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The importance of molecular classification of endometrial carcinomas in clinical practice: how to apply it and difficulties in application

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Abstract

Classification of endometrial carcinomas (EC) based solely on histological features is not sufficient for the prognostic and therapeutic guidance of patients. Furthermore, the existence of EC in which the histological type cannot be determined clearly and the poor reproducibility of histological typing have led to difficulties in clinical management. However, molecular classification of EC is very promising because of the high reproducibility and good correlation with clinical outcome. Within the scope of "the Cancer Genome Atlas Project", EC were divided into four different genomic subtypes, and molecular classification models for EC were developed based on these molecular subcategories. The prognostic differences between these molecular subgroups and the benefit for guidance for adjuvant therapy have been clearly demonstrated in studies. In this article, the importance of molecular classification for EC is discussed and its use in clinical practice is reviewed.

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Introduction

More than 50% of endometrial carcinomas (EC) present at an early stage, are in the low-risk group and can be treated with surgery alone, but there is a significant proportion of patients with an aggressive disease course. There is a need for an accurate and useable risk stratification model and for the identification of predictive biomarkers that will determine the extent of surgery, the need for post-operative adjuvant therapy and, if needed, the type of adjuvant therapy for optimal management of these agressive cases.

According to the traditional dualistic model, defined by Bokhman (1) in 1983, ECs have been categorized into two main groups, type 1 and type 2 carcinomas, in terms of clinical, endocrine and histopathological features. Based on this classification, endometrioid type ECs (EEC) constitute the majority of type 1, are associated with excess unopposed estrogenic stimulation of the endometrium and have a favourable prognosis. However, type 2 ECs, which include non-endometrioid histotypes, such as serous and clear cell carcinoma, have a worse prognosis and don't respond well to hormonal therapy. While some ECs will be prototypic examples of type 1 and type 2 ECs, many of them, particularly high grade [International Federation of Gynecology and Obstetrics-(FIGO) grade 3] ECs often do not fit into either category. Clear cell endometrial carcinomas (CCEC) generally fall into the type 2 category, but not all CCECs show the expected aggressive course of type 2 ECs. Histological type of the EC is an important factor in the selection of appropriate adjuvant therapy. However, there is a morphological overlap, especially in high-grade ECs, which even complicates the distinction between the two main clinically significant histological categories; endometrioid or non-endometrioid. Furthermore, it has been clearly seen that patient management models based solely on histological subtypes are inadequate, both for the very rare and little-known EC subtypes (2-4), and

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for the more common and well-known EC subtypes, which may show prognostic inter-patient heterogeneity.

The original dualistic model does not fully reflect the clinical diversity of ECs, and recent molecular developments have highlighted the importance of incorporating molecular features into risk grouping algorithms, including for patients with EC, as has been done for some other cancer types. The Cancer Genome Atlas (TCGA) project categorised ECs into four distinct genomic subtypes through integration of mutational analysis, copy number variation, and mRNA expression results in 2013 (5).

In this review, the role of the TCGA molecular classification in the management of patients with EC are discussed, including how it is applied in clinical practice and the difficulties that may be encountered.

The Cancer Genome Atlas genomic classification of endometrial carcinoma

TCGA performed a genome-wide analysis of 373 ECs, including EEC (n=307), serous endometrial carcinomas (SEC) (n=13), and mixed endometrioid and serous (n=13) carcinomas. Based on the data obtained from the genomic, transcriptomic, and proteomic analysis of these cases, four distinct molecular subgroups of ECs with different clinical, pathological and molecular features, have been identified (5). Then, molecular classification models based on TCGA molecular subcategories of ECs were developed for further adaptation for clinical practice (6,7). The prognostic differences between these molecular subgroups, and the benefit for guidance for adjuvant therapy have been clearly demonstrated (8).

These molecular subgroups are the ultramutated subtype with mutation in the exonuclease domain of DNA polymerase epsilon (POLE) (7% of cases); the hypermutated-microsatellite instable (MSI) subtype characterized by deficiency of ≥1 mismatch repair proteins (MMRd) (28%); the copy number-high subtype/serous-like characterized by TP53 mutations (26%) and the copy number-low subtype [no specific molecular profile (NSMP)] (39%), which doesn't fit any of these molecular subclasses mentioned above. The molecular classification of ECs has provided a better clinicopathological approach to patients with EC in many regards, including tumor histological typing, prognostic and therapeutic guidance, and elucidation of hereditary carcinomas.

1. POLE-ultramutated endometrial carcinomas: During DNA replication, the synthesis of the DNA strand and the rereading of the synthesized DNA strand plays a crucial role in correcting errors. DNA polymerase is one of the key molecules in DNA replication. The *POLE*mut subgroup of ECs is characterized by pathogenic mutation in the exonuclease domain of the *POLE* gene, which is a DNA polymerase, leading to high tumour mutation load (exceeding 100 mutations per megabase) (9,10). This category includes 7-12% of ECs. Patients tend to be younger and have a normal body mass index. Although *POLE*mut ECs often have high-risk pathological features, such as extensive lymphovascular invasion (LVI) and high tumor grade, they have a very favourable clinical course, regardless of the tumor histotype and histological grade. Most, but not all, tumors in this group are of the endometrioid histotype. In the TCGA tumor cohort, 6.4% of low-grade EEC and 17.4% of high-grade EEC, but none of the mixed histology or serous carcinomas were *POLE*mut EC (5). High tumour grade, scattered tumour giant cells and prominent lymphocytic infiltrate are characteristic histological features. They may show intratumoral morphological heterogeneity and ambigious morphology with coexistence of both endometrioid and serous-like histological features (11).

Detection of *POLE* mutation in EC appears to lead reduction in therapy, as these carcinomas have a very favourable prognosis. These patients may also be candidates for anti-*PD1*/*PDL1* immune checkpoint inhibitory (ICI) therapies, when they are at advanced stage or have recurrent disease.

2. MSI/MMRd-hypermutated endometrial carcinomas: DNA mismatch repair (MMR) system repairs errors that occur during DNA replication, and has two heterodimers consisting of *MutS protein homologue 2 (MSH2)*/*MutS protein homologue 6 (MSH6)* and *MutL homologue 1 (MLH1)*/*PMS1 homologue 2 (PMS2)* (12). The MMR is responsible for the maintenance of genomic stability by correcting DNA replication errors (base mismatches or insertion-deletion errors) (13). MMR deficiency is characterized by deficiency of ≥1 MMRd. Microsatellites are short repeated DNA sequences found throughout the genome and DNA polymerases are more prone to make mistakes in these regions. Dysfunction of any *MMR* gene is first manifested by variations in the length of these microsatellite repeats, termed MSI. MSI is an indirect indicator of MMR dysfunction.

Initially, MSI/MMRd ECs were defined as hereditary cases, associated with Lynch syndrome (LS), a hereditary cancer syndrome characterized by autosomal dominant heterozygous germline mutations in one of the four major *MMR* genes and which has a 60% lifetime risk of developing EC (14). LS-related cancers develop following somatic loss of function of the other intact allele of the affected *MMR* gene. Only 10% of MMR deficiencies in ECs are inherited, and associated with LS. In the remaining sporadic cases, epigenetic deletion of the MLH1 promoter region by hypermethylation is the main mechanism preceding the majority of sporadic MMR deficiencies, and some are associated with somatic mutations in the *MMR* genes. MMR deficiency contributes to high tumor mutation load (>10 mutations/megabase), therefore ECs in this molecular subgroup are highly immunogenic tumors, though not as immunogenic as *POLE*mut ECs.

The MSI/MMRd EC subgroup accounts for 25-30% of all ECs, and consists predominantly of high grade EECs (15). In the TCGA tumor cohort, 28.6% of low-grade EEC and 54.3% of high-grade ECC were MSI/MMRd EC (5). Mucinous differentiation, "microcystic, elongated and fragmented" pattern of myometrial invasion and LVI are common histological features. Dense peritumoral and intratumoral lymphocytic infiltration usually accompany the tumor cells (16). This tumor type tends to involve the lower uterine segment and occur in a wide age range; those associated with LS occur at a younger age than sporadic cases.

MSI/MMRd ECs constitute the group with intermediate prognosis, among the four molecular subgroups, in which *POLE*mut ECs have the best prognosis and those with TP53 mutation have the worst. CCECs with MMR deficiency have been reported to have a more favourable prognosis than MMR proficient CCECs. Although they are in the non-endometrioid EC group which is expected to have a poor prognosis, they behave more like MMRd EECs and thus patients with MMRd CCEC are recommended to be managed in the same manner as MMRd EECs (17). Hormonal therapy, given in the context of a fertility-preserving approach, is not suitable for the MMRd group of ECs. As MMRd ECs have high propensity for LVI, sentinel or other lymph node procedures may be required (18,19). They respond well to radiotherapy (RT), but patients do not benefit from platinum-based chemotherapy (CT). They may be good candidates for ICI therapy. *PD-1/PD-L1* inhibitor therapy has been approved by The Food and Drug Administration for patients with recurrent or advanced MMRd EC and who do not have any other treatment option (20).

3. *P53* abnormal EC (*P53*abn EC)/copy number-high/serous-like: *P53*abn EC is characterized by the mutation of the tumor supressor gene-*TP53* and constitutes the most aggressive group among the four molecular subgroups, with a high number of somatic copy-number alterations. Most of the tumors in this group are high-grade tumors and have serous morphology. In the TCGA study, 97.7% of SECs, 75% of ECs with mixed histology, 19.6% of high-grade EECs, and 5.0% of low-grade EECs were included in this group (5). A relationship between SEC and hereditary breast and ovarian cancer syndrome, which is associated with germline mutations in the *BRCA1* or *BRCA2* genes, has been reported (21). For women with SEC, who have a family history of hereditary breast and ovarian cancer syndrome-related malignancy, this relationship should be considered and patients should be referred for germline *BRCA1/2* screening.

*P53*abn ECs tend to be seen at an older age in comparison to other molecular subgroups and are more likely to present at an advanced stage. They can spread to the adnexa and peritoneum without deep invasion of the uterine wall.

*P53*abn ECs have the worst prognosis within the four molecular subgroups. However, no significant prognostic difference was observed between *p53* wild type and *p53*abn CCECs, and thus similar management of these patients is recommended (17).

P53 abn ECs respond well to the combination of platinum-based CT and RT. Targeted therapies based on *Human epidermal growth factor receptor 2 (HER2)* (trastuzumab) or homologous recombination defects (HRD) poly-ADP ribose polymerase [(PARP) inhibitors] may provide treatment options (11).

4. No specific molecular profile endometrial carcinomas/copy number-low: Tumors in this molecular group do not harbor specific molecular features of other EC molecular groups, any pathogenic *POLE* mutation, MMR defect, or *p53* abnormality. Hence, after the exclusion of these molecular features, the tumor should be included in this molecular category, which is also the most common one, accounting for almost half of all ECs. Somatic copy number alterations and mutation load are low in this group (22).

The majority of NSMP ECs are typically low grade (grade 1 or 2), early stage EECs, that develop on the basis of endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia and may respond to hormone therapy.

NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers to identify tumors with a high propensity for disease recurrence and thus requiring aggressive treatment, complicates the management of this patient group. A potential biomarker is β-catenin (CTNNB1) mutation status. Studies have shown that low-grade EECs harboring mutations in exon 3 of the *CTNNB1* gene have more aggressive outcome, with higher recurrence rates (23). Further studies are needed to elucidate predictive biomarkers that can identify patients with NSMP EC who require adjuvant therapy. NSMP ECs may be suitable candidates for hormonal therapy and they respond well to adjuvant therapy, which is given when there are poor prognostic features.

Distribution of EC histological types based on molecular subgroups is illustrated in Figure 1 (24). Most of the low-grade (FIGO grades 1 and 2) EECs correspond to the NSMP and MMRd molecular subgroups. In contrast, high-grade EECs show a heterogeneous molecular profile with a similar distribution rate across all genomic categories. All SECs are encompassed by the *p53*abn group.

Application of molecular classification in clinical practice

Molecular classification models based on TCGA molecular subcategories of ECs were developed to be adapted to clinical practice (6,7). Talhouk et al. (6) proposed a clinically applicable method for the molecular classification of ECs, the proactive molecular risk classifier for endometrial cancer (ProMISE). In the context of their study, consisting of 319 ECs,

four distinct prognostic molecular subgroups with significantly different survival profiles were identified using methods of MMR immunohistochemistry (IHC) to identify the MMRd group, *p53* IHC to identify *p53*abn group, and sequencing for POLE exonuclease domain mutations to identify *POLE*mut group (6). Thus, the molecular subtype of ECs can be successfully determined with high interobserver reproducibility (25), and a high level of compatibility is achieved between the molecular subclasses defined in endometrial biopsy and hysterectomy specimens (26). Studies of ECs with dual or multiple molecular classes (consistent with more than one molecular subgroup) have suggested a sequential algorithmic approach to identify the exact molecular subtype that will provide prognostic and therapeutic guidance in these tumors. This approach starts with *POLE* mutation analysis as the first step, MMRd/MSI analysis follows this and p53 mutation analysis is the last step (Figure 2) (11). Since *POLE* mutation analysis, the first step, is an expensive and not widely available test, it is usually not possible in routine practice to apply this algorithmic approach in this sequence. Hence, the *POLE* mutation test can be used in a limited fashion, only for patients in whom adjuvant therapy is planned. This may remain the situation until a cheaper and easier method, such as an IHC assay, is developed to investigate pathogenic *POLE* mutations.

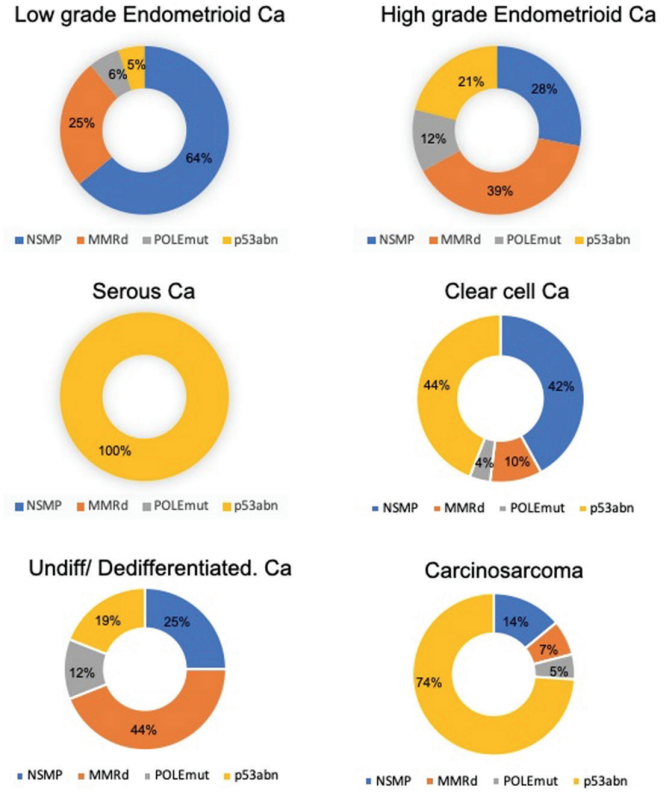

Figure 1. Distribution of endometrial carcinoma histological types by molecular subgroups
Ca: Carcinoma, MMRdef: Mismatch repair deficiency, POLEmut: Polymerase ε mutant, NSMP: No specific molecular profile

While the majority of EC are compatible with one of these four molecular categories, 3-6% show features of more than one molecular group and are termed "multiple classifier ECs" (27). Multiple classifier ECs harbor molecular features of different combinations of two molecular groups (*POLE*mut + MMRd/MMRd + *P53*abn/*POLE*mut + *P53*abn) or a combination of three groups (*POLE*mut + MMRd + *P53*abn). Concomitant TP53 mutation in *POLE*mut or MMRd ECs has been shown to be a passenger mutation that does not affect biological behavior of the tumor. It has been elucidated that these tumors do not have such a poor prognosis as single-classifier *p53*abn ECs and thus intensive treatment is not required. Since MMRd ECs with pathogenic *POLE* mutations are also found to have

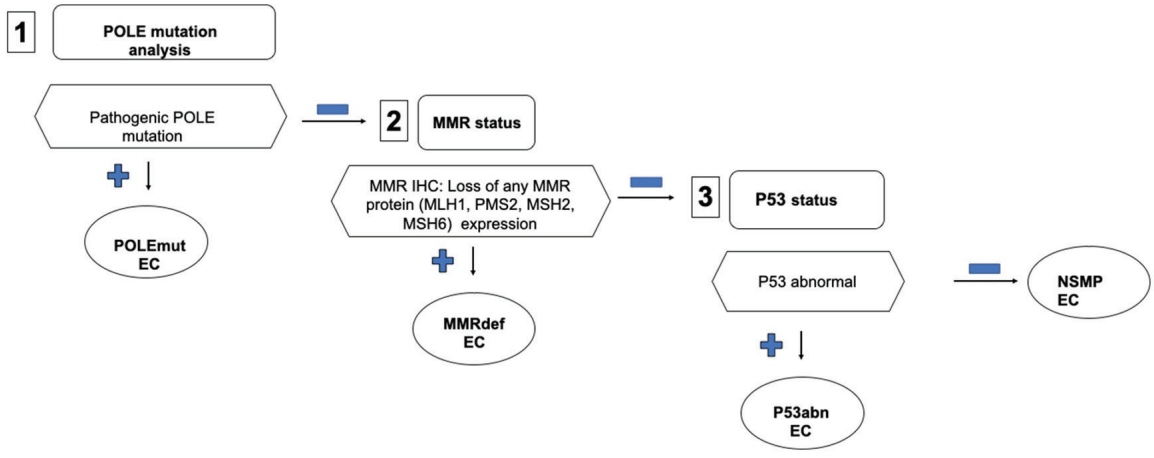

Figure 2. The proactive molecular risk classifier for endometrial cancer (ProMisE) technique to define the molecular class of endometrial carcinoma
EC: Endometrial carcinoma, POLE: Polymerase ε, POLEmut: Polymerase ε mutant, MMR: Mismatch repair, MMRdef: Mismatch repair deficiency, IHC: Immunochemistry

a good prognosis, like single classifier-*POLE*mut ECs, it is recommended to classify these tumors as single classifier *POLE*mut ECs (27). Therefore, *POLE* mutation analysis is the first step in the recommended algorithm to define the molecular subgroup of ECs.

a. *POLE* mutation testing: *POLE*mut ECs are diagnosed by detection of one of the 11 different pathogenic somatic missense mutations in the exonuclease domain of the *POLE* gene (Table 1) (28), P286R and V411L being the most common hot spot mutations. The method currently in use is DNA extraction from the tumor and sequencing of exons 9, 13, and 14 (or exon 9 through 14) by next-generation sequencing (NGS) or Sanger sequencing. Currently, there is no immunohistochemical assay to detect pathogenic *POLE* mutations, for the diagnosis of *POLE*mut ECs.

Table 1. Pathogenic mutations in the *POLE* gene, leading to the diagnosis of *POLE*mut EC

Protein change	Nucleotide substitution
P286R	c.857C>G
V411L	c.1231G>T/C
S297F	c.890C>T
S459F	c.1376C>T
A456P	c.1366G>C
F367S	c.1100T>C
L424I	c.1270C>A
M295R	c.884T>G
P436R	c.1307C>G
M444K	c.1331T>A
D368Y	c.1102G>T

POLE: DNA polymerase epsilon, *POLE*mut EC: *POLE* mutant endometrial carcinoma

b. MMR/MSI testing: It is recommended to peform MMR/MSI testing for all ECs, due to its diagnostic, prognostic and therapeutic contributions in the management of patients with EC (Figure 3). One of the purposes of MMR/MSI testing is the detection of the MMRd EC molecular subgroup, which has its own characteristics, in terms of treatment response and alternative treatment approaches. Since ICI inhibitor therapy has been approved for all advanced MMRd or MSI-high solid tumors, detection of MMR deficiency or high MSI in an EC raises the option of targeted therapy with ICI. Screening for LS is another indication. EC is often the first carcinoma type detected in patients with LS. Therefore, screening by MMR/MSI testing in ECs enables earlier detection of this syndrome, and provides follow-up of these patients, in terms of the development risk for more fatal carcinomas in the future (frequently colorectal carcinoma), and thus reduces the cancer-related mortality (29,30).

MMR defect is mainly detected using two methods. The first is IHC in which four major MMR protein expressions (*MLH-1, MSH-2, MSH-6, PMS-2*) are evaluated in tumor cells. The second method is a polymerase chain reaction (PCR) based technique, in which MSI analysis is performed. These two methods have approximately 95% compatibility, and IHC is the leading method with several advantages, such as being cheaper and more widely accessible, and with the capability to identify which *MMR* gene is likely defective.

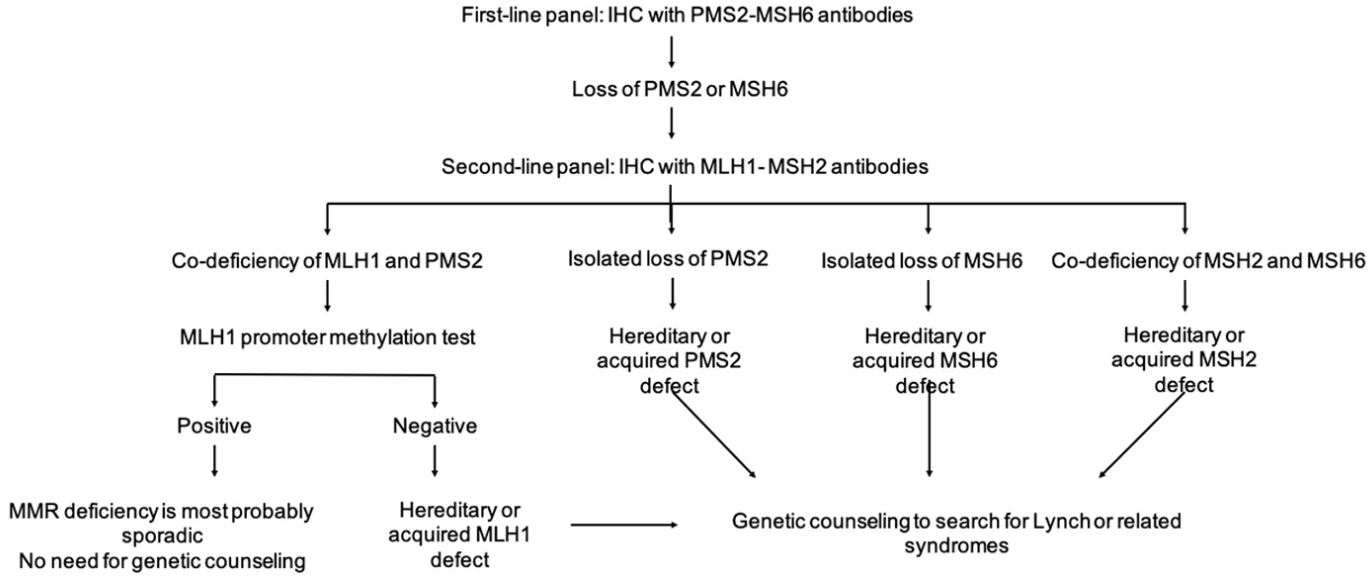

Figure 3. Steps of the MMR immunohistochemistry test and interpretation of possible results
IHC: Immunochemistry, MSH2: MutS protein homologue 2, MSH6: MutS protein homologue 6, MLH1: MutL homologue 1, PMS2: PMS1 homologue 2, MMR: Mismatch repair

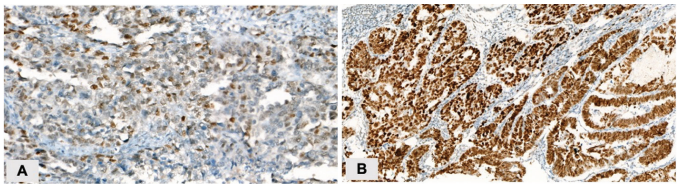

Figure 4. *p53* immunohistochemistry (A) "Wild type" *p53* staining patern. (B) *p53* overexpression (strong nuclear expression in more than 80% of tumor cell nuclei)

MMR proteins exist as heterodimers in which *MSH2* pairs with *MSH6* and *MLH1* pairs with *PMS2*. *MLH1* and *MSH2* can maintain their stability by forming heterodimers with other proteins in the cell, even in the absence of their own counterpart. However, *PMS2* and *MSH6* can maintain their stability only in the presence of *MLH1* and *MSH2* in the cell. Therefore, in order to reduce the cost of MMR IHC, it is recommended to perform a first-line panel consisting of only two antibodies first (*PMS2* and *MSH6*), and, if a defect is detected in any of these, to perform

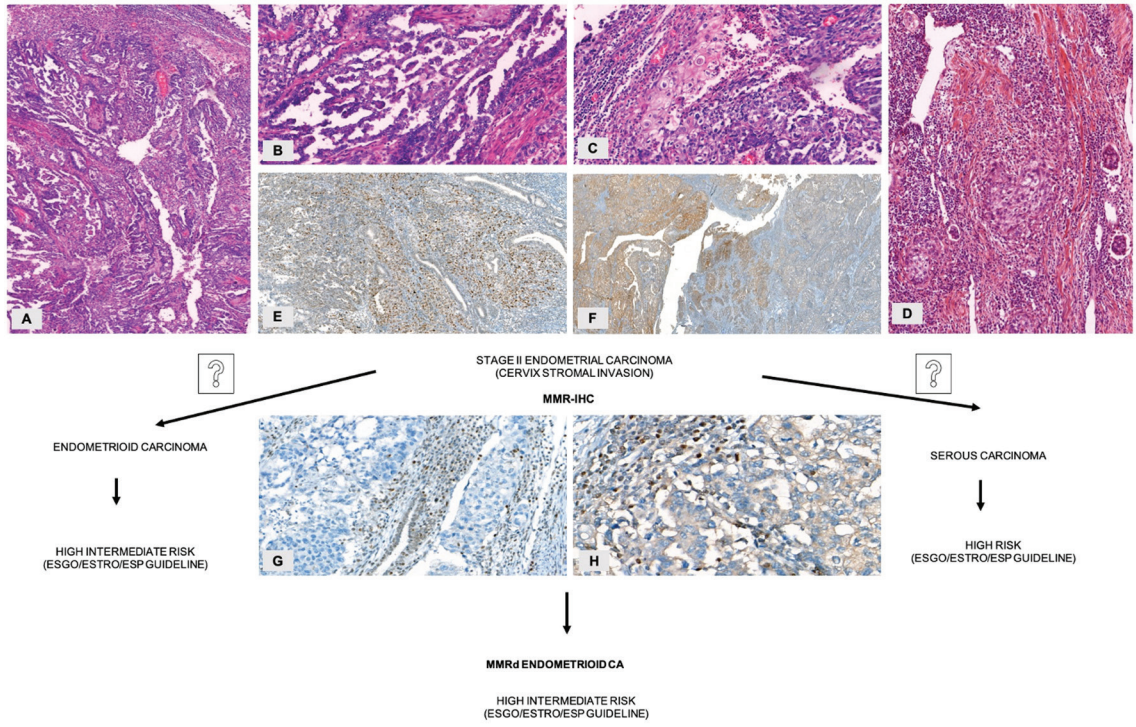

Figure 5. A 59 year old female patient. An exophytic mass with a long diameter of 9.5 cm in the uterine cavity. Endometrial carcinoma infiltrating the outer half of the myometrium and the cervical stroma is detected. (A, B) Serous carcinoma-like morphology. Tubulopapillary structures lined with cuboidal, polygonal tumor cells. However, the high degree of cytological atypia expected in serous carcinoma is not observed. (C) Squamous differentiation, which is not an expected histological feature for serous carcinoma, can be clearly seen in the tumor. (D) The tumor cells are accompanied by dense infiltration of immune cells and extensive lymphovascular invasion can be easily seen. (E, F) Immunohistochemical examination revealed "wild type" expression with p53 antibody, and patchy, scattered staining with p16 antibody. Immunohistochemical features were not supportive of serous carcinoma. Histological features are not consistent with endometrioid carcinoma, clear cell carcinoma, or any other type of endometrial carcinoma. With these findings, the tumor was classified as "stage 2 endometrial carcinoma" due to the invasion of cervix stroma, and a further histological typing could not be made, which is a required parameter for the ESGO/ESTRO/ESP guideline used for patient risk stratification in the management of ECs. According to this guideline, the patient will be evaluated in the "high-intermediate risk group" in case the tumor type is endometrioid carcinoma, and in the "high-risk group" in case of the tumor type is serous carcinoma. (G, H) MMR IHC is performed in the tumor and co-deficiency of MLH1 and PMS2 is detected, as shown in figures G and H, respectively. Since MMR deficiency is not an expected finding in serous carcinoma, the morphological suspicion of serous carcinoma is definitively ruled out and the tumor is reported as "MMRd endometrioid carcinoma". Thus, the tumor is included in the "high-intermediate risk group" according to the molecular classification integrated version of the ESGO/ESTRO/ESP guideline. In this case, the significant contribution of molecular classification to the clinical management of patients with endometrial carcinoma is clear, both to determine the histological type and the risk group of the patient.

ESGO/ESTRO/ESP: European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology, EC: Endometrial carcinomas, MMRd: Deficiency of ≥1 mismatch repair proteins, IHC: Immunohistochemistry, MLH1: MutL homologue 1, PMS2: PMS1 homologue 2

the second step in which the other two antibodies (*MLH1* and *MSH2*) are added. Two-step IHC has been reported to have similar accuracy to a single-step four-antibody test (31).

Accordingly, isolated loss of *MSH6* or isolated loss of *PMS2* indicates hereditary or acquired *MSH6* or *PMS2* defects, respectively. On the other hand, the co-deficiency of *MLH1* and *PMS2* indicates hereditary or acquired *MLH1* defect; co-deficiency of *MSH2* and *MSH6* indicates hereditary or acquired *MSH2* defect. When reporting the MMR IHC result in the pathology report, the terminologies "normal" or "abnormal/defective/deficient" should be used. The use of ambiguous terminologies such as "positive, negative, present, absent, preserved, lost" should be avoided.

MSI PCR testing is a highly accurate and sensitive test, but in terms of its higher cost and poorer availability, it cannot be the first choice in routine practice, as the MMR IHC test is a cheaper and easily accessible test, and has a similar accuracy to the MSI test. As there is a high concordance of these two tests, co-administration of these two tests in all cases is unnecessary. It is recommended that the MSI PCR test is performed when an unexpected or unclear result is obtained with MMR IHC.

c. *P53* testing: Immunohistochemically different *p53* results are related to different types of *TP53* mutations (missense, frameshift, truncating mutations) (32). *TP53* missense mutations result in degradation resistant mutant proteins which accumulate in the tumor cell nucleus and reveal *p53* overexpression immunohistochemically (strong nuclear expression in more than 80% of tumor cell nuclei) (Figure 4). In contrast, non-sense or frameshift mutations result in premature termination codons that terminate translation and appear as complete loss of *p53* expression (null pattern) in tumor cells. The much rarer cytoplasmic *p53* expression pattern is usually caused by TP53 mutations that impair the nuclear localization of the protein. In the absence of TP53 mutation, a "normal, wild type" staining pattern is observed immunohistochemically. The "wild-type" staining pattern is characterized by varying rate (from a few positive tumor cells to the positivity of most tumor cell nuclei) and varying intensity of *p53* staining in tumor cells (Figure 4). In the "wild-type" staining pattern, the extent of *p53* staining varies from a few positive tumor cells to the positivity of most tumor cell nuclei and unlike the mutation immunophenotype of *TP53* gene, the intensity of *p53* staining differs intercellularly. The level of "wild type" expression depends on the differentiation status and proliferative activity of tumor cells. Highly proliferating tumors may show high levels of wild-type *p53* expression, and this profile may be difficult to distinguish from IHC *p53* overexpression seen in TP53 missense mutations. The sensitivity of *p53* IHC in the detection of TP53 mutation is quite high. The concordance of NGS and IHC for the detection of TP53 mutation is 88% (33). A small percentage of ECs harbouring TP53 mutation (truncating mutation) show "wild type" *p53* expression pattern immunohistochemically (32).

The significance of molecular classification in the clinical management of endometrial carcinomas and aspects that require improvement

1. It has been elucidated that accurate and reproducible histotyping, and even grading, of ECs, is not always possible by an approach based solely on histological features, and this issue is more problematic in high-grade ECs. The inclusion of molecular features in the risk stratification scheme appears to make a significant contribution to the clinical approach to patients with ECs, specifically the decision about whether any adjuvant treatment is needed or determination of the appropriate treatment approach (Figure 5). However, prospective validated clinical data are needed to provide therapeutic guidance from molecular classification in routine clinical practice.

2. Molecular subgroups also guide the therapy of patients with ECs. Since MMRd ECs tend to have high LVI, a conservative approach with hormonal therapy is not a good option. In addition, RT should be preferred to CT as a choice of adjuvant therapy in MMRd ECs, as these tumors do not respond well to CT. These patients may also be candidates for anti-PD-1/PD-L1 ICI therapies, when they are at advanced stage or have recurrent disease.

As *POLE*mut ECs often show aggressive histological features, such as higher histological grade, deeper invasion, or LVI, most of the studies regarding *POLE*mut ECs report results from patients who have already received adjuvant therapy. Despite the aggressive features of these tumours, patients with *POLE*mut EC show almost no recurrence or death. It is not clear whether this good clinical course is a result of a good response to treatment or regardless of the treatment (34). However, a recent meta-analysis revealed that most of the *POLE*mut ECs did not exhibit any recurrence or death, and neither type of adjuvant therapy (RT or CT) was associated with clinical outcome in these patients (35). For now, it has been suggested to reduce the treatment of *POLE*mut ECs and thus protect the patient from the toxicity of an unnecessary treatment. However, this approach again needs to be supported by evidence from prospective studies. Anti-PD-1/PD-L1 ICI therapy may be a good treatment option in recurrent or advanced *POLE*mut ECs, as in MMRd ECs.

The *p53*abn group benefits from platinum-based CT and RT. Among the four molecular subgroups, *p53*abn ECs get most benefit from the addition of CT to RT in adjuvant therapy, even at an early stage (36). *HER2/neu* amplification is closely related to the *p53*abn EC group, regardless of the histology.

Therefore, tumors with *TP53* abnormality and *HER-2/neu* amplification may benefit from the addition of trastuzumab to therapy, even in non-serous histology. Moreover, the success of PARP inhibitors in ovarian carcinomas has prompted the consideration of their use in the treatment of *p53*abn ECs. Studies are ongoing to identify the appropriate patient group with HRD for this treatment.

3. Current findings indicate that the presence of a pathogenic *POLE* mutation in an EC is the most important prognostic determinant among these molecular features, and in the presence of MMRd or *POLE*mut, p53 mutation is a passenger mutation that does not appear to affect prognosis. In addition, MMRd ECs also harboring pathogenic *POLE* mutations (multiple classifiers) were found to have a good prognosis, similar to single classifier *POLE*mut tumors. Therefore, the accepted best current knowledge is that the presence of any of the reported 11 POLE pathogenic variants in an EC can be considered the driver genomic feature for molecular classification and take precedent over other added molecular features as the prognostic determinant. Thus, there is a great need for more widely available and cheaper methods, such as IHC, to detect *POLE* mutations. Currently, the only method available for *POLE* mutation analysis is sequencing by NGS or Sanger, and this remains relatively expensive and difficult. Therefore, POLE sequencing is not suitable for all patients with ECs and its use may be limited to patients who are scheduled to receive adjuvant therapy. On the other hand, the presence of any *POLE* mutation other than the 11 reported pathogenic mutations has no prognostic effect and these tumors cannot be considered in the *POLE*mut EC category.

4. NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers to identify those with a high propensity for disease recurrence and thus requiring aggressive treatment, complicates the management of this patient group. it has been shown that NSMP ECs containing β-catenin (CTNNB1) mutations show a more aggressive course. Low-grade EECs harbouring mutations in exon 3 of *CTNNB1* gene have more aggressive outcome, with higher recurrence rate (23). Therefore, further studies are needed to identify prognostic subcategories in the molecular group of NSMP ECs. The presence of the CTNNB1 mutation provides a therapeutic option as well as these patients have been shown to benefit most from Bevacizumab treatment (37).

Conclusion

Molecular classification provided a major improvement in the management of patients with EC across diagnostic, prognostic and therapeutic aspects. Molecular classification has also been integrated into the patient risk stratification guidelines for ECs. MMR and *p53* analysis by IHC should be routinely performed in all ECs. Presence of any pathogenic *POLE* mutation in an EC plays a driver role in the determination of the molecular subgroup and constitutes the first step in the algorithmic approach, in which the MMRd/MSI and *p53* tests are subsequent steps to be performed. However, as *POLE* mutation analysis is expensive and not widely available, this test may be reserved for patients with EC who will be given adjuvant therapy, until a cheaper and easier method, such as an IHC assay, is developed. The significance of molecular classification of ECs should be validated prospectively and improved with further studies.

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