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REVIEW ARTICLE

Targeting immune checkpoints in gynecologic cancer: updates & perspectives for pathologists

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Checkpoint inhibitor-based immunotherapy is increasingly used in the treatment of gynecologic cancers, and most often targets the PD-1/PD-L1 axis. Pathologists should be familiar with the biomarkers required to determine candidacy for these treatments based on existing FDA approvals, including mismatch repair protein immunohistochemistry, microsatellite instability testing, tumor mutation burden testing, and PD-L1 immunohistochemistry. This review summarizes the rationale behind these treatments and their associated biomarkers and delivers guidance on how to utilize and readout these tests. It also introduces additional biomarkers which may provide information regarding immunotherapeutic vulnerability in the future such as neoantigen load; *POLE* mutation status; and immunohistochemical expression of immunosuppressive checkpoints like LAG-3, TIM-3, TIGIT, and VISTA; immune-activating checkpoints such as CD27, CD40, CD134, and CD137; enzymes such as IDO-1 and adenosine-related compounds; and MHC class I.

Modern Pathology (2022) 35:142–151; https://doi.org/10.1038/s41379-021-00882-y

INTRODUCTION

The interactions between the immune system and malignancy have long been appreciated and, over the course of the last two decades, have successfully been harnessed for therapeutic purposes in cancers, including some gynecologic tumors[1–4]. Gynecologic cancers include a diverse array of malignancies of ovarian, uterine, cervical, and vulvar origin and show variable vulnerability to both established and emerging immunotherapies[5–7]. Their responses to some of these therapies appear to be tied in part to genetic and protein expression characteristics including mismatch repair status, tumor mutational burden, and checkpoint ligand expression.

Immunotherapy is a broad term that encompasses a variety of techniques including immune checkpoint inhibitors and stimulators, therapeutic cancer vaccines, and adoptive cellular transfer. While some of these treatments are now commonly enlisted in the clinical setting, others remain largely restricted to the research sphere. We herein discuss the current state of immune checkpoint-based therapy across gynecologic cancer types, with an emphasis on the pathologist's role in reading out these biomarkers and triaging tissue specimens to optimize the selection of gynecologic oncology patients for immunotherapeutic access.

THE THERAPEUTIC RATIONALE FOR TARGETING IMMUNE CHECKPOINTS

Manipulation of immune checkpoints represents the most successful and widely enlisted strategy of immunotherapy across tumor types. These drugs capitalize on the inveterate "checks and balances" built into the human immune system: immune checkpoints are co-signaling pathways that can either enhance or suppress the immune response when T cells engage antigen-presenting cells—including tumor cells that have co-opted this system for their own benefit. In a normally functioning immune response, the equilibrium between immune-activating and immunosuppressive checkpoints ensures that reactions to immunogenic stimuli do not propagate inexorably, protecting the host against autoimmunity and promoting self-tolerance. Malignancies, however, may hijack these pathways to avoid recognition and attack by the host's immune system.

Although checkpoint-based immunotherapy can target either immune-activating or immunosuppressive molecules, immunosuppressive checkpoint blockers have seen far more clinical success thus far. The most clinically relevant immunosuppressive checkpoint receptors are programed cell death (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), each of which tamps down the immune response through interaction with its ligands (PD-L1/PD-L2 and B7-1/B7-2, respectively)[3,8]. Therapies targeting these pathways include antibodies directed at both the receptors and their ligands. Anti-CTLA-4 drugs were developed first and continue to play a role in the care of melanoma patients, however, they are associated with significant immune-related toxicities[9]. Although there is some evidence that they can synergize with PARP inhibition in BRCA1-deficient ovarian cancers[10], overall anti-CTLA-4 therapies have failed to gain significant traction in the treatment of gynecologic tumors. Antibodies targeting PD-1 and its ligand PD-L1 have proven more broadly effective and less toxic, showing tolerability and durable responses among patients with a variety of malignancies including melanoma, non-small cell lung carcinoma, and urothelial carcinomas[11–13]. More recently, these drugs have shown to benefit a subset of gynecologic cancer patients, including some women

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Received: 24 March 2021 Revised: 23 July 2021 Accepted: 23 July 2021
Published online: 7 September 2021

with endometrial cancer[14–17]; cervical squamous cell carcinoma and adenocarcinoma[18,19]; vulvar squamous cell carcinoma[20]; ovarian carcinoma[21,22]; gestational trophoblastic tumors[23]; and even occasional uterine sarcomas[24–27]. (Table 1) Among endometrial cancers, mismatch repair-deficient/microsatellite unstable cancers are particularly vulnerable to anti-PD-1 therapy[28], although mismatch repair-intact/microsatellite stable advanced and recurrent cancers can also benefit[17], particularly in the context of polymerase epsilon (*POLE*) mutations[29,30].

THE PATHOLOGIST'S ROLE: CURRENT FDA-APPROVED BIOMARKERS FOR CHECKPOINT INHIBITOR CANDIDACY IN GYNECOLOGIC CARCINOMA

Pathologists play a critical role in identifying which gynecologic cancers will be candidates for checkpoint inhibition. At present, there are Food & Drug Administration (FDA)-approved pathways for treatment with the PD-1 inhibitor pembrolizumab based on the presence of mismatch repair-deficiency/high-level microsatellite instability[15,31] or high tumor mutational burden[32,33] in any advanced solid tumor—including gynecologic cancers. The anti-PD-1 drug dostarlimab is also approved for advanced mismatch repair-deficient endometrial cancers using a specific companion diagnostic assay (Ventana MMR RxDx)[34]. In addition, recurrent and metastatic cervical carcinomas are FDA-approved pembrolizumab candidates if they have positive PD-L1 expression using the companion diagnostic 22C3 pharmDX assay[35]. Details regarding the rationale behind and application of these biomarkers are detailed below.

Mismatch repair deficiency/microsatellite instability

In 2017 Le et al. published data in Science showing that over half of progressive mismatch repair-deficient solid tumors treated pembrolizumab demonstrate appreciable responses, including over 20% with complete response[15]. This led to the groundbreaking FDA approval of pembrolizumab in advanced solid tumors demonstrating mismatch repair deficiency or high-level microsatellite instability, representing the agency's first tumor type and site-agnostic recommendation tied to a molecular feature[15,31] (Tables 1–2). These impressive immunotherapeutic responses make biologic sense: when malignancies are unable to successfully repair DNA mismatches they rapidly accumulate mutations (often on the order of 50–100 per megabase) which leads to enhanced neoantigen production[36]. This high neoantigen load, in turn, triggers increased immune recognition that drives adaptive resistance through the expression of PD-L1 and other immunosuppressive molecules[36–40]. Although these adaptations help the tumor thrive despite a cytotoxic T cell-enriched milieu, they also render the tumor vulnerable to immune attack when checkpoint inhibitors such as pembrolizumab are applied[15].

Table 1. The current state of checkpoint inhibitors in gynecologic carcinoma (as of May 2021).

Tumor type	Current state
All solid tumors	• Advanced/metastatic solid tumors are FDA-approved candidates for pembrolizumab in the setting of MMRd/MSI-H. Across solid tumors in this category, responses have been observed in 53% with complete responses in 21%[14,15]. • Advanced/metastatic solid tumors are FDA-approved candidates for pembrolizumab in the setting of high TMB on (FoundationOne assay, ≥10 mutations per megabase). 29% of high TMB solid tumors have shown response to this therapy including 4% with complete responses[32].
Endometrial carcinoma	• Many advanced endometrial cancers qualify for pembrolizumab based on the FDA approval in MMRd/MSI-H solid tumors, with 78% of qualifying cases showing either response or stable disease following this therapy[14,15]. • Dostarlimab is FDA-approved for advanced MMRd endometrial cancers using a specific companion diagnostic assay (Ventana MMR Dx)[34]. • Pembrolizmuab is FDA-approved in combination with the VEGFR kinase inhibitor lenvatinib for recurrent/advanced endometrial cancers irrespective of biomarker status; trial data demonstrated response in 40% of patients who received this combination[17]. • Trials in PD-L1-positive endometrial cancer which did not require MMRd/MSI-H showed partial response to pembrolizumab in 13% with stable disease in another 13%[16]. • *POLE*-mutated endometrial cancers have shown complete regression following treatment with nivolumab[29,30].
Cervical carcinoma	• Pembrolizumab is FDA-approved for PD-L1-positive (CPS ≥ 1) recurrent/advanced cervical carcinomas. • The majority (~80%) of cervical squamous carcinomas and adenocarcinomas will meet the threshold for PD-L1-positivity. • 14% of PD-L1-positive advanced tumors show some response but <3% demonstrate complete response[18,19]. • 2.6% of cervical cancers are MMRd/MSI-high and 14.9% are TMB-high, offering additional avenues for pembrolizumab access;[51,63] however, the majority of these cases would already qualify based on PD-L1 expression.
Vulvar carcinoma	• No tumor-specific FDA approvals exist. • Responses to pembrolizumab have been reported in recurrent squamous cell carcinoma of the vulva[20].
Ovarian carcinoma	.• No tumor-specific FDA approvals exist. • Up to 10% of endometrioid and clear cell ovarian carcinomas and 1–2% of ovarian serous carcinomas will qualify for pembrolizumab based on MMRd/MSI-H[49–52]. • Pembrolizumab and nivolumab have been shown to have anti-tumor activity in some cases of platinum-resistant ovarian cancer[21,22]
Gestational trophoblastic tumors	• No tumor-specific FDA approvals exist. • Avelumab provided cures for 50% of patients with chemotherapy-resistant gestational trophoblastic tumors in a recent trial[23].
Uterine sarcomas	• No tumor-specific FDA approvals exist. • When administered as a single-agent, nivolumab did not show benefit among previously treated women with advanced uterine leiomyosarcoma[24]. • Trials from sarcomas across anatomic sites have shown rare cases of uterine leiomyosarcoma with therapeutic response or disease stabilization following anti-PD-1 treatment[25–27].

FDA food & drug administration, *MMRd* mismatch repair-deficient, *MSI-H* microsatellite instability-high, *TMB* tumor mutational burden.

Table 2. FDA-approved biomarkers for checkpoint inhibitor candidacy in gynecologic carcinoma (as of May 2021).

Biomarker	FDA-approved drug(s)	Companion diagnostic	Positivity threshold	Tumor type(s)
MMR IHC	Pembrolizumab[31]	N/A	Total loss of expression in tumor nuclei	Any solid tumor
	Dostarlimab[34]	Ventana MMR RxDx	Total loss of expression in tumor nuclei	Endometrial carcinoma
MSI testing	Pembrolizumab[31]	N/A	MSI-High	Any solid tumor
TMB testing	Pembrolizumab[33]	FoundationOne CDx	≥10 mutations per megabase	Any solid tumor
PD-L1 IHC	Pembrolizumab[35]	22C3 pharmDX	CPS ≥ 1	Cervical carcinoma

FDA food & drug administration, *IHC* immunohistochemistry, *MMR* mismatch repair, *MSI* microsatellite instability, *TMB* tumor mutational burden

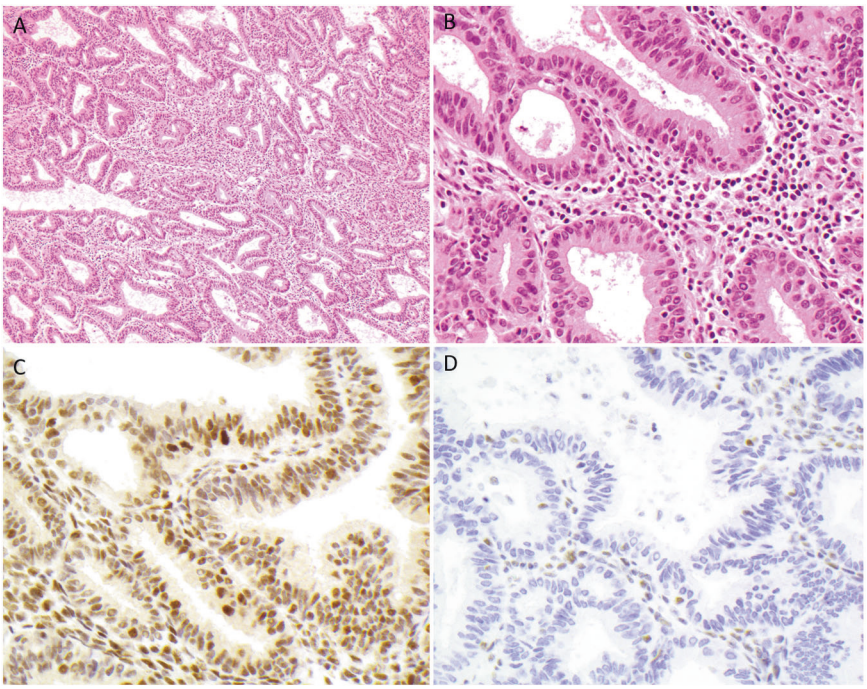

Fig. 1 Mismatch repair-deficient endometrial carcinoma. This endometrioid carcinoma (**A**) demonstrates robust tumor-associated lymphocytes (**B**), which are attributable to the elevated mutation burden and high neoantigen load associated with underlying mismatch repair deficiency. Although MLH1 (**C**), MSH2 (not pictured), and MSH6 (not pictured) expression was intact, PMS2 (**D**) shows total loss of expression within tumor cell nuclei with preserved positive internal control staining in background stroma and associated lymphocytes. Genetic testing in this patient revealed a germline *PMS2* mutation. Based on the presence of mismatch repair deficiency, this tumor would qualify for anti-PD-1 checkpoint inhibitor therapy.

While this FDA approval significantly expanded the pool of cancer patients who could receive immunotherapy, confusion quickly ensued regarding which test was optimal for selecting potential responders as the approval was not tied to a companion assay or even a specific kind of test[41]. Many used "mismatch repair deficiency" and "microsatellite instability" interchangeably, however, these terms refer to results produced from entirely different assays which have variable performance characteristics in different tumor types. Mismatch repair deficiency is conventionally defined by immunohistochemical loss of expression of one or more of the four main mismatch repair proteins (MLH1, PMS2, MSH2, and MSH6). (Fig. 1) Microsatellite instability, in contrast, is a PCR or NGS-based test that evaluates repetitive regions of the genome (microsatellites) which are vulnerable to expansion and contraction in the context of an improperly functioning mismatch repair system. Moreover, some labs can perform DNA sequencing which can directly identify aberrations in mismatch repair gene sequences. Not surprisingly, pathologists and oncologists have been left wondering which assay is most appropriate.

To ameliorate this uncertainty, in 2018 the College of American Pathologists (CAP) convened an expert panel to review data on mismatch repair-associated biomarkers and immunotherapeutic response in solid tumors[42]. Because there is a paucity of direct data relating mismatch repair biomarker results to immunotherapy response, the panel relied largely on data derived from the Lynch syndrome literature, assessing biomarker results in comparison to a gold-standard of germline-confirmed mismatch repair mutations. While the final iterations of these guidelines are under development, preliminary recommendations were released for open comment from February 19-March 13 2020[43].

The CAP panel's drafted recommendations stated that mismatch repair immunohistochemistry is an acceptable assay for checkpoint inhibitor access across tumor types—including gynecologic cancers. Moreover, immunohistochemistry is preferred over microsatellite instability testing in non-colorectal carcinomas as commercially available microsatellite instability assays are optimized for colorectal carcinoma, and have been less well-validated outside of this tumor type. In addition, microsatellite instability testing has decreased sensitivity for *MSH6* mutations, which are enriched in endometrial cancers when compared to colorectal cancers[44,45]. Importantly, only complete loss of expression in the presence of adequate internal control staining

constitutes true deficiency using immunohistochemistry. Although subclonal loss patterns can be observed[46], their significance with regard to immunotherapeutic vulnerability is unknown. It is also unclear whether retesting on recurrence specimens is warranted, as mismatch repair status changes over time have not been well studied and cases with mismatch repair status evolution were not included in checkpoint inhibitor trials. Finally, the panel cautioned that while DNA sequencing represents a promising methodology for the detection of mismatch repair gene defects, there are limited data regarding the performance characteristics of this approach in this specific context. The final iteration of the CAP's Guidelines for mismatch repair resting for immunotherapy access are expected by late 2021, and should be consulted for final updates.

It is worth noting that while the 2017 FDA approval for pembrolizumab in mismatch repair-deficient/microsatellite instability-high tumors did not require a specific assay[31], more recently the Ventana MMR RxDx assay has been linked to the PD-1 inhibitor dostarlimab as a companion diagnostic in endometrial carcinoma[34]. It remains to be seen whether future FDA approvals will include such companion diagnostic requirements, and pathologists should be aware that this may impact their ability to determine candidacy for certain drugs based on mismatch repair status without confirming assay interchangeability.

At present, mismatch repair-related biomarkers are typically applied based on the treating clinician's request as checkpoint inhibitors are not currently considered front-line in any gynecologic carcinoma types. At many institutions, including our own, immunohistochemical staining for mismatch repair proteins is already performed reflexively on all endometrial carcinomas and some ovarian carcinomas (specifically those with clear cell and endometrioid histologies) as part of universal Lynch syndrome screening[47,48], therefore in these tumor types mismatch repair immunohistochemistry need not be repeated for immunotherapeutic access. In other gynecologic cancers where this testing is not performed reflexively, mismatch repair immunostaining may be requested for patients who have progressed on standard of care chemotherapy and may provide a novel treatment option for occasional patients[49–52].

PD-L1 expression

In 2017, data from the KEYNOTE-158 study revealed that 14% of PD-L1-positive cervical carcinomas that had failed prior therapy respond to pembrolizumab, including just under 3% with complete responses[18,35]. This led to the 2018 FDA approval of pembrolizumab to treat advanced cervical cancers that express PD-L1 on immunohistochemistry (22C3 pharmDX kit, Agilent)[35] (Tables 1–2). Because all responders expressed PD-L1 with a Combined Positive Score (CPS) ≥1, PD-L1 immunohistochemistry was approved as a biomarker for pembrolizumab access at this positivity threshold. The CPS PD-L1 scoring system was originally developed for upper gastrointestinal tumors and accounts for both tumor cell and tumor-associated immune cell staining using the following equation: [(total number of PD-L1-positive tumor cells, lymphocytes, and macrophages) / (total number of viable tumor cells)] x 100[53,54]. (Fig. 2) The threshold lower limit (CPS = 1) requires only an average of one PD-L1 positive tumor cell, lymphocyte, or macrophage per 100 tumor cells. Although many cervical carcinomas are much more flagrantly PD-L1 positive, it is not clear that increased expression correlates with improved response to therapy. Details regarding how to properly readout a PD-L1 CPS are provided elsewhere[53–56].

Importantly, the FDA approval for pembrolizumab in cervical cancer is specifically tied to the 22C3 pharmDX Kit on the Dako Autostainer. Although PD-L1 expression has been shown to vary somewhat across different antibody clones and staining instruments, there is also evidence that some assays can produce comparable results at clinically significant thresholds[57–59]. It may therefore be reasonable to consider validating alternate PD-L1 biomarker assays with the same or different anti-PD-L1 primary antibody clones to facilitate testing in settings where the FDA-approved assay is unavailable. However, it is key that the validation design is appropriate for predictive immunohistochemical biomarker assays and that relevant guidelines for laboratory-developed tests are followed, including both technical and clinical validation[60,61]

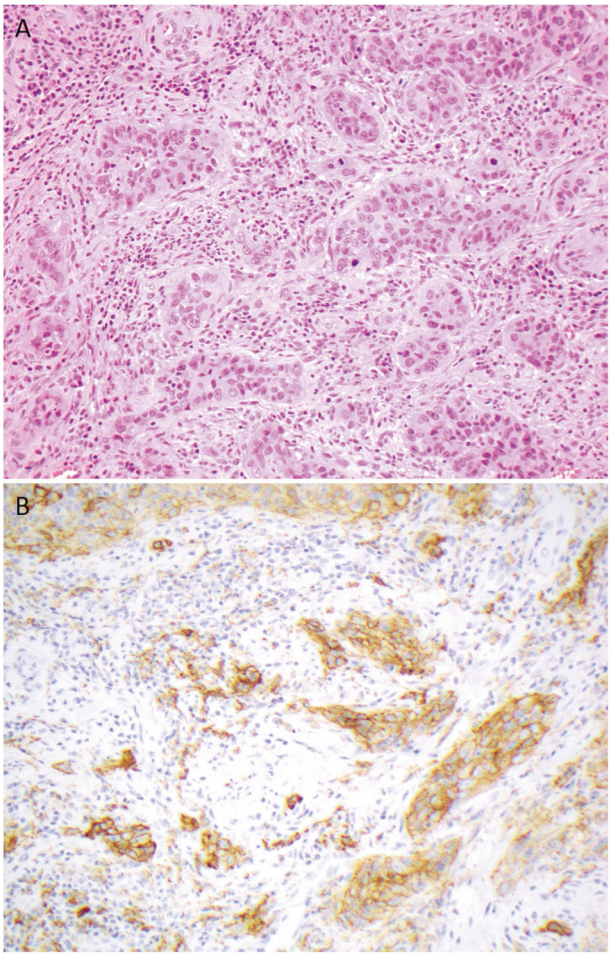

Fig. 2 PD-L1 expression in cervical squamous cell carcinoma. This cervical squamous cell carcinoma (**A**) demonstrates strong diffuse membranous PD-L1 expression (**B**) on tumor cells as well as cytoplasmic and membranous staining in tumor-associated macrophages and lymphocytes. Given that the stain is positive on essentially every tumor cell, this carcinoma qualifies for the maximum combined positive score (CPS) of 100. Because a minimum score of only 1 is required for FDA-approved access to anti-PD-1 immunotherapy, this tumor readily clears the threshold for treatment candidacy.

At the time of writing, cervical carcinoma is the only gynecologic cancer in which PD-L1 immunostaining is enlisted for checkpoint inhibitor access based on PD-L1 CPS status, however, that may change in the future. Vulvar squamous cell carcinomas, in particular, represent attractive candidates for PD-L1 biomarkers as these tumors have considerable biologic overlap with cervical squamous cell carcinomas and can show impressive responses to pembrolizumab[20]. Given the rapidly expanding role of PD-L1 immunohistochemistry as a biomarker for checkpoint inhibitor access in other organ systems, it would be not be surprising if a new FDA approval tied to this biomarker emerged. Fortunately, the principles discussed for PD-L1 in cervical carcinoma are likely to be transferable to other gynecologic sites, provided the CPS remains the preferred scoring methodology as it has in most other organ systems.

Tumor mutational burden

In 2020 the KEYNOTE-158 study revealed that 29% of patients with solid tumors showing high-level tumor mutational burden (defined as ≥10 mutations per megabase and using the FoundationOne CDx assay) showed durable responses to monotherapy with pembrolizumab, including 4% with complete responses[32]. Their data revealed that while high tumor mutation burden often overlapped with high-level microsatellite instability and/or PD-L1 immunohistochemical expression, this correspondence was not absolute. This molecular signature therefore became an additional FDA-approved avenue for anti-PD-1 access among solid tumors in 2020[33]. (Tables 1–2).

Although high tumor mutational burdens are seen in roughly 10% of gynecologic malignancies[62,63], tumor mutational burden testing for immunotherapeutic access has not yet penetrated routine practice for gynecologic cancer care in most settings. This is in part because endometrial cancers and cervical cancers are often candidates for checkpoint inhibition through other FDA-approved avenues and the other most common gynecologic cancer type, high-grade ovarian serous carcinoma, has alternate therapeutic options such as PARP inhibitors. Nonetheless, pathologists should be aware that oncologists may request that formalin-fixed tumor tissue be sent for this testing, and should be prepared to select tumor-containing blocks as appropriate.

FUTURE DIRECTIONS

Although FDA-approved assays like mismatch repair testing and PD-L1 immunohistochemistry identify many gynecologic cancer patients as candidates for checkpoint inhibitor therapy, the majority still fail to demonstrate durable treatment responses. Moreover, these FDA-approved biomarkers do not necessarily capture all responders. The potential reasons for this are myriad due to the incredible intricacy of the tumor-host immune interaction. Additional biomarkers that further characterize this interaction may therefore be of value in optimizing the patient selection for immunotherapy. Emerging biomarkers that could help identify strong candidates for checkpoint inhibition include neoantigen load and *POLE* mutation status as well as immunohistochemistry for other immunosuppressive checkpoints, immune-activating checkpoints, immunosuppressive enzymes, and molecules necessary for adaptive immune recognition.

Neoantigen load

Cancer-specific neoantigens are accrued due to genetic alterations that lead to novel sequences of amino acids which have not been previously recognized by the immune system, and DNA sequencing is to infer the overall burden of these neoantigens using bioinformatics algorithms[64]. Neoantigen loads have been shown to vary dramatically across gynecologic tumors[64] but are particularly high among mismatch repair-deficient endometrial cancers[36] and *BRCA*-mutated high-grade serous ovarian cancers[65]. Moreover, higher neoantigen loads are generally correlated with higher tumor mutational burdens, higher levels of immune infiltration, and better overall survival[64]. However, while neoantigen load often runs parallel to tumor mutational burden, the two are not interchangeable, and neoantigen load may theoretically identify tumors with lower mutational burdens in which mutations are particularly immunogenic. While it is not yet well established that neoantigen load significantly contributes to the identification of responders when compared to related technologies like tumor mutational burden testing, preliminary data suggests that it could have independent value in predicting clinical benefit to immunotherapy[66], and this test may have significance as a biomarker in the future.

Polymerase epsilon (POLE) status

Mutations in *POLE*—the gene encoding the DNA polymerase epsilon catalytic subunit—also impart vulnerability to checkpoint inhibitor-based immunotherapy. Hotspot mutations in *POLE* are fairly common in endometrial carcinoma with up to 10% of all endometrial cancers falling into the molecular subgroup defined by these mutations[67]. These DNA proofreading defects lead to an impressive accumulation of mutations that exceeds even what is seen in the context of mismatch repair deficiency, with *POLE*-mutated tumors demonstrating as many as 500 mutations per megabase[67]. Multiple trials investigating PD-1/PD-L1 inhibitors in POLE-mutated gynecologic cancers are underway (clinicaltrials.gov: NCT04463771; NCT04774419; NCT04267939; NCT03012581), and initial data suggests that the anti-PD-1 drug nivolumab has promise in mismatch repair-intact endometrial cancers with pathogenic *POLE* mutations[29,30]. *POLE* mutations can also be found in 3% of cervical carcinomas and 1–2% of ovarian carcinomas and may eventually represent a useful future immunotherapeutic biomarker in these tumor types as well[68].

Targetable checkpoints

The PD-1/PD-L1 axis is one of many immunosuppressive checkpoint pathways that malignancies can enlist to evade the host immune response. Other immunosuppressive checkpoint molecules that have been identified in gynecologic carcinomas include lymphocyte-activation gene (LAG-3)[39,69–71], T cell immunoglobulin and mucin domain (TIM-3)[40,72–75], T cell immunoreceptor with Ig and ITIM domains (TIGIT)[76–78], and V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA)[79–81]. (Table 3) Expression of some of these molecules and of their ligands is particularly prominent in the setting of mismatch repair deficiency, and they are often co-expressed with PD-L1[39,40,71,72]. This suggests that for many gynecologic malignancies, anti-PD-1/anti-PD-L1 monotherapy may be inadequate, and that combination approaches targeting multiple immune checkpoint pathways may find more success. Multiple trials investigating drugs targeting these pathways are underway in gynecologic cancers (clinicaltrials.gov: NCT03250832; NCT03489343; NCT03708328; NCT02817633; NCT04475523; NCT04693234; NCT04693234; NCT04570839) and may inform our approach to checkpoint inhibitor therapy and biomarker testing in the future.

There may also be a role for biomarkers and drugs targeting immunostimulatory checkpoints. Examples of immunostimulatory checkpoints that have shown early promise in the investigative setting include CD27, CD40, CD134 (OX40), CD137 (4-1BB); these molecules are in the immunoglobulin (Ig) and tumor necrosis factor (TNF) families and interact with their ligands to enhance T cell survival, proliferation, and differentiation[82–84] (Table 3). Clinical trials investigating the role of immunostimulatory checkpoint agonism are ongoing for a variety of solid tumors—including gynecologic cancers (clinicaltrials.gov: NCT04406623; NCT01644968; NCT02410512; NCT02335918), and may help to identify successful combination immunotherapy approaches in these tumors.

Targetable enzymes

Immunosuppressive enzymes may also confound attempts to enhance the anti-tumoral response using checkpoint inhibitors. Enzymes that have shown promise as immunotherapeutic targets include indoleamine dioxygenase 2, 3 and adenosine-related compounds. Indoleamine dioxygenase 2, 3-1 (IDO1) is an immunoregulatory enzyme induced by interferon-gamma[85–92]. Its immunosuppressive function is tied to its metabolization of tryptophan, which lymphocytes require for survival, and the generation of a toxic metabolite known as kynurenine. It plays a key role in blocking T-cell expansion and enhancing the immunosuppressive properties of some dendritic cells and is critical for promoting fetal tolerance and in curbing autoimmunity[87,89,90,93–95]. IDO1 expression has been demonstrated in

Table 3. Emerging immunohistochemical biomarkers for checkpoint inhibitor vulnerability.

Biomarker	Function	Ligand(s)	Staining characteristics in the tumoral microenvironment	Clinical trials in gynecologic carcinoma
LAG-3	Immunosuppressive checkpoint	MHC Class II, Gal3, LSECtin	Expressed on lymphocytes	NCT03250832
TIM-3	Immunosuppressive checkpoint	Gal9, Cecam-1, HMGB-1, phosphatidyl serine	Expressed on lymphocytes, macrophages, and some tumor cells	NCT03489343; NCT03708328; NCT02817633
TIGIT	Immunosuppressive checkpoint	CD112, CD115	Expressed on lymphocytes	NCT04693234; NCT04570839
VISTA	Immunosuppressive checkpoint	VSIG-3, PSGL-1	Expressed on lymphocytes	NCT04475523
CD27	Immune-activating checkpoint	CD70	Expressed on lymphocytes, NK cells	NCT02335918
CD40	Immune-activating checkpoint	CD40 Ligand	Expressed on macrophages, dendritic cells	NCT04406623
CD134 (OX40)	Immune-activating checkpoint	OX-40 Ligand	Expressed on lymphocytes	NCT01644968; NCT02410512
CD137 (4-1BB)	Immune-activating checkpoint	4-1BB Ligand	Expressed on lymphocytes	NCT02179918; NCT04442126; NCT02410512
IDO-1	Immunosuppressive enzyme	N/A	Expressed on tumor cells, macrophages, other immune cells	NCT03459222; NCT04106414
A2AR	Adenosine receptor	Adenosine, GS protein	Expressed on tumor cells, lymphocytes	NCT03629756; NCT03719326
CD73 (NT5E)	Immunosuppressive enzyme in the c-AMP pathway	AP-3, SMAD proteins, SP-1	Expressed on tumor cells, lymphocytes, myeloid-derived suppressor cells	NCT04148937, NCT03255252; NCT03267589
MHC class I	Ubiquitous on nucleated human cells; required for adaptive immune recognition and engagement	N/A	Complete membranous expression is normal in nucleated cells; a subset of tumors show complete or subclonal loss	N/A

Fig. 3 IDO expression in high-grade ovarian serous carcinoma. This high-grade ovarian serous carcinoma (**A**) strongly expresses the immunosuppressive enzyme indoleamine dioxygenase 2, 3 (IDO) (**B**). Drugs targeting this enzyme both alone and in combination with checkpoint inhibitors are currently under investigation in a variety of clinical trials.

endometrial, cervical, and ovarian cancers, with particularly high expression in the settings of high-level PD-L1 expression and mismatch repair deficiency[38,96–100]. (Fig. 3) Drugs targeting IDO are in clinical trials in a variety of carcinoma types and are of particular interest in combination with anti-PD-1/PD-L1 check point inhibitors (clinicaltrials.gov: NCT03459222; NCT04106414). The related molecule indoleamine dioxygenase 2, 3-2 (IDO2) may also have a role in immunotherapy but has been less well-studied[101,102].

Enzymes related to adenosine production have also shown promise in immunotherapy. Adenosine is a nucleoside derivative of adenosine triphosphate (ATP) which accumulates in response to cellular stress and breakdown. It has anti-inflammatory function through the generation of its secondary messenger, cyclic adenosine monophosphate (cAMP), which upregulates transforming growth factor-beta (TGF-ß) and immune-inhibitory checkpoints such as PD-1[103]. Immunotherapeutic targets of interest in the adenosine pathway include the adenosine A 2 A receptor (A2AR, ADORA2A) and CD73 (NT5E)[103–106]. Clinical trials of immunotherapies directed at these targets are underway in a variety of solid tumor types, including gynecologic cancers. (NCT03719326; NCT03629756; NCT03719326; NCT04148937, NCT03255252; NCT03267589).

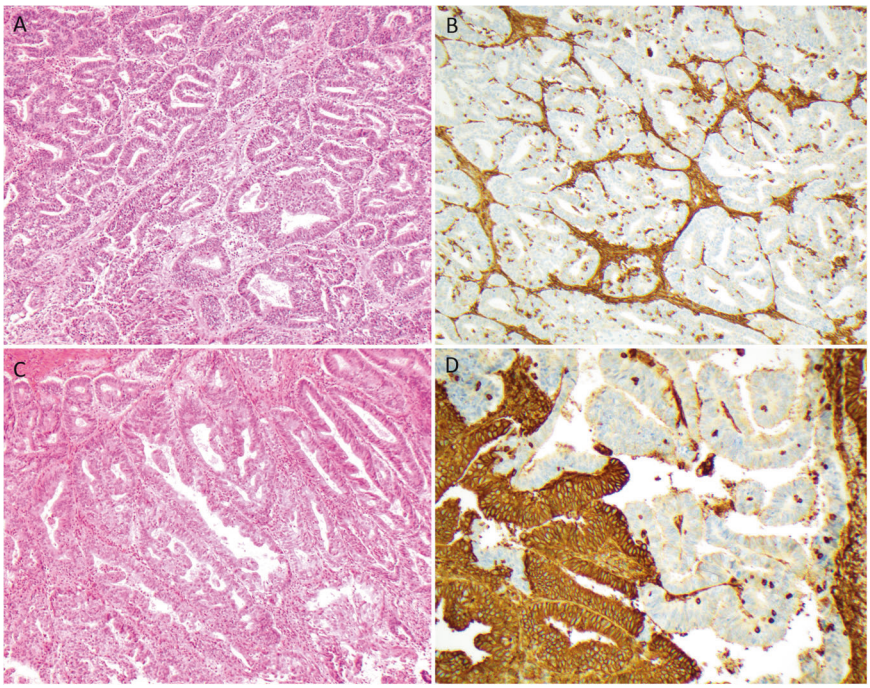

Fig. 4 Loss of MHC class I expression in endometrial carcinomas. Although all normal nucleated human cells express MHC Class I, loss of expression can be seen in malignancies and represents a mechanism of adaptive immune evasion. Loss may be total, as seen in the endometrial carcinoma depicted in (**A**, **B**), or partial, as in the endometrial carcinoma illustrated in (**C**, **D**). Notably, both these cancers were mismatch repair-deficient. Evidence from other tumor types suggests that MHC class I loss may confer resistance to checkpoint inhibitor-based immunotherapy, and studies are underway to determine the significance of this biomarker in the gynecologic tract.

Other mechanisms of immune evasion

Finally, some gynecologic cancers appear capable of evading the host's adaptive immune system altogether through the loss of major histocompatibility (MHC) class I. MHC class I is expressed on the surface of all nucleated human cells and functions like a flag pole for the display of intracellular antigens, interacting with T cells to promote a cytotoxic response when foreign antigens are present[107–109]. The presence of MHC class I on a tumor cell's surface is thus critical for enlistment of a CD8+ T cell adaptive anti-tumoral immune response, and a decrease or loss of MHC class I expression represents a putative mechanism of immune evasion in some cancers[110–113]. Among gynecologic malignancies, nearly half of endometrial carcinomas and more than a third of HPV-associated cervical squamous carcinomas have been shown to have complete or partial loss of MHC class I, potentially limiting the efficacy of adaptive immunotherapeutic approaches in these cancers irrespective of other biomarker statuses[114,115]. (Fig. 4) Interestingly, loss of MHC class I should render tumors more vulnerable to recognition by the innate immune system, particularly NK cells, due to the "non-human" nature of MHC class I-deficient cells[116]. Although innate immune responses are thought to be inadequate for the control for well-established tumors, MHC class I deficiency may suggest increased vulnerability to emerging immunotherapies that enhance innate immune effectors such as NK cells[116,117]. In addition, recent evidence suggests that MHC class I loss may confer increased susceptibility to chimeric antigen receptor T cell-based immunotherapy[118]. Such approaches, however, are currently limited to the investigative sphere.

In summary, although tumor responses to checkpoint inhibitors can be influenced by far more than currently FDA-approved biomarkers like mismatch repair status, tumor mutational burden, and PD-L1 expression, it remains to be seen whether any additional biomarkers become relevant for routine clinical practice. One could certainly imagine a diagnostic setting in which additional molecular assays or a panel of immunohistochemical stains could be used to optimize immunotherapy candidate selection and curate tumor-specific combination immunotherapeutic approaches, however, clinical trials are needed to critically assess the value of such an approach. In the short term, it is worthwhile for pathologists to understand the rationale behind current FDA-approved checkpoint inhibitor therapies, know how to apply and readout their associated biomarkers, and appreciate that many new biomarkers may be on the horizon.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: Review conception: A.M; Draft paper preparation: A.M; Revisions and critical appraise for intellectual content: T. N.B., K.L.R. All authors reviewed and approved the final version of the paper.

FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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