Update in the molecular classification of endometrial carcinoma

Alicia Léon-Castillo

ABSTRACT

The pathological classification of endometrial carcinomas, one of the cornerstones in patient clinical management, has traditionally been based on morphologic features. However, this classification system does not fully reflect the biological diversity of endometrial carcinomas and has limited reproducibility. In the last decade, several studies have reported the strong prognostic value of the molecular endometrial carcinoma subgroups and, more recently, its potential to inform adjuvant treatment decisions. This has in turn resulted in a transition from a purely morphological classification towards an integrated histological and molecular system in the latest World Health Organization (WHO) classification of tumors of female reproductive organs. The new European treatment guidelines combine the molecular subgroups with traditional clinicopathological features in order to guide treatment decision-making. Accurate molecular subgroup assignment is therefore essential for adequate patient management. This review aims to address caveats and evolution of molecular techniques relevant in the implementation of the molecular endometrial carcinoma classification, as well as challenges in the integration of the molecular subgroups with traditional clinicopathological features.

INTRODUCTION

The molecular endometrial carcinoma classification introduced by The Cancer Genome Atlas (TCGA) has elicited a transition from a purely morphological classification towards an integrated morphological- and molecular-based system. In the TCGA landmark study, the molecular subgroups (POLE, microsatellite unstable, copy-number low and copy-number high) had distinct molecular landscapes and also significant differences in their clinical outcomes. In subsequent years, surrogate markers that are easy to apply on formalin-fixed, paraffin-embedded tissue were developed, allowing the identification of subgroups analogous to the ones described by the TCGA (Table 1). Through sequencing of the exonuclease domain of the DNA polymerase epsilon (POLE) and assessment of the expression of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6) and p53 by immunohistochemistry, endometrial carcinoma could now be classified into POLE-ultramutated (POLEmut), MMR-deficient (MMRd), p53-abnormal (p53abn), or no specific molecular profile (NSMP). Using this highly reproducible methodology several studies have proved the strong prognostic value of the molecular endometrial carcinoma classification, independent of other clinicopathological factors such as stage or histology: POLEmut endometrial carcinomas have an indolent behavior, p53abn endometrial carcinomas have a poor clinical outcome, and MMRd and NSMP endometrial carcinomas have an intermediate prognosis. Furthermore, the molecular subgroups also have the potential to guide adjuvant treatment, as they show different benefit from adjuvant chemotherapy and radiotherapy. This has ultimately resulted in a turning point in the clinical care of patients with endometrial carcinoma.

The clinical relevance of the molecular endometrial carcinoma classification has led to a novel combined histological and molecular classification in the latest World Health Organization (WHO) classification of tumors of female reproductive organs. Additionally, the 2021 European Society of Gynaecological Oncology (ESGO)/European Society for Radiotherapy and Oncology (ESTRO)/European Society of Pathology (ESGO/ESTRO/ESP) guidelines and the most recent European Society of Medical Oncology (ESMO) clinical practice guidelines have integrated the molecular subgroups with traditional clinicopathological features into a novel risk stratification system to assess relative risk of recurrence, with subsequent impact on adjuvant treatment decisions. The European guidelines have therefore encouraged the implementation of the molecular classification in all endometrial carcinomas. However, implementation and interpretation of the surrogate markers in clinical practice can be challenging, especially for the assessment of POLE variants. Additionally, different pathological features, as well as potential biomarkers, may hold distinct prognostic value within each specific molecular subgroup. This review aims to discuss caveats and advances on the implementation of the molecular endometrial carcinoma classification and the novel integrated clinicopathological and molecular risk model.

IMPLEMENTATION OF SURROGATE MARKERS

The identification of surrogate markers has made feasible the implementation of the molecular endometrial carcinoma classification in everyday practice. Currently this entails performing immunohistochemical stains for MMR proteins and p53, and tumor DNA sequencing to identify POLE exonuclease domain
mutations. However, in order to ensure an accurate subgroup allocation, it is important to be aware of caveats and pitfalls of these techniques.

**Interpretation of Somatic** POLE **Exonuclease Domain Variants**

There are five hotspot POLE exonuclease domain mutations that are widely recognized as pathogenic (in this context meaning causal of an ultramutated phenotype and, ultimately, of a favorable clinical outcome): P286R, V411L, S297F, S497F, and A456P. However, it is challenging to determine the effect that rarer non-hotspot variants will have on the tumor’s biology. Although POLEmut endometrial carcinomas have characteristic genomic features (high mutational burden, high proportion of C>A substitutions, low proportion of C>G, and almost no indels, as well as COSMIC mutational signature 10), these parameters were ill defined, hampering the annotation of non-hotspot POLE variants.

Additionally, these genomic correlates can only be properly assessed after performing whole genome or whole exome sequencing, techniques that are currently not (or only rarely) available in clinical practice. This represented an important hurdle in the adoption of the molecular endometrial carcinoma classification.

<p>| Table 1 Molecular and clinicopathological features of molecular subgroups |
|--------------------------------------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th><strong>POLEmut EC</strong></th>
<th><strong>MMRd EC</strong></th>
<th><strong>NSMP EC</strong></th>
<th><strong>p53abn EC</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency</strong></td>
<td>5–15%</td>
<td>20–30%</td>
<td>30–60%</td>
</tr>
<tr>
<td><strong>Surrogate markers</strong></td>
<td><strong>NGS (POLE sequencing)</strong></td>
<td><strong>MMR proteins IHC: PMS, MSH6 (MLH1, MSH2)</strong></td>
<td><strong>p53-IHC</strong></td>
</tr>
<tr>
<td><strong>Molecular features</strong></td>
<td><strong>Ultramutated (&gt;100 mut/Mb)</strong></td>
<td><strong>Hypermutated (&gt;10 mutations/ Mb)</strong></td>
<td><strong>Low TMB</strong></td>
</tr>
<tr>
<td><strong>Somatic copy number alteration-low</strong></td>
<td><strong>Somatic copy number alteration-low</strong></td>
<td><strong>Somatic copy number alteration-low</strong></td>
<td><strong>Somatic copy number alteration-high</strong></td>
</tr>
<tr>
<td><strong>20% with MMR deficiency or MSI</strong></td>
<td><strong>MSI</strong></td>
<td><strong>MSS</strong></td>
<td><strong>MSS</strong></td>
</tr>
<tr>
<td><strong>20% with p53 mutant-expression/TP53 mutations</strong></td>
<td><strong>10% with p53 mutant-expression/TP53 mutations</strong></td>
<td><strong>TP53 wild-type</strong></td>
<td><strong>TP53 mutated</strong></td>
</tr>
<tr>
<td><strong>PTEN mutations</strong></td>
<td><strong>Frequent homologous recombination deficiency</strong></td>
<td></td>
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<td><strong>PI3CA mutations</strong></td>
<td><strong>20–25% Her2 amplification</strong></td>
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<td><strong>CTNNB1 mutations</strong></td>
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**Associated histological features**

- Mostly high-grade endometrioid
- Mostly high-grade endometrioid
- Mostly low-grade endometrioid
- Mostly high-grade, all histologies
- Substantial LVSI
- Squamous metaplasia
- Substantial LVSI
- MELF-like invasion
- ER/PR positive
- High-grade atypia

**Associated clinical features**

- Low BMI
- High BMI
- High BMI
- Low BMI
- Early stage
- Advanced stage
- Younger patients
- Older patients
- Local recurrences
- Distant recurrences

**Prognosis**

- Excellent
- Intermediate
- Intermediate-poor; stage and histologic-grade dependent
- Poor

**Potential biomarkers for prognosis refinement**

- TLS
- CD8 intra-epithelial lymphocytes
- CD8 intra-epithelial lymphocytes
- Molecular mechanism (MLH1 promoter methylation vs germline mutations)
- L1CAM
- CTNNB1 mutations
- ER/PR expression

as an incorrect annotation of a POLE variant could lead to an erroneous allocation of an endometrial carcinoma within the POLEmut subgroup, and thus impact the prognostic assessment and clinical management of the patient. This was highlighted by the study by Imboden et al, in which patients with an endometrial carcinoma with one of the five POLE hotspot exonuclease domain mutations had a significantly better clinical outcome than those with non-POLEmut endometrial carcinoma. However, this difference was no longer observed when comparing patients with an endometrial carcinoma with any POLE variant with patients with a POLE wild-type carcinoma.

Analysis of the TCGA endometrial carcinoma cohort using cancers with a hotspot POLE exonuclease domain mutation as a truth set revealed genomic features that allowed generation of a scoring system, with well-defined cut-off points, to assess POLE variants’ pathogenicity. Furthermore, this scoring system identified a set of 11 POLE exonuclease domain mutations eliciting an ultramutated phenotype and causative of indolent clinical behavior (Box 1). This has provided a pragmatic guideline for the interpretation of POLE variants, facilitating the implementation of POLE testing in clinical practice. Importantly, only 11 of the 21 different POLE exonuclease domain variants in the TCGA cohort qualified as pathogenic, proving that the sole presence of a POLE variant is insufficient to classify an endometrial carcinoma as POLEmut. This was further illustrated by the individual patient data meta-analysis by McAlpine et al, where patients with endometrial carcinomas with non-pathogenic POLE variants (a variant different from the 11 POLE exonuclease domain mutations previously mentioned) had a significantly poorer outcome than those with a POLEmut. Of note, as whole genome/exome sequencing techniques become more widely available and more endometrial carcinomas undergo extensive sequencing, the initial list of 11 POLE exonuclease domain mutations causative of ultramutation is bound to be expanded in upcoming years.

**Testing for MMR Deficiency in Endometrial Carcinomas**

Identification of endometrial carcinomas with MMR deficiency has a threefold value: it (1) serves to detect patients at higher risk of presenting a Lynch syndrome, (2) provides prognostic information as a surrogate marker for MMRd endometrial carcinomas (once POLE pathogenic mutations have been excluded, see later in the text), and (3) holds predictive value, as patients with MMRd endometrial carcinomas benefit from check-point inhibitor treatment. Furthermore, it can aid in the histological subtyping of endometrial carcinomas as MMRd endometrial carcinomas have most frequently an endometrioid morphology.

Approximately 10% of MMRd endometrial carcinomas and 3% of all endometrial carcinomas are due to Lynch syndrome, a cancer susceptibility syndrome caused by germline mutations in the MMR genes (MLH1, PMS2, MSH2, MSH6) or EPCAM. Although MMRd endometrial carcinomas are enriched for high-grade endometrioid subtype, occasionally non-endometrioid cancers are encountered, also in the context of Lynch syndrome. For example, in the study by Post et al, 14% and 8% of patients with endometrial carcinoma and Lynch syndrome presented with a serous or clear cell histology respectively, while Ryan et al reported two (12.5%) carcinomas, two (12.5%) mixed endometrial carcinoma, and one differentiated carcinoma (6.3%) in a group of patients with endometrial carcinoma and Lynch syndrome. Those carcinomas arising in the context of Lynch syndrome can also be encountered at any age, with approximately 17% and 86% of cases presenting in women over 70 and 50 years of age, respectively. Accordingly, current guidelines for Lynch syndrome screening recommend staining for MMR proteins on all endometrial carcinomas, regardless of histologic type or patient’s age.

In order to classify MMRd endometrial carcinomas, either immunohistochemistry or DNA-based techniques (panels of microsatellite markers for detection of microsatellite instability) can be used. MMR immunohistochemistry consists of staining for the four major MMR proteins: MLH1, PMS2, MSH2, and MSH6. MMRd endometrial carcinomas will have loss of one or more MMR protein. Alternatively, it is possible to use a two-marker approach (immunohistochemistry stain for only PMS2 and MSH6) to identify MMRd endometrial carcinomas. MMR proteins occur as heterodimers in the cell. While MLH1 and MSH2 can stabilize in the cell by forming heterodimers with different partners in the absence of PMS2 and MSH6, respectively, this is not the case for PMS2 and MSH6. As a result, there will always be loss of PMS2 expression in absence of MLH1, and MSH6 will always be lost in absence of MSH2. Accordingly, it is possible to use a two-antibody approach to identify MMRd endometrial carcinomas. In cases of PMS2 or MSH6 loss, MLH1 and MSH2 should be performed, followed by MLH1 promoter methylation analysis in endometrial carcinomas with MLH1-PMS2 loss in order to screen for Lynch syndrome carriers. This strategy has been established to be sufficient in endometrial carcinoma to detect MMRd cases.

The concordance between MMR immunohistochemistry and microsatellite instability analysis is high (93–95%). Discrepant results between both techniques can occur in the context of subclonal MMR deficiency (a well-delimited area of the tumor with loss of expression of a major MMR protein), as these MMRd areas can be overlooked by using microsatellite instability analysis. Additionally, cancers with MSH6 mutations, which are frequently encountered in endometrial carcinoma, can present as microsatellite stable. It is also important to note that correct interpretation of microsatellite instability assay results requires highly trained personnel, as changes in endometrial carcinoma can be more subtle than those described in colorectal cancer. Since immunohistochemistry can identify the defective protein, allows correlation with morphology, and is a widely available and cheaper
technique, its use is currently favored in clinical practice over that of DNA methods.

**Surrogate Markers for p53abn Endometrial Carcinomas**

p53 immunohistochemistry or *TP53* sequencing is used currently as surrogate markers for the identification of p53abn endometrial carcinomas. There are four distinct p53 mutant-expression patterns: mutant overexpression (diffuse and strong nuclear positivity) associated with missense mutations, null mutant (complete absence of expression) with frequent frameshift or nonsense mutations, cytoplasmic (overexpression in the cytoplasm) due to mutations in the tetramerization or C-terminal domain, and subclonal (a well-delimited area of the tumor with mutant expression of p53 in a background of wild-type expression). No differences in recurrence rates have been observed between the different mutant-expression patterns or mutation types.

Subclonal mutant p53 expression is frequently defined with a lower threshold of 10% of the tumor’s area with mutant-expression pattern. However, tumors with minimal and multifocal areas with mutant expression have also been described, a finding occurring most frequently in the context of *POLE* mut or MMRd endometrial carcinomas. The clinical value of these small mutant areas is still unknown. Since a lower-limit 10% threshold has been used in previous studies for molecular subgroup assignment, for uniformity purposes it is advisable to use this as a lower-limit cut-off point.

Both p53 immunohistochemistry and *TP53* sequencing have a high concordance rate (90.7–92.3%). Discrepancies are most frequently encountered in *POLE* mut or MMRd endometrial carcinoma (multiple classifier endometrial carcinomas: *POLE* mut-p53abn, MMRd-p53abn, or *POLE* mut-MMRd-p53abn), where *TP53* mutations are not driving events and do not confer a poor prognosis. Once *POLE* mut and MMRd endometrial carcinomas are excluded, the concordance between both techniques reaches 94.5–95.1%. Discrepancies between both methods can also be caused by suboptimal pre-processing or staining of the sample, or to misinterpretation of the immunohistochemistry staining pattern. Pathologists must therefore be trained in the variety of mutant-expression patterns and be aware of possible pitfalls.

A small remaining group of MMR proficient and *POLE* wild-type endometrial carcinomas will show true discrepancies between both techniques. Given that most studies assessing the prognostic value of the molecular subgroups have been based on p53 immunohistochemistry, and the affordable price and wide availability of this technique, p53 immunohistochemistry is overall favored as the preferred surrogate marker for p53abn endometrial carcinomas.

**Molecular Subgroup Diagnosis**

Correct molecular subgroup allocation requires all surrogate markers to be performed in order to correctly categorize carcinomas with more than one classifying feature (multiple classifier endometrial carcinomas), accounting for approximately 3–7% of all endometrial carcinomas. A simple stepwise diagnostic algorithm now allows molecular subgroup assignment of these endometrial carcinomas (Figure 1). First, *POLE* exonuclease domain is sequenced to classify *POLE* mut endometrial carcinomas. Carcinomas without a pathogenic *POLE* exonuclease domain mutation are then stained for MMR proteins to identify MMR-deficient endometrial carcinomas. Finally, MMR-proficient endometrial carcinomas are stained for p53, to classify p53abn cancers.

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**Figure 1** Diagnostic algorithm for the integrated molecular endometrial carcinoma classification. Adapted from Vermij et al. EC, endometrial carcinoma; EDM, exonuclease domain mutation; MMR, mismatch repair; MMRd, mismatch repair-deficient; MMRp, mismatch repair-proficient; NOS, not otherwise specified; NSMP, no specific molecular profile; p53abn, p53-abnormal; *POLE*, polymerase epsilon; *POLE* mut, polymerase epsilon-ultramutated. Pathogenic *POLE* mutations include p.Pro286Arg, p.Val411Leu, p.Ser297Phe, p.Ala456Pro and p.Ser459Phe. MMR deficiency is defined by loss of one or more MMR proteins (MLH1, PMS2, MSH2 and MSH6). p53 immunohistochemistry is an acceptable surrogate marker for *TP53* mutation status in MMR-proficient, *POLE* wild-type endometrial carcinoma. Permission to use figure under a creative commons (CC) by license, Wiley https://creativecommons.org/licenses/by/4.0/.
INTEGRATED CLINICOPATHOLOGICAL AND MOLECULAR RISK MODEL

Using the surrogate marker approach, several studies have repeatedly proved the strong prognostic significance of the molecular subgroups. Whether addressing selected cohorts of patients, such as (high-)intermediate risk or high-grade endometrial carcinomas, or unselected cohorts, POLE mut endometrial carcinomas show an indolent behavior with barely any recurrences or endometrial carcinoma-related deaths, patients with p53abn endometrial carcinoma have a poor clinical outcome, and patients with MMRd and NSMP have an intermediate prognosis. Even in high-risk subgroups, resulting in a more accurate risk stratification and selection of adjuvant treatment for patients with endometrial carcinoma.2–4 34 35  Even in high-risk cohorts, including non-endometrioid histology and stage III disease, the molecular classification is predictive of recurrence and overall survival, independently of other clinicopathological factors.5

The latest European clinical practice guidelines have adopted a model integrating clinicopathological features and molecular subgroups, resulting in a more accurate risk stratification and selection of adjuvant treatment for patients with endometrial carcinoma.2–4 34 35  This approach is currently being tested in patients with (high-)intermediate risk disease in the ongoing phase III clinical trial, PORTEC-4a (Figure 2).36 In this trial, patients with (high-)intermediate risk endometrial carcinoma will be randomized to a standard adjuvant treatment arm (vaginal brachytherapy) or an experimental arm where adjuvant treatment will be dependent on pathological and molecular features, including substantial lymphovascular space invasion (LVI), molecular subgroups, CTNNB1 mutations, and L1 cell adhesion molecule expression.36

Importantly, the prognostic weigh of traditionally relevant pathological features (such as stage, International Federation of Gynecology and Obstetrics (FIGO) grade, histotype, and LVSIV) and biomarkers will differ across the molecular subgroups.

Prognostic Role of Stage Within the Molecular Classification

Stage is one of the most important prognostic factors for recurrence and survival in endometrial carcinoma. In multivariable analyses including the molecular subgroups and pathologic characteristics, stage showed a strong and independent prognostic feature.5 35 Stage is a particularly relevant prognostic factor for MMRd and NSMP endometrial carcinomas, for whom it is a chief feature for risk assignment and clinical management decision making.8 9

Considering the higher frequency of advanced stage disease at diagnosis observed in p53abn endometrial carcinomas as compared with the other molecular subgroups,2 3 4 36 and the fact that there were no studies addressing the clinical outcome of the molecular subgroups in patients staged by lymphadenectomy, it had been suggested that the poor outcome of p53 endometrial carcinoma could partly be due to undetected lymph node metastases (undetected FIGO stage IIC disease). In a recent study analyzing a cohort of high-grade endometrial carcinomas staged by lymphadenectomy, multivariable analysis including the molecular subgroups and relevant clinicopathological features rendered p53abn as a strong prognostic factor for poor survival and recurrence, independently of stage.36 Moreover, a subanalysis of patients staged by lymphadenectomy as stage I confirmed a poor clinical outcome for lymph node-negative patients with p53 endometrial carcinoma (28.1% 5-year recurrence rate and 66.2% 5-year overall survival).6

POLEmut endometrial carcinomas present most frequently with early-stage disease (stage I–II). Few stage III POLEmut endometrial carcinoma have been described in the literature. In the meta-analysis by McAlpine et al only two of the 25 patients with stage III POLEmut endometrial carcinoma had a recurrence and only one died due to endometrial carcinoma.14 In PORTEC-3, 23.5% of 51 POLEmut endometrial carcinomas were stage III, of which only one patient with advanced lymphatic disease at presentation had recurrence and ultimately died due to her cancer. Of note, this patient had a supraclavicular node identified at the time of the first external beam radiotherapy session. These data suggest that the outstanding clinical outcome of POLEmut endometrial carcinoma shown in previous studies could also hold true for stage III endometrial carcinoma and would support adjuvant treatment de-escalation. Prospective studies, like the blue trial in the Refining Adjuvant treatment IN endometrial cancer Based On molecular features.
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(RAINBO) program, a single-arm trial where patients with POLEmut endometrial carcinoma will receive no/de-escalation of adjuvant treatment and be prospectively registered, will help to define the adequate adjuvant treatment (if any) for these patients.37

Prognostic Role of FIGO Grade and Histotype in the Molecular Classification

NSMP endometrial carcinoma is generally considered to have an intermediate prognosis. However, when addressing cohorts enriched for high grade histologies, particularly clear cell carcinomas, NSMP endometrial carcinoma shows a poor clinical outcome, similar to that of p53abn endometrial carcinomas.38–40 A recent study addressing the prognostic value of FIGO grading in the context of the molecular subgroups found a lower risk of recurrence for low-grade NSMP endometrial carcinoma as compared with high-grade NSMP (82.4% vs 55.2% for low-grade NSMP; p=0.007).41 The variation in clinical outcome of low-grade versus high-grade NSMP endometrial carcinoma could mirror differences in their molecular background. Momeni-Boroujeni et al reported three distinct molecular clusters in NSMP endometrial carcinoma, cluster C3 presenting a higher proportion of non-endometrioid and high-grade histologies as compared with the other two clusters.42 While clusters C1 and C2 were characterized by PTEN mutations combined with PIK3R1 or PIK3CA mutations, respectively, cluster C3 was defined by KRAS mutations and 1q high level gain or CTNNB1 mutations in combination with AKT1 mutations, as well as a higher fraction of their genome altered as compared with the other two clusters. Additionally, cluster C3 had a higher proportion of estrogen receptor and progesterone receptor negative endometrial carcinomas. A subanalysis of PORTEC-3 patients with NSMP endometrial carcinoma revealed a poorer clinical outcome for those patients with estrogen receptor and progesterone receptor negative NSMP endometrial carcinoma.43 These estrogen receptor/progesterone receptor negative tumors more often had high-risk features such as high-grade histology and L1 cell adhesion molecule expression.

Importantly, serous p53abn endometrial carcinomas do not show significant difference in survival as compared with p53abn of other histologies.44–46 Accordingly, there are similarities in the mutational landscape between p53abn with different histologies, and a lack of molecular features exclusive to any histologic type.45 Similar results have been reported regarding POLEmut and MMRd endometrial carcinomas when comparing low-grade and high-grade carcinomas.41

Prognostic role of Lymphovascular Space Invasion in the Molecular Classification

LVSI is an important prognostic feature for endometrial carcinomas. Several studies have reported the relevance of the extension, rather than the mere presence, of LVSI. Substantial LVSI, defined as more than four to five vessels with tumor emboli, has proved to be an important predictor of lymph node and distant metastases as well as reduced overall survival.46–47 Furthermore, substantial LVSI is an adverse prognostic factor independent of molecular features in (high-)intermediate risk as well as in high-grade endometrial carcinoma.16–18 It is therefore an excellent candidate to further refine the risk assessment of molecularly classified endometrial cancer.

A higher proportion of substantial LVSI is frequently described by p53abn and MMRd endometrial carcinomas. It is probably within the MMRd and particularly the NSMP group, given its heterogeneous clinical outcomes in the different cohort studies, that substantial LVSI will play a major role. This was highlighted by the study on (high-)intermediate risk presented by Stelloo et al.4 More limited cases are available in literature, substantial LVSI does not seem to have an impact on the prognosis of patients with POLEmut endometrial carcinoma.

Biomarkers in the Context of the Molecular Subgroups

Although the integration of the molecular subgroups with clinicopathological features enhances risk stratification of patients with endometrial carcinoma, new biomarkers are still needed to provide an even more accurate prognostic assessment beyond the improved integrated clinicopathological and molecular model. A promising biomarker for further prognostic refinement is anti-tumor immune infiltrate, for which several studies have reported its prognostic value.48–49

More recently, two research groups have addressed the value of T-cell response in the context of the molecular classification.50–51 As previously reported, both studies showed a high prevalence of POLEmut and MMRd endometrial carcinoma in cases with high immune infiltrate.52 More surprisingly though, they also found a significant group of p53abn and NSMP endometrial carcinomas with a high-density immune infiltrate (60% and 37% of p53abn and NSMP endometrial carcinomas with tumour-infiltrating lymphocytes-high, and 40.8% and 36.0% of p53abn and NSMP endometrial carcinomas with immune high densities as per hierarchical clustering analysis).50–51 In the multivariable analysis presented by Talhouk and colleagues including the molecular subgroups, immune clusters, and tumour-infiltrating lymphocytes, only the molecular subgroups proved to have an independent prognostic significance.51 In contrast, the study presented by Horeweg et al did find a significant prognostic value of intra-epithelial CD8 lymphocytes, independent of the molecular subgroups.50 Furthermore, exploratory analysis on the predictive value for recurrence of intra-epithelial CD8+ lymphocytes found a significant effect within the p53abn and NSMP endometrial carcinomas, although weaker in this last subgroup. These discrepancies could be due to differences in methodology, sample size, or cohort characteristics (while the study of Talhouk et al used a retrospective cohort, Horeweg and colleagues used material from the clinical trials PORTEC-1 and 2).

More recently, tertiary lymphoid structures have also awakened interest in the field of endometrial carcinoma.53 These structures consist of B-cell aggregates with germinal B-cells centers and dendritic cells surrounded by T-cells, and associated with high endothelial venules. These aggregates can be more easily identified with L1 cell adhesion molecule immunohistochemistry, facilitating its implementation in clinics. Importantly, tertiary lymphoid structures could have a favorable predictive value for recurrence and death related to endometrial carcinoma, independent of molecular subgroups and clinicopathological features, particularly in MMRd cancers.53

MMRd endometrial carcinomas are most frequently due to MLH1 promoter methylation (70–75%) or somatic mutations (15–20%), with a minority of cases arising in the context of germline mutations in MMR genes (10%).18 These different molecular mechanisms might have an impact on the mutational landscape of MMRd endometrial carcinomas and ultimately on their risk of recurrence.
MMRd endometrial carcinomas due to MLH1 promoter methylation could be associated with enrichment of JAK1 mutations, lower tumor mutational burden, and lower tumor-infiltrating lymphocytes densities, compared with MMRd endometrial carcinomas with germline mutations. Furthermore, MMRd endometrial carcinomas with MLH1 promoter methylation could have shorter progression-free survival compared with MMRd endometrial carcinomas with germline mutations, although this difference in survival was not found significant in multivariable analysis including clinicopathological features such as stage, age, or LVSI.

The need for prognostic refinement is particularly relevant in the context of NSMP endometrial carcinomas. These are defined by absence of pathogenic POLE exonuclease domain mutations, MMR proficiency, and p53 wild-type staining pattern. In other words, NSMP endometrial carcinomas do not have a distinct molecular feature but a heterogeneous molecular landscape, and thus a heterogeneous clinical outcome, with potential biomarkers with prognostic value. CTNNB1 exon 3 mutations are a candidate molecular feature to further refine the molecular classification, as several studies have reported a poorer clinical outcome in low-grade, early-stage (NSMP) endometrial carcinomas carrying these mutations. Several studies have also suggested a prognostic role for L1 cell adhesion molecule expression (assessed through immunohistochemistry stain) as a biomarker for poor clinical outcome. Although this marker frequently co-exists with p53abn expression, the prognostic value of L1 cell adhesion molecule is independent of the latter. Additionally, lack of expression of estrogen receptor could identify a subgroup of NSMP endometrial carcinomas at higher risk of recurrence. The clinical role in the decision-making about adjuvant treatment of a molecular-integrated risk model, including CTNNB1 exon 3 mutations and L1 cell adhesion molecule expression, is being tested in the ongoing PORTEC-4a clinical trial.

COST-EFFECTIVENESS OF THE MOLECULAR ENDOMETRIAL CARCINOMA CLASSIFICATION

Reducing the Costs of Molecular Testing

The new ESGO/ESTRO/ESP and ESMO guidelines have integrated the molecular subgroups into the new endometrial carcinoma prognostic risk groups and encouraged the implementation of the molecular classification in all endometrial carcinoma. This would entail performing immunohistochemistry stains for MMR proteins and p53, and tumor DNA sequencing to identify POLE exonuclease domain mutations. Immunohistochemistry stains for MMR proteins and p53 are used regularly in clinical practice and are easy to perform. As previously discussed, MMR immunohistochemistry is a valuable screening tool to identify patients at risk of having Lynch syndrome and should therefore be performed on all endometrial carcinomas regardless of histologic type or patient’s age. p53 immunohistochemistry is a useful marker to identify serous carcinomas with pseudoglandular morphology that could otherwise be misdiagnosed as grade 1 endometrioid cancers. However, lack of resources may not allow sequencing of all endometrial carcinomas to identify POLE exonuclease domain mutations.

The implementation challenge for the molecular classification lies therefore in POLE testing. Sequencing methods such as Sanger and next generation sequencing panels can be used to identify POLE mutations in the exonuclease domain (exons 9–14). Nevertheless, these techniques are time consuming and costly, and require expertise. Sequencing methods restricted to the analysis of the hotspot POLE exonuclease domain mutations, easier to interpret and implement, with faster turn-around time, and more affordable price, could be a valid alternative to Sanger or next generation sequencing techniques and would facilitate a broad implementation of the molecular classification on all endometrial carcinomas. Nevertheless, it is important to point out that even the use of in-house or comprehensive next generation sequencing panels offered by commercial companies for the molecular profiling of high-risk, early-stage endometrial carcinoma has been shown to be cost-effective in a first study.

Should Molecular Testing be Performed on All Endometrial Carcinomas?

Currently, it is recommended that molecular classification is carried out on all endometrial carcinomas. However, until rapid, reliable, and cheap testing techniques for the identification of POLE exonuclease domain mutations are developed, it might not be possible to perform POLE testing on all tumors. Hence, in a setting with limited resources, it is important to establish which patients with endometrial carcinoma would benefit most from molecular profiling and should therefore be prioritized for molecular testing.

In recent years it has become apparent that the molecular endometrial carcinoma classification has prognostic value and can also guide adjuvant treatment, potentially reducing overtreatment and undertreatment. The molecular profiling of high-risk endometrial carcinomas in PORTEC-3 translational study demonstrated differences in prognosis and also in treatment benefit between the molecular subgroups. Patients with p53abn endometrial carcinoma had a significant and clinically relevant benefit from addition of chemotherapy to external beam radiotherapy (absolute difference of 22.4% for 5-year recurrence-free survival). These results are in line with the clinical trial results, where patients with serous endometrial carcinoma had a greater absolute benefit from adjuvant chemotherapy and radiotherapy. There was no benefit from added chemotherapy in patients with POLEmut endometrial carcinoma, as both treatment arms had an excellent prognosis. Small studies have also evaluated the sensitivity of POLEmut endometrial carcinomas (the majority of them with low-grade endometrioid endometrial carcinomas) to adjuvant radiotherapy. Analysis of patients in the observation arm of PORTEC-1 revealed a 10-year recurrence-free survival of 100% for POLEmut endometrial carcinoma versus 80.1% for POLE wild-type endometrial carcinoma (HR=0.143, p=0.049). Similar results were reported by McConkey et al: patients with POLEmut endometrial carcinomas and no adjuvant treatment had no endometrial carcinoma-related events and had a favorable outcome compared with those patients with POLE wild-type endometrial carcinomas. In a large cohort of high-grade endometrial carcinomas, patients with a POLEmut high-grade endometrial carcinoma not receiving adjuvant treatment had an excellent clinical outcome, presenting no recurrences or endometrial carcinoma-related deaths. This is in line
with a meta-analysis based on individual patient data on the treatment effect on POLEMut endometrial carcinomas, where patients with POLEMut endometrial carcinoma did not seem to benefit from adjuvant treatment.14 These data suggest that the omission of adjuvant radiotherapy would also be safe for patients with high or (high-) intermediate risk POLEMut endometrial carcinoma.14 64 65

Given the potential of the molecular subgroups to improve patient management, the most recent European treatment guidelines have included specific recommendation for POLEMut and p53abn endometrial carcinomas: no adjuvant treatment should be considered for patients with POLEMut stage I–II, while adjuvant external beam radiation and chemotherapy are recommended for patients with p53abn with myometrial invasion.8 9 Risk assignment and adjuvant treatment for patients with MMRd or NSMP further depend on stage, histotype, FIGO grade and LVSI. Patients with high-grade advanced stage II or higher or substantial LVSI should be therefore prioritized for molecular subtyping, as these results will be relevant to guide adjuvant treatment decisions and avoid overtreatment or undertreatment. This strategy was considered cost-effective, as reported by Orellana et al.62

The added clinical value of the molecular classification for women with low-risk endometrial carcinoma (stage IA, low-grade endometrioid endometrial carcinoma without focal LVSI) is unclear. Given the low recurrence risk of carcinoma in these patients (approximately 5%),68 the identification of POLEMut endometrial carcinomas would have limited clinical value. Since NSMP and MMRd endometrial carcinomas represent most of low-risk endometrial carcinomas,4 the findings of previous studies regarding survival of patients at low risk are largely applicable to these molecular subgroups. Nevertheless, p53abn subgroup might hold prognostic value in the context of low-risk cancers, although there are few survival data available.

A small subanalysis of low/low-intermediate risk endometrial carcinomas (n=242) within the molecularly profiled PORTEC-1 and 2 cohorts revealed a trend towards significance for a higher risk of distant recurrences and reduced overall survival for patients with p53abn in univariable analysis (distant recurrence HR=3.939, 95%CI 0.941 to 16.487, p=0.061; overall survival HR=1.989, 95%CI 0.977 to 4.048, p=0.058).4 Importantly, p53abn endometrial carcinoma represented approximately only 9% of low/low-intermediate risk endometrial carcinomas.4 In order to avoid the extra cost of POLE testing in low-risk endometrial carcinomas, p53 immunohistochemistry could be used as a screening method in this group of patients (only tumors with a mutant p53 staining pattern and MMR-proficient would be tested for POLE exonuclease domain mutations).67 Future studies assessing the clinicopathological and molecular features of low-risk p53abn endometrial carcinomas will elucidate the best clinical management for these patients. Until then, treatment decisions for patients with low-risk p53abn endometrial carcinomas should be made on an individual basis.69 Of note, the use of only MMR and p53 immunohistochemistry (no POLE testing) is insufficient to molecularly classify endometrial carcinomas, and consequently the terms NSMP/MMRd/p53abn endometrial carcinoma should be avoided.

CONCLUSION
Clinicopathological features are the basis of high-risk stratification in endometrial carcinoma, which has guided the adjuvant treatment of these patients. In recent years, there has been a paradigm shift with the incorporation of the molecular endometrial carcinoma classification. The strong prognostic value of the molecular classification and its potential to guide adjuvant treatment has prompted its integration with traditional clinicopathological features into endometrial carcinoma risk groups in the latest European clinical practice guidelines. The specific prognostic value and size effect of pathological variables within each molecular subgroup should be evaluated in coming years to further improve risk assessment and patient management. In order to further refine prognosis assessment and to provide patients with tailored adjuvant therapy options, future clinical trials should address the molecular endometrial carcinoma subgroups in their designs.

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ORCID iD Alicia Léon-Castillo http://orcid.org/0000-0003-0873-5362

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