chemotherapy on TME of ovarian cancer mouse model using flow cytometry and expression profiling (61). The analysis revealed an acute immunosuppression after paclitaxel and carboplatin treatment, leading to a rationale of co-targeting both the innate and adaptive immunity after chemotherapy. Combination involving anti-IL-10 and 2′3’-cGAMP (target innate immunity) as well as anti-PD-L1 (adaptive immunotherapy) reversed immunosuppression and promoted immune activation. This combination of immunotherapeutic and chemotherapeutic agents significantly increased survival of mice compared to chemotherapy alone.

The ability of PARP inhibitor to modulate TME of ovarian cancer has been convincingly demonstrated (57). PARP inhibitor BMN 673 caused an accumulation of cytosolic DNA and activation of the cGAS–STING–TBK1–IRF3 innate immune pathway to induce type I interferon, thereby rendering susceptibility of ovarian cancer cells to anti-PD-L1 antibody (57). Remarkably, the response to the combination treatment was independent of *BRCA* mutation status. Another study showed that CLTA-4 blockade acted effectively with PARP inhibitor ABT-888 in a *BRCA1*-deficient immunocompetent murine ovarian cancer model through upregulating interferon-gamma (INF-γ) secretion in the peritoneal TME to enhance tumor cell cytotoxicity (62). A subsequent phase I clinical trial revealed tolerable use of anti-CLTA-4 antibody and PARP inhibitor in *BRCA*-associated ovarian cancer patients. Therapeutic responses were evident by decreases in tumor size and CA-125 level in all patients who received the combination therapy (63). Combination of pembrolizumab (anti-PD-1 antibody) and niraparib (PARP inhibitor) was safe and provided an antitumor activity in patients with recurrent platinum-resistant ovarian cancer in a phase I/II clinical trial (64) (Table 2). Pembrolizumab and niraparib resulted in an ORR of 18% and a disease control rate (DCR) of 65%, which were higher than expected in monotherapy with either agent. Echoing the study by Shen *et al* (57), the responses of combination therapy in patients were independent of their *BRCA* mutation status in this trial, suggesting the expanded use of PARP inhibitor in *BRCA*-proficient patients in combination with PD-1/PD-L1 blockade.

The immunosuppressive TME in ovarian cancer is also maintained by angiogenic factors. In particular, VEGF not only promotes angiogenesis, but also suppresses the T cell activation and induces the immunosuppressive MDSCs (58) , giving grounds for combinatorial targeting of VEGF signaling and immune checkpoints. In fact, there are ongoing clinical trials evaluating these combinations and previous trials revealed exciting results. A phase I trial demonstrated durvalumab (anti-PD-L1 antibody) combined with cediranib was tolerable and provided evidence of clinical activity of the treatment approach in recurrent gynecological cancer patients (65). Another phase II

clinical trial examining the combination of nivolumab (anti-PD-1 antibody) and bevacizumab in platinum-sensitive or platinum-resistance relapsed ovarian cancer patients. Results revealed differential outcomes depending on platinum status. While the ORR was 28.9% across all patients. it was 16.7% and 40% in platinum-resistant or platinum sensitive patients respectively (66). The median PFS for the entire cohort was 9.4 months, with a median PFS of 12.1 months in platinum-sensitive patients but only 7.7 months in platinum-resistant patients. In contrast to platinum status, PD-L1 level was not indicative of the response.

4.2 Cancer vaccine

Cancer vaccines which boost antigen-specific antitumor immune responses represent another major development in immunotherapy. Peptide vaccine targeting tumor-associated antigen (TAA) is currently the most studied type of cancer vaccines. Examples of ovarian cancer-associated TAAs evaluated in early clinical trials as single agents include NY-ESO-1, p53 and HER2. To potentiate the effects of cancer vaccines, combination therapies are being explored to overcome the challenges caused by immunologic tolerance as well as low or heterogeneous expression of TAAs.

One strategy exploited by combination therapy enhances TAA presentation. A regimen consisting of a combination of decitabine (DNA methyltransferase inhibitor) and NY-ESO-1 vaccine in addition to the existing PLD treatment enhanced the efficacy of the vaccine through upregulation of NY-ESO-1 expression (67). This phase I clinical trial recorded a DCR of 60% with antigen-spreading evident by the induction of immune responses against a wide range of other tumor antigens, which was not observed in previous monotherapy trials. Another strategy to enhance vaccine efficacy is to simultaneously target the immunosuppressive cells in the TME. Monoclonal antibody against CD11b depletes myeloid cells by targeting MDSCs and immunosuppressive macrophages. Immunization of ID8 murine model with MIS416 vaccine consisting of stimulatory ligands for innate receptors followed by anti-CD11b treatment delayed tumor progression compared to vaccination alone, demonstrating that broad myeloid depletion enhances vaccine efficacy (68). Apart from myeloid cells, Tregs also suppress antitumor immunity. In a phase II trial, potency of a p53-synthetic long peptide vaccine inducing p53-specific T cell immunity was enhanced upon pre-treatment with low-dose cyclophosphamide which eliminates Tregs (69).

In addition to peptide vaccines, dendritic cell-based vaccine is under investigation for combination therapy due to its ability to present tumor antigens and induce potent antitumor T cell responses. For example, clinical efficacy of personalized vaccine made of autologous DCs loaded with autologous tumor lysate could be augmented by VEGF-A blocking antibody bevacizumab and cyclophosphamide (70). The 2-year OS of these patients was higher (78%) than that of patients in a previous cohort who received bevacizumab/cyclophosphamide but no vaccine (44%) (log-rank P=0.046). These studies together have provided encouraging proof-of-principle results of combination therapy to raise the efficacy of cancer vaccines. Nevertheless, clinical responses of cancer vaccines in ovarian cancer patients remain obscure compared to other immunotherapy combinations. Optimization of these combinatorial approaches are keenly anticipated to achieve therapeutic purposes in the clinic.

4.3 Oncolytic virus

Oncolytic viruses are modified viruses designed to infect and destroy cancer cells through activating the immune system upon the release of cancer antigens during oncolysis. Previous study has demonstrated an oncolytic vaccinia virus expressing a T-cell attracting chemokine, CXCL11, enhanced T-cell infiltration into the tumor and induced PD-L1 expression on tumor cells. These effects are possibly the consequences of enhanced cytokines/chemokines production and hypoxic TME upon virus infection (71). Combination of vaccinia virus and anti-PD-L1 antibody elicited a significant antitumor effect in a murine ID8 model (71). Moreover, enhanced therapeutic effects were seen when combining oncolytic virus with chemotherapy compared with single agent (72, 73). It is suggested that chemotherapy-resistant tumors may contain cancer stem-like cells, which display stem cell markers such as nestin. Oncolytic virus 34.5ENVE with an anti-angiogenic gene encoding Vasculostatin-120 (VStat120) driven by a nestin promoter was engineered to target nestin-positive ovarian cancer cells (72). Concurrent treatment of doxorubicin and 34.5ENVE synergistically induced apoptosis (72). Alternatively, paclitaxel and carboplatin-resistant ovarian cancer cells were shown to be susceptible to combination of doxorubicin and oncolytic vaccinia virus expressing an antagonist of C-X-C chemokine receptor type 4 (CXCR-4) (73). Similar to nestin, the expression of CXCR4 receptor was elevated in chemo-resistant cells. Targeting CXCR4 receptor signaling, which promotes tumorigenesis partially through immune-suppression, induced antitumor immune responses (73). The interaction between the vaccinia virus and doxorubicin leading to the augmented antitumor immunity remains to be characterized. Another recent study has revealed an interesting modality in which an antiangiogenic polypeptide 3TSR was combined with an oncolytic virus (Newcastle disease virus F3aa) (74). Treatment with 3TSR prior to oncolytic virus delivery diminished the vascular shutdown caused by the virus, facilitating virus infiltration and tumor regression. As only preclinical data are presented thus far, the translational relevance of these oncolytic virus combinations has to be examined in clinical settings.

4.4 ACT

ACT approach involves in the activation and expansion of TILs isolated from patients *ex vivo*, followed by reinfusion of TILs into patients with the cytokine interleukin-2 after lymphodepleting chemotherapy. Alternatively, a more recent approach has focused on chimeric antigen receptor T (CAR-T) cells, which are engineered to recognize tumor-associated antigens. There are only few reported studies on the efficacy of ACT in ovarian cancer (75). The effects of these strategies have been limited and short-term. The combination of chemotherapy with T cell administration may promote antitumor effects. Among the first evidence of such combination is the abrogation of platinum resistance by unengineered effector CD8+ T cells which produce effector cytokine IFNγ. IFNγ in turn increased the therapeutic efficacy of cisplatin through reversing fibroblast-mediated metabolic changes and chemo-resistance (76). A subsequent independent study has demonstrated that combinatorial treatment of carboplatin and CAR-T cells targeting ErbB dimers expressed in ovarian cancer cells enhanced the cytotoxicity of immunotherapy (77). Combination therapy of oncolytic viruses and CAR-T cells have also been evaluated in multiple solid cancers. In a study utilizing an ovarian cancer cell line, the *in vitro* efficacy of combining CAR-T cells targeting folate receptor alpha and oncolytic virus armed with bispecific T-cell engager (an immunotherapeutic molecule) was shown to be superior to single targeting (78).

5. Future Perspectives

Currently, DNA-damaging agents represent the most widely used therapeutic options for ovarian cancer. Combinational strategies associating with DNA-damaging drugs have achieved better treatment outcomes than monotherapy. These strategies are effective in *BRCA*-deficient and/or *BRCA*-proficient tumors, HRD-positive tumors or PARP inhibitor-resistant tumors, covering wide population of ovarian cancer patients. Biomarkers are the keys for realizing the potential of targeted therapies because data thus far showed additive or synergistic antitumor effects of the combinatory approaches in selected populations. Immunotherapy represents a new era in cancer treatments and combination therapy appears to be critical for immunotherapy to work effectively in ovarian cancer. As immunotherapy requires the engagement of the host immune system, the composition and interaction of immune cells in the TME may be important determinants of the effectiveness. Tumor mutational burden is low in most of the ovarian tumors, alternative markers predictive of immunotherapy responses remain to be explored. Deeper understanding of the biology of ovarian cancer, including the signaling mechanisms, TME and immune responses, would refine treatment strategies for the disease. Further, another emerging clinical challenge is to determine dosing schedule of the therapeutic agents to achieve optimal survival benefit of the combinations.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Table 1. Selected preclinical studies of combinatorial treatment approaches

Drug 1	Drug 2	Study model	References
Veliparib (PARPi)	**Carboplatin** (chemotherapy)	Chinese hamster ovary (CHO) cells *in vitro* and xenograft	12
Carboplatin (chemotherapy)	**Ganetespib** (HSP90i)	Human ovarian cancer cell lines *in vitro* and xenograft	16
Olaparib (PARPi)	**MK-8776** (CHK1i) or **ceralasertib** (ATRi)	Human ovarian cancer cell lines *in vitro* and *in vivo* PDX	17
Talazoparib (PARPi)	**Adavosertib** (WEE1i)	Human ovarian cancer cell lines *in vitro* and xenograft; Human ovarian cancer PDX	20
Olaparib (PARPi)	**JQ1** (BRD4i)	Human ovarian cancer cell lines *in vitro* and xenograft	21
Talazoparib (PARPi)	**Navitoclax** (BCL-2/xLi)	Human ovarian cancer cell lines *in vitro*	22
Cisplatin, Carboplatin, or **Doxorubicin** (chemotherapy)	**APR-246** (p53 reactivating)	Primary cells isolated from ascitic fluid of human ovarian, fallopian tube, or peritoneal cancer patients	24
Cediranib (VEGFRi)	**Olaparib** (PARPi)	Human ovarian cancer cell lines *in vitro* and xenograft	29
Erlotinib (EGFRi)	**Olaparib** (PARPi)	Human ovarian cancer cell lines xenograft *	37

PF-04691502 (PI3Ki/mTORi)	**PD-0325901** (MEKi)	Human ovarian cancer cell lines *in vitro* and xenograft	44
Saracatinib (SRCi)	**Selumetinib** (MEKi)	Human ovarian cancer cell lines *in vitro* and xenograft	47
Cobimetinib (MEKi)	**Navitoclax** (BCL-2/xLi)	Human ovarian cancer PDX	48
MK-2206 (AKTi)	**C188-9** (STAT3i)	Human ovarian cancer cell lines *in vitro* and xenograft	49
Buparlisib (PI3Ki)	**Olaparib** (PARPi)	Human ovarian cancer cell lines *in vitro*; *ex vivo* culture of human primary ovarian cancer tissues	50
Anti-PD-L1 antibody	**Talazoparib** (PARPi)	Murine ovarian cancer cell line xenograft *	57
Anti-PD-1 or **PD-L1** antibody	**Paclitaxel** (chemotherapy)	Murine ovarian cancer cell line xenograft *	59
Anti-PD-1 antibody	**Trabectedin** (chemotherapy)	Murine ovarian cancer cell line xenograft *	60
Anti-PD-L1 antibody, **anti-IL-10** antibody and **2′3’-cGAMP**	**Paclitaxel** and **Carboplatin** (chemotherapy)	Murine ovarian cancer cell line xenograft *	61
Anti-PD-1 or **PD-L1** or **CTLA-4** antibody	**Veliparib** (PARPi)	Murine ovarian cancer cell line xenograft *	62

Cancer vaccine MIS416 consisting of stimulatory ligands (TLR9 and NOD-2)	**Anti-CD11b** antibody	Murine ovarian cancer cell line xenograft *	68
Oncolytic virus expressing CXCL11	**Anti-PD-L1** antibody	Murine ovarian cancer cell line xenograft *	71
Oncolytic virus 34.5ENVE	**Doxorubicin** (chemotherapy)	Human ovarian cancer cell line *in vitro* and xenograft	72
Oncolytic virus expressing CXCR4 antagonist	**PLD** (chemotherapy)	Human or murine ovarian cancer cell lines *in vitro* and xenograft *	73
Oncolytic virus NDV(F3aa)	**3TSR** (anti-angiogenic polypeptide)	Murine ovarian cancer cell line xenograft *	74
CD8+ T cells	**Cisplatin** (chemotherapy)	Human ovarian cancer cells *in vitro* and xenograft *	76
CAR-T cells targeting ErbB dimers	**Carboplatin** (chemotherapy)	Human ovarian cancer cell line xenograft *	77
CAR-T cells targeting folate receptor alpha	**Oncolytic virus** armed with bispecific T-cell engager	Human ovarian cancer cell line *in vitro* *	78

PDX, patient-derived xenograft; PLD, pegylated liposomal doxorubicin.
The listed combinations are synergistic except studies marked with *, in which synergy score was not provided.

Table 2. Selected clinical studies of combinatorial treatments

Phase	Evaluable patients	Drug 1	Drug 2	Outcome	References
II	n=162; platinum-sensitive, recurrent	**Olaparib** (PARPi)	**Paclitaxel** and **Carboplatin** (chemotherapy)	Median PFS in combo group: 12.2 months (95% CI 9.7–15.0) vs. chemo alone group: 9.6 months (95% CI 0.34–0.77); P=0.0012	13
I	n=26; metastatic	**Olaparib** (PARPi)	**PLD** (chemotherapy)	ORR: 50% (ORR in platinum-resistant and platinum-sensitive patients was 25% and 71%, respectively)	14
I	n=54; advanced or metastatic	**Veliparib** (PARPi)	**Carboplatin** and **Gemcitabine** (chemotherapy)	ORR: 69% of *BRCA*-mutated patients. Median PFS in *BRCA*-mutated patients: 8.6 months (95% CI: 7.1–11.7) vs. *BRCA*-wild type/unknown patients: 5.9 months (95% CI: 4.1–9.9)	15
II	n=21; *TP53*-mutated, refractory or resistant to first-line chemotherapy	**Carboplatin** (chemotherapy)	**Adavosertib** (WEE1i)	ORR: 43%. Median PFS: 5.3 months (95% CI, 2.3 to 9.0 months). Median OS: 12.6 months (95% CI, 4.9 to 19.7)	19
III	n=484; platinum-sensitive, recurrent	**Bevacizumab** (anti-VEGF-A antibody)	**Gemcitabine** and **Carboplatin** (chemotherapy)	Median PFS in combo: 12.4 months vs. chemo alone: 8.4 months. ORR in combo: 78.5% vs. chemo alone: 57.4%. DOR in combo: 10.4 months vs. chemo alone: 7.4 months	25

III	n=361; platinum-resistant, recurrent	**Bevacizumab** (anti-VEGF-A antibody)	**Paclitaxel** or **PLD** or **Topotecan** (chemotherapy)	Median PFS in combo: 6.7 months vs. chemo alone 3.4 months. ORR in combo: 11.8% vs. chemo alone 27.3% (P<0.001). Median OS in combo: 16.6 months vs. chemo alone: 13.3 months	26
II	n=90; platinum-sensitive, relapsed or had a deleterious germline BRCA1/2 mutation	**Cediranib** (VEGFRi)	**Olaparib** (PARPi)	Median PFS in combo: 16.5 months vs. Olaparib alone: 8.2 months. Median OS in combo: 44.2 months vs. Olaparib alone: 33.3 months	28
III	n=806; advanced	**Bevacizumab** (anti-VEGF-A antibody)	**Olaparib** (PARPi)	Median PFS in combo: 22.1 months vs. bevacizumab alone: 16.6 months. (HR, 0.59; 95% CI, 0.49 to 0.72; P<0.001)	30
III	n=156; platinum-resistant	**Pertuzumab** (anti-HER2 antibody)	**Topotecan** or **Paclitaxel** or **Gemcitabine** (chemotherapy)	Median PFS: 4.3 months in combo arm vs. 2.6 months in chemo alone arm. ORR: 13.1% in combo arm vs. 8.7% in chemo alone arm	39
II	n=223; platinum-resistant or refractory	**Seribantumab** (anti-HER3 antibody)	**Paclitaxel** (chemotherapy)	Median PFS: 3.75 months in combo vs. 3.68 months in paclitaxel alone. Tumors with low HER2 had better treatment benefit from combination compared with paclitaxel alone (PFS HR, 0.37; 95% CI, 0.18 to 0.76; P=0.007)	41
Ib	n=21; with *RAS* or *BRAF* mutation	**Buparlisib** (PI3Ki)	**Trametinib** (MEK1/2)	ORR: 29%. Median PFS: 7 months	46

I	n=46; recurrent	**Buparlisib** (PI3Ki)	**Olaparib** (PARPi)	ORR: 29%	51
Ib	n=28; 26 (93%) were platinum-resistant or refractory	**Alpelisib** (alpha-specific PI3Ki)	**Olaparib** (PARPi)	ORR: 33% in patients with germline *BRCA* mutations, and 31% in those with germline wild type *BRCA*	52
I/II	n=60; advanced	**Pembrolizumab** (anti-PD-1 antibody)	**Niraparib** (PARPi)	ORR: 18%	64
II	n=38; relapsed	**Nivolumab** (anti-PD-1 antibody)	**Bevacizumab** (anti-VEGF-A antibody)	ORR: 28.9%. Median PFS: 9.4 months	66
I	n=10; relapsed	**Cancer vaccine** NY-ESO-1 vaccine	**Decitabine** (DNA methyltransferase inhibitor)	DCR (SD or PR): 60%	67
II	n=10; relapsed	**Cancer vaccine** p53-synthetic long peptide	**Cyclophosphamide** (Treg-depleting chemotherapy)	SD: 20%	69
I	n=10; relapsed	**Cancer vaccine** autologous dendritic cells with autologous tumor lysate	**Bevacizumab** (anti-VEGF-A antibody) and **Cyclophosphamide** (Treg-depleting chemotherapy)	OS at 2 years: 78%	70

PLD, pegylated liposomal doxorubicin; HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; OS, overall survival; ORR, objective response rate; SD, stable disease; PR, partial response; DOR, duration of response; DCR, disease control rate. All patients received chemotherapy prior to the trials.