Immune disorder in endometrial cancer: Immunosuppressive microenvironment, mechanisms of immune evasion and immunotherapy (Review)

LEI ZHAN[1,2], XIAOJING LIU[2], JING ZHANG[2], YUNXIA CAO[1] and BING WEI[2]

[1]Department of Obstetrics and Gynecology, Reproductive Medicine Center, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230022; [2]Department of Gynecology and Obstetrics, The Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230601, P.R. China

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Abstract. Immunotherapy is an emerging clinical approach that has gained traction over the past decade as a novel treatment option for lung cancer and melanoma. Notably, researchers have made marked improvements in the treatment of endometrial cancer (EC), and potential immune responses have been identified in patients with EC, thereby offering the possibility of exploring immunotherapy for EC. Nevertheless, various needs remain unmet, and immunotherapy applications in EC have yielded limited success, as only a minority of patients exhibited a clinical response. Therefore, further understanding of immune dysfunction associated with EC is still required. The present review describes recent findings regarding the immunosuppressive microenvironment of EC, with emphasis on immune evasion mechanisms and immunotherapy in EC.

Correspondence to: Professor Bing Wei, Department of Gynecology and Obstetrics, The Second Affiliated Hospital of Anhui Medical University, 678 Furong Road, Hefei, Anhui 230601, P.R. China
E-mail: 499329901@qq.com

Professor Yunxia Cao, Department of Obstetrics and Gynecology, Reproductive Medicine Center, The First Affiliated Hospital of Anhui Medical University, 218 Jixi Road, Hefei, Anhui 230022, P.R. China
E-mail: caoyunxia6@126.com

Abbreviations: COX, cyclooxygenase; CTL, cytotoxic T lymphocyte; EC, endometrial cancer; ER-α, estrogen receptor α; FasL, Fas ligand; JAK1, Janus kinase 1; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; LNM, lymph node metastasis; MMR, mismatch repair; MSI, microsatellite instability; MHC, major histocompatibility complex; NK, natural killer; NLR, nucleotide oligomerization domain-like receptor; NLRC5, NLR family, caspase recruitment domain containing 5; OS, overall survival; PBL, peripheral blood lymphocyte; PD-1, programmed cell death-1; PD-L1, PD-1 ligand 1; PFS, progression-free survival; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; RCAS1, receptor-binding cancer antigen expressed on SiSo cells; TAM, tumor-associated macrophage; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cell; VISTA, V-domain Ig suppressor of T cell activation

Key words: endometrial cancer, immune response, immunosuppressive microenvironment, immune evasion mechanisms, immunotherapy

Contents

1. Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in the developed world. The latest estimates indicated that there were 61,880 new cases and 12,160 EC-related deaths in 2019 in the USA (1). Obesity, hypertension and diabetes are the major risk factors for the development of EC in developed nations (2,3). Furthermore, Lynch syndrome is known to lead to the development of EC (4,5). In 1983, EC was first classified into the type I and II subgroups based on clinicopathological characteristics. Type I EC represents the most common form (70-80%). At least 90% of tumors express estrogen receptor (ER) moderately or strongly. By contrast, type II EC is estrogen-independent and predominantly represents serous carcinoma (6-8). However, this histological classification of EC has its limitations, such as poor reproducibility and overlapping morphological and immunohistochemical features (9,10). By performing comprehensive genomic analysis, The Cancer Genome Atlas program, which was first funded by the National Cancer Institute, aimed to classify EC on the basis of survival outcomes. The percentage of cases found for each type were as follows: i) Polymerase ε (POLE) ultramutated, 7%; ii) microsatellite instability (MSI) hypermutated, 30%; iii) copy-number low (microsatellite stable), 65%; and iv) copy-number high

(predominantly serous histology), 26%. However, The Cancer Genome Atlas classification guidelines are not suitable for clinical application due to differences in demographic and clinical characteristics other than race/ethnicity and age, the members of The Cancer Genome Atlas classification guidelines not being systematically characterized, and no specific diagnosis criteria for each cancer type being present in the guidelines (11). Talhouk *et al* (12) developed a more practical technique, the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), which utilizes immunohistochemistry to identify mismatch repair (MMR) proteins, including mutL homolog 1, PMS1 homolog 2, mismatch repair system component, mutS homolog 2 and mutS homolog 6, as well as p53 expression and DNA sequencing to identify POLE mutations. The feasibility of the ProMisE system has recently been validated in 452 EC cases (12-14).

The standard therapeutic approach for EC is surgical resection of the uterus by total hysterectomy (15). During the past decade, considerable advances made in the field of cancer cell-mediated immune evasion in the tumor microenvironment have invigorated the field of immuno-oncology (16,17). The success of immunomodulating strategies, such as the use of immune checkpoint inhibitors in lung cancer and melanoma, has generated great interest regarding their potential in the treatment of other solid tumors (18,19). Recently, immunotherapeutic approaches for the treatment of EC have been extensively evaluated. However, the developed treatment strategies have not been successful (20-22). In the present review, PubMed (https://pubmed.ncbi.nlm.nih.gov/) was used to search for peer-reviewed publications using the following search terms: 'endometrial cancer', 'endometrial carcinoma', 'immune response', 'immunosuppressive', 'immune evasion mechanisms' and 'immunotherapy' in combination with other keywords related to the subject area. Relevant articles published until March 2020 are critically discussed. An overview of the immunosuppressive microenvironment of EC is presented first. The well-characterized mechanisms of immune evasion in EC are also described. Finally, preclinical studies and clinical trials involved in the development of immunotherapies for EC are reviewed.

2. Immunosuppressive microenvironment in EC

Immunoregulation in the endometrium is associated with the balance of the immune system in the endometrial microenvironment (20). The endometrium serves various immunological roles and acts as a physical barrier that prevents infection (20). The endometrium also establishes an immunosuppressive microenvironment that is essential for gestation and fetal development (20,23). The immunosuppressive microenvironment in EC is induced either through cell-mediated mechanisms or through molecular targets.

Cell-mediated mechanisms

$CD8^+$ T cells. Pascual-García *et al* (24) analyzed 35 neoplastic and 23 non-neoplastic endometrial samples, as well as corresponding peripheral blood samples, and demonstrated that the number of $CD8^+$ T cells was lower in the endometrium of patients with EC than in the endometrium of control subjects. Furthermore, there was a lower number of $CD8^+$ T cells in the peripheral blood from patients with endometrioid grade 3 EC, who had not received radio- or chemotherapy before surgery, compared with that in the healthy group. Additionally, these data also indicated that CD8 expression was downregulated in EC (24). In another study involving 90 patients with EC, Kondratiev *et al* (25) demonstrated that an increase in the number of $CD8^+$ T cells at the invasive border of the tumor epithelium is a favorable prognostic factor for patients with EC. Patients with a higher number of intraepithelial $CD8^+$ lymphocytes at the invasive border of the tumor epithelium had improved overall survival (OS) time compared with patients with a lower number of intraepithelial $CD8^+$ lymphocytes. Survival analysis demonstrated that cancer stage, vascular invasion, tumor grade and the number of intraepithelial $CD8^+$ lymphocytes at the invasive border were independent predictors of OS time (25).

Regulatory T cells (Tregs). Chang *et al* (26) studied 57 patients with stage I-IV EC and observed that the $CD4^+CD25^+$ T cell population was considerably larger in tumor-infiltrating lymphocytes (TILs) than that in peripheral blood lymphocytes (PBLs). Correlation analysis suggested that the upregulation of CD4 and CD25 expression in T cells in the cancer microenvironment was positively associated with high tumor grade, stage and myometrium invasion (26). Forkhead box P3 (Foxp3) expression in $CD4^+CD25^+$ Tregs is lower in PBLs than in TILs (26). Additionally, both granzyme B and perforin are rarely expressed in peripheral Tregs, but are widespread in Tregs in the tumor milieu (26). However, $CD8^+$ cytotoxic T lymphocytes (CTLs), derived from PBLs, express higher levels of granzyme B and perforin than TILs, and T helper 1 cytokines and cytotoxic molecules are simultaneously increased in $CD8^+$ CTLs, suggesting that, in the EC microenvironment, Tregs restrict $CD8^+$ T cell activity in a granzyme B- and perforin-dependent manner (26). Low levels of both T helper 1 cytokines and cytotoxic enzymes contribute to the Treg-mediated inhibition of tumor clearance (26). Similar results were also obtained by Yamagami *et al* (27), who reported that the $CD4^+Foxp3^+$ cell count and $CD4^+Foxp3^+/CD8^+$ ratio were novel prognostic factors for EC.

Macrophages. Macrophages, which can be polarized to the M1 (classical) or M2 (alternative) phenotypes, are one of the most abundant stromal immune cell types. In the tumor microenvironment, tumor-associated macrophages (TAMs) polarize to the M2 phenotype, which promotes immunosuppression, tumor progression and metastasis (28-30). The frequency of $CD68^+$ macrophages is higher in the epithelial and stromal cells of type I and II EC than in those of the benign endometrium (31). Furthermore, patients with EC who have high $CD68^+$ macrophage counts in the intra-tumoral border have worse progression-free survival (PFS) and OS time than patients with low $CD68^+$ TAM density (32). Weber *et al* (33) demonstrated that the density of $CD163^+$ M2 macrophages increased to a high level in the advanced stages of endometrioid adenocarcinoma of the uterus. Consistent with these findings, Kübler *et al* (34) reported that there is a positive association between the expression of TAMs and advanced stage, higher tumor grade, lymphovascular space invasion and lymph node metastasis (LNM) in type I EC. Furthermore, TAMs are independently associated with recurrence-free survival and OS in type I EC (34).

Table I. Immunosuppressive microenvironment in EC: Cell-mediated approach.

Cell type	Regulation	Specimen	Clinical application	Refs.
$CD8^+$ T cells	Down	Blood and tumor tissue	The presence of intraepithelial $CD8^+$ lymphocytes at the invasive border are an independent predictor of survival in EC.	(23,24)
Treg cells	Up	Blood and tumor tissue	The presence of $CD4^+CD25^+$ T cells in the EC milieu is associated with the tumor grade, stage and myometrium invasion. $CD4^+Foxp3^+$ count and $CD4^+$ $Foxp3^+/CD8^+$ ratio are novel prognostic factors for EC.	(25,26)
Macrophages	Up	Tumor tissue	Worse PFS and OS time are observed in patients with high $CD68^+$ density at the invasive margin. The number of TAMs strongly correlate with advanced stages, higher tumor grade, LVI and LNM, and are independently associated with recurrence-free survival and OS in type-I EC.	(31-33)
NK cells	Down	Blood and tumor tissue	Decrease in NK cell activity is associated with the depth of myometrial invasion. NK cell activity is related to myometrial invasion and immunoreactivity of proliferating cell nuclear antigen in stage I EC. The number of NK cells and HLA-E expression are associated with EC survival and prognosis.	(35,36,38)
Dendritic cells	Down	Tumor tissue	Dendritic cell markers S100 and HLA-DR have functions related to the delay of tumor progression and LNM in EC.	(39-41)
B lymphocytes	Up	Tumor tissue	Low expression levels of markers for tumor-associated T lymphocytes (CD3), NK cells (CD57) and macrophages (CD68), and an increased expression of markers for tumor-associated B lymphocytes (CD20) and dendritic cells (S100) are associated with poor survival outcomes in EC.	(42)

EC, endometrial cancer; HLA, human leukocyte antigen; PFS, progression-free survival; OS, overall survival; TAMs, tumor associated macrophages; LNM, lymph node metastasis; LVI, lymphovascular space invasion; NK, natural killer.

Natural killer (NK) cells. NK cells are effector cells involved in antitumor and antiviral innate immune responses. NK cell activation is impaired in the tumor microenvironment, including the EC microenvironment. Garzetti *et al* (35) suggested that locally advanced stage I and II ECs had significantly lower mean values of NK cell activity compared with healthy controls. Furthermore, a decrease in NK cell activity increased the depth of myometrial tumor invasion (35). NK cell activity also diminished with increased nuclear grade in stage I EC. In another study involving 40 patients with stage I EC who underwent radical surgery, NK cell activation was negatively associated with histopathological features of stage I EC, including myometrial tumor invasion and proliferating cell nuclear antigen immunoreactivity (36). Recently, Versluis *et al* (37) suggested that the upregulation of human leukocyte antigen (HLA)-E predicted improved disease-free survival and disease-specific survival time in EC. The number of NKp46 positive cells predicted a good clinical outcome, when the HLA-E levels were upregulated. However, the prognosis was poor when HLA-E levels were normal (Hazard ratio, 13.4; 95% confidence interval, 1.70-106.14).

Dendritic cells (DCs). DCs are a major part of the tumor microenvironment and serve an essential role in antitumor immunity by processing and presenting antigens to antigen-specific T cells. Disruption of DC activity is associated with EC progression (38). DC invasion has been observed in endometrial endometrioid adenocarcinoma (38). The DC markers S100-DR and HLA-DR serve a positive role in inhibiting EC progression and LNM (39). Jia *et al* (40) reported that the expression levels of CD80, CD86 and CD40 on DCs in the normal human endometrium were significantly higher than those on DCs in endometrioid adenocarcinoma. Morphological differences have also been observed between tumor-infiltrating DCs and those in the normal human endometrium. These findings suggested that the morphological differences and low expression levels of CD80, CD86 and CD40 on DCs in endometrioid adenocarcinoma could reflect functional changes in tumor-infiltrating DCs, affecting antigen uptake and presentation, thereby possibly promoting tumor immune escape.

B lymphocytes and others. Zinovkin and Pranjol (41) studied 82 patients with endometrioid adenocarcinoma at stages I-III and demonstrated that the downregulation of lineage-specific markers in T lymphocytes (CD3), NK cells (CD57) and macrophages (CD68), and the upregulation of markers in B lymphocytes (CD20) and DCs (S100) in endometrioid adenocarcinoma, indicated a poor clinical outcome. Furthermore, as

cancer-associated fibroblasts (42) and adipocytes (43) serve an important role in the genesis of an immunosuppressive microenvironment and in the malignant progression of hyperplasia, their functions in EC need to be studied further in the future. Cell-mediated mechanisms are summarized in Table I.

Molecular targets

Programmed cell death-1 (PD-1)/PD-1 ligand-1 (PD-L1) and PD-L2. The B7 family of immune checkpoint inhibitors is divided into three subgroups: Group I consists of B7-1, B7-2, CD28, cytotoxic T-lymphocyte-associated protein 4 and B7H, group II includes PD-1/PD-L1/PD-L2, and group III includes B7-H3, B7-H4, HERV-H LTR-associating 2, and transmembrane and immunoglobulin domain-containing protein 2. The members of the B7 family serve a critical role in the immune response (44). PD-1 was first discovered in 1992 and is expressed on the surface of T cells (45). PD-1 has two known ligands, PD-L1 and PD-L2 (46). PD-L1, which has been extensively studied over the last few years, is the most well-known immune checkpoint inhibitor (47). Overall, 67-100% of primary, recurrent and metastatic EC cases express PD-L1 (48). Mo *et al* (49) suggested that PD-1, PD-L1 and PD-L2 expression in all tumor-infiltrating immune cells is more frequent in moderately and poorly differentiated EC and non-endometrioid EC than in well-differentiated EC and endometrioid EC. Recently, Kim *et al* (50) proposed the prognostic significance of PD-1 and PD-L1 in patients with EC, and indicated that high PD-L1 levels were an independent adverse prognostic factor for PFS, especially for subgroups of patients with an MSI mutation. Analysis of immune markers suggested that high PD-L1/CD8 and PD-L1/PD-1 ratios were independently positively associated with shorter PFS times.

B7-H3 and B7-H4. B7-H3 and B7-H4, two novel members of the B7 family, have been suggested to serve an immune function in the tumor microenvironment (51). Brunner *et al* (52) studied 99 patients with type I or II primary EC and observed that patients with advanced tumors had markedly higher B7-H3 levels than patients with low-grade tumors. Additionally, expression analysis of B7-H3 in the vascular endothelium of the tumor tissue suggested a positive association with EC grade. Furthermore, there was a strong association between B7-H3 expression in tumor cells and frequency of $CD8^+$ positive TILs. Univariate survival analysis indicated that B7-H3 overexpression in cancer cells was associated with shortened OS time (52). Similarly, B7-H4 was upregulated in hyperplastic and malignant endometrial epithelium, and associated with the frequency of T cells, suggesting that B7-H4 overexpression reflects more aggressive EC, leading to EC tumor cell evasion (53). Additionally, a recent study indicated that B7-H4 expression was consistent across the various molecular subtypes of EC, suggesting that B7-H4 expression is independent of EC grade, histological type and infiltrating-immune cell type (54).

Indoleamine 2,3-dioxygenase (IDO). IDO is an enzyme that catalyzes the metabolism of the essential amino acid tryptophan in the initial and rate-limiting steps of the kynurenine pathway (55). Accumulating evidence has indicated that cancer tissue contains higher IDO levels than normal tissue (55). Ino *et al* (56) reported that high IDO expression in EC cells was present in 37/80 cases and was positively associated with surgical stage, myometrial invasion status, lymphovascular space involvement and LNM, but not with histological grade. Patients with EC expressing high levels of IDO had significantly worse PFS and OS time than patients with EC with low or no IDO expression. Multivariate analysis has suggested that IDO expression is an independent predictor of PFS (56). Furthermore, high levels of IDO in EC correspond to a low density of TILs and NK cells (57,58), and high levels of PD-L1 (59).

HLA. The HLA class I system serves an important role in the tumor immune response. This system comprises the classical HLA-A, -B and -C antigens, and the non-classical HLA-E, -F and -G antigens (60). Cancer cells can escape the CTL response by inhibiting HLA class I molecules (61,62). de Jong *et al* (63) reported the loss of HLA-A and/or HLA-B/C in 41.3% of patients in a study conducted on a cohort of 486 patients with sporadic endometrioid EC. Furthermore, the downregulation of HLA-B/C has been observed to occur more frequently in high-grade EC (63). Barrier *et al* (64) demonstrated that HLA-G protein was localized in the glandular epithelium and expressed in a substantial proportion of endometrial adenocarcinoma cases. However, overexpression of HLA-G in EC is not associated with clinical variables, disease-free survival or disease-specific survival (65). Non-classical HLA-G comprises four membrane-bound isoforms (HLA-G1 to HLA-G4) and three soluble isoforms (HLA-G5 to HLA-G7) (66). All HLA-G isoforms are detectable in the early and advanced stages of EC (67). The plasma levels of soluble HLA-G are significantly higher in patients with EC compared with that in healthy individuals. Additionally, soluble HLA-G5 molecules are more frequently observed than membrane-bound HLA-G1 molecules in patients with EC (67). Notably, the level of soluble HLA-G is higher in the early stages of EC compared with that in high-grade EC (67).

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1). RCAS1 is expressed on immune cells and serves as a ligand for a receptor of RCAS1 present on various human cell lines and normal peripheral lymphocytes such as T, B and NK cells (68,69). In hepatocellular carcinoma and pancreaticobiliary cancers, high levels of RCAS1 result in aggressive tumor behavior in humans, widely invasive type more frequently overexpressed RCAS1 than the minimally invasive type, and the incidences of RCAS1 overexpression increased with carcinoma dedifferentiation (70). Sonoda *et al* (71) was the first to report that the expression levels of RCAS1 were higher in endometrioid adenocarcinoma than in the normal and hyperplastic endometrium, and that RCAS1 exhibited significantly higher expression in grade III tumors than in grade I or II tumors. By contrast, RCAS1 expression is independent of clinical stage, myometrial invasion status, lymph-vascular space invasion and LNM in EC (71). Subsequently, the same authors (72) studied 147 patients with uterine EC and demonstrated that RCAS1 was expressed in 106 patients. Furthermore, 30/147 patients exhibited RCAS1 overexpression, which was positively associated with age at surgical resection, cancer stage, extent of myometrial invasion and positive peritoneal cytology results. Additionally, RCAS1 expression and metastasis were clinically

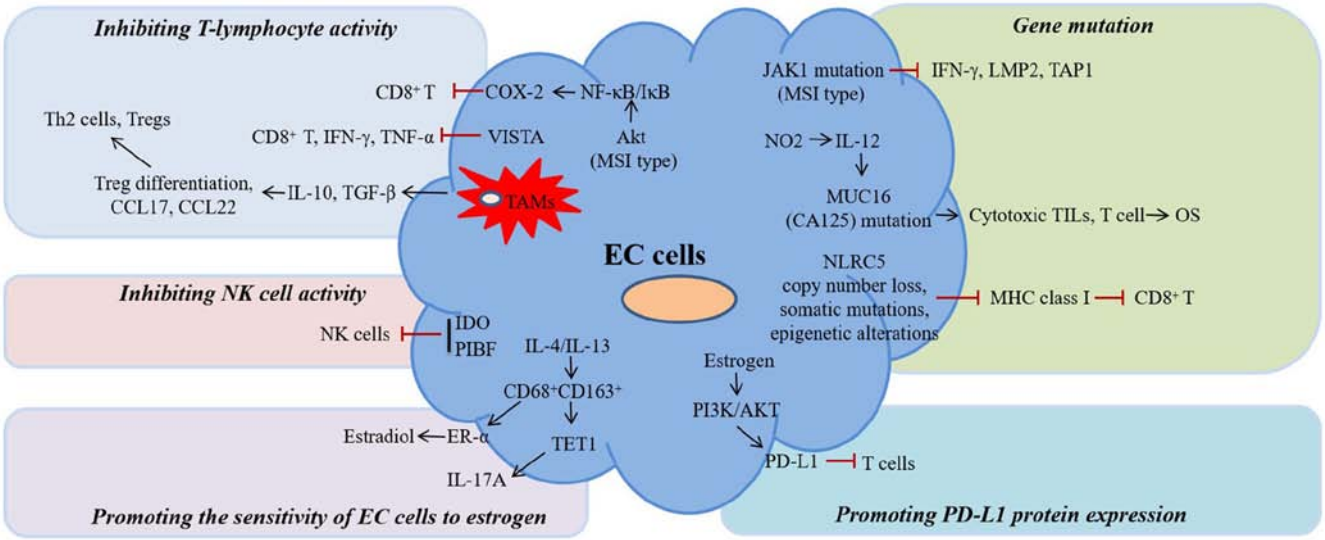

Figure 1. Mechanisms of immune evasion in EC. The mechanisms mainly include the inhibition of T-lymphocyte activity by the AKT/NF-κB/IκB/COX-2 signaling pathway, VISTA and TAMs; the inhibition of NK cell activity by IDO and PIBF; gene mutation (JAK1, MUC16 and NLRC5); the promotion of EC cell sensitivity to estrogen by IL4/IL13-induced $CD68^+CD163^+$ macrophages; and the promotion of PD-L1 protein expression by estrogen. CTL, cytotoxic T lymphocyte; EC, endometrial cancer; ER-α: estrogen receptor-α; JAK1, Janus kinase 1; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; MSI, microsatellite instability; MHC, major histocompatibility complex; NK, natural killer; NLRC5, NLR family, caspase recruitment domain-containing 5; PD-1, programmed cell death-1; PD-L1, PD-1 ligand 1; Treg, regulatory T cell; Th, T helper cell; IFN, interferon; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; IκB, inhibitor of κB; COX-2, cyclooxygenase-2; MUC16, mucin 16; TIL, tumor-infiltrating lymphocyte; OS, overall survival; PI3K, phosphoinositide 3-kinase; TAM, tumor-associated macrophage; VISTA, V-set immunoregulatory receptor; LMP-2, latent membrane protein 2; TAP-1, Transporter-1, ATP-binding cassette subfamily B member; PIBF, progesterone-induced blocking factor.

significant predictors of OS according to multivariate analysis(72). Recently, Szubert *et al* (73) suggested that high levels of RCAS1 in post-surgery serum are an independent p redictor of shortened OS time in patients with EC.

Cyclooxygenase-2 (COX-2). COX is the rate-limiting enzyme in the synthesis of prostaglandins. COX exists both as a constitutively expressed isoform (COX-1) and a regulated isoform (COX-2) (74). COX-2 enables tumor cells to escape immunological surveillance (75,76). Ohno *et al* (77) studied 70 patients with EC and proposed that COX-2 overexpression was positively associated with EC stage and myometrial invasion status. There was also an inverse association between the levels of COX-2 and the frequency of $CD8^+$ T cells in tumor cells. Furthermore, univariate analysis indicated that COX-2 levels were predictive of EC recurrence (77). A previous study demonstrated that patients with MSI-positive EC and high COX-2 expression had a worse prognosis than patients with MSI-positive EC and low COX-2 expression (78).

Fas and Fas ligand (FasL). Fas (CD95) and its ligand FasL are expressed in various types of cancer and have been implicated in immune evasion mechanisms in cancer cells (79). In a previous study, Fas mRNA expression was markedly lower in EC tissue, compared with that in normal endometrial tissue. However, no significant difference in FasL mRNA expression was detected (80). Jia *et al* (81) reported that Fas expression was significantly lower in tumor-infiltrating DCs and significantly higher in endometrioid adenocarcinoma than in the normal endometrium, resulting in tumor immune escape (81).

Survivin. Immune responses to survivin have been described in several types of tumors (82,83). Survivin upregulation in patients with EC leads to the inhibition of apoptotic proteins and promotes multi-drug resistance. High levels of survivin have been suggested to be an independent prognostic factor of EC associated with poor PFS time (84,85). Furthermore, survivin downregulation by curcumin-loaded amphiphilic mixed micelles has been demonstrated to improve immunochemotherapy in EC (86).

Interleukin-6 (IL-6). IL-6 is a pro-inflammatory cytokine that is involved in the modulation of the immune response (87). Bellone *et al* (88) reported that IL-6 mRNA expression was significantly upregulated in uterine serous papillary carcinoma. Furthermore, IL-6 expression levels were significantly higher in patients with EC and uterine papillary serous carcinoma than in healthy females (88). The molecular targets described in this section are summarized in Table II.

3. Mechanisms of immune evasion in EC

The mechanisms of immune evasion in EC can be broadly grouped into five categories: i) Gene mutations; ii) inhibition of T lymphocyte activity; iii) inhibition of NK cell activity; iv) promotion of PD-L1 protein expression; and v) promotion of EC cell sensitivity to estrogen (Fig. 1). However, the actual mechanisms of immune evasion in EC are likely more complex. The available evidence is reviewed in the following sections.

Gene mutations. Ren *et al* (89) detected 50 Janus kinase 1 (JAK1)-truncating mutations in 5.67% of gynecological tumors, using the Total Cancer Care® tumor bank resource. Furthermore, frame-shift mutations at K142, P430 and K860 have been suggested to be hotspot mutation sites. Functional

Table II. Immunosuppressive microenvironment in EC: Molecular target approach.

Molecular target	Regulation	Specimen	Clinical application	Refs.
PD-1/ PD-L1/ PD-L2	Up	Tumor tissue	High PD-L1 is an independent adverse prognostic factor for PFS in all patients and in the MSI subgroup. Immune marker ratios indicate independently shorter PFS times for high PD-L1/CD8 and PD-L1/PD-1 ratios. Therapeutic strategies targeting the immune checkpoint inhibitors PD-1/PD-L1 are in clinical application, and a number of clinical trials are underway.	(47-49)
B7-H3/ B7-H4	Up	Tumor tissue	B7-H3 expression is associated with EC cells and TILs; overexpression of B7-H3 in EC cells is associated with shortened OS times. B7-H4 expression is independent of grade, histology and immune cell infiltration in EC. Inhibiting B7-H3/B7-H4 represents a promising treatment in EC, and clinical trials targeting B7-H3/B7-H4 are in progress.	(52-54)
IDO	Up	Tumor tissue	In EC, high IDO expression is positively associated with surgical stage, myometrial invasion, lymph-vascular space involvement and LNM. Patients with high IDO expression have significantly impaired OS and PFS time. IDO expression is an independent prognostic factor for PFS. Inhibiting IDO represents promising treatment in EC, and the clinical trials targeting IDO are in progress.	(56-59)
HLA	HLA-A/B/C, down; HLA-G, up	Blood and tumor tissue	Patients with grade-III EC express more soluble HLA-G than patients with low grade. HLA-G5 is only expressed in high-grade EC as well as in early stages, suggesting that inhibiting HLA-G is a promising treatment in EC, but no clinical trials targeting HLA-G in EC are under investigation. Patient HLA type may influence immunotherapy.	(63-67)
RCAS1	Up	Blood and tumor tissue	RCAS1 overexpression is associated with age at surgery, stage, extent of myometrial invasion and positive peritoneal cytologic results. RCAS1 expression is a clinically significant prognostic factor for OS. High post-surgery serum RCAS1 levels are an independent indicator of shortened OS time in patients with EC. Inhibiting IDO may be a promising treatment in EC. No clinical trial involving RCAS1 is currently in progress.	(71-73)
COX-2	Up	Tumor tissue	COX-2 is associated with EC FIGO stage and myometrial invasion, and COX-2 is a significant predictor of disease relapse. The prognosis is poorer in patients with MSI-positive EC with high COX-2 expression, compared with that in those with low COX-2 expression. A clinical trial using the COX-2 inhibitor celecoxib is currently in progress.	(77,78)
Fas/FasL	Fas, down; FasL, up	Tumor tissue	The role of Fas/FasL in tumor immune escape suggests a promising treatment in EC. However, no clinical trial involving Fas/FasL is currently underway.	(80,81)
Survivin	Up	Tumor tissue	Survivin is an independent prognostic factor in EC, suggesting that the inhibition of survivin could be a promising treatment option for EC. However, no clinical trial investigating survivin in EC is currently underway.	(84-86)
IL-6	Up	Blood	IL-6 levels represent a promising marker in tumor immunotherapy, and inhibiting IL-6 provides a promising immunotherapy approach for EC.	(88)

EC, endometrial cancer; PFS, progression-free survival; OS, overall survival; HLA-E, human leukocyte antigen E; PD-1, programmed cell death-1; PD-L1/2, PD-1 ligand 1/2; MSI, microsatellite instability; TILs, tumor-infiltrating lymphocytes; IDO, indoleamine 2,3-dioxygenase; RCAS1, receptor-binding cancer antigen expressed on SiSo cells; COX-2, cyclooxygenase 2; FIGO, Federation of Gynecology and Obstetrics; FasL, Fas ligand; IL-6, interleukin 6.

experiments revealed cancer cells lacking JAK1 in the interferon-γ-driven induction of low-molecular weight protein-2 of the proteasome and the MHC class I (MHC I) pathway protein transporter associated with antigen processing-1

expression (89). Furthermore, the loss of low-molecular weight protein-2 and transporter associated with antigen processing-1 inhibits the presentation of tumor antigens and contributes to tumor immune evasion in EC (89). Recently, Albacker *et al* (90) demonstrated that JAK1 frameshift mutations were associated with high tumor mutation burden and MSI mutations in EC. Furthermore, endometrial and stomach adenocarcinomas with JAK1 frameshift mutations displayed low interferon response levels and an increase in antitumor immunological reactions. These results suggested that mutations in JAK1 were associated with the loss of interferon response and tumor immune escape in MSI-type EC (90).

Mucin 16, cell surface-associated (MUC16), also known as CA125, is a diagnostic serum marker and an indicator of adverse prognosis in gynecological cancer types (91). Recently, the MUC16 gene was found to be frequently mutated in EC. Patients with EC harboring somatic MUC16 mutations had longer OS times compared with non-MUC16 mutated patients with EC. In addition, MUC16 mutations promoted antitumor immune responses in these patients. Furthermore, the upregulation of the NO_2-dependent IL-12 signaling pathway indicated the higher rate of MUC16 mutations in NK cells and some surface proteins in CTLs. These patients also had significantly longer survival times. In addition, patients with EC harboring MUC16 mutations have an elevated level of cytotoxic TILs, thereby rescuing T cell antitumor immunity in the EC microenvironment as well as prolonging OS (92).

MHC I molecules inhibit cancer by activating the immune response, and MHC I inhibition leads to cancer immune evasion (93). Nucleotide oligomerization domain-like receptor (NLR) family, caspase recruitment domain-containing 5 (NLRC5) has recently been recognized as a crucial transcriptional coactivator of MHC I expression (94). Yoshihama *et al* (95) revealed that copy-number loss-, somatic mutation- and epigenetic alteration-mediated NLRC5 downregulation was associated with the expression of MHC I molecules and cytotoxic T cell markers in uterine cancer. Furthermore, overexpression of NLRC5 contributes to the activation of $CD8^+$ CTLs and patient survival in uterine cancer (95).

Inhibition of T-lymphocyte activity. COX-2 upregulation suppresses antitumor immunity in several types of cancer (96,97). High levels of COX-2 are implicated in immunosuppression in EC. Ohno *et al* (77) reported that COX-2 attenuated the infiltration of $CD8^+$ T cells into the EC tumor milieu, thereby allowing cancer cells to escape immunosurveillance. St-Germain *et al* (98) demonstrated that AKT signals induce COX-2 expression via the activation of the NF-κB/IκB signaling pathway in EC (98).

V-domain Ig suppressor of T cell activation (VISTA) is a newly identified immune checkpoint inhibitory molecule. VISTA is overexpressed in EC. Upregulation of VISTA in EC suppresses T cell proliferation and IFN-γ and tumor necrosis factor-α expression *in vitro*, and reduces the number of tumor-infiltrating $CD8^+$ T cells *in vivo* (99). Furthermore, treatment with allophycocyanin-conjugated anti-VISTA antibody prolongs the survival of tumor-bearing mice (99).

TAMs serve an important role in immunosuppression mechanisms in EC (100). A previous study demonstrated that TAMs could mediate immune suppression by inhibiting T-cell activation and promoting the expression of immunosuppressive cytokines, as well as C-C motif chemokines 17 and 22 (101).

Inhibition of NK cell activity. Yoshida *et al* (102) demonstrated that, in a mouse xenograft model of EC, tumor growth was faster in nude mice bearing IDO-overexpressing xenografts than in control mice. Splenic NK cell counts were lower in mice bearing an IDO-overexpressing xenograft compared with those in control xenografted mice. Furthermore, IDO-overexpressing cell cultures greatly decreased the lytic activity of NK cells in nude mice. Oral administration of the IDO inhibitor 1-methyl-D-tryptophan and paclitaxel to mice bearing an IDO-overexpressing xenograft promoted the antitumor effect of paclitaxel and led to markedly longer survival times. These results indicated that IDO overexpression in human EC cells may lead to tumor development *in vivo* by restricting NK cell activity (102). In addition, Check and Cohen (103) demonstrated that progesterone-induced blocking factor (PIBF) may be associated with the production of an immunomodulatory protein through the inhibition of NK cell cytotoxicity in cancer (103). Nevertheless, the role of PIBF in EC needs to be studied further.

Promotion of PD-L1 protein expression. Yang *et al* (104) reported that 17β-estradiol could increase the levels of PD-L1 by activating the PI3K/AKT signaling pathway in ER-α-positive Ishikawa cells. In a co-culture of Ishikawa cells and T cells, the expression levels of interferon-γ and IL-2 were downregulated, and the expression levels of B cell lymphoma 2 were upregulated following treatment with 17β-estradiol, suggesting that ER-α-positive EC cells inhibit T-cell function by promoting PD-L1 expression in the tumor microenvironment (104).

Promotion of EC cell sensitivity to estrogen. Ning *et al* (105) demonstrated that IL-4- and IL-13-induced $CD68^+CD163^+$ macrophages enhance the effects of estradiol on EC cell proliferation by upregulating ER-α. The infiltrating $CD68^+CD163^+$ macrophages upregulate the expression levels of inflammatory cytokines, such as IL-17A, through ten-eleven translocation 1-mediated epigenetic modulation. The increased estrogen sensitivity of the EC cells stimulates cancer cell proliferation by activating the PI3K/AKT signaling pathway.

4. Immunotherapy for EC

Based on the findings on the immunosuppressive microenvironment and the mechanisms of immune evasion in EC, several immunotherapeutic strategies were explored in EC. These approaches can be broadly subdivided into three categories: i) Anticancer vaccines; ii) immune checkpoint inhibitors; and iii) immunomodulatory agents (Fig. 2).

Anticancer vaccines. Vaccine-based treatments for cancer are designed with the aim of activating immune responses against tumor antigens. In a phase I trial by Kaumaya *et al* (106), which enrolled 24 patients with metastatic cancer (5 with breast cancer, 5 with ovarian cancer, 5 with colorectal cancer, 2 with EC, 1 with cervical cancer, 1 with pancreatic cancer, 1 with adrenal cancer, 1 with gastrointestinal stromal cancer,

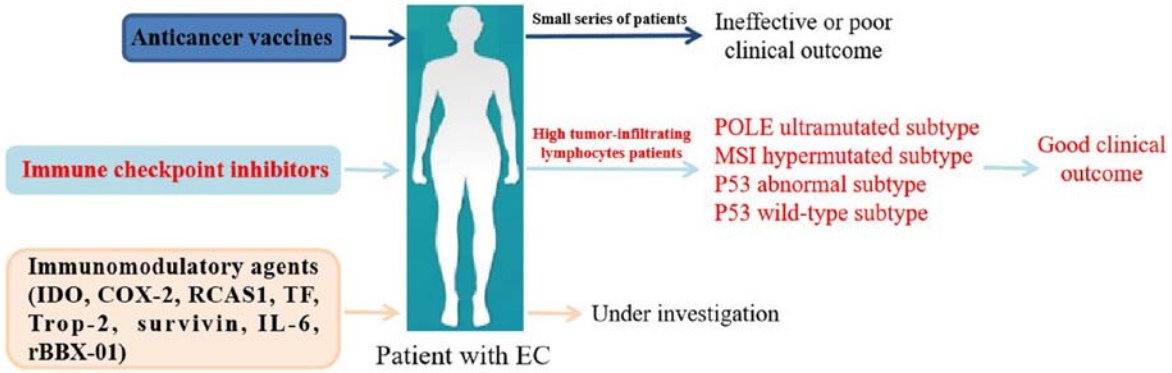

Figure 2. Immunotherapy for EC. Anticancer vaccines are largely limited to a small number of patients, and the clinical outcomes are ineffective or poor. Immune checkpoint inhibitors have been widely investigated in EC; at present, it is suggested that patients with the POLE-ultramutated and MSI-hypermutated phenotypes, p53 abnormalities and p53 wild-type EC, with high tumor-infiltrating lymphocytes, have good clinical outcomes to immune checkpoint inhibitors. Immunomodulatory agents regulate IDO, COX-2, RCAS1, TF, Trop-2, survivin, IL-6 and rBBX-01 in EC and are currently under investigation. COX-2, cyclooxygenase-2; EC, endometrial cancer; IDO, indoleamine 2,3-dioxygenase; IL-6, interleukin-6; MSI, microsatellite instability; POLE, polymerase ε; RCAS1, receptor-binding cancer antigen expressed on SiSocells; rBBX-01, a recombinant form of the Eimeria protein; TF, tissue factor, Trop-2, trophoblast-cell surface marker.

1 with leiomyosarcoma, 1 with non-small cell lung cancer and 1 with an unspecified squamous cell cancer), a dose escalation (range, 0.5-3.0 mg) study was designed with a combination vaccine. The vaccine was a mixture of two chimeric, human epidermal growth factor receptor 2 B cell epitopes fused to a promiscuous T cell epitope. After receiving three inoculations of the intended dose, 62.5% of patients raised an antibody response with no serious adverse events, autoimmune disease or cardiotoxicity. These results suggested that the peptide vaccine safely induced the generation of IgG antibodies in a population of patients who have metastatic disease, including EC (106). Nonetheless, to date, immunotherapeutic approaches, such as the use of vaccines in patients with EC (107,108), are largely limited to a handful of patients owing to the prognostic relevance of TILs, data related to TILS remains controversial with a higher level of TILs associated to low grade lesions by some authors (21) and to a high grade by others (22).

Immune checkpoint inhibitors. As aforementioned, PD-1 and PD-L1 are frequently expressed in EC. Overexpression of PD-1 and PD-L1 inhibits the activation of tumor-infiltrating $CD4^+$ and $CD8^+$ T cells in the EC microenvironment (48). Previous studies have indicated that POLE-ultramutated EC and MSI-hypermutated EC exhibit high neoantigen expression and a high number of TILs, as well as upregulation of PD-1 and PD-L1 (109). Therefore, targeting the PD-1 signaling pathway is a potentially useful approach to accelerate the antitumor immune response in the POLE-ultramutated and MSI-hypermutated subtypes of EC. Mehnert *et al* (110) described the case of a 53-year-old patient who presented with irregular vaginal bleeding and was subjected to a hysterectomy as a result of the diagnosis of a pT1b, pN0, stage IB, Federation of Gynecology and Obstetrics (10) grade III endometrial adenocarcinoma of the high-grade endometrioid type, with extensive necrosis, lymphovascular invasion and myometrial invasion. Furthermore, the primary tumor tissue and LNM were subjected to genomic profiling and both samples exhibited POLE mutations (110). The patient was then enrolled in a phase Ib trial (NCT02054806). In the phase Ib trial, 24 patients with advanced and PD-L1-positive EC were administered an intravenous humanized monoclonal antibody targeting PD-1 (pembrolizumab) at 10 mg/kg every 2 weeks for up to 24 months. Among the 24 patients, 3 patients with EC had a partial clinical response, while stable disease was observed in 2 patients with EC. Furthermore, the overall response rate was 13%. The 6-month PFS and OS rates were 19.0 and 68.8%, respectively. Only mild adverse effects, such as fatigue, pruritus, pyrexia and anorexia, were observed in 54.2% of patients (111). In a phase II trial involving pembrolizumab, objective response rates reached 71% in 2 patients with MSI^{high} EC (112). Recently, Makker *et al* (113) conducted a study involving 54 patients with EC (unscreened for MSI or PD-L1 expression) and analyzed 53 patients in an open-label, single-arm, phase 2 study (NCT02501096). In this study, 21 patients had an objective response, with an acceptable safety profile at week 24 after they were administered 20 mg oral lenvatinib daily, plus 200 mg intravenous pembrolizumab every 3 weeks. However, an increased frequency of hypothyroidism was observed (113). Other humanized antibodies of checkpoint inhibitors targeting PD-1, such as atezolizumab, durvalumab, tremelimumab, ipilimumab and nivolumab, were also studied in EC with a POLE-ultramutated and MSI-hypermutated phenotype. A significant clinical response was observed in these clinical trials (111,113-116). These clinical trials involving immunotherapy for EC are available at https://clinicaltrials.gov, and are listed in Table III.

Patients with Lynch syndrome-associated MSI^{high} EC exhibit a lower response rate to single anti-PD-1 or anti-PD-L1 therapy than patients with sporadic MSI^{high} EC (117). These data suggested that clinical trials assessing the effect of immunotherapy in patients with EC must assess Lynch-related and sporadic MSI^{high} EC independently (117). However, POLE-ultramutated status has been revealed as an early and, possibly, initiating event in EC (118). These results provide a strong theoretical basis for further research of checkpoint inhibitors in EC with a POLE-ultramutated and MSI-hypermutated phenotype (119). Notably, a recent study by Talhouk *et al* (120) revealed that TIL^{high} tumors harbor dense T- and B-lineage infiltrates and multiple immunosuppressive

Table III. Ongoing and completed clinical trials involving immunotherapy in EC.

Study title	Brief study description	Intervention	Phase	Status	Enrollment, n	NCT number	Refs.
Safety and immune response to a multi-component immune-based therapy (mkc1106-pp) for patients with advanced cancer	A dose comparison of a multi-component active immunotherapy designed to stimulate an immune reaction to specific tumor-associated antigens that are highly expressed on a large number of solid tumors	PSMA/PRAME	I	Completed	12	NCT00423254	NA
Screening of biomarkers on endometrial cancers	To evaluate the role of mesothelin and elucidate the potential of mesothelin as a target antigen for immunotherapy	Surgery	NA	Unknown	250	NCT00674349	NA
Immunotherapy using TILs for patients with metastatic cancer	To select a specific subset of white blood cells from the tumor and to test whether selected cells can cause digestive tract, urothelial, breast or ovarian/endometrial tumors to shrink, and to see if this treatment is safe	Young TIL + aldesleukin + cyclophosphamide + fludarabine + pembrolizumab	II	Recruiting	332	NCT01174121	115
Tisotumab vedotin (humax®-tf-adc) safety study in patients with solid tumors	To establish the tolerability of Tisotumab vedotin in a mixed population of patients with specified solid tumors	Tisotumab vedotin (HuMax-TF-ADC)	I, II	Completed	195	NCT02001623	116
Study of pembrolizumab (mk-3475) in participants with advanced solid tumors (mk-3475-028/keynote-28)	To assess the efficacy and safety of pembrolizumab (MK-3475) administered to participants with incurable advanced biomarker-positive solid tumors that have not responded to current therapy or for which current therapy is not appropriate	Pembrolizumab	I	Active, not recruiting	477	NCT02054806	112
Phase 1b/2 trial of lenvatinib (e7080) plus pembrolizumab in subjects with selected solid tumors	To determine and confirm the maximum tolerated dose for lenvatinib in combination with pembrolizumab in participants with selected solid tumors and evaluate the safety and efficacy of the combination	Lenvatinib + pembrolizumab	I, II	Recruiting	360	NCT02501096	114
Mk-3475 immunotherapy in endometrial carcinoma	To evaluate the mechanism of action of pembrolizumab (MK-3475) on the endometrial cancer tumor environment	Pembrolizumab	I	Active, not recruiting	10	NCT02630823	NA
Pilot study of durvalumab and Vigil in advanced women's cancers	To determine the effects of Vigil and durvalumab in advanced women's cancers	Vigil + durvalumab	II	Enrolling by invitation	15	NCT02725489	NA
A phase ii of nivolumab plus ipilimumab in non-resectable sarcoma and endometrial carcinoma	To determine whether nivolumab plus ipilimumab are effective and safe in the treatment of sarcoma and endometrial carcinoma patients with somatic deficient MMR as a selection tool	Ipilimumab + nivolumab	II	Not yet recruiting	60	NCT02982486	NA

Table III. Continued.

Study title	Brief study description	Intervention	Phase	Status	Enrollment, n	NCT number	Refs.
Study of pembrolizumab, radiation and immune modulatory cocktailin cervical/ uterine cancer	To discover the antitumor immune response by a combination of PD-1 blockade, radiation and immune/environmental -targeting compounds in cervical and uterine cancer	Pembrolizumab + radiation + vitamin D + aspirin + lansoprazole + cyclophosphamide + curcumin +	II	Recruiting	43	NCT03192059	117
Durvalumab, radiotherapy in gynecological cancer	To evaluate the safety and efficacy of Durvalumab and Tremelimumab in combination with radiation therapy as a possible treatment for recurrent or metastatic gynecological cancer	Durvalumab + tremelimumab + radiation	I	Recruiting	32	NCT03277482	NA
Multiorgan metabolic imaging response assessment of abemaciclib	A screening program for Abemaciclib efficacy in multiple platinum-resistant tumor types by using metabolic imaging (PERCIST) and RECIST v1.1 criteria	Abemaciclib	II	Recruiting	85	NCT03339843	NA
Synergy-AI: artificial intelligence-based precision oncology clinical trial matching and registry	To evaluate the feasibility and clinical utility of an Artificial Intelligence-based precision oncology clinical trial matching tool, powered by a virtual tumor boards program, and its clinical impact on patients with advanced cancer to facilitate clinical trial enrollment, as well as the financial impact, and potential outcomes of the intervention	Clinical Trial Matching	NA	Recruiting	1,500	NCT03452774	NA
Lenvatinib in combination with pembrolizumab versus treatment of physician's choice in participants with advanced endometrial cancer (mk-3475-775/e7080-g000-309 per merck standard convention (keynote-775)	To evaluate the treatment outcome between pembrolizumab (MK-3475) in combination with lenvatinib and doxorubicin or paclitaxel	Pembrolizumab + lenvatinib + paclitaxel + doxorubicin	III	Recruiting	780	NCT03517449	NA
Atezolizumab trial in endometrial cancer-attend	To determine the effects of atezolizumab in advanced endometrial cancer	Atezolizumab + placebos + paclitaxel + carboplatin	III	Recruiting	550	NCT03603184	NA

Table III. Continued.

Study title	Brief study description	Intervention	Phase	Status	Enrollment, n	NCT number	Refs.
A study to evaluate the safety and tolerability of immunotherapy combinations in participants with advanced malignancies	To evaluate the safety, tolerability, pharmacokinetic, pharmacodynamic and clinical activity of AB928 in combination with AB122 (an anti-PD-1 antibody) in participants with advanced malignancies	AB928 + AB122	I	Recruiting	58	NCT03629756	NA
A phase 2 study of mirvetuximab soravtansine (imgn853) and pembrolizumab in endometrial cancer	To evaluate Mirvetuximab Soravtansine (IMGN853) and Pembrolizumab in combination as a possible treatment for endometrial cancer	Pembrolizumab + IMGN853	II	Not yet recruiting	35	NCT03835819	NA
Pembrolizumab in addition to paclitaxel and carboplatin for treating patients with stage iii-iv or recurrent endometrial cancer	To evaluate how well the combination of pembrolizumab, paclitaxel and carboplatin works compared with paclitaxel and carboplatin alone in treating patients with endometrial cancer that is stage III or IV, or has come back (recurrent)	Carboplatin + paclitaxel + pembrolizumab + placebo	III	Not yet recruiting	810	NCT03914612	NA
Frontline immunotherapy combined with radiation and chemotherapy in high-risk endometrial cancer	To evaluate the feasibility of pembrolizumab combined with radiation administered to the upper part of the vagina (vaginal cuff brachytherapy) followed by three cycles of pembrolizumab and chemotherapy in patients with endometrial cancer	Pembrolizumab + radiation	I	Not yet recruiting	20	NCT03932409	NA

AB, Arcus Biosciences; EC, endometrial cancer; MMR, mismatch repair; NA, not applicable; NCT, national clinical trial; PSMA, prostate-specific membrane antigen; PRAME, preferentially expressed antigen of melanoma; TIL, tumor-infiltrating lymphocytes.

features, and are more common among the molecular subtypes of EC associated with high mutation load (MMR and POLE) than ECs with a low mutation load (p53 abnormalities and p53 wild-type). Furthermore, TIL^{low} tumors are generally devoid of immunological features and are more prevalent in ECs harboring p53 abnormalities and wild-type p53, although they are also seen in MMR and POLE subtypes. Additionally, in multivariate models involving a ProMisE subtype, T-cell markers and TIL clusters, only ProMisE was associated with independent prognostic significance (120). These findings suggested that the assessment of an immune response rather than the molecular subtype may better predict a clinical response to immunotherapy (120,121). Nevertheless, as aforementioned, targeting the PD-1 signaling pathway has great potential in enhancing antitumor immune responses, as accumulating evidence demonstrates that antitumor functions are induced following PD-1 and PD-L1 engagement in cancer cells *in vivo* (50,112-117). These multifunctional PD-1 signaling pathways need to be made available for the treatment of additional types of cancer.

Immunomodulatory agents in EC. Immunomodulatory agents, other than immune checkpoint inhibitors, may emerge as promising therapeutic agents in the future. Mills *et al* (59) demonstrated that IDO levels were upregulated in endometrial carcinoma and diffuse staining was principally more common in MMR-deficient cancer, particularly Lynch syndrome-associated cases, suggesting that targeting IDO may be a promising treatment approach for MMR-deficient endometrial carcinoma.

Other immunotherapies currently under investigation for the treatment of EC involve immunomodulatory agents, such as COX-2 (122), RCAS1 (123), tissue factor (124), human trophoblast-cell surface marker (125), survivin (126), IL-6 (127) and rBBX-01 (128). These targets are still in preclinical or early clinical development and have achieved promising clinical results, indicating that these immunotherapies are potential strategies in the treatment of EC.

5. Conclusions and future perspectives

The aforementioned research efforts, which aimed to investigate the role of the immune response in EC, including the immunosuppressive microenvironment, immune evasion mechanisms and immunotherapy, offer a strong rationale for immunotherapeutic approaches in the treatment of EC. Indeed, the use of checkpoint inhibitors and cancer vaccines for EC treatment has yielded good clinical outcomes. Although key milestones have been reached, numerous efforts have proven ineffective and the efficacy of immunotherapy in EC needs to be further demonstrated in the future.

Firstly, the induced immunosuppressive microenvironment in EC is influenced by multiple factors, including immune cells, immune checkpoint inhibitors, and immunomodulatory agents and their interactions. Therefore, it is necessary to understand the manner in which the tumor immunosuppressive microenvironment can be modulated to enhance the immune response against EC. Furthermore, significant knowledge gaps need to be filled through the use of molecular, cellular and structural biology approaches, in order to identify appropriate targets for immune cells, immune checkpoint inhibitors and immunomodulatory agents in immunotherapeutic applications.

Secondly, the exact immunosuppressive or immune evasion mechanisms involved in EC remain to be determined. Indeed, only a few candidate neoantigens selected by the current neoantigen-prediction algorithms trigger an antitumor response. Mechanisms of immunosuppression and immune evasion proposed on the basis of preclinical studies on immune checkpoint inhibitors and immunomodulatory agents represent a promising clinical application. However, these have yet to be tested on patients in EC, with the results pending for numerous clinical trials.

Lastly, a single immunotherapy approach may be ineffective against advanced metastatic or recurrent EC. Evidence from preclinical research indicates that combinatorial modalities, targeting different facets of the immune response, lead to improved therapeutic efficacy; early clinical studies suggest that such therapeutic approaches can be more effective. However, side effects must be considered when using such combinations. The sequence, timing, dosage and choice of drugs should be designed well to achieve optimal antitumor and minimal off-target side effects. Furthermore, a combinatorial therapy approach may be appropriate to synergize the effects of conventional therapies and immunotherapy against EC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LZ wrote the manuscript and drafted the figures. XL and JZ revised the manuscript. YC and BW reviewed and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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