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

Duygu Enneli. Molecular classification in endometrial carcinomas

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Abstract
Classification of endometrial carcinomas based on solely histological features is not sufficient for prognostic and therapeutic guidance of patients. The presence of endometrial carcinomas in which the histological type cannot also be determined clearly and the poor reproducibility of the histological typing lead difficulties on the management of these patients. Molecular classification is very promising due to its high reproducibility and good correlation with clinical outcome of the endometrial carcinoma. Within the scope of “The Cancer Genome Atlas Project”, endometrial carcinomas were divided into four different genomic subtypes, and molecular classification models for endometrial carcinomas were developed based on these molecular subcategories. The prognostic differences of these molecular subgroups and their guiding role for adjuvant therapy have been clearly demonstrated in studies. In this article, the importance of molecular classification for endometrial carcinoma is discussed with different aspects and its use in clinical practice is reviewed.

Keywords: Endometrial carcinoma, molecular classification, mismatch repair, POLE, p53

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Introduction
The majority of endometrial carcinomas (EC) more than 50% 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 course. There is a need for a well-functioning risk stratification model and predictive biomarkers that will determine the extent of surgery or the need type of post-operative adjuvant therapy for the accurate management of these aggressive cases.

According to the traditional dualistic model, defined by Bokhman in 1983, ECs have been categorized into two main groups as type I and type II carcinomas, in terms of clinical, endocrine and histopathological features[1]. According to this classification, endometrioid type ECs (EEC) constitute the majority of type I category, are associated with excess unopposed estrogenic stimulation of the endometrium and have a favourable prognosis. On the other hand, type II ECs, which include non-endometrioid histotypes (such as serous, clear
cell carcinoma), have a worse prognosis and don’t respond well to hormonal therapy. While some ECs are the prototypic examples of type I and type II ECs, many of them, particularly high grade (International Federation of Gynecology and Obstetrics-FIGO grade 3) EECs often do not fit into either category. Clear cell EC (CCEC) is involved in the type II category, but not all CCECs show the expected aggressive course of type II carcinomas. 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 complicates even the distinction between the two main clinically significant histological categories as 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 endometrial carcinoma subtypes[2-4], and for the more common and known EC subtypes, which may show prognostic inter-patient heterogeneity.

As this dualistic model doesn’t fully reflect the clinical diversity of ECs, and recent molecular developments have highlighted the importance of incorporating molecular features into risk grouping algorithms, also 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 by the 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 with different aspects, including how it is applied in clinical practice and the difficulties in its application.

**TCGA 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 achieved 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 to be adapted to clinical practice[6, 7]. The prognostic differences of these molecular subgroups, and their guiding role 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 aspects, including tumor histological typing, prognostic and therapeutic guidance, and elucidation of the hereditary carcinomas.

1. **POLE-ultramutated EC (POLEmut EC)**: During DNA replication, the synthesis of the DNA strand and the rereading of the synthesized DNA strand play crucial role in correcting errors. DNA polymerase is one of the key molecules in DNA replication. POLEmut subgroup of ECs is characterized by pathogenic mutation in the exonuclease domain of the POLE gene, which is a DNA polymerase, leading high tumour mutation load (exceeding 100 mutations per megabase)[9, 10]. 7-12% of ECs are involved in this category. Patients tend to be younger and have a normal body mass index. Although POLEmut 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% low-
grade EEC and 17.4% high-grade EEC, but none of the mixed histology or serous carcinomas were POLEmut EC[5]. High tumour grade, scattered tumour giant cells and prominent lymphocytic infiltrate are characteristic histological features. They may show intratumoral morphological heterogeneity and ambiguous morphology with coexistence of both endometrioid and serous-like histological features[11].

Detection of POLE mutation in EC appears to lead to 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 ec (msi/mmr ec): DNAMismatch repair system (MMR) repairs errors that occur during DNA replication, and has two heterodimers consisting of MutS protein homolog 2 (MSH2)/MutS protein homolog 6 (MSH6) and MutL homolog 1 (MLH1)/PMS1 homolog 2 (PMS2)[12]. It 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 mismatch repair protein. On the other hand, 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, which is called microsatellite instability (MSI). MSI is an indirect indicator of MMR dysfunction.

Initially, MSI/MMRd ECs were defined as hereditary cases, associated with Lynch syndrome, which is a hereditary cancer syndrome characterized by autosomal dominant heterozygous germline mutations in 1 of the 4 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 Lynch syndrome. 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 high as POLEmut ECs.

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% low-grade EEC and 54.3% high-grade ECC were MSI/MMRd EC[5]. Mucinous differentiation, “microcystic, elongated and fragmented (MELF)” pattern of myometrial invasion and LVI are common histological features. Dense peritumoral and intratumoral lymphocytic infiltration usually accompany the tumour cells[16]. They tend to invovle lower uterine segment and occur in a wide age range (those associated with Lynch syndrome occur at a younger age than sporadic cases).

MSI/MMRd ECs constitute the group with intermediate prognosis, among the four molecular subgroups, in which POLEmutECs 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 likely as MMRd EECs and thus patients with MMRd CCEC are recommended to be managed like MMRd EECs[17]. Hormonal therapy, given in the context of fertility-preserving approach, is not suitable for the MMRd group of ECs. As MMRd ECs have high propensity for LVI, sentinel or other nodal procedures may be required[18, 19]. They respond well to radiotherapy (RT), but do not get 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 (FDA) for patients with recurrent or advanced MMRd EC and who don’t have any other treatment option[20].

3. **P53 abnormal ec (P53abn ec)/ copy number-high/ serous-like:**

*P53*abn EC is characterized by the mutation of the tumour suppressor gene-TP53 and constitutes the most aggressive group among the four molecular subgroups, with 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, 5.0% of low-grade EECs were involved in this group[5]. A relationship between SEC and hereditary breast and ovarian cancer syndrome, which is associated with the 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 to 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, thus similar management of these patients is recommended[17].

*P53* abnormal ECs respond well to the combination of platinum-based CT and RT. Targeted therapies based on *HER2* (trastuzumab) or homologous recombination defects (PARP inhibitors) may provide treatment options[11].

4. **No specific molecular profile (nsmp) ec/copy number-low:** Tumors in this molecular group do not harbour 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 tumour is involved in this molecular category, which is 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 (EAH)/endometrioid intraepithelial neoplasia (EIN) and may respond to hormone therapy. NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers, to identify those with a high propensity of disease recurrence and need 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 harbouring mutations in exon 3 of *CTNNB1* gene have more aggressive outcome, with higher recurrence rate[23]. Further studies are needed to find 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 applied in the presence of poor prognostic features.

Distribution of EC histological types as to molecular subgroups is illustrated in Fig.1[24]. Most of the low-grade (FIGO grades 1 and 2) EECs correspond to the NSMP and MMRd molecular subgroups. Whereas, high-grade EECs show a heterogeneous molecular profile with a similar distribution ratio across all genomic categories. All SECs are involved in 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., proposed a clinically applicable method for the molecular classification of ECs – “Proactive molecular risk classifier for endometrial cancer – ProMISE”. In the context of their study consisting of 319
ECs, 4 distinct prognostic molecular subgroups with significantly different survivals have been identified, by using methods of MMR immunohistochemistry (IHC) (to identify MMRd group), p53 IHC (to identify p53abn group), and sequencing for POLE exonuclease domain mutations (to identify POLEmut 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 on 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.

It starts with POLE mutation analysis as the first step, MMRd/MSI analysis follows this and p53 mutation analysis is the last step (Fig. 2)[11]. Since POLE mutation analysis, which is in the first step, is an expensive and inaccessible test, it is usually not possible in routine practice to apply this algorithmic approach in this order. Hence, POLE mutation test can be applied limitedly, only for the patients who are planned to be given adjuvant therapy, until a cheaper and easier method, such as an IHC assay, is developed to investigate pathogenic POLE mutations.

While the majority of endometrial carcinomas are compatible with one of these 4 molecular categories, 3 to 6% of them show features of more than one molecular group and are called "multiple classifier ECs"[27]. Multiple classifier ECs harbour molecular features of different combinations of two molecular groups (POLEmut + MMRd / MMRd + P53abn / POLEmut + P53abn) or combination of three groups (POLEmut + MMRd + P53abn). Concomitant TP53 mutation in POLEmut or MMRd ECs has been shown to be a passenger mutation that does not affect biological behavior of the tumour. It has been elucidated that these tumours do not have a poor prognosis as single-classifier p53abn ECs and thus intensive treatment is not required. Since MMRd ECs with pathogenic POLE mutations are also found to have a good prognosis like single classifier-POLEmut ECs, it is recommended to classify these tumors as single classifier POLEmut 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:** POLEmut 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. Currently, there hasn’t been any immunohistochemical assay to detect the pathogenic POLE mutations, for the diagnosis of POLEmut ECs.

**b. MMR/MSI testing:** MMR/MSI testing is recommended to apply for all ECs, due to its diagnostic, prognostic and therapeutic contributions in the management of patients with EC (Fig. 3). One of the purposes of the 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 Lynch syndrome is another indication. Endometrial carcinoma is often the first carcinoma type detected in patients with Lynch syndrome. Therefore, screening by MMR/MSI testing in ECs enables earlier detection of Lynch syndrome, and provides follow-up of these patients, in terms of the development risk of more fatal carcinomas in the future (frequently colorectal carcinoma), and thus reduces the cancer-related mortality[29, 30].

MMR defect is detected mainly by 2 methods, one is immunohistochemistry (IHC) in which 4 major MMR protein expressions (MLH-1, MSH-2, MSH-6, PMS-2) are evaluated in tumour...
cells and the other is 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 accessible, and revealing also which MMR gene is likely defective.

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 with 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 the second step in which the other two antibodies (MLH1 and MSH2) are added. Two-step immunohistochemistry 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 not being easily accessible, it cannot be the first choice in routine practice, as MMR IHC test, is a cheaper and easily accessible test, and has a similar accuracy to MSI test. As there is a high concordance of these two tests, co-administration of these two tests in all cases is unnecessary and expensive. The MSI PCR test is recommended to be 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) (Fig. 4). On the other hand, non-sense or frameshift mutations result in premature termination codon that terminate translation and reveal complete loss of p53 expression (null pattern) in tumour 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 tumour cells (Fig.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 the immunohistochemical 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 in 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 its aspects needed to be improved
1. It has been elucidated that accurate and reproducible histotyping, and even grading, of ECs, is not always possible by an approach based on solely histological features. And this issue is more problematic in high-grade ECs. The inclusion of molecular features in the risk stratification scheme seems to make a significant contribution to the clinical approach of patients with ECs, on the decision whether any adjuvant treatment is needed or the determination of the appropriate treatment approach (Fig.5). However, prospective validated clinical data are needed to benefit from therapeutic guidance of molecular classification in routine clinical practice.

2. Molecular subgroups also guide 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 POLEmut ECs often show aggressive histological features such as higher histological grade, deeper invasion, LVI, it can be seen in the literature that most of the studies regarding POLEmut ECs reveal results of the patients who have already received adjuvant therapy. Despite the aggressive features of these tumours, patients with POLEmut 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 POLEmut ECs didn’t show any recurrence or death, and any type of adjuvant therapy (RT or CT) was not associated with clinical outcome of these patients[35]. For now, it has been suggested to reduce the treatment of POLEmut ECs and thus protect the patient from the toxicity of an unnecessary treatment. However, this approach needs to be supported by prospective studies. Anti-PD-1/PD-L1 ICI therapy may be a good treatment option in recurrent or advanced POLEmut ECs, as in MMRd ECs.

The p53abn group benefits from platinum-based CT and RT. Among the 4 molecular subgroups, P53abn ECs get most benefit from the addition of CT to RT in adjuvant therapy, even at an early stage[36]. Human epidermal growth factor receptor 2 (HER2)/neu amplification is closely related to the P53abn EC group, regardless of the histology. Therefore, tumors with TP53 abnormality and Her-2/neu amplification may get benefit from the addition of Trastuzumab to therapy, even in non-serous histology. Moreover, the success of poly-ADP ribose polymerase (PARP) inhibitors in ovarian carcinomas has prompted the consideration of their use in the treatment of p53abn ECs. Studies are ongoing to identify the appropriate patient (with homologous recombination deficiency-HRD) for this treatment.

3. Current findings indicate that the presence of 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 direct the prognosis. In addition, MMRd ECs also harbouring pathogenic POLE mutations (multiple classifiers) were found to have a good prognosis similar to single classifier POLE mut tumors. That is, to our current knowledge, 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 precedes other added molecular features as a prognostic determinant. Thus, there is a great need for a cheaper and accessible method such as IHC to detect POLE mutation. Currently, the only method available for POLE mutation analysis is sequencing by NGS or Sanger, and as it is an expensive and difficult method, it cannot be possible to apply or its application may only be limited to patients who are scheduled to receive adjuvant therapy. On the other hand, the presence of any POLE mutation other than 11 reported pathogenic mutations has no such prognostic effect and these tumors cannot be considered in the POLEmut EC category.
4. NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers, to identify those with a high propensity of disease recurrence and need aggressive treatment, complicates the management of this patient group. One of the limited data on this problem is 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. These patients compose the group that benefited most from Bevacizumab treatment[37].

**Conclusion**

Molecular classification provided a major improvement in the management of patients with EC in diagnostic, prognostic and therapeutic aspects. It has also been integrated into the patient risk stratification guidelines of 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 stepwise algorithmic approach in which the MMR/d MSI and p53 tests are performed respectively. However, as POLE mutation analysis is an expensive test, it can be performed limitedly to patients with EC who are planned to be given adjuvant therapy, until a cheaper and easier method, such as an immunohistochemical assay, is developed. The significance of molecular classification of ECs should be validated prospectively and improved with further studies.

**References**


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POLE: DNA polymerase epsilon, POLEmut EC: POLE mutant endometrial carcinoma
Figure 1. Distribution of endometrial carcinoma histological types as to molecular subgroups

Ca= Carcinoma; MMRdef= Mismatch repair deficiency; POLEmut= Polymerase ε mutant; NSMP= No specific molecular profile
Figure 2. The ProMisE (proactive molecular risk classifier for endometrial cancer) technique to define the molecular class of endometrial carcinoma.

EC = Endometrial carcinoma; POLE = Polymerase ε; POLE\text{mut} = Polymerase ε mutant; MMR = Mismatch repair; MMR\text{def} = Mismatch repair deficiency; IHC = Immunochemistry.

Figure 3. Steps of MMR IHC 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.

Figure 4. p53 IHC. A. “Wild type” p53 staining pattern. B. p53 overexpression (Strong nuclear expression in more than 80% of tumor cell nuclei).
Figure 5. 59 years 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 myometrium and the cervical stroma is detected. A, B. Serous carcinoma-like morphology. Tubulopapillary structures lined with cuboidal, polygonal tumor cells. However, 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 noticed in the tumour. 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 in favour of serous carcinoma. Histological features are not consistent with endometrioid carcinoma, clear cell carcinoma, or any other type of endometrial carcinoma.

With these findings, we can only call it as “Stage 2 Endometrial carcinoma” due to the invasion of cervix stroma, and cannot make a further histological typing, 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 of 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 tumour and co-deficiency of MLH1 and PMS2 is detected, as respectively shown in the figures G and H. 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, we have seen the significant contribution of molecular classification to the clinical management of patients with
endometrial carcinoma, both to determine the histological type and the risk group of the patient.

ESGO/ESTRO/ESP= European Society of Gynaecological Society/ European Society for Radiotherapy and Oncology/ European Society of Pathology; MMR= Mismatch repair; IHC= Immunohistochemistry; MLH1= MutL homologue 1; PMS2= PMS1 homologue 2