Past, present and future targets for immunotherapy in ovarian cancer

Ovarian cancer is the leading cause of death from gynecologic malignancy in the US. Treatments have improved with conventional cytotoxic chemotherapy and advanced surgical techniques but disease recurrence is common and fatal in nearly all cases. Current evidence suggests that the immune system and its ability to recognize and eliminate microscopic disease is paramount in preventing recurrence. Ovarian cancer immunotherapy is targeting tumors through active, passive and adoptive approaches. The goal of immunotherapy is to balance the activation of the immune system against cancer while preventing the potential for tremendous toxicity elicited by immune modulation. In this paper we will review the different immunotherapies available for ovarian cancer as well as current ongoing studies and potential future directions.

Keywords: adoptive transfer • CA125 • EpCAM • mesothelin • NY-ESO-1 • ovarian cancer • p53 • tumor associated antigens • tumor vaccines • WT-1

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Ovarian cancer is the leading cause of death from gynecologic cancer in the US [1]. The majority of patients diagnosed with ovarian cancer have stage III or IV disease at the time of diagnosis because of lack of effective screening modalities. Fortunately, most patients will have an initial response to surgery and platinum/taxane-based chemotherapy. Patients with International Federation of Gynecology and Obstetrics (FIGO) stage III–IV disease who have an initial complete response to surgery and chemotherapy still have a recurrence rate as high as 85%. Survival rates for advanced disease are poor. Data suggest that the presence of tumor infiltrating lymphocytes at the time of diagnosis portends a nearly eightfold improvement in 5-year survival rate (38 vs 4.5%) [2]. These data suggest that the immune system plays a crucial role in the elimination of ovarian cancer and improved patient survival.

The ability of the immune system to recognize and eliminate foreign entities is tremendous. Cancers tend to be less immunogenic. The lack of immunogenicity is likely due to initial slow development lead-

ing to immune ignorance, tumor-mediated immunosuppression and eventual tumor growth and escape from recognition. Taken together these concepts have been termed immunoediting [3]. Tumors often downregulate the expression of MHC-I, or secrete proteins that inhibit NK and T-cell function, thereby preventing the activation and/or efficacy of NK and T-lymphocytes [4–8]. A number of tumors also secrete proteins that promote the development of regulatory T-cells that suppress immune function [9,10]. Other tumor mechanisms of immune evasion include the ability to downregulate intravascular cell adhesion molecules necessary to mount an immune response, as well as to alter molecules which are normally responsible for apoptosis signaling, thereby inhibiting cell death [11,12]. Ovarian cancers may stimulate endogenous immune responses and the differential production of immune cell subsets in the tumor microenvironment leading to tumor rejection or progression depending on which subset of immune cells becomes prevalent. Tumor-infiltrating CD8⁺ effector T cells have been associated

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with favorable disease outcome while other cell subsets such as Tregs and tumor-associated macrophages may promote disease progression through multiple mechanisms [2]. Furthermore data also suggest that improved outcomes may be related to co-infiltration of B cells into tumors allowing for improved cytolytic immune responses and increased survival [13].

The association of intra-tumoral T and B cells with increased survival, and Treg with worse survival, strongly suggests that ovarian cancers could respond to immune therapy. However, recent data also suggest that various histotypes of ovarian cancer, or even tumors within the same histological type (i.e., high-grade serous ovarian cancer), may potentially differ in their immunogenicity. Consistent with this view, data analysis from TCGA studies demonstrated through gene expression profiling the presence of an 'immune-reactive' subtype [14] which differ from the 'differentiated', 'proliferative' and 'mesenchymal' ones on the basis of the expression of the T-cell chemokine ligands CXCL11 and CXCL10 and the receptor CXCR3 gene [14]. Tothill *et al.* used microarray gene profiling to identify six subtypes of ovarian cancer. They described at least two subtypes of high-grade ovarian cancer that seemed to have the ability to restrict infiltration of lymphocytes into the tumor itself, which correlates with worse survival compared with more immunogenic subtypes [15]. These data suggest that there is a complicated interplay between ovarian cancer subtypes and the immune system leading which further challenges the development of effective immunotherapy in the future.

Over the past 40 years research scientists and physicians have made progress toward the development of therapeutic interventions to improve the recognition of and elimination of cancer by the immune system. The goal of immunotherapy is to balance the activation of the immune system against cancer while preventing the potential for tremendous toxicity elicited by immune activation and modulation. In this paper we will review the different approaches to immunotherapy, currently available treatments and potential future directions in relation to ovarian cancer.

Active immunotherapy

As early as the 1970s, trials were designed to help understand the role of active immunotherapy in the treatment of ovarian cancer. Initially, trials focused on using immunomodulating conjugates, including BCG, in combination with chemotherapy. These studies were based on the premise that if the immune system was activated in the presence of dying cells undergoing apoptosis, a more robust immune response would occur and lead to increased tumor clearance [16]. In

1986 the first large-scale clinical trial was published that tested this concept. Gall *et al.* showed that administration of *Corynebacterium parvum* with concomitant chemotherapy did not improve overall survival (OS) or progression-free interval in Gynecologic Oncology Group (GOG) study 25 [17].

As research continued to focus on harnessing the power of the immune system to clear disease, many were attempting to sensitize the immune system to antigens it was accustomed to through chronic exposure as tumor grows. There are currently four main active approaches to sensitizing the immune system, they include exposure to inactivated autologous whole tumor, exposure to tumor antigens, exposure to tumor antigens with pulsed antigen presenting cells (dendritic cells, DCs), or exposure to epitopes contained in a vector. Each of these approaches relies on the same premise, sensitizing the immune system to single or multiple antigens that are produced in large quantities by ovarian tumors. Some examples of these individual antigens include mesothelin, NY-ESO-1, p53, HER2/Neu and WT-1.

Mesothelin

Mesothelin is overexpressed in many ovarian cancers [18]. The protein is also expressed in few normal tissues with the exception of the peritoneum, pericardium and the pleura of the lung [19]. The differential expression of the protein makes it a potential target for active immunotherapy. Several clinical trials have been designed to test different compounds developed to target mesothelin. Most of these targeted therapies are passive in their mechanism of action including SS1P (recombinant immunotoxin of anti-mesothelin Fv linked to a pseudomonas exotoxin that causes cell death) and MORAb-009 (Chimeric IgG1 antibody). Of the mesothelin-directed compounds, CRS-207 is the most immunogenic and thus considered an active form of immunotherapy [20]. It utilizes a live attenuated facultative intracellular bacterium, *Listeria monocytogenes*, as a vehicle to deliver the mesothelin antigen to kupffer cells [20–22]. Once the bacteria undergo phagocytosis, both innate and adaptive immune responses occur. Ultimately, this leads to mesothelin-specific T-cell response directed toward malignant cells over-expressing mesothelin. A recent Phase I study of the CRS-207 examined the tolerability of the vaccine in 17 patients with mesothelin overexpressing mesothelioma, lung, pancreatic or ovarian cancers. Both patients with ovarian cancer included in the trial were heavily pretreated with 8 and 10 previous therapies. One patient was evaluable at the end of the study and had a survival of only 4 months despite a measured mesothelin response [20]. Obviously no conclusions regarding

the efficacy of CRS 207 in the treatment of ovarian cancer can be drawn, but the study indicated that CRS 207 was able to generate immune responses to mesothelin and was relatively well tolerated in patients [20].

NY-ESO-1

A vaccine which has drawn increased attention is directed at the protein NY-ESO-1. This antigen has been shown to be differentially expressed in many ovarian tumors compared with normal tissues and to induce CD4, CD8 and humoral immune responses [23,24]. As a result vaccines against NY-ESO-1 have been studied in the Phase II setting. Odunsi *et al.* published the findings of a Phase II study in 2014. Data revealed that of 22 patients (82% with serous histology) with NY-ESO-1 positive epithelial ovarian cancer, who had undergone debulking and completed adjuvant platinum-based chemotherapy, an improved survival occurred in those who had a measurable immune response after vaccination [25]. More recent data in predominantly high-grade serous ovarian cancer suggest that responses may be enhanced when NY-ESO-1 methylation is inhibited in tumor cells by using DNA methyltransferase inhibitors [26]. Currently a Phase I clinical trial is examining the safety and potential applicability of NY-ESO-1 vaccine given with an immunogenic agent glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE) in patients with a variety of solid tumors including ovarian cancer (NCT02015416). Results are expected in November of 2014.

p53

Other molecular targets currently being used to develop an ovarian cancer vaccine include the tumor suppressor gene *p53*. Ovarian cancer often expresses a mutant form of *p53* early in its development making it a potential target for immunotherapy [27]. Early pre-clinical studies showed that immune responses could be generated to *p53* [28]. These data coupled with evidence of possible improved OS in patients who develop antibodies to *p53* naturally make it a target of interest [29]. As a result, the GOG examined the immunogenicity of the *p53* vaccine in patients with advanced stage ovarian cancer. The vaccine was given with IL-2 as well as both GM-CSF and montanide or pulsed DCs. Results indicated that *p53*-specific immune responses could be generated with either regimen and that IL2 induced upregulation of Treg. This led the authors to conclude that IL2 should be left out of the regimen. [30]. While evidence supports the safety of the vaccine, its efficacy has yet to be determined.

WT1 & HER2/neu

Vaccine development has also focused on targeting the antigens WT1 and HER2. Both proteins can be

found by immunohistochemical staining at the time of pathologic review. What makes them particularly attractive as targets are the frequency with which they are found in ovarian cancer and the association HER2 expression has with more aggressive phenotype of tumor [31,32]. A Phase I study showed that vaccination with HER2 peptides could induce a strong CD4 and CD8 specific response and that these responses were long lived [33]. However, clinical data regarding the efficacy of the vaccine to treat or prevent ovarian cancer are lacking. The WT1 vaccine has been studied in a number of different tumor types and has shown some promise in their treatment [34–37]. A subsequent Phase I study designed to determine the safety and potential efficacy of the WT1 vaccine in gynecologic malignancies was reported by Ohno *et al.* in 2009. The WT1 vaccine induced at least partial responses in 4 of 13 patients enrolled in the study with few side effects and 50% of the aforementioned patients had ovarian cancer [38]. More recently a case report was published which detailed a durable 1-year response for a patient with ‘intractable’ serous ovarian cancer [39]. Again data from a large prospective randomized controlled trial are lacking.

Whole tumor lysates & embryonic stem cell vaccines

All of the aforementioned proteins are targets for the development of vaccines. If considered in total, nearly 94% of ovarian cancers express at least one of these proteins [40]. This conclusion has led to the study of whole tumor lysates and the possible use of multivalent vaccines or embryonic stem cells.

Preclinical studies using DCs exposed to pulsed ovarian cancer tumor lysates demonstrated T-cell activation. These cells when exposed to autologous tumor showed tumor specific cytotoxicity, while T cells exposed to DCs or lysate alone were unable to kill tumor cells [41]. These data suggest that therapy with pulsed DCs may improve tumor-associated immune responses. A Phase I study reported in 2001 reported the safety of the therapy and showed that 3/6 patients with recurrent ovarian carcinoma treated with tumor lysate pulsed DCs had stable disease following vaccination [42]. It has been proposed that better responses to whole lysate pulsed DCs would be seen in patients with ovarian cancer if Treg were inhibited with cyclophosphamide and VEGF receptors blocked by co-administration of bevacizumab [43]. Data from large clinical trials are not yet available to determine the potential efficacy of such a regimen.

It is well known that cancer cells are transformed to assume a less differentiated form compared with the tissues from which they are derived. The less-differentiated tumor cells become the more properties they begin

to share with stem cells including many of the same stem cell surface markers or antigens [44]. These cells allow for self-renewal and propagation of tumor growth because they are slower growing and more resistant to the cytotoxic effects of chemotherapy [44–46]. Although the presence of stem cells within ovarian cancers has not been firmly established, it is thought that with repeated cycles of chemotherapy these tumor stem cells may be preferentially selected and continue to replicate producing new tumor cell populations which are more resistant to chemotherapy. Targeting these cancer stem cells is challenging because of the aforementioned properties. Vaccination with noncancerous human stem cells has been suggested to cause an immune response which may help eliminate these tumor stem cells [47]. A large preclinical trial indicated that vaccination with embryonic stem cells effectively induced antitumor effects in animals carrying ovarian cancer xenografts. This study also suggested that stem cells carried many proto-oncogenes and tumor suppressor genes held in common with the ovarian cancer cell line including HER2, C-myc, p53 and nm23 [47]. These findings will likely be the basis for Phase I studies in humans.

Passive immunotherapy

In 1975 a landmark paper was published by Kohler and Milstein describing a method through which specific monoclonal antibodies could be made [48]. Since that time the use of monoclonal antibodies has evolved tremendously and is now the cornerstone of passive immunotherapy. Antibodies directed at differentially expressed tumor antigens allow for targeting of tumors specifically either by modulating immune responses, inhibiting signaling or delivering cytotoxic drugs.

Anti-B7-H4 antibodies

B7-H4 has been detected in significant quantities on the surface of ovarian cancer cells [49]. B7-H4 acts to inhibit T-cell activation and typically exists as a transmembrane protein expressed on the surface of APCs. Previous evidence in renal cell carcinoma lesions have indicated that the quantity of B7-H4 on the surface of cancer cells increases in response to signals from the microenvironment and may as a result promote immune evasion [49,50]. Anti-B7-H4 recombinant antibodies may be used to reverse the inhibition of tumor-specific T cells. The use of these antibodies targeting the B7-H4 pathways may potentially extend the survival of cancer patients by restoring T-cell-mediated antitumor responses.

EpCAM-antiCD3 conjugates

The EpCAM gene is located on chromosome 4q and contains nine exons. It is a calcium-independent trans-

membrane, glycoprotein cell-adhesion molecule, with a molecular weight of 39–42 kDa [51]. EpCAM promotes cell adhesion and the formation of EpCAM-mediated adhesions has negative regulatory effect on adhesions conferred by cadherin's. EpCAM is abundantly expressed on human cancers and EpCAM overexpression has been associated with poor prognosis in patients with ovarian, breast, prostate and gallbladder carcinoma, both functioning as an oncogene and suppressing CD4⁺ T-cell-dependent immune responses [52,53]. Köbel *et al.* studied biomarkers in four ovarian cancer subtypes (high-grade serous, clear-cell, endometrioid and mucinous tumors) and EpCAM demonstrated consistent high expression across subtypes [54]. EpCAM also has the advantage of being a stable antigen and preserving its expression levels in primary, metastatic and recurrent disease, including malignant ascites and pleural effusions [55,56].

Catumaxomab is a trifunctional monoclonal antibody with two different antigen-binding sites and a functional Fc domain [57]. The two specific antigen-binding sites bind to epithelial tumor cells via EpCAM and to T cells via CD3. Catumaxomab also activates Fcγ-receptor positive accessory cells such as DCs, macrophages and NK cells via its functional Fc domain [57,58] and as a result produces a comprehensive and complex immune reaction. Eventually, tumor cells die as a result of cell lysis via perforin/granzyme-B, antibody-mediated cell death and phagocytosis [58,59]. Tumor cells in malignant ovarian cancer-associated ascites have been shown to express EpCAM in 70–100% of cases, while the mesothelial cells lining the peritoneal cavity lack expression [60]. With the basis in mind, catumaxomab has broad applicability for ovarian cancer patients, particularly those with advanced/extensive disease.

A Phase I study of EpCAM/CD3-bispecific antibody in patients harboring advanced tumors is currently ongoing [61]. The first clinical intraperitoneal application of a trifunctional anti-EpCAM antibody (catumaxomab/Removab®) has recently shown effective tumor cell destruction, substantially decreased ascites accumulation and reduced necessity of paracentesis in advanced ovarian carcinoma patients harboring malignant tumors refractory to salvage chemotherapy [62].

Recently, a prospective, randomized Phase II/III study was conducted comparing the efficacy of catumaxomab plus paracentesis with paracentesis alone in the treatment of malignant ascites [60]. The primary efficacy endpoint was puncture-free survival. Secondary efficacy parameters included time to next paracentesis, ascites signs and symptoms, and OS. Puncture-free survival was significantly longer in the catumaxomab group (median 46 days) than in the con-

trol group (median 11 days; hazard ratio [HR]: 0.254; $p < 0.0001$), as was median time to next paracentesis (77 vs 13 days; $p < 0.0001$). Within the ovarian cancer cohort, median puncture-free survival was 52 days in the catumaxomab arm versus 11 days in the placebo arm (HR: 0.205; $p < 0.0001$). Catumaxomab-treated patients displayed fewer signs and symptoms of ascites than control patients [60]. As a result, catumaxomab has recently received market approval by the EMA for this indication.

Anti-CA125 antibodies

CA 125 is a high-molecular weight glycoprotein over-expressed on ovarian cancer cells surface. It is currently used as a tumor marker for ovarian cancer as it is actively shed into the bloodstream. High levels of CA 125 at the time of diagnosis tend to suggest disseminated disease [63,64]. CA125 biological role is still uncertain. Previous studies suggest that it is involved in cell adhesion, migration and invasion, and may have immunosuppressive properties [65].

In order to target CA125, Wagner *et al.* generated an IgG1 murine monoclonal anti-idiotypic antibody to CA125 (ACA 125). Following multiple injections of this antibody 9/16 patients developed anti-anti-idiotypic responses to ACA 125. Treatment with ACA125 improved progression-free survival from 8 to 11 months compared with those not receiving any therapy [66]. Immune modulation was noted after administering ACA125 through increases in TH1-associated cytokines including $\text{INF-}\gamma$, IL2 and IL4 as well as an increase in delayed TH2 response [67]. Similarly, Abagovomab is an anti-idiotypic antibody produced by a mouse hybridoma and generated against OC125 epitope of CA 125. The murine monoclonal antibody recognizes the tumor-associated antigen (TAA) CA-125 and the induction of both humoral and cellular immune responses was confirmed in preclinical studies, and a Phase I/II trial with 119 patients with CA125-positive ovarian, tubal or peritoneal cancers showed that 68.1% of patients that received Abagovomab showed an association between prolonged survival in patients who demonstrated an anti-idiotypic antibody (Ab3) response to vaccination versus those who did not. Ab3 responders had a significantly longer OS (23.4 months) compared with Ab3 nonresponders (4 months; $p < 0.0001$). ADCC, a possible mechanism of Abagovomab antitumor effect, was also observed in some patients (26.9%) and they had significantly longer median survival than those that did not (25 vs 10 months; $p = 0.0126$) [68,69].

Given the previous antitumor effects, Abagovomab was administered to patients with predominantly serous histology (81.5%), FIGO stage III–IV ovarian cancer

in complete clinical remission after primary surgery and platinum- and taxane-based chemotherapy in a Phase III, double-blind, placebo-controlled, multicenter study. Abagovomab was given as repeated monthly injections and was found to be safe and induced a measurable immune response. However, the trial found that Abagovomab used as maintenance therapy for patients with ovarian cancer in first remission does not prolong recurrence-free survival or OS [70].

Oregovomab is a high-affinity murine monoclonal antibody specific for CA-125. Oregovomab forms complexes with circulating CA-125 and as a result leads to immune processing of antibody and antigen [71]. Oregovomab does not directly inhibit tumor growth and it has not been found to induce CDC or ADCC by itself; however, it elicits tumor-specific T-cell responses.

In a trial involving 184 patients with ovarian cancer (FIGO stages I–IV), a single treatment of Oregovomab caused a brisk reduction in serum levels of CA125 owing to the formation of immune complexes [72]. After 1–10 infusions, 26 of 60 (43%) patients had a greater than threefold increase in anti-CA125 antibody levels. The level of anti-CA125 antibody increase also correlated with the amount of circulating CA125 at the time of injection. Anti-CA125 responders also had a longer survival compared with nonresponders (22.9 vs 13.5 months; $p = 0.0089$). Also 53% of patients showed an increase in T-cell proliferation in response to CA125 and had a significantly longer survival than nonresponders (>84 vs 13.2 months; $p = 0.0202$).

The therapeutic effect of combining Oregovomab with conventional chemotherapies, carboplatin and paclitaxel was investigated in a Phase II clinical trial [73]. In that study, 40 patients with FIGO stages III/IV were separated into two groups and received Oregovomab either simultaneously or 1 week after carboplatin and paclitaxel treatment. The authors concluded that the immune responses triggered by the chemo-immunotherapy were more robust than those measured in previously published mono-immunotherapy protocols. Chemo-immunotherapy is attractive because chemotherapies directly induce apoptosis of tumor cells and as a consequence release antigens to drive immune responses. Furthermore, Some data support the use of low-dose cyclophosphamide and fludarabine to selectively deplete and suppress regulatory T-cell activity, rendering tumors cells more susceptible to immune-mediated cytotoxicity [74].

Antibody-drug conjugates targeting (mesothelin/CA125)

Mesothelin, a glycosylphosphatidylinositol anchored cell surface protein, is a potential target for antibody-based cancer therapy due to its high expression in mesothelioma, ovarian cancer, pancreatic cancer, chol-

angiocarcinoma and other cancers [75]. Mesothelin may play a role in peritoneal implantation and metastasis of tumors through its interaction with mucin MUC16 (also known as CA125). Mesothelin expression has also been associated with resistance to certain chemotherapy drugs such as TNF- α , paclitaxel, and a combination of platinum and cyclophosphamide [75].

The high frequency of mesothelin and CA 125 expression has made them potential targets for antibody-drug conjugate (ADC) therapy. A previous study investigated BAY 94–9343, a novel ADC consisting of a human anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4 via a disulfide-containing linker. This study found that the antitumor efficacy of BAY 94–9343 correlated with the amount of mesothelin expressed in xenograft models including an ovarian cancer model [76]. It was also found that this therapy was generally superior to that of standard-of-care regimen resulting in complete tumor eradication in most of the models [76]. Anti-mesothelin and anti-CA 125 ADC therapies may hold potential for management of advanced or recurrent ovarian cancer.

Anti-CD25 antibodies

CD25 is the IL-2 receptor α chain and is expressed with CD4 by Tregs [77]. Malignant ascites from previously untreated ovarian cancer patients have been shown to contain significant numbers of CD4⁺ CD25⁺ CD3⁺ T cells and to the contrary these cells were infrequently seen in nonmalignant ascites [78]. Advanced stage ovarian cancers were also shown to have higher numbers of CD4⁺ CD25⁺ cells than stages I–II tumors [78]. Also, higher tumor infiltration of Tregs correlated with reduced patient survival [79]. These data suggest that Tregs may have a role in ovarian cancer progression, and approaches that block or deplete these cells may be novel strategies for treating cancer patients.

Daclizumab is a cell-depleting humanized IgG1 monoclonal antibody specific for CD25. Daclizumab is currently being evaluated in clinical trials as an immunotherapy agent for management of a number of cancers, including ovarian cancer [80]. Data from clinical trials of Ontak, another form of anti-CD25 therapy, are promising. Ontak is an engineered protein combining IL-2 with diphtheria toxin causing apoptosis of CD25⁺ cells and is US FDA approved for the treatment of cutaneous T-cell leukemia. Ontak is in clinical trials as a potentially effective therapeutic option for other cancer types, including metastatic ovarian, renal and breast cancers [81].

Ipilimumab

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a homologue of CD28 expressed on the surface

of T cells upon activation. CTLA-4 inhibits the activation of T cells by actually competing with CD28 for its ligands on APCs and also may deliver inhibitory signals to T cells [82]. As such, CTLA-4 functions as an immune-checkpoint receptor. CTLA-4 is also constitutively expressed on Tregs and is a means by which Tregs mediate immune suppression [83]. The use of therapeutic agents such as Ipilimumab, to hinder CTLA-4 function, prevents immune inactivation and is a logical approach to induce effective antitumor immune responses.

Ipilimumab (MDX-CTLA-4) is a humanized IgG1 monoclonal antibody, which binds to and blocks the activity of CTLA-4. It has received FDA approval for the treatment of advanced melanoma. The data from several studies suggest that the antitumor effect of Ipilimumab is mediated by immune modulation. In support of this, response rates in some clinical trials have been significantly higher in patients who developed immune-related adverse events such as enterocolitis [84], pneumonitis or hypophysitis than those patients who did not. The majority of Ipilimumab trials to date have been conducted mainly in patients with melanoma rather than ovarian cancer. A Phase II safety and efficacy study of Ipilimumab monotherapy following completion of chemotherapy in recurrent platinum-sensitive ovarian cancer patients with residual measurable disease is now underway [NCT01611558]. Results are expected in late 2016.

Anti-PD-1/PD-L1

MDX-1105 and MDX-1106 are two antibodies designed to target the programmed cell death 1 (PD-1) axis. The stimulation of the PD-1 axis acts as a negative regulator of immune function. Antibodies directed at these regulatory molecules on immune cells block binding of receptor ligands which would normally diminish immune function. Blocking PD-1 results in a more effective and lasting antitumor immune response. Preclinical studies have demonstrated that PD-1 blockade guards against growth of ovarian cancer in murine models [85]. In early-phase clinical trials, the antitumor activity of anti-PD-L1 monoclonal antibodies has been observed in patients with melanoma, renal cell carcinoma, non-small-cell lung cancer and ovarian cancer. Larger clinical trials in ovarian cancer are needed to better understand the safety and efficacy of this therapy.

Anti-folate receptor α antibody

Farletuzumab, a humanized IgG1k antibody that binds to folate receptor α , is significantly expressed on 90% epithelial ovarian cancers but not on normal ovarian tissues [86]. A Phase II clinical trial was conducted with plati-

num-sensitive ovarian cancer patients experiencing a first relapse and showed that Farletuzumab in combination with carboplatin/taxane improved objective response rates compared with historical controls, and increased duration of second remission compared with first remission in some patients [87]. Conversely, a recent Phase III trial (FAR-122, NCT00738699) of Farletuzumab in combination with paclitaxel in advanced platinum-resistant ovarian cancer was discontinued as interim analyses showed a limited survival benefit for patients.

Adoptive immunotherapy

Adoptive T-cell therapy

Adoptive transfer of *ex vivo* activated autologous tumor-specific T cells holds great promise. Lymphocytes are first harvested, often from ascites or the tumor itself (TILs, tumor-infiltrating lymphocytes) and its draining lymph nodes. These cells are enriched for particular antigen specificity or genetically modified to express a naturally occurring or chimeric T-cell receptor and expanded with IL-2 prior to reinfusion [88]. Responses may be augmented with preparative chemoradiotherapy-mediated lymphodepletion [89], antibody blockade of inhibitory pathways such as PD-1 [90], or co-infusion of pro-inflammatory cytokines [91], each of which counters the immunosuppressive tumor microenvironment at time of reinfusion.

Natural-occurring T-cell therapies

In preclinical models of ovarian cancer, T cells against specific antigen targets such as sperm protein 17 [92] and TADG-12 [93], among others, have resulted in highly effective antitumor activity. Aoki *et al.* was among the first to perform clinical trials of adoptive T-cell therapy in 17 patients with advanced or recurrent ovarian cancer. Of the 17 patients in the study, 12 were noted to harbor tumors of serous histology. This group reported one complete and four partial responses across seven patients who received *ex vivo* expanded TILs, with durability of response as long as 3–5 months. Among 10 patients who received adoptive transfer in conjunction with cisplatin, there were seven complete responses and two partial responses; four of the seven complete responses, all were serous in histology and remained disease-free for at least 15 months [94]. In a separate investigation of patients with epithelial ovarian cancer and no evidence of disease after completion of cisplatin-containing therapy, those who received adoptive TIL therapy had improvements in 3 year estimated overall (100 vs 67.5%, $p < 0.01$) and disease-free survival (82.1 vs 54.5%, $p < 0.05$) compared with controls [95].

Several investigations have focused upon intracavitary reinfusion. Dobranski *et al.* showed that peripheral blood T-cell stimulation and intraperito-

neal reinfusion produced markers of systemic memory that correlated with diminished serum CA125 tumor marker levels and enhanced patient survival [96]. Another pilot study among seven patients with recurrent ovarian cancer using intraperitoneal infusion of cytotoxic T lymphocytes harvested by leukapheresis then primed against MUC-1 resulted in a median OS of 11.5 months [97].

Genetically modified T-cell therapies

As pioneered by Gross *et al.*, T cells may be genetically modified to recognize TAA independent of antigen processing [98]. This can be accomplished using viral vectors encoding for a tumor-specific T-cell receptors or chimeric antigen receptors (CARs). CARs contain a fragment length antibody containing heavy/light chain variable domains targeting a TAA, in conjunction with a transmembrane domain of either CD8 (first generation) or CD28 (second generation) fused to a cytoplasmic signaling domain of the T-cell receptor. Third-generation CARs are further modified to contain cytoplasmic signaling domains of co-stimulatory receptors such as CD28-OX-40 or CD28-4-1BB [99]. For ovarian cancer, preclinical studies of genetically modified T cells have been successful in exploiting folate receptor- α [100,101], Lewis-Y [102,103], Her2/Neu [104], the ErbB family [105], MUC-16 [106], WT-1 [107] and L1CAM [108] antigens. The first Phase I study of adoptive gene-modified autologous T cells was conducted in 2006, using T cells with reactivity against the ovarian cancer-associated antigen folate receptor- α linked to the signaling domain of the Fc receptor gamma chain. Unfortunately, outcomes were plagued by poor durability; at 1 month after transfer, CARs were barely detectable and no patient had reduction in tumor burden [109].

Advancement of adoptive T-cell therapies suffers from their complexity and labor-intensive manufacture, as well as toxicity from cross-reactivity and antigenic mimicry [110]. Accordingly, trials in ovarian cancer are limited. A Phase I study of adoptive transfer of autologous CD4⁺ antigen-specific T-cell clones stimulated with DCs pulsed with NY-ESO-1 in patients with stage III/IV ovarian or primary peritoneal cancer is ongoing (NCT00101257). A Phase I trial of adoptive transfer of vaccine-primed CD3/CD28-costimulated autologous T cells combined with bevacizumab for recurrent ovarian, fallopian tube or primary peritoneal cancer previously vaccinated with autologous tumor vaccine is currently recruiting (NCT01312376). The adoptive transfer of folate receptor- α redirected autologous T cells in lymph-depleted recurrent ovarian cancer patients has been proposed but not yet accruing [111,112].

DC therapies

DCs originate from the bone marrow and may be of plasmacytoid or myeloid lineage. They undergo distinct stages of development: immature DCs exhibit high antigen uptake but low T-cell stimulatory potential; semi-mature DCs express high amounts of major histocompatibility complex but little cytokine; mature DCs present antigen and migrate toward lymph nodes. Given a potency of antigen presentation that is 10–100 times that of monocytes or B cells [113], there is immense interest in exploiting DCs for immunotherapy.

A variety of DC strategies have been developed with encouraging preclinical results. Some groups have shown successful antitumor activity with reinfusion of unloaded DCs [114] or DCs that are transformed to overexpress various chemokines (such as IL-12 [115–117], CCL21 [118], or IL-23 [119]) or antigen (p53 [120], MART [121], MUC-1 [122], or gp100 [123]). In other instances, DCs loaded *ex vivo* with TAA [114,115] or pulsed with RNA [124–126] have been reinfused with good effect. *In vivo* loading of DCs with TAA [127–129] or use of DC cell fusion exosomes [130–132] has also been promising.

Clinical investigations of DC-based immunotherapy in ovarian cancer remain relatively nascent. Among these reports, Chu *et al.* (2012) published the experience with vaccination using DC cells loaded with human telomerase reverse transcriptase (hTERT), Her2/neu and pan HLA DR- binding epitope (PADRE) peptides with or without cyclophosphamide in 11 patients with advanced ovarian cancer in remission. Only two recurred during vaccination, and six had no evidence of disease at 36 months [133]. While survival data were encouraging, the objective measurement of T-cell responses to Her2/neu and hTERT were modest at best.

GOG protocol 9910 compared the subcutaneous administration of p53 peptide vaccine to intravenous administration of p53-pulsed DCs in 13 patients, all of whom also received IL-2 [30]. Objective immune responses were detected in 69% vs 83%, respectively. Median overall and progression-free survivals were 40.8 vs 29.6 and 4.2 vs 8.7 months, respectively. There was minimal toxicity. Unexpectedly, IL-2 was noted to be detrimental by causing stimulation of Tregs. The authors concluded that the less cumbersome subcutaneous administration was superior.

Hernandez *et al.* reported on six patients with progressive ovarian carcinoma and two with progressive uterine sarcoma who received 23 subcutaneous injections of DC pulsed with KLH (keyhole limpet hemocyanin) and autologous tumor lysate every 10–28 days. Three patients demonstrated disease stabilization lasting to 6–11 months; five progressed within

the first 14 weeks. Objective immunologic response to vaccination was documented in eight patients [42].

Loveland *et al.* described remarkable disease stabilization for >3 years in two (one ovarian and one renal cell carcinoma) of 10 patients with clearly progressive disease at entry using DCs pulsed with mannan-MUC-1 fusion protein injected every month [134]. The authors attribute the sustained response to the targeting of the mannose receptor to allow enhanced proteolytic processing and class I/II presentation to CD8⁺ and CD4⁺ T cells.

Though existing data for ovarian cancer patients treated with DC therapies is scant, interest continues to grow. From July 2012 to 2013, a total of 29 trials utilizing DC-based therapy were launched [130]. Trials specific to patients with ovarian, fallopian tube or primary peritoneal cancers are listed in Table 1.

Conclusion & future perspective

Immunotherapy has continued to develop and be refined over the past 30 years. The balance between immune system activation toward ‘nearly self’ cancerous tissue and benign tissue remains a challenge. Multiple clinical trials are currently ongoing which will help better define the role immunotherapy will play in both the treatment of ovarian cancer and prevention of recurrence. The future of immunotherapy lies not only in improving current treatments and investigating potential therapies, but also in continuing to explore available mechanisms for enhanced immune surveillance and disabling initial tumor immune evasion. Advancing technologies will continue to pursue further delineation of available tumor-associated and tumor-specific antigens as well as the identification of additional immune checkpoints to serve as immunotherapeutic targets. The way in which immunotherapeutic interventions are administered, in addition to their medium, vector and frequency, as well as adjuvant therapies, will further evolve with a goal of achieving higher levels of immunogenicity. Forthcoming targets of immunotherapy will bring together the aforementioned broader divisions of the field into synthesized therapies, in order to pursue a more integrative approach to both the fundamental inhibition of oncogenesis, as well as the prevention of tumor progression.

Current research has focused predominantly on each individual immunotherapeutic modality as either an adjunct to standard treatments or as single-agent maintenance therapy. As improvements in progression-free survival and OS are seen using immunotherapeutic approaches they may potentially be combined in the future. It may be possible at the time of surgery for portions of tumor to be taken to a lab and processed to harvest tumor-infiltrating lymphocytes, create whole

Table 1. Currently active dendritic cell-based immunotherapy trials in ovarian, primary peritoneal and fallopian tube carcinoma.

Phase	Status	Intervention	Patient population	Trial number
0	Recruiting	DC vaccine with autologous oxidized tumor cells administered intranodally alone or in combination with metronomic cyclophosphamide or metronomic cyclophosphamide and bevacizumab	Recurrent ovarian, fallopian tube or primary peritoneal cancer	NCT01132014
0	Recruiting	Folate receptor α loaded DC vaccination	Stage IIIC/IV ovarian cancer without evidence of disease after completion of initial cytoreduction and chemotherapy	NCT02111941
I/II	Recruiting	DCs loaded with amplified ovarian cancer stem cell mRNA, hTERT and survivin	Relapsed and platinum-sensitive epithelial ovarian carcinoma patients with response to chemotherapy in recurrent disease setting	NCT01334047
II	Recruiting	Arm 1: DC vaccination with KLH-loaded immature DCs into inguinal lymph nodes Arm 2: Ontak (denileukin diftitox, a mixture of IL-2 and diphtheria toxin) Arm 3: DC vaccination with KLH-loaded immature DCs into inguinal lymph nodes plus Ontak (denileukin diftitox, a mixture of IL-2 and diphtheria toxin)	Recurrent ovarian cancer	NCT00703105
I	Active, not recruiting	DCs loaded with TERT-mRNA and survivin	Stage III ovarian cancer	NCT01456065
II	Active, not recruiting	Arm 1: DC/tumor fusion vaccination with GM-CSF Arm 2: DC/tumor fusion vaccination with GM-CSF and imiquimod	Stage III or IV ovarian, fallopian tube or primary peritoneal serous carcinoma	NCT00799110
II	Active, not recruiting	MUC-1 DC vaccine	Stage III or IV epithelial ovarian, primary peritoneal or fallopian tube cancer who have previously undergone surgical cytoreduction and received first- or second-line conventional chemotherapy and are currently in complete remission based on clinical and radiologic studies	NCT01068509

DC: Dendritic cell; KLH: Keyhole limpet hemocyanin.

tumor lysates and to analyze tumors for expression of mutated proteins that could be targeted through the use of available vaccines. Through this approach treating physicians could potentially tailor treatment for each patient based on each individual's tumor biology. The potential barriers to this approach are cost and the cumbersome nature of the work that would be involved for each patient. As technology evolves to fill the needs brought about through immunotherapeutic successes in the treatment of ovarian cancer, these processes will become more streamlined and feasible for a large number of patients. Furthermore, the impressive results obtained in the last few years with the use of

immune checkpoint blockade using anti-CTLA-4 Ab or anti-PD-1 Ab as effective option for cancer therapy in multiple type of human cancers [135] strongly suggest that combination therapies using anti-CTLA-4 Ab and anti-PD-1 Ab may represent some of the most promising approaches for immunotherapeutic treatment of ovarian cancers in the near future.

The role immunotherapy will play in the prevention of ovarian cancer is yet to be determined. The data presented here show that significant advances are being made toward the implementation of immunotherapy in the management of patients with ovarian cancer. Immunotherapy will likely play an important

role in the treatment and prevention of ovarian cancer in the future.

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Executive summary

Ovarian cancer epidemiology & standard treatment

- Ovarian cancer is typically diagnosed after it is of an advanced stage (Federation of Gynecology and Obstetrics stage III or IV).
- Most patients will have recurrence and die from disease despite aggressive cytotoxic chemotherapy and surgery.
- Tumor-associated lymphocytes are associated with improved survival. Immune clearance and surveillance in ovarian cancer are key components to survival.

Active immunotherapy

- Ovarian cancer vaccine development has focused on the differentially expressed cancer specific antigens mesothelin, NY-ESO-1, p53, HER2/neu and WT1.
- There is a current focus on the use of 'multivalent vaccines'. These include the use of whole tumor lysates and embryonic stem cells. This approach may improve survival through targeting differentiated tumor cells and tumor stem cells.

Passive immunotherapy

- Antibody-mediated immunomodulation is achieved through blockade of T-cell inhibitor B7-H4, inhibition or lysis of Treg (Daclizumab, Ontak), inhibition of CTLA-4 (Ipilimumab) and blocking the PD-1 axis.
- Tumor cells may be targeted through EpCAM with catumaxomab, a trifunctional antibody, which complexes with immune cells to mediate destruction.
- Antibody–drug complexes may be implemented to target tumors specifically with highly toxic chemotherapy and fewer side effects using mesothelin and CA-125 as targets.
- Sensitization of the immune system to antigens using antibodies to CA-125 (Abagovomab and Oregovomab) may improve survival.

Adoptive immunotherapy

- Removal of T cells and dendritic cells from a patient and exposure of these immune effector cells to ovarian cancer associated antigens, followed by reinfusion through either the intravenous or intraperitoneal route, can help effectively eliminate tumors and may improve overall survival.
- A large number of trials are planned and currently ongoing.

Conclusion & future perspective

- Multiple large clinical trials are ongoing, which will help to clarify the important role immunotherapy will play in ovarian cancer treatment.
- The future of immunotherapy in ovarian cancer will likely rely upon identification of new general ovarian cancer antigens to target as well as each patient's own specific tumor antigens.
- Ultimately future therapies will likely rely on integration of multiple immunotherapy strategies in combination with surgery and cytotoxic chemotherapy.

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