HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2020 August 25.

Published in final edited form as:

Nat Rev Cancer. 2019 September ; 19(9): 510–521. doi:10.1038/s41568-019-0177-x.

Clinical Actionability of Molecular Targets in Endometrial Cancer

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Abstract

Endometrial cancer accounts for ~76,000 deaths amongst women worldwide. Disease mortality and the increasing number of new diagnoses make endometrial cancer an important consideration in women's health, particularly in industrialized countries, where the incidence of this tumor type is highest. Most endometrial cancers are carcinomas, with the remainder being sarcomas. Endometrial carcinomas can be classified into several histological subtypes including endometrioid, serous and clear cell carcinomas. Histological subtyping is currently routinely used to guide prognosis and treatment decisions for endometrial cancer patients, while ongoing studies are evaluating the potential clinical utility of molecular subtyping. In this review we summarize the over-arching molecular features of endometrial cancers and highlight recent studies assessing the potential clinical utility of specific molecular features for early detection, disease risk stratification, and directing the use of targeted therapies.

Introduction

Uterine corpus cancer is the 6th leading cause of cancer death amongst women in the US and the 8th leading cause of cancer-related death amongst European women[1,2]. Most uterine cancers are endometrial carcinomas (ECs), originating from the uterine epithelium (Fig. 1A). The vast majority of ECs are sporadic, with an estimated 5% occurring in the context of inherited cancer susceptibility syndromes[3], most commonly Lynch Syndrome (Box 1)[4–6]. ECs are classified into various histological subtypes, including endometrioid EC (EEC), serous EC (SEC), clear cell EC (CCEC), mixed EC, and uterine carcinosarcoma **[G]** (UCS), which differ in their frequency, clinical presentation, prognosis and associated epidemiological risk factors (BOX 1)[7–9]. Importantly, the incidence of EC is rising in the US and more than 20 other countries[10]; recent data correcting for hysterectomy **[G]** rates in the US and Denmark indicates that the incidence of non-EECs, which are generally more clinically aggressive, is increasing while the incidence of EECs has remained stable or decreased over time[11,12].

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

Dr. Bell receives royalty income from US patent No.7,294,468 "Method to determine responsiveness of cancer to epidermal growth factor receptor targeting treatments", which is licensed to Esoterix Genetic Labs LLC. Dr. Urick has no competing interests.

EECs constitute more than 80% of newly diagnosed EC cases, are generally estrogen-dependent tumors, and have a mean age at diagnosis of 62 years[8]. In contrast, SECs and CCECs are relatively uncommon, accounting for ~10% and 3% of newly diagnosed ECs, are generally estrogen-independent, and are diagnosed later in life (mean of 66.5 and 65.6 years, respectively)[8]. Uterine carcinosarcomas (UCSs), which are biphasic tumors composed of both carcinomatous and sarcomatous cells, represent <2% of ECs[13]. The prognosis for most newly diagnosed EC patients is good, with a relative 5-year survival rate of 81.1% (2008–2014)[14]. The generally high survival rate for EC is largely driven by the frequent early detection of EECs, coupled with the effectiveness of surgery for treating many early-stage, low-grade EECs. However, considerably poorer outcomes are associated with high-grade, recurrent, or metastatic EEC, as well as certain non-endometrioid histologies (SEC, CCEC and UCS).

Research conducted over the last two decades, fueled by the need to identify biomarkers to predict disease recurrence and druggable targets, has revealed critical insights into the molecular landscape of ECs (Table 1). We now know that low grade EECs and SECs have distinguishing molecular features, while the mutational profiles of CCECs overlap with that of EECs and SECs[15–25]. Although UCSs most closely resemble SECs molecularly, these highly aggressive tumors exhibit unique somatic changes including whole genome doubling and variable epithelial-to-mesenchymal transition **[G]** (EMT) gene signature scores[16]. Herein, we review the major molecular characteristics of ECs and UCSs, as well as recent efforts to translate this knowledge into clinical actionability.

The molecular etiology of endometrial cancers

Endometrioid endometrial cancer

The development of EEC is strongly associated with epidemiological risk factors leading to an excess of estrogen relative to progesterone (Box 1)[8,9,26–28]. Unopposed estrogen stimulation of the uterine epithelium can result in the outgrowth of endometrial hyperplasia, which may further evolve into complex atypical hyperplasia **[G]** (CAH), the precursor lesion for EEC (FIG. 1B). Molecular studies of EEC, its precursor lesions, and morphologically normal endometrial glands have inferred the timing of somatic mutations in tumor initiation and progression, which proceeds via branched evolution[29–3233–35]. Such studies have shown that *PTEN* mutation, the most frequent somatic mutation among EECs (Table 1), is an early but insufficient event in the initiation of tumorigenesis. This observation is corroborated and complemented by genetically engineered mouse models of EC, which have demonstrated that biallelic *Pten* loss leads to development of CAH, whereas biallelic *Pten* loss together with mutational activation of *Pik3ca* results in progression of CAH to EC[36]. These findings add context to the fact that *PTEN* mutations commonly co-occur with *PIK3CA* and *PIK3R1* mutations in human EECs[15,37–40]. Genetically-engineered mouse models have also inferred co-operativity between *Pten* loss and *Ctnnb1* (which encodes β-CATENIN) mutation (exon 3 deletion, resulting in β-catenin stabilization) or *Mlh1* inactivation. Specifically, *Ctnnb1* exon 3 deletion synergizes with biallelic *Pten* loss and *Pik3ca* activation to promote EC and myometrial invasion in the setting of ovarian insufficiency **[G]**[41], and endometrial tumorigenesis is accelerated in $Pten^{+/-}/Mlh1^{-/-}$ mice as compared to $Pten^{+/-}$ mice[42]. In the

context of human EC, gain-of-function missense mutations in *CTNNB1* exon 3, resulting in β-catenin stabilization, and epigenetic silencing of *MLH1,* leading to mismatch repair **[G]** deficiency (MMR-D), are frequent aberrations in EEC that often independently co-occur with *PTEN* inactivating mutations (Table 1)[15,43]. The pathogenicity of MMR-D in EC is further underscored by the inherited predisposition to EC in MMR gene mutation carriers in Lynch Syndrome families (Box 1). Another common aberration among EECs is mutational inactivation of the *ARID1A* tumor suppressor gene **[G]** (Table 1). Although loss of *Arid1a* is insufficient to induce endometrial hyperplasia or carcinoma in mouse models[44,45], immunohistochemical analyses of human CAHs have shown that endometrial glands with loss of both ARID1A and PTEN expression have higher proliferative indices than adjacent glands with loss of only PTEN[46]. This finding has led to the proposal that *ARID1A* acts as a "gatekeeper" to suppress the transition of *PTEN*-deficient CAH to EEC[46].

Serous endometrial cancers

SECs are generally estrogen-independent and arise in the setting of the atrophic endometrium **[G]** (FIG. 1C). They are high-grade tumors, preceded by serous endometrial intraepithelial carcinoma **[G]** (SEIC) (FIG. 1C), that are often diagnosed at a late stage with a high risk of recurrence. The occurrence of *TP53* mutations and/or p53 stabilization in SEIC, is evidence for this aberration being an early event in SEC pathogenesis[47,48]. Consistent with this idea, aged transgenic mice with conditional deletion of *Trp53* in the genitourinary tract develop SEC, as well as other ECs[49]. However, this phenotype is in contrast to the lack of endometrial tumors in mice with endometrial-specific deletion of *Trp53*[50]. One possible explanation for this difference is the variable ages at which mice were assessed for tumor development in each model (1 year versus 5 months, respectively). The frequent occurrence of *FBXW7*, *PIK3CA*, and *PPP2R1A* somatic mutations (Table 1) as well as *CCNE1* amplification in SEC[15,17,24,37,51–53], and in adjacent SEIC, indicates that these are also early events in disease pathogenesis[24,51]. The functional impairment of SEC-associated recurrent mutations in *FBXW7*, *PIK3CA*, and *PPP2R1A* has been established experimentally[5453,55], and the pathogenicity of cyclin E dysregulation in cancer is well-recognized. However, the precise role of these events in the initiation and progression of SEC, both by themselves and in conjunction with *TP53* mutations, would be aided by the development of appropriate mouse models.

Uterine carcinosarcomas

Most (but not all) UCSs are believed to be monoclonal, originating from high grade EEC, SEC or other aggressive histotypes that have undergone a metaplastic transition to form the sarcomatous component of these biphasic tumors (FIG. 1B,C). Supporting their derivation from aggressive ECs, the most frequently somatically mutated genes in UCSs are also commonly mutated in other ECs (Table 1)[16,18,20,23,56]; in fact, UCSs most closely resemble SECs molecularly[15,16,18,20,56–58]. However, unlike most other subtypes of EC (except grade 3 EEC) where *PTEN* and *TP53* mutations tend to be mutually exclusive[15,19], a majority of UCSs with *PTEN* mutations also have *TP53* mutations[16,20,56]. The co-occurrence of *PTEN* and *TP53* mutations is not likely due to the biphasic nature of UCSs since the carcinoma **[G]** and sarcoma **[G]** components of most UCSs share mutations[23,56,59,60].

Also distinguishing UCSs from other tumor types, whole-genome-doubling occurs in 90% of UCSs, and UCSs exhibit highly variable EMT transcriptomic gene signature scores[16], which may reflect the transient nature of EMT and/or the presence of intermediate EMT states[61]. Although EMT scores have not been shown to correlate with outcome in UCSs[16], EMT has been associated with metastasis and therapy resistance in other tumor types[61,62], thus maintaining the possibility that EMT contributes to the poor prognosis for women with UCSs.

Clear cell endometrial cancers

The exact molecular etiology of CCECs remains unclear but multiple studies have shown that clinically diagnosed CCECs share mutational features with both EECs and SECs[19,21,63,64] (Table 1). However, a clinical diagnosis of CCEC represents a conundrum because accurate histopathological classification of this tumor is challenging, even for specialty gynecologic pathologists, and some shared molecular features likely reflect a misclassification[65]. CCECs have been the subject of targeted gene sequencing and exome sequencing studies[19,21,63,64,66], but thus far have not been scrutinized using integrated multi-platform analyses akin to methods used by The Cancer Genome Atlas **[G]** (TCGA).

Molecular classification

The main molecular characteristics of EECs, SECs, and UCSs have been revealed through targeted molecular studies, whole exome sequencing, and TCGA's integrated genomic analyses (Table 1). While UCSs were characterized by TCGA independently[16], TCGA assimilated EECs and SECs into four distinct molecular subgroups with prognostic significance[15]: The first subgroup is formed by *POLE*-mutated (ultramutated) tumors, which were characterized by *POLE* exonuclease domain (ED) mutations, predominantly recurrent $POLE^{P286R}$ or $POLE^{V411L}$, and an excess of G:C>T:A transversions. Women in this subgroup exhibited the best progression-free survival **[G]** (PFS). The second subgroup is formed by hypermutated tumors, which were characterized by microsatellite instability **[G]** (MSI) and hypermethylation of the *MLH1* promoter. Forming the third subgroup, copy number low/microsatellite stable (MSS) tumors were characterized by low copy number aberrations, MSS, and exhibited frequent *CTNNB1* (β-catenin) mutation. Tumours in the fourth subgroup were copy number high (serous-like) tumors and were characterized by frequent *TP53* mutation and high-level somatic copy number alterations. Women in this subgroup exhibited the worst PFS[15].

EECs were distributed among all four subgroups, whereas SECs were almost exclusively in the serous-like subgroup (FIG. 1D). Paradoxically, the *POLE*-mutated subgroup exhibited the best PFS but was enriched for high-grade EECs[15]. Several subsequent studies confirmed significant associations between *POLE*-ED mutations and favorable clinical outcomes for high-grade EEC[15,67–71]. *POLE*-mutated ECs have high neoantigen **[G]** loads and markers of enhanced immune response[72,73]. Whether the favorable prognosis associated with *POLE*-mutated EC is attributed to these characteristics, aggressive therapeutic regimens administered to high-grade patients, or increased sensitivity to chemotherapy remains unclear[72–77]. However, recent studies indicate that the favorable prognosis for *POLE*-mutant

ECs is not likely due to differential treatment response or tumor immunogenic phenotype[78,79].

Early detection of endometrial cancer

Early detection of EC can increase the likelihood of women achieving disease-free survival **[G]**. The most common symptom of EC is post-menopausal vaginal bleeding (PMB). However, although 90% of women with EC (irrespective of tumor stage) exhibit PMB, this symptom is not a specific indicator of the disease: only 9% of women with PMB are diagnosed with EC[80]. Similarly, methods used to screen for EC, most commonly cytology and transvaginal ultrasound, lack specificity[22]. Thus, accurate screening tests that detect EC in women with early stages of the disease are needed.

In 2013 it was reported that EC-associated mutations could be detected in DNA extracted from specimens collected during routine Papanicolaou (Pap) tests **[G]**[22]. This led to the development of a prototype test (the “PapGene” test) that sequenced frequently mutated regions of 12 genes, with the potential to be incorporated into routine medical exams at a cost equivalent to HPV analysis[22]. In 2018, this test was advanced closer to commercial availability under the name of the “PapSEEK” test, which detects mutations in targeted regions of 18 genes as well as aneuploidy; a test is positive for cancer if a mutation or abnormal chromosome arm number is detected (Fig. 2)[81]. Of 382 women with EC, 81% tested positive with PapSEEK (78% with stage I/II tumors, 92% with stage III/IV tumors, and 85% with high-grade ECs confined to the endometrium). Furthermore, 93% of 123 EC patients sampled with Tao brushes **[G]** tested positive with PapSEEK (90% of stage I/II tumors, 98% of stage III/IV tumors, and 89% of high-grade tumors confined to the endometrium). The most commonly mutated genes detected by PapSeek in EC patient Pap and Tao brush samples **[G]** were *PTEN*, *TP53*, *PIK3CA*, and *PIK3R1*; the most commonly altered chromosomal arms in Pap samples were 4p, 7q, 8q, and 9q. Importantly, in 125 women without cancer, 0% of Tao brush and 1.4% of Pap samples, respectively, tested positive using PapSEEK, indicating increased specificity over alternate screening methods[81]. These results confirm earlier reports of sensitivity and specificity ranges for EC detection in Tao brush samples of 95–100% and 66–100%, respectively[82–85]. The PapSEEK test now needs to be evaluated in prospective studies.

The sensitivity and specificity of PapSEEK testing of Tao brush samples are currently the best reported values, but other promising methods for early detection of EC are also in development. The PapSEEK test has shown increased specificity over the use of next generation sequencing **[G]** (NGS) on uterine lavage **[G]** samples[86,87] (FIG.2), but it is possible that a “false positive” may reflect the detection of somatic driver aberrations in non-cancerous endometrium, a phenomenon reported by several groups[86,88–90], or actually may be accurate early identification of EC. For example, *PTEN* mutations were detected in the uterine lavage of an asymptomatic woman with no clinical evidence of cancer 10 months prior to the identification of a single microscopic focus of EC[91]. An endometrial tumor <1mm in diameter contained within a polyp was also identified in the lavage fluid of another woman[86], highlighting the sensitivity of genomic analysis of uterine lavage fluid for early detection of EC. Another potentially promising uterine sampling method is vaginal

tampon[92,93]. Increased methylation of 11 genes has been detected in DNA extracted from vaginal tampons and may be particularly useful to identify stage I EECs, although it is unclear what methylation levels would be used to distinguish ECs from non-cancerous endometrium and whether increased methylation would be detectable in women pre-biopsy[92,93]. Furthermore, it is unclear if other aberrations are detectable from vaginal tampon samples. Despite the needed fine-tuning of this method, the potential use of tampons as screening tools for high risk women is particularly enticing because it could enable samples to be mailed in for testing similar to currently available Cologuard® **[G]** tests for colorectal cancer screening. Collectively, recent advancements indicate that the addition of genomic analyses to minimally invasive uterine sampling is a move in the right direction toward early detection of women with EC.

Molecular tests for risk stratification

While early detection of EC is ideal, the current reality is that the most clinically aggressive subtypes of EC are commonly diagnosed at advanced stages and identification of women at risk of developing aggressive disease is arguably a more pressing challenge than early detection. These facts have driven the quest to identify actionable genomic aberrations in ECs, which may ultimately be revolutionary for risk stratification and treatment of women with EC. The prognostic significance of TCGA-based molecular subgroups represented a paradigm-shifting development towards the use of molecular information to refine EC risk stratification. Independent groups have confirmed the prognostic significance of the TCGA-based subclasses[66,94,95], and have proposed classification systems that are more feasible for routine clinical use than TCGA's comprehensive molecular analysis (FIG. 3). Although an extensive expanse of literature describes molecular biomarkers for EC risk stratification, here we focus on the two most current pragmatic molecular classification schemes: the Translational Research in Post-Operative Radiation Therapy in EC (*Trans*PORTEC) molecular classification system and the Proactive Molecular Risk Classifier for EC (ProMisE) (FIG. 3).

The original *Trans*PORTEC model stratified high-risk EC patients into four subgroups, which are defined as p53 mutant, MSI, *POLE*-mutant, or No Specific Molecular Profile (NSMP)[66]. Patients were not classified if molecular testing was only partially performed or if they harbored more than one abnormality. When *Trans*PORTEC was used to classify 116 EC patients deemed high-risk based on clinicopathological features, only patients in the p53 mutant (n=39) and NSMP (n=44) groups were shown to be truly high-risk; they exhibited significantly higher rates of distant metastases and lower 5-year RFS compared to those in the *POLE*-mutant (n=14) and MSI (n=19) groups (who had favorable prognoses)[66] (FIG. 3C). The *Trans*PORTEC molecular classification system was subsequently revised to integrate clinicopathological factors[96], and is now being prospectively tested as a means to stratify women with high-intermediate risk EEC for radiotherapy in the phase III PORTEC-4a trial[97] (FIG. 3A). While the results of PORTEC-4a are undoubtedly highly anticipated, it is also of great interest to determine whether *Trans*PORTEC could be useful for prospective stratification of EC patients for treatments other than radiotherapy. In the meantime, the *Trans*PORTEC model continues to evolve; the most recent version was refined to incorporate markers of DNA damage repair[98], but is not yet being tested clinically.

ProMisE classifies EC patients based on testing of specimens for aberrations in the order of MMR-D, *POLE* mutation, and *p53* mutation[99] (FIG. 3B). Patients are not classified if they advance to a step for which they are unable to be tested, and those that harbor more than one aberration are classified based on the first positive test. ProMisE was shown to retrospectively enhance prediction of outcome in first a discovery cohort of 143 EC patients[94], then on a confirmation cohort of 319 EC patients when combined with the European Society of Medical Oncology (ESMO) risk-stratification system[99]. In a validation cohort of 452 ECs, ProMisE was a significant prognostic marker of progression and disease-specific survival, even after adjustment for known risk factors[100] (FIG. 3C). Enhanced retrospective prognostic ability of ProMisE was observed when ESMO risk stratification or clinicopathological parameters were added. Most recently, ProMisE was retrospectively significantly correlated with overall and disease-specific survival in a cohort of 257 young (<50 yo) EC patients[101]. Compared to the other non-age-stratified cohorts tested, this younger cohort was distributed more in p53 WT and *POLE* mutated subgroups, and less in p53 abnormal and MMR-D (FIG. 3C)[101].

The retrospective data for *Trans*PORTEC and ProMisE indicates that either has potential to be implemented as standard practice for risk stratification of EC patients, but neither currently has clinically proven *prospective* utility. Although both classification schemes utilize similar core molecular features (FIG. 3), differences can be considered. For example, while *Trans*PORTEC completes all molecular testing prior to patient stratification, ProMisE follows sequential molecular testing and stratification. *Trans*PORTEC is already being tested prospectively in the PORTEC-4a trial (described above), but ProMisE was developed, confirmed, and validated following the US Institute of Medicine guidelines and is now ready to be tested in prospective trials[100]. ProMisE was validated to be performed on diagnostic biopsies; *Trans*PORTEC was also shown to produce concordant results between diagnostic and hysterectomy specimens[102], but is being prospectively tested on hysterectomy samples. The *Trans*PORTEC model being tested in PORTEC-4a incorporates molecular testing beyond that used in ProMisE (*TP53* and *CTNNB1* sequencing, LSVI quantification, and MLH1, MSH2, and L1-CAM immunohistochemistry) (FIG. 3a) although key clinicopathological parameters available at diagnosis are being evaluated for use with ProMisE[103]. The inclusion of *CTNNB1* sequencing reflects findings that *CTNNB1* exon 3 mutations have emerged as a prognostic marker for increased risk of disease recurrence among patients with low-grade and early-stage EEC[104,105]. However, substantive intratumor heterogeneity for *CTNNB1* mutations observed in the molecular evolution of low-grade EECs from precursor lesions has been noted, prompting caution on the choice of clinical tissue sampling approaches for this marker[34].

Implementation of either *Trans*PORTEC or ProMisE would involve surmounting several challenges that include, but are not limited to, the cost and technical training required to perform and interpret genomic sequencing (with *POLE* being particularly challenging[103]), the development of methods for translation of genomic data to patients (most likely with the aid of genetic counselors), as well as risk of patient attrition while awaiting molecular profiling; pilot results of the PORTEC-4a trial indicated an average time of 10.2 days between randomization and molecular profile determination[106]. Furthermore, neither *Trans*PORTEC or ProMisE currently incorporates histology, stage, or grade. Along these

lines, it is important to note that these pragmatic models were originally designed to recapitulate TCGA's prognostic subgroups of EECs and SECs; therefore, it remains to be determined how prognostic these classifications are for other EC histological subtypes, including mixed and undifferentiated ECs. For now, the current recommendation for reporting of genomic classifiers are to include histology, stage, and grade[107].

Matching patients to therapies

The practice of guiding cancer therapy based on molecular aberrations is gaining momentum across the field of oncology and has shown potential to improve patient outcomes, despite logistical hurdles. In addition to those mentioned in the preceding paragraph, common challenges of matching patients to targeted therapies are lack of availability of therapies or clinical trials, impaired geographic accessibility of trials, and lack of insurance coverage[108–113]. Cost increase, another potential challenge associated with matched targeted therapies, are mainly being attributed to increased treatment duration[113]. Further complicating the interpretation and translation of genomic results is the fact that therapies targeting identical aberrations in different tumor types have shown differing efficacy; likewise, different aberrations within the same gene have been shown to produce distinctive functional consequences. The contextual importance of mutations in preclinical design and interpretation is highlighted by the variability in synthetic lethal effects observed between EC cell lines with gain-of-function and loss-of-function *TP53* mutations[114–117]. Despite the challenges associated with molecularly guiding therapies, three quarters of 1,281 US physician survey respondents in 2017 reported using NGS tests to guide treatment decisions[118], and treatment of solid tumors based on matching actionable mutations to targeted therapies has resulted in improved outcomes of patients with advanced cancers[109,110,119–123].

Counter to this optimistic data, clinical trials targeting aberrations in the PI3K pathway or ERBB2 (also known as HER2), which are some of the most common clinically actionable aberrations in advanced ECs[112,123], have yielded modest results[124]. Lack of response could be due in part to initial or acquired drug resistance; in this respect it is noteworthy that a majority (70%; 14/20) of *ERBB2* amplified ECs also harbor PI3K pathway aberrations[15], raising the potential for combination therapies. Along these lines, whereas preclinical studies have produced variable reports of sensitivity to PARP inhibitors in PTEN-deficient EC[125–127], treatment of a mouse model of EC with combined PARP and PI3K inhibitors has resulted in synergistic effects[128]. Relatedly, in an inducible PTEN knockout mouse model, a CDK4/6 inhibitor exhibited antitumor activity[129].

Reliable biomarkers of response to therapies targeting the PI3K pathway or ERBB2 also need to be identified for EC patients. Biomarker identification could potentially be improved if trials are designed to accrue patients and report results by molecular and/or histological subtypes. For example, a recent study that accrued only SEC patients who overexpressed ERBB2, reported decreased risk of progression and increased PFS for patients treated with carboplatin-paclitaxel-trastuzumab **[G]** compared to those treated with carboplatin-paclitaxel[130]. Independent of this study, effectiveness of trastuzumab in *ERBB2* amplified SEC patients has been reported: one heavily pretreated woman achieved a durable complete

response to trastuzumab[112], while another woman with recurrent disease achieved a complete response following the addition of trastuzumab to her carboplatin-paclitaxel regimen[131]. These results are evidence that trials designed to incorporate histological and/or molecular stratification could help identify EC patient populations most likely to respond to targeted therapies.

In this regard, it is noteworthy that in a recent prospective analysis, 47% (16/34) of EC patients who matched to a therapy after NGS panel tumor profiling experienced clinical benefit[132], including 40% (2/5) of MSI-H patients treated with immune checkpoint inhibitors and 42% (8/19) of patients matched based on *PIK3CA* and *PTEN* mutations[112]. Of 189 EC patients within this study (75% of which had grade 3 EEC, SEC, CCEC or UCS), 67% had at least one alteration for which a therapy was either FDA-approved or under clinical investigation. The most common clinically actionable aberrations among the entire cohort were *PIK3CA* or *PTEN* mutation, MSI, and *ERBB2* amplification[112]. Importantly, of 4 patients with matched primary and metastatic samples within this study, the mutational profiles differed between primary and metastatic sites; in 2 cases, metastases acquired potentially actionable mutations in *MTOR* and *PIK3R1*[114]. Indeed, discordance in mutations, and changes in the dominant mutational signature, between matched primary and metastatic ECs have been reported[133,134,135]. EC metastases gain aberrations in genes in multiple functional groupings including the PI3K pathway, WNT signaling, RAS-RAF pathways, transcriptional regulation, DNA damage response, and FBXW7-related genes (FIG. 4). Additionally, recurrent or metastatic EECs exhibit ~7% higher frequencies of MSI and/or MMR-D compared to matched primary tumors[136,137]. Discordance between aberrations found between primary tumors and metastases may reflect lack of representation of tumor heterogeneity in primary tumor biopsies, or the acquisition of novel aberrations, potentially due to a change in microenvironment encountered by metastatic lesions. Regardless of the biological explanation for this descrepancy, comprehensive molecular profiling of metastatic lesions may be key for treatment stratification of EC patients. Encouragingly, a recent mutational analysis of metastases from 20 untreated cancer patients (including 4 ECs lacking *POLE* mutation) indicated that all metastases within a patient share functional driver mutations[138]. If this holds true for other aberrations and for patients in relapse, it would decrease the need to evaluate multiple metastases from a single patient.

Targeted treatment of additional EC cohorts is needed to help assess the utility of matched therapies in this patient population. Treatment arms of the National Cancer Institute's Molecular Analysis for Therapy of Choice (MATCH) trial comprise one such cohort[139]. Another is patients enrolled in the American Society of Clinical Oncology's (ASCO's) Targeted Agent and Profiling Utilization Registry (TAPUR) trial[140]. Importantly, and relevant to the next section of this review, EC patients that are MMR-D that enroll in the MATCH trial and those in the TAPUR trial that harbor *POLE/POLD1* mutations, have a high mutational load, or are MSI-H have the potential to match to immunotherapies.

Immunotherapy for endometrial cancer

As of November 2018, clinicaltrials.gov[141], which encompasses the US and 20 other countries worldwide, listed over 50 clinical trials testing various forms of immunotherapy

for which advanced EC patients were potentially eligible (Supplementary Table 1). Immune therapies have shown particular efficacy in solid tumors that are MSI-H, MMR-D and/or those with high concentrations of Tumor Infiltrating Lymphocytes **[G]** (TILs). In fact, the programmed cell death 1 (PD1) antibody pembrolizumab **[G]**, received US food and drug administration (FDA) approval in 2017 for treatment of MSI-H or MMR-D cancers, regardless of tumor type. This landmark approval could be particularly beneficial for EC patients, given that 16–17% are MMR-D as detected by NGS[112,142], ~34% of EECs have MSI[15,143–145], 48–100% express PD1 ligand 1 (PDL1) or PDL2[146,147], and equivalently high numbers of TILs are found in subsets of all molecular subgroups of EC[79].

Pembrolizumab has been remarkably effective in small numbers of EC patients (Table 2), but definitive biomarkers of response remain elusive, a need several clinical trials are currently attempting to address (Supplementary Table 1). Treatment with pembrolizumab achieved a noteworthy 53% (8/15) overall response rate (ORR) in MMR-D EC patients[142], and 43% (3/7) ORR when combined with the indoleamine 2,3-dioxygenase (IDO1) inhibitor epacadostat in ECs with unreported biomarker status[148]. Two separate cohorts of advanced EC patients each responded with ORRs of 13% to single-agent pembrolizumab and the PD-L1 antibody atezolizumab **[G]**. The combined results of these cohorts may indicate that hypermutation or high TILs combined with PD-L1 positivity may predict response to PD-1 blockade: of patients responding to atezolizumab, one exhibited 70% TIL, while the other was hypermutated and a patient that exhibited a prolonged (>14 month) partial response to pembrolizumab harbored *POLE* mutations; all three patients were PD-L1 positive (Table 2)[149,150].

Information revealed in recent studies considering ECs along with other cancer types, so-called “pan-cancer studies”, has supported a potential importance of immune response in ECs and may also aid in identification of novel treatment strategies[151]. For example, unsupervised clustering of TCGA’s Pan-Gyn cohort **[G]** based on 16 molecular features revealed that 16.5% of SEC-like EECs, SECs, and UCSs group within a cluster characterized by high leucocyte infiltration, which supports immunotherapy as a potential treatment option. It was further speculated that tumors within two other clusters encompassing 32.5% and 36.9% of SEC-like EECs, SECs, and UCSs might respond to HER2 targeted therapy (discussed above as a potential promising treatment strategy for *ERBB2* amplified SECs) or therapies targeting the DNA damage response[151]. A second pan-cancer clustering based on 5 immune suppression gene signatures revealed that the vast majority of CN-high ECs, SEC-like ECs, and UCSs populated “wound healing” and “IFNγ dominant” clusters, raising the possibility that molecular targets involved in the physiological response to wounds or IFNγ signaling could be therapeutically relevant for clinically aggressive ECs[152]. Finally, a large-scale functional genomics screen of pan-cancer cell lines revealed that WRN (Werner syndrome RecQ like helicase) is a synthetic lethal target in MSI (but not *POLE* mutated[153]) ECs[153–156]. These discoveries open up promising new avenues for future preclinical exploration of rational drug development for ECs.

Concluding remarks

The current molecular portraits of the most common ECs, and of rare but clinically aggressive forms of the disease, have revealed shared and distinguishing features, as well as prognostically distinct subgroups. This knowledge has inspired ongoing efforts to develop diagnostic tests to facilitate the early detection of EC and clinically feasible molecular classifiers that may be used for disease risk stratification, to prevent both under- and over-treatment of women with EC. Future challenges in the field include: overcoming difficulties associated with incorporation of molecular subtyping in the clinic; more extensive genomic characterization of CCECs and of EC metastases; functional characterization of mutations in novel driver genes; proteomic studies to provide a global view of the net impact of genomic, transcriptomic and translational perturbations in ECs; and high throughput screens for druggable targets and synthetic lethal interactions in this disease. It is hoped that these efforts, together with strategies to reduce obesity, will ultimately reduce the impact of EC on women's health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We apologize to those authors whose work we could not cite due to space limitations. This work was supported by the Intramural Research Program of the National Human Genome Research Institute at NIH (HG200338 and HG200379) to DWB.

Glossary

atezolizumab
Humanized, monoclonal antibody of programmed cell-death ligand 1.

atrophic endometrium
Thin layer of nonpoliferative epithelial cells lining the uterus; characteristic of postmenopausal women

carboplatin
Chemotherapy drug that inhibits cell growth and/or causes apoptosis by inducing DNA-DNA and DNA-protein crosslinks.

Carcinoma
Cancer caused by uncontrolled proliferation of epithelial cells.

Carcinosarcoma
Tumor comprised of both carcinoma and sarcoma.

clear cell endometrial cancer
(CCEC) rare histopathological subtype of endometrial cancer that typically arises from atrophic endometrium and includes large clear eosinophilic cells (basic cells that stain with the acidic dye eosin).

Cologuard® test
Colorectal cancer screening test which enables patients to collect stool samples in-home; samples are mailed to a lab where they are analyzed for presence of blood and DNA abnormalities.

complex atypical hyperplasia of the endometrium
(CAH) precancerous changes in the epithelial cells lining the uterus characterized by abnormal growth and acquisition of somatic genomic aberrations.

driver genes
Pathogenic aberrations of these genes contribute to the initiation and/or progression of cancer.

disease-free survival
Length of time a patient lives without signs of disease.

endometrioid endometrial cancer
(EEC) most common histopathologic subtype of endometrial cancer.

epithelial-to-mesenchymal transition
(EMT) process by which epithelial cells acquire characteristics of mesenchymal cells, including but not limited to, decreased cell-to-cell adhesion, decreased polarity and increased motility.

Hysterectomy
Surgical removal of the uterus.

lymphovascular space invasion
(LSVI) spreading of cancer to the lymphatic system or blood vessels.

microsatellite instability
(MSI) alteration of the number of short, repeated sequences of DNA because of a defect in DNA mismatch repair.

mismatch repair
(MMR) type of DNA repair that corrects base-base mismatches and insertions/deletions.

Neoantigen
Antigens not previously recognized by the immune system.

next generation sequencing
(NGS) high-throughput technologies (also known as massively parallel or deep sequencing) that enable faster determination of DNA or RNA base pair codes than previously-used technologies (e.g. Sanger Sequencing).

ovarian insufficiency
Loss of normal ovarian function.

Paclitaxel

Chemotherapy drug that binds tubulin and inhibits cell division; also induces apoptosis through binding and inhibition of Bcl-2 (B-cell leukemia 2).

Papanicolaou (Pap) test
Routine screening tool in which cervical cells are collected using a small brush and analyzed microscopically for signs of disease (e.g. irregular cell morphology).

pap brush
Flexible brush used to sample the inside of the cervix.

Pembrolizumab
Humanized, monoclonal antibody of programmed cell-death ligand 1.

progression-free survival
(PFS) length of time a patient lives without objective worsening of disease.

Sarcoma
Cancer caused by uncontrolled proliferation of connective tissue.

serous endometrial cancer
(SEC) rare histopathologic subtype of endometrial cancer that typically arises in atrophic endometrium, usually with well-formed papillae.

serous endometrial intraepithelial carcinoma
(SEIC) noninvasive malignant precursor to SEC (serous endometrial cancer).

somatic aberration
Genomic change that occurs spontaneously; is not present in germline.

Synthetic lethality
Occurs when multiple genomic aberrations combine to cause cell death, while the independent aberrations do not.

Tao brush
Flexible brush used to sample the inside of the uterus.

The Cancer Genome Atlas
(TCGA) NIH (National Institutes of Health)-funded initative that molecularly characterized over 20,000 primary cancer and matched normal samples covering 33 cancer types.

The Cancer Genome Atlas' Pan-Gyn cohort
1,087 invasive breast carcinomas, 308 endocervical adenocarcinomas, 579 high-grade serous ovarian cystadenocarcinomas, 548 uterine corpus endometrial carcinomas, and 57 uterine carcinosarcomas molecularly characterized by TCGA.

Trastuzumab
Recombinant human monoclonal HER2 (human epidermal growth factor receptor 2) antibody.

tumor infiltrating lymphocytes

White blood cells (immune cells) found within tumor tissue.

tumor suppressor gene

Gene which normally functions to prevent uncontrolled growth of cells.

uterine lavage

Process by which the uterus is flushed with a sterile solution.

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Box 1.

Epidemiological and genetic risk factors for endometrial cancer

Epidemiological risk factors:

Increased risk for developing endometrioid endometrial cancer (EC), an estrogen dependent tumor type, is associated with obesity, diabetes, unopposed estrogen use, nulliparity, early menarche, and late menopause[8]. Increasing age is a risk factor for serous and clear cell ECs[8]. Tamoxifen use increases risk of developing EC; histological subtypes enriched in users of tamoxifen are serous EC, high-grade endometrioid ECs and carcinosarcomas[9].

Genetic risk factors:

Increased genetic risk for developing EC is associated with Lynch Syndrome, Polymerase Proofreading Associated Polyposis, and Cowden Syndrome.

- Lynch Syndrome is a highly penetrant, autosomal dominant cancer predisposition syndrome caused by monoallelic germline mutation in a mismatch repair gene, specifically *MLH1, MSH2, MSH6* or *PMS2*, or by germline deletion within *EPCAM* that leads to epigenetic silencing of the adjacent *MSH2* gene[4]. Mutation carriers are at increased risk of developing colorectal cancer and ECs, the two major component tumors of Lynch Syndrome, as well as cancers of the ovary, stomach, kidney, urinary tract, biliary tract, small intestine and skin[4]. Approximately 2–6% of ECs are attributed to Lynch Syndrome[157].
- Polymerase Proofreading Associated Polyposis is an autosomal dominant cancer susceptibility syndrome attributed to germline mutations in the exonuclease domain of *POLD1* or *POLE*. *POLD1* mutation carriers are at increased risk of developing attenuated adenomatous polyposis of the colorectum and cancers of the colorectum, endometrium, breast, and brain[5]. *POLE* mutation carriers are at increased risk of developing colorectal cancer. Predisposition to EC has not been established in *POLE* mutation carriers.
- Cowden syndrome is a condition in which *PTEN* mutation carriers have an increased predisposition for developing multiple hamartomas, and cancers of the breast, thyroid, endometrium, colorectum, kidney, and skin[6].

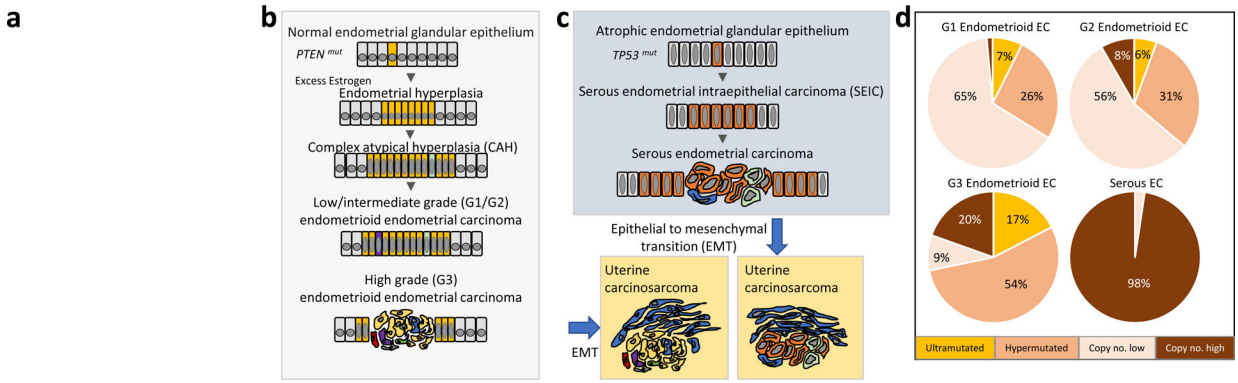

Figure 1. Overview of endometrial carcinoma origin, development, and molecular classification.

(a) The image in this panel was deleted to comply with *Nature Reviews Cancer* policy on self-archiving. Schematic depiction of the initiation and progression of endometrioid (B) and serous (C) endometrial carcinomas (ECs) from the normal and atrophic endometrial glandular epithelium, via precursor lesions (CAH and SEIC). Columnar epithelial cells that have acquired somatic mutations are colored; intratumoral heterogeneity is depicted by differentially colored epithelial cells. *PTEN* mutation and *TP53* mutation are, respectively, early events in the etiology of many endometrioid and serous endometrial carcinomas. In some instances, carcinomas, particularly high-grade carcinomas, undergo an epithelial to mesenchymal transition to give rise to uterine carcinosarcomas, which are biphasic tumors consisting of epithelial carcinoma cells and sarcoma cells (blue). (D) Pie charts showing the distribution (% of tumors) of low grade (grade 1 and grade 2) endometrioid EC, high grade (grade 3) endometrioid EC, and serous EC among the four molecular subgroups delineated in The Cancer Genome Atlas[15].

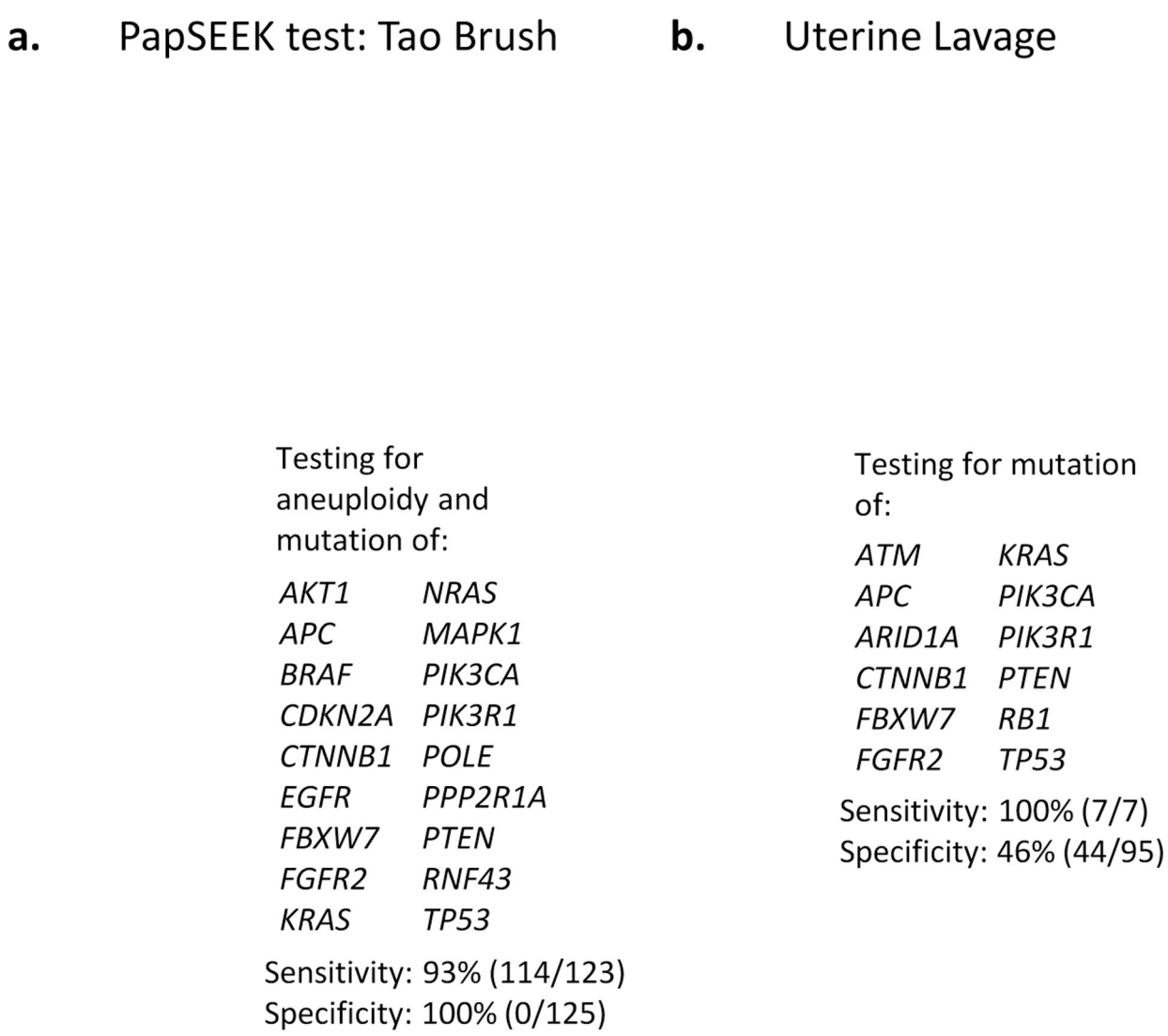

Figure 2. Minimally invasive sampling methods for endometrial cancer (EC) patients. (a.) Tao brush sampling with PapSEEK test:

women testing positive for aneuploidy in any of ~38,000 loci of long interspersed nucleotide elements or mutation in any of 18 genes would be sent for confirmatory testing[81]. PapSEEK testing of Tao brush samples accurately detected EC in 93% of women tested with EC; out of 125 women without EC, none tested positive[81]. (b.) **Uterine lavage** samples analyzed on a 12 gene next generation sequencing panel detected cancer in 7 women with EC; mutations in the 12 genes were also detected in 51 of 95 women with a non-cancerous uterus[86]. Two images in this Figure were deleted to comply with *Nature Reviews Cancer* policy on self-archiving.

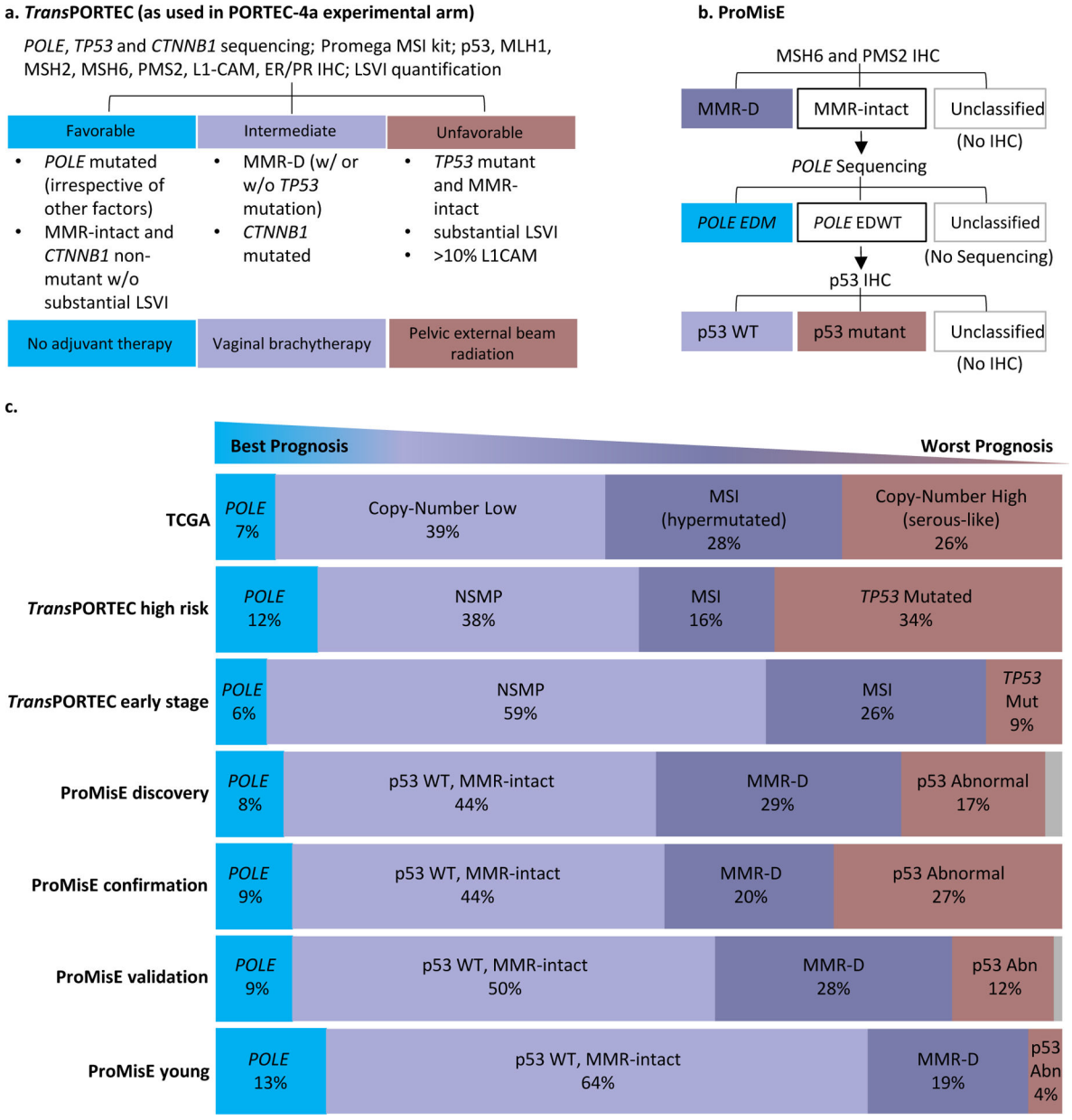

Figure 3. Molecular-based risk/treatment stratification strategies for endometrial cancer (EC) patients.

(a.) The *Trans*PORTEC molecular classification system currently being tested in the PORTEC-4a clinical trial[97] and (b.) the Proactive Molecular Risk Classifier for EC (ProMisE)[100]. (c.) Distribution of endometrial cancer patients in molecular subgroups. Each cohort consisted of the following EC patients: **TCGA** (n=232: 186 EEC, 42 SEC, 4 Mixed ECs)[15], **TransPORTEC high risk** (n=116: 86 high risk EEC, 12 SEC, 18 CCEC)[66], **TransPORTEC early stage** (n=834 early stage EEC)[98], **ProMisE discovery** (n=143: 119 EEC, 15 SEC, 8 mixed, 1 undif; 64 ESMO high risk)[94], **ProMisE confirmation** (n=319; 215 EEC, 5 CCEC, 89 SEC, 10 other; 173 ESMO high risk)[99], **ProMisE validation** (n=452; 397 EEC, 34 SEC, 21 CCEC/mixed; 131 ESMO high risk)[100], **ProMisE young** (n= 257 <50 yo: 225 EEC, 17 NEEC, 15 unknown; 21 ESMO high risk)[101].

Abbreviations: Clear Cell Endometrial Cancer (CCEC); Catenin Beta 1 (*CTNNB1*); Exonuclease Domain Mutation (EDM); Exonuclease Domain Wildtype (EDWT); Endometrioid Endometrial Cancer (EEC); European Society of Medical Oncology (ESMO); Immunohistochemistry (IHC); lymphovascular space invasion **[G]** (LVSI); MicroSatellite Instable (MSI); Mismatch Repair-Deficient (MMR-D); No Specific Molecular Profile (NSMP); polymerase-ε mutated (*POLE*); Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE); Serous Endometrial Cancer (SEC); The Cancer Genome Atlas (TCGA); Translational Research in Post-Operative Radiation Therapy in Endometrial Carcinoma (*Trans*PORTEC); tumor protein 53 (p53); Undifferentiated (undif); wild-type (WT); year-old (yo).

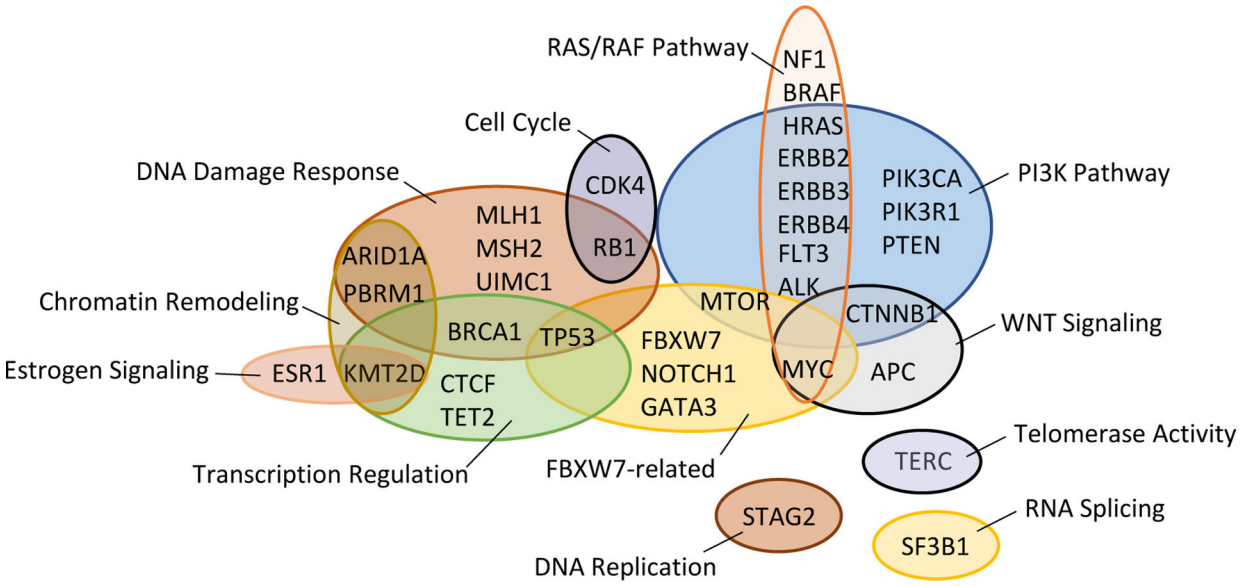

Figure 4. Functional grouping of genes in which aberrations are acquired in metastases of endometrial cancer (EC)
112,133–135,138.

Table 1.

Somatic aberration [G] frequencies for major driver genes in endometrial carcinomas

Somatic Aberration	Potential Clinical Actionability	EEC	SEC	UCS	CCEC	Refs
Mutated *PTEN*	• PI3K-AKT pathway inhibition • Synthetic lethality **[G]** with PARP inhibition • CDK4/6 inhibition	• G1-G3, 64–80% • G1/G2, 52–82% • G3, 62–90%	2–3%	11–33%	0–21%	15,16,19,21–23,25,38,53,56,63,64,158
Mutated *PIK3CA*	PI3K-AKT-mTOR inhibition	• G1-G3, 22–59% • G1/G2, 38–54% • G3, 45–59%	15–35%	22–40%	24–36%	15–19,21–25,38,43,52,53,56,158
Mutated *PIK3R1*	PI3K-AKT-mTOR inhibition	• G1-G3, 9–43% • G1/G2, 19–38% • G3, 31–41%	5–8%	6–20%	7–18%	15,16,19,21–23,37,38,56,64
Mutated *KRAS*	MEK inhibition	• G1-G3, 19–43% • G1/G2, 17–23% • G3, 7–33%	2–6%	10–17%	2–14%	15,16,19,21–23,25,38,43,52,53,56,64,158
Mutated *FGFR2*	FGFR inhibition	• G1-G3, 10–18% • G1/G2, 11–13% • G3, 14–16%	8%	0–2%	0%	15,16,22,38,43,56,64,159
Mutated *CTNNB1*	Adverse prognosis in low-grade EEC	• G1-G3, 19–37% • G1/G2, 24–28% • G3, 19–40%	0–3%	0–5%	0%	15,16,21,22,25,43,56,158
MSI (MMR-D)	• Immunotherapy • Synthetic lethality with WRN depletion	• G1-G3, 34–35% • G1/G2, 34% • G3, 44%	0–3%	3–6%	11–14%	15,16,18,19,43,63,144

Somatic Aberration	Potential Clinical Actionability	EEC	SEC	UCS	CCEC	Refs
Mutated *ARID1A*	Multiple potential synthetic lethal interactions	• G1-G3, 39–55% • G1/G2, 39–47% • G3, 39–60%	7–11%	10–24%	14–21%	15,16,19,22,25,56,64,158
Mutated *POLE*	• Immune checkpoint inhibition • Favorable prognosis in high grade EEC	• G1-G3, 13–16% • G1/G2, 11% • G3, 15–20%	0–2%	3–4%	2–7%	15,18,19,21,56,69,94,95,100,101,151
Mutated *TP53*	• Adverse prognosis • Synthetic lethality with G2/M checkpoint inhibition and chemotherapy	• G1-G3, 5–14% • G1/G2, 6–10% • G3, 21–35%	59–93%	44–91%	28–46%	15–19,21,23–25,38,48,52,56,64,112,158,160
Mutated *FBXW7*	Undetermined	• G1-G3, 10–12% • G1/G2, 11–15% • G3, 0–14%	15–29%	11–39%	13–25%	15–18,21,23,24,52,56
Mutated *PPP2R1A*	Undetermined	• G1-G3, 7–8% • G1/G2, 7% • G3, 10–13%	19–43%	13–28%	7–21%	15–19,23–25,52,56,64,158
Amplified *ERBB2*	ERBB2 inhibition	• G1-G3, 1% • G1/G2, 3% • G3, 4%	26% −44%	9%	11%	15,16,21,52

Table 2.

Immunotherapy clinical trial results for endometrial cancer (EC) patients

Immunotherapy	Patient Population	ORR	Responding Biomarker Status (response)	Refs
Anti-PD1 (Pembrolizumab)	MMR status: 100% (15/15) MMR-D	53% (8/15)	All MMR-D; other biomarker status not reported 3 (CR) 5 (PR) 3 (SD)	142,161
Anti-PD1 (Pembrolizumab) plus IDO1 inhibitor (epacadostat)	No information for EC patients	43% (3/7)	Biomarker status not reported 1 (CR) 2 (PR)	148,162
Anti-PD1 (Pembrolizumab)	MSI status: 5% (1/19) MSI-H 95% (18/19) MSS Histology: 74% (17/23) EEC 9% (2/23) SEC 4% (1/23) UCS 13% (3/23) other PDL1 status: 100% (23/23) +	13% (3/23)	1 PD-L1+, *POLE* muts (PR >14months) 1 PD-L1+, MSS (PR) 1 PD-L1+ (PR)	146,149,163
Anti-PDL1 (Atezolizumab)	MSI status: 6% (1/15) MSI-H 47% (7/15) MSS 47% (7/15) unknown histology: 33% (5/15) EEC 33% (5/15) SEC 7% (1/15) Leiomyosarcoma 27% (4/15) Unknown PDL1 status: 33% (5/15) + 67% (10/15) -	13% (2/15)	1 PD-L1+, MSS, 70% TIL (PR) 1 PD-L1+, MSI, 10% TIL, hypermutated (PR)	150,164

IDO1, Indoleamine 2, 3-dioxygenase 1; MMR= Mismatch Repair; MSI= Microsatellite Instability; EEC= endometrioid EC; SEC= serous EC; UCS= uterine carcinosarcoma; PD1, programmed cell death 1; PDL1= PD1 Ligand 1; ORR=overall response rate; CR= complete response; PR= partial response; SD= stable disease.