Molecular Targeted Therapy in Endometrial Cancer: Basis and Therapeutics

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Abbreviations

AKT	Protein kinase B
AMPK	Activated mitogen protein kinase
ARID1A	AT-rich interactive domain 1A
ARID5B	AT-rich interactive domain 5B
BER	Base excision repair
BRCA	Breast cancer type
CDK	Cyclin-dependent kinase
CHK-1	Checkpoint kinase 1
COX-2	Cyclooxygenase 2
CTNNB1	Catenin beta-1
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ERα	Estrogen receptor α
ERBB	Erythroblastic leukemia viral oncogene
FBXW7	F-box and WD repeat domain containing protein 7
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GOG	Gynecologic oncology
HER-2	Human epidermal growth factor 2
HR	Homologous recombination
IGF-1	Insulin growth factor-1
IGF-1R	Insulin growth factor-1 receptor
IgG	Immunoglobulin G

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JAK	Janus kinase
K-RAS	Kirsten rat sarcoma
MLH-1	MutL homolog 1
MMR	Mismatch repair
MSI	Microsatellite instability
MSH-2	MutS protein homolog 2
MSH-6	MutS homolog 6
MSS	Microsatellite stable
mTOR	Mammalian target of rapamycin
PARP	Poly-ADP ribose polymerase
PD	Programmed death
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PDL	Programmed death ligand
PGE-2	Prostaglandin E2
PI3K	Phosphatidylinositol 3 kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit 1
PIP3	Phosphatidylinositol triphosphate
PMS-2	PMS1 Homolog 2, Mismatch Repair System Component
PPP2R1A	Protein phosphatase 2 scaffold subunit A alpha
PR	Progesterone receptors
PTEN	Phosphatase and tensin homolog
Raf	Rapidly accelerated fibrosarcoma
Ras	Rat sarcoma
RPL22	Ribosomal protein
SSB	Single strand breaks
STAT	Signal transducer and activator of transcription
TKI	Tyrosine kinase inhibitors
TNF-α	Tumor necrosis factor
TP53	Cellular tumor antigen p53
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

12.1   Introduction

Endometrial cancer (EC) is the third most common gynecologic cancer in India after cervical and ovarian cancer. In 2018, an estimated 13,328 new cases were diagnosed in India, and approximately 5000 deaths were attributed to this disease [1]. The increasing incidence in India and worldwide is thought to be related in part to the rise of obesity and diabetes. EC is going to become a more prominent health-care concern in the near future.

Majority of women with EC are diagnosed at an early stage, which carries an excellent outcome. However, women with advanced stage and those with recurrent disease have extremely poor outcomes, with 5-year survival rates close to 20% [2].

The mainstay of treatment for EC is surgery (staging with hysterectomy, bilateral salpingo-oophorectomy, and with or without lymphadenectomy). Adjuvant radiotherapy is considered for a subset of high-risk cases. Radiation decreases local recurrence rates but does not affect relapse at distant sites or increase overall survival (OS). Adjuvant chemotherapy is given for advanced stages. The most active systemic agents are platinum compounds, taxanes, and anthracyclines, all of which produce a response rate of 20–30% [3]. However, response rates for metastatic and recurrent disease are lower, and there are no standard second-line therapies. Only one new drug, pembrolizumab has been approved in the last three decades for recurrent/advanced EC, and that too for a very small patient subset. There is an urgent need for new therapeutic approaches. As in many cancers, one such approach could be studying the tumor biology of this disease with targeting of specific molecular pathways.

Advances in understanding of molecular events leading to EC and molecular classification of EC have generated new avenues for targeting the disease. Common agents include drugs that affect apoptosis, signal transduction, epigenetic modification, drug resistance, cell cycle progression, hormone receptor activity, and angiogenesis. This is the basis of PORTEC-4a (Postoperative radiation therapy in endometrial carcinoma) trial which is comparing standard vaginal brachytherapy with different adjuvant treatments based on the integrated molecular profile [4]. The trial is ongoing and final results are awaited. Several other studies are exploring the role of immunotherapy in combination with paclitaxel and carboplatin, the role of metformin, and the role of PARP inhibitors. Many of these strategies appear promising in the treatment of recurrent or advanced disease.

In this chapter we will discuss the potential molecular targets and their therapeutic possibilities for EC.

12.1.1 Types of Endometrial Cancers

Historically, endometrial adenocarcinoma was divided into two histologic categories—type I and type II, as originally described by Bokhman in 1983 (Table 12.1) [5].

Table 12.1 Histologic classification of endometrial cancer

	Type I	Type II
Phenotype	Younger age Obese	Older age Nonobese
Pathogenesis	Estrogen dependent	Estrogen independent
Histology	Endometroid	Non-endometroid
Prognosis	Good	Poor
Molecular alterations	KRAS, PTEN, MSI, PI3K/AKT	p53, HER-2, Aneuploidy

- Type I: These tumors account for 70–80% of all new cases. They are usually seen in younger, obese, and premenopausal women. They have endometrioid histology, are low grade, and are estrogen driven. Patients with type I endometroid adenocarcinoma have high rates of K-RAS and PTEN loss or mutations, as well as MSI.
- Type II: This subtype comprises non-endometroid histology, high grade, estrogen-independent tumors, that usually have a poor outcome. These tumors are seen in women of older age group. They have high rates of p53 mutations, may overexpress HER-2, and show aneuploidy.

Although useful in many ways, there are limitations to this classification. Recently, The Cancer Genome Atlas (TCGA) project in 2013 reclassified EC based on genomic, transcriptomic, and proteomic differences studied in 373 primary EC surgical specimens (Table 12.2). The four subtypes described as per TCGA are: (1) polymerase epsilon catalytic subunit (POLE) ultramutated, (2) microsatellite instability (MSI)-hypermutated, (3) copy number low (CNL)-microsatellite stable, and (4) copy number high (CNH)-serous-like [6].

Table 12.2 TCGA genomic characterization of endometrial cancer

	POLE ultramutated	MSI-hypermutated	Copy number low	Copy number high
Histological features	Endometroid, broad front invasion, peri-tumor lymphocytes	Endometroid, lymphovascular invasion, lower uterine segment involvement	Endometroid, low grade, squamous differentiation, ER/PR	Serous, mixed histology, grade 3, high nuclear atypia
Clinical features	Lower BMI, early stage	Higher BMI, Lynch syndrome	Higher BMI	Lower BMI, advanced stage
Prognosis	Good	Intermediate	Variable	Poor
Suggested treatment options	Immune checkpoint inhibitors	Immune checkpoint inhibitors	Hormonal therapy, mTOR inhibitors	Small molecule activators of p53, PARP inhibitors
Mutation frequency per megabase (Mb)	>100/Mb	100-10/Mb	<10/Mb	<10/Mb
Microsatellite stability	Mixed	Instable	Stable	Stable
Frequent molecular alterations	POLE, PTEN, PIK3CA PIK3R1, FBXW7, ARID1A, KRAS, ARID5B	PTEN, RPL22, KRAS, PIK3CA, PIK3R1, ARID1A	PTEN, CTNNB, PIK3CA, PIK3R1, ARID1A	TP53, PPP2R1A, PIK3CA

1. POLE-ultramutated: This subgroup is characterized by very high somatic mutation rate, endometrioid histology, and is associated with good prognosis. It makes up only 1% of recurrent disease. The most commonly seen mutations in this subgroup are PTEN, PIK3CA, PIK3R1, FBXW7, ARID1A, KRAS, and ARID5B.
2. MSI-hypermutated: This subgroup is characterized by MSI due to dysfunctional mismatch repair genes, and mostly have an endometrioid histology. This subgroup comprises around 25% cases of recurrent disease. The most commonly seen mutations in this subgroup are PTEN, RPL22, KRAS, PIK3CA, PIK3R1, and ARID1A.
3. CNL-microsatellite stable: This subgroup is characterized by lower mutation rates, microsatellite stable, low-grade tumors, with endometrioid histology. The most common mutations in this subgroup are PTEN, CTNNB1, PIK3CA, PIK3R1, and ARID1A.
4. CNH-serous like: This subgroup is characterized by the lowest mutation rates, serous-like histology, chromosomal instability, and worse prognosis. The most common mutations seen in this subgroup are TP53, PPP2R1A, and PIK3CA.

12.2 Therapeutic Strategies

12.2.1 Obesity and Anti-inflammatory Agents

Obesity is an important risk factor for EC. It is also associated with an increased risk of recurrence and mortality from EC. An excess adipose tissue in obesity may increase the risk of cancer development by a number of mechanisms, like chronic inflammation, dysregulation of sex hormones, insulin resistance, altered immune response, and abnormal secretion of cytokines. Adipose tissue is an endocrine organ, producing the enzyme aromatase which leads to increased production of estrone from androstenedione. The increased estrogen levels lead to direct stimulation of endometrial cells by activating estrogen receptor alpha (ERα). Hyperinsulinemia seen in obesity decreases the levels of sex hormone-binding globulin (SHBG) by inhibiting its production in the liver. Lower levels of SHBG result in elevation of bioavailable estrogens, thus stimulating endometrial cells. Secondly, hyperinsulinemia leads to decreased levels of insulin like growth factor (IGF)-binding proteins, which results in elevated levels of free IGF-1. IGF-1 receptors are present in endometrial tissue and have been shown to stimulate endometrial cell proliferation. The binding of IGF-1 receptor ligand leads to autophosphorylation and subsequent activation of multiple downstream signaling pathways. Of these, the most important is PI3K/AKT/mTOR pathway (to be discussed in detail below). There is inactivation of AMPK pathway, which is commonly seen in obesity. This further leads to hyperactivity of mTOR and tumorigenesis in the endometrium (Fig. 12.1) [7, 8].

Adipose tissue also secretes adipokines like leptin, and pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), and interleukins. These inflammatory agents cause hyperactivation of PI3K/AKT pathway and increased production
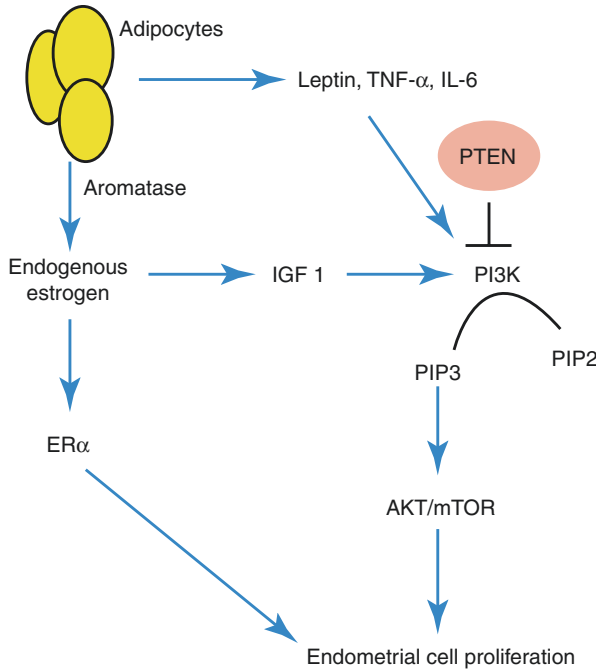
Fig. 12.1 Obesity and endometrial cancer. Adipose tissue produces pro-inflammatory adipokines leading to insulin resistance and stimulation of PI3K/AKT/mTOR pathway resulting in endometrial cell proliferation. Hyperestrogenism produced by peripheral conversion of androstenedione to estrogen by aromatase enzyme in adipocytes leads to direct stimulation of endometrial cells. *TNF* Tumor necrosis factor, *IL* interleukin, *PTEN* phosphatase and tensin homolog, *PI3K* phosphatidylinositol 3 kinase, *PIP2* phosphatidylinositol biphosphate, *PIP3* phosphatidylinositol triphosphate, *AKT* protein kinase B, *mTOR* mammalian target of rapamycin, *IGF* insulin growth factor, *ER* estrogen receptor

of COX-2 and PGE-2. Overexpression of COX-2 and PGE-2 has been linked to carcinogenesis, as they inhibit apoptosis, and promote angiogenesis [9]. In opposition to the pro-inflammatory adipokines, adiponectin reverses insulin resistance and acts as an anti-inflammatory agent. It inhibits tumor progression by inhibiting PI3K/AKT/mTOR signaling pathway [8]. Anti-inflammatory agents like aspirin and COX-2 inhibitors are therefore being investigated as possible therapeutic options in EC [10].

12.2.2 Hormonal Treatment

Hormonal therapy is not recommended routinely in the adjuvant setting, but it is still used for the management of recurrent and metastatic low-grade endometrioid EC. Megestrol acetate, a progestin that has been in use for over 40 years, was the first U.S. Food and Drug Administration (FDA) approved targeted therapy in

EC. However, the efficacy of megestrol acetate has been inconsistent, and according to a 2010 Cochrane review there has been no survival benefit for women receiving endocrine therapy for advanced EC [11]. The main predictors of response to hormonal agents are type I estrogen-dependent endometroid variety, well-differentiated histology, and expression of ER/PR receptors.

Various strategies have been tried to exploit the hormonal dependence of EC. Hormonal agents used for the treatment of EC have included systemic progestins (megestrol acetate, medroxyprogesterone acetate), levonorgestrel intrauterine device, selective estrogen receptor modulators (SERM) like tamoxifen, aromatase inhibitors (anastrozole, letrozole), and selective estrogen receptor downregulators (SERD) like fulvestrant. They can be used alone or in combination.

(A) *Single Agents*
  1. Progestins: These agents counter the hyperestrogenism associated with EC. Single-agent progestins have yielded overall response rates between 20 and 25%. Studies have suggested that ERα or PR expressing cancers are more likely to respond, although the overall data are still inconsistent [12]. Oral medroxyprogesterone acetate 200 mg/day has shown favorable results in well-differentiated, PR-positive advanced or recurrent EC [13]. High-dose megestrol acetate in advanced and recurrent EC showed a clinical response of 24%, but the responses were short-lived [14]. The short duration of response was attributed to the downregulation of the PR. Levonorgestrel releasing intrauterine device has been used in women with early-stage and low-grade endometrioid EC who want to preserve fertility [15]. The results have been encouraging and it is now the preferred treatment in women desiring fertility with grade 1, endometrioid EC, with disease limited to the endometrium.
  2. Aromatase inhibitors: In postmenopausal women, estrone produced by aromatase conversion of androstenedione is the main source of estrogen. Aromatase enzyme inhibitors—anastrazole and letrozole—have been used in EC, but with limited efficacy [16, 17]. They have shown some benefits in early-stage EC, but not in advanced or recurrent stage [18]. There is a need for newer generation aromatase inhibitors with fewer side effects and higher receptor specificity.
  3. Fulvestrant: It is the only compound among SERDs approved by the FDA for use in the treatment of EC. Phase I/II trials have been performed, and have shown a good tolerability profile. It may be clinically efficacious due to its pure estrogen antagonist properties. However, the reported response rates (RR) have been low [19]. Further trials are ongoing to validate the dosing and also to study its use in combination with mTOR inhibitors [20].
(B) *Combination strategies*
  1. Megestrol and tamoxifen: Tamoxifen increases the expression of progesterone receptors, thereby increasing the efficacy of megestrol acetate. This has been the basis of the GOG-153 study which evaluated the response of tamoxifen 20 mg twice daily every 3 weeks alternating with megestrol ace-

tate at 80 mg twice daily every 3 weeks. An RR of 27% was obtained with this strategy [21]. This combination also showed an increased durability of response, with more than half the responses lasting more than 20 months.

2. Hormonal agents and mTOR inhibitors: Hyperestrogenism leads to stimulation of PI3K/AKT/mTOR pathway (as explained above). Targeting this pathway has been proposed as a mechanism to overcome resistance to hormonal therapy. A phase II trial of everolimus (an mTOR inhibitor) and letrozole in patients of recurrent EC resulted in an objective RR of 32%, and median progression-free survival (PFS) of 3 months [22]. The limited response was due to incomplete blockade of mTOR complex by the mTOR inhibitors, and due to intra-pathway feedback loops. Another strategy to increase the efficacy of hormonal therapy efficacy would be to target multiple pathways. However, a phase II study of letrozole, everolimus, and metformin resulted in RR of only 29% [23]. Another study on the combination of temsirolimus (an mTOR inhibitor), megestrol, and tamoxifen, also reported an RR of only 14% and a high incidence of venous thromboembolism [24].

3. Hormonal agents and cyclin-dependent kinase (CDK) inhibitors: The combination of CDK4/6 inhibitors (to be discussed later) with hormonal therapy is a proven beneficial strategy in metastatic breast cancer. Elevated CDK4 expression has been seen in 34–77% of endometrioid EC [25]. Currently, studies are ongoing evaluating the role of palbociclib (CDK4/6 inhibitor) in combination with letrozole (NCT 02730429) and ribociclib (cyclin D1 and CDK4/6 inhibitor) in ER-positive advanced EC (NCT 02657928).

To summarize, hormonal therapy is recommended in recurrent or advanced stages of low-grade endometrioid EC, preferably in patients with small tumor volume or indolent growth rate. Medroxyprogesterone acetate, megestrol acetate, and levonorgestrel intrauterine device have also been recommended for hormonal treatment in women with EC desiring fertility (NCCN category IIA recommendations) [26].

12.2.3 Metformin

Metformin is an oral biguanide. It has recently gained importance as a potential anticancer agent in EC. There is epidemiological data suggesting that metformin use decreases the rate and risk of cancer deaths among diabetic patients [27, 28]. Studies have shown that metformin inhibits cellular proliferation and induces apoptosis, potentially by inhibiting the mTOR pathway. This is through the activation of AMPK and suppression of IGF-1/PI3K/AKT pathway. An indirect mechanism for metformin effect may be the inhibition of liver gluconeogenesis resulting in a decrease in insulin levels and reduced hyperglycemia. In vitro studies have also shown inhibition of EC cells treated with metformin [29, 30].

Various studies have shown improved OS and recurrence-free survival with metformin in diabetic EC patients, in combination with chemotherapy [31, 32]. Although some authors did not find any effect of metformin exposure on survival

parameters [33], a 2017 meta-analysis supports a higher OS rate in metformin users with EC compared to non-metformin users and nondiabetic patients [34]. Another meta-analysis of 28 studies reported that metformin use was associated with decreased all-cause mortality in patients with concurrent diabetes for several cancer types, including EC [35].

Currently, metformin is being evaluated in recurrent/advanced EC in combination with standard cytotoxic chemotherapy (NCT 02065687) and with hormonal and mTOR agents (NCT 01797523). Overall, studies have shown promise for metformin as an adjunctive treatment for EC. However, further studies are needed to define its exact therapeutic role in EC.

12.2.4 PTEN/PI3K/AKT/mTOR Pathway Inhibitors

The phosphatidylinositol 3 kinase (PI3K) pathway is the most frequently altered pathway in human tumors, and EC has a very high incidence of PI3K pathway alterations. Approximately 40% of serous EC and over 70% of endometrioid EC have PI3K pathway aberration [36]. PTEN located on chromosome 10 encodes a phospholipid called phosphatase and tensin homolog and acts as a tumor suppressor gene by inhibiting the PI3K signaling pathway. PTEN expression in endometrium is regulated by estrogen and progesterone levels in blood. PTEN inactivation has been implicated in the development of EC. Inactivation of PTEN usually occurs as a result of deletional or mutational events, and less by promoter methylation. When PTEN is suppressed, there is upregulation of PI3K and mTOR activity which leads to increased tumor cell proliferation, migration, and invasion.

PI3K signaling pathway is initiated through multiple receptor tyrosine kinases, including EGFR, HER-2, IGF-1R, VEGFR, PDGF, and Src family kinases. PI3K in turn activates protein kinase B, also called AKT. Through a series of downstream effectors AKT leads to activation of mTOR. mTOR is a serine-threonine protein kinase that ultimately triggers cell proliferation through several downstream moieties. It forms the core of two regulatory complexes—mTORc1 and mTORc2. As will be discussed later, everolimus and the newer mTOR inhibitors mainly target the mTORc1. PI3K inhibition potentially targets multiple aspects of tumor biology, including angiogenesis, inflammation, epithelial to mesenchymal transition, and metastasis. Its integral role in immune modulation may make PI3K inhibitors ideal partners for immune checkpoint inhibitors [37–39].

Rapamycin is an antibiotic derived from *Streptomyces hygroscopicus* and is known to inhibit the proliferation of endometrial cancer cells in vitro. The rapamycin analogs (rapalogs) used in clinical trials include everolimus, temsirolimus, deforolimus, and ridaforolimus [40–43]. These agents inhibit cytokine and growth-factor dependent cell proliferation through the inhibition of mTORc1. Increases in mTORc2 may be a means of therapeutic resistance to these agents. Thus efforts are underway to develop more potent dual mTORc1 and mTORc2 inhibitors.

Clinical experience with the rapamycin analogs has shown modest results. In a study of 54 patients, temsirolimus showed a response rate of 14% in

chemotherapy-naïve patients [44]. NCCN panel has recommended (level IIa evidence) temsirolimus for the treatment of EC patients who have progressed on previous chemotherapy [26].

Targeting the PI3K/AKT/mTOR pathway alone or in combination remains an active area of research in EC. A study is going on to examine the role of the PI3K inhibitor, copanlisib, in patients with PI3KCA hot spot mutations in their EC (NCT02728258). Some of the studies combining hormonal agents with inhibitors of mTOR pathway have been mentioned in the section on hormonal agents above.

12.2.5 PARP Inhibitors

Poly-ADP ribose polymerases (PARPs) are a family of nuclear enzymes that regulate the repair of DNA single-strand breaks (SSBs) through the base excision repair (BER) pathway. Upon DNA damage, PARP cleaves nicotine adenine dinucleotide (NAD) to generate poly-ADP-ribose (PAR) polymers, which are then added on to DNA, histones, and DNA repair proteins. These processes lead to the recruitment of the cellular repair machinery which facilitates the BER process.

BRCA-1 and BRCA-2 genes encode proteins involved in the homologous recombination (HR) repair of double-stranded breaks. Tumors with mutations in BRCA-1 and BRCA-2 are dependent on the BER rescue pathway for DNA damage repair. Inhibition of PARP leads to the accumulation of DNA double-strand breaks in HR-deficient, BRCA-1/2 mutated tumor cells. This induces cellular apoptosis. Hence, PARP inhibitors may be effective in tumor subtypes with BRCA mutations, and this mechanism of targeted therapy with PARP inhibitors in BRCA mutant tumors has been named "synthetic lethality." PARP inhibitors like olaparib, rucaparib, and niraparib have been approved for clinical use in BRCA-1/2 mutated ovarian cancers. HR deficiency due to BRCA-1/2 mutation occurs in many EC, especially in non-endometroid, TP53-mutant tumors.

As described above, PTEN acts as a tumor suppressor gene by inhibiting the PI3K/AKT/mTOR pathway. PTEN also plays a tumor-suppressive role in the nucleus by maintaining genome integrity. Loss of PTEN impairs CHK1 function, leading to the accumulation of DNA double-strand breaks and genomic instability. PTEN also regulates the expression of RAD51, a key protein in HR repair of DNA double-strand breaks. PTEN deficiency may also be predictive of sensitivity to PARP inhibitors like BRCA-1/2 mutation [45, 46]. However, there are conflicting results regarding the synthetic lethal targeting of PTEN-deficient EC cells with PARP inhibitors.

Olaparib has shown good results in a single study [47]. However, another study has shown that some PTEN-mutated EC cell lines were not sensitive to this agent [48]. The reasons for the reported difference in responses are not yet clear. Niraparib is being studied in patients with recurrent/advanced EC (NCT 03016338). PARP inhibitors in combination with cytotoxic agents have also shown promising results in the treatment of advanced/recurrent EC [49]. Studies are ongoing to look for newer PARP inhibitor drugs (BMN-673) which may be more efficacious [50]. Currently, PARP inhibitors are not a part of the routine treatment of EC.

12.2.6 Antiangiogenic Therapy

Angiogenesis is a crucial process involved in the growth and progression of solid tumors. Vascular endothelial growth factor (VEGF) generated from the cancer cells induces new blood vessel formation by stimulating endothelial cell proliferation and migration. The activated endothelial cells release matrix metalloproteinases to break down the surrounding extracellular matrix to promote new vessel formation [51, 52].

VEGF family includes three transmembrane receptors—VEGF1, VEGF2, and VEGF3. Each of these three receptors serve distinct biological functions. Binding of the VEGF ligand to its receptor activates the downstream PI3K/AKT/mTOR pathway. VEGF has been correlated with high-grade histology, lymphovascular space invasion, deep myometrial invasion, and lymph node metastases in EC [53].

Bevacizumab has been the most studied antiangiogenic drug. It is a recombinant humanized IgG2 monoclonal antibody that binds to circulating VEGF, and prevents it from binding to its receptors. The drug also normalizes tumor vessels that are structurally and functionally abnormal. This may enhance the effect of other chemotherapeutic drugs also. Adverse events are hypertension, proteinuria, and major gastrointestinal toxicities like perforation and fistula formation. Bevacizumab has been approved for ovarian cancer patients in both primary and recurrent settings. It has also shown favorable response in cervical cancer. A GOG phase II trial of bevacizumab as monotherapy in recurrent/persistent EC in 2011 found it to be well tolerated and showed PFS of 6 months [54].

A limitation with using a monoclonal antibody is that they cannot directly target the downstream intracellular pathways, which are usually redundant with multiple converging stimuli. To target other elements in the VEGF signaling cascade, bevacizumab has been combined with other small molecules, including TKI. Phase II studies have shown improved PFS with bevacizumab in combination with mTOR inhibitors [55]. Other retrospective studies have shown high PFS and OS in patients who received bevacizumab, paclitaxel, and carboplatin regimen as first-line therapy in advanced and recurrent EC [56–58]. Although no antiangiogenic drug has been approved by the FDA for therapy of EC, the NCCN panel considers bevacizumab as an appropriate single-agent therapy for patients who have progressed on previous cytotoxic chemotherapy [26]. Recently, the addition of bevacizumab to radiotherapy has been found to be beneficial in improving local disease control [59, 60].

12.2.7 CDK Inhibitors

Cyclins and cyclin-dependent kinases (CDK) are the main regulators of progression through the cell cycle. CDKs are serine/threonine protein kinases that phosphorylate nuclear target proteins involved in the cell cycle. Different types of cyclins are specific for each phase of the cell cycle. They are synthesized during one cycle phase and subsequently degraded during the succeeding phase. A cyclin forms a complex with its corresponding CDK, which leads to the activation of CDK. CDK 4/6 promote the G1/S phase transition by phosphorylating and inactivating the

retinoblastoma protein (Rb), and lead to cell cycle progression. Cyclin D1 is the upstream activator of CDK 4/6. Cyclin D1 amplification is observed in more than one-third of endometrioid ECs [61].

CDK inhibitors can arrest the cell cycle, and CDK4/6 targeted therapy has become an important strategy in endocrine-resistant breast cancer. Currently, phase II trials are ongoing to evaluate the role of CDK 4/6 inhibitor palbociclib, and cyclin D1 and CDK 4/6 inhibitor ribociclib in ER-positive advanced or recurrent EC (NCT 02730429 and NCT 02657928). Ribociclib is currently under phase II trial for use in advanced/recurrent EC, in combination with letrozole and the mTOR inhibitor temsirolimus (NCT 03008408).

12.2.8 Tyrosine Kinase Inhibitors

Tyrosine kinase receptors (TKR) are a family of transmembrane glycoproteins that are usually activated by a variety of growth factors. The important TKRs involved in EC include HER-2, EGFR, FGFR2, and VEGFR. Kinases play a crucial role in major cell functions like cell cycle progression, signal transduction, and transcription. As a result, TKRs have become an important target for cancer therapy. TKIs interfere with intracellular signaling pathways by preventing kinases from catalyzing the transfer of the γ phosphate group from adenosine triphosphate to target proteins. Multiple oral TKIs have become available for a variety of tumors. Their role in EC is also under evaluation.

Geftinib, erlotinib, lapatinib, and imatinib are small molecule inhibitors of EGFR-tyrosine kinase pathway. A phase II trial of erlotinib in recurrent or metastatic EC showed a response rate of 12.5% [62]. Cediranib, a TKI targeting VEGF, PDGF, and FGF receptors has been studied in recurrent or persistent EC. The phase II trial of this study showed a median OS of 12.5 months with no severe toxicities [63].

12.2.9 ERBB-2/HER-2 Inhibitors

HER-2 receptor belongs to the EGFR family. EGFR family consists of four distinct cell surface receptors—ERBB-1, ERBB-2/HER-2, ERBB-3, and ERBB-4. Upon ligand binding, these transmembrane proteins form homo- or heterodimers that lead to activation of their intracellular tyrosine kinase domain. Downstream signaling pathways of the HER-2 receptors include the Ras/Raf/AMPK, PI3K/AKT/mTOR, and JAK/Stat pathways [64]. These three pathways govern key cellular functions such as cell proliferation, survival, and apoptosis, and also cell migration and metastases. HER-2 is amplified in 21–47% of serous ECs, found in the TCGA CNH subgroup, and in 3–21% of endometrioid ECs [65, 66].

Trastuzumab is a humanized monoclonal antibody that binds to the extracellular domain of the HER-2 receptor, leading to inhibition of downstream signaling. The clinical efficacy of trastuzumab has been reported in several case reports, in patients

with recurrent or advanced EC [67, 68]. However, a phase II study evaluating the role of trastuzumab for HER-2 expressing recurrent/advanced EC found no objective response [69]. Similarly, a phase II study of lapatinib (HER-2 inhibitor) in unselected patients with recurrent or persistent EC observed limited clinical activity [70].

A recent randomized phase II study examined the effect of adding trastuzumab to carboplatin/paclitaxel for patients with advanced HER-2 positive serous EC. They found significant improvement in PFS without an increase in overall toxicity [71]. However, the patient number in this study was small ($n= 63$) and further phase III studies with higher patient population are needed, which may be difficult considering the low incidence of serous EC. Currently, phase II studies are undergoing to evaluate the role of afatinib (a pan ERBB inhibitor) and ado-trastuzumab emtansine (NCT 02491099 and NCT 02675829) in EC.

In summary, the role of HER-2 and ERBB targeted therapy in combination with cytotoxic drugs in EC is under evaluation, and future studies will define its role in recurrent and metastatic EC.

12.2.10   Immunotherapy and Microsatellite Instability

T cells are stimulated to elicit response to neoantigens through binding of T cell receptors (TCR) to major histocompatibility antigens (MHC) on the surface of antigen presenting cells (APC). Binding of CD28 on T cells and B7 on APC serves a co-stimulatory function. To modulate the immune response, T cells express programmed death 1 (PD-1), and cytotoxic T-lymphocyte (CTLA-4) antigens. PD-1 has two potential ligands on APCs, namely PD-L1 and PD-L2, while CTLA-4 binds to the B7 antigen on APC. These interactions promote T cell anergy.

Tumors may evade immune surveillance by various mechanisms like loss or alteration of specific antigens, promotion of an immune tolerant microenvironment by manipulation of cytokines, or by upregulation of immune checkpoint molecules such as PD-L1. Upregulation of PD-1/PD-L1 signaling enables tumors to "turn off" T cells and evade immune recognition [72].

The PD-1/PD-L1 complex is expressed on tumor-infiltrating immune cells of 60–80% of primary ECs and in 100% of metastatic EC [73]. The high mutation load in the POLE-mutated and MSI-H subgroups is also correlated with PD-1 and PD-L1 expression. These subgroups of EC patients may be appropriate candidates for immune checkpoint inhibitor therapy [74].

Immune checkpoint inhibitors include CTLA-4 inhibitors, PD-1 inhibitors, and PD-L1 inhibitors (Fig. 12.2). Pembrolizumab is a monoclonal antibody to PD-1, and promotes tumor cell apoptosis by binding to T cell PD-1 receptors. It has been shown to be effective in many solid tumors, especially those with MSI (described later). In the preliminary results from KEYNOTE-028 study, there was an objective RR of 13% in 24 patients with advanced PD-L1 positive EC who were treated with pembrolizumab. The drug demonstrated a favorable safety profile and durable anti-tumor activity in treatment-experienced patients with advanced disease [75].
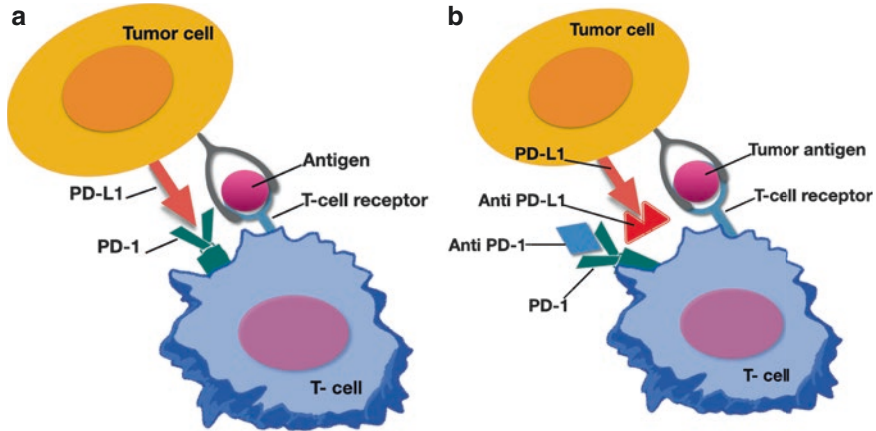
Fig. 12.2 (**a**) PD-L1/PD-1 binding inhibits T cell killing of tumor cells. (**b**) Blocking of PD-1 or PD-L1 enables T cell killing of tumor cells. *PD-1* programmed death 1, *PD-L1* programmed death ligand-1

Avelumab, a monoclonal antibody to PD-L1 is being studied in a phase II trial along with carboplatin and paclitaxel in recurrent/advanced EC (NCT03503786).

Microsatellites are noncoding sections of the DNA that consist of repeats of short sequences of nucleotides. Because of their repetitive nature, microsatellites have a tendency to develop errors during DNA replication. The mismatch repair genes are responsible for repairing these errors during DNA replication [76]. Tumors with a defect in DNA mismatch repair (MMR) mechanism show MSI. MMR abnormalities are usually due to a lack of MLH-1, MSH-2, MSH-6, and PMS-2 protein(s), which are essential for the process of repairing mismatch errors during DNA replication. MSI can be due to germline mutations involving the MMR genes (as in Lynch syndrome) or epigenetic defects [77, 78]. Epigenetic defects are due to MLH-1 promoter hypermethylation with consequent epigenetic silencing.

Lynch syndrome is an autosomal dominant disorder characterized by germline mutations in one of the DNA MMR genes and can be diagnosed by direct gene sequencing as directed by the tumor immunohistochemistry results. Lynch syndrome accounts for approximately 5% of EC cases. The lifetime risk of developing EC with Lynch syndrome varies with age and mutation of the specific MMR gene. Patients with MSH-6 mutations are at a higher risk (64–71% lifetime risk) for developing EC than those with MSH-2 or MLH-1 mutations (40–50% lifetime risk). MSI is not seen exclusively in Lynch syndrome. 15–25% of sporadic EC are MSI high (MSI-H), because of promoter-hypermethylation of MLH-1 gene as an epigenetic event [79, 80].

According to the TCGA, MSI is present in 30–40% of endometroid EC. EC with MSI has a propensity for lower uterine segment involvement, intratumoral heterogeneity, and intense peritumoral lymphocytic infiltration [81].

MSI status may be used to guide therapy in recurrent and metastatic EC. The incidence of somatic mutations is higher in tumors with MSI, and these tumors express significantly more neoantigens in comparison with microsatellite stable (MSS) EC [82, 83]. This high rate of somatic mutations as well as neoantigens makes MSI-H tumors an attractive target for immune-based therapies. MSI-H status may be a marker for response to anti-PD-1/PD-L1 antibodies [84, 85]. Recently, based on data from five single-arm, multicenter clinical trials including 149 patients, pembrolizumab has been granted accelerated approval by the FDA for tissue or site-agnostic use in unresectable or metastatic MSI-H solid tumors, including EC [86, 87]. It is recommended that recurrent EC cases should be tested for MSI status or defective MMR, if not done previously [88].

One recent multicenter study has reported a significant beneficial effect of pembrolizumab in combination with lenvatinib (a multi-kinase inhibitor of VEGFR 1−3, FGFR 1−4, PDGFR α, RET, and KIT) in 54 patients with previously treated, metastatic EC. An objective RR of 50% was found at 24 weeks. The patients enrolled in this study were not preselected based on MSI or PD-L1 status, and included 3 MSI-H, 43 MSI-low, and eight unknown MSI status patients. These encouraging results led FDA to grant "breakthrough therapy" designation for lenvatinib and pembrolizumab combination for the treatment of patients with advanced/metastatic non-MSI-H/proficient MMR EC who have progressed following at least one prior systemic therapy. A randomized, international, two-arm phase III study of pembrolizumab and lenvatinib combination in recurrent EC is underway [89, 90].

Other studies are evaluating the combination of immunotherapy with cytotoxic chemotherapy in EC. There is an ongoing phase II trial examining pembrolizumab plus carboplatin/paclitaxel in advanced or recurrent EC (NCT02549209). Thus, while single-agent immune checkpoint blockade has been successful in a subset of MSI-H and POLE EC patients, combination strategies will be necessary to overcome resistance to immunotherapy in the majority.

12.3   Conclusion

EC has a favorable prognosis in stages I and II. However, the outcome is poor in advanced/metastatic/recurrent disease. The optimal adjuvant treatment for these high-risk cases is largely unsettled. Platinum-based chemotherapy is being used currently in clinical practice, but the results are not very encouraging. The recent genomic characterization of EC has provided new insights and new potential opportunities. There is a need to integrate this molecular and histologic tumor stratification into the management strategy for EC. The paradigm of cancer treatment is moving from "one-size-fits-all" strategy to personalized therapy. Targeted therapies like antiangiogenic agents, TKIs, PARP inhibitors, and immunotherapy agents have shown promise in the treatment of EC. Increasing knowledge in cancer biology will allow the development of new treatments tailored to a particular signaling pathway, while minimizing the side effects.

12.4 Key Points

1. Endometrial cancer (EC) was initially divided into two types based on histology: type-I, endometroid variety, and type-II, non-endometroid variety. Based on molecular profiling, EC is now divided into four varieties: POLE ultramutated, MSI-hypermutated, copy number low, and copy number high.
2. Hormonal therapy is recommended in recurrent or advanced stages of low-grade endometrioid EC, preferably in patients with small tumor volume or indolent growth rate. Medroxyprogesterone acetate, megestrol acetate, and levonorgestrel intrauterine device have also been recommended as hormonal treatment in women with EC desiring fertility.
3. Metformin—an oral biguanide, is an effective drug for the management of diabetes which is a major risk factor for EC. It has also shown good results as an adjunctive drug for the management of EC, alone and in combination with standard chemotherapy. However, it is not yet an established adjuvant treatment of EC.
4. The PI3K/AKT/mTOR pathway is an important pathway for EC. Drugs targeting this pathway alone, or in combination are an active area of research in the treatment of EC.
5. PARP inhibitors—olaparib and niraparib have shown good results in the treatment of recurrent/advanced EC. However, they are not a part of routine treatment currently.
6. Bevacizumab, an antiangiogenic drug is recommended for use in recurrent EC cases who have progressed on previous cytotoxic chemotherapy.
7. CDK inhibitors like palbociclib, and ribociclib, and TKIs like gefitinib, erlotinib, lapatinib, and cediranib, and the HER-2 receptor inhibitor trastuzumab are currently under trial for use in advanced/recurrent EC.
8. Pembrolizumab, an immune checkpoint blocker, has been found to be successful in MSI-H and POLE subset of EC patients. It is recommended for use in metastatic EC cases with MSI-H status.
9. MSI testing is becoming increasingly important in many cancers including EC. MSI can be sporadic or associated with Lynch syndrome. NCCN guidelines recommend universal testing of all EC cases for MSI status.

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