Improving Outcomes through Immune System Modulation in the Treatment of Gynecological Malignancies

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Abstract

The treatment of gynecological cancers continues to evolve with combination of different therapies. There has been a significant effort to induce stimulation of the immune system through treatment with interferons and interleukins in the past. More recently, the remarkable results of clinical trials demonstrating efficacy of checkpoint inhibitor immunotherapies in multiple cancer types has generated considerable interest within the gynecological community. Here, we review the findings of efforts to augment the humoral immune system and review the pre-clinical and clinical evidence for checkpoint inhibitors. The Abscopal effect, a phenomenon whereby localized radiation therapy results in immune mediated tumor regression in distant sites is currently also discussed in the context of gynecological cancers. The combination of various immunotherapies in gynecological cancer and emerging clinical evidence for the combinations may lead to improved treatment outcomes.

Keywords: Gynecological cancers; Tumor; Immunotherapies; Immune system; Malignancies

Overview

There have been significant improvements in the treatment of gynecological malignancies in the recent past owning to advances in surgical techniques, radiation treatment and chemotherapy delivery. While the backbone of systemic treatment for gynecological malignancies has focused on cytotoxic treatments to cancer cells, investigations have been on-going to determine whether incorporation of strategies to alter tumor-host interaction can improve on our current clinical outcomes. One clinically relevant example of this was incorporation of anti-angiogenic agents to alter the tumor microenvironment. Another aspect of tumor-host interaction that is actively being explored both in gynecologic malignancies, as well as cancer treatment in general, is the interaction between tumor and the immune system. There has been a recent flurry of excitement over the prospect of altering the immune system to recognize and aid in cancer treatment. With a focus on gynecological malignancies, we will 1) review early efforts to harness the immune system for treatment, 2) discuss findings from the early 2000's focusing on more targeted immune therapies and 3) present recent findings on the use of antigen specific therapies and discuss current and potential avenues of incorporation of these therapies.

Non-Specific Immune System Modulators

Initial efforts to harness the effect of the immune system focused on the use of cytokines that were isolated from endogenous sources and found to alter the activation of the immune system. Cytokines are soluble secreted proteins that alter various innate and adaptive immune cell properties and can be released by immune and non-immune cells. Within gynecologic malignancies, multiple endogenous cytokines have been explored, both in the pre-clinical and clinic arena.

Interferons

Interferons can be divided into two major classes, i.e. Class I (alpha and beta) and Class II (gamma). The original function attributed to the IFN family was protection of naive cells from viral infection. Further work demonstrated that these cytokines play important roles linking and regulating adaptive and innate immunity. During characterization of their function, extracts containing type I IFNs significantly increased survival times in mice transplanted with syngeneic tumors [1,2]. They bind to a cell surface receptor, and activate signal transducer and activator of transcription (STAT) complexes, thereby playing a significant role in protecting normal cells causing the production of intracellular molecules, which in turn interfere with viral RNA and DNA production. Furthermore, they stimulate MHC 1

molecule expression on the cell surface, which can increase immunogenic response *via* cytotoxic T lymphocytes. Interferon gamma, on the other hand, is produced largely by T cells and NK cells, and acts through IFN gamma specific receptors, but also serves to activate the JAK-STAT cascade [3]. Clinical trials across a variety of sites have suggested the efficacy of interferons in malignancy.

Type I Interferons: Interferon alpha (IFN-α) was initially one of the more successful immunomodulatory drugs used in the treatment of cancer. Randomized clinical trials demonstrated that IFN-α reduces the recurrence of malignant melanoma following surgical excision of localized lymph node metastases [4-6]. IFN-α was initially found to be active against ovarian cancer *in vitro* [6] and subsequently reported to show clinical promise in small patient numbers with refractory disease [7,8].

Thereafter, IFN-α has been utilized in Phase I/II clinical trials in ovarian cancer. In early clinical studies by Berek et al., interferon alpha was utilized as an adjunct to cisplatin as an intraperitoneal salvage agent in women with advanced refractory epithelial ovarian cancer after primary systemic chemotherapy with a cisplatin based combination regimen. They enrolled 24 patients in a prospective non-randomized study with attempts at escalating cisplatin and interferon alpha dosage. Primary side-effects included malaise, fever, and other flu-like symptoms, as is often the case with interferon therapy [9]. On the basis of that study, a Gynecologic Oncology Group study was initiated for those with minimal residual evidence of ovarian cancer on second look laparotomy. Berek et al., enrolled 92 patients in a multi-institutional, prospective, Phase II study of 12 cycles of interferon alpha delivered intraperitoneally [10].

Interferon beta: IFN-β, while less well studied in the context of gynecological malignancies, has multifaceted antitumor properties, including enhancement of antitumor function of dendritic cells, macrophages, NK cells and T cells along with direct induction of apoptosis [11]. Initial phase I trials using recombinant systemic administration of IFN-β were limited by short half-life, toxicity and minimal efficacy [12,13]. Efforts to harness the properties of IFN-β culminated in identifying improved drug delivery mechanisms. One such method, using replication deficient adenovirus, demonstrated effective immune system activation and tumor regression with intracavitary delivery in mice harboring tumors [14]. These findings led to a phase I clinical trial for intrapleural adenovirus based delivery of human IFN-β for patients with malignant pleural effusions. Toxicity was minimal. While most of the patients had mesothelioma, a complete cytological and objective response was achieved in the peritoneum and pleura of a patient with platinum resistant recurrent ovarian cancer with one injection of adenovirus vector [15].

Type II Interferons: Interferon-γ (IFN-γ) is structurally unrelated to type I interferons and is produced by activated effector T cells and NK cells after recognition of a target and highlights the role that IFN-γ plays linking the adaptive and innate immune responses. Initial studies in gynecological malignancies focused on whether IFN-γ led to increased antigen presentation. In pre-clinical models, both *in vitro* and *in vivo*, IFN-γ administration to ovarian tumor cells led to upregulation

of HLA class I and Class II antigen presentation receptors [16]. Further work demonstrated that IFN-γ led to increased T cells migrating into tumors, suggestive of an increased invasion [17]. IFN-γ is known to have antiproliferative activity on ovarian cancer cells, *in vitro* [18,19]. When combined with cisplatin and doxorubicin, the effect of IFN-γ was synergistic [20].

Early clinical investigations with IFN-γ were performed in combination with chemotherapy or as intraperitoneal monotherapy. Clinical evidence for efficacy of IFN-γ in ovarian cancer treatment was initially suggested by Welander et al., in 1988, who reported on 14 patients treated with IFN-γ 1b for relapsed ovarian cancer, observing a response in 4 patients (29%) [21]. Subsequent early investigations with intraperitoneal IFN-γ in patients with residual or recurrent disease led to responses in a small but significant proportion of patients [22,23]. This led to a larger trial with 108 patients with residual ovarian cancer treated with intraperitoneal IFN-γ at second look laparotomy which documented a 23% complete response with 3 year survival of 62% in responders [24]. In an effort to combine the benefits of IFN-γ into multi agent regimens, a phase II trial of recurrent platinum sensitive ovarian and fallopian tube primary cancer, was conducted with the addition of GM-CSF and paclitaxel. On 54 evaluable patients, Schmeler et al., report a response rate of 56% with 9 (17%) completed responses [25].

This led to 2 large phase III trials testing IFN-γ in the initial treatment of ovarian cancer [26,27]. Addition of subcutaneous IFN-γ to cisplatin and cyclophosphamide led to a statistically significant increase in PFS from 17 to 48 months with a non-statistically significant increase in OS from 58% to 74% at 3 years. Patients who received IFN-γ had high mild flu like symptoms [26]. This trial was closed early with 148 patients because of a change to carboplatin and paclitaxel as the standard of care chemotherapy. A Phase I/II trial of IFN-γ with carboplatin and paclitaxel in newly diagnosed stage III/IV ovarian cancer with 34 patients did not observe dose limiting toxicities and had an overall response rate of 72% with a clinical complete response in 54% [28]. Thereafter, a randomized phase III trial of carboplatin and paclitaxel plus or minus subcutaneous IFN-γ enrolled 847 patients and was stopped early due to shorter OS in the patients receiving IFN-γ with a HR=1.45 and an increase in serious adverse events [27]. The differences between the above phase 3 trials highlight the interplay between immunomodulators and chemotherapy, as paclitaxel requires use of steroids, which are known to blunt the immune response.

Interleukins

Interleukins are cytokines secreted by immune cells which signal through cell surface receptors to other immune cells and lead to changes in the target cells properties and/or function. Interleukin-2 (IL-2) was the first interleukin to be extensively characterized and was initially isolated after the discovery that leukocyte stimulated media promoted subsequent lymphocyte proliferation. Further investigation identified IL-2 as an important mediator of proliferation and differentiation of Natural Killer cells (NK cells) and plays a crucial role regulating T cell effector functions, such as cytokine production and cytolytic activity. After the isolation of individual or subpopulations of

cytokines, they quickly became an attractive target for use in the treatment of cancer.

IL-2 is a growth factor responsible for the clonogenic expansion of antigen-stimulated T lymphocytes and was the first interleukin approved by the FDA for treating metastatic melanoma and metastatic renal cell carcinoma and has shown durable complete responses lasting many years in a small percentage of patients. In the initial phase II clinical trial, 255 patients with metastatic renal cell carcinoma received multiple cycles of which resulted in 7% complete responders and a 15% overall response rate [28,29]. IL-2 has been broadly studied in treating cancer with chemotherapeutic agents (cisplatin and dacarbazine) [30], other cytokines (i.e., IFN-a, TNF), monoclonal antibodies (antiCTLA4, Ipilimumab, see below) [31] and as a monotherapy. With the further identification of specific Interleukins, efforts to take advantage of their specific properties were also investigated in the sole and combinatorial treatment of cancer. Here, we will focus on the use of Interleukin 2 and other Interleukins trialed in gynecological malignancies.

Pre-clinical studies suggested that cytotoxic NK and T cells exhibit deficient function within ovarian tumors [32]. IL-2, when added to cell culture experiments, led to the activation of lymphocytes and cytotoxic killing of ovarian cancer cells [33,34]. Thereafter a clinical trial of intraperitoneal IL-2 was completed in patients with recurrent or persistent peritoneal ovarian cancer. 41 patients had weekly or 7 day infusions of IL-2. Of 35 assessable patients, there were 6 laparotomy-confirmed complete responses and 3 partial responses [35]. In another phase II study, 65 patients were treated with IL-2 and oral retinoic acid after the completion of chemotherapy for ovarian cancer. IL-s improved NK and T cell counts and led to a decrease in VEGF serum levels. Five year PFS and OS were 29% and 38%, respectively, and statistically significantly improved compared to matched control patients [36,37]. Peripheral blood T-cell functional suppression prior to therapy, as measured by low expression of CD3-zeta chain, predicted for poor response to IL-2 therapy [38]. Regulatory T cells (Tregs) are also activated with IL-2 treatment, activating their suppressor function, and their infiltration into ovarian tumors has been shown to be a negative prognostic factor [39]. After IL-2 was withdrawn, those patients who experienced a clinical response were observed to also have decreases in their Tregs, suggesting that IL-2 works through activation of pre-treatment anti-tumor T-cells [40].

The use of IL-2, however, has limitations related to its activation of the immune system. It is typically used at MTD, which induces a systemic inflammatory response and resulting organ toxicity and capillary leak syndrome [41]. Therefore, alternate cytokines that activate effector T cells without promoting Treg cells may have an improved therapeutic function. Possible alternatives to IL-2 include IL-7, IL-12, IL-15, IL-18 and IL-21, some of which have been used in the context of gynecological malignancies.

IL-18 enhances cellular immunity through activation of key immune effector cells including T lymphocytes and NK cells as well as directing infiltration of these cell types in tumors in preclinical models [42]. Using a mouse model of ovarian cancer, in combination with pegylated liposomal doxorubicin, IL-18 treatment led to antitumor activity and generated long-term protective immunity [43]. Recently, Simpkins et al., reported on a phase I trial using recombinant IL-18 with pegylated liposomal doxorubicin in recurrent ovarian cancer. 16 patients received increasing doses of IL-18 which led to upregulation of Th1 cytokines and modest response while demonstrating an acceptable safety profile with plans for a phase II trial to determine efficacy [44].

IL-12 and IL-21 have also been shown to elicit an antitumor activity in animal based tumor models and have been studied in early clinical trials. IL-12 exerts effective anti-metastatic and antitumor effects in murine tumor models and *in vitro* and *in vivo* human tumor cells systems [45]. A phase I trial of refractory ovarian cancer patients were treated with IL-12 enrolled 28 patients and reported partial response in one (3.8%) patient and stable disease in 13 (50%) or patients with tolerable toxicity [46]. IL-21 has been shown to enhance CD8^{+} T and NK cell cytotoxicity, inhibit the Treg-mediated suppression, and induce antibody production [47]. In ovarian cancer, preclinical data using mesenchymal stem cells to deliver IL-21 in ovarian mouse models led to increased immune system activation and delayed tumor growth and prolonged survival [48].

In patients with cervical intraepithelial neoplasia (CIN) and cervical cancer, administration of IL-12 and IL-21 to isolated peripheral blood mononuclear cells isolated led to increased cytotoxicity to cervical cancer cells [49]. Also highlighting the potential of IL-12 in cervical cancer, 10 patients with CIN without prior treatment were given intralesional IFN-α and those with increase in serum concentrations of IL-12 correlated with good pathological responses [50]. These findings highlight that the use of interleukins could provide potential benefits in multiple gynecological malignancies.

While the majority of combinatorial approaches using interferons have used systemic agents, IL-24 (also known as melanoma differentiation associated gene-7) has been shown to radiosensitize various cancer cells [51]. Using adenovirus mediated delivery in pre-clinical models, IL-24 has been shown to induce ovarian cancer cell apoptosis [52] and displays radiosensitization properties when combined with ionizing radiation in ovarian cancer cells [53]. IL-24 exerts a tumor suppressor activity on multiple cancer types and, in conjunction with cisplatin, also inhibits cervical cancer growth in mouse models [54]. The precise understanding of IL-24 function requires further investigation, and clinical efficacy remains to be determined within gynecological malignancies.

Targeted Therapies

Checkpoint inhibitors

Findings from studies with cytokines, as described above, set the stage for implicating the adaptive immune response in controlling outgrowth of neoplastic transformation [55]. T-cell receptor recognition by cognate antigen presented on MHC molecules triggers T-cell activation. In addition to this T-cell clonal specific interaction, T-cell activation is regulated by

complex co-stimulatory (CD28 and ICOS) and inhibitory (CTLA-4, PD-1 and BTLA) signals downstream of the family of CD28 receptors. Produced by CD8^{+} T-cells, CTLA4 competes with CD28 to bind either CD80 or CD86 on antigen-presenting cells (APCs). Endogenously, this prevents detrimental over-activation of cytotoxic T cells. Similarly, The PD-1 receptor-ligand and PD-1 interaction is endogenous functions to down-regulate excessive immune system responses. Both of these pathways are hijacked by tumor cells to decrease cancer cell recognition by the endogenous immune system.

This ability of cancer cells to evade immune system function led to the development and trial of monoclonal antibodies against to disrupt the CTLA-4 and PD-1 inhibitory pathways. Here, we will review the initial clinical trials demonstrating efficacy and highlight pre-clinical and clinical attempts to harness their function in gynecological malignancies.

CTLA-4

CTLA-4 was the first endogenous protein discovered to inhibit T-cell stimulation. Allison et al. generated antibodies to block CTLA4 signaling and demonstrated tumor rejection *in vivo* [56]. Their work led to the development of humanized antibodies against CTLA4 (ipilimumab, tremelimumab). In a phase III trial, ipilimumab administration in patients with advanced-stage melanoma had improved overall survival (10.1 months), as compared to those treated with gp-100 vaccine (6.4 months) (p=0.003) [57]. Since ipilimumab was shown to have clinical efficacy in metastatic melanoma, pre-clinical experiments and clinical trials have been undertaken in many cancer types with promising results [58].

In gynecologic malignancies, however, to date there is little evidence of clinical efficacy of blockade of CTLA-4. In an investigation of ovarian cancer patients treated with ipilimumab, significant clinical response was seen in a minority of patients. Initially, a single infusion of ipilimumab was administered to 2 women, which was well tolerated and triggered a decrease or stabilization of CA-125 that lasted several months [59]. Nine additional women were treated with ipilimumab to define to toxicity and anti-tumor effects. As was the case with the initial melanoma patients, all patients had previously received GM-CSF modified irradiated autologous tumor cells vaccine. Two cases of grade 3 inflammatory gastrointestinal toxicity were observed manifested by significant diarrhoea. One patient achieved a significant decrease in CA-125 levels several months after the initial dose of ipilimumab. Although the response was not sustained, a second treatment led to a more prompt decrease in CA-125 levels. In their report, Hodi et al., report continued treatment with 9 infusions over the following four years with maintained disease control. This patient also had radiographic response with regression of a large hepatic mass and mesenteric lymphadenopathy. Three additional patients were reported to have stable disease for several months in the absence of serious toxicities [60]. Of note, tumor regression was correlated with an increase in the CD8^{+}/Treg ratio, which suggests other forms of therapy to deplete Tregs may prove to be effective treatments.

CTLA-4 and CD28 are also implicated in cervical cancer. In 22 patients with advanced cervical cancer, peripheral blood T cells had increased proportions of CTLA-4 expression than controls [61]. Additionally, CTLA-4 inhibition with ipilimumab is currently under clinical investigation for the treatment of recurrent cervical cancer in a phase I/II trial.

PD1/PD-L1

Identification and characterization of programmed death-1 (PD-1, CD279) and its ligand PD-L1 (CD274) led to additional immunomodulatory targets. PD-1 is expressed principally on T cells and is an inducible suppressor of T-cell activity. PD-L1 is expressed on both APCs and T cells and binding of PD-1 by PD-L1 leads to an inhibitory signal in activated T cells and promotes T-cell anergy and apoptosis [62,63]. Similar to targeting of CTLA-4, monoclonal antibodies targeting PD-1 and PD-L1 induce immunologically mediated tumor regression and prolonged disease stabilization in malignancies, including tumors thought not to be sensitive to immunotherapy like non–small cell lung cancer [64,65]. These findings have led to the broadening of studies which target inhibition of PD-1 and PD-L1 in additional cancers, including gynecological malignancies.

In ovarian cancer, Curiel et al., removed dendritic cells from ascitic fluid and tumor-draining lymph nodes and observed high expression of PD-L1, compared to controls. Treatment with an anti-PD-L1 antibody resulted in T cell activation with increased expression of IFN-γ, IL-2 and IL-12 and a down-regulation of IL-10 [66]. In murine models, up-regulation of PD-L1 and PD-1 on dendritic cells was observed in ovarian cancer, suggesting that PDL1/PD-1 may be responsible for T cell anergy [62]. PD-1 blockade acts synergistically with triggering of OX40, a co-stimulatory receptor, to protect against growth in a mouse ovarian cancer model [67]. In a syngeneic mouse model, PD-1 ligand expression correlated with T cell exhaustion and blockade yielded increased T cell proliferation, inhibition of Tregs and generation of precursor memory T cells [68]. Abiko et al., have shown a direct effect in murine ovarian carcinoma cells of increasing PD-L1 leading to suppression of the CD8 T cell response [69]. Similarly, another report shows that CD8 T cells have enhanced activity with PD-1 blockade in both mouse models of ovarian and colon cancer. In addition to activation of CD8 T cells, PD-1 blockade resulted in attenuation of Treg-mediated suppression. PD-L1 was found to be highly expressed on monocytes and T cells from patients with advanced ovarian cancer, as compared to patients with earlier stage disease. *Ex vivo* studies on these samples also demonstrate suppression of T cell responses [70].

There is also early evidence for a role of PD-1/PD-L1 in cervical cancer. PD-1 was demonstrated to be expressed on more than 50% of the infiltrative CD8 T cells in a cohort of 115 cervical cancer patients *via* immunohistochemistry [71]. In a study by Yang et al., PD1 and PD-L1 in cervical intraepithelial neoplasia demonstrated increased expression of the inhibitory PD-1/PD-L1 signals which could contribute to progression to cervical cancer [72].

In a recent study presented at the ASCO Annual meeting, Nivolumab demonstrated encouraging results in gynecological cancers particularly cervical cancer, after first line of treatment, with manageable safety profile [73].

Oregovomab

Uses of monoclonal antibodies directed at CA-125 have also been tried in combination with chemotherapy. Oregovomab is an anti-CA-125 monoclonal antibody that forms complexes with circulating CA125 resulting in cross presentation of CA125 to T-cells [74]. Observations of enhanced humoral and cellular immune responses are associated with favorable clinical outcomes [75-77]. A phase III study failed to demonstrate a clinical benefit of oregovomab in post chemotherapy maintenance administration [77]. A concurrently completed randomized phase II adding oregovomab to carboplatinum/paclitaxel for advanced ovarian cancer demonstrated immune responses that were schedule dependent [78]. Like the findings with IFN-γ, these studies underscore the importance of the interaction between standard cytotoxic agents and immunotherapies.

Abscopal Effect

Radiation is traditionally regarded as a local therapy. Emerging evidence suggests there may be alternate pathways that are activated by radiation which affect systemic disease. While the previous sections have focused on the ability to use immune modulators alone or as an adjunct to Radiation, there is also some evidence to suggest radiation alone stimulates an immune response that can impact disease control.

Escalated immunologic responses have been demonstrated in murine experiments using high dose per fraction treatments [79,80]. Camphausen et al., showed that this effect may also be related to p53, and further revealed a dose dependency (10 Gy x 5 Fractions *vs.* 2 Gy x 12 Fractions) for the abscopal effect80. They also demonstrated that such a response at the distant sites is not related to weight loss, scatter dose, or irradiated circulating tumor cells [80].

Such effects have been reported in humans as well. Takaya et al. reported on a 69 year old female with metastatic cervical cancer to para-aortic lymph nodes that was treated palliatively to the pelvis, but was not on any systemic therapies. She was noted to have dramatic response in the primary as well as the para-aortic regions which were outside the field of radiation [81].

Conclusion

Over the past decade, our understanding of the immune system in tumorogenesis has paved the way for generation of novel therapeutic agents that can improve outcomes through enhanced activation of anti-tumor immune response. These novel therapies have demonstrated varying responses in gynecological malignancies, especially in ovarian cancer. Promising data with immune checkpoint inhibitors may allow for incorporation of these agents in combination with other treatment modalities and could lead to improvements in outcomes. With numerous immunotherapy drugs entering clinical practice at an accelerating rate, considerable work lies ahead in order to determine the most effective therapeutic combinations for tumor types and individual patients. The use of

radiotherapy for optimum activation of anti-tumor activity is an innovative concept, but much remains to be learned to understand the mechanism and ways to integrate tumor immunology with radiation biology. Such understanding will be critical to rationale development of combinatorial immunotherapies in the future.

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