Immunotherapy for Gynecologic Malignancies: The Way Forward

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Abstract

Further understanding of tumor immune escape mechanisms has allowed targeting of specific immunosuppressive pathways that are ubiquitous among different tumor types, thus allowing the treatment of gynecologic malignancies to benefit from basic science and clinical research established in other solid tumors. Discovery of novel inhibitors targeting tryptophan metabolism, various immune checkpoint T cell receptors and their corresponding ligands, as well as other immunomodulatory agents using viral proteins have created exciting new treatment possibilities that harness a patient's own immune system to better recognize tumor cells.

Background

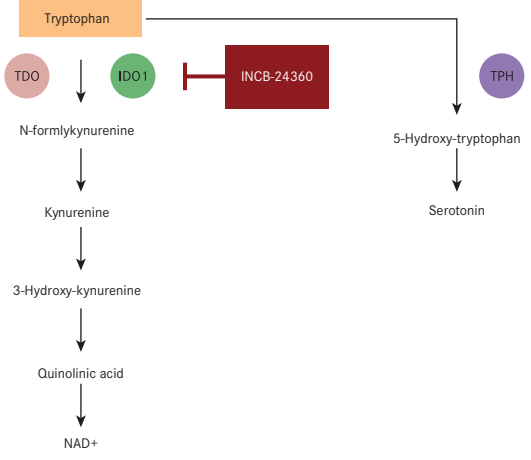
Recent advances in understanding the microenvironment of T cells and their intricate stimulatory and inhibitory interactions with other cells have allowed new immunomodulatory agents to be at the forefront of cancer therapy development. Until the last decade, most immunotherapeutic strategies had focused on stimulating immune effector cells with tumor-specific antigens or exogenous cytokines to activate the host's immune system with limited benefit. In contrast, a more recent understanding of tumor immune escape mechanisms has allowed targeting of specific immunosuppressive pathways that are often present among different tumor types. These new immunomodulatory agents have yielded durable results in preliminary clinical studies. These agents are potentially useful in malignancies not traditionally thought to be responsive to immunotherapy. This review describes new immune checkpoint pathway inhibitors and other compounds with novel mechanisms of action that have shown clinical activity and may serve as the basis for new combination strategies in the treatment of gynecologic cancers.

Historically, certain gynecologic malignancies such as epithelial ovarian cancers and human papilloma virus- (HPV-) associated cervical cancers have been considered immunogenic tumors.[1-3] In ovarian cancer, cytotoxic T cells have demonstrated antitumor activity, and the presence of tumor-infiltrating lymphocytes (TIL) have been associated with improved survival.[4-7] Despite encouraging laboratory findings, strategies to enhance antigen presentation to T cells with tumor-specific peptide vaccination, antigen-pulsed dendritic cells (DC), or antibodies targeting tumor antigens have been of limited clinical benefit.[8-10] Understanding the mechanisms of immune regulation are essential to understanding how tumors are able to escape the host immune surveillance.

Role of IDO1 in Immune Tolerance

One mechanism of immune tolerance involves indoleamine 2,3-dioxygenase-1 (IDO1), an intracellular enzyme that catalyzes the rate-limiting step in metabolizing tryptophan, an essential amino acid (**Figure 1**).[11] Prior to identifying IDO1, tryptophan 2,3-dioxygenase (TDO) was initially isolated in the liver and found to metabolize tryptophan.[11] TDO is a liver-specific enzyme that regulates dietary tryptophan catabolism. IDO1, on the other hand, remains absent or inactive in cells of the immune system until activated or induced in macrophages and specific DC subsets by cytokines, particularly interferon-gamma (IFN-ɣ).[12-15]

FIGURE 1. Overview of Tryptophan Biochemistry



Tryptophan concentrations are regulated by various enzymes including tryptophan 2,3-dioxygenase (TDO), a liver-specific enzyme that metabolizes dietary tryptophan, and tryptophan hydroxylase (TPH), an enzyme that metabolizes tryptophan in a separate pathway leading to serotonin production. Indoleamine 2,3-dioxyogenase 1 (IDO1) is an intracellular enzyme in cells of the immune system that catalyzes the rate-limiting step of tryptophan metabolism. Immune suppression occurs when local tryptophan concentrations decreases and immunosuppressive metabolites such as kynurenine accumulates in the microenvironment. INCB-24360 is an oral IDO1 inhibitor that is currently being investigated in various clinical trials.

IDO1: indoleamine 2,3-dioxygenase 1; TDO: tryptophan 2,3-dioxygenase; TPH: tryptophan hydroxylase; INCB-24360: oral inhibitor of IDO1.

Munn and colleagues demonstrated the biologic significance of the role of IDO1 in immune tolerance by demonstrating the fundamental importance of IDO1 for maternal-fetal immune tolerance in the placenta of pregnant mice.[16] The activity of IDO1 in depleting the local placental microenvironment of tryptophan is critical to establishing maternal T-cell tolerance of fetal alloantigens.

Additional studies showed that IDO1-induced immune suppression not only by depleting tryptophan from the local microenvironment but also by accumulating immunosuppressive metabolites such as kynurenine. T cells undergoing antigen-dependent activation are extremely sensitive to local tryptophan concentrations such that a decrease in tryptophan can lead to effector T-cell cycle arrest and anergy.[17] Downstream tryptophan metabolites, such as l-kynurenine, have also been shown to modulate natural killer (NK) cell cytotoxicity by selectively interfering with NK receptors and thereby modifying NK cell responses.[18]

Uyttenhove and associates first demonstrated that the expression of IDO1 in tumor cells allowed the malignant cells to resist host immune rejection by preventing activation of alloreactive T-cells.[19] Several studies further demonstrated that IDO1 expression is inversely correlated with the presence of TILs,[20,21] suggesting IDO1 expression may be associated with poor prognosis due to IDO1-mediated TIL and/or NK suppression.

Increased IDO1 expression is associated with poor clinical outcomes in multiple solid tumors including melanoma, renal cell, colon, pancreatic, hepatic, and squamous cell carcinomas.[21-28] Increased IDO1 expression in tumors of patients with gynecologic cancers has been correlated with a worse prognosis compared to those patients whose tumors have limited or negative IDO1 expression. Overexpression of IDO1 in patients with serous ovarian tumors has been correlated with paclitaxel resistance and poor survival outcomes.[29,30] High IDO1 expression in one study was found in over 70% of patients with stage II-IV disease and was significantly correlated with low intratumoral CD8+ TILs.[31] Additionally, in vitro studies have shown that IDO1-expressing ovarian cancer cells suppress T-cell proliferation.[32] Increased IDO1 expression was correlated with a poor prognosis in endometrial and cervical cancer patients as well.[20,33-35] Taken together, these findings as well as similar findings in other solid tumors suggest that IDO1 plays a key role in creating an immunosuppressive microenvironment potentially tolerant to tumors.[36]

Preclinical mouse studies demonstrated IDO1 inhibitors could slow tumor growth and potentiate cancer chemotherapy.[31,37] Inaba and co-investigators showed increased peritoneal metastases in mice bearing IDO1 transfected SKOV3 ovarian cancer xenografts compared to control mice bearing IDO1 negative xenografts. Administering an oral IDO1 inhibitor, 1-methyltryptophan (1-MT), abrogated the effect. Additionally, prolonged survival was found when IDO1 inhibition was combined with chemotherapy compared to chemotherapy alone.[31]

A significantly more potent IDO1 inhibitor, INCB-24360, was investigated in a phase I study in patients with advanced malignancies; results were presented at the American Society of Clinical Oncology (ASCO) 2013 Annual Meeting.[38] Most of the enrolled patients had colorectal (55.8%) cancer or melanoma (13.5%). Although no patients experienced a complete or partial response, 15 patients experienced stable disease for at least 8 weeks, and 8 patients experienced stable disease for at least 16 weeks. In 10 patients, the duration of INCB-24360 stable response exceeded that of their last prior therapy, including ipilimumab in 2 patients with melanoma. In this phase I study, the maximum tolerated dose (MTD) was not obtained; however, doses of ≥300mg twice daily were able to inhibit IDO1 activity by more than 90% at all time points and were found to effectively normalize kynurenine plasma concentrations. Common adverse events (AEs) were grade 1-2 fatigue and gastrointestinal disturbances. Two patients had grade 3-4 ALT or AST elevations that did not appear to be dose related. The recommended phase II dosage is 600 mg orally twice daily.

There currently is an ongoing trial in women with ovarian, fallopian tube, or primary peritoneal cancer who have had a biochemical recurrence defined as two successively increasing CA 125 values that are greater than the upper limit of normal and without evidence of disease by RECIST 1.1 (NCT01685255). These patients are being randomized in a 1:1 fashion to receive oral medications INCB-24360 (600 mg) or tamoxifen (20 mg) twice daily.

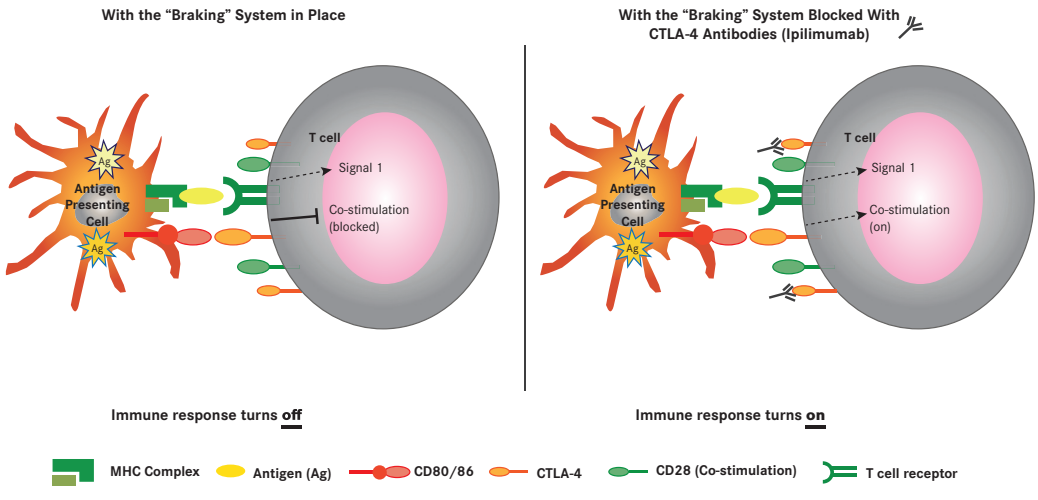
Targeting CTLA-4

Immunotherapy involving T cells provides long-lasting tumor responses in patients with melanoma.[39] However, less than 20% of patients achieve an objective response, and the addition of cytokine-based treatments were found to either increase the toxicity profile or to not be effective. To generate antitumor responses, T cells must be both specific for cancer cell antigens and have the potential to exert effector activity. Thus, in addition to T cell receptor (TCR) recognition of specific tumor antigens, a second costimulatory signal, such as the one between receptor CD28 and B7 ligand, is needed for full activation of T cells.[40] This costimulation is tightly regulated through specific stimulatory and inhibitory receptor-ligand relationships. Recently, several inhibitory receptors and ligands found on antigen presenting cells (APCs), T cells, and tumor cells have been identified as targets for cancer immunotherapy, as they play critical roles in immune suppression within the tumor microenvironment.[41]

These novel immunotherapy strategies targeting negative regulatory pathways in T-cell activation are considered immune checkpoint inhibitors. These checkpoint inhibitors interfere with endogenous T-cell regulation in order to prevent the development of immune tolerance to tumors. Ipilimumab was the first immune checkpoint inhibitor that the US Food and Drug Administration (FDA) approved for clinical use. Ipilimumab, a human monoclonal IgG1 antibody, binds and blocks inhibitory signaling mediated by cytotoxic T-lymphocyte antigen-4 (CTLA-4) found on T-cell surfaces (**Figure 2**).[42] As the mechanism of

action is not specific to one tumor type and because preclinical data support immunotherapy as a potential treatment for various malignancies, ipilimumab is actively being investigated as a treatment option for patients with prostate, breast, renal, and lung cancers, in addition to other tumor types including cervical cancer.[43,44]

FIGURE 2. CTLA-4 and Ipilimumab



Normally, cytotoxic T-lymphocyte antigen-4 (CTLA-4) provides a T-cell "braking" system that initially mutes activating antigen signals via CD80/86. Ipilimumab, a fully humanized monoclonal antibody against CTLA-4, binds CTLA-4, thus allowing the co-stimulatory molecule CD28 to continue biding to CD80/86. This results in having an immune response left in the active state.

CTLA-4 is a critical negative regulator of early T-cell expansion, opposing the actions of CD28 receptor co-activation when bound to B7-1 (CD80) and B7-2 (CD86) ligands (anergy).[45] CTLA-4 induces inhibitory downstream T-cell receptor signaling while also upregulating CTLA-4 expression and competitively inhibiting CD28 co-activation.[46] CTLA-4 is also expressed on CD25+FOXP3+ T regulatory cells (T_{reg}) and is important to T_{reg} function.[47] CTLA-4 interactions occur more centrally at an earlier step of interaction between T cells and APCs in lymphatic tissue. Specifically, CTLA-4 blockade with ipilimumab leads to T-cell activation and intratumoral T_{reg} depletion.[47]

In 2010, Hodi and co-investigators reported a landmark phase III trial in patients with recurrent unresectable melanoma. Patients were randomized to ipilimumab 3 mg/kg with or without gp100 peptide vaccine versus gp100 peptide vaccine alone. Although all patients received prior treatment, patients who received the ipilimumab with or without gp100 had a significant overall survival (OS) advantage of approximately 3.7 months (10.1, 10.2 months, respectively) to those in the gp100 control arm (6.4 months, hazard ratio [HR] = 0.68, P <.001).[48]

A subsequent phase III trial in patients with untreated melanoma comparing dacarbazine +/- ipilimumab showed improved OS in the regimen containing ipilimumab. Increased liver toxicity was noted, potentially due to additive or synergistic enhancement of known single-agent hepatotoxicity for each drug. The median OS in the ipilimumab-dacarbazine group was 11.2 months compared with 9.1 months in the dacarbazine-placebo patients (HR = 0.72, P = .006).[49] One of the impressive findings of CTLA-4 blockade has been the durability of objective tumor responses that are found in approximately 10% of patients with melanoma. Monoclonal antibodies targeting programmed death protein-1 (PD-1) and programmed death ligand-1 (PD-L1), which are earlier in their development, seem to follow a similar pattern (discussed in the next section).

The most frequently reported AEs associated with treatment with ipilimumab were immune-related, grade 1-2, and primarily affected the skin (pruritis, rash), and gastrointestinal tract (diarrhea, nausea, vomiting, and colitis). A dose-dependent increase in immune-related AEs of any grade was seen with increasing dosages of ipilimumab. In the study by Hodi and colleagues, grade 3 or 4 immune-related AEs occurred in 10% to 15% of patients who received ipilimumab and resolved over a median time of 4.9 weeks (95% CI, 3.1 to 6.4 weeks).[48] It is important to also be aware of endocrinopathies such as thyroiditis and hypophysitis that can develop when treating with immunotherapy agents. Most high-grade AEs were able to be medically treated and resolved in approximately 4 weeks.

GOG 9929 is an ongoing phase I study investigating the use of ipilimumab for the primary treatment of high-risk cervical cancer after chemoradiation (NCT01711515). Patients must have squamous, adenosquamous, or adenocarcinoma histology and at least stage IB2 or IIA disease with positive para-aortic lymph nodes or stage IIB and higher with positive pelvic and/or para-aortic lymph nodes. HPV contains immunogenic viral E6 and E7 oncogenic proteins that are capable of inducing an immune response in most immunocompetent patients. However, failure to generate an effective immune response in some women facilitates HPV persistence, which ultimately increases the risk of cervical cancer development.[50,51] After enrolled patients are treated with pelvic and extended field radiation with concurrent cisplatin (40 mg/m^2) weekly and intracavitary brachytherapy, dif-

ferent cohorts then receive increasing doses of ipilimumab to determine the MTD, feasibility of treatment, dose-limiting toxicities, and disease outcomes. A subcohort of patients will continue to be treated with an extended regimen for an extra 48 weeks, with a dose given every 12 weeks for 4 weeks. The underlying hypothesis of the trial is that chemoradiation would induce an antigen release in patients whose immune response would be boosted by receiving ipilimumab. There is a second National Cancer Institute (NCI) trial that involves administering single agent ipilimumab in patients with metastatic or recurrent HPV-related cervical cancer (NCT01693783).

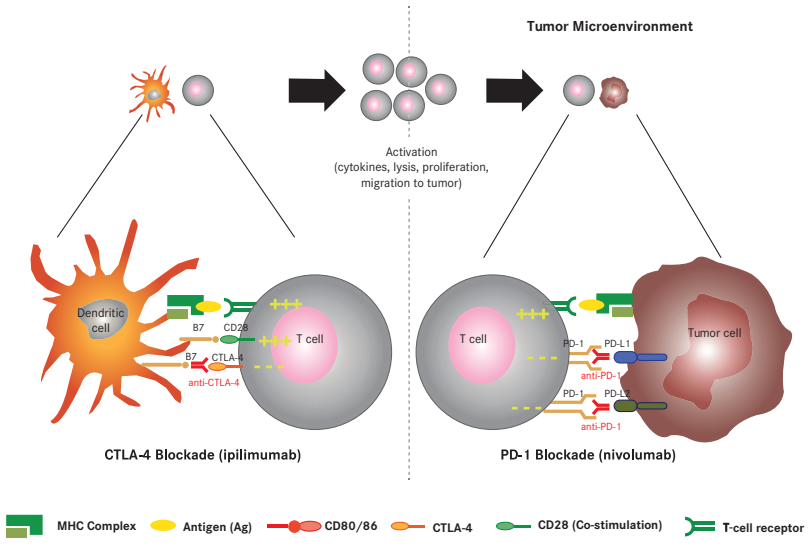
Anti-PD-1/Anti-PD-L1 Inhibitors

While CTLA-4 is involved in early T-cell activation in lymphatic tissues, PD-1 receptor signaling functions in regulating T cell activation in peripheral tissues (**Figure 3**). PD-1 is an immunoinhibitory receptor expressed on numerous cell types that have had long-term exposure to antigens including activated T cells, T_{regs}, activated B cells and NK cells (**Figure 4**). The primary ligand of PD-1, PD-L1 (also known as B7-H1 or CD274), is frequently expressed within the tumor microenvironment, including tumor cells and tumor-infiltrating macrophages. PD-1/PD-L1 interactions decrease the risk of collateral tissue damage by activated T cells.[52,53] On the other hand, PD-L2 (also known as B7-DC or CD273) is the second ligand of the PD-1 receptor and is restricted largely to APCs.[54] The interaction of PD-1 with its two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), occurs mainly in peripheral tissues within the tumor microenvironment, which lead to apoptosis and downregulation of T-cell effector function.[55] Tumors expressing PD-L1/L2 have been found to suppress TILs by activating PD-1/PD-L1, L2 interactions.[56] Targeting these interactions with therapeutic antibodies against PD-1/PD-L1 enhanced the T-cell response and stimulated antitumor activity.[57]

The first anti-PD-1 inhibitor to be evaluated was nivolumab, a human IgG4 monoclonal inhibiting antibody directed against the PD-1 protein.[58] A phase I study in patients with selected advanced solid tumors showed that nivolumab was tolerable and effective with an objective response rate of 16% to 31% in heavily pretreated patients across diverse tumor types. Also notable was the durability of objective responses for >1 year after treatment. These results demonstrated that immunotherapy via PD-1 blockade could be expanded beyond targeting usual immunogenic tumor types, such as melanoma and renal cell cancer, to include treatment-refractory metastatic non-small cell lung cancer (NSCLC), particularly squamous cell carcinoma. These unexpected findings emphasized the possibility that any tumor type could be immunogenic with the appropriate immune activation.[58] Similar to findings with ipilimumab, some patients experienced apparent progression or stable disease prior to ultimately responding to therapy, and responses have been observed with re-induction therapy.[59] Measuring objective responses to immunotherapeutic agents has proved to be quite different from measuring responses to traditional cytotoxic chemotherapeutic agents. Thus, Wolchok and colleagues have summarized new immune-related response criteria that are considered an appropriate alternative to traditional methods for measuring objective responses mediated by these new immunomodulatory agents.[60]

Although AEs and efficacy with ipilimumab seem to be dose dependent, this correlation was not observed in patients treated with nivolumab, which may be explained by high receptor-antibody occupancy even at smaller doses.[61] In 2 clinical trials, common AEs associated with anti-PD-1 blockade included fatigue (30%), rash (21%), pruritus (21%), diarrhea (20%), and myalgia (12%), with some rare serious AEs including pneumonitis (4%) and interstitial nephritis (4%).[62,63]

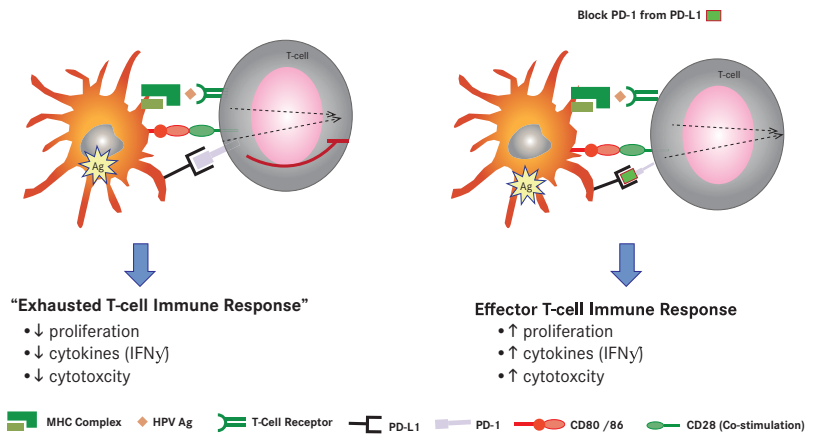
FIGURE 3. Simultaneous Inhibition of CTLA-4 and PD-1 Pathways



CTLA-4 acts to dampen the immune response at the level of the APC and the T-cell, thus decreasing the early activation of T-cells. PD-1, on the other hand, helps modulate T-cell activity in peripheral tissues via its interactions with PD-L1 and PD-L2. Since the two pathways act at different points in the immune response, therapeutically blocking both pathways with ipilimumab and nivolumab, monoclonal antibodies targeting CTLA-4 and PD-1 respectively, may have a synergistic effect.

Two anti-PD-L1 inhibitory antibodies, BMS-936559 and MPDL3280A, have been clinically investigated. These agents are thought to function by specifically blocking PD1/PD-L1 signaling. Unlike PD-1 antibodies, PD-L1 antibodies spare potential interactions between PD-L2 and PD-1, but additionally block interactions between PD-L1 and CD80.[64] The therapeutic significance of these particular interactions remains to be determined.

FIGURE 4. PD-1 and Anti-PD-1



Programmed death protein-1 (PD-1) is an immunoinhibitory receptor expressed on multiple immune cells, including activated T-cells, B-cells, NK cells, and regulatory T cells. By blocking the interaction between PD-1 and its primary ligand, PD-L1, T-cell receptor signaling is able to proceed with effector functions.

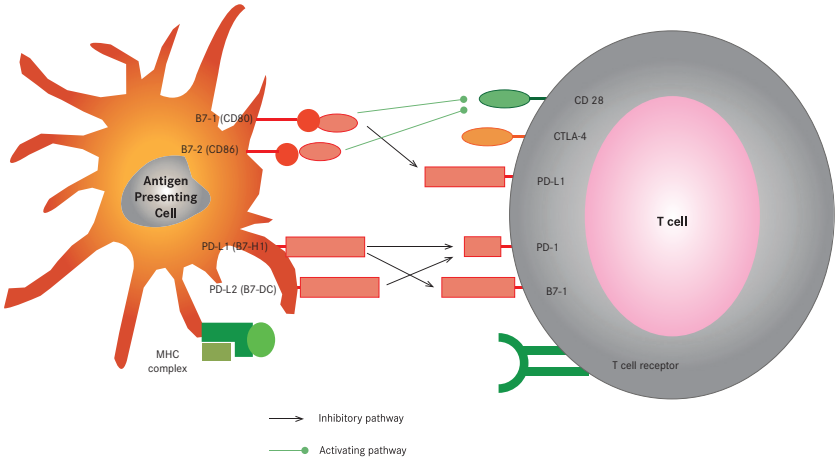
A multi-institutional phase I study showed that BMS-936559 was tolerable and clinically active across multiple advanced tumor types.[61] Blocking the immune inhibitory ligand PD-L1 with a monoclonal antibody produced both objective tumor regression with an objective response rate (ORR) of 6% to 17% and a durability of response across tumor types in these heavily pretreated patients. Anti-PD-L1 blockade generated 1 response in 17 (6%) enrolled patients with recurrent ovarian cancer. Also the 10% ORR was observed in patients with advanced NSCLC who received anti-PD-L1 therapy.

Although the 2 studies targeting anti-PD-1[58] and anti-PD-L1[61] are similar in patterns of clinical activity, the molecular interactions involved are not identical. For example, PD-L1 exerts inhibitory signals to T cells through PD-1 and B7-1.[65] Thus, an antibody that specifically blocks PD-1 would inhibit the interaction between PD-1 and its two ligands PD-L1 and PD-L2. However, PD-L1 would be able to send inhibitory signals through B7-1. In contrast, an antibody only directed at PD-L1 would block the inhibitory signals through PD-1 and B7-1, however, PD-L2 would still be available to bind to PD-1 (**Figure 5**). The latter interaction has been found to downregulate T cell responses in vitro and in vivo.[64,66,67] As immune checkpoint inhibitor research continues, the potential benefit of combining immunotherapeutic agents is being considered. Although monotherapeutic approaches to PD-1 blockade have shown some success, preclinical models indicate that combination therapies may generate greater clinical impact.[68,69] For example, when combining ipilimumab with nivolumab, more rapid and greater magnitude responses were seen in patients treated with the combination regimen compared to that seen with either agent alone with up to 15% grade 3 or 4 toxicity depending on the type of AE.[70]

Application of Lm-LLO for HPV-Associated Disease

HPV-associated cervical cancer is one of the most well-established associations in medicine where an infection with a virus is the cause of malignancy. Normal cell cycle regulation becomes disrupted when HPV oncoprotein E6 complexes with the tumor inhibitor gene p53, and HPV oncoprotein E7 complexes with the tumor suppressor protein retinoblastoma (pRb).[71] These events lead to genomic instability and subsequent neoplasia.[72] Immunologic activation of the HPV proteins expressed by transformed

FIGURE 5. Multiple Immunoinhibitory Receptor Interactions



Antigen presenting cells have complex interactions with T cells that include multiple immunoinhibitory receptor interactions. The various interactions between the programmed death protein-1 (PD-1) and its ligands PD-L1 and PD-L2 are illustrated. For example, PD-L1 exerts inhibitory signals to T cells through PD-1 and B7-1. Antibody blockade of PD-1 would only inhibit the interaction between PD-1 and PD-L1/L2. PD-L1 continues to be able to send inhibitory signals through B7-1. In contrast, antibody blockade of PD-L1 would inhibit the signals through PD-1 and B7-1; however, PD-L2 would still be available to bind to PD-1 thereby downregulating T cell responses.

TABLE. New Immune Checkpoint Pathways and Other Novel Mechanisms

Immunomodulating Target/ Pathway	Corresponding Target or Ligand	Mechanism of Action and Notable Features	Investigational Immunotherapy Agent Examples
IDO1 *Indoleamine 2,3-dioxygenase 1*	Tryptophan	• Catalyzes rate-limiting step of tryptophan metabolism and causes accumulation of immunosuppressive kynurenine metabolites in local microenvironment	INCB 24360 (IDO1 inhibitor)
CTLA-4 *Cytotoxic T lymphocyte-associated antigen-4*	B7-1 (CD80) B7-2 (CD86)	• Co-inhibitory molecule that binds to B7-1 and B7-2 with higher affinity and avidity than CD28 in order to downmodulate the early stages of central T-cell activation in lymphatic tissue • Member of Ig superfamily and expressed on surface of CD4+ T-cells and T_{regs}	Ipilimumab (Anti-CTLA-4 IgG1) Tremelimumab (Anti-CTLA-4 IgG2)
PD-1 *Programmed death protein-1*	PD-L1 (B7-H1 or CD274) PD-L2 (B7-DC or CD273)	• Cell surface protein that downregulates T-cell effector functions and suppresses TILs in peripheral tissue • Member of extended CD28 superfamily of T-cell regulators • Expressed innately during thymic development; inducibly expressed on peripheral CD4+ and CD8+ T-cells, B-cells, NK T-cells, monocytes, some DCs	Nivolumab (BMS 936558) (Anti-PD-1 IgG4) MDX1105 (BMS 936559) (Anti-PD-L1)
HPV16-E7 *Human Papilloma Virus 16 - E7 oncoprotein*	*Lm*-LLO-E7	• Bioengineered live attenuated *Listeria monocytogenes* (*Lm*) vector that secretes an antigen-adjuvant (*Lm*-LLO) fused to HPV16-E7 • *Lm*-LLO-E7 induces E7-specific cytotoxic T cells and mature dendritic cells while decreasing intratumoral regulatory T cells and inhibiting angiogenesis	ADXS11-001

Toxicity based on Wieberdink regional toxicity scale.[28]
CR, complete response; HILP, hyperthermic isolated limb perfusion; ILI, isolated limb infusion; LE; lower extremity; N/A, not applicable; TNF, tumor necrosis factor; UE, upper extremity.

cells have been associated with increased numbers of CD8+ T cells and a high ratio of CD8+ T cells to FOXp3+ T_{regs}.[73-75] A similar therapeutic change in the ratio of CD8+ TILs to T_{regs} has been seen after the administration of *Listeria monocytogenes* protein listeriolysin O (*Lm*-LLO) in various models.[76,77]

Studies have also demonstrated that bioengineered *Listeria monocytogenes* (*Lm*) is a potent vector in both infectious diseases and when applied to cancer immunotherapy.[78-80] *Lm*-LLO-E7 (ADXS11-001) is a live attenuated Lm-based immunotherapy agent for the treatment of HPV-associated diseases that secretes an antigen-adjuvant fusion (*Lm*-LLO) protein consisting of a truncated fragment of the *Lm*-LLO fused to HPV16-E7.[78] *Lm* stimulates innate immunity and infects APCs where stimulation of both the CD4+ and CD8+ lymphocytes occur. Immunization of mice with *Lm*-LLO-E7 induces regression of E7-expressing established tumors and confers long-term protection.[81] Additionally, *Lm*-LLO-E7 was able to overcome immunological tolerance and limit the development of autochthonous tumors in an E7 transgenic murine model.[82] The therapeutic efficacy of *Lm*-LLO-E7 is attributed to its ability to induce E7-specific cytotoxic T-lymphocytes and mature DCs while reducing the number of intratumoral T_{regs} and inhibiting tumor angiogenesis.[83]

A phase I study investigating the safety and feasibility of ADXS11-001 was performed in 15 patients with previously treated metastatic, recurrent, or refractory cervical carcinoma who had failed chemotherapy, radiotherapy and/or surgery.[84] Patients in the first 2 dose levels of 1 x 10^9 CFU and 3.3 x 10^9 CFU experienced a tolerable safety profile. Dose-limiting toxicities of grade 2 hypotension occurred in the 1 x 10^{10} CFU group within hours after receiving the *Lm*-LLO-E7 infusion, requiring therapeutic intervention and resulting in study discontinuation as per protocol. Patients were given ampicillin to clear the *Lm* vector. No patients manifested any serious symptoms of *Lm* infection. All 15 patients experienced AEs during the study with the most common being pyrexia (100%), vomiting (60%), chills (53%), headaches (53%), anemia (53%), nausea (47%), tachycardia (47%),

and musculoskeletal pain (28%). Six (40%) patients experienced grade 3 toxicities considered related to receiving *Lm*-LLO-E7: 3 (20%) were related to pyrexia, 2 (13%) had significant transaminitis, and 1 (7%) fatigue. These toxicities resolved in the first 12 hours after treatment. No drug-related grade 4 AE occurred. In this heavily pre-treated population, there was 1 (7%) patient who had a partial response and 7 (47%) patients who experienced stable disease. Additional phase II studies are currently active or will be initiated in the near future.

Conclusions

The future clinical application of immunotherapy in gynecologic malignancies is upon us. Several trials with immune checkpoint inhibitors should start late this year in ovarian and cervical cancers in response to the recent mass solicitation by the Cancer Therapy Evaluation Program (CTEP) of the NCI. With increased understanding of the tumor microenvironment and the complex immunoregulatory interactions between tumor cells and the host immune system, clinical trials involving immune checkpoint inhibitors and other immune regulating agents in gynecologic cancers are already under way (**Table**).

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