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PD-1/PDL-1 INHIBITORS AS IMMUNOTHERAPY FOR OVARIAN CANCER

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BACKGROUND

Programmed death 1 (PD-1) is a member of the B7-CD28 immunoglobulin (Ig) superfamily. It encodes a 55 kDa type I transmembrane monomeric glycoprotein that is expressed on activated T and B lymphocytes, natural killer (NK) cells as well as myeloid cells.1-5 Unlike other members of the B7-CD28 family, PD-1 is a monomeric glycoprotein lacking an extracellular cysteine which prohibits covalent dimers.6 Its extracellular region contains a single Ig-like variable IgV domain,7 while its cytoplasmic region consists of an immunoreceptor tyrosine-based inhibitory motif (ITIM).2,8 The ligands for PD-1 include PD-L1 (aka B7-H1 and CD274) and PD-L2 (aka B7-DC and CD273).9,10 PD-L1 expression is seen in normal tissues such as the heart, pancreas, placenta, vascular endothelium, liver, lung, and skin.9,10 Additionally, CD80 (B7-1) can act as receptor for PD-L1 and induces inhibitory T-cell signals when bound.11,12 PD-L2 also has a second receptor, repulsive guidance molecule b, which functions in respiratory immunity.13

PD-1 is structurally similar to another member of the B7 family, cytotoxic T lymphocytes-associated antigen 4 (CLTA-4), which binds B7-1 and B7-2 and is involved in maintenance of T-cell homeostasis. PD-1 ligation, like CLTA-4, is involved in inhibition of lymphocyte proliferation.9 In another similarity with CLTA-4, PD-1 is also expressed on regulatory T cells (Tregs) and helps to enhance and sustain their proliferation.13 Since PD-1 is expressed not only on activated T cells but also B cells and NK cells, blockade will lead to augmented effector T-cell activity in both the periphery as well as tumor microenvironment, increased NK cell activity in tumor or tissues, and increased antibody production.14,15

Unlike its name, PD-1 does not directly cause cell death. Rather, when bound to its ligands PD-1 inhibits T-cell signaling and cytokine production, as well as limits effector T-cell proliferation and increases their susceptibility to apoptosis.15 The role of PD-1 was initially evaluated by Nishimura et al. with deficient mice who were found to have consistently mild splenomegaly secondary to proliferation of lymphoid and myeloid cells, selected augmentation in IgG3 antibody response to type 2 T-independent antigen, as well as increased in vitro response to anti-IgM stimulation. All this suggested that PD-1 plays a negative regulatory role in the immune response.16 PD-1 engagement with its ligands directly inhibits TCR signaling via the Zap-70 and Ras pathways.17,18 Binding also has downstream effects on the PI3K pathway. Specifically, the cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) have
been shown to act as negative regulators of tyrosine kinase-based signaling pathways of immunological receptors. Phosphorylation of the ITIM and ITSM leads to recruitment of tyrosine phosphatases such as SHP-1 and SHP-2. These phosphatases lead to inactivation of PI3K/AKT and MAPK signaling pathways, therefore blocking cell-cycle progression in immune cells, impairing proliferation, blunting cytokine production, and increasing apoptosis.

Additionally, PD-1 limits the effector activity of T cells in peripheral tissues to prevent excessive inflammatory damage in the setting of infection and works to limit autoimmunity by inducing T-cell exhaustion. These effects of PD-1 were described in PD-1 deficient mice who developed various autoimmune syndromes depending on the strain of mouse. T-cell exhaustion was demonstrated in viral infection models, where high PD-1 expression was correlated with increased viremia due to the dysfunctional proliferation and cytokine secretion of T cells, which lead to an ineffective immune response. This early research showed that deficiency of PD-1 leads to dysfunction of peripheral self-tolerance at the T-cell level, which has since been shown to occur via inhibition of TCR, lymphocyte proliferation, and cytokine secretion.

**PD-1/PD-L1 IN CANCER**

Based on these initial findings, the role of PD-1/PD-L1 has been extensively studied in cancer immunology (Figure 10.1). PD-1 was initially found on many of the tumor infiltrating lymphocytes (TILs) in the tumor microenvironments of various types of cancer including melanoma and prostate. The ligands for PD-1 can be upregulated on the tumor cell surface of many different cancers as well as tumor-associated macrophages (TAMs), myeloid derived suppressor cells,

![Image: Diagram of PD-1/PD-L1 interaction in cancer immunotherapy]

**Figure 10.1** Anti-PD-1 receptor and anti-PD-L1/L2 antibodies as cancer immunotherapy. Antigen-presenting cells present antigens (Ag) released from cancer cells to T cells within the tumor microenvironment. Cancer cells can also present Ag directly to activated T cells through the use of the MHC. Upon T-cell activation, PD-1 receptors are expressed on T cells. Engagement with its ligands, PD-L1 and PD-L2 on APCs and PD-L1 on cancer cells, leads to inhibited immune responses. Blockade of the PD-1/PD-L1/PD-L2 pathway can enhance anti-tumor immunity.

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dendritic cells (DCs), T cells and B cells.\textsuperscript{32} Certain solid tumors have been shown to express PD-L1, including melanoma, lung, ovarian, breast, glioblastoma, esophagus, the gastrointestinal tract, and squamous cell carcinoma of head and neck.\textsuperscript{33-36} Meanwhile, PD-L2 is more commonly found to have increased expression on B cell lymphomas like primary mediastinal B cell lymphoma, follicular lymphoma, and Hodgkin lymphoma.\textsuperscript{37}

Iwai et al. first observed that PD-1 engagement with its ligand PD-L1 lead to inhibited anti-tumor cytolytic activity of CD8+ T-cells.\textsuperscript{38} Furthermore, they showed that deficient mice were unable to mount an immune response, which leads to tumor growth and metastatic suppression.\textsuperscript{39,40} Two different mechanisms have been described in which tumor cells use PD-1 and its ligands to evade the human immune system, known as the innate and adaptive immune resistance. Innate immune resistance relies on constitutive signaling of the upregulated PD-L1 expression on tumor cells independent of inflammatory or cytokine signals in tumor microenvironment.\textsuperscript{16} This signaling is conducted through the AKT and STAT3 pathways. This has been exhibited in glioblastomas which demonstrate increased expression in the setting of PTEN deletion, suggesting involvement of PI3K-AKT pathway.\textsuperscript{40} Another model has been shown with constitutive anaplastic lymphoma kinase signaling in lymphoma and lung cancer due to signal transduction and activation of STAT3.\textsuperscript{41} Amplification of JAK2 and 9p24.1 copy number variation has been seen in classical Hodgkin lymphoma and mediastinal large B cell lymphoma.\textsuperscript{42} There have been some reports of MAPK signaling pathway controlling PD-L1 expression in anaplastic large cell lymphoma and Hodgkin lymphoma; however, Atoui et al. did not identify this association or other mutations in PI3K/AKT pathways either.\textsuperscript{43}

Normally, certain cytokines, such as interferon-gamma (IFN-γ), are secreted in the setting of inflammation leading to PD-L1 expression as a negative feedback cycle to dampen the activity of PD-1+ T-cells. Tumors have been found to hijack this normal host negative feedback system which is physiologically used to prevent autoimmunity and protect peripheral tissue damage from inflammation by using similar mechanisms to protect itself from antitumor immune response. This is part of the adaptive immune response. PD-L1 is not automatically expressed, rather it is induced in response to inflammatory signals such as interferons, mainly IFN-γ, in the microenvironment. Taube et al. demonstrated that IFN-γ was only seen in setting of PD-L1+ but not in PD-L1−/− tumors, and more specifically it was seen at the junction of TILs and the PD-L1+ tumors. This suggests that TILs produce inflammatory and cytokine factors which upregulate PD-L1 expression likely as a negative feedback mechanism; however, this also inadvertently leads to decreased anti-tumor immunity.\textsuperscript{44} Other cytokines that have been suggested to be involved in this process include interleukin (IL)-2, IL-6, IL-7, IL-10, IL-15, IL-32γ, and common γ-chain cytokines,\textsuperscript{45-47} with the different cytokines inducing upregulation on distinct cell types. Additionally, hypoxia has been shown to induce PD-L1 expression via the hypoxia-inducible factor 1 alpha pathway.\textsuperscript{48}

**PD-L1 PROGNOSTIC VALUE IN CANCERS**

PD-L1 expression has varying prognostic values in different solid tumors. High expression is associated with improved survival in melanoma,\textsuperscript{49} merkel cell,\textsuperscript{50} breast,\textsuperscript{51} and cervical carcinomas\textsuperscript{52} as opposed to a poor prognosis seen in non-small cell lung cancer (NSCLC),\textsuperscript{53} renal cancers,\textsuperscript{54-58} esophageal,\textsuperscript{59} gastric,\textsuperscript{60} and bladder cancer.\textsuperscript{61} In an effort to predict response to PD-1/PD-L1 blockade, Teng et al. revised a prior classification system\textsuperscript{49} of the tumor microenvironment depending on TIL presence and PD-L1 expression in melanoma and suggested a treatment stratification based on this framework (Figure 10.2).\textsuperscript{44} TILs alone...
have been investigated as independent prognostic factors. In some studies, presence of TILs in patients with colorectal, ovarian, pancreatic, esophageal, and small-cell lung carcinoma were associated with a better prognosis, as opposed to TILs in patients with renal cell carcinoma, who were associated with a poor prognosis. Type 1 was classified as tumors exhibiting adaptive immune resistance with presence of TILs driving PD-L1 expression. These tumors are suggested to be the type most likely to respond to immune checkpoint inhibitor therapy as they already have TILs present in the microenvironment which have been inactivated by the PD-L1+ tumor cells. Therefore the use of checkpoint inhibitors would be able to reactivate those TILs which are already present and allow them to attack the tumor cells. Type 2 is represented by the absence of TILs and no PD-L1 expression, indicating an immunologic resistance. These tumors have been shown to have poor prognosis in melanoma and are unlikely to have a response to immune checkpoint blockade in the setting of the absence of TILs. However, combination therapy which could attract effector T-cells into the tumor might be successful as the anti-PD-1/PD-L1 therapy could then act on those newly present TILs. Some combination therapy to be considered in conjunction with an PD-1/PD-L1 inhibitor in these situations can include the use of vaccines to increase immunogenicity and recruit TILs, induction of type I IFN response, or CTLA-4 inhibitors. Type 3 includes intrinsic induction with lack of TILs but +PD-L1 expression via oncogenic signaling. These tumors are unlikely to have a response, highlighting that presence of PD-L1 alone cannot predict response to anti-PD-1/PD-L1 therapy as without the presence of inactivated TILs to reactivate, inhibition of PD-1/PD-L1 will not have an anti-tumor response. Therefore, these types of tumors also require enlisting lymphocytes in order to have clinical benefit. Type 4 exhibits presence of TILs but lack of PD-L1 expression leading to immune tolerance, which suggests a possible role of other suppressors driving tolerance. Therefore other immune checkpoint inhibitors might be successful in this situation.
However, this treatment stratification framework is not perfect, as some studies have shown that there is a role for anti-PD-1/L1 therapy even in the setting of PD-L1 negative tumors.63–72 Additionally, immunohistochemistry protocols are not standardized, therefore presence or absence of PD-1/PD-L1 is based on different stains and the analysis is somewhat subjective with different studies using different cutoffs73 and antibodies used in the various studies and a degree of intratumoral heterogeneity suggesting possible sampling bias. Despite these limitations and the fact that this framework was based on melanoma, this can help us better understand the tumor microenvironment and rationale for the use of anti-PD-1/PD-L1 therapies and combination therapies in ovarian cancer.18

**PD-1/PLD1 IN OVARIAN CANCER**

As already discussed, the tumor microenvironment is not the same in all cancers, with different immune cells present and differing proteins or cytokines and therefore varying actions which leads to distinct responses to therapy and prognoses. The tumor microenvironment in ovarian cancer has been shown to contain TILs which recognize tumor antigens and have cytolytic activity.74–76 Initially, the presence of CD3+ TILs in ovarian cancer were demonstrated to have a significantly improved median progression free survival (22.4 vs. 5.8 months, \( p < 0.001 \)) and overall survival (50.3 vs. 18.0 months, \( p < 0.0001 \)) compared to tumors without presence of TILs.46 Subsequent studies indicated that the types of T cells present and the ratio of regulator versus effector T cells impact outcomes.77,78 For example, an increase in intraepithelial CD8+ TILs and a high CD8 T cell to Treg cell ratio was associated with improved survival,77 while an increased proportion of CD4+CD25+FoxP3+ Tregs and NK cells was shown to be a poor prognostic factor.61 Tregs have been shown to curb CD4 and CD8 activity as well as activate the immunosuppressive effect of macrophages, which would add to this decreased survival.82–84 Tumors are often found to have high levels of infiltrating Tregs, which likely help dampen effector response leading to tumor immune escape; PD-1 blockade likely enhances anti-tumor effect by depleting Treg population in the tumor microenvironment. Some investigators are examining the effect of depleting FoxP3+ Tregs on anti-tumor response.85–89

Different cancers are not only classified differently based on these criteria due to the varying immune infiltrative cells in their respective tumor microenvironments, but they also as a result have diverse prognoses and response to immunotherapy. Immune responses differ not only based on the overall type or cancer, but they have been seen to change based on histologic subtype within certain cancers. For example, when analyzing by stage some studies showed that advanced stage epithelial ovarian cancer (EOC) with presence of Tregs were associated with increased survival88 as opposed to the studies which use heterogeneous populations including different ovarian cancer subtypes. Similarly, when analyzing by histologic subtype, high-grade serous cancer, the most common and fatal form of EOC, is associated with favorable TIL response, and presence of Tregs were found to be associated with increased survival as opposed to other subtypes.79,91–94 The authors theorize that this increase in survival seen with Treg presence might be indicative of a stronger CD8 response which overshadows the suppressive effects of Tregs,89 but more research is needed to better understand these differences. Some proposed reasons for these inconsistencies in survival include varying study methodology, specifically in the use of various antibodies or markers and their subjective scoring criteria, and analyzing subtypes separately as opposed to grouping them all in one.92,94 Another reason for further investigation and detailing of the specific types of infiltrative cells and their locations in the microenvironment is that these elements might be able help direct
future therapy. This thinking is extrapolated from the ovarian cancer mouse model findings in which certain chemotherapies were found to lead to activation of these specific cells but other treatments did not elicit an immune response in those infiltrative cells.

Other criteria which alter prognosis include surgical outcome, effect of neoadjuvant chemotherapy, and TIL differentiation. While surgical outcomes are an independent prognostic indicator of ovarian cancer, they have been seen to be related to presence of TILs. Specifically, the presence of TILs has been suggested to control disease burden allowing for suboptimal debulking and cytotoxic therapy whereas those without TILs present required maximal surgery to maintain outcomes. However, these distinctions still require further investigation and validation. Wouters et al. have complemented these findings, explaining that surgical outcomes in ovarian cancer can negate the prognostic value of TILs, as incomplete resection outweighed presence of positive prognostic CD8+ TILs. In terms of TIL differentiation, the authors found that patients with tumors infiltrated by less differentiated CD8+ TILs, measured by CD27 expression, had better outcomes in those with maximal cytoreductive surgery, possibly indicating an activated tumor-reactive environment. As a result, these different factors including prior treatment regimens, surgical outcome, and the type or location of TILs all must be evaluated together when thinking about patient prognosis and future treatment plans.

In addition to TILs, other antigen presenting cells are of interest as they too play a role in the PD-L1/PD-L1 pathway, specifically macrophages. Macrophages in ovarian cancer have been described to be differentiated to an M2 phenotype, consisting of IL-10 and TGF-b expression, which is believed to lead to tumor progression likely due to IL-10’s effect of inducing PD-L1. Additionally, macrophages have been known to produce CCL22, which attracts Tregs into the tumor microenvironment. Webb et al. demonstrated that PD-L1 was seen to be upregulated on TAMs as compared to tumor cells, and all the tumors that had PD-L1 expression also had associated TAMs. One possible explanation for increased expression of PD-L1 on TAMs is that while they scurry the tumor microenvironment they ingest large amounts of proteins and antigen loads which they subsequently present, making them recognizable by T cells. In such a setting, PD-L1 upregulation allows for a means of self-defense and protection from T-cell mediated killing. In that study, PD-L1+ TAMs were not only noted to be present but also found to have positive association of PD-L1+ TAMs as a marker of favorable prognosis. However, immunohistochemical staining has been inconsistent in different studies with some demonstrating significant tumor staining and others showing staining limited to TAMs.

Another immune cell involved in the PD-1/PD-L1 axis are DCs, which have been shown to constitute up to 40% of the tumor microenvironment. DCs have the ability to shift their immune response depending on the cytokines in the microenvironment and have shown a role in the ability to express PD-1 as ovarian cancer progresses. When expressing PD-1, NF-kB activity of DCs is inhibited, therefore affecting downstream activities such as suppressing its co-stimulatory function and cytokine production.

**PD-L1 AS PROGNOSTIC FACTOR IN OVARIAN CANCER**

In early studies of PD-L1 in cancer Hamanishi demonstrated that PD-L1 was associated with a poor prognosis in EOC, with a five-year survival rate of 52.6% in patient with high expression of PD-L1 versus 80.2% in those with low expression (p = 0.016). This data goes along with the thought that PD-1/PD-L1 engagement would render these local T cells ineffective, which leads to tumor survival and the poor prognosis seen here. Subsequently, other studies have also explored the prognostic indication of PD-L1 expression including...
Maine et al., who demonstrated that PD-L1+ monocytes in the ascites and blood of patients with malignant ovarian cancer were more common than those with benign or borderline disease. However, more recently, some studies have found the opposite with improved prognosis associated with PD-L1 expression. Darb-Esfahani investigated PD-1/PD-L1 expression on cancer cells where CD3+, PD-1+, PD-L1+, and TIL densities were all positive prognostic factors. One theory to explain this improved prognosis is that the increased PD-L1 expression is a result of a compensatory upregulation in the setting of the adaptive immune reaction's attempt at combating the tumor with cytokines which have induced marker expression. Similarly, Webb et al demonstrated a positive association between PD-L1 and TIL presence in HGSC. They theorized that these contradictory studies were due to differences in staining antibody protocols, the histologic subtypes evaluated in each study, and the degree prior surgical debulking in the representative studies. Another distinction between these studies is the innate differences in varying histological subtypes of EOC, as Kobel et al. reported that various subtypes have significant molecular, immunological, and clinical differences and therefore have varying associations of biomarkers with outcomes. This study did not specifically look at PD-1/PD-L1 as a biomarker, their hypothesis can be extrapolated to the PD-1/PD-L1 axis in ovarian cancer subtypes and might explain why some studies had opposing findings. PD-L1 expression status on tumor cells alone is not enough to use as a marker of prognosis or response to immunotherapy; instead, PD-L1 expression might be an indicator of a setting in which immunotherapy will be of use since PD-L1 is likely suppressing Tregs and other immunosuppressive cells.

**IMMUNOTHERAPY**

Durable objective responses and improved overall survivals with PD-1 inhibitors have been documented in 19% to 44% of patients with multiple tumor types including melanoma, NSCLC, and renal cancers. Based on these studies two PD-1 inhibitors, Nivolumab and Pembrolizumab, both IgG4 monoclonal antibodies, were granted Food and Drug Administration (FDA) approval for these cancers and with continued trials in other cancer types will likely gain approval in the near future. However, not all cancers have exhibited responses to anti-PD-1/PD-L1 therapy, including prostate and colorectal cancer, and even within those cancers which have shown response, not every patient has experienced benefit.

In their Phase I study of humanized IgG1 monoclonal antibody to PD-1 in hematologic malignancies, Berger et al. found the antibody to be safe and well tolerated. They also observed clinical benefit in 33% of patients, one with complete remission (CR). A subsequent Phase I study of anti-PD-1 in solid tumors, including melanoma, NSCLC, prostate, renal, and colorectal cancers was carried out. In this study the observed cumulative response rates were 18% in NSCLC, 28% in melanoma, and 27% in renal cell. Durable responses were seen in 20 out of 31 patients, and responses were noted at one year or more. In 2012 Brahmer et al. investigated the use of Nivolumab, which was FDA approved for treatment of melanoma, NSCLC, renal cell carcinoma, and Hodgkin lymphoma. This was a multicenter Phase I trial of anti-PD-L1 therapy in advanced solid tumors. Seventeen patients with ovarian cancer were enrolled, and the investigators observed one partial remission (PR) and three patients with stable disease, with a disease control rate of 23.5%. More recently, Hamanishi et al. published the results of their single-center Phase II trial using Nivolumab in 20 patients with platinum-resistant, recurrent, or advanced ovarian cancer with a history of at least two chemotherapy regimens including platinum and taxane agents. Patients were treated with either 1mg/kg or 3mg/kg of Nivolumab every two weeks until disease progression was identified or up to
48% of patients including melanoma cancers. The approved PD-1 inhibitors, pembrolizumab, and nivolumab, both IgG4 monoclonal antibodies, were granted Food and Drug Administration (FDA) approval on the basis of continued trials showing they gain approval for a broad variety of cancers. Not all cancers progress well with anti-PD-1/PD-L1 monoclonal antibodies and colorectal cancers were included in the cancers which were effective in every patient has a variable.

Antitumoral activity of humanized IgG4 monoclonal antibodies is often found in hematologic malignancies but had limited activity in solid tumors. They also observed a 93% of patients, specifically, complete response (CR). A subset of the patients with anti-PD-1 in solid tumors, non-small-cell lung carcinoma, NSCLC, and other solid tumors was observed to have a 18% in NSCLC, 60% in renal cell carcinoma, and 20% in 20 out of 26 patients were noted at the time of first response. Brahmer et al. also observed pembrolizumab, which was effective in the treatment of melanoma, lung cancer, and lymphoma. The randomized multicenter Phase 1 study in advanced melanoma patients with variant TNM stage IIIB with 11% of patients having a durable response (CR) and 11% of patients having a durable response, with a median follow-up of 12 months. More recently, the results of clinical trials using Nivolumab (anti-PD-1) in resistant, recurrent, and metastatic melanoma cancer with a histological pattern of treatment regimens in melanoma and in combination with platinum and taxane agents. Nivolumab alone at 1mg/kg or 3mg/kg every two weeks until disease progression or until to the maximum of 48 weeks maximum. Their primary endpoint was best overall response which was assessed by RECIST 1.1 criteria. They observed grade 3 or 4 treatment-related adverse events in eight patients (20%) and serious events in two patients (one with disorientation and gait abnormality in the setting of a month of fevers; the other developed fever and deep vein thrombosis). The best overall response rate was 15% with a disease control rate of 45%. A complete response was observed in two patients in the 2mg/kg group, and prolonged stable disease was noted in four patients. These response rates were similar to those of patients treated with platinum chemotherapy in patients with platinum-resistant tumors; however, the durable anti-tumor response seen has been clinically encouraging in this pretreated population. Hamaishi et al. also evaluated expression levels of PD-L1, which were not significantly correlated with objective response. Sixteen of the patients' tumors (80%) were classified as having high expression of PD-L1, but only two of those patients with high expression showed objective response, and one of the patients with low levels of expression showed a response to therapy.

Pembrolizumab, another anti-PD-1 humanized IgG4 monoclonal antibody, has been FDA approved for treatment of melanoma and NSCLC. In the non-randomized multicohort Phase 1b (KEYNOTE-028, NCT02054806) trial of Pembrolizumab in patients with solid tumors, 26 patients with ovarian cancer enrolled. Using 10mg/kg of Pembrolizumab every two weeks, best overall response rate was assessed using RECIST 1.1 criteria. The objective response rate observed was 11.5% with one CR, two PR, and 23% stable disease with a durable response. Avelumab was the first anti-PD-L1 inhibitor to be evaluated. It is a humanized selective IgG1 monoclonal antibody to PD-L1 (which does not affect PD-L2-PD-1 interaction). In a Phase Ib study 124 recurrent or refractory ovarian cancer patients were treated with 10mg/kg every two weeks until progression or toxicity. Twelve patients were observed to have PR with an objective response rate (ORR) 9.7%. While 55 patients reported to have stable disease burden and the disease control rate was 54%, the ORR in PD-L1+ tumors was 12.3% and in PD-L1- ORR was 5.9%. They did not demonstrate statistically significant difference between PFS or OS based on PD-L1 expression status.

Of these published studies using anti-PD-1/PD-L1 as immunotherapy, they were all in patients with platinum-resistant ovarian cancer, the ORR at best was 15% and a few had durable long-lasting disease control. While these results were encouraging, the hope is to continue improving outcomes. Therefore, the next step in many upcoming trials is using these agents as combination therapy, first with standard chemotherapy and then with other immunotherapies.

Standard treatments like chemotherapy or radiation leads to apoptosis and subsequent dispersal of antigens which can affect other tissues or tumors in the body. Recently, presented preclinical models showed that standard chemotherapy with platinum and taxanes increased expression of PD-L1 therefore adding to chemoresistance. Similarly, Mesnage showed that both TIL and PD-L1 expression were augmented after standard neoadjuvant chemotherapy in patients with EOC. Bohm et al. also documented the effect of neoadjuvant chemotherapy with platinum therapy in boosting the immune response. However, this stimulation of the immune response has not been shown to alter outcomes. Other chemotherapies aside from platinum and taxanes are also involved in augmenting anti-tumor response. Maniashaldone et al. evaluated the use of pegylated liposomal doxorubicin (PLD) in patients with BRCA mutated cancers which lead to a heightened immune response with increased TIL recruitment and vulnerability to T-cell cytotoxicity. Therefore, Bohm and others suggest the incorporation of immunotherapy to harness this enhanced immune response and further improve disease control. Current trials are evaluating these combinations. For example, combination therapy of platinum
chemotherapy with anti-PD-L1 immunotherapy in vivo has been shown to control and reduce tumor burden.\textsuperscript{124,125,129} And given that BRCA mutated tumors are deficient in homologous recombination and rely on the imperfect poly (ADP-ribose) polymerase (PARP) mediated pathway for double-stranded break repair,\textsuperscript{130,131} further research is needed on the combination of PD-1/PD-L1 immunotherapy with PARP inhibitors.

Other therapies aside from standard chemotherapy are being used for combination therapies. Vascular endothelial growth factor (VEGF) inhibitors which affect angiogenesis play a role in ovarian cancers,\textsuperscript{64} affecting the T-cell immune response, and inversely correlate with TIL infiltration.\textsuperscript{56,260} Durvalumab, an Fc optimized IgG1 monoclonal antibody to PD-L1 which has been FDA approved for urothelial bladder cancer, is being used in combination with VEGF inhibitors. In an ongoing Phase I/II trial, Durvalumab is being studied in combination with Olaparib (PARP inhibitor) or Cediranib (VEGFR inhibitor). Recently presented results showed one partial response in nine evaluable ovarian cancer patients lasting over six months with Durvalumab/Olaparib and one PR in five evaluable ovarian cancer patients treated with Durvalumab/Cediranib.\textsuperscript{18,132} Some trials are currently exploring the combination of immune-checkpoint blockers such as anti-CLTA-4 therapy Ipilimumab with Nivolumab based on its promising results in melanoma.\textsuperscript{113}

The results of these combination therapies are promising and encouraging, leading to many more trials which are ongoing and further research into other related possible pathways. Table 10.1 highlights the open clinical trials as of January 2017 using PD-1 and PD-L1 inhibitors. Another active agent being studied is the use of DNA methyltransferase inhibitors. Studies have shown that solid tumors treated with DNA methyltransferase inhibitors exhibit upregulation in genes involved in the interferon pathway.\textsuperscript{133,134} More recent studies have gone on to show that pretreatment with a DNA methyltransferase inhibitor can sensitize melanoma to immune checkpoint inhibitors like anti-CLTA-4.\textsuperscript{135} Given these findings and Wrangle et al., who showed that treatment with Azacytidine led to upregulation of PD-L1, future work should include trials using PD-1/PD-L1 inhibitors in combination with DNA methyltransferase inhibitors.\textsuperscript{136}

**GENETIC VARIABILITY AND NEOANTIGENS**

As therapy evolves, and more combinations are being studied, we need further detailed subclassifications based on tumor subtypes. While there are some classification frameworks such as the one suggested by Teng et al., which was described earlier, more recent research suggests adding a molecular layer to include certain mutations. Genetic variability and mutational load has also been suggested to play a role in PD-1/PD-L1 expression. Recent research (budczies)\textsuperscript{136} has suggested that PD-L1 copy number variation correlates with mRNA expression and with poor prognosis in many cancers including ovarian cancer. T cells recognize foreign peptide epitopes presented on major histocompatibility complexes, providing the initial signal for T cell activation. These epitopes can either be non-mutated proteins from the tissue, for which there is incomplete tolerance, or mutated peptides which are novel compared to the human genome, called neoantigens.\textsuperscript{137} There is evidence suggesting that increased mutational load leads to increases in neoantigens in the tumor microenvironment which recruits TILs and leads to increased response to PD-1/PD-L1 therapies.\textsuperscript{119,138} and with low genetic alterations there have been decreased response to immunotherapy.\textsuperscript{120} However, there are a limited number of mutant epitopes to which T cells react, possibly due to immune editing or ineffective priming and tolerance of the T cells.\textsuperscript{138} Based on this, current research includes the use of vaccination with neoantigen peptides in an attempt to increase the breadth of T cell reactivity and hopefully
TABLE 10.1 ONGOING CLINICAL TRIALS USING ANTI-PD-1/PD-L1 THERAPIES

<table>
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<tr>
<th>NCT</th>
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<th>Combination Therapy</th>
<th>Phase</th>
<th>Population</th>
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A Dose Escalation and Cohort Expansion Study of Anti-CD27 (Varilumab) and Anti-PD-1 (Nivolumab) in Advanced Refractory Solid Tumors

Anti-PD-1 Antibody in Combination with Low-Dose Decitabine in Relapsed or Refractory Malignancies

A Combination Clinical Study of PLX3397 and Pembrolizumab to Treat Advanced Melanoma and Other Solid Tumors

Study of Niraparib in Combination with Pembrolizumab (MK-3475) in Patients with Triple-Negative Breast Cancer or Ovarian Cancer

A Combination Clinical Study of PLX3397 and Pembrolizumab to Treat Advanced Melanoma and Other Solid Tumors

Study of Pembrolizumab Plus Chemotherapy in Patients with Advanced Cancer (PembroPlus)

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<td>NCT02341625</td>
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<td>BMS-986148 (anti-mesothelin antibody-drug conjugate)</td>
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<td>Ipilimumab, Cobimetinib</td>
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<td>Durvalumab</td>
<td>Olaparib (PARP-Inhibitor), Cediranib (anti-VEGF)</td>
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<td>Phase I/II Study of the Anti-Programmed Death Ligand-1 Antibody MEDI4736 in Combination with Olaparib and/or Cediranib for Advanced Solid Tumors and Advanced or Recurrent Ovarian, Triple Negative Breast, Lung, Prostate and Colorectal Cancers</td>
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<td>A Phase I/II Study of Motolimod (VITX-2337) and MEDI4736 in Subjects with Recurrent, Platinum-Resistant Ovarian Cancer for Whom Pegylated Liposomal Doxorubicin (PLD) is Indicated</td>
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<td>NCT02915523</td>
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<td>Entinostat (histone deacetylase inhibitor)</td>
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<td>Phase Ib/II Study of Avelumab with or without Entinostat in Patients with Advanced Epithelial Ovarian Cancer</td>
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<td>NCT02963831</td>
<td>Durvalumab</td>
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<td>I/II</td>
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<td>A Phase I/II Study to Investigate the Safety, Biologic and Anti-Tumor Activity of ONCOS-102 in Combination with Durvalumab in Subjects with Advanced Peritoneal Malignancies</td>
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| NCT02734004 | Durvalumab | Olaparib                             | I/II  | Advanced solid tumor              | A Phase I/II Study of MEDI4736 in Combination with Olaparib in Patients with Advanced Solid Tumors |


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A Phase I/II Study to Investigate the Safety, Biologic and Anti-Tumor Activity of ONCOS-102 in Combination with Durvalumab in Subjects with Advanced Peritoneal Malignancies

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A Phase I/II Study of MEDI4736 in Combination with Olaparib in Patients with Advanced Solid Tumors.

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Matched Paired Pharmacodynamics and Feasibility Study of Durvalumab in Combination with Chemotherapy in Frontline Ovarian Cancer

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Olaparib, Durvalumab, and Tremelimumab in Treating Patients with Recurrent or Refractory Ovarian, Fallopian Tube, or Primary Peritoneal Cancer with BRCA1 or BRCA2 Mutation

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Phase II: Pembrolizumab/Carboplatin/Taxol in Epithelial Ovary Cancer

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A Study of Pembrolizumab with Standard Treatment in Patients with Recurrent Platinum-Resistant Ovarian Cancer

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<tr>
<th>NCT03029403</th>
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Phase II Study of Pembrolizumab, DPX-Survivac Vaccine and Cyclophosphamide in Advanced Ovarian, Primary Peritoneal, or Fallopian Tube Cancer

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<tr>
<th>NCT02834975</th>
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Pembrolizumab, Paclitaxel, and Carboplatin in Patients with Advanced Stage Epithelial Ovarian Cancer (EOC).

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Study of the Effects of Pembrolizumab in Patients with Advanced Solid Tumors

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Nivolumab and Ipilimumab in Treating Patients with Rare Tumors

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<td>NCT02520154</td>
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<p>| NCT02669914 | Durvalumab            | II | Patients with brain metastasis from epithelial-derived tumors | MEDI4736 (Durvalumab) in Patients with Brain Metastasis From Epithelial Tumors |</p>
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<td>Patients with brain metastasis from epithelial-derived tumors</td>
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* Based on clinicaltrials.gov, January 2017.
improve immunogenicity against the endogenous tumor. In the setting of this heightened immunogenicity and neoantigen load from these vaccines, enhancement of neoantigen-specific reactive T cells can recognize the tumors epitopes, and therefore combination therapy with immunotherapy may be synergistic. A variety of tumors with microsatellite instability or mismatch repair mutations, specifically those with POLD, POLE, or MYH mutations, have been shown to be strongly associated with improved clinical response to anti-PD-1 therapy, likely due to their subsequent increase in neoantigen load. Mutational load has been shown to be a positive prognostic value in melanoma and bladder cancer as well as a predictor of sensitivity to PD-1 immunotherapy in NSCLC. Additionally, based on their analysis of multiple solid tumor types, Danilova et al. showed that PD-L1 expression was independent from BRAF, PTEN, and NRAS mutations; their group therefore suggest these as other future mutational targets which might be able to serve as biomarkers. They also highlight other targetable mutations such as LAG-3, IDO, ICOS, and Tim-3, to name a few, some of which are already part of ongoing clinical trials. Despite all this, the mutational landscape is constantly changing from diagnosis, as a result of treatment, and at time of relapse, leaving the field with more research to be done to continue to understand these pathways.

Data on genetic variability previously documented in other cancers may be extrapolated to ovarian cancer. Up to 29% of ovarian cancers have somatic mismatch repair mutations, and in those without mismatch repair mutations the likely abnormality is copy number alteration. One such gene being studied is BRCA mutations, which are known to have increased mutational load due to changes in homologous recombination repair. With increased number of mutations comes genetic variability, which is suggested to lead to development of tumor-specific neoantigens. These neoantigens lead to increased mobilization of TILs which then stimulates upregulation of immunocheckpoints like PD-1/PD-L1. In their preliminary work Strickland et al. observed an improved survival associated with tumors with higher neoantigen load as a result of their DNA repair mutations, suggesting that BRCA mutational status may be more sensitive to PD-1/PD-L1 immunotherapy. More specifically, tumors with BRCA mutations contained higher CD8+ TILs, indicating they likely have higher sensitivity to checkpoint inhibitors. However, according to recently presented data, there were no responses seen in the BRCA mutation group treated with Avelumab, though we continue to wait for further results and research.

Another pathway under investigation includes PI3K/Akt/mTOR mutational status. Recent studies have shown that the PI3K/Akt/mTOR pathway is often altered in clear cell carcinoma (CCC), and preclinical data from NSCLC suggest that mutations in this pathway have been correlated with increased PD-L1 expression in tumor cells, which is the suspected reason why CCC patients showed benefit with Nivolumab. While these are some of the relevant mutations, more are being discovered, and in the future we will likely need panels of markers to assess expression levels, which taken together can guide combination therapies specific to that patient.

**FUTURE WORK**

We still require reliable or validated predictive biomarkers to guide patient-directed therapy and as a predictor or indicator of response. Without standardized protocols, we cannot definitively develop appropriate markers. For example, when evaluating the prognostic value of TILs, some studies qualify all CD3+ cells as TILs as opposed to other studies which specifically target CD8+ cytotoxic T-cells. Furthermore, as discussed previously, not every patient with an otherwise responsive tumor type will actually have
benefit, and the contrary is true too: some patients with generally unresponsive cancers might show some response depending on the presence of certain factors. PD-L1 expression on tumor cells has not proven to be a reliable marker of response to immunotherapy as it is inconsistent when comparing different tumor subtypes. Specifically, Hamanishi reported 88% of tumors analyzed as expressing PD-L1 whereas Webb et al. and Gottlieb et al. only showed a small portion with expression (13.2% and 8.2%, respectively), and both detected simultaneous PD-L1+ TILs or TAMs. Gottlieb et al. explains this discrepancy due to TAMs admixing with tumor cells and given their histologic characteristics they are easily confused for tumor cells. Therefore in their study they distinguish tumor cells from macrophages with concurrent CD68 staining. If one was able to reliably differentiate between tumor cells and macrophages, PD-L1 expression on TAMs could be another area to investigate and validate as a possible prognostic marker. In their cohort of 51 patients, Qu et al. showed that PD-L1+ CD68 macrophages in tumor tissue as well as in peripheral blood were elevated compared to healthy controls and patients with benign ovarian cysts. Similarly, Maine et al. showed comparable results of PD-L1 expression on monocytes in ascites and peripheral blood. Some preliminary studies, which still require validation, are looking into the role of postchemotherapy TILs and PD-L1 expression as predictors of immunotherapy benefit to be used for treatment stratification. And future trials will hopefully evaluate PD-L1 expression both on tumor cells but also on specific immune infiltrates in the tumor microenvironment. Abiko et al. have been evaluating both tumors as well as other related cells. They investigated the relationship of PD-L1+ tumors and the resulting cytology of peritoneal ascites with preclinical models of PD-L1 inhibition associated with reduced peritoneal tumor growth and improved survival. Based on their results, they suggested future evaluation of primary tumor PD-L1 expression as a marker of response but also the possibility of PD-L1 status in ascites.

Additionally, checkpoint expression and immune infiltrates are a moving target. Spatial and temporal heterogeneity in ovarian cancers is a problem, and therefore the current genomic scene cannot be used as a prognostic biomarker. For example, clinically this is seen with tumor sampling, as one might miss focal expression or infiltrates at the edge of the tumor. Recognizing its influence and accounting for this heterogeneity in future studies can help better understand outcomes in immunotherapy.

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