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Immunity and immune suppression in human ovarian cancer

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Abstract

Clinical outcomes in ovarian cancer are heterogeneous, independent of common features such as stage, response to therapy and grade. This disparity in outcomes warrants further exploration into tumor and host characteristics. One compelling issue is the response of the patient's immune system to her ovarian cancer. Several studies have confirmed a prominent role for the immune system in modifying disease course. This has led to the identification and evaluation of novel immune-modulating therapeutic approaches such as vaccination and antibody therapy. Antitumor immunity, however, is often negated by immune suppression mechanisms present in the tumor microenvironment. Thus, in the future, research into immunotherapy targeting ovarian cancer will probably become increasingly focused on combination approaches that simultaneously augment immunity while preventing local immune suppression. In this article, we summarize important immunological issues that could influence ovarian cancer outcome, including tumor antigens, endogenous immune responses, immune escape and new and developing immunotherapeutic strategies.

Keywords

antibody therapy; myeloid-derived suppressor cells; regulatory T cells; vaccines

Ovarian cancer has the highest mortality rate of the cancers unique to women. According to the 2010 cancer statistics, there was going to be an estimated 21,880 new cases of ovarian cancer and an estimated 13,850 deaths [1]. The majority (65–75%) of women with ovarian cancer is diagnosed with advanced-stage disease (III and IV) and only approximately 15–20% of these are free of recurrence at 10 years [2–4]. While these statistics are sobering, there are, nevertheless, unique features about ovarian cancer that raise the possibility of increasing the cure rates. First, because it is localized to the peritoneal cavity, ovarian cancer is accessible for initial cytoreductive surgery, which dramatically reduces tumor volume. Second, most ovarian cancers are sensitive to chemotherapy, such that 80% of optimally debulked patients will achieve a complete response to first-line platinum–paclitaxel chemotherapy [3]. Unfortunately, at a median of 16–18 months following chemotherapy,

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recurrences will manifest, and by 5 years from diagnosis, the majority will have recurred [5,6]. The major determinants of outcome in ovarian cancer are tumor-specific features such as grade, histology, sensitivity to chemotherapy and the extent of the primary surgical debulking. As is evident from high recurrence rates following therapy, minimal residual disease remains in most patients, consisting of cells resistant to initial chemotherapy. It is this minimal residual disease state that can be targeted with innovative therapeutic strategies (e.g., immune-based therapies).

Clinical outcomes in ovarian cancer are quite heterogeneous, with approximately 15% of patients dying of the disease within the first year, and approximately 25% surviving over 5 years from diagnosis [7]. This disparity in outcomes warrants further exploration into tumor and host characteristics. One compelling, and potentially modifiable arena, for study is the response of the patient's immune system to her ovarian cancer. Thus, research into ovarian cancer has become increasingly focused on the function of the immune system. Despite the observation that ovarian cancer is an immune reactive malignancy, and there are notable regressions in response to immune modulation, there has not been a therapy that has advanced to the point of routine clinical use. Although there are many reasons for this inability to implement effective immune-based treatments, one major reason is that ovarian tumors establish a complex multilayered immune suppression network that effectively neutralizes most attempts at augmenting antitumor immunity (Figure 1). In this article, we present an updated summary of the immunological mechanisms of ovarian cancer including natural immune response, mechanisms of immune escape in the ovarian microenvironment and current immune-based therapies for ovarian cancer being studied in the clinical setting.

Basic principles of immunity

The immune system is constituted by a heterogeneous population of both cellular and molecular effectors that function in an organized and integrated manner to eradicate disease and maintain the overall health of the host, while minimizing off-target activity (e.g., autoimmunity). The immune system is typically divided into two different, but interacting, systems referred to as the innate and the adaptive mechanisms. The innate immune system is the first line of defense that consists of a group of cellular and humoral factors. The cellular component of innate immunity includes NK T cells, mast cells, eosinophils, basophils, macrophages, neutrophils and dendritic cells (DCs), while the humoral factors primarily include cytokines and complement. The major functions of the innate immune system include recruiting immune cells into sites of infection, activation of complement, identification of foreign substances and preparation and activation of the adaptive immune systems. Innate responses are immediate, maximal and antigen independent. In the absence of stimulation, nonactivated innate cells are well known to maintain tolerance and prevent inappropriate immune activation. The adaptive immune response involves both T and B lymphocytes. T lymphocytes produce cytokines or cytolytic molecules, while B cells produce antibodies. Primary adaptive immune responses usually develop after 1 week and are highly antigen-specific. Like the innate immune system, subsets of lymphocytes (e.g., Tregs) are also known to regulate immunity and prevent autoimmunity. Finally, another key feature of the adaptive immune response is generation of immune memory and the resulting rapid and robust responses to previously encountered antigens, a fundamental reason for the widespread clinical success of vaccines.

Natural immune responses to ovarian cancer influence the clinical course of the disease

Observations that date back over two decades have established that there are endogenous immune responses to ovarian tumors; more recently, it has been shown that the quality of

the immune responses can have a significant impact on the clinical course of the disease. Although infiltration of T cells and other immune effectors was observed in ovarian cancers as early as 1982 by Haskill and colleagues [8], it would not be until nearly two decades later that the prognostic significance of these cells was appreciated. In their landmark publication in 2003, Zhang and colleagues showed that T-cell infiltration into ovarian tumors was associated with improved survival [9]. For example, among 74 patients with a complete clinical response after debulking and platinum-based therapy, the 5-year survival rate was 73.9% among those patients with $CD3^+$ T cells within their tumor compared with 11.9% among patients without infiltrating T cells [9]. This study also revealed that in tumors with high numbers of tumor-infiltrating T cells the expression of monokines induced by IFN-γ, macrophage-derived chemokines and secondary lymphoid-tissue chemokines were significantly increased as compared with tumors lacking T cells, indicating that these chemokines may be involved in the antitumor response [9]. Although this study did not elucidate the nature of the CD3 T-cell infiltrate with respect to the different subsets of T cells (CD4 T helper [Th] cells, CD8 cytotoxic T cells and CD4 Treg cells), it bolstered the hypothesis that the immune infiltrate has an active role in the clinical course of ovarian cancer.

Whereas helper and cytotoxic T cells, collectively known as effector T cells, exhibit antitumor immune functions, the regulatory T-cell subsets would suppress immunity [10]. Studies in recent years have increasingly focused on whether effector T cells are associated with improved survival, and, if so, what is their phenotype and function. For example, Sato and colleagues studied 117 ovarian cancer cases finding improved survival in patients who had higher numbers of intraepithelial $CD8^+$ T cells compared with patients without intraepithelial CD8 T cells (median survival 55 vs 26 months) [11]. These findings were largely confirmed by Leffers and colleagues in an independent cohort [12]. CD8 cytotoxic T cells are generally thought to be the primary mediators of antitumor immune responses. These cells recognize antigens displayed in the context of MHC (HLA) class I molecules expressed on ovarian cancer cells. Upon recognition of their cognate antigen, CD8 T cells release, among several mediators, perforin and granzyme, which induce apoptosis in target cells [13]. In recent years, however, the identification of regulatory subsets of CD8 T cells has clouded interpretations of tumor-infiltrating CD8 cells [14]. Nonetheless, the interpretation that the vast majority of CD8 T cells are cytotoxic seems reasonable given the recent study by Milne and colleagues who showed a strong positive correlation between levels of CD8 T cells and granzyme B within tumors [15]. Other studies seeking to understand the mechanisms behind lymphocyte recruitment to tumors have used gene expression profiling of serous ovarian tumors with high and low CD8 T-cell infiltration finding two genes differentially expressed in tumors with high versus low CD8 T-cell infiltration: interferon regulatory factor (*IRF*)-1 and chemokine receptor (*CXCR*)6 [16,17]. Upregulation of *IRF-1* results in the induction of the MHC class I-dependent pathway and activation of transcription factors for MHC class II gene expression. Upregulation of *CXCR6*, along with other chemokine receptor genes, has been associated with metastasis of several tumors including epithelial cancers. Thus, these studies provide insight into specific genes and pathways associated with recruitment of cytotoxic T cells in ovarian tumors and give possible paths for further immune therapies designed to influence recruitment of helpful T-cell subsets.

In contrast to CD8 cytotoxic T cells, the role of CD4 helper T-cell infiltration is less clear owing to a high prevalence of the CD4 marker on Tregs. Both Sato and Milne observed similar outcomes among patients with or without $CD4^+$ T-cell staining of tumors [11,15]. Kryczek and colleagues found that high levels of IL-17 were associated with greatly improved outcome suggesting that a subset of CD4 Th cells, called Th17 and producing IL-17, may have a direct role in eradicating tumors [18]. Given the abundant expression of

IL-17 among innate immune effectors, it is difficult to draw a conclusion as to whether intratumoral Th17 cells might impact the clinical course of the disease [19]; however, the fact that levels of Th17 cells correlate strongly with other prognostic cells (e.g., Tregs) suggests that they might also be predictive of outcome [18].

Other specific subsets of antitumor immune effectors have also been studied but with less than clear results. One example is the subset of NK cells, a group of cytotoxic lymphocytes that have two different mature phenotypes: $CD16^{+}CD56^{dim}$ NK cells, found in the periphery, which have high cytolytic function, and $CD16^{-}CD56^{bright}$ NK cells found in the secondary lymphoid tissue, with inefficient cytotoxicity [20]. These cells have two types of surface receptors: activating (i.e., NKG2) receptors and inhibitory (i.e., KIR) receptors. NK cells have the ability to lyse cells without first having to recognize specific antigens [21]. The balance between inhibitory and activating signals through the different NK receptors is important in the NK cell activation process. NK cells can also be activated in an antigen-dependent manner to mediate antigen-dependent cellular cytotoxicity, which involves binding of cell bound antibodies to Fc receptors on the NK cells. The activating NKG2 receptors bind to stress ligands such as MICA, MICB and ULBP1–3, while KIR receptors bind to MHC class I and class I associated ligands [21,22]. Like other cancers, nearly all ovarian cancers express MICA, MICB and ULBP2 [23]. Furthermore, higher NK cell activity in the peripheral blood of ovarian cancer patients at the time surgery is predictive of improved progression-free survival (PFS) [24]. Despite this, increased numbers of NK cells in peritoneal and pleural effusions of metastatic ovarian carcinoma have been associated with poorer prognosis [25]. The generation of antibody responses to ovarian cancer is a common observation suggesting a role for B cells in disease protection [26–28]. Although antibody-secreting B cells do not need to be at the tumor site to exert antitumor activity, studies evaluating whether B-cell infiltration is associated with improved survival show mixed results [15,25].

Antigen specificity of the ovarian cancer immune response

Since the 1990s, reports have shown heterogeneity of human ovarian cancers with respect to infiltration by immune effectors, particularly effector T cells, and often associated with favorable outcome leading to speculation that ovarian cancer activates an antigen-specific immune response, which kills tumor cells and/or blocks growth. Whether or not immune effectors in the tumor are antigen specific and, importantly, which antigens are tumor rejection antigens remain largely unanswered. Despite this, several ovarian cancer antigens have been identified and in recent years, several studies have demonstrated that patients who have ovarian cancer respond naturally to these antigens, as measured in the peripheral blood or in ascites fluid.

One of the first suggestions that the effector T cells associated with ovarian cancers were specific for antigens overexpressed in the tumor came from Ioannides and colleagues [29]. They found that ascites-derived HLA-$A2^{+}$ contains CD8 T cells capable of recognizing the human EGF receptor (EGFR)2 (HER-2/neu)-derived peptide, p971–980 [29]. HER-2/neu protein, also known as HER-2, ErbB-2 and c-erbB2, is a transmembrane glycoprotein (185 kDa) that is part of the EGFR family that also includes EGFR-1, HER-3 and HER-4. HER-2/neu comprises a large extracellular domain, a short hydrophobic transmembrane domain and a cytoplasmic intracellular domain containing tyrosine kinase activity [30]. Her2/neu is an attractive immunologic target because of its low-level expression in peripheral tissues and its biologic relevance. HER-2/neu activates signaling pathways that are involved in cellular differentiation, proliferation, migration and apoptosis. Estimates of the percentage of ovarian tumors with HER-2/neu expression have been variable, ranging from 5 to 66% [31–34]. Data in the past decade have been somewhat contradictory with

regard to the prognostic relevance of *HER-2/neu* gene amplification or protein overexpression in ovarian cancer. Some studies show HER-2/neu overexpression and amplification as a poor prognostic factor [32,35], while other studies show no prognostic value associated with this protein [34]. The immune system naturally targets HER-2/neu in ovarian cancer patients, as shown by Karyampudi and colleagues [36]. In that study, peripheral blood T cells from ovarian or breast cancer patients and age-matched women who had not had cancer were tested for immune reactivity against a panel of fifteen epitopes that were predicted to bind to several distinct allelic forms of HLA-DR. Of those epitopes, four were targets of T cells in cancer patients but not healthy donors [36].

Folate receptor (FR)α, previously known as folate-binding protein, is a glycosyl-phosphatidylinositol-linked membrane protein overexpressed in many epithelial cancers [37–39]. Expression of FRα in nonmucinous ovarian tumors is increased approximately 90-fold in comparison with normal epithelial cells [40]. Its expression is also retained on metastatic lesions and recurrent ovarian tumors [41]. Expression of FRα is also relatively limited to a few specific tissues, notably the apical surface of kidney tubule epithelium where it is involved in recovery of folate from the urine [42]. Interest in targeting FRα was initially established by Peoples and colleagues. In their studies, they found that tumor-associated lymphocytes isolated from the malignant ascites of ovarian cancer patients recognized naturally processed and presented HLA-A2 (MHC class I) peptides derived from FRα [43,44]. Using a $CD4^+$ T-cell epitope prediction algorithm in another study, we predicted promiscuous epitopes of FRα and tested for immunity in 30 breast or ovarian cancer patients and 18 healthy donors using ELISPOT [27]. A total of 14 peptides were predicted, and it was found that more than 70% of patients demonstrated immunity to at least one epitope. Patients responded to an average of three epitopes, whereas healthy donors responded to only one. Five of the 14 peptides were recognized by more than 25% of patients, and responses to three peptides were higher in patients than in healthy donors, suggesting that the presence of the tumor augmented immunity. Finally, patients demonstrated elevated levels of FRα antibodies, consistent with a coordinated immune response. Thus, FRα is a promising therapeutic target not only due to its tumor specificity and high-level expression, but also because it is naturally immunogenic.

IGF binding proteins (IGFBPs) are a family of six binding proteins that have 50% homology with each other and have binding and regulatory properties for IGF-1 and −2, therefore having a role in cell proliferation, differentiation and apoptosis. All six family members are secreted and expressed in normal ovarian tissue, although previous studies have shown IGFBP-2 serum levels and expression to be significantly increased in ovarian cancer tissue in comparison with controls [45–48]. We revealed for the first time that IGFBP-2 elicits an *in vivo* antigen-specific CD4 T-cell immunity in patients with breast and ovarian cancer [49]. In this study, T cells from more than 15% of the patients elicited a response against four HLA-DR-degenerate IGFBP-2 epitopes compared with controls. Moreover, this pool of four IGFBP-2 peptides should cover the majority (~80%) of the patients with tumors with IGFBP-2 overexpression based on the allelic frequencies of the HLA-DR homologs; however, the significant patient response was only 35% [49]. These results implicate IGFBP-2 as a target for vaccine-based therapeutics against ovarian cancer.

Transmembrane mucins are a family of heavily glycosylated proteins with high molecular weights, produced by epithelial tissues, which are involved in coating, lubrication and protection [50]. There are two types of mucins: the extracellular complex mucins found in gastrointestinal and respiratory tracts, and the transmembrane mucins found in glandular and ductal epithelial cells [50]. Of the 11 transmembrane mucins identified to date, three (MUC1, MUC4 and MUC16) have been well characterized and shown to be overexpressed in many types of cancer including ovarian carcinomas [51–54]. MUC16 is the largest

membrane-bound mucin protein; it is expressed on the surface of ovarian cancer cells and shed into the bloodstream and peritoneal cavity after proteolytic cleavage. MUC16 contains the CA-125 peptide epitope that is used as a tumor marker for monitoring growth and recurrence of epithelial ovarian cancer [55,56]. MUC1 has been shown to be overexpressed in 50–80% of ovarian tumors and is a prognostic factor for resistance to platinum-based chemotherapy in ovarian cancer [50,53,57]. Although MUC4 is overexpressed in human ovarian cancer, much less is known about its biology. MUC4 is a large glycoprotein that is aberrantly expressed in over 90% of malignant ovarian tumors with very low to an undetectable expression in the normal ovary [53]. Recent studies clearly suggest a pathologic role of MUC4 in ovarian cancer by mediating epithelial to mesenchymal transition, which is involved in metastasis and enhanced tumor aggression [58]. MUC4 also activates HER-2/neu and enhances the motility of ovarian cancer cells [59]. Despite association of MUC4 with pathologic features, there is no association of expression with outcome in ovarian cancer patients [53]. Overexpression and aberrant glycosylation of the mucins are recognized by the immune system. For example, some studies have shown the presence of anti-MUC1 antibodies in healthy individuals as well as in ovarian cancer patients and the levels of anti-MUC1 antibodies inversely correlate with ovarian cancer risk factors [60,61].

Mutation of the *p53* gene is one the most common, single genetic alterations in sporadic human epithelial ovarian carcinoma [62]. Either loss of wild-type p53 function, gain of oncogenic function or the ability to activate p53 severely compromises controlled cellular proliferation and growth [63]. As a result of mutation, p53 is overexpressed in nearly 50% of ovarian cancers. In a study of 104 women with ovarian cancer, Goodell and colleagues assessed the levels and clinical impact of anti-p53-specific IgG antibodies. Multivariate analyses showed that the presence of p53 antibodies to be an independent predictor of survival. Specifically, the median survival for antibody positive patients was 51 months compared with 24 months for patients without antibodies [26]. Consistent with the development of IgG antibodies, ovarian cancer patients also develop p53-specific T-cell memory [64].

Epithelial cell adhesion molecule (EpCAM) is a type I membrane glycoprotein constructed by three domains, an extracellular domain, with EGF and thyroglobulin repeat-like domains, a transmembrane domain and an intracellular domain (26-amino acid) termed EpICD [65]. This molecule is expressed in almost all epithelial cell membranes but not on mesodermal or neural cell membranes. EpCAM has a high-level expression on human epithelial cancers with a negative prognostic potential for survival of patients [65]. In a retrospective study carried out by Kobel and colleagues on 500 ovarian cancer patients, EpCAM was highly expressed across all ovarian cancer subtypes [66]. Furthermore, EpCAM-positive tumor cells and ascites-derived exosomes containing EpCAM molecules have also been detected in the majority of ascites samples from ovarian cancer patients [67–69]. Although EPCAM expression in normal epithelial tissue has been detected [67], the expression of this molecule in ascites is highly tumor specific, because normal cells in the peritoneal cavity originate from the mesothelium and do not express EpCAM on their surface. EpCAM is naturally targeted by the immune system but the extent and nature of the immune responses remain undefined [70].

Cancer testis antigens (CTAs) are a group of tumor-associated antigens expressed in normal testis and in some female reproductive organs, trophoblasts and different types of tumors. There are approximately 140 members in 70 families, and the expression of some of these antigens have been studied in different types of tumors and some have been shown to be immunogenic, specifically recognized by cytotoxic T lymphocytes [71]. Some members of the CTA family are expressed in ovarian cancer, including the *MAGE*, *BAGE*, *LAGE*,

GAGE, sperm protein 17 (*SP17*) and the synovial sarcoma X (*SSX*) genes [72–75]. CTAs are biologically relevant antigens. For example, Zhang and colleagues showed that moderate to high expression of MAGE-1 and MAGE-3 in ovarian cancer tissue (37–57%) positively correlated with tumor differentiation and clinical stage, while GAGE-1/2 and BAGE had relatively low expression, although ovarian cancer patients with ascites had significantly higher BAGE expression [73]. It was found that overexpression of SP17 in ovarian cancer cells results in enhanced migration and chemoresistance [76]. The immune system responds to CTAs in many cancers, including ovarian cancer. Among the CTAs in ovarian cancer, immunity to NY-ESO-1 is best characterized and clinically targeted. Nearly 25% of patients with the disease develop detectable, natural antibody and T-cell immune responses [77,78]. Recent studies show that NY-ESO-1-specific cytotoxic T cells infiltrate into ovarian tumors where they appear to be disabled through the coinduction of programmed death (PD)-1 and LAG-3 by factors in the tumor microenvironment, thus providing a mechanism for tumor growth despite measurable immunity [77]. Collectively, these results show that antitumor immunity is naturally elicited against ovarian cancer and impacts the clinical course of disease. However the antitumor response, as alluded previously with the NY-ESO-1 T cells, is blunted by a hostile immune suppressive microenvironment.

Immune suppression in ovarian cancer

Immune evasion in ovarian tumors involves a complex array of immune suppressive factors and cells that effectively halt the generation and clonal expansion of antitumor immunity. Genetic changes also occur, permitting the tumor cells to be ignored by the immune response. Immune suppression is mediated by factors released from the tumor or by infiltration of the tumors by a variety of either lymphoid or myeloid-derived suppressor cells (MDSCs) or regulatory cells.

The role of CD4 Tregs in the immune evasion of ovarian cancers is well characterized. Tregs are a heterogeneous T-cell subpopulation whose primary function is immune regulation by blocking the function of activated T cells. $CD4^+$ Tregs can be divided into subsets: the thymus-generated naturally occurring Tregs that have a $CD4^+CD25^+Foxp3^+$ phenotype, and the induced (adaptive) Tr1 Treg and Th3 Tregs that have CD25 variable expression [10,79]. While induced Tregs produce immune- suppressive soluble mediators such as TGF-β and IL-10 to mediate their inhibitory activities and block T-cell proliferation, natural Treg cells use cytokine-dependent, cell contact- dependent or a cytokine/cellular contact-dependent mechanisms to halt T-cell responses [10]. Three other subpopulations of $CD4^+$ Tregs have been proposed that can be differentiated by their level of expression of Foxp3, CD25 and CD45RA [80]. These subpopulations are functionally and phenotypically different: resting Tregs ($CD45RA^+CD25^{inter}Foxp3^{low}$) show more active proliferation upon stimulation and increased cytotoxic T lymphocyte antigen-4 expression; activated Tregs ($CD45RA^-$ $CD25^{high}$ $FOXP3^{high}$) die after proliferation but can suppress the proliferation of resting Tregs; and nonregulatory Tregs ($CD45^-CD25^{inter}$ $Foxp3^{low}$) that produced the highest levels of IL-17 when compared with naive non-Treg cells [80]. There are several mechanisms by which tumors, aided by Tregs, can halt the immune response. Tumors can increase the numbers of Tregs in the peripheral blood of cancer patients as reported for several tumor types, including ovarian [81–83]. Tumors can also recruit or induce Treg tumor infiltration as shown by numerous studies that demonstrate intratumoral localization of $CD4^+CD25^+Foxp3^+$ Tregs in several human cancers. The connection between pathogenesis of Tregs and prognosis in ovarian cancer was first suggested by Curiel and colleagues who showed that accumulation of intratumoral Tregs was associated with poor patient survival [84]. In that study, Tregs, as measured with immunohistochemistry, were associated with a high mortality rate. Two subsequently published manuscripts also demonstrated the importance of Tregs in ovarian cancer pathogenesis and outcome. Wolf

and colleagues showed that patients with low levels of intratumoral Foxp3 had substantially improved survival compared with patients with high levels (77 vs 30 months) [85]. Sato and colleagues also determined that the presence of $CD4^+$ Tregs influences the antitumor activity of intratumoral cytotoxic $CD8^+$ T cells [11].

Dendritic cells are classified into two subtypes according to their lineage: plasmacytoid DCs ($CD123^+$, $CD45RA^+$, $CD8^+$, $CD11c^-$, $ILT3^+$, $ILT1^-$ and Lin^-) and myeloid DCs (MDCs) that express CD11c and CD33, but lack CD45RA and CD123 [86]. Under quiescent conditions, they are present in the body in an immature form and are responsible for detecting danger and sampling of antigens. Upon detection of danger through their pathogen-associated molecular pattern (PAMP) receptors or danger-associated molecular pattern (DAMP) receptors, DCs will mature and migrate to the lymph nodes to activate Th cells and cytotoxic T lymphocytes [86]. Although tumors are able to produce danger signals, they are ineffective in inducing DC maturation and trafficking to lymph nodes, which is thought to be due to tumor-induced alterations in DC differentiation, thus reducing the number of functional cells available for effective T-cell activation [87]. For example, ovarian cancers secrete large amounts of IL-10, which promotes differentiation of DC to $CD14^+CD1a^-$ macrophage-like cells with reduced T-cell activation properties [87].

Human ovarian cancers are known to contain plasmacytoid and MDCs as well as their precursors. CXCR4 plasmacytoid precursor cells (preDC2s) are attracted into the tumor microenvironment by tumor derived stroma-derived factor (SDF)-1. SDF-1, also known as *CXCL12*, is a small chemokine that belongs to the intercrine family and CXC subfamily that activate leukocytes and is often induced by proinflammatory stimuli such as lipopolysaccharide, TNF or IL-1 [88]. SDF-1 exists as two variants, SDF-1α and SDF-1β, and has a very specific receptor CXCR4 with unique recognition of SDF-1. This receptor is a G-protein-coupled receptor that is expressed in a wide group of cells including T lymphocytes, B lymphocytes, monocytes and macrophages [88]. Bignotti *et al.* have identified *CXCL12*, along with other integrins, among the most highly differentiated genes in metastatic sites of papillary ovarian carcinomas [89]. PreDC2 recruited into the ovarian cancer micro environment induce T cells to release large amounts of IL-10, preventing local T-cell activation [90]. Induction of IL-10 is due, at least in part, to local induction of $IL\text{-}10^+$ regulatory CD8 T cells [91]. Although rare in the blood, plasmacytoid DCs and their precursors preferentially accumulate in ovarian cancers. By contrast, MDCs are the dominant DCs in the blood under normal health conditions. Some early studies appear to suggest that there is little accumulation of MDCs in ovarian cancer [90], but more recent studies demonstrate their infiltration. Their role in local immune suppression remains unclear, particularly in the solid tumor mass as opposed to the ascites fluids [92]. MDCs in their immature states are promoters of angiogenesis and vasculogenesis during tumor growth [93–95]. One study suggests that MDCs mediate local immune suppression in ovarian cancer by suppressing the effector function of T cells through the engagement of MDC-expressed B7-H1 [96]. B7-H1, also known as PD-L1 or CD274, is the PD-1 receptor ligand and it is thought that they help in the regulation of T-cell activation [97]. A group showed that DCs expressing B7-H1 inhibit T-cell proliferation directly and also indirectly by promoting the induction of $CD25^{high}FoxP3^+$ Tregs, thus suggesting that B7-H1 is a key player in the tumor micro environment immune suppression [98]. B7-H1 is expressed on the surface of several gynecological cancers, including ovarian, and is associated with poor overall survival in ovarian cancer [99–101]. PD-1 is also shown to be expressed on various adaptive immune effectors, notably CD4 and CD8 T cells, where it negatively regulates cell activation in cancers including ovarian [77,102–104]. The molecular interactions, particularly those associated with PD-1, remain elusive [96]. MDCs can mediate local immune suppression not only by expressing PD-1 and B7-H1 but also by the generation and increased activity of other mediators including arginase, indoleamine 2,3-dioxygenase, nitric

oxide (NO) and reactive oxygen species (ROS) [105–107]. Overall, the DC component of ovarian cancers remains an intriguing and underexplored area of ovarian cancer research and could represent a therapeutic target. This concept is borne out by recent murine modeling studies demonstrating improved anti-tumor immunity following specific depletion of DCs [108].

Apart from plasmacytoid DCs and MDCs, there is another subset of myeloid-derived cells, termed MDSCs, which have an increased ability to block local and systemic immune activation [109]. This is a heterogeneous population consisting of macrophages, DCs and granulocytes at early stages of differentiation [95]. MDSCs are classified into two subpopulations, granulocytic and monocytic MDSCs that, in mice, but not humans, are uniquely identified by coexpression of CD11b and Gr1 [110]. MDSCs are known to expand dramatically under various health disturbances such as tumor growth, inflammation and infection [95,111,112], and may have a role in immune suppression of ovarian cancer in murine models; however, their relevance in human ovarian cancers remains uncertain [113].

Neutrophils are potent initiators of non-infectious inflammation, and although recent studies have associated them with tumor progression and metastasis, the mechanism is still not well understood but may be due to blockade in adaptive immune responses. A study carried out by Klink and colleagues evaluated the interactions between neutrophils and ovarian cancer cells [114]. They showed that direct contact between ovarian cancer cells and neutrophils elicited enhanced ROS production, increased adhesion ability and upregulation of CD11b/CD18 expression in neutrophils from ovarian cancer patients compared with control neutrophils [114]. The neutrophil-to-lymphocyte ratio (NLR) serves as an indirect measurement of inflammatory status. Many recent studies showed that elevated NLR is an independent prognostic factor associated with an increase in disease recurrence in several cancers [115–119]. A recent study by Cho and colleagues evaluated the prognostic significance of NLR in patients with ovarian cancer compared with patients with benign gynecological tumors and healthy controls [116]. They showed that patients with advanced ovarian cancer and high preoperative NLR had decreased overall survival compared to patients with low NLR [116]. Overall, these results showed that neutrophils have a potential immune deregulating role in ovarian cancer and, in the future, they may become targets for immune-based therapies for advanced and metastatic ovarian cancer.

Aside from the recruitment of suppressive cells into the tumor microenvironment, tumor cells themselves express a variety of molecules that directly block immune responses. Studies show that MUC16 (the protein source of CA-125) facilitates peritoneal metastasis of ovarian tumors and adds to the immune suppressive tumor microenvironment by inhibiting the activity of NK cells [120–123]. A recent study by Gubbels and colleagues sheds more light onto the role and importance of MUC16 on cancer cells [122]. For example, they showed that MUC16 acts as an inhibitor of the NK–tumor conjugation of ovarian cancer cells. NK cells killed cells expressing low levels of MUC16 more effectively, with approximately 20% more NK-cell lysis and a two- to three-fold increase in NK leukemia cell lysis when compared with lysis of cells with high MUC16 expression [122]. Furthermore, a recent study by Krockenberger and colleagues showed that macrophage migration inhibitory factor (MIF; inducer of inflammation and promoter of tissue repair) inhibits the antitumor immune response against ovarian cancer cells by downregulating NKG2D receptor in NK cells [124].

Recent immunological approaches for treatment of ovarian cancer

Passive antibody therapies

HER-2/neu antibodies, namely trastuzumab and pertuzumab, have been extensively studied as therapeutics for the small subset of patients with tumors that overexpress HER-2/neu (Table 1). Trastuzumab (Herceptin® [Genentech, CA, USA]) is a humanized monoclonal antibody that targets the HER-2/neu extracellular domain and inhibits HER-2/neu-positive tumor cell proliferation. This antibody is the standard of care for patients with HER-2/neu$^+$ breast cancer [125]. Few clinical trials have been reported on the use of trastuzumab in ovarian cancer. One study performed on 41 patients with advanced epithelial ovarian cancer showed that trastuzumab administered as a single agent therapy yielded a low response rate (~7%) and a median progression-free interval of only 2 months in patients with recurrent or advanced ovarian cancer [31]. A Phase II clinical study looked at the efficacy and tolerance of trastuzumab when combined with paclitaxel and carboplatin in seven patients with taxane/carboplatin- resistant ovarian cancer and HER-2/neu overexpression [126]. In this study the combination treatment elicited a clinical response in three patients with a median PFS of 2.9 months and overall survival of 12.3 months. Another clinical study performed on 33 patients with mucinous ovarian carcinoma showed approximately 18% HER-2 expression, and of the three patients treated with chemotherapy and trastuzumab, one had complete effective response and another had partial response, suggesting that trastuzumab may be effective for this subset of individuals [127].

A recent preclinical study by Wilken and colleagues explored the mechanism behind the trastuzumab resistance in four HER-2$^+$ ovarian cancer cell lines. They showed that ovarian cancer cells treated with trastuzumab for 12 weeks and later treated with a HER-1-targeted drug, such as cetuximab or gefitinib, were effectively growth-inhibited [128]. This study suggests that trastuzumab may increase its effect when combined with a second HER-2/neu-targeted drug. In this case, it appears that trastuzumab induces the cells to rely on other growth factor receptors such as the EGFR and, after inhibiting this second pathway, the cancer cell's growth can be effectively halted.

Pertuzumab (rhuMAb 2C4, Omnitarg [Genentech, CA, USA]) is a HER-2/neu targeted monoclonal antibody that binds to a different epitope than trastuzumab, inhibiting the formation of HER-2/neu heterodimers with other HER-family receptor and halting the proliferation of ovarian cancer cells [129,130]. Pertuzumab is the HER-2 inhibitor that has been mostly studied in clinical trials for ovarian cancer (Table 1). The first clinical study was carried out by Gordon and colleagues and showed that pertuzumab as a monotherapy elicited a 4.3% partial response, 6.8% stable disease for 6 months and 14.5% of total clinical activity, with a median PFS of 6.6 weeks in patients with recurrent ovarian carcinoma [129]. A study carried out in platinum-resistant patients showed that the combination therapy of pertuzumab with gemcitabine resulted in a response rate of 13.8% with 2.9 months PFS as compared with a 4.6% response rate and PFS of 1.5 months in the gemcitabine and placebo group [131]. A recent ongoing study of 152 platinum- sensitive patients evaluated the activity of pertuzumab and carboplatin-based therapy compared with carboplatin therapy alone [132]. Preliminary results demonstrated that pertuzumab with carboplatin is well tolerated, has a 64% response rate and PFS of 34.1 weeks in comparison with 52% response rate and PFS of 31.3 weeks in the single-agent carboplatin group [132,133].

Several antihuman FRα antibodies have been previously studied in ovarian cancer. Farletuzumab (MORAb-003) is a humanized monoclonal antibody, optimized from the original murine LK26 antibody, which has high affinity for FRα [134]. Preclinical *in vivo* and *in vitro* studies have shown that farletuzumab binds specifically to ovarian cancer cells, has high efficacy in decreasing tumor growth with very low to no toxicity, and is a useful

antibody for radioimmunoscintigraphy of ovarian cancer cells expressing FRα [135,136]. A clinical study performed by Konner and colleagues reported that farletuzumab as a single therapy in platinum-resistant ovarian cancer patients was safe and well tolerated at doses between 12.5 and 400 mg/m^2 [137]. Moreover, 35% of the patients treated with one cycle (four weekly doses) had stable disease and three of these patients received extended therapy showing 3–17% mean decreases in tumor size. Another recent clinical study showed the results of farletuzumab as single-agent therapy or in combination with platinum and taxane in platinum-sensitive ovarian cancer patients with first recurrence of disease [138,139]. In this study, the group treated with farletuzumab combination therapy showed a 7% complete response, 63% partial response and 89% of the patients achieved normal CA-125. Moreover, four patients still remain in remission with median extension of the secondary remission being 11 months [140]. Ongoing and future Phase III studies include adjuvant use of farletuzumab as a monotherapy and in combination with chemotherapy in patients with recurrent ovarian cancer.

Catumaxomab is a trifunctional antibody with bispecificity for EpCAM in tumor cells and CD3 antigen in T cells. These new types of antibodies induce a tri-cell complex of tumor cells, T cells and accessory immune cells (e.g., macrophages, DCs and NK cells) owing to their unique Fc-composition of mouse IgG_{2a} and rat IgG_{2b}[141]. This drug has been approved in Europe since 2009 for the treatment of malignant ascites with EpCAM-positive carcinomas where first-hand therapy is not available or no longer viable. This drug has also been tested in the clinical setting as a new therapeutic strategy for ovarian cancer patients with symptomatic malignant ascites. A clinical study from Burges and colleagues showed good tolerance of catumaxomab in patients with recurrent ascites due to ovarian cancer [142]. They demonstrated a significant decrease in production of ascites in all 23 patients (only one patient required paracentesis at day 28) with a decrease in the number of intraperitoneal $EpCAM^+$ tumor cells from 540,000 per 10^6 analyzed cells prior to treatment to 39 per 10^6 following the last infusion. Another recent clinical trial using catumaxomab in patients with malignant ascites due to epithelial cancers (129 ovarian and 129 nonovarian) assessed the efficacy and safety of catumaxomab and paracentesis compared with paracentesis alone [143]. This study showed that catumaxomab reduced the ascites volume and the time between paracentesis in both cancer groups, but most significantly in the ovarian cancer group along with an overall survival of 110 days compared with 81 days in the control group. Moreover, the catumaxomab-treated group elicited decreased $EpCAM^+$ tumor cell count and increased $CD45^+$ leukocytes in the ascites at the end of treatment when compared with the paracentesis alone.

Adoptive T-cell therapies

Adoptive T-cell therapy involves *ex vivo* activation, expansion and reinfusion of antigen-specific T cells [144]. The approach has been used successfully for the treatment of malignant melanoma and is envisioned to be useful for ovarian cancer based on the importance of T cells in relapse [145]. From a technical and infrastructure standpoint, adoptive T-cell therapy remains a challenge. Thus far, only two clinical trials of adoptive T-cell therapy in ovarian cancer have been reported. The first clinical trial by Kershaw and colleagues tested the safety and feasibility of infusion of T cells genetically modified to express a FRα-receptor antibody coupled to the signaling motif of the Fc receptor γ-chain [146]. The study was performed in patients with recurrent disease. While the infusions were shown to be safe, no clinical activity was observed, probably due to two notable problems. First, the infused T cells did not persist for long periods of time in large numbers, as required for tumor eradication in melanoma [145]. Second, an inhibitory factor developed in half of the patients, which significantly reduced the ability of the gene-modified T cells to respond against the tumor. The second clinical trial involved the infusion of nonmodified

MUC1-specific Th1 effector cells [147]. MUC1-specific T cells were derived by repeated stimulation and expansion of leukapheresis-derived cells with the 20-mer MUC1 peptide, GSTAPPAHGVTSAPATAPAP. Three doses of between 10^8 and 10^9 cells (largely CD4 T cells) were administered intraperitoneally into seven patients. Three discontinued treatment due to local inflammation or obstruction at the intraperitoneal port; in the four remaining patients, treatment was shown to increase systemic MUC1 immune responses, which could have contributed to the long-term survival observed in two of the patients.

Vaccine therapies

Peptide vaccines are a very attractive type of active immunotherapy that offer several advantages including specificity, stability and the ability to be manufactured to increase their immunogenicity. To date, a few clinical studies have been published using peptide vaccines for ovarian cancer focusing on the safety and immunogenicity of the vaccines (Table 1). One study of a multipeptide vaccine constructed from three ovarian cancer-associated antigens, HER-2/neu (amino acids 369–377 and 754–762), MAGE-A1 (amino acids 161–169 and 96–104) and FRα (amino acids 191–199), for the treatment of advanced ovarian cancer was reported to be safe and well tolerated [148]. This study demonstrated that this vaccine was immunogenic with detection of $CD8^+$ T-cell response in all treated patients, one detected *ex vivo* and eight after *in vitro* stimulation. Despite the $CD8^+$ T-cell response, 89% of the patients had progression of their disease. In a Phase I trial by Diefenbach and colleagues, NY-ESO-1b peptide (amino acids 157–165) vaccine was studied as an adjuvant therapy in nine patients with high-risk ovarian cancer [149]. Results showed no *ex vivo* $CD8^+$ T-cell responses; however, after *in vitro* presensitization with either NY-ESO-1b peptide or recombinant adenovirus, 75% of the treated patients showed $CD8^+$ T-cell immunity and remained present 3 weeks after vaccination. In another study, a p53 synthetic long-peptide (SLP) vaccine in patients with recurrent ovarian cancer showed that this vaccine is safe with only grade 1 or 2 adverse events observed in all patients after four immunizations [150]. This vaccine was capable of inducing proliferation of p53-specific $CD4^+$ T cells, predominantly of the Th2 phenotype, in 82.4% of the patients, although only 10% of the patients had stable disease and the rest showed disease progression.

While most of these vaccines being tested target the generation of T cells, the fact that antibody immunity correlates with disease outcomes in patients makes antibody- inducing vaccines a logical option. To that end, Kaumaya determined the activity of a HER-2/neu antibody- inducing chimeric peptide (amino acids 628–647 and 316–339) vaccine with a promiscuous T-cell epitope (amino acids 280–302) from the measles virus fusion protein in patients with metastatic solid tumors including patients with advanced ovarian cancer [151]. This study demonstrated that this vaccine is moderately well tolerated (20% severe adverse events) and elicits IgG antibodies at all doses. Moreover, of six patients that showed clinical benefit, two were patients with ovarian cancer who had a high antibody response to the vaccine [151]. Overall, the few vaccine trials that have been conducted reveal that patients can mount immunity safely to tumor antigens paving the way for advanced clinical trials designed to test clinical efficacy. Optimism remains that vaccines can eradicate residual tumor to prevent recurrence or disease progression. Indeed, Hernando and colleagues recently published a case report demonstrating vaccine potency in a patient with recurrent metastatic ovarian cancer [152]. The 61-year-old patient has been previously treated twice with surgical debulking and platinum based therapy. At third recurrence 4 months following the last therapy, the patient presented with a bulky axillary lymph node metastasis and a para-aortic mass. At that time, a vaccination regimen with autologous DCs engineered to express FRα was initiated, and ten immunizations were given at 4-week intervals. CT scans before and 3 months after the last treatment showed a marked, albeit partial, shrinkage in tumor. CA-125 levels, which were rising just prior to vaccine, immediately declined to

baseline following the first two vaccinations. A follow-up CT scan at 16 months following the start of vaccination showed greater than 50% regression. Immune monitoring revealed that the patient developed strong FRα-specific T-cell immunity to the vaccine. Remarkably, at 11 months following the last vaccine, CA-125 levels remained at baseline showing that vaccines can induce long-term remission.

Future perspective

There is some agreement in the field of cancer immunotherapy that combination approaches could lead to synergism and greater efficacy. A number of strategies have emerged, some of which are being tested clinically. For example, the efficacy of trastuzumab therapy may be improved by combining with agents that boost T-cell immunity. In one study, zum Buschenfelde found that pretreatment of HER-2/neu^{+} ovarian tumor cells with trastuzumab enhanced the cytolytic activity of HER-2/neu-specific T cells against the HER-2/neu-overexpressing tumors *in vitro* [153]. Although the mechanism is unclear, it is possible that trastuzumab promotes the internalization and degradation of HER-2/neu, resulting in increased presentation of HER-2/neu MHC class I epitopes, which may lead to greater activation and expansion of HER-2/neu- specific T cells. A clinical trial of combination trastuzumab and HER-2/neu vaccination in breast cancer was recently reported by Disis and colleagues [154]. A total of 22 patients with stage 4 HER-2/neu-overexpressing breast cancer receiving trastuzumab therapy were vaccinated with an HER-2/ neu T-cell peptide-based vaccine. The patients had either no evidence of disease or stable masses at the time of therapy. The combination was well tolerated with 15% of patients experiencing an asymptomatic decline in left ventricular ejection fraction below the normal range during combination therapy. While many patients had pre-existing immunity to HER-2/neu when treated with trastuzumab alone, that immunity could be significantly boosted and maintained with vaccination. Importantly, at a median follow-up of 36 months from the first vaccine, the median overall survival in the study population has not been reached.

In summary, circulating or tumor-associated Tregs may block the immune response; subsequently, there is interest in preconditioning patients to inhibit Tregs in order to augment immunity. Agents such as cyclophosphamide, which augment immune-based therapies in both human and mouse studies are thought to involve Treg depletion [155]. Other strategies that are being explored to inhibit the function of Tregs include anticytotoxic T lymphocyte antigen-4 monoclonal antibodies and CD25-targeted agents such as denileukin diftitox [156]. Alternatively, targeted therapeutics, such as small molecule inhibitors, could also work in concert with vaccines. An example is inhibitors of TGF-β, a growth factor with multiple roles in cancer [157,158]. TGF-β promotes tumor progression, invasion and metastasis by inducing epithelial-to-mesenchymal transition, migration and release of VEGF [157]. TGF-β also directly inhibits cytotoxic actions of tumor-infiltrating CD8 T cells [159]. Thus, an agent that simultaneously blocks immunosuppression and tumor progression may be more effective by providing sufficient time for the immune system to expand and destroy residual tumor burden. Many combinatorial strategies are currently being tested and time will tell whether such mixing will be therapeutically effective.

Executive summary

Basic principles of immunity

- The innate immune system is the first line of defense that consists of:
  - A cellular component that includes NKT cells, mast cells, eosinophils, basophils, macrophages, neutrophils and dendritic cells.

- Humoral factors that include mainly cytokines and complement.

- The adaptive immune system involves both T and B lymphocytes.
- Adaptive immune responses usually develop after 1 week, are highly antigen specific and capable of generating immunologic memory.

Natural immune response to ovarian cancer

- There is a natural immune response associated with ovarian cancers shown to have a significant impact on the clinical course of the disease.
- $CD8^+$ T cells tumor infiltration is shown to improve survival in ovarian cancer patients.

Ovarian cancer antigens

- Several ovarian cancer antigens have been identified and studied (i.e., folate receptor α, HER2/neu, IGF binding protein 2, p53, mucins, epithelial cell adhesion molecule and NY-ESO-1) demonstrating that ovarian cancer patients respond naturally to these antigens.

Immune suppression in ovarian cancer

- Despite the fact that antitumor immunity is naturally elicited against ovarian cancers, the antitumor response is blunted by a complex immune suppressive microenvironment.
- Immune suppression is mediated by factors released from the tumor or by infiltration of the tumors by a variety of either lymphoid or myeloid-derived suppressor or regulatory cells.

Immunological treatments

- Immunological approaches for ovarian cancer have been studied in recent clinical trials that include antibody therapies (i.e., farletuzumab, pertuzumab and catumaxomab), adoptive T-cell therapies and vaccines with promising results.

Conclusion

- Ovarian cancer is an immune reactive malignancy with a complex immune microenvironment.
- Future direction of this field should focus on a synergistic combination of therapies that can generate immunity and target immune suppression, hopefully with greater results.

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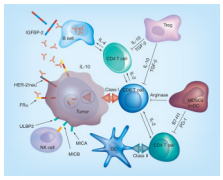

Figure 1. Immune microenvironment in ovarian cancer

Despite the presence of various immune effector cells (i.e., CD4 T cells, CD8 T cells and NK cells) that can attack the tumor, the presence of a complex immune suppressive network including Tregs, ImDCs and MDSCs along with their mediators (i.e., IL-10, TGF-β, B7-H1, PD-1 and arginase) effectively halts the antitumor immunity.

DC: Dendritic cell; FR: Folate receptor; IGFBP: IGF binding protein; ImDC: Immature DC; MDSC: Myeloid-derived suppressor cell; PD: Programmed death.

Table 1

Immune-based clinical trials for ovarian cancer.

Agent	Antigen targeted	Patient population	Patients (n)	Clinical description	Ref.
Passive antibody therapies					
Trastuzumab	HER-2/neu	Recurrent or persistent ovarian or primary peritoneal cancer, 2+ or 3+ Her-2/neu overexpression	41	Single agent, group wide, Phase II; overall objective RR: 7.3%; SD: 39%; PFS: 2 months	[31]
Trastuzumab + TC	HER-2/neu	Recurrent TC-resistant ovarian cancer + Her-2/neu overexpression	7	Open-label, noncomparative, prospective, Phase II; CR: 43%; PFS: 2.9 months; OS: 12.3 months	[126]
Pertuzumab	HER-2/neu	Refractory or recurrent platinum-resistant ovarian cancer	117	Open-label, single agent, Phase II; overall RR: 4.3%; overall SD: 6.8%; overall PFS: 6.6 weeks; OS: 52.7 weeks; cohort 1 PFS: 7.6 weeks; OS: 46.7 weeks; cohort 2 PFS: 6.1 weeks; OS not yet reached	[129]
Pertuzumab + gemcitabine	HER-2/neu	Platinum-resistant ovarian, fallopian tube or primary peritoneal cancer	130	Randomized, double-blind, Phase II; gemcitabine + placebo: PFS: 2.6 months; PR: 4.6%; OS: 13.1 months; gemcitabine + pertuzumab PFS: 2.9 months; PR: 13.8%; OS: 13 months	[131]
Pertuzumab + carboplatin	HER-2/neu	Platinum-sensitive ovarian cancer	149	Ongoing Phase II, carboplatin + paclitaxel or gemcitabine: RR: 52%; PFS: 31.3 weeks; pertuzumab + carboplatin: RR: 64%; PFS: 34.1 weeks	[132,133]
Farletuzumab	FRα	Platinum-resistant or refractory ovarian, fallopian tube or primary peritoneal cancer	25	Open-label, dose-escalation, Phase I, well tolerated at 12.5–400 mg/m^2; SD: 35%; three patients with SD had extended treatment with 3–17% decrease in tumor size from baseline	[137]
Farletuzumab + taxane/carboplatin	FRα	Platinum-sensitive ovarian cancer patients with first recurrence	44	Open-label, Phase II, farletuzumab alone: frequent SD but no objective RR; farletuzumab + taxane/carboplatin: 89% normalized CA-125, RR: 64% in first progression-free interval <12 months, RR: 71% in first progression-free interval >12 months	[138–140]
Catumaxomab	EpCAM	Recurrent ascites from pretreated refractory ovarian cancer	23	Open-label, dose-escalation, Phase I/II, safe and well tolerated at 10–200-μg doses, 100% decrease in ascites and EpCAM$^+$ tumor cells	[142]
Catumaxomab + paracentesis	EpCAM	Symptomatic malignant ascites with epithelial ovarian and nonovarian cancers	258†	Open-label, randomized, Phase ll/lll, paracentesis alone: puncture-free survival: 11 days; TP: 11 days; OS: 81 days; catumaxomab + paracentesis: puncture-free survival 52 days; TP: 71 days; OS: 110 days	[143]
Adoptive T-cell therapies					
Autologous T-cell specific for FRα	FRα	Recurrent or residual epithelial FRα$^+$ ovarian cancer with metastasis	14	Phase I, cohort 1 = T cells + IL-2, cohort 2 = T cells + allogeneic PBMCs; well tolerated in both groups, no clinical response observed	[146]

Agent	Antigen targeted	Patient population	Patients (n)	Clinical description	Ref.
Autologous MUC1-stimulated T cells	MUC-1	Residual or recurrent epithelial ovarian cancer	7	Phase I, four patients completed three cycles: one patient is disease free + maintained low CA-125, one patient had 16-month survival + decrease in CA-125 and two patients had 3–5-month survival + increase CA-125	[147]
Vaccine therapies					
Multipeptide vaccine	HER-2/neu, FRα, MAGE-A1	Primary peritoneal, fallopian tube or ovarian cancer with HLA-A1$^+$ HLA-A2$^+$ or HLA-A3$^+$	9	Phase I, well tolerated, 89% had progression of disease, one patient disease-free after 19 months; CD8$^+$ T-cell response in all nine patients	[148]
NY-ESO-1b peptide vaccine	NY-ESO-1b	High-risk epithelial ovarian cancer in first clinical remission	9	Phase I, well tolerated, after *in vitro* presensitation 75% had CD8$^+$ T-cell response, 67% patients recurred, PFS: 13 months, three patients remain disease-free	[149]
p53 synthetic long peptide vaccine	p53	Recurrent epithelial ovarian cancer	20	Phase I, well tolerated, p53-specific CD4$^+$ T-cell proliferation in 82.4% patients, SD: 10%	[150]

†Ovarian cancer group = 129; non-ovarian cancer group = 129.

CR: Complete response; EpCAM: Epithelial cell adhesion molecule; FR: Folate receptor; MUC: Mucin; OS: Median overall survival; PBMC: Peripheral blood mononuclear cell; PFS: Median progression-free survival; PR: Partial response; RR: Response rate; SD: Stable disease; TC: Paclitaxel/carboplatin; TP: Median time to next therapeutic paracentesis.