Immune Checkpoints in Endometrial Cancer

Subjects: Oncology

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Endometrial cancer (EC) occurs when cancer cells proliferate in the lining of the uterus, called the endometrium. This disease primarily occurs in post-menopausal women and often shows symptoms such as abnormal vaginal bleeding and discharge or severe pelvic pain. Due to poor outcome with conventional therapy, novel treatment options such as immune checkpoint inhibitors are urgently needed for advanced/ recurrent disease EC.

Keywords: endometrial cancer ; immune checkpoint inhibitors ; clinical trials ; combination therapy ; adverse effects ; disease improvement.

1. Introduction

Endometrial cancer (uterine corpus endometrial cancer; EC) is the most frequently occurring gynecologic cancer in the Western world. It is estimated that in the United States, there will be 65,620 new cases of this disease in 2020, and a projected 12,590 deaths due to this disease [1]. EC is generally diagnosed at an early stage and is well managed with surgery, radiation, and/or chemotherapy. More than 80% of patients diagnosed with early stage EC achieve a 5-year overall survival rate [1]. However, EC patients with advanced/recurrent disease have a poor outcome, such that those with distant metastasis have a five-year survival rate of approximately 16% [1]. This latter category of EC patients responds poorly to current management treatments, and hence, the use of novel therapies such as immune checkpoint inhibitors (ICI) is often considered for these advanced/recurrent disease EC patients.

Immune checkpoint synapses consist of several co-inhibitory molecules that are primarily responsible for limiting T-cell receptor signaling and abrogating immune responses. This strategic process set in place by the immune system is useful to halt immune responses in individuals after microbial infections are resolved, or in the development of self-tolerance to limit autoimmune disease [2]. However, in cancer, high levels of immune checkpoint molecules on immune cells or on tumor cells are often associated with exhausted T cells, which are incapable of developing aggressive anti-tumor responses, as well as with resistance to several classes of therapy [3][4][5].

For clinical practice, the first Food and Drug Administration (FDA)-approved monoclonal antibody targeting immune checkpoints was ipilimumab, which targets cytotoxic T lymphocyte associated protein-4 (CTLA-4). This treatment was followed by FDA approval of antibodies blocking the programmed death-1 (PD-1)/PD-l ligand (PD-L1) axis, treatment which has revolutionized the therapy of many solid cancers [6]. This review will focus primarily on outlining the molecular and cellular parameters which may influence the outcome of EC to ICI.

2. Immune Checkpoint Synapses in Cancer Immunotherapy

Immune checkpoints (IC) present potent immune-suppressive mechanisms in cancer, and blocking of two of these pathways in particular has provided useful therapeutic alternatives to improve survival in many cancer types. Briefly, the binding of CD28 on T cells to B7-1/B7-2 (CD80/CD86) on antigen-presenting cells (APC) results in co-stimulatory anti-tumor responses. However, co-inhibitory molecule CTLA-4 on T cells has a higher affinity for B7-1/B7-2 molecules than does CD28, and the preferential binding of CTLA-4 to B7-1/B7-2 blocks IL-2 release from T cells and limits T cell proliferation. In cancer, the development of an antibody to block the CTLA-4 to B7-1/B7-2 ligation leads to potent anti-tumor responses [7][8][9][10]. The first such CTLA-4 blocking antibody, ipilimumab, was FDA approved for metastatic melanoma in 2011 [6][11][12]

Immune checkpoint molecule PD-1 (CD279) is predominantly expressed on T cells, and PD-L1 (CD274) is primarily expressed on antigen-presenting cells (APC), immunosuppressive macrophages, and tumor cells. These molecules generally show higher density in tumors, and the cross-linking of PD-1 to PD-L1 is a critical immune-suppressive component in the tumor microenvironment (TME). In cancers, including endometrial cancer, the linkage of PD-1 to PD-L1

parallels with an environment rich in CD4+ CD25 high FoxP3+ T regulatory cells (T regs), high myeloid-derived suppressor cell (MDSC) activity, low cytotoxic T cell potential, and many other immunosuppressive parameters that trend to tumor progression [4][13][14][15].

In 2014, the FDA approved a monoclonal antibody pembrolizumab, blocking PD-1, for use in patients with metastatic melanoma. Pembrolizumab is now approved for several other cancer types including non-small cell squamous cell carcinoma, recurrent head and neck squamous cell cancer, and solid cancers with high microsatellite instability (MSI-H) or mismatch repair (MMR) gene defects, including EC [16][17]. There are now several other FDA-approved antibodies targeting the PD-1 axis [6][18], many of which are in single and combination therapy clinical trials for EC and other gynecologic malignancies [19][20].

3. Characterization of Endometrial Cancer

Cancer of the endometrium is the most commonly diagnosed gynecologic malignancy in the United States **(Table 1)**, and it comprises approximately 7% of new cancers in women [1]. Endometrial carcinomas (EC) are a collection of distinct histologic subtypes. Traditionally, endometrial cancers have been classified into two Bokhman histopathologic categories, based on pathologic features, endocrine and metabolic factors, and prognosis [21]. Type 1 endometrial neoplasms represent International Federation of Gynecology and Obstetrics (FIGO) grade 1 and 2 EC and constitute about 80% of EC. These tumors are generally hormonally mediated and sensitive to estrogen, associated with obesity, and may be preceded by a precursor lesion such as endometrial intraepithelial neoplasia. Type 2 endometrial cancers account for 10 to 20% of EC and include FIGO grade 3 EC and non-endometrioid, clinically aggressive histologies such as clear cell, serous, mixed cell, and undifferentiated. These tumors lack estrogen sensitivity, are not associated with obesity, tend to be high grade tumors, and are associated with diagnosis at a later stage and poorer prognosis. Tumors in the type 2 subgroup are associated with a higher rate of p53 mutations and overexpression of HER2/neu [22][23].
Recently, the Cancer Genome Atlas analysis stratified EC into four distinct molecular subtypes as follows: polymerase ε (POLE)-mutant ultramutated, microsatellite instability high (MSI-H, hypermutated), copy number low, and copy number high [24]. The hypermutated group generally carries a high number of mismatch repair (MMR) defects [24][25]. The usefulness of these molecular classifications to correlations of patient outcome to ICI therapy will be addressed in subsequent sections.

Table 1. Estimated new cases of female gynecologic cancer diagnoses and estimated deaths in the U.S. in 2020.

Organ	New diagnosis	Deaths	Deaths x 100% / New diagnosis[1]
All	113,520	33,620	21.4
Uterine corpus (endometrial)	65,620	12,590	19.2
Ovary	21,750	13,940	64.1
Uterine cervix (cervical)	13,800	4,290	31.1
Vulva	6,120	1.350	22.1
Vagina and others	6,230	1,450	23.3

[1]The table shows the relative percentage of deaths for female gynecologic cancers based on the numbers of these cancers newly diagnosed. The uterine cervix is classified as cervical cancer, and the uterine corpus is classified as endometrial cancer (adapted from Siegel et al., 2020) [1].

In EC, survival is primarily controlled by disease stage at the time of diagnosis, histologic subtype, and tumor grade. Generally, in this disease, the majority of women present with uterine bleeding and are diagnosed at an early stage. Early stage disease is highly amenable to treatment with surgical resection, followed by adjuvant therapy with radiation and/or cytotoxic chemotherapy based on clinico-pathologic factors such as disease stage, histology of the tumor, grade, and

tumor size. About 67% of EC are diagnosed with disease confined to the uterus, resulting in a high survival rate of 95% at five years [1][26]. However, prognosis is significantly worse for patients diagnosed with regional or distant metastasis, with 69% and 16% five-year survival, respectively [1]. Patients with metastatic and/or recurrent EC often have low response rates to chemotherapy, and they have essentially run out of effective therapy management options. Therefore, this underscores the need to develop novel therapeutic approaches such as the administration of ICI for patients with advanced disease EC.

4. Cellular and Molecular Factors Regulating Endometrial Cancer Outcome

Similar to most cancer types, the TME of EC consists of immunosuppressive lymphoid and myeloid cells, soluble molecules, and other pro-tumor elements that may limit patient success to novel and conventional therapies [27][28]. In a study of endometroid adenocarcinoma (EA) patients, investigators studied the relationship of inflammatory immune cells including lymphocytes, macrophages, and dendritic cells with disease outcome. These investigators evaluated archived histological material of 82 patients with stage I to III EA, with good (survival) and poor (disease progression and death) outcome. Outcome status was retrospectively determined from their patient study database [29]. All cases were stained with antibodies to identify CD3 (T cells), CD20 (B cells), CD57 (NK cells), CD68 (macrophages), and S100 (dendritic cells) by immunohistochemistry. Expressions of CD3, CD57, and CD68 were significantly higher in archived tissue in the good outcome group ($p < 0.001$) compared with the poor outcome group, whereas there was no significant difference between CD20 and S100 in the two groups [29]. High levels of immune cells, notably CD3 T cells in cancer tissue, and good outcome is consistent with the findings of other investigators [30][31][32].

However, in the case of EC, there are overriding considerations that shape the outcome of responses to ICI, and the subsequent text will focus on these parameters. The Cancer Genome Atlas classification (2013) of EC is particularly useful for the prediction of disease prognosis. As earlier mentioned, these molecular groupings are polymerase ε (POLE)-mutant ultramutated, microsatellite instability high (MSI-H, hypermutated), copy number low, and copy number high [24]. The MSI-H hypermutated group carries a high number of MMR defects and is most easily regulated by immunotherapeutic agents [33][34].The function of the MMR pathway is to repair single-strand breaks, mispairings, as well as small insertions or deletions that occur during DNA replication. Germline MMR deficiencies of one of four DNA MMR genes (*MLH1, PMS2, MSH2*, or *MSH6*) are associated with Lynch syndrome [35], which affects between 2 and 6% of EC patients [36][37]. Yet, most of the MMR pathways deficiencies are due to somatic mutations [38][39].

In tumors, a high CD3+ and CD8+ T cell density indicates an active immune response against cancer cells and correlates with better prognosis in several cancers, including EC. POLE-ultramutated and MSI/MMR deficiency (MMRd) tumors generally have high CD3+ and high cytotoxic CD8+ T cells, correlating with the best outcome of the four EC groups [30][31][32]. Importantly, EC was found to have the highest prevalence of MSI of 30 tumor types. About 30% of primary EC are MSI-H, whereas 13% to 30% of recurrent EC are MSI-H or MMRd [33][40][41][42][43].

Tumor mutational burden (TMB) is the total amount of somatic (acquired) mutations in a tumor [44]. Highly mutated tumors generally have an abundance of tumor-specific mutant epitopes, which act as neoantigens and are recognized as non-self and provoking immune responses [44][45]. Immune checkpoint inhibitors have shown promising efficacy against hypermutated cancers such as melanomas, lung cancers, and EC [16][46][47]. These cancer types have more tumor-specific neoantigens that stimulate the recruitment of more immunocompetent tumor-infiltrating lymphocytes (TILs) to augment anti-tumor immunity. Tumors with higher neoantigen load are associated with improved overall survival and increased tumor cell cytotoxicity parameters, including the expression of T cell receptor (TCR), interferon-γ (IFN-γ), and tumor necrosis factor (TNF) receptor pathway genes [48].

One report showed that POLE-ultramutated and MSI-H EC tumors also have an overexpression of PD-L1 [40]. Interestingly, there is still great uncertainty concerning the interpretation and relevance of PD-L1 expression on immune or tumor cells across several tumor types and its relationship to ICI efficacy in cancer [49]. However, it is believed that tumors such as EC, with elevated TIL numbers and high tumor mutational burden, are more easily recognized and targeted by T cells **[33][40][41][50][51]**. Such tumors typically respond well to ICI therapy [52], with patients showing significant disease improvement and improved overall survival (O/S), as is often the case for EC patients selected for ICI therapy.

5. Immune Checkpoint Inhibitor Therapy in Endometrial Cancer

Immune checkpoint inhibitors have shown efficacy in multiple advanced solid tumors, predominantly among MMRd and MSI-H cancers and those with a high tumor mutational burden, such as EC (Table 2). Some of these studies will be discussed in the subsequent text in relation to EC treatment.

An early signal of clinical activity of ICI in advanced EC was seen in a phase 2 study of 41 heavily pretreated patients with metastatic carcinoma with or without MMRd. Subjects were treated with pembrolizumab, which is a fully humanized immunoglobulin monoclonal antibody against PD-1. The treatment was associated with an immune-related objective response rate (ORR) of 71% and an immune-related progression-free survival (PFS) of 67% in MMRd non-colorectal cancers. A total of two patients with EC were enrolled. One of these exhibited a complete response, and the other exhibited a partial response [53]. High somatic mutation burden was associated with prolonged PFS ($p = 0.02$) [53].

The phase II KEYNOTE-158 study evaluated the anti-tumor activity and safety of pembrolizumab in previously treated, advanced non-colorectal MSI-H/MMRd cancer [54]. Patients were treated with a fixed dose of pembrolizumab 200 mg IV once every three weeks for two years or until disease progression, unacceptable toxicity, or patient withdrawal. Among patients with a broad range of solid tumors including 27 tumor types, there were 49 patients with EC (21% of the treatment population). In the cohort of patients with EC, the ORR was 57.1%, with eight patients (16%) achieving a complete response and 20 patients (41%) achieving a partial response. The median PFS was 25.7 months. In the entire study cohort of 233 patients, 64.8% of patients had treatment-related adverse events and 14.6% had grade 3 to 5 treatment-related adverse events, with one grade 5 event related to pneumonia. The most common treatment-related adverse events were fatigue, pruritus, diarrhea, and asthenia. This study further indicated that MSI/MMRd status could be a predictor of the response to PD-1 blockade in EC [54].

Pembrolizumab was subsequently approved by the FDA in 2017 for the treatment of MSI-H or MMRd solid tumors, regardless of tumor type, with progression following treatment and for which there are no satisfactory alternative treatment options [16]. In June of 2020, the FDA labeling was extended to include patients with unresectable or metastatic tumor mutational burden-high solid tumors (TMB-H; ≥10 mutations/megabase [mut/Mb]) after prior therapy and in the absence of other treatment options. Simultaneously, the FDA approved the FoundationOne® CDx (Foundation Medicine) test as the companion diagnostic for pembrolizumab to identify patients with solid tumors that are TMB-H (≥10 mutations/megabase) (pembrolizumab FDA package insert, June 06/20; https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125514s071s090lbl.pdf).

Table 2 summarizes some ICI monotherapy trials blocking the PD-1 axis in EC patients, evaluating the success of agents other than pembrolizumab. Overall, even though there is good outcome in some monotherapy trials blocking PD-1 or PD-L1 in EC (up to 57.1% ORR), a greater cohort of EC patients may benefit in combination therapy designs using ICI and other agents, which may potentially alleviate suppressor mechanisms in the tumor TME. Combination therapy has the potential to afford additive or synergistic benefits, as compared to single agent treatment, as well as to overcome resistance mechanisms that are observed with ICI monotherapy administration, due to the upregulation of alternative immune checkpoint molecules or to emerging resistance caused by the presence of cells such as myeloid-derived suppressor cells (MDSCs) [55][56][57][58][59]. Currently, several clinical trials are ongoing with ICI treatment in EC patients, which are used in combination with cytotoxic chemotherapy, other ICI, vaccines and other immunotherapies, or targeted therapies [19][20][23][26][60].

Table 2. Clinical data for select immune checkpoint inhibitors evaluated as monotherapy in endometrial cancer.

Treatment [1]	Study Phase	Endometrial Cancer Study Population	ORR	Reference
Anti-PD-L1 antibody				
Atezolizumab	Phase Ia	*n* = 15 Advanced or recurrent EC	Entire cohort—13%	Fleming [61]
Avelumab	Phase II	*n* = 31 Advanced or metastatic EC	MMRd tumors—26.7% MMRp tumors—6.25%	Konstanti-nopoulos [62]
Durvalumab	Phase II	*n* = 71 Advanced EC	MMRd tumors—40% MMRp tumors—3%	AntiII [63]
Anti-PD-1 antibody				

Dostarlimab	Phase I/II	*n* = 110 Advanced or recurrent EC	MSI-H tumors—48.8% MSS tumors—20.3%	Oaknin [64]
Nivolumab	Phase II	*n* = 23 Advanced or recurrent EC	Entire cohort—23%	Hasegawa [65]

[1] The clinical efficacy for multiple monoclonal antibodies targeting the PD-1/PD-L1 axis has been investigated in phase I/II trials, enrolling patients with advanced or recurrent EC. Abbreviations: EC, endometrial carcinoma; ORR, objective response rate; MSI-H, microsatellite instability-high; MSS, microsatellite stability; MMRd, mismatch repair deficient; MMRp, mismatch repair proficient.

For example, ICI treatment has also been combined with targeted therapy agents such as lenvatinib. Lenvatinib is an oral multikinase inhibitor of vascular endothelial growth factor receptor 1–3 (VEGFR1-3), fibroblast growth factor receptors (FGFR) 1–4, platelet-derived growth factor receptor (PDGFR) alpha, c-Kit, and RET proto-oncogene. Pre-clinical data suggest that this agent induces immune activation via decreasing tumor-associated macrophages, which may lead to an increase in CD8+ T cells and enhanced anti-tumor activity [66].

KEYNOTE-146/Study 111 was a single-arm, open label, phase Ib/II study to evaluate the safety and efficacy of lenvatinib plus pembrolizumab in advanced solid tumors, including endometrial carcinoma [67]. Patients received lenvatinib 20 mg once daily orally plus pembrolizumab 200 mg IV once every three weeks, based on the recommended dosing from the phase Ib portion of the study. The final primary efficacy analysis was reported for the patient cohort with advanced endometrial carcinoma. The primary endpoint was ORR at 24 weeks (ORR_{Wk24}). The ORR_{Wk24} was 38% in the cohort of 108 patients who were previously treated with conventional therapy. For 94 patients with MSS/MMRp tumors, ORR as measured by immune-related RECIST (irRECIST) was 37.2% versus 63.6% for 11 patients with MSI-H/MMRd tumors [67].

The safety profile of lenvatinib plus pembrolizumab was generally similar to that previously reported for each drug alone with the exception that hypothyroidism was reported at higher rates than previously observed for either monotherapy. Grade 3/4 treatment-related adverse events were seen in 68% of patients, and 17.7% of patients discontinued one or both therapies because of treatment-related adverse events. Overall, 19 patients (15.3%) discontinued lenvatinib, 15 (12.1%) discontinued pembrolizumab, and 11 (8.9%) discontinued both study drugs [67]. Based on the outcome of these studies, lenvatinib in combination with pembrolizumab was granted accelerated approval by the FDA in September of 2019 for the treatment of patients with advanced endometrial carcinoma that is not MSI-H or MMRd, and who have disease progression following prior systemic therapy and are not candidates for curative surgery or radiation [67].

From the studies summarized in the preceding text, it is evident that blocking the PD-1 axis in EC patients with monotherapy treatment may result in an ORR as high as 57.1%. ICI therapy in combination with targeted therapy lenvatinib had a better outcome of 63.6%. Based on these and other studies, ICI treatment is generally regarded as very promising as an alternative therapy option for advanced/recurrent EC. A more detailed list of ongoing EC clinical trials using monotherapy and combination therapy regimens are summarized elsewhere [19][20][23][26].

6. Adverse Immune Effects to immune checkpoint inhibitor therapy

ICI are associated with a broad spectrum of unique immune-mediated toxicities, requiring expert management, as these toxicities may occasionally be life threatening [68]. Immune-mediated toxicities can affect most organ systems and are believed to arise from autoimmune inflammatory complications of ICI treatment. Immune-related adverse events encompass dermatologic/mucosal, gastrointestinal, hepatic, endocrine, and pulmonary toxicities. Other less common but important immune-mediated toxicities include rheumatologic, cardiovascular, hematologic, ocular, neurologic, and renal manifestations.

The management of immune-mediated adverse events depends on the nature and severity of the toxicity and has been discussed elsewhere in more detail [69][70]. Treatment of higher-grade toxicities usually involves immunosuppression with glucocorticoids. An escalation of therapy may include tumor necrosis factor-alpha antagonists, mycophenolate mofetil, or other immunosuppressive agents. Depending on the specific toxicity and grade, moderate and severe immune-related adverse events may require interruption of the checkpoint inhibitor and close monitoring while glucocorticoid immunosuppression is introduced. In such cases, the ICI should not be resumed until toxicities are down to grade 1 or less. For severe or life-threatening toxicities, a permanent discontinuation of ICI therapy is usually indicated along with immunosuppression.

7. Conclusions

To date, a myriad of combination clinical trials are in progress investigating the response of gynecologic cancers to treatment blocking PD-1 ligation. Endometrial cancer is encouragingly responsive to ICI therapy. Achieving a high response rate of 57.1% with single therapy anti-PD-1 antibody (pembrolizumab) for EC strongly suggests that agents blocking the PD-1 axis may be useful and strategic alternatives for first-line chemotherapy failures. A better understanding of cellular and molecular parameters guiding response rates and survival in patients will be paramount to the optimization of future combination therapy options for the improved management or cure of advanced/ recurrent disease EC, resulting in significantly improved survival in patients with this diagnosis.

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