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Immunotherapy in ovarian cancer

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Introduction

Epithelial ovarian cancer (EOC) is the most common cause of gynecological cancer-associated death in woman¹. To date, there are no effective means of screening or early detection for this disease, and consequently, the majority of patients have advanced stage disease involving metastasis at the time of initial diagnosis². The first-line therapy of EOC includes surgical debulking and platinum-based chemotherapy³. Although initial response is often excellent, patients oftentimes develop resistance to chemotherapy and succumb to their disease. Therefore, it is critical to develop novel treatment strategies to improve clinical outcomes.

Current immunotherapy, including checkpoint blockade and adoptive T-cell transfer (ACT), has become a clinically effective treatment modality for a wide variety of cancer types. However, the application of immunotherapy in EOC is still being tested in clinical trials. Tumor-infiltrating lymphocytes (TILs), positively correlating with patient survival, have now been recognized as a predictive biomarker for immunotherapy and chemotherapy responses^{3,4}. In ovarian cancer, the presence of CD3⁺ T-cell and its correlation with improved clinical outcome had been observed in an early study, which establishes the validity of utilizing immunotherapy for EOC management⁵. Later studies demonstrated the

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prognostic significance of FOXP3⁺ T_{reg} cells⁶ and/or the ratio of CD8⁺ T cells versus FOXP3⁺ T_{reg} cells in ovarian cancer, and confirmed CD8⁺ T cells as important antitumor effectors^{6,7}. CD8⁺ effector T cells, are poised to eliminate tumor cells after recognizing tumor associated antigens and particularly the neoantigens presented on the surface of tumor cells⁸. However, their activation and effector functions are often held in check and attenuated by inhibitory signals in the tumor microenvironment, which include inhibitory cytokines, immunosuppressive metabolites, and immunoinhibitor B7 family member expressed on the antigen presenting cells (APCs) and tumor cells^{9,10}. Checkpoint blockade can restore the antitumor activity of effector T cells and mediate tumor regression¹¹. Adoptive transfer of ex vivo-expanded tumor-specific T cells can directly increase the number of CD8⁺ effector T cells in tumor¹². Cancer vaccine may activate TAA-specific CD8⁺ T cell response through APCs¹³. These immunotherapeutic strategies have been tested or are under development in ovarian cancer. The progress of immunotherapy in EOC will be discussed in this review.

Checkpoint blockade

Several checkpoint molecules have been identified, including cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), PD-1, and PD ligand 1 (PD-L1, B7-H1). Antibodies targeting these checkpoints can block their inhibitory function, thus unleashing the antitumor activity of effector T cells and mediating tumor regression¹⁴. There are currently 6 immune checkpoint inhibitors approved by the Food and Drug Administration (FDA): one CTLA-4 antibody (Ipilimumab), two PD-1 antibodies (Pembrolizumab and Nivolumab) and three PD-L1 antibodies (Avelumab, Atezolizumab and Durvalumab). These antibodies have demonstrated remarkable clinical benefits in a wide range of tumor types, such as melanoma, NSCLC, renal cell and urothelial cancer¹¹.

Although anti-CTLA-4 and anti-PD-1/PD-L1 both enhance anti-tumor immune response through increasing the number of tumor-infiltrating T cells and recovering the effector function of cytotoxic T cells, their underlining cellular and molecular mechanisms are distinct. CTLA-4 is a receptor on T cells and shares the same set of ligands with costimulatory receptor CD28 but with a much higher affinity, therefore providing a competitive inhibition¹⁵. Antibody targeting CTLA-4 blocks the ligation of CTLA-4 with its ligands, which prevents inhibitory signal transduction and results in increased CD28-mediated co-stimulation. CTLA-4 blockade has been shown to directly enhance the proliferation and activation of tumor-specific CD8⁺ T cells¹⁶.

PD-1 is another inhibitory receptor expressed on T cells. Unlike CTLA-4, which regulates T-cell activation at the priming phase, PD-1 inhibits effector T-cell activity mainly in the effector phase in peripheral tissue and tumor microenvironment¹⁷. Upon activation by its ligands PD-L1 or PD-L2, PD-1 becomes phosphorylated and recruits the inhibitory phosphatases SHP-2, which rapidly dephosphorylates CD28 and inactivates the costimulatory signaling¹⁸. Of the two ligands, PD-L1 is considered to be more relevant in the tumor microenvironment because of its expression on tumor, stromal and immune cells¹⁹. Antibodies targeting either PD-1 or PD-L1 result in abrogation of the negative signal, thus restoring the function of tumor-infiltrating T cells and mediating tumor regression²⁰.

Single-agent checkpoint inhibitor in ovarian cancer

Several antibodies targeting PD-1, PD-L1 and CTLA-4 have been tested in patients with recurrent ovarian cancer. Overall, objective response (OR) (complete response (CR) + partial response (PR)) rates range from 5.9% to 15% (Table 1).

In an early phase I trial, 207 patients with selected advanced cancers, including 17 with EOC, received anti-PD-L1 antibody (BMS-936559). Among the 17 women, 1 (5.9%) had a PR and a total of 4 women (23%) had disease control. This is the first data suggesting immune checkpoint blockade as a potentially valuable therapeutic approach in ovarian cancer²¹. Later, a phase II trial of an anti-PD1 antibody (nivolumab) was conducted in 20 patients with platinum-resistant EOC. 2 patients experienced a CR, 1 had a PR and 6 had a stable disease (SD), leading to a disease control rate of 45%²². Pembrolizumab, another anti-PD1 antibody, was shown to have an OR rate of 11.5% and a disease control rate of 34.6% in 26 patients with advanced EOC. Patients were selected with a PD-L1 expression level of 1% of tumor cells for trial entry, and 85% of patients were heavily pre-treated²³.

Atezolizumab is a fully humanized monoclonal antibody against PD-L1 and has been approved for metastatic non-small cell lung cancer (NSCLC) treatment. In a phase I study (), 12 patients with recurrent ovarian cancer were treated with atezolizumab until loss of clinical benefit. Of 9 response-evaluable patients, 2 patients (22%) had a PR and 5 patients had PD. PD-L1 status on immune cells (IC) was found to correlate with response²⁴. More recently, a study assessed the safety and activity of another anti-PD-L1 antibody (avelumab) in 124 women with recurrent or refractory EOC (). This is the largest study of anti-PD(L)1 agents in patients with EOC to date. Avelumab achieved an OR of 9.7% and a disease control rate of 54.0%. Again, in this cohort more than two-thirds of the patients had received previous lines of therapy. This study showed that single-agent avelumab was clinically active in heavily pretreated patients with EOC²⁵.

In addition to PD(L)1 blockade, the activity and safety of anti-CTLA-4 antibody in EOC was studied. In a phase II trial, 40 patients with recurrent platinum-sensitive EOC received an anti-CTLA-4 antibody ipilimumab (). The OR rate was 10.3% and adverse events (AEs) of grade 3 occurred in 50% of the patients²⁶.

In summary, these studies suggest that single-agent checkpoint inhibitor, in particular PD(L)-1 blockade, is promising in epithelial ovarian cancer, although the OR rate is much lower compared with the response rates achieved in melanoma, NSCLC, renal cell and urothelial cancer. Although potential mechanisms of resistance toward immune checkpoint blockade are under investigation, combining checkpoint inhibitors with other biological agents to enhance the anti-tumor immune response has been rationally illustrated and numerous combinatorial trials have entered the clinic. The combination of two checkpoint blockades (nivolumab + ipilimumab) was firstly tested in melanoma²⁷. In EOC, the same combination () and another combination of durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA-4) () are both being investigated.

Combination with PARP inhibitor

Tumor mutational burden (TMB) is a recently suggested biomarker of response to PD(L)-1 blockade therapy²⁸. Tumor-specific mutations can result in immunogenic neoantigens, therefore high TMB correlates with high neoantigen load. This higher neoantigen load correlated with an increased number of tumor-infiltrating lymphocytes (TILs), which is counterbalanced by PD-1/PD-L1 signaling²⁹. Melanoma, lung cancer and a subtype of colorectal cancer with mismatch repair deficiency have a significantly higher TMB, and they all have a remarkable response to PD-1 inhibition³⁰. In ovarian cancer roughly the 25–30% of the high grade serous (HGS) harbor a mutation in the BRCA1 or BRCA2 genes, which have integral functions in DNA homologous recombination (HR) repair. The presence of a HR-deficiency is associated to a higher mutation burden and increased TILs compared to the BRCA-wild type tumors³¹. Thus, BRCA-mutated HGS ovarian cancer could represent a subtype with potential increased response to immune checkpoint blockade alone or in combination with poly (ATP-ribose) polymerases (PARP) inhibitors. Several clinical trials evaluating the combination of Olaparib (PARP inhibitor) and checkpoint blockade in patients with BRCA-mutated ovarian cancer are currently ongoing (Table 2). An ongoing phase II trial (), the combination of olaparib and durvalumab (anti-PD-L1) was found to induce objective responses in over 70% of patients with relapsed, platinum-sensitive, *BRCA*-mutated ovarian cancer³². Another phase I/II trial evaluating the combination Olaparib with anti-CTLA-4 and anti-PD-L1 in patients with BRCA1/BRAC2-mutated EOC () is also ongoing.

Combination with VEGF inhibitor

Another interesting novel approach is to combine vascular endothelial growth factor (VEGF) inhibitor and checkpoint blockade. VEGF is primarily known as a mediator of angiogenesis; however, emerging studies have also recognized VEGF as a critical mediator of immune suppression in the tumor microenvironment³³. Inhibitors targeting VEGF or VEGF receptors (VEGFR) have been shown to decrease the numbers of regulatory T cells and myeloid-derived suppressor cells (MDSC) and enhance the infiltration of effector T cells^{33,34}. Several clinical trials are currently ongoing to evaluate the safety and efficacy of the combination of bevacizumab (anti-VEGF) and checkpoint blockade in melanoma, NSCLC and renal cell cancer. For EOC, a phase II trial focused on platinum-resistant disease, in which atezolizumab (anti-PD-L1) is given together with bevacizumab and acetylsalicylic acid (). In another study, durvalumab combined with olaparib or the VEGFR inhibitor cediranib in EOC is being tested ().

Combination with chemotherapy

Chemotherapy can be used in combination with checkpoint blockade to amplify the antitumor immune response. In addition to direct cytotoxic effects on tumor cells, a proportion of chemotherapy agents may actually induce immunogenic cell death, expand neoantigen repertoire, increase antigen presentation, change the inflammatory milieu of tumor microenvironment and/or decrease the numbers of immunosuppressive cells, thereby inducing or enhancing the anti-tumoral immune response^{35,36}. There are now more than 200

clinical trials testing immune checkpoint inhibitors in combination with various chemotherapeutic agents across different cancer types.

In ovarian cancer, there are currently 5 randomized phase III trials adding PD-L1 blockade to chemotherapy: 2 are with avelumab and 3 are with atezolizumab. JAVELIN Ovarian 200 () is the first one to compare avelumab alone or in combination with pegylated liposomal doxorubicin in patients with platinum-resistant/refractory recurrent ovarian, fallopian tube or peritoneal cancer. The second trial is JAVELIN Ovarian 100 (), which will assess the efficacy of avelumab in combination with conventional chemotherapy carboplatin and paclitaxel in patients with previously untreated ovarian cancer. The other 3 trials with atezolizumab involve adding both chemotherapy and bevacizumab (anti-VEGF) to atezolizumab. The trial will assess the efficacy of atezolizumab in combination with bevacizumab plus platinum-based chemotherapy in patients with ovarian cancer who have platinum-sensitive relapse (platinum-free interval > 6 months). The trial will test the activity of atezolizumab in combination with bevacizumab paclitaxel and carboplatin in participants with newly diagnosed ovarian cancer. The trial combines atezolizumab with bevacizumab and pegylated liposomal doxorubicin in platinum resistant ovarian cancer.

Similarly, another PD-L1 inhibitor Durvalumab and a PD-1 inhibitor in combination with chemotherapy are also being tested in several phase I/II clinical trials in ovarian cancer. These include Durvalumab combined with PLD (), Pembrolizumab combined with paclitaxel and carboplatin () or with dose dense paclitaxel ().

Based on the published results and preliminary data, combinational therapy including PD-1/PD-L1 inhibitors and chemotherapy certainly has the potential to be superior to chemotherapy alone^{37,38}. However, there are some ongoing challenges. Cytotoxic chemotherapy has historically been considered immunosuppressive because most chemotherapeutic agents indiscriminately impair cellular division and thus impact not only tumor cells, but also effector lymphocytes and innate leukocytes³⁹. Moreover, recent studies demonstrated that certain chemotherapy agents might attenuate the anti-tumor immune response^{40,41}. Therefore, to thoroughly assess the combination with chemotherapy, selection of chemotherapy agents, optimization of the sequencing, timing, dose, and management of concurrent toxicities will be required.

Adoptive T-cell therapy (ACT)

Adoptive T cell therapy consists of the infusion of ex vivo-expanded tumor-specific T cells that can recognize and attack tumor cells resulting in tumor regression¹². There are currently two major forms of ACT being used for cancer treatment: isolating and expanding tumor-infiltrating lymphocytes (TIL) or using genetically modified T cells that express a specific TCR or a chimeric antigen receptor (CAR)⁴². Both strategies have been tested in preclinical ovarian cancer models, and currently being investigated in early phase trials.

TIL therapy

TIL therapy has been shown to induce long-lasting and complete regression of metastatic tumor in patients with melanoma⁴³. Generally, ACT of TIL consists of several steps:

1. isolation of TILs from fresh tumor biopsy;
2. selection and expansion of tumor-specific T-cell population ex vivo;
3. lymphodepletion of immunosuppressive components by chemotherapy or radiation; and
4. infusion of expanded T cells to patients with IL-2 administration.

During this long-term procedure, both CD4⁺ and CD8⁺ T cells are expanded ex-vivo and are subsequently infused back into the patient⁴⁴.

TIL therapy in melanoma response rates can be from 47% to 72%⁴⁵. The efficacy of TIL therapy in EOC has also been investigated in several early clinical studies (Table 3). The first trial of TIL therapy in EOC enrolled 17 patients with advanced or recurrent platinum-resistant disease. Among 7 patients received TIL infusion them, 1 CR and 4 PR were reported and tumor regression lasted for 3–5 months. Another group of 10 patients received TIL infusion combined with cisplatin-based chemotherapy. 7 had a CR, 2 had a PR, and 1 had a SD. The duration of response ranged from 13 to more than 26 months⁴⁶. Later, a pilot study was conducted to determine the feasibility and clinical effects of TIL infusion plus low-dose IL-2 in patients with platinum-resistant ovarian cancer. Among 8 patients received the treatment: 2 had reduction in ascites, 1 had reduction in tumor burden and CA-125 and 1 had surgically confirmed stable disease⁴⁷. In 1995, Fujita et. al tested the efficacy of maintenance therapy with TIL in ovarian cancer patients with no evidence of disease after debulking surgery and adjuvant platinum-based chemotherapy. 13 patients received TIL and 11 additional patients (control group) received standard treatment. The TIL group had significant improved 3-year OS (100% vs 65%, $p < 0.01$) and 3-year disease free survival (DFS) (82.1% vs 54.5%, $p < 0.05$) compared to the control group⁴⁸. In summary, these early trials showed limited efficacy of TIL therapy in EOC. However, it is notable that none of these trials used current chemotherapy regimens for lymphodepletion before TIL infusion, and the methods for ex-vivo TIL expansion have changed considerably. Therefore there is still significant biological and clinical rationale to test TIL therapy in ovarian cancer (Table 4). An ongoing phase II trial is assessing the anti-tumor efficacy of TIL in metastatic digestive tract, urothelial, breast, or ovarian/endometrial cancer (). In this study, short-term cultured autologous TIL will be used after a lymphocyte depleting regimen, and checkpoint blockade pembrolizumab will be administrated at time of progression. More recently, a phase I study focused upon patients with platinum resistant high-grade serous ovarian, fallopian tube, or primary peritoneal cancer () was opened. Patients will receive an infusion of autologous TIL which will be stimulated ex-vivo with autologous dendritic cells (DCs) and OKT3 (anti-CD3 antibody). This “re-stimulation” is believed to make autologous TIL work better.

TCR-engineered T cells

Autologous T cells can be genetically modified to express a cloned TCR directed towards a specific antigen. T cells isolated from patient peripheral blood are used for transduction with a viral vector encoding TCR, and then can rapidly be expanded to a great number for infusion within a short time. Similarly to the endogenous TCR-expressed T cells, TCR-

engineered T cells recognize tumor-specific antigen in a MHC-dependent manner and its function can be negatively regulated by the immunosuppressive signals in the tumor microenvironment⁴⁹.

The choice of antigen to be targeted determines the specificity of TCR-engineered T cells. To date, most of the studies have engineered TCRs to target tumor-associated antigens, such as Melan-A/MART-1 and gp100, or cancer germline antigens such as NY-ESO-1 and MAGE^{50,51}. Although these TCR-engineered T cells have resulted in objective responses in the majority of treated patients, especially in melanoma patients, they also caused “off-tumor-on-target” toxicity by reacting to healthy organs that expressed the targeted antigen⁵². In EOC, so far, there are no completed clinical trials using TCR-engineered T cells and several phase I studies are on-going (Table 4). The study evaluates the safety and effect of MAGE-A4-specific TCR gene transduced T cells in HLA-A*24:02 positive patients with refractory ovarian cancer that express the antigen MAGE-A4. Another 3 trials (, ,) all use T cells engineered with NY-ESO-1-specific TCR to treat ovarian cancer patients with the HLA-A*02:01 or HLA-A*02:06 allele and whose tumor expresses the NY-ESO-1 antigen. NY-ESO-1 is highly expressed in various types of cancer, including EOC. While in normal somatic tissues its expression is restricted to the germline cells, which lack HLA molecules and cannot be recognized by T cells. Therefore, NY-ESO-1 has been selected as an attractive target for ACT in clinical trials. Most recently, efforts have been focused on the development of T cells engineered with TCRs specific to particular tumor neoantigens. A new pilot study has demonstrated the feasibility of the process from identification of neoantigen to production of the neoantigen-specific cytotoxic TCR-engineered T cells for ovarian cancer⁵³.

CAR T cells

CAR is another mean for providing recognition specificity to engineered T cells. CAR molecule combines the antigen-binding domain of a single-chain variable fragment (scFv) derived from an antibody with cytoplasmic signaling motifs. The scFv fragment recognizes antigens that present on tumor cell surface. The cytoplasmic signaling motifs mediate T-cell activation, consisting of a CD3-zeta activation domain and two co-stimulatory domains, CD28 and CD137/4-1BB. Upon antigen encounter, intracellular domains of the CAR transduce the activation signals, leading to T-cell activation with cytotoxic function^{54,55}. Recently, the first two CAR T-cell therapies (axicabtagene ciloleucel and tisagenlecleucel) have been approved for the treatment of refractory diffuse large B-cell lymphoma (DLBCL) and refractory B-cell precursor acute lymphoblastic leukemia (ALL). They both use anti-CD19-redirected CAR T cells and have achieved impressive response rates. Thus, the field of CAR T-cell therapy is now booming and over 200 CAR-T clinical trials are running.

In EOC, the first study of adoptive transfer of folate receptor- α (FR α)-specific CAR T cells in ovarian cancer patients showed no clinical benefit probably because of low expression of the CAR and poor persistence of the transferred T cells⁵⁶. Another phase I trial () tested the mesothelin-specific CAR T cells in six recurrent serous ovarian cancer patients. The treatment was feasible and safe and trafficking of CAR T cells to the tumor site was observed. All six patients showed stable disease after one month treatment⁵⁷. The effect of

mesothelin-specific CAR T cells is promising and being tested in two more phase I/II clinical trials (,) in mesothelin expressing cancers, including ovarian cancer. In addition to FR α and mesothelin, some other CAR T cells targeting tumor-associated antigens are under investigation in clinical trials, including Mucin-16 (), Epidermal growth factor receptor (EGFR) (), Human epidermal growth factor receptor-2 (HER-2) (), CD133 () and Natural killer group 2D (NKG2D) (). Identifying the tumor-specific surface antigens is the priority to manufacture effective CAR T cells against solid tumors. Moreover, the immunosuppressive environment within a solid tumor also inhibits the trafficking and effector function of CAR T cells. Therefore, various combinations of CAR T cells with other therapies would be expected to achieve greater persistence and response.

Cancer vaccine

The biological principle of cancer vaccines is to provoke a tumor-specific immune response that involves both innate and adaptive immunity. Therapeutic cancer vaccine theoretically could increase TAA presentation by APCs and then generate TAA-specific CD8 $^{+}$ T cells to eradicate tumor cells. Moreover, vaccine-induced immune response has the ability of immunological memory that can persist for long periods of time⁵⁸. In EOC, many clinical studies have revealed induced antigen-specific T cell response and improved survival in different degrees by using different vaccines, including recombinant protein or peptide vaccines and whole tumor lysate or dendritic cell (DC)-based autologous vaccines.

Protein/peptide-based vaccine

Protein/peptide-based vaccines are usually based on the well-defined TAAs and administered together with an adjuvant to improve their presentation by endogenous DCs. The ideal TAA should be selective expression by tumor cells relative to normal tissue and high immunogenicity. Among antigens targeted for vaccination, NY-ESO-1 is one of the most spontaneously immunogenic tumor antigens that have been tested in clinical trials in various solid tumors, including ovarian cancer⁵⁹. Vaccination of HLA-A*0201-positive EOC patients with NY-ESO-1 peptide (NY-ESO-1 157–165) and Montanide, an incomplete Freund's adjuvant, induced specific T-cell immune response in patients with both NY-ESO-1-positive and NY-ESO-1-negative tumors⁶⁰. Another study used the peptide NY-ESO-1 157–170 that contains both CD4 $^{+}$ and CD8 $^{+}$ T cell epitopes for vaccination in advanced EOC patients. Long-lived and functional vaccine-elicited CD4 $^{+}$ and CD8 $^{+}$ T cells were detected in patients with no evidence of disease (NED) for >6 months⁶¹. Overlapping long peptides (OLP) derived from NY-ESO-1 was also tested in various adjuvant combinations in patients with EOC. NY-ESO-1-specific antibody and CD8 $^{+}$ T cell responses were detected in 6 of 13 (46%) and 8 of 13 (62%) of patients after vaccination with OLP+ Montanide, and both were detected in 10 of 11 (91%) of patients receiving OLP + Montanide+ poly-ICLC⁶². Recombinant poxviruses (vaccinia and fowlpox) expressing the whole protein of NY-ESO-1 was evaluated in a phase II clinical trials in 22 ovarian cancer patients. Integrated NY-ESO-1-specific antibody and CD4 $^{+}$ and CD8 $^{+}$ T cells were induced in a high proportion of EOC patients⁶³. The expression of NY-ESO-1 is suppressed by DNA methylation and can be enhanced by the DNA methyltransferase (DNMT) inhibitor decitabine. A phase I trial combined the NY-ESO-1 peptide vaccine with escalating doses of

decitabine and doxorubicin liposome chemotherapy in 12 patients with relapsed EOC. Increased NY-ESO-1-specific antibodies and CD8⁺ T cells were detected in the majority of patients. Among 10 evaluable patients, 5 patients had SD and 1 had a PR⁶⁴. Other antigens such as p53, Her2 and FR α have also been tested for vaccination in clinical studies of EOC. A p53 synthetic long peptide (p53-SLP) vaccine combined with low-dose cyclophosphamide was tested in a phase 2 single-arm trial in EOC. p53-specific effector T cells were observed in 90% of patients and stable disease was observed in 20% of patients⁶⁵. Many different peptides derived from Her2 have also been tested in ovarian cancer. However, no immunogenicity was observed in most studies using single or mixed Her2 peptides and no clinical data was obtained⁶⁶. FR α peptide vaccine was tested in a recent phase I clinical trial enrolling patients with EOC who showed no evidence of disease after conventional therapy. Vaccination was well tolerated in all patients and induced immune response in more than 90% of patients⁶⁷.

DC-based vaccine

DC is the most potent APC population for activating anti-tumor T-cell response. Also because it rarely presents immune-related toxicities, DC has become the most frequently used cellular therapeutic in clinical trials. DC can be given alone after cytotoxic chemo- or radiotherapy or loaded *ex vivo* with various antigens, such as proteins, peptides or the whole tumor lysate, before reinfusion into patients⁶⁸.

Protein- or peptide-loaded DC

Similar with recombinant protein/peptide vaccine, this strategy is also based on well-defined TAAs. Autologous DCs were pulsed with recombinant protein or peptides before reinfusion. An early phase I study evaluated immunization with autologous DC pulsed with mannan-MUC1 fusion protein to treat patients with advanced cancer, including 2 EOC patients. T cell response was observed in all patients and 1 of 2 EOC patients in progression at entry was stable after therapy for at least 3 years⁶⁹. Later, Lapuleucel-T, the Her-2-GM-CSF fusion protein loaded DC vaccine was tested in 4 patients with Her2-positive ovarian cancer. Her-2-specific T cell response was detected after vaccination and 2 patients had SD⁷⁰. Peptide-loaded DC vaccines were also tested in ovarian cancer. A phase I trial enrolled 10 patients including 3 EOC patients to receive immunization with DC pulsed with Her-2- or MUC-1-derived peptides. Antigen-specific T cell responses were detected in 5 of 10 patients⁷¹. In another phase I trial, 11 EOC patients were immunized with DC loaded with Her2, hTERT and PADRE peptides with low-dose cyclophosphamide. Her2- and hTERT-specific T-cell responses were observed and the 3-year overall survival was 90%⁷².

Whole tumor lysate-loaded DC

The advantage of whole tumor cells as a source of antigens for DC based vaccine is that the entire repertoire of antigens associated with a particular tumor, including the specific neoantigens, may be processed and presented to the immune system. Therefore, this strategy is predicted to generate a better immune response and prevent tumor immune escape through antigen-loss variants or mutations. An earlier phase I study used DC loaded with crude

whole tumor lysate to treat 8 patients, including 6 with recurrent EOC. 3 patients showed stable disease lasting 25 to 45 weeks and tumor antigen specific immunity was detected in 2 of 6 evaluable patients⁷³. Later, oxidized whole tumor lysate with enhanced immunogenicity were used to load DC. A recent pilot trial tested the autologous oxidized lysate-loaded autologous DC in recurrent ovarian cancer patients. DC vaccine was administered alone (cohort 1, n = 5), in combination with bevacizumab (cohort 2, n = 10), or bevacizumab plus low-dose cyclophosphamide (cohort 3, n = 10). 3 patients had SD in cohort 1; 1 patient had PR and 4 patients had SD in cohort 2; and in cohort 3, 1 patient had PR and 6 patients had SD. Vaccination induced T cell responses to tumor antigen, which were associated with significantly prolonged survival⁷⁴.

The FANG vaccine is another whole tumor cell-based vaccine. Autologous tumor cells are electroporated with FANG vector, a plasmid expressing GM-CSF and a bifunctional shRNA against furin⁷⁵. This vaccine has been tested in a Phase I study recruiting 27 patients with advanced cancer, including 5 ovarian cancers⁷⁵. Currently, this vaccine is being tested in a phase II study in patients with high-risk stage III/IV ovarian cancer ().

Conclusion and future directions

Checkpoint blockade combination therapy

To date, checkpoint blockade is the most promising and studied immunotherapy in ovarian cancer. The combinations of PD(L)-1 antibodies with PARP inhibitors or conventional chemotherapy have achieved good response in clinical trials and demonstrate excellent clinical potential. Tumor cells create an immunosuppressive microenvironment through multiple mechanisms^{10,76,77}, therefore combinatorial therapeutic strategies will be required to unleash the maximal antitumor immune response. Epigenetic modulators, including the inhibitors targeting HDAC, enhancer of zeste homolog 2 (EZH2), DNA methyltransferase 1 (DNMT1) and histone demethylase LSD1 are recently found to have the ability to regulate the anti-tumor immune response^{78–80}. It has been showed that inhibitors of EZH2 and DNMT1 could induce chemokine expression in tumor cells, increase CD8+ T cells tumor infiltration and augment antitumor efficacy of PD-L1 blockade in an EOC preclinical model⁷⁸. Two ongoing clinical trials in ovarian cancer are testing the combinations of PD-1 blockade pembrolizumab with epigenetic modulators: one is a DNMT1 inhibitor guadecitabine () and another is a DNA methyltransferase inhibitor azacitidine (). Targeting tumor metabolism represents another promising strategy to combine with PD blockade. Growing evidence has shown that the tumor microenvironment supports metabolic reprogramming that dampens T cell function. Indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan catabolism has been recognized as a mechanism of immunosuppression in many types of cancer, including EOC. It has been well established that IDO inhibition could enhance antitumor immune response⁸¹. A current clinical trial involving EOC patients is assessing the combination between IDO inhibitor epacadostat and PD-L1 blockade nivolumab.

In addition to the PD-L1/PD-1 axis, other immunosuppressive molecules, such as T cell immunoglobulin mucin 3 (Tim-3), lymphocyte activation gene 3 protein (LAG3) and T cell immunoglobulin and ITIM domain (TIGIT), are all potential targets for developing

immunotherapy⁸². The combinations of PD blockade with LAG3 blockade are under testing in clinical trials ().

Neoantigen vaccine

Another promising approach is the development of EOC neoantigen vaccine. Cancer neoantigens are a class of HLA-bound peptides that are generated by tumor missense mutations. They are exclusively tumor-specific and highly immunogenic, thus can be recognized by host immune system⁸³. Neoantigens-derived vaccine has been recently tested in several clinical studies in patients with melanoma. The vaccine consisting of synthesized long peptides targeting up to 20 neoantigens per patient plus poly-ICLC (Hiltonol) was evaluated in a phase I study in patients with advanced cutaneous melanoma⁸⁴. Vaccine-induced both CD4⁺ and CD8⁺ T cells were detected against unique neoantigens. In 6 immunized patients, 4 had no recurrence at 25 months after vaccination. 2 recurrent patients were further treated with PD1 blockade and experienced complete tumor regression. In another study, a RNA-based poly-neo-epitope vaccine was tested in patients with advanced cutaneous melanoma⁸⁵. Similarly, both CD4⁺ and CD8⁺ T-cell responses were detected against 66% and 25% of the candidate neoepitopes, respectively. These studies indicate that neoantigen vaccine can induce expansion of the repertoire of neoantigen-specific T cells and mediate tumor regression, which represents a novel strategy of personalized immunotherapy for patients with cancer, including EOC.

In summary, the tumor microenvironment is the main battleground between tumor cells and the host immune system. Major immunosuppressive networks have been identified in the human epithelial ovarian cancer microenvironment. Current checkpoint therapy targets the cancer immunosuppressive mechanisms and generates clinical therapeutic efficacy in a fraction of patients. However, it seems that EOC patients are relatively insensitive to the single checkpoint therapy. Nonetheless, tumor associated effector T cells are positively associated with ovarian cancer patient survival and response to chemotherapy³. As effector T cell tumor trafficking is controlled by the cancer epigenetic pathway and T cell effector function may be additionally affected by metabolic pathway, the combination therapy by targeting the immunosuppressive networks (e.g. PD-L1/PD-1 pathway) and qualitatively supporting specific T cells may be a potential successful immunotherapy approach for ovarian cancer patients.

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KEY POINTS

1. The application of immunotherapy in ovarian cancer is being tested.
2. Checkpoint blockade can restore the antitumor activity of effector T cells and mediate tumor regression.
3. Adoptive transfer of ex vivo-expanded tumor-specific T cells can directly increase the number of CD8+ effector T cells in tumor.
4. Cancer vaccine may activate tumor associated antigens-specific CD8+ T cell response through antigen presentation cells.

SYNOPSIS

The phenotype and functionalities of the major immune cell subsets including myeloid cells, macrophages, dendritic cells, and T cells are altered in the ovarian cancer microenvironment. Immunosuppressive networks including inhibitory B7 family members and regulatory T cell-associated adenosine pathway have been defined in human ovarian cancer. In this review, the authors integrate emerging information on immunosuppressive mechanisms and T cell phenotype, and discuss strategies of immunotherapeutic and vaccine regimens. Finally, crucial points regarding design of immuno-oncology clinical trials are reviewed.

Table 1.

Clinical studies of checkpoint blockade in EOC

Target	Agent	Phase	Trial identifier	Disease status	Patients	Response
PD-1	Nivolumab	II	UMIN000005714	Platinum-resistant EOC	20	CR 2, PR 1, SD 6
PD-1	Pembrolizumab	I		Recurrent EOC	26	CR 1, PR 2, SD 6
PD-L1	BMS-936559	I		Recurrent EOC	17	CR 0, PR 1, SD 3
PD-L1	Avelumab	I		Recurrent EOC	124	CR 0, PR 12, SD 55
PD-L1	Atezolizumab	I		Recurrent EOC	9	CR 0, PR 2, SD 0
CTLA-4	Ipilimumab	I, II		Recurrent EOC	40	10% best response rate

NCT, National Clinical Trial; CR, complete response; PR, partial response; SD, stable disease

Table 2.

Ongoing trials of checkpoint inhibitors in combination with other biological agents in EOC

Combined agents	Combination	Treatment setting	Study population	Phase	Trial identifier
Checkpoint inhibitor	aCTLA-4 +aPD-1	Nivolumab vs nivolumab + ipilimumab	Recurrent or persistent EOC	IIa	
	aPD-L1 +aCTLA-4	Durvalumab + tremelimumab		I	
PARP inhibitor	aCTLA-4 +PARPi	Tremelimumab + olaparib	BRCA-deficient Ovarian Cancer	I/II	
	aCTLA-4 +PARPi	Tremelimumab vs tremelimumab + olaparib	Recurrent or Persistent EOC	I/II	
	aPD-L1 +PARPi	Durvalumab + olaparib vs durvalumab + cediranib	Recurrent EOC and germline BRCA1 or BRCA2 mutation	I/IIa	
	aCTLA4 +aPD-L1 +PARPi	Durvalumab + tremelimumab + olaparib	Recurrent EOC and germline or somatic BRCA1 or BRCA2 mutation	I/II	
VEGF inhibitor	aPD-L1+Bev	Atezolizumab + bevacizumab	Recurrent Platinum Resistant Ovarian Cancer	IIa	
Chemotherapy	aPD-L1 +PLD	Avelumab+ Pegylated Liposomal Doxorubicin	Platinum Resistant/ Refractory Ovarian Cancer	IIIa	
	aPD-L1 + TC	Avelumab+ carboplatin+ paclitaxel	Advanced EOC with no prior treatment	III	
	aPDL1 +Bev +TC/PLD	atezolizumab + avastin + platinum-based chemotherapy (Carboplatin combined with gemcitabine or paclitaxel or pegylated liposomal doxorubicin)	Recurrent platinum-sensitive EOC	III	
	aPD-L1 +Bev +TC	Atezolizumab+ Paclitaxel, Carboplatin + Bevacizumab	Newly-Diagnosed EOC with no prior treatment	III	
	aPD-L1 +Bev +PLD	Atezolizumab + Pegylated Liposomal Doxorubicin Hydrochloride + or Bevacizumab	Platinum-resistant EOC	III	
	aPD-L1 +PLD	Durvalumab + PLD	Recurrent, Platinum-Resistant Ovarian Cancer	I/II	
	aPD-1 + TC	Pembrolizumab + paclitaxel + carboplatin	Frontline Ovarian Cancer	II	
	aPD-1 + ddT	Pembrolizumab + dose-dense paclitaxel	Recurrent, Platinum-Resistant Ovarian Cancer	II	

aCTLA4, anti-CTLA-4; Bev, bevacizumab; ddT, dose-dense paclitaxel; NCT, National Clinical Trial; PARPi, PARP inhibitor; PLD, pegylated liposomal doxorubicin; TC, paclitaxel + carboplatin

Table 3.

Completed clinical ACT therapy trials in EOC

Agent	Target antigens	Trial identifier	Disease status	Patients	Response
TIL	-	-	Advanced or recurrent EOC	17	CR 8, PR 6, SD 1
TIL	-	-	Recurrent EOC	8	CR 0, PR 0, SD 1
TIL	-	-	Advanced OC after surgery and chemotherapy, 100% disease-free	13	Improved overall survival
CAR	Folate receptor- α		Recurrent FR ⁺ EOC	8	No clinical benefit
CAR	mesothelin		EOC	6	All patients showed stable disease at one month after treatment

ACT, adoptive T-cell therapy; TIL, tumor-infiltrating lymphocytes; CAR, chimeric antigen receptor; FR, Folate receptor- α

Table 4.

Ongoing ACT clinical trials in EOC

Agent	Phase	Target antigen	Infused cells	Patients	Trial identifier
TIL	II	-	short-term cultured autologous TIL	Metastasis ovarian cancer	
TIL	I	-	Re-stimulated T cells	Platinum-resistant high grade serous ovarian cancer	
TCR	I	MAGE-A4	MAGE-A4 specific TCR gene transferred T lymphocytes	HLA-A*24:02 positive MAGE-A4 expressing EOC	
TCR	I	NY-ESO-1	NY-ESO-1 specific TCR gene transduced T lymphocytes	HLA-A*02:01 or HLA-A*02:06 positive NY-ESO-1 expressing EOC	
TCR	I/II	NY-ESO-1	NY-ESO-1 (C259) transduced autologous T cells	Patients with HLA-A201, HLA-A205, and/or HLA-A206 allele and whose tumor expresses the NY-ESO-1	
TCR	I	NY-ESO-1	Anti-NY ESO-1 TCR-transduced T cells	Patients with HLA-A2+NY-ESO-1-expressing tumor	
CAR	I/II	mesothelin	Anti-mesothelin CAR transduced PBL	Metastatic or unresectable cancer that expresses mesothelin	
CAR	I	mesothelin	Anti-mesothelin CAR transduced T cells	Refractory or relapsed mesothelin expressing tumor	
CAR	I	MUC16	4H11-28z/fIL-12/EGFRt+ genetically-modified T cells	EOC with MUC16ecto antigen expression	
CAR	I/II	EGFR	anti-EGFR-CAR transduced autologous T cells	Chemotherapy resistant or relapsed EOC with EGFR expression	
CAR	I/II	ErbB2/Her2	anti-HER-2-CAR transduced autologous T cells	Chemotherapy resistant or relapsed EOC with Her2 expression	
CAR	I	CD133	anti-CD133-CAR vector-transduced T cells	Chemotherapy refractory or relapsed CD133-positive EoC	