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Review article

Checkpoint inhibitors in ovarian cancer: A review of preclinical data

David W. Doo[a], Lyse A. Norian[b], Rebecca C. Arend[a,*]

[a] Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Alabama at Birmingham, Birmingham, AL, United States of America
[b] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, United States of America

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ABSTRACT

Ovarian cancer is the deadliest gynecologic malignancy, and relapse after initial treatment is frequently fatal. Although ovarian cancer typically has an immunosuppressive tumor microenvironment, a strong intratumoral T cell presence is associated with an improved response to chemotherapy and better overall prognosis. Given the success of checkpoint inhibitors in the treatment of other malignancies, there has been an attempt to replicate these results in ovarian cancer clinical trials. Preclincal studies in ovarian cancer have also been conducted over the past decade, and most of the focus has been on the use of programmed cell death protein 1 (PD-1). Several other checkpoint inhibitors have also been investigated in various combinations with chemotherapy, oncolytic vaccines, co-stimulatory molecules, poly ADP ribose polymerase (PARP) inhibitors, and other checkpoint inhibitors. Unfortunately, these successes have yet to translate to the clinical realm. Whether this is because the drug class is truly ineffective in ovarian cancer, or simply because the research is lacking is unclear. Either way, it is evident that preclinical data on the use of checkpoint inhibitors is woefully deficient in ovarian cancer and more research is urgently needed to inform the translation of immune checkpoint blockade into successful clinical use. In this review, we discuss the results from preclinical studies using checkpoint inhibitors to treat ovarian cancer, with a focus on strategies that show potential for clinical use.

1. Background

Ovarian cancer is the deadliest gynecologic malignancy in the United States, with an estimated 22,240 new cases diagnosed and 14,070 deaths in 2018 (Siegel et al., 2018). Although incremental advances in the treatment of this disease have been made, major breakthroughs are lacking. One area that has garnered substantial interest is the field of immunotherapy, which has proven effective in the treatment of other malignancies such as melanoma and non-small-cell lung cancer. Immune checkpoint blockade therapeutics, in particular, have become more ubiquitous due to their ease of administration, favorable side effect profile, and effectiveness in certain tumor types. The concept of harnessing the immune system for the elimination of cancer is appealing, but there are many hurdles unique to ovarian cancer that make implementation of this technology more challenging. Although preclinical experiments have been ongoing for the past decade in ovarian cancer models, the number of actual published reports is fairly small. Unsurprisingly, these results have yet to translate to the clinical realm, wherein immunotherapy has thus far been ineffective. This review will focus on preclinical experiments using checkpoint inhibitors in *in vivo* models of ovarian cancer. We will also discuss the unique hurdles that must be overcome for the successful implementation of immunotherapy in the clinical treatment of ovarian cancer.

2. Methods

Original articles published between 2010 and 2018 were retrieved from PubMed. The search included Medical Subject Heading (MeSH) terms “ovarian neoplasm” and “immunotherapy,” as well as title/abstract searches for “preclinical” or “mouse” or “model.” References were also reviewed for additional sources. A total of 392 articles were initially screened under these criteria. Sources were then reviewed for the following inclusion criteria: (a) preclinical *in vivo* experiments published in English; (b) use of a checkpoint inhibitor alone or in combination for treatment of a preclinical ovarian cancer model; (c) use of a syngeneic tumor model in mice with an intact immune system. Articles were excluded if they published *in vitro* data only. A total of 18 primary research articles were found to meet these criteria and were included in this review.

* Corresponding author at: Division of Gynecologic Oncology, University of Alabama at Birmingham, 619 19th Street South, 176F Rm 10250, Birmingham, AL 35249, United States of America.
E-mail address: rarend@uabmc.edu (R.C. Arend).

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3. The role of the immune system in ovarian cancer

The primary function of the immune system is to provide protection from pathogens while remaining unresponsive or "tolerant" to the myriad proteins that comprise an individual's normal "self". The immune system consists of two interconnected compartments, designated innate and adaptive. Innate immunity is considered the first line of defense against invading pathogens and is composed of cellular and soluble/protein mediators. The cellular component consists of mast cells, eosinophils, basophils, natural killer (NK) cells macrophages, neutrophils, and dendritic cells (DCs). Its roles are to identify pathogens, provide immediate control of a nascent infection, and prepare the host for a subsequent adaptive immune response. The adaptive immune system is highly antigen-specific and consists of cellular responses involving T lymphocytes and B cells that lead to the production of long-lived immunologic "memory" against pathogens.

In attempting to harness the immune system to fight cancer, processes originally developed to control infections must be adapted for this new use. Furthermore, immunological checkpoints intended to prevent immune recognition of "self" antigens must be overcome. Because adaptive immunity is antigen-specific, and T cells are capable of killing target cells that express those antigens, most cancer immunotherapies are designed to promote tumor antigen-specific T cell immunity. Four main steps, involving both innate and adaptive immunity, must be successfully completed to achieve this goal: (1) uptake and processing of tumor antigens by DCs, (2) presentation of tumor antigens by DCs expressing required co-stimulatory proteins to naive T cells within lymphoid tissues, (3) trafficking of differentiated effector T cells into the tumor site, and (4) maintenance of a tumoricidal, effector T cell responses within the tumor microenvironment (Mellman et al., 2011). A disruption at any of these points can lead to immune tolerance for growing tumors, shutting down the cytolytic T cell response and resulting in uncontrolled tumor progression.

In order to shift the intratumoral immune response toward a more protective phenotype, immune checkpoint inhibitors have been investigated in both the clinical and preclinical settings. After naive T cells are activated by DCs, they increase expression of checkpoint proteins that limit the scope and duration of the T cell response, as a means of preventing unintended damage to self tissues. This transition from activation to exhaustion is a continuum that can be assessed by evaluating the patterns and expression levels of different checkpoint proteins on the T cell surface. Co-stimulatory receptors that promote the activity of T cells include CD28, OX40, GITR, CD137, CD27, and HVEM. Checkpoint receptors that decrease T cell activity include PD-1, CTLA4, TIM-3, BTLA, VISTA, and LAG-3 (Fig. 1) (Mellman et al., 2011). The ligands that bind to and activate both categories of receptors are commonly found on the surface of other cells, and the ligands for checkpoint receptors tend to be found at a much higher rate on malignant cells. Immune checkpoint blockade therapeutics work by preventing these negative receptor/ligand interactions, and restoring the function of exhausted T cells (Sakuishi et al., 2010).

The most thoroughly studied immune checkpoint receptor is programmed cell death protein 1 (PD-1). After binding to one of its ligands, programmed cell death ligand-1 or 2 (PD-L1 or PD-L2), the PD-1 receptor suppresses T cell responses in peripheral tissues at the site of infection or other immune stimulus. This can cause a significantly blunted anti-tumor effect, and expression of PD-L1 or PD-L2 on the surface of tumor cells and tumor-associated macrophages is therefore a major mechanism of immune evasion (Gottlieb et al., 2017). PD-1 is also highly expressed on Tregs, and activation of this checkpoint receptor on Tregs increases their suppressive activity.

CTLA4 is another immune checkpoint receptor that is exclusively found on T cells, where it directly competes with the co-stimulatory receptor CD28 to downregulate the early phases of T cell activation. The ligands for CTLA4 and CD28 are the same, CD80 and CD86, but the affinity of CTLA4 for these ligands is much higher than that of CD28. Binding of CTLA4 to one of its ligands delivers inhibitory signals to the T cell, while simultaneously preventing CD80 and CD86 from binding to CD28. CD80 and CD86 are subsequently removed from the surface of the antigen-presenting cell to prevent the possibility of further engagement of CD28 (Pardoll, 2012). Additional actions of CTLA4 include reduced function of helper T cell activity and upregulation of Treg-mediated immunosuppression.

Ovarian cancer poses unique challenges to the immune system, due to its intraperitoneal dissemination and predictable omental involvement. Adipocytes in the omentum secrete growth factors and other bioactive molecules that facilitate tumor cell proliferation and viability (Wagner and Dudley, 2013). Tissue resident macrophages promote regulatory T cell (Treg) expansion, which suppresses the activity of effector T cells (McCaw et al., 2019). Abundant myeloid derived suppressor cells (MDSCs) further inhibit the ability of effector T cells to eradicate their targets. Collectively, these suppressive cells create a tolerogenic environment that is difficult to overcome (Meza-Perez and Randall, 2017).

Despite the multitude of ways that ovarian cancer can evade the immune system, a robust immune response is particularly important in ovarian cancer recurrence and survival. Several studies have shown that the presence of tumor infiltrating lymphocytes (TILs) is associated with platinum sensitivity, longer progression free survival, and longer overall survival (Zhang et al., 2003; Mariya et al., 2014). Conversely, tumor infiltration by immunosuppressive Tregs portends a poor prognosis for patients with this disease (Curiel et al., 2004; Sato et al., 2005). T cells clearly play a critical role as mediators of tumor regression *versus* progression and therapies to alter their presence and activity are urgently needed to treat ovarian cancer going forward.

4. Checkpoint inhibitor use in ovarian cancer clinical trials

Despite their success in other tumor types, the clinical use of checkpoint inhibitors in ovarian cancer has thus far been disappointing. The objective response rates of single-agent checkpoint inhibitors ovarian cancer clinical trials are generally low, around 6–15%, and there has yet to be an FDA approval for any immune checkpoint blockade therapeutic for ovarian cancer, other than the use of pembrolizumab for those tumors with high microsatellite instability (MSI-H) (Hamanishi et al., 2015; Hinchcliff et al., 2018). However, there are

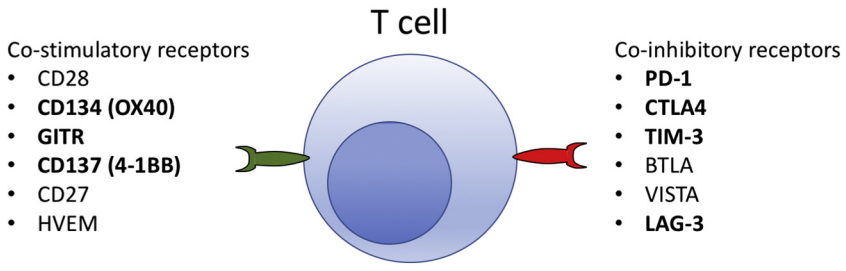

Fig. 1. Co-stimulatory and co-inhibitory receptors expressed on the surface of T cells. Bold indicates therapies that have successfully targeted these receptors in mouse models of ovarian cancer.

many more trials ongoing that are testing checkpoint inhibitors in combination with chemotherapy and other agents. Additionally, the failures of previous clinical trials may have more to do with the timing of drug administration than they do with the drugs themselves. These concerns are being addressed in ongoing clinical trials (NCT02728830, NCT03249142, NCT02520154, NCT02766582) that are designed with immunotherapy given as part of the primary treatment as opposed to later in the disease course.

5. Preclinical results of checkpoint inhibitors in ovarian cancer

5.1. Murine models of ovarian cancer

Much of the preclinical data informing the use of various therapeutics for the treatment of ovarian cancer come from murine models. Experimental design varies between publications, ranging from the subcutaneous injection of tumor cells that are grown *in vivo* to implantation of these cells into the peritoneal cavity. Furthermore, patient-derived xenograft (PDX) models and genetically engineered mouse models (GEMMs) provide additional experimental options. Each method has its advantages and disadvantages, and most investigators testing immune checkpoint inhibitors in ovarian cancer have chosen to use either subcutaneous or intraperitoneal models in their research. Subcutaneous inoculation of ovarian cancer cells has the advantage of having a palpable tumor that can be directly measured over time to evaluate response to treatment. However, these models are limited by the lack of normal tumor stroma that is normally encountered in an orthotopic model. This is particularly important in testing immunotherapy because stromal components such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) have been shown to be integral to the immunosuppressive state that characterizes ovarian cancer.

Due to these factors, most papers testing checkpoint inhibitors in ovarian cancer have used an IP injection model in order to more closely recapitulate the human ovarian cancer tumor microenvironment. Results from these papers are more convincing than those that utilize SC tumor models, but all data should be considered when designing clinical trials for human ovarian cancer patients. Experimentation with PDX models may also be interesting as the implanted tumors reflect the heterogeneity of human ovarian cancer than those that use cell lines. Alternatively, the use of humanized mouse models may be even more intriguing as these mice contain human immune cells and more closely mimic the behavior of the human immune system toward ovarian cancer. Humanized models would be particularly useful for testing immune checkpoint inhibitors and may be more valid than the use of syngeneic mice.

5.2. PD-1/PD-L1

PD-1 blockade is the most commonly studied immune checkpoint therapeutic in ovarian cancer, and its use has been studied in murine models both individually and in combination with a number of other interventions. The use of PD-1 antibody alone has mixed results, with tumor regression and improvement in T cell function found in one study (Table 1) (Krempski et al., 2011), but a lack of response reported in another (Wei et al., 2013). In contrast, when PD-L1-deficient ovarian cancer cell lines are used, tumor regression and survival do improve significantly, which suggests that this regulatory pathway is very important for immune evasion in ovarian cancer (Abiko et al., 2013). Moreover, that the complete absence of PD-L1 expression in cell lines produces a robust response but administration of a PD-1 antibody sometimes does not suggests that the timing or dosage of antibody administration needs to be optimized in future studies.

It should be noted that the majority of these studies used ID8 cell lines, which behave like ovarian cancer in that they produce ascites and peritoneal metastases when injected IP. However, this cell line lacks a p53 mutation, which is present in the vast majority of epithelial ovarian cancers. Additionally, the ID8 cell line does not normally express PD-L1, although expression of PD-L1 can be induced in the presence of IFN-γ (Abiko et al., 2013). These limitations must be considered in the interpretation of data generated with this cell line as its behavior may be significantly affected by these differences in gene expression, particularly when investigating immunotherapy agents.

5.3. PD-1 combined with other checkpoint inhibitors

The greatest benefit of PD-1/PD-L1 blockade occurs when it is used in combination with other agents, especially other checkpoint inhibitors. When combined with CTLA4 antibodies, PD-1 blockade caused tumor rejection in 50% of mice that were inoculated subcutaneously with the ovarian cancer cell line ID8 modified to express vascular endothelial growth factor (VEGF). This rate was boosted to 75% when combined with a vaccine consisting of irradiated granulocyte macrophage colony-stimulating factor (GM-CSF)-transduced tumor cells (Duraiswamy et al., 2013a). The PD-1/CTLA4 combination was also tested in conjunction with activating antibodies to the co-stimulatory receptor CD137, which activates CD8 and CD4 lymphocytes and NK cells. The triple antibody combination improved survival of mice threefold *versus* controls treated with 2A3 antibody following intraperitoneal tumor challenge with parental ID8 tumor cells. This treatment also decreased the frequency of intraperitoneal suppressive Tregs and MDSCs, and increased activation of intraperitoneal T lymphocytes as measured by IFNγ and TNFα production (Dai et al., 2013). The combination of PD-1 and CD137 inhibition, without CTLA4, doubled survival but did not have as profound of an effect as the triple therapy (Wei et al., 2013).

The importance of combining multiple immune checkpoint inhibitors in ovarian cancer was further highlighted in a study that examined the expression of PD-1, CTLA4, and LAG-3 by flow cytometry in an IE9mp1 murine model. In this study, many of the TILs that were examined expressed at least 2 checkpoint inhibitors when isolated from ovarian tumors. When any one of these markers were inhibited, there was a compensatory increase in the expression of the other checkpoints, allowing for persistent immune suppression. This was overcome when two or three of these antibodies were used in combination, resulting in increased infiltration of CD8 T cells, higher expression of effector cytokines, and a significant increase in survival (Huang et al., 2017).

5.4. PD-1 combined with chemotherapy

Blockade of PD-1 in combination with chemotherapy has increased the efficacy of immune checkpoint blockade in multiple studies of murine ovarian cancer. PD-L1 antibodies have been tested in combination with carboplatin, and showed increased CD4 and CD8 T cells in the peritoneal cavity of mice, as well as decreased the number of suppressive cells such as Tregs and MDSCs (Zhu et al., 2018). One group that combined carboplatin, PD-1 antibody, and a 'Stimulator of Interferon Genes' (STING) agonist showed improved survival *versus* any single agent use (Ghaffari et al., 2018). Use of PD-1 blockade with paclitaxel is also a promising combination, as paclitaxel alone increased intratumoral PD-L1 expression in a mouse model of ovarian cancer (Peng et al., 2015). In fact, when the combination was employed, mice survived longer than with paclitaxel alone (Peng et al., 2015). The strategy of upregulating PD-L1 expression of tumor cells with chemotherapy prior to initiation of immunotherapy may be even more effective than using them concurrently. For this reason, further studies investigating the timing of immunotherapy with other treatments are warranted. PD-1 blockade has also been combined with trabectedin, which both has direct cytotoxic effects and has been shown to deplete tumor-associated macrophages (TAM) and myeloid derived suppressor cells (MDSCs) (Germano et al., 2013). This combination resulted in decreased tumor growth, increased CD4 and CD8 T cell populations in

Table 1
PD-1.

Year	First author	Model	Design	Results
PD-1/PD-L1 checkpoint blockade				
2011	Krempski	ID8 cells injected IP into C57BL/6 mice	PD-1 antibody	Enhanced T cell immunity, reduced tumor burden
2013	Wei	ID8 cells injected IP into C57BL/6 mice	Multiple antibodies tested alone and in combination (PD-1, CTLA4, TIM-3, LAG-3, CD40, CD137)	PD-1 alone ineffective, with CD137 abs double survival, increased T cells. Further improvement with cisplatin. Other combinations were less effective
2013	Abiko	HM-1 or ID8 injected IP into C57BL/6 mice	PD-L1 expression up-regulated or knocked out in tumor cells	Decreased tumor growth and prolonged survival with PD-L1 depleted cells
PD-1 combined with other checkpoint inhibitors				
2013	Duraiswamy	ID8-VEGF cells injected SC into C57BL/6 mice	PD-1, CTLA4 antibodies combined with cellular ID8 vaccine expressing GM-CSF	Reversal of CD8 T cell dysfunction and tumor rejection in 3/4 of mice
2013	Dai	ID8 cells injected IP into C57BL/6 mice	PD-1, CTLA4, and CD137 antibodies	Prolonged survival and improved immunity with triple ab, no difference with single ab
2017	Huang	IE9mp1 cells injected IP into C57BL/6 mice	PD-1, CTLA4, LAG-3 antibodies in various combinations	PD-1/CTLA4 or PD-1/CTLA4/LAG-3 combinations had the highest rate of tumor-free survival
PD-1 combined with chemotherapy				
2018	Zhu	ID8 cells injected IP into C57BL/6 mice	PD-1 antibody +/− carboplatin	Improved survival with combo/carbo alone along with increased tumor immunity
2018	Ghaffari	ID8-*Trp53*$^{-/-}$ cells injected IP into C57BL/6 mice	PD-1 antibody, STING agonist, carboplatin	Combination treatment resulted in prolonged survival, decreased tumor burden
2015	Peng	ID8 cells injected IP into C57BL/6 mice	PD-1/PD-L1 antibody + paclitaxel	Paclitaxel and PD-1/PD-L1 antibody prolonged survived better than paclitaxel alone, improved immune response
2015	Guo	ID8 cells injected IP into C57BL/6 mice	PD-1 antibody + Trabectedin	Strong antitumor response with combined therapy
PD-1 combined with oncolytic viruses				
2017	Liu	ID8-luc cells injected IP into C57BL/6 mice	Oncolytic vaccinia virus + PD-L1 antibody	Decreased tumor burden, improved survival, enhanced immunity
2018	Kowalsky	ID8-luc cells injected IP into C57BL/6 mice	Oncolytic virus expressing IL-15 + PD-1 antibody	Decreased tumor burden, improved survival
PD-1 combined with costimulatory molecules				
2014	Guo	ID8 cells injected IP into C57BL/6 mice	PD-1 antibody + OX40	Combined treatement inhibited tumor growth in 60% of mice. CD4/CD8 T cells increased, Tregs/MDSCs decreased
2014	Lu	ID8 cells injected IP into C57BL/6 mice	PD-1 antibody, GITR antibody, or combination. Also added cisplatin or paclitaxel	Combination with decreased tumor growth and increased immune response. Further improvement with chemo
2013	Duraiswamy	ID8 injected IP into C57BL/6 mice	PD-1/PD-L1, PD-L2 antibodies combined with cellular ID8 vaccine, α-4-1BB or TLR9 ligand	Rejection of ID8 tumors in 75% of mice, increased CD8+ T cells, decreased Tregs and MDSCs, generation of T memory cells

Table 2
CTLA4.

Year	First author	Model	Design	Results
2015	Wang	BR5FVB1-Akt cells injected SC or IP into FVB mice	CTLA4 antibody + decitabine	Increased cytokine production, improved survival
2015	Higuchi	BR5-Akt (BRCA1-deficient) or ID8 cells were injected IP or into ovarian bursa	PD-1/PD-L1 or CTLA4 antibodies + PARP inhibitor	CTLA4 but not PD-1/PDL-1 blockade synergized therapeutically with PARP inhibitor resulting in long term survival in majority of animals.

the peritoneum, and a change in genetic expression of the tumor microenvironment (TME) from a suppressive to a stimulatory state (Guo et al., 2015).

5.5. PD-1 combined with oncolytic viruses

Oncolytic viruses are another class of immunotherapy that work directly by selectively killing cancer calls by oncolysis, as well as indirectly by providing danger signals that activate the immune system. In a mouse model of ovarian cancer, an oncolytic pox virus induced PD-L1 expression on tumor cells. When a PD-L1 antibody was then added there were more tumoral CD4 and CD8 T cells and higher effector cytokine expression, as well as decreased MDSC, TAM, and Treg populations. Additionally, tumor burden was reduced and survival of these mice improved significantly (Liu et al., 2017). Another experiment used an oncolytic vaccinia virus that expressed superagonist IL-15, which is a combination of the immunostimulatory cytokine IL-15 along with the α subunit of IL-15 receptor. This combination prolongs the half-life and bio-availability of IL-15 *in vivo (*Van den Bergh et al., 2017*)*. When this virus was used in combination with PD-1 blockade it resulted in tumor regression and prolonged survival (Kowalsky et al., 2018).

5.6. PD-1 combined with costimulatory agents

Costimulatory biologics represent another class of agents that have been tested along with PD-1/PD-L1 inhibition, and evidence exists to suggest that these agents may further increase the potency of immune checkpoint blockade therapeutics. OX40 is a costimulatory molecule belonging to the tumor necrosis factor (TNF) receptor family that is expressed primarily on activated effector T cells and naïve regulatory T cells and can lead to T cell clonal expansion, activation, and cytokine expression. Combinations of an OX40 agonist antibody with PD-1 antibody resulted in decreased tumor growth, an increase in CD4 and CD8 T cells, and a decrease in Tregs and MDSCs in the peritoneal cavity (Guo et al., 2014). Glucocorticoid-induced TNFR related protein (GITR) is another costimulatory protein of the TNA family that has been shown to increase CD4 and CD8 T cell activation, expansion, and cytokine expression. When a PD-1 blocking antibody was combined with an antagonistic GITR antibody, mice had decreased tumor growth and prolonged survival. The TME again was shifted into an immunostimulatory state, with increased cytokine-expressing effector T cell and decreased frequency of Tregs and MDSCs (Lu et al., 2014). In this study cisplatin or paclitaxel chemotherapy was also added to the PD-1/GITR antibody combination with even further improvement in tumor-free long term survival. A third study combined PD-1 or PD-L1 blockade with the costimulants α-4-1BB (CD137), which is in the TNF receptor gene family, or an agonistic TLR9 ligand, and a vaccine from irradiated ID8 cells expressing GM-CSF or Flt3-ligand. Treated mice that had ID8 cells injected IP had tumor rejection rates of 75% (Duraiswamy et al., 2013b). This again was associated with reprogramming of the immune environment to a more stimulatory phenotype, characterized by increased antigen-specific CD8 T cells and effector cytokines, and downregulation of suppressive Tregs and MDSCs.

5.7. CTLA4

CTLA4 blockade has not been studied as thoroughly as PD-1 in preclinical ovarian cancer models, but there have been several studies that have evaluated its effectiveness. When tested alone, CTLA4 blockade has shown minimal activity in ovarian cancer models (Wei et al., 2013). When used in combination with other agents, however, anti-CTLA4 had improved anti-tumor activity. One drug that has been tried in combination with anti-CTLA4 is decitabine, which is in the class of DNA methyl transferase inhibitor drugs that can alter the expression of immunostimulatory genes. When combined with CTLA4 antibodies to treat a syngeneic model of ovarian cancer using BR5FVB1-Akt tumor

cells, mice had an increased survival that was associated with heightened percentages of memory T cells in peritoneal fluid and increased production of effector cytokines by CD8 T cells and NK cells (Table 2) (Wang et al., 2015). In another experiment using a BRCA1 knockout mouse model of ovarian cancer, the PARP inhibitor Veliparib was used in combination with CTLA4 antibodies, which resulted in prolonged survival compared to the PARP inhibitor alone. This effect was T cell-mediated and resulted in the establishment of immunologic memory (Higuchi et al., 2015). Other combinations using CTLA4 often also utilize PD-1/PD-L1 antibodies (Wei et al., 2013; Duraiswamy et al., 2013a; Dai et al., 2013; Huang et al., 2017), which were covered previously.

5.8. TIM-3

T cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a checkpoint inhibitor that plays a role in inhibiting Th1 responses and overexpression of this molecule correlates with T cell exhaustion (Das et al., 2017). Though blockade of TIM-3 is less potent than others that have been studied, it does have some activity in ovarian cancer models. When combined with CD137 it inhibited tumor growth and increased immune stimulation (Guo et al., 2013). However, when combined with PD-1 and CTLA4 antibodies in another study it had no effect on tumor growth compared to PD-1 and CTLA4 alone (Wei et al., 2013). While the activity seems modest, examination of the role of TIM-3 blockade in ovarian cancer has just begun. Further studies are required to establish if this treatment modality may be effective in this disease.

6. Conclusions

The use of checkpoint inhibitors in the treatment of ovarian cancer has been fairly promising in preclinical studies, particularly when combined with other checkpoint inhibitors, immunostimulatory molecules, or cytotoxic agents. PD-1/PD-L1 blockade has been the most thoroughly studied checkpoint inhibitor in mouse models of ovarian cancer and also has had the most positive results. The modest success that has been seen with checkpoint inhibitors in mouse models has yet to translate into the clinical realm, however, with minimal or no benefit seen in several clinical trials. Though this is likely in part due to biologic differences between preclinical models and human patients, it may also be due to differences in treatment regimens. Early clinical studies have included only patients with recurrent cancer and have given checkpoint inhibitors as single agent treatment, while preclinical data suggest that primary treatment with a checkpoint inhibitor in combination with other agents would be a more successful strategy. After these initial failures, more sophisticated therapeutic combinations are currently under investigation in clinical trials. Additionally, the timing of immunotherapy is likely an important factor, as it would be preferable if administration coincided with the maximum concentration of TILs in the TME. In fact, evaluation of the intratumoral immune response to priming agents such as chemotherapy, PARP inhibitors, or angiogenesis inhibitors may be an effective way to predict who might respond to immunotherapy. As we continue to elucidate the role of checkpoint inhibition in the treatment of ovarian cancer, preclinical results should continue to guide clinical trial design to determine when and how checkpoint inhibitors should be given for maximum clinical benefit. However, the preclinical data is extremely limited thus far, and further exploration of novel combinations of immune checkpoint inhibitors with other therapeutic modalities, alterations in timing, dose, and route of administration, and optimization of those protocols that show benefit are required.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

Author contributions

DWD was responsible for reviewing primary sources and initial manuscript development. LAN and RCA were responsible for revisions and key oversight of the project.

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