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REVIEW

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Predictive biomarkers in endometrial carcinomas: a review of their relevance in daily anatomic pathology

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Abstract

Endometrial carcinoma (EC) is the most common gynecologic malignancy in high-income countries, with its incidence and mortality rising globally, particularly in countries undergoing rapid socioeconomic transitions. Over the past decade, the management of EC has shifted towards molecular classification and biomarker-driven therapies. This shift began in 2013 with the discovery of the 4 prognostic and molecular EC subgroups by The Cancer Genome Atlas (TCGA). Following the TCGA discovery, the Proactive Molecular Risk Classifiers for Endometrial Cancer (ProMisE) and the TransPORTEC initiative have provided 4 pragmatic molecular classifiers by combining next-generation sequencing (NGS) and surrogate immunohistochemical markers to TCGA's categorization: Mismatch Repair Deficient (MMRd); p53-abnormal (p53abn); No Specific Molecular Profile (NSMP); and *POLE*-mutant (*POLE*mut). These subgroups not only provide insights into the biological behavior of EC but also have strong clinical relevance and prognostic implications. In line with these advancements, the World Health Organization (WHO) endorsed molecular classification in 2020, advocating for its integration into EC pathology reports. In 2023, the Federation of International Gynecology and Obstetrics (FIGO) proposed a new staging system that integrates histological parameters and molecular profiles into routine pathology practice. The use of complete molecular classification surrogates in all EC cases, whenever resources permit, is intended to promote prognostic risk-group stratification, data collection, Lynch Syndrome (LS) screening, and potentially influence adjuvant and systemic treatment decisions, as well as predict the efficacy of Immune Checkpoint Inhibitors (ICI). Consequently, the evolving landscape of predictive biomarkers in EC has gained importance in daily oncology practice, profoundly changing the role of pathologists. Pathologists are now crucial in interpreting molecular information and participating as members of multidisciplinary teams in therapeutic decisions. This review article aims to emphasize the importance of molecular classification in EC and encourage pathologists to become familiar with the use of predictive biomarkers in their daily anatomical pathology practice.

Keywords Predictive biomarkers, Molecular classification, Endometrial carcinoma, Precision medicine

Introduction

Endometrial carcinomas (ECs) rank as the sixth most common neoplasm in women worldwide and the second most commonly diagnosed cancer of the female genital organs, with 420,242 new cases reported in 2022 (Bray et al. 2024). Both incidence and mortality rates are rising globally, particularly in countries undergoing rapid socioeconomic transitions (Lortet-Tieulent et al.

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2018). Increased risk factors include exposure to higher total concentrations of estrogens, such as earlier age at menarche, later age at menopause, nulliparity, obesity, tamoxifen use, polycystic ovary syndrome, and estrogen-producing ovarian tumors (Ali 2014).

The traditional categorization of EC, based on the Bokhman classification (endometrioid-type and serous-type), has been central to risk stratification and guiding clinical decisions for decades (Alexa et al. 2021). Bokhman's dual-stratified pathway divided ECs into type I and type II tumors based on pathological, clinical, and epidemiological features (Bokhman 1983). Type I tumors, such as endometrioid histotypes, account for over 70% of EC cases and are associated with high estrogen levels and favorable outcomes. In contrast, type II tumors make up approximately 10% of EC cases, including serous and clear cell types, which are not associated with high estrogen levels and have a worse prognosis, with recurrence rates exceeding 50% even in early stages (Inoue et al. 2021).

In 2013, the results from the TCGA project were published, marking a paradigm shift by describing four molecular subgroups of ECs with distinct clinical, pathological, and molecular features (Cancer Genome Atlas Research Network et al. 2013). The TCGA performed an integrated genomic, transcriptomic, and proteomic characterization of ECs and elegantly demonstrated the molecular diversity of EC based on mutational burden and somatic copy number variations. Subsequently, several large studies have confirmed the strong clinical and prognostic relevance of these molecular subgroups, leading to their incorporation into the management of EC (Vermij et al. 2020).

Following the TCGA discovery, the ProMisE and the TransPORTEC consortium—two research groups—independently developed a pragmatic molecular classification by combining next-generation sequencing (NGS) for detection of pathogenic *POLE* mutations and surrogate immunohistochemical markers to TCGA's categorization: Mismatch Repair Deficient (MMRd); p53-abnormal (p53abn); No Specific Molecular Profile (NSMP); and *POLE*-mutant (*POLE*mut). In contrast to TCGA methods, which rely on freshly frozen tissue and require costly and complex methodologies, these research groups replicated the four TCGA molecular subgroups using lower-cost, clinically available diagnostic testing methods applied to formalin-fixed, paraffin-embedded (FFPE) tissues (Kommoss et al. 2018; Stelloo et al. 2016).

The development of these pragmatic classifiers with strong prognostic and clinical significance prompted a revision of the 2020 WHO Classification of Female Genital Tumours, 5th edition (Léon-Castillo 2023; Concin et al. 2021). The 2020 WHO Classification endorsed incorporating well-established molecular parameters into endometrioid endometrial carcinomas (EECs) as relevant prognostic information, encouraging their adoption through biomarkers such as targeted *POLE* sequencing, MSH6, PMS2, and p53 immunohistochemistry (IHC) (WHO Classification of Tumours Series, 5th ed.; vol. 4). The availability of surrogate markers for all molecular subtypes, which can be easily applied to FFPE tissues, facilitates the identification of subgroups analogous to those previously described by TCGA (Léon-Castillo, 2023; Concin et al., 2021).

Following the 2020 WHO classification, the Federation of International Gynecology and Obstetrics (FIGO) proposed a new staging system in 2023 (Berek et al. 2023). The updated system focuses on diagnostic parameters such as histological type and grade, lymphovascular space invasion (LVSI), and molecular alterations, to better reflect the improved understanding of the complex nature of the various types of endometrial carcinoma and their underlying biological behavior (Berek et al., 2023).

The rapid advancement in molecular biological technologies has enhanced our understanding of the multistage process of endometrial carcinogenesis, extending beyond the knowledge of mutations in single oncogenes and tumor suppressor genes (Banno et al. 2014). Over the last two decades, research has increasingly focused on epigenetic mechanisms, such as DNA methylation, histone modification, and non-coding RNAs (Banno et al., 2014). Understanding the genetic and epigenetic events involved in endometrial carcinogenesis has become integral to advances in biomarker-driven cancer therapies (Eskander et al. 2018).

Tumor biomarkers, which are substances produced by tumors or by the body's response to tumors during tumorigenesis and progression, have demonstrated critical and promising value in screening and early diagnosis, prognosis prediction, recurrence detection, and monitoring therapeutic efficacy (Zhou et al. 2024). Over the past decades, continuous efforts have been made to discover novel and cost-effective biomarkers for application in clinical cancer management. Biomarker-based anticancer targeted therapies have advanced significantly, promoting personalized medicine and improving outcomes for cancer patients (Zhou et al., 2024).

The aim of this review is to provide a better understanding of new approaches to EC management, reinforce the importance of molecular classification, and encourage pathologists to become familiar with the use of key predictive biomarkers in daily anatomical pathology practice.

An overview of molecular classification in clinical decisions

Since the publication of the EC TCGA in 2013, significant attention has been given to incorporating molecular classification into treatment decision algorithms (Cancer Genome Atlas Research Network et al. 2013; Concin et al. 2021). Following the ProMisE approach, which is more pragmatic, many retrospective and prospective cohorts have confirmed the prognostic value of molecular classification (Talhouk et al. 2015). Although there are no randomized clinical trials, some studies have demonstrated that molecular classification can indeed predict the benefits of escalating or de-escalating adjuvant treatment. In the PORTEC-3 trial, external beam radiotherapy was compared to a combination of chemoradiation followed by four cycles of carboplatin and paclitaxel in a population of high intermediate/high-risk patients (De Boer et al. 2018). The study achieved its primary endpoint, showing an increase in progression-free and overall survival with the experimental arm. A special retrospective subgroup analysis was performed after applying molecular classification to the entire patient cohort. In p53 aberrant tumors, a clear benefit was observed with the addition of chemotherapy to radiotherapy. Conversely, patients with *POLE* mutations had excellent overall survival regardless of the treatment received (León-Castillo et al. 2020). Based on these findings, in 2020, the European Societies of Gynecological Oncology (ESGO), Radiotherapy (ESTRO), and Pathology (ESP) recommended, for the first time, the inclusion of molecular classification to guide treatment decisions when the molecular classification is known (Concin et al., 2021). This recommendation has been endorsed by many international guidelines (Oaknin et al. 2022). Another important retrospective analysis was conducted in the PORTEC-1 and PORTEC-2 studies (Creutzberg et al. 2023). After applying molecular classification, p53 aberrant tumors benefited from external beam radiation, NSMP tumors from brachytherapy, and *POLE* tumors did not appear to benefit from any form of adjuvant radiotherapy. The prospective RAINBO study will further elucidate how molecular classification can be incorporated. In this study, patients with POLE mutations will be observed, p53 aberrant patients will be randomized to receive olaparib or placebo maintenance after chemotherapy, non-specific molecular profile patients will receive hormonal therapy, and mismatch repair patients will receive immunotherapy (RAINBO Research Consortium 2022).

In the palliative setting, understanding molecular classification has also enhanced patient management. For patients who progress on platinum-based therapies, the standard of care includes the combination of pembrolizumab and lenvatinib for those with mismatch repair proficient tumors (MMRp), and anti-PD-L1 monotherapy (pembrolizumab or dostarlimab) for mismatch repair deficient tumors (MMRd), based on the results of the Keynote 775, Keynote 158, and GARNET trials, respectively (Makker et al. 2023; O'Malley et al. 2022; Oaknin et al. 2022). With the success of immunotherapy in the recurrent setting, four recent randomized clinical trials have also demonstrated the benefit of adding anti-PD-1 (dostarlimab or pembrolizumab) and anti-PD-L1 (durvalumab or atezolizumab) therapies in terms of progression-free survival (Mirza et al. 2023; Eskander et al. 2023; Colombo et al. 2024; Westin et al. 2024). The benefit was observed in the overall population, but the reduction in progression or death was particularly significant in the MMRd cohort.

An overview of molecular classification in pathological practice

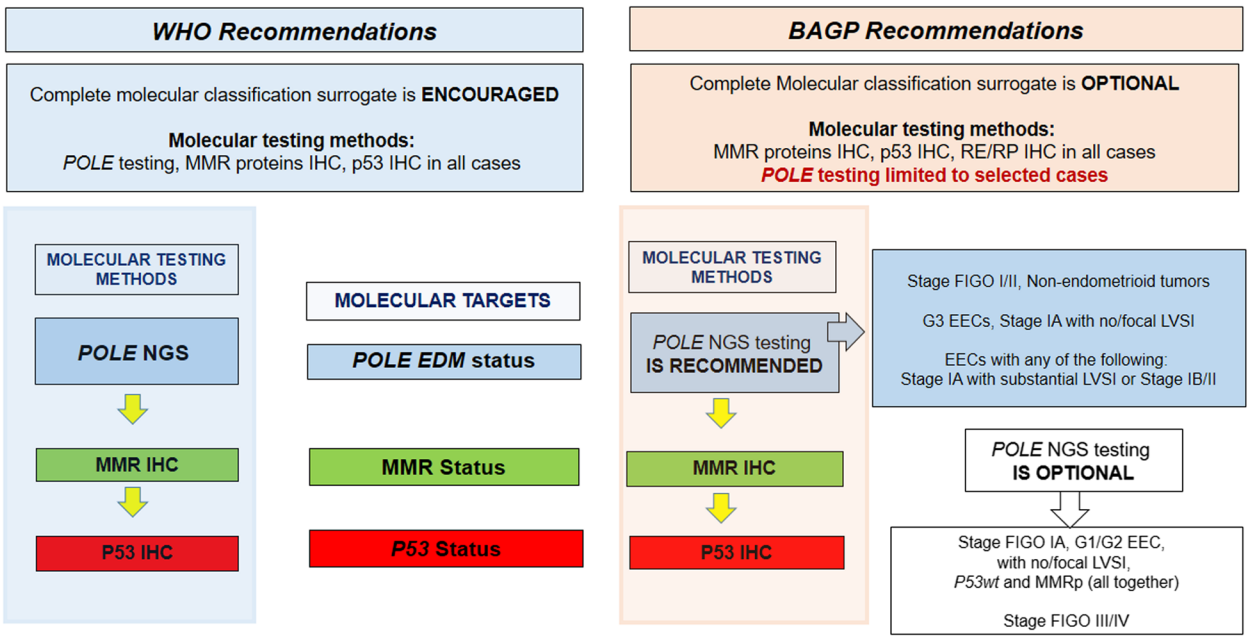
The decision to apply molecular classification to all ECs, especially those that are high-grade or high-risk, depends on resource availability and the decisions made by the multidisciplinary team at each center (Concin et al. 2021). A complete molecular classification surrogate (*POLE*mut, MMRd, NSMP, p53abn) aids in prognostic risk-group stratification, data collection, LS screening, and serves as potential factors influencing adjuvant and systemic treatment decisions, as well as for predictive value regarding ICI (Addante et al. 2024; Chacon et al. 2024).

In early-stage ECs (stages I and II), the presence of *POLE* mutations (*POLE*mut) or p53 mutations (p53mut) can influence the FIGO stage based on surgical, anatomical, and histological findings. In contrast, advanced stages III and IV of ECs are not typically modified by additional molecular analyses. *POLE*mut ECs are rare at advanced stages (Yasuda 2024). Irrespective of the data supporting the usefulness of identifying *POLE* mutations in ECs, this sequencing technique is not widely available, and its relatively high cost may constrain its routine use in pathology (Yasuda, 2024). Due to the limitations of *POLE* mutation analysis, it is suggested that morphological screening of particularly ambiguous histological features and rare bizarre nuclei can be useful for selectively enriching ECs for *POLE* mutation analysis (Keyhanian et al. 2024). Recently, it has been shown that the presence of morules or nuclear expression of β-catenin can effectively rule out the *POLE* mutation, which may help in deciding when to withhold further *POLE* mutation testing based on morphological aspects (Fan et al. 2024).

WHO Recommendations

The WHO encourages the use of complete molecular classification surrogate for all ECs, whenever resources

Table 1 Algorithm for molecular classification in ECs (WHO and BAGP)



allow (WHO Classification of Tumours Series, 5th ed.; vol. 4, 2020). Molecular testing for EC, including mutational analysis for *POLE* and surrogate IHC for MMR proteins and p53, is particularly recommended for high-grade endometrioid endometrial cancers (EECs) and intermediate-high risk ECs (Addante et al. 2024).

British Association of Gynecological Pathologists (BAGP) Recommendations

The BAGP provides an algorithm for implementing molecular classification that closely aligns with the daily practices of gynecological oncologists at their hospital. According to this algorithm, all EC biopsies, regardless of histotype, are tested by IHC to determine mismatch repair (MMR) and p53 status. In contrast to WHO recommendations, *POLE* next-generation sequencing (NGS) testing is reserved for cases where it is essential for patient care, such as: (1) when *POLE* testing is indicated due to abnormal MMR and/or p53 IHC results; (2) in Stage I-II non-endometrioid EC or any grade endometrioid EC at stage IA with lymphovascular space invasion (LVSI) or stage IB/stage II regardless of LVSI; (3) when *POLE* testing is not indicated in Stage III-IV EC unless directed by the multidisciplinary team or based on patient choice (British Association of Gynaecological Pathologists 2022).

Table 1 Comparison of Molecular Classification Algorithms (WHO vs. BAGP).

Main features of the molecular subtypes

*POLE*mut group

Frequency and clinicopathologic features

POLE mutations are significantly more frequent in high-grade ECs (12.1%) compared to low-grade ECs (6.2%) (Travaglino et al. 2020). Morphological heterogeneity and marked atypia are common features of *POLEmut* ECs, which may also present with giant anaplastic cells (Soslow et al. 2019). A relatively high frequency of *POLE* mutations has been observed in undifferentiated/dedifferentiated carcinoma (UDC/DDC) (12.4%), whereas lower frequencies are found in clear cell carcinoma (CCC) (3.8%) and carcinosarcoma (CS) (5.3%) (Travaglino et al., 2020). Notably, *POLE* mutations have been identified in a significant proportion of mixed endometrioid-serous carcinomas in young women (16%). These tumors are thought to originate as EECs that secondarily develop a serous morphology with or without *p53* mutations. Both high-grade features and *p53* mutations result from a high mutational load but have no additional clinical significance (Casanova et al. 2024). A small subset of tumors (3–5%) exhibits more than one molecular feature (*POLEmut* and *p53mut* or MMRd and *p53mut*), and these are referred to as "multiple classifiers" (León-Castillo et al. 2020). Figure 1 illustrates a case with histological features indicative of a high probability of *POLE*mut EC.

The mutational load itself induces a strong immune response due to exposure to multiple neoantigens, which is reflected in the lymphocytic infiltration observed in

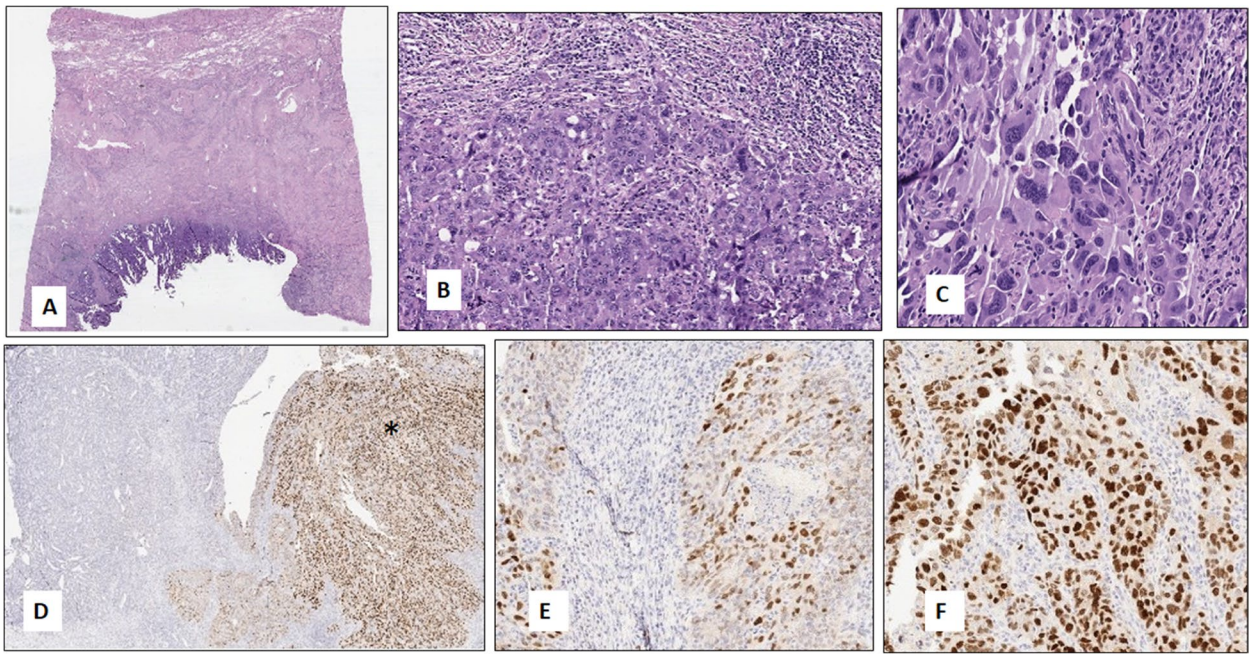


Fig. 1 **A** A case of mixed EC (endometrioid-serous type) at stage 1 (HE, 4X). **B** Image showing the high-grade atypia and lymphocytes (TILs). (HE, 10X) (**C**) Giant anaplastic cells (HE, 40X). D p53 aberrant staining in the serous component (*) and p53 wild type (p53wt) in the endometrioid component (IHC, 10X). **E** p53wt (IHC, 40X). **F** p53abn (IHC, 40X)

most (79%) *POLE*mut ECs and may contribute to their favorable prognosis (Van Gool et al. 2015). Given the prominent lymphocytic infiltrates found in most *POLEmut* ECs, it is reasonable to hypothesize that these tumors may benefit from immunotherapy (Talhouk et al. 2019).

Molecular features: POLE exonuclease domain mutational status as a biomarker

POLE mutations tend to cluster in the exonuclease domain (*POLE*-exo), especially in the conserved residues 286 to 411 (Billingsley et al. 2016; Bellone et al. 2017; Barbari et al., 2017). Somatic pathogenic variants in the exonuclease domain (ED) of *POLE* have been identified in 2–8% of colorectal cancer (CRC), 7–15% of endometrial tumors, and more rarely in other tumor types (Barbari et al., 2017).

POLE is critical for DNA proofreading and replication. Located on chromosome 12, the *POLE* gene encodes the catalytic subunit of DNA polymerase epsilon, which has both DNA polymerase activity (polymerase domain) essential for DNA elongation and 3'-5' exonuclease proofreading activity (exonuclease domain) responsible for correcting mismatched bases (Yao et al. 2024). Mutations in *POLE* are associated with hypermutated tumors and a favorable response to ICI therapy. The detection of *POLE* mutations in tumors has significant implications for treatment decisions. Cancer patients with somatic pathogenic variants in the *POLE*-ED typically have an excellent prognosis and a robust response to immune checkpoint inhibition, likely due to the strong immune response elicited by the large number of neoantigens produced as a result of hypermutation (Talhouk et al. 2019).

The occurrence of two proofreading-inactivating events in *POLE* is extremely rare, suggesting that *POLE* may not act as a classical tumor suppressor gene (Heitzer et al. 2014). Pathogenic variants of *POLE* (only the *POLE* hotspot mutations were defined as *POLE* mutated (Léon-Castillo 2023) can have different functional effects – whose functional studies were carried out mostly *e.g.* by yeasts. Different *POLE* mutation sites can lead to different degrees of tumor mutation burden (TMB).

Somatic *POLE* exonuclease domain mutations (EDMs) are found in sporadic ECs (6–10%), but rarely, germline *POLE* mutations can occur (0,25 – 4%). Both the germline and the somatic DNA polymerase EDMs can cause an 'ultramutated' type of cancer, sometimes leading to over a million base substitutions per tumor (Briggs et al. 2013). *POLE*-mut ECs are associated with striking CD8 + lymphocytic infiltrate, a gene signature of T-cell infiltration, and marked upregulation of cytotoxic T-cell effector markers. Because of their remarkable mutation burden, *POLE* proofreading-mutant cancers are predicted to display substantially more antigenic peptides than other tumors, providing a possible explanation about the strong association between cytotoxic

lymphocyte infiltration and favorable outcome in multiple cancers (Inge et al. 2015; Howitt et al. 2015; Bourdais et al. 2017).

Germline heterozygous missense mutations affecting the *POLE* exonuclease domain are associated with Polymerase Proofreading-Associated Polyposis (PPAP), a syndrome that increases the risk for the development of multiple colorectal adenomas and colorectal cancer, which exhibits a high TMB and an immunotherapy response (Allgäuer et al., 2018; Endris et al., 2019). An even more immunogenic phenotype is observed when a heterozygous POLE-ED mutation is associated with a loss-of-function (LoF) genetic event in comparison with EC with single *POLE*-ED alterations. (Rosa et al. 2020).

Truncating mutations in *POLE* gene are unlikely to lead to PPAP phenotype, since a successful DNA synthesis must occur before the proofreading activity of Pol- (Heitzer et al. 2014). By itself, the germline frameshift mutation does not confer a genetic predisposition to EC and cannot lead to a mutator phenotype in the tumor, but might contribute to increase the mutational load because only proofreading-deficient Pol- will replicate DNA in these tumor cells. Normally, tumors that harbor the S459F mutation in *POLE* were found to be microsatellite stable (Shinbrot et al. 2014; Andrianova et al. 2017; Barbari et al., 2017).

Detection of a combination of *POLE*-ED and LoF *POLE* mutations could be considered as prognostic or therapeutic marker, even if a minority of tumors with *POLE*-ED shows LOH or other inactivating mutations that could act as 'second hits' (Heitzer et al. 2014), since the absence of a wild-type *POLE* allele can lead to a high TMB (Rosa et al. 2020). The complex relationship between *POLE* hotspot mutation and dMMR/MSI-H in EC was fully explored. EC with both *POLE* hotspot mutation and MSI-H is rare, occurring only in 4.3% of cases. However, the associated TMB is high (median TMB is 339.0 mut/MB), which is consistent with that of cases with both *POLE* hotspot mutation and MSS. The median TMB of tumors with *POLE* non-hotspot mutation and MSI is 207.1 mut/MB. The median TMB of EC with *POLE* non-exonuclease domain mutation and MSI is 48.5 mut/MB (Ma X et al. 2022).

Molecular testing methods: Next Generation Sequencing (NGS)

Although mutational signatures are preferably determined by genomic analysis (Jamieson et al. 2024), such as whole genome sequencing (WGS) and whole exome sequencing (WES)—*see Supplementary Material 1* – some authors have identified some mutational signatures that can be extracted from sequencing data derived from a small gene panel (e.g. 63 genes) in tumors that are highly mutated (Van Hoeck et al. 2019).

MMRd group

Frequency and clinicopathologic features

The MMRd group represents approximately 30% of ECs and is characterized by distinct histopathological features, including: (1) origin in the lower uterine segment; (2) endometrioid differentiation; (3) severe nuclear atypia with an undifferentiated component; (4) high mitotic index; (5) high tumor-infiltrating lymphocytes (TILs) and/or peri-tumoral lymphocytes (≥40 TILs/10 HPFs); (6) high morphological heterogeneity; (7) substantial LVSI; (8) deeper myometrial invasion; and (9) synchronous ovarian cancer, particularly in clear cell or endometrioid variants (Addante et al. 2024; Yasuda 2024).

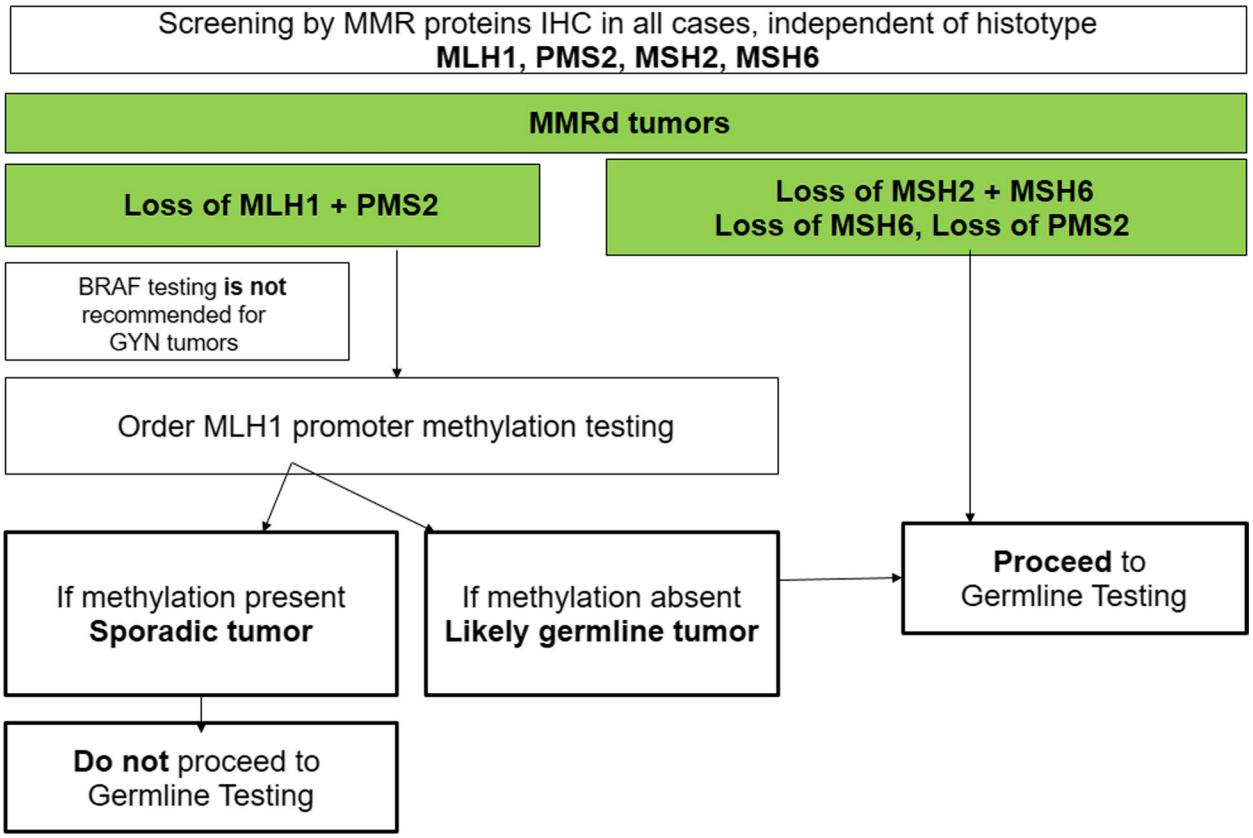
Regarding the prevalence of MMRd ECs across different histotypes, UDC/DDC is the most common MMRd subtype (44%), followed by neuroendocrine carcinoma (42.9%), high-grade EEC (39.7%), mixed forms (33.3%), low-grade EEC (24.7%), CCC (9.8%), and CS (7.3%). Only sporadic cases of serous carcinoma and mesonephric-like carcinoma have been reported to show MMR deficiency (Addante et al. 2024; Vermij et al. 2020).

Compared to *POLE*mut ECs, MMRd ECs seem to be more prognostically affected by clinicopathological variables, although not as much as NSMP ECs. The ESGO-ESTRO-ESP guidelines substratify MMRd ECs into different risk groups based on pathological features, such as the depth of myometrial invasion, LVSI and histotype. It seems that grading does not matter in the MMRd molecular group (Concin et al. 2021). The overall prognosis of MMRd ECs is intermediate (Vermij et al. 2020).

Molecular features: MMRd/MSI status as a biomarker

The MMRd group is characterized by microsatellite instability (MSI) and displays a high mutational rate with frequent insertions and deletions but low copy-number variations. MSI is a condition of genetic hypermutability resulting from a defective DNA mismatch repair process, and the terms MSI and MMRd are often used interchangeably (Addante et al. 2024). The MMR system is one of the DNA repair pathways responsible for recognizing and repairing erroneous insertions, deletions, and mis-incorporations of bases that can occur during DNA replication. Deficiency in the MMR system leads to the accumulation of DNA mismatches in repetitive sequences known as microsatellites. These mismatches integrate into the genetic code as mutations, resulting in high levels of microsatellite instability (MSI-H). This signature arises from primary biallelic defects in genes that regulate DNA MMR, characterized by the loss of

Table 2 Lynch syndrome testing algorithm in ECs



function of at least one of the following genes: MLH1, PMS2, MSH2, or MSH6 (Addante et al. 2024; Mendiola et al. 2023).

Sporadic and germline MMRd ECs

MSI can be caused by somatic or germline alterations. Somatic alterations, accounting for 85% of cases, include biallelic epigenetic MLH1 hypermethylation (in about 77% cases of sporadic ECs); downregulation of MMR genes by microRNAs; biallelic mutations; one somatic mutation and LOH; and secondary epigenetic MSH6 silencing induced by neoadjuvant Radiotherapy and Chemotherapy (RT/CHT) (Addante et al. 2024). Approximately 10% of MMRd ECs and 3% of all endometrial ECs are due to LS, a cancer susceptibility syndrome caused by germline mutations in the MMR genes (MLH1, PMS2, MSH2, MSH6) or EPCAM (Léon-Castillo 2023). Indeed, germline mutations in the MMR genes can determine two different types of clinical syndrome: (1) Constitutional mismatch repair deficiency (CMMRD), a rare childhood cancer predisposition syndrome with recessive inheritance, due to a biallelic MMR gene mutation in which MMR defects (occurring in MLH1, PMS2, PMS1, MSH2 or MSH6) are inherited from both parents; (2) LS, an autosomal dominant disorder characterized by the occurrence of multiple cancers, resulting from constitutional germline mutations, affecting the DNA MMR genes MLH1, MSH2, MSH6 and PMS2; constitutional MLH1 hypermethylation; or deletion of the stop codon (3′ end truncating) of the EPCAM gene causing the epigenetic silencing of the neighboring MSH2 (Addante et al. 2024).

The identification of MMR pathogenic variants in germline sequencing is the gold standard for the diagnosis of LS. However, the first step for LS screening in EC is represented by IHC (Addante et al. 2024). Universal tumor screening in endometrial cancer (EC) is increasingly being adopted to identify individuals at risk of LS (Kaya et al. 2024). Table 2 shows an algorithm of Lynch Syndrome Testing.

Molecular testing methods: MMRP IHC, MSI testing and next generation sequencing

EC is one of the types of cancer most commonly associated with the MMRd/MSI-H phenotype. There is currently a strong recommendation to assess the MMR status in EC, and the main guidelines recommend IHC for analyzing this biomarker (Mendiola M et al. 2023;

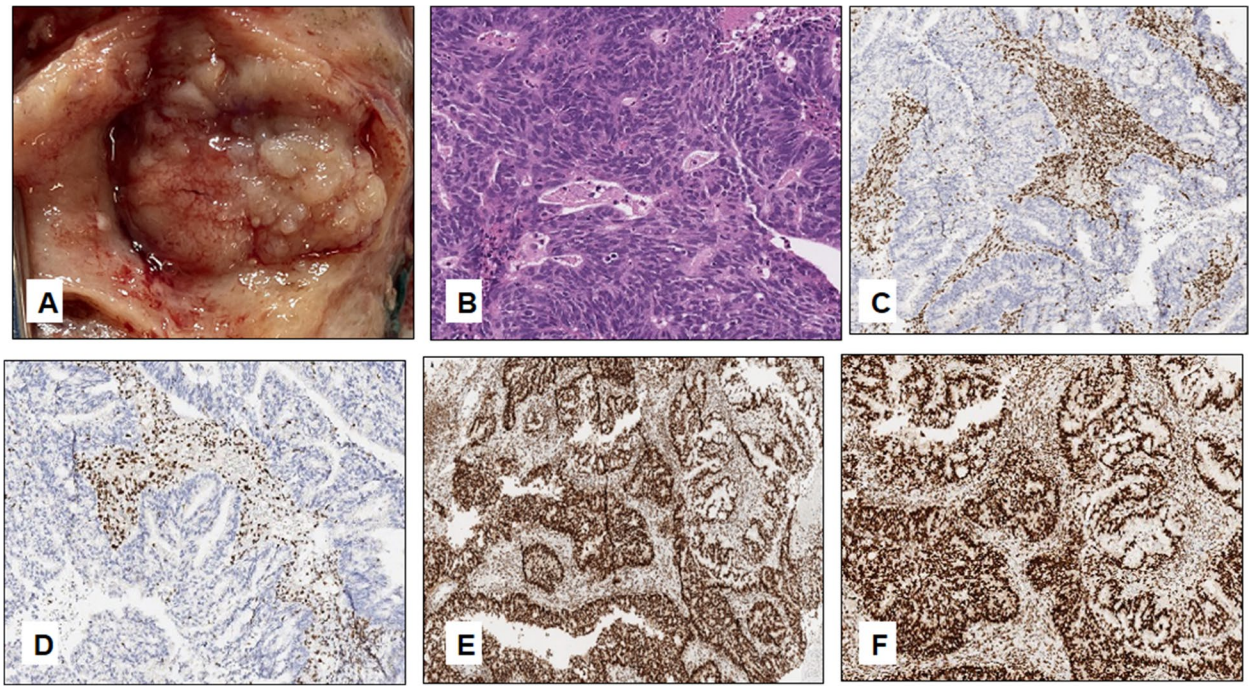


Fig. 2 Low grade EC. **A** Exophytic tumor located n the fundus of the endometrial cavity. **B** Endometrioid histology (HE, 40X). **C** MLH1: loss of nuclear expression. Postive internal control (IHC 40X). **D** PMS2: loss of nuclear expression. Positive internal control (IHC, 40X). **E** MSH2: intact nuclear expression (IHC, 40X). F MSH6: intact nuclear expression (IHC 40X)

Casey et al. 2021). Compared with others approaches, IHC is widely available and not expensive. The presence of stained non-neoplastic cells in the sample represents an internal positive control that easily identifies false negative results. Another advange of IHC over PCR is that the loss-of-expression pattern provides information on the particular altered gene, guiding later sequencing to rule out LS (Mendiola et al., 2023; Casey et al., 2021).

Identification of ECs with MMRd/MSI-H status has a threefold value: it (1) serves to detect patients at higher risk of presenting a LS, (2) provides prognostic information as a surrogate marker for MMRd EC (once *POLE* pathogenic mutations have been excluded), and (3) holds predictive value, as patients with MMRd endometrial carcinomas benefit from check-point inhibitor treatment (Léon-Castilho 2023; Mendiola et al. 2023).

In order to classify MMRd ECs, either IHC or DNA-based methods, such as panels of microsatellite markers for detecting MSI, can be used (Léon-Castilho 2023). Since MSI typically results from a loss of function in the MMR system, leading to decreased corresponding protein expression, IHC for MMR proteins can serve as a surrogate test to identify the MMRd group (Addante et al. 2024). MMR IHC for MLH1, PMS2, MSH2, and MSH6 is the gold standard surrogate testing method and the MMRd ECs will have loss of nuclear expression of one or more of these four key MMR proteins, while the retention of expression of these four markers is known as MMR proficiency (Addante et al. 2024). Tumors with microsatellite stability (MSS) harbor an intact MMR machinery, which is call by the terminology "MMR proficiency" (MMRp). Dual loss of MLH1 and PMS2 is the most frequent pattern observed in daily IHC results (Addante et al. 2024). Figure 2 illustrates an example of dual loss of MLH1 and PMS2, in a case of low-grade endometrioid EC (likely sporadic tumor) and Fig. 3 shows a case of mixed carcinoma with isolated loss of MSH6 (likely germline tumor).

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. Alternatively, it is possible to use a two-marker approach (IHC stain for only *PMS2* and *MSH6*) to identify MMRd ECs (Léon-Castilho 2023).

The accordance between MMR IHC and MSI analysis is high (93–95%), but discrepancies can occur due to (1) subclonal MMR deficiency (a well-delimited area of the tumor with loss of expression of a major MMR protein can be overlooked by using MSI analysis), (2) ECs with *MSH6* mutations that may appear as microsatellite stable (MSS), and (3) incorrect interpretation of MSI

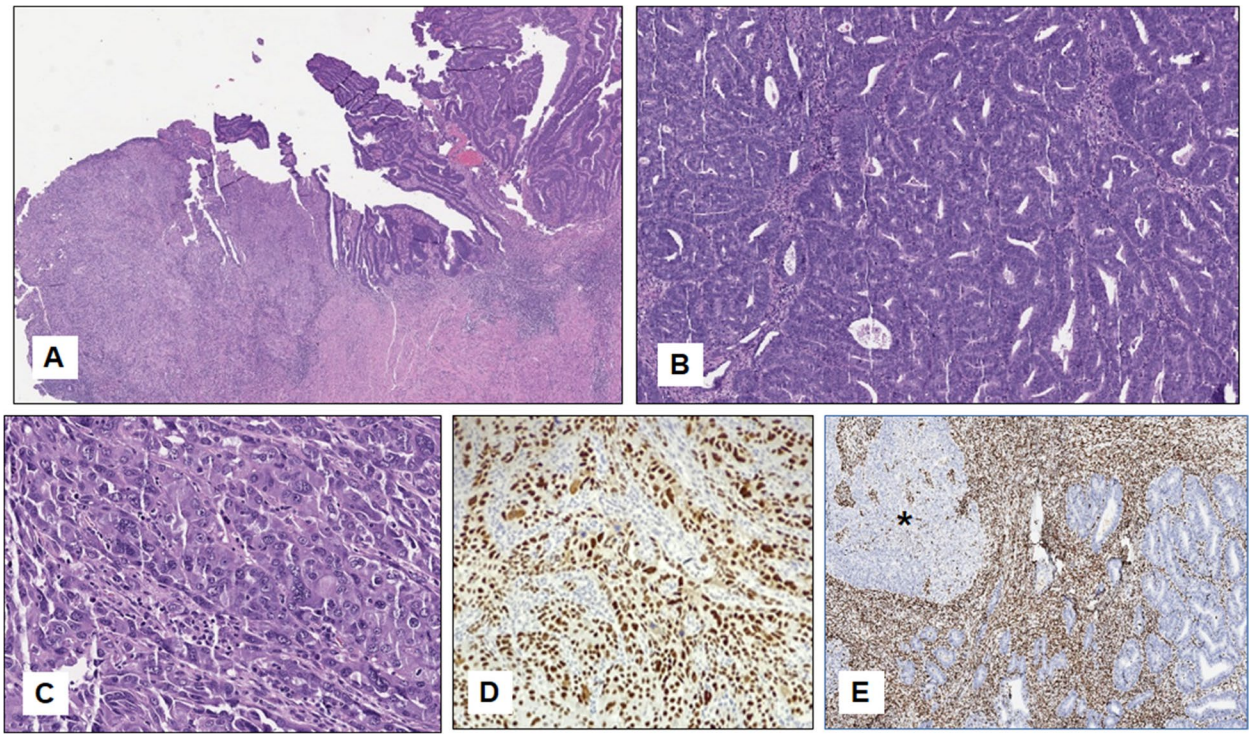


Fig. 3 **A** Mixed EC (endometrioid-serous histotype) (HE, 4X). **B** Endometrioid component (HE, 10X). **C** Serous solid component (HE, 40X). **D** p53-abn with nuclear overexperssion in serous component (IHC, 40X). **E** MSH6: loss of expression in the two tumor components espacially distincts. (*) serous solid pattern (IHC, 10X)

assay results, which requires highly trained personnel (Léon-Castilho 2023).

However, there are specific conditions requiring PCR or NGS instead of MMR protein IHC, namely: (1) Whenever IHC shows indetermined/ambiguous/equivocal results; (2) False negative IHC results due to pre-analytical tissue poor fixation; (3) Whenever IHC shows aberrant patterns (e.g., cytoplasmic, dot-like and perinuclear staining); (4) Whenever IHC shows the loss of only one heterodimer subunit, i.e., only MLH1 or PMS2 and not both (Addante et al. 2024).

Polymerase chain reaction (PCR) represents the first-line molecular analysis for MSI testing, also performed on FFPE tissue slides (Walsh et al. 2023). MLH1 promoter methylation testing, also performed on FFPE tissue slides, is another relevant molecular testing technique used in cases of absent MLH1 or absent MLH1 and PMS2 IHC. Most laboratories utilize a methylation-specific real-time PCR assay to determine the presence of methylation. It is used in cases of absent MLH1 or absent MLH1 and PMS2 IHC to differentiate between somatic loss due to promoter methylation and potential germline loss. Defective MMR in sporadic endometrial cancer is most often due to inactivation of the *MLH1* gene promoter by methylation, by epigenetic silencing (Walsh et al., 2023).

NGS represents another type of molecular test that allows efficient sequencing of the entire genome, also performed on FFPE tissue slides. NGS has the advantage of allowing coupling MSI analysis with the determination of tumor mutational burden (Walsh et al. 2023).

MSI, PD-1/PD-L1, TMB and immunotherapy in EC

Concerning therapeutic options, MMRd ECs are considered optimal candidates for immunotherapy due to their high mutation load and rich immune infiltrate (Addante et al. 2024). All MMRd tumors exhibit high microsatellite instability (MSI-H), with a rapid accumulation of genomic mutations and high mutational load (H-TMB: 10–100 mutations per megabase). These tumors harbor hundreds to thousands of mutations and are highly immunogenic, with strong expression of immune checkpoints and high levels of lymphocyte infiltration. Cells from MMRd tumors may express programmed death ligand 1 (PD-L1) on their membranes, and infiltrating lymphocytes display upregulated checkpoint proteins, including programmed death 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and

lymphocyte-activation gene 3 (LAG-3) (Marabelle et al. 2020). Immune cell infiltration may result from the high number of mutations found in MMRd tumors, specifically frameshift mutations, which produce mutant protein neoantigens (Addante et al. 2024).

The Phase II KEYNOTE-158 study demonstrated the clinical benefit of anti–programmed death-1 (PD-1) therapy with pembrolizumab in patients with previously treated unresectable or metastatic MMRd colorectal cancer, showing a response rate of 48% and a median progression-free survival of 13.1 months. Other studies with immunotherapies such as dostarlimab, durvalumab, and avelumab in similar scenarios have also shown good clinical benefits. Pembrolizumab is a humanized immunoglobulin G4 monoclonal antibody that binds to the inhibitory immune checkpoint receptor PD-1 expressed on lymphocytes, blocking the binding of its ligands PD-L1 and PD-L2, thereby allowing reactivation of T-cell–mediated tumor destruction (Marabelle et al., 2020). PD-L1 expression by IHC may predict the response to anti-PD-1/PD-L1 monoclonal antibodies (Addante et al., 2024; Mendiola et al. 2023). Given that MMRd/MSI status may serve as a suitable biomarker for response to PD-1/PD-L1 immunotherapy with pembrolizumab (Marabelle et al. 2020), ESGO/ESTRO/ESP guidelines recommend IHC for MMR proteins in all EC cases as a universal screening test to assess MMRd/MSI status and identify patients who could benefit from immunotherapy (Concin et al. 2021).

Tumor mutational burden (TMB) is a biomarker defined as the total number of somatic mutations per coding area of a tumor genome, obtained through NGS. Tumors with high TMB (TMB-H) often harbor elevated levels of neoantigens, making them targets for activated immune cells and potentially leading to superior and/or prolonged responses to ICIs, including anti-PD-1 agents. Cancers with > 10 mutations/Mb are referred to as TMB-H (Wash et al. 2023). *POLE*-mutant ECs and MMRd ECs are examples of TMB-H tumors, characterized by immunogenic microenvironments (Marabelle et al. 2020). A recently published biomarker analysis from KEYNOTE-158 reported that tumors with TMB-H status had a higher objective response rate to pembrolizumab monotherapy compared to non-TMB-H tumors, leading to the FDA approval of pembrolizumab for TMB-H solid tumors (Marabelle et al. 2020). TMB is a useful biomarker for selecting EC patients for ICI therapy (Walsh et al. ; Marabelle et al. 2020).

p53-abnormal (p53abn) group

Frequency, clinicopathologic and molecular features

Somatic mutations of the TP53 gene on chromosome 17p13.1 are present in up to 28% of all endometrial cancers (ECs) (Jamieson et al. 2021). Given that p53-abnormal (p53-abn) ECs represent the most aggressive molecular subtype with a poor prognosis, *TP53* mutation status is a suitable biomarker for predicting unfavorable outcomes in EC patients (Jamieson et al. 2021; Arciuolo et al. 2022).

This group is characterized by a low mutation rate (TMB < 10 mutations per megabase) and high somatic copy-number alterations, with *TP53* mutations present in 90% of cases (Jamieson et al. 2021). The majority of p53-abn ECs are high-grade serous carcinomas, along with other histotypes such as carcinosarcomas, mixed ECs, and clear-cell carcinomas (Jamieson et al., 2021). These other histotypes exhibit similarly aggressive biological behavior, resulting in the worst outcomes (Jamieson et al., 2021). Consequently, ESGO-ESTRO-ESP guidelines classify all p53-abn ECs as high-risk, except for non-myoinvasive cases (Arciuolo et al. 2022).

Molecular testing methods

The assessment of p53 by IHC has a high concordance with *TP53* mutational status, making it a suitable surrogate method for *TP53* mutation detection (Köbel et al. 2019; Köbel et al. 2021). Although p53 IHC is not a perfect substitute for copy-number analysis, its accuracy in routine practice remains adequate. Since 2020, the WHO has endorsed the inclusion of p53 IHC in diagnostic algorithms due to its accessibility, simplicity, and cost-effectiveness. However, the correct interpretation of p53 IHC by pathologists is crucial for accurate diagnostic reports, and the terminology used must be clearly understood by the multidisciplinary team (Arciuolo et al. 2022).

In a pathologist's daily routine, the majority of *TP53-mutant* cases shows p53 overexpression on IHC, defined by a diffuse and uniformly strong nuclear staining in 80–100% of tumor cells. This aberrant pattern is commonly linked to missense mutations in the DNA-binding domain of *TP53* (Vermij et al., 2022). Others aberrant patterns include complete loss of p53 nuclear expression (with a positive internal control) and unequivocal cytoplasmic expression (Jamieson et al. 2021). The "null" pattern often results from frameshift or nonsense mutations encoding truncated p53 protein, and the cytoplasmic overexpression is caused by mutations in the tetramerization or C-terminal domain of *TP53*. Köbel et al. (Köbel et al. 2019 and 2021) have described a heterogenous pattern ("subclonal"), where a distinct geographic area of the tumor exhibits both a abnormal p53 staining and a wild-type p53 expression (Vermij et al., 2022). Subclonal patterns are frequently associated with MMRd or *POLE*mut, where p53 abnormalities lack prognostic value (Arciuolo et al. 2022). Another subset of *TP53*-mutant tumors (~ 5%) harbor truncated *TP53* mutations that do

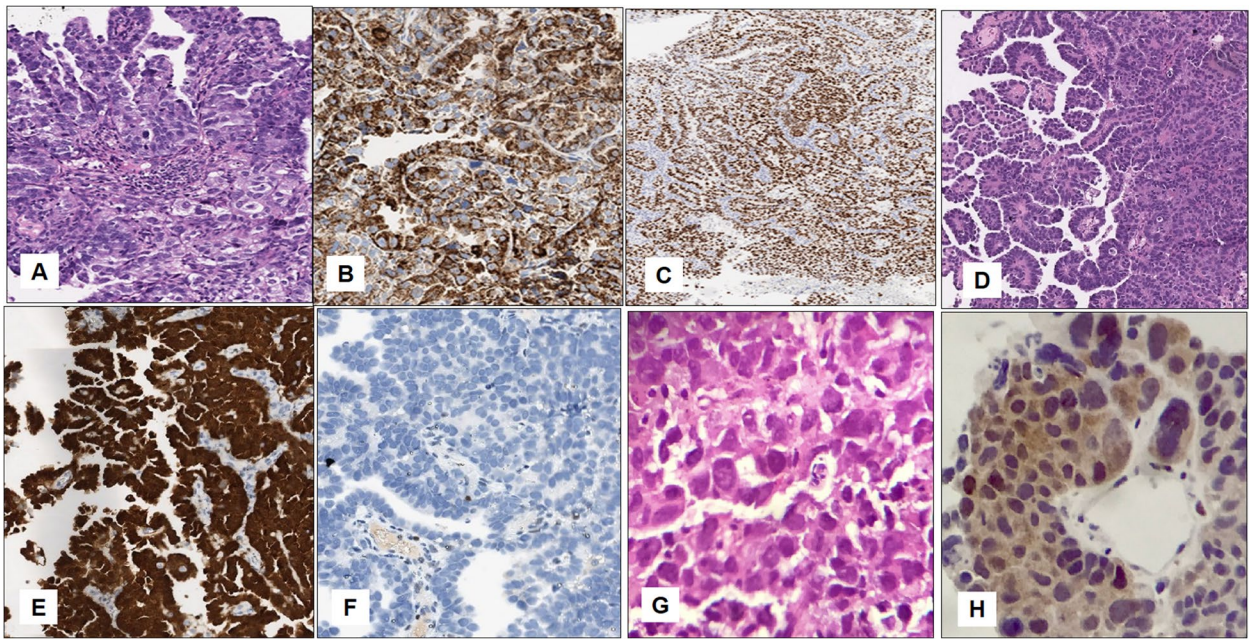


Fig. 4 **A** Case 1: CCC (HE, 40X). **B** CCC: NAPSIN A: Positive stain (granular citoplasmatic stain) (IHC, 40X). **C** CCC: p53 aberrant, nuclear overexpression (IHC, 40X). **D** Case 2: Serous carcinoma (ESC) (HE, 40X). **E** ESC: p16 overexpression (IHC, 40X). **F** ESC: p53 aberrant stain, null type. Positive estromal cells (IHC, 40X). G Case 3: ESC (HE, 40X). H ESC: p53 aberrant, nuclear and citoplasmatic stain (IHC, 40X)

not manifest abnormal p53 expression on IHC (Arciuolo et al., 2022). Additionally, some copy number-high EC cases, defined solely by molecular analysis of copy-number variations, do not exhibit *TP53* mutations (Arciuolo et al. 2022). Figure 4 illustrates three examples of abnormal p53 IHC patterns, considered indicative of mutation-type staining (overexpression, null-type and citoplasmatic stain). Figure 5 shows two cases of

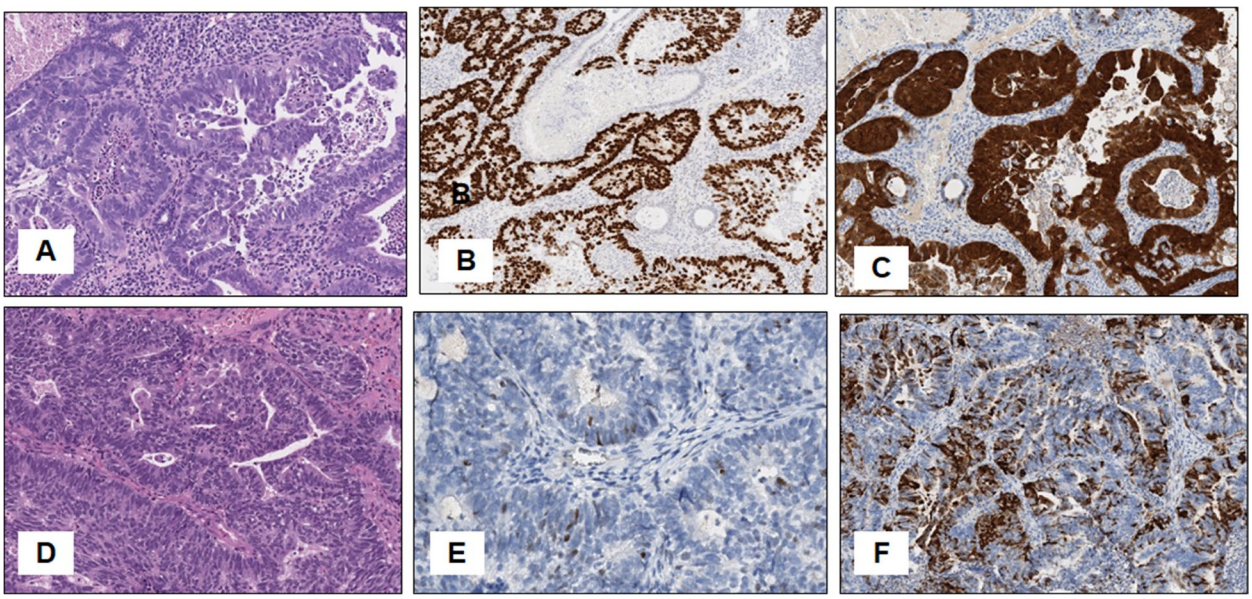


Fig. 5 Two cases of low grade endometrioid tumor. **A** Case 1: G2 endometrioid tumor (HE, 40X). **B** Case 1: p53-abn (nuclear overexpression) (IHC, 40X). **C** Case 1: p16 aberrant, nuclear and citoplasmatic overexpression (IHC, 40X). **D** Case 2: G1 endometrioid tumor (HE, 40X). **E** Case 2: p53 wild type (IHC, 40X). **F** Case 2: p16 mosaic type (IHC, 40X)

low-grade endometrioid tumors (same histology), with different p53 status (abnormal/mutated type and normal/wild type).

Due to the aggressive nature of p53-abnormal (p53-abn) tumors, adjuvant treatment is often recommended for this group. For all myoinvasive cases, chemoradiotherapy is indicated (Arciuolo et al. 2022; Jamieson et al. 2021). Additionally, targeted therapy options may be available for a subset of cases based on specific characteristics such as homologous recombination deficiency (HRD) or human epidermal growth factor receptor 2 (*HER2*) overexpression/amplification. Patients with these features are potential candidates for HER2 inhibitors or PARP inhibitors (Arciuolo et al., 2022; Jamieson et al., 2021).

No Specific Molecular Profile (NSMP) group

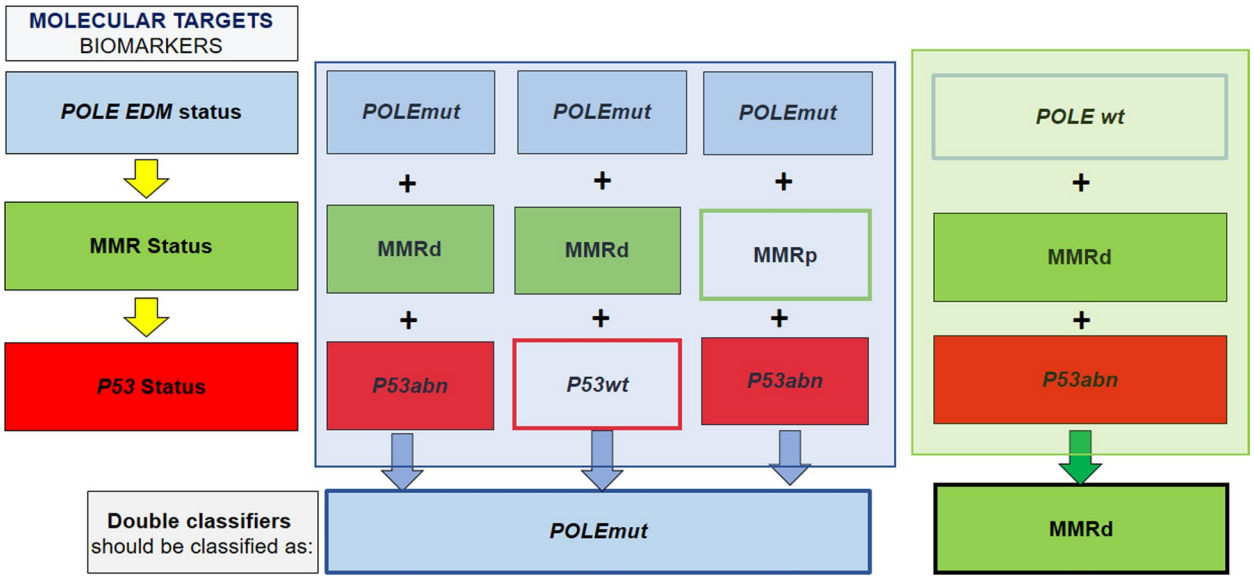
Most endometrial tumors (about 50%) with no molecular signatures are placed in this group. These tumors neither exhibit high mutational burden nor significant copy-number variations, been similar to the TCGA copy-number low group (Arciuolo et al. 2022). NSMP ECs represent a diagnosis of exclusion, assigned based on the absence of MMR deficiency, pathogenic *POLE* mutations, and p53 abnormalities. This group shows diverse clinicopathological and molecular features and is generally associated with intermediate patient outcomes, with strikingly different outcomes at the individual level (Aro et al. 2024). NSMP ECs encompass young women with low-grade indolent ECs arising in settings of obesity/excess estrogen and thin older women with rare histotypes historically categorized as high-risk and with unfavorable disease trajectories. A study showed that after the removal of high-risk NSMP cases, the low-grade (grade 1 or 2) and ER-positive endometrioid carcinomas, accounting for 84% of the NSMP group and corresponding to prototypical type 1 EC, was associated with a very favorable prognosis (Jamieson et al. 2023). Low ER expression is associated with hightumour grade, lymph node metastasis, deepmyo-invasion, high tumour stage, increased risk of disease recurrence, decreased overall survival and decreased disease-specific survival (Alafraidi et al. 2024). It seems that NSMP non-endometrioid tumors have a unfavored prognosis (like p53-abnormal ECs), while NSMP endometrioid ECs demonstrate a highly heterogeneous prognosis, ranging from favorable (like *POLE*-mutant ECs) to unfavorable (like p53-abnormal ECs) (Arciuolo et al., 2022). There is a suggestion that ER IHC can serve as a biomarker for stratification of NSMP carcinomas into two clinically distinct groups: low-risk carcinomas with high ER expression and an excellent prognosis similar to that of *POLE*mut endometrial carcinoma, and high-risk carcinomas with low ER expression (Alafraidi et al., 2024).

A model of sub-stratifying is currently being evaluated in the PORTEC-4a study (Van den Heerik et al. 2020), which integrates TCGA-based molecular subgroups with L1-CAM overexpression, substantial LVSI, and CTNNB1-exon 3 mutations. Such sub-stratification appears to be more applicable to the NSMP tumors (Arciuolo et al. 2022; Van den Heerik et al. 2020). This trial global study applies a molecular-integrated risk profile to the high-intermediate risk cohorts of the PORTEC-1 and -2 trials, utilizing L1CAM and CTNNB1 and delineating three risk categories (favorable, intermediate, and unfavorable) with significantly different recurrence-free survival rates. L1CAM serves as an independent risk factor for locoregional and distant spread and is associated with TP53 mutations, high tumor grade, and LVSI. L1CAM is a crucial indicator of high-risk disease. L1CAM expression is most frequent in p53-abnormal (p53-abn) tumors but also predicts worse outcomes among tumors with NSMP (Oaknin et al. 2022). CTNNB1-exon 3 mutations prognosticate a higher risk of recurrence, particularly in the copy-number-low group (Van den Heerik et al. 2020; Wash et al. 2023). In summary, L1CAM overexpression and CTNNB1 mutations appear to be significant prognostic biomarkers with clinical implications for deciding adjuvant treatment in patients with EC (Van den Heerik et al., 2020). ER positivity and L1CAM negativity are strongly associated with low grade, which limits the ability of these biomarkers to further improve prognostication in these patients. Notably, risk assessment for high-risk–advanced-metastatic NSMP and MMRd subtype carcinomas can be refined by ER status (Aro et al. 2024).

Tumors with multiple classifying alterations

Leon-Castillo et al. reported that approximately 3–5% of ECs are classified as "multiple classifiers," exhibiting more than one molecular signature. They demonstrated that double classifiers such as MMRd/*p53abn* tumors behave like MMRd EC and *POLE*mut/p53abn behave like *POLE*mut EC in terms of morphology, prognosis and clinical behavior, and not like p53abn ECs (León-Castillo et al. 2020). The multiple classifiers represent a rare proportion (3–10%) of molecularly classified ECs from various previously published studies, such as the ProMisE confirmation and TransPORTEC early-stage EC cohorts, and efforts have been directed towards understanding their prognostic implications (León-Castillo et al. 2020; De Vitis et al. 2024). The most frequent subgroup was MMRd-p53abn, followed by *POLE*mut-MMRd, *POLE*mut-p53abn, and *POLE*mut-MMRd-p53abn (De Vitis et al.,

Table 3 Multiple classifiers



2024). Indeed, studies indicate that *POLE* mutations demonstrate the most profound prognostic significance, surpassing both MMRd and *TP53* mutations. Following *POLE* mutations, MMRd exhibits a lesser prognostic impact, followed by *TP53* abnormalities. Therefore, both prognosis and genomic data strongly support that *POLE*mut trumps both MMRd and p53abn, and MMRd trumps p53abn (León-Castillo et al., 2020). These findings endorse the prioritization of *POLE* sequencing over MMR protein and p53 IHC, in the algorithms of molecular classification proposed in clinical oncology guidelines (León-Castillo et al., 2020).

Given the high mutational burden observed in *POLE*mut and MMRd tumours, secondary *TP53* mutations/p53 IHC abnormalities can be found. These findings suggest the *TP53* mutation is a later event during tumour progression in *POLE*mut and MMRd tumours and does not affect the clinical outcome. This also highlights the importance of interpreting p53 and MMR IHC in the context of *POLE* mutation status to avoid overtreatment in these patients with 'multiple classifiers'. The 2020 ESGO/ESTRO/ESP guidelines for the management of patients with EC classify stage I-II *POLE*mut EC as low-risk and omission of adjuvant therapy should be considered (Concin et al. 2021). Information on p53abn status alone is not enough on its own to guide treatment decision-making as a proportion of these are double-classifiers with significant prognostic and predictive implicantions (León-Castillo et al. 2020). Table 3 shows subgroups of double classifiers and Table 4 shows a summary of the four-histomolecular groups, including their prognosis and clinical relevance.

Other selected biomarkers in EC

Human epidermal growth factor receptor 2 *(HER2, HER2/Neu or ERBB2)*

HER2/Neu, the human epidermal growth factor receptor 2, also called ERBB2, has been established as an important biomarker with both prognostic and therapeutic implications in breast and gastric cancers (Pina et al. 2024). Endometrial serous carcinoma (ESC) is an aggressive, high grade endometrial cancer subtype associated with poor clinical outcomes and significant mortality. Approximately 30% of ESC overexpress HER2 (Fader et al. 2020). Figure 6 illustrates an example of ESC with 3+HER2 positivity.

ESC is typically treated with hysterectomy and surgical staging followed by platinum/taxane combination chemotherapy. Despite overexpression or amplification of *HER2* in this subtype of cancer carries a poor prognosis (Ferriss et al. 2021), anti-HER2 therapy has emerged as an effective targeted treatment approach for patients with advanced stage and recurrent ESC, resulting in significantly prolonged progression-free and overall survival when combined with the standard chemotherapy regimen (Fader et al. 2020).

Most cases of HER2 overexpression are caused by *HER2* gene amplification and can be detected either by IHC for the gene product, by fluorescent in situ hybridization (FISH) to determine the gene copy number, and by NGS (McNamara et al. 2023).

Table 4 Histomolecular classification of ECs

Molecular groups	*POLE*mut	MMRd	NSMP	P53abn
Molecular targets	**Pathogenic *POLE mutations***	**MMR deficiency**	**Abscent**	***TP53* mutations**
Molecular features	High TMB (> 100 mut/Mb), Very low SCNAs, MSS	TMB (10–100 mut), Low SCNAs, MSI	Low TMB (< 10 mut/Mb), Low SCNAs, 30–40% with *CTNNB1* mutations	Low TMB (< 10 mut/Mb), High SCNAs, MSS
Histological features	Often high-grade endometrioid, ambiguous morphology with scattered tumour giant cells, prominent TILs	Often high-grade endometrioid, prominent TILs, mucinous differentiation, MELF-type invasion, LVSI	Endometrioid histotype, Mostly low-grade with frequent squamous differentiation or morule, absence of TILs	Non-endometrioid, serous histotype Mostly high-grade with cytonuclear atypia; glandular and solid forms exist
Testing methods	NGS	MMR-IHC; MSI assay; NGS for MMR genes	MMRp/MSS, absence of pathogenic *POLE* or TP53 mutations	p53 IHC: aberrant staining, NGS for *TP53*
Prognosis/Stage	**Excellent Frequent stage I** (I > II-IV)	Intermediate (I > II-IV)	Intermediate to excelente ER-negative cases: poor-outcomes (I > II-IV)	Poor Advanced stage at presentation (I=II-IV)
Clinical and therapeutic relevances	Lower body mass index (BMI) Observation/De-escalation Candidates to Immunotherapy	Lynch syndrome-associated Candidates to Immunotherapy Radiation	Higher BMI High proportion estrogen-driven Endocrine therapy if ER-positive De-escalation in selected stage I	Lower BMI, older Chemotherapy Substratification to target HRD, HER2 overexpression, imune-angiogenesis, or CCNE1 pathways

Tumors with intense complete or lateral/basolateral membranous HER2 immunostaining in more than 30% of tumor cells are considered positive and assigned a 3+score, 2+score, equivocal, is assigned when intense complete or lateral/ basolateral membrane staining was seen in 30% or less of tumor cells, or weak to moderate staining in greater than or equal to 10% of tumor cells. 0 score (no staining observed) and 1+score (incomplete membrane staining that is faint/barely perceptible in any proportion of cells or weak complete staining in less than 10% of tumor cells) are considered negative. FISH is performed only on tumors with a 2+immunohistochemical score on a large tumor area in direct correlation with the HER2 immunostained slide. HER2/CEP17 ratio of 2.0 or HER2/CEP17 ratio less than 2.0 with average HER2, copy number equal to or greater than 6 per nucleus is considered amplified (Buza 2021; Buza 2022). Table 5 shows the reporting results of HER2 testing by IHC and by FISH (dual-probe assay) for ESC.

HER2 intratumoral heterogeneity by IHC is defined by the presence of at least two-degree difference in staining intensity (0 vs 2+, 1+vs 3+, or 0 vs 3+) involving at least 5% of tumor cells (Ross et al. 2022). With a tumor harboring both 3+and 0/1+HER2 tumor cells, HER2-directed therapy may not work up front or may diminish as HER2-negative tumor cells outgrow HER2-positive tumor cells due to trastuzumab's selective pressure in HER2 heterogenous tumors over time (Buza 2021). Discordant HER2 status has also been observed between paired primary and metastatic tumors in close to 20% of breast, gastric, and most recently ECs (Buza 2021). HER2 testing of multiple specimens (endometrial biopsy, curetting, hysterectomy, and metastatic foci) is stimulated, as it may increase the rate of HER2 positivity and eligibility for targeted therapy (Rottmann et al. 2021).

Primary results from the DESTINY-PanTumor02 phase II assessed the efficacy of Trastuzumab deruxtecan (T-DXd), using ASCO/College of American Pathology guidelines for scoring HER2 in gastric cancer, and showed benefits observed in the endometrial, cervical, and ovarian cohorts. In the endometrial cohort, 77.5% of patients had ≥ two prior lines of therapy. The objective response rate in patients with HER2 IHC 3+expression was 84.6%. In all patients with endometrial cancer, median PFS and OS were 11.1 months and 26.0 months, respectively. The clinically significant response and survival rates observed in this study are encouraging for HER2-expressing ECs, which are typically associated with high risk for progression and poor survival rates (Meric-Bernstam et al. 2024). Table 6 shows IHC scoring for HER2 in gastric and gastro-oesophageal junction cancer.

In high copy number/ p53abn group, patients can have mutations in *FBXW7*, *PIK3* and *PPP2R1A*. *PI3K/AKT/mTOR* pathway is affected in 50–60% and ERBB2 alterations are observed in 25% of the patients, which can be potential targets for new combinations (Pina et al. 2024). Other notable recurrent genetic alterations included PIK3CA activating mutations (40%) and *ERBB3* amplification (10%), both of which have been reported to impact the efficacy of anti-HER2 therapy in other cancer types

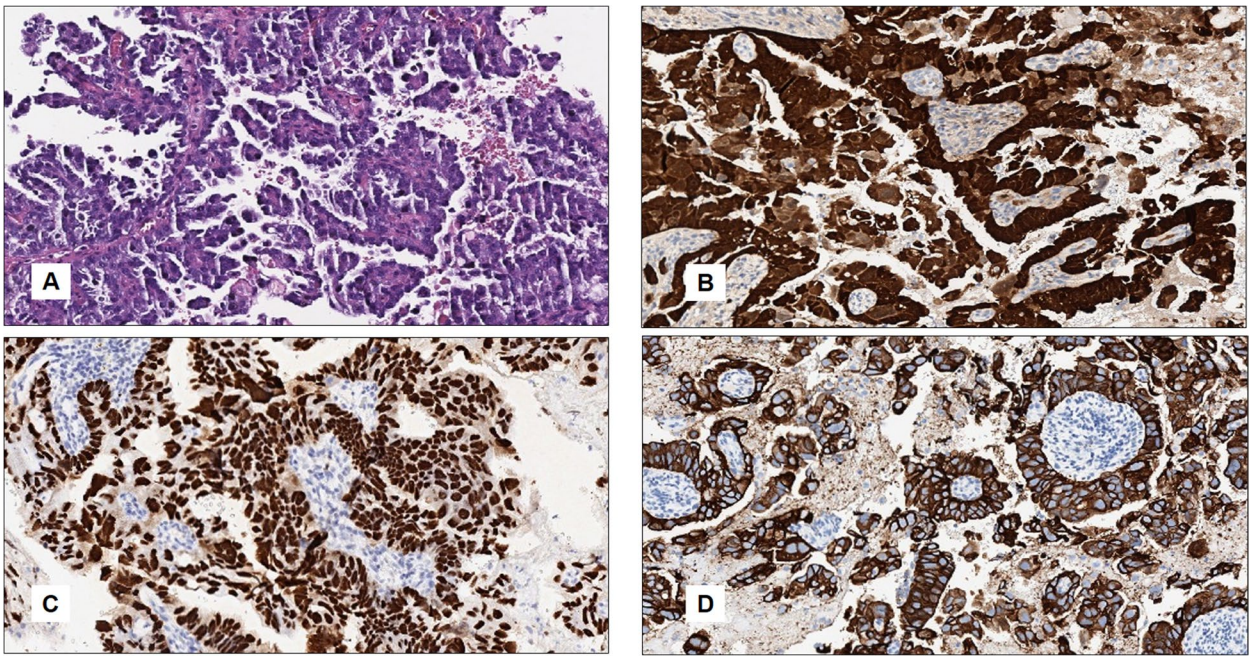


Fig. 6 **A** SEC (HE, 40X). **B** p16: nuclear and citoplasmatic overexpression (IHC, 40X). **C** p53-abn: nuclear overexpression (IHC, 40X). **D** HER2: intense and complete membranous expression score 3 + (IHC, 40X)

Table 5 Reporting results of HER2 testing by IHC and FISH (dual-probe assay) for ESC

HER2 testing by IHC:
Negative (Score 0): No staining observed.
Negative (Score 1+): Incomplete membrane staining that is faint/barely perceptible in any proportion of cells or weak complete staining in less than 10% of tumor cells.
Equivocal (Score 2+): Intense complete or basolateral/lateral membrane staining in 30% or less tumor cells or weak to moderate staining in greater than or equal to 10% of tumor cells.
Positive (Score 3+): Intense complete or basolateral/lateral membrane staining in over 30% of tumor cells.

HER2 testing by FISH:
Negative: FISH HER2/CEP17 ratio less than 2.0 and average HER2 copy number less than 6 per nucleus.
Positive: FISH HER2/CEP17 ratio greater than or equal to 2.0 or FISH HER2/CEP17 ratio less than 2.0 with average HER2 copy number equal to or greater than 6 per nucleus.

Table 6 IHC scoring for HER2 in gastric and gastro-oesophageal junction cancer

Negative (Score 0): No staining or membranous staining in <10% of tumor cells.
Negative (Score 1+): Faint or barely perceptible membranous staining in ≥10% of tumor cells; cells are stained only in part of their membrane.
Equivocal (Score 2+): Weak to moderate complete, basolateral/lateral membranous staining in ≥10% of tumor cells.
Positive (Score 3+): Strong complete, basolateral/lateral membranous staining in ≥10% of tumor cells.

(Ross et al. 2022). *HER2*-directed Antibody–Drug Conjugates show substantial promise and stand to provide significant improvements in effectivity and tolerability of cytotoxic therapy (McNamara et al. 2023). The correlation between *HER2* positivity and p53abn EC is significantly stronger than the correlation between HER2 positivity and serous histology (Karpel et al. 2023; Vermij et al. 2020). It suggests that subsets of high-grade ECs with aberrant p53 expression may also be *HER2* amplified, and these patients could benefit from the addition of targeted therapy (Joehlin-Price et al. 2023, Ross et al., 2022).

Estrogen and progesterone receptors (ER/PR)

Along the years, hormonal therapy has been employed in ER and PR-positive ECs, as viable management option, particularly in cases of recurrent or metastatic disease within the NSMP group, where the majority of endometrioid tumors exhibiting overexpression of ER and PR are placed (Karpel et al. 2023).

The hormone receptor status is determined in FFPE tissue sections using IHC and despite the absence of a consensus regarding the reporting of ER and PR IHC results, the College of American Pathologists (CAP) advocates for a standardized reporting approach in Ecs, akin to protocols employed in breast cancer diagnosis. The assessment involves the proportion of positive tumor cells and the intensity of immunoreactivity, akin to protocols employed in breast cancer diagnosis. Positivity is defined by nuclear staining in at least 1% of tumor cells (Walsh et al. 2023; Longacre et al. 2017).

Conclusion

The rise of molecular classification has profoundly changed the approach to EC management, with the emergence of biomarker-driven therapies guided by diagnostic, predictive, and prognostic biomarkers. The integration of these biomarkers as molecular tools in pathological reports represents a new histomolecular concept for EC. This integration enables clinicians to perform individualized risk stratification based on molecular profiles and to define tailored treatment strategies. This molecular approach is so applicable that expanding clinical trials are in progress, and new molecular biological tools, such as liquid biopsy biomarkers, are expected to emerge. Novel AI-based methods have been applied to enhance the molecular classification of EC, highlighting a broader range of morphological findings that might otherwise be classified as dichotomous by the human eye (Fremond et al. 2023). Additionally, clonal expression of markers or heterogeneous findings could be better integrated through computational imaging tools (Darbandsari et al. 2024). In the era of molecular pathology, pathologists have a new role, integrating molecular knowledge into pathological reports and guiding treatment decisions in collaboration with multidisciplinary teams, which is crucial for the success of precision medicine.

Abbreviations

AKT	Protein Kinase B
BAGP	British Association of Gynecological Pathologists
BMI	Body Mass Index
CAP	College of American Pathologists
CCC	Clear Cell Carcinoma
CHT	Chemotherapy

CNH	Copy Number High
CNL	Copy Number Low
CTNNB1	Catenin Beta 1
DSS	Disease-specific survival
EC	Endometrial Carcinoma
ECs	Endometrial Carcinomas
EEC	Endometrioid Endometrial Carcinoma
EECs	Endometrioid Endometrial Carcinomas
ER	Estrogen Receptor
ERBB2	Human Epidermal Growth Factor Receptor 2
ESC	Endometrial Serous Carcinoma
ESGO	European Society of Gynecological Oncology
ESTRO	European Society for Radiotherapy and Oncology
ESP	European Society of Pathology
FDA	Food and Drug Administration
FFPE	Formalin-Fixed, Paraffin-Embedded
FISH	Fluorescent In Situ Hybridization
FBXW7	F-Box and WD Repeat Domain Containing 7
HER2/Neu	Human Epidermal Growth Factor Receptor 2
HRD	Homologous Recombination Deficiency
ICIs	Immune Checkpoint Inhibitors
IHC	Immunohistochemistry
L1CAM	L1 Cell Adhesion Molecule
LOH	Loss of Heterozygosity
LS	Lynch Syndrome
LVSI	Lymphovascular Space Invasion
MMR	Mismatch Repair
MMRd	Mismatch Repair Deficient
MMRp	Mismatch Repair Proficient
MSI	Microsatellite Instability
MSI-H	Microsatellite Instability-High
MSS	Microsatellite Stability
NGS	Next-Generation Sequencing
NSMP	No Specified Molecular Profile
OS	Overall survival
p53abn	p53 Abnormal
PARP	Poly (ADP-Ribose) Polymerase
PCR	Polymerase Chain Reaction
PI3K	Phosphatidylinositol 3-Kinase
PIK3	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase
POLE	Polymerase Epsilon
*POLE*mut	Polymerase Epsilon (POLE) Mutations
*POLE*wt	Polymerase Epsilon (POLE) Wild Type
PFS	Progression-free survival
PPP2R1A	Protein Phosphatase 2 Regulatory Subunit A Alpha
PR	Progesterone Receptor
RT	Radiotherapy
SCNA	Somatic copy number alterations
T-DXd	Trastuzumab Deruxtecan
TCGA	The Cancer Genome Atlas
TILs	Tumor-Infiltrating Lymphocytes
TMB	Tumor Mutational Burden
TP53	Tumor Protein 53
UDC/DDC	Undifferentiated/Dedifferentiated Carcinoma
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
JN contributed to the project by providing expertise on the MMR-d group, p53abn group, NSMP group, and tumors with multiple classifying alterations. She wrote the text and elaborated figures and tables pertaining to these data. RBP contributed to the project by providing expertise on the *POLE*mut group. He wrote the text related to *POLE*mut and elaborated on the Supplementary Material 1 titled "*POLE*-rides in May-sunshine". PSDB contributed to the project by providing expertise on the HER2 biomarker and the NSMP group. She wrote the text related to NSMP group and HER2 and elaborated the tables and figures pertaining to this biomarker. EP and MDPED, the oncologists of the group, contributed to the project by providing clinical data and writing comprehensive information regarding the relevance of clinical trials in EC management. All authors read and approved the final manuscript.

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Consent for publication
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References

Addante F, d'Amati A, Santoro A, Angelico G, Inzani F, Arciuolo D, Travaglino A, Raffone A, D'Alessandris N, Scaglione G, Valente M, Tinnirello G, Sfregola S, Padial Urtueta B, Piermattei A, Cianfrini F, Mulè A, Bragantini E, Zannoni GF. Mismatch repair deficiency as a predictive and prognostic biomarker in endometrial cancer: a review on immunohistochemistry staining patterns and clinical implications. Int J Mol Sci. 2024;25(2):1056.

Alafraidi M, Hoang L, Howitt BE, Longacre TA, McAlpine JN, Jamieson A, Singh N, Gilks CB, Pors J. The spectrum of oestrogen receptor expression in endometrial carcinomas of no specific molecular profile. Histopathology. 2024;85(4):660–70.

Allgäuer M, Budczies J, Christopoulos P, Endris V, Lier A, Rempel E, et al. Implementing tumor mutational burden (TMB) analysis in routine diagnostics-a primer for molecular pathologists and clinicians. Transl Lung Cancer Res. 2018;7(6):703–15. https://doi.org/10.21037/tlcr.2018.08.14.

Ali AT. Reproductive factors and the risk of endometrial cancer. Int J Gynecol Cancer. 2014;24(3):384–93.

Alexa M, Hasenburg A, Battista MJ. The TCGA Molecular classification of endometrial cancer and its possible impact on adjuvant treatment decisions. Cancers (Basel). 2021;13(6):1478.

Andrianova MA, Chetan GK, Sibin MK, Mckee T, Merkler D, Narasinga RK, Ribaux P, Blouin JL, Makrythanasis P, Seplyarskiy VB, et al. Germline PMS2 and somatic POLE exonuclease mutations cause hypermutability of the leading DNA strand in biallelic mismatch repair deficiency syndrome brain tumors. J Pathol. 2017;243:331–41.

Arciuolo D, Travaglino A, Raffone A, Raimondo D, Santoro A, Russo D, Varricchio S, Casadio P, Inzani F, Seracchioli R, Mollo A, Mascolo M, Zannoni GF. TCGA molecular prognostic groups of endometrial carcinoma: current knowledge and future perspectives. Int J Mol Sci. 2022;23(19):11684.

Aro K, Pasanen A, Bützow R, Loukovaara M. The impact of estrogen receptor and L1 cell adhesion molecule expression on endometrial cancer outcome correlates with clinicopathological risk group and molecular subgroup. Gynecol Oncol. 2024;189:9–15.

Banno K, Yanokura M, Iida M, Masuda K, Aoki D. Carcinogenic mechanisms of endometrial cancer: involvement of genetics and epigenetics. J Obstet Gynaecol Res. 2014;40(8):1957–67.

Barbari SR, Shcherbakova PV. Replicative DNA polymerase defects in human cancers: consequences, mechanisms, and implications for therapy. DNA Repair (Amst). 2017;56:16–25.

Bellone S, Bignotti E, Lonardi S, Ferrari F, Centritto F, Masserdotti A, Pettinella F, Black J, Menderes G, Altwerger G, Hui P, Lopez S, de Haydu C, Bonazzoli E, Predolini F, Zammataro L, Cocco E, Ferrari F, Ravaggi A, Romani C, Facchetti F, Sartori E, Odicino FE, Silasi DA, Litkouhi B, Ratner E, Azodi M, Schwartz PE, Santin AD. Polymerase ε (POLE) ultra-mutation in uterine tumors correlates with T lymphocyte infiltration and increased resistance to platinum-based chemotherapy in vitro. Gynecol Oncol. 2017;144(1):146–52.

Berek JS, Matias-Guiu X, Creutzberg C, Fotopoulou C, Gaffney D, Kehoe S, Lindemann K, Mutch D, Concin N, Endometrial Cancer Staging Subcommittee, FIGO Women's Cancer Committee. FIGO staging of endometrial cancer: 2023. Int J Gynaecol Obstet. 2023;162(2):383–94.

Billingsley CC, Cohn DE, Mutch DG, Hade EM, Goodfellow PJ. Prognostic significance of POLE exonuclease domain mutations in high-grade endometrioid endometrial cancer on survival and recurrence: a subanalysis. Int J Gynecol Cancer. 2016;26(5):933–8.

Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10–7.

Bourdais R, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, Baumgaertner I, Neuzillet C, Tournigand C. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. Crit Rev Oncol Hematol. 2017;113:242–8.

Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63.

Briggs S, Tomlinson I. Germline and somatic polymerase ε and δ mutations define a new class of hypermutated colorectal and endometrial cancers. J Pathol. 2013;230(2):148–53. https://doi.org/10.1002/path.4185. PMID: 23447401; PMCID: PMC3709119.

British Association of Gynaecological Pathologists. POLE NGS Testing guidance for Endometrial Cancer (v1.2-july 2022). https://www.thebagp.org/wp-content/uploads/download-manager-files/BAGP-POLE-testing-in-Endometrial-cancer-v1.2-July-2022.pdf.

Buza N. HER2 testing in endometrial serous carcinoma. Arch Pathol Lab Med. 2021;145(6):687–91.

Buza N. Immunohistochemistry in gynecologic carcinomas: Practical update with diagnostic and clinical considerations based on the 2020 WHO classification of tumors. Semin Diagn Pathol. 2022;39(1):58–77.

Casanova J, Duarte GS, da Costa AG, Catarino A, Nave M, Antunes T, Serra SS, Dias SS, Abu-Rustum N, Lima J. Prognosis of polymerase epsilon (POLE) mutation in high-grade endometrioid endometrial cancer: Systematic review and meta-analysis. Gynecol Oncol. 2024;182:99–107.

Casey L, Singh N. POLE, MMR, and MSI testing in endometrial cancer: proceedings of the ISGyP companion society session at the USCAP 2020 annual meeting. Int J Gynecol Pathol. 2021;40(1):5–16.

Chacon E, Boria F, Lyer RR, Fanfani F, Malzoni M, Bretová P, Luzarraga Aznar A, Fruscio R, Jedryka MA, Tóth R, Perrone AM, Kakkos A, Cristóbal Quevedo I, Congedo L, Zanagnolo V, Fernandez-Gonzalez S, Ferro B, Narducci F, Hovhannisyan T, Aksahin E, Cardenas L, Oliver MR, Nozaleda G, Arnaez M, Misiek M, Ferrero A, Pain FA, Zarragoitia J, Diaz C, Ceppi L, Mehdiyev S, Roldán-Rivas F, Guijarro-Campillo AR, Amengual J, Manzour N, Sanchez Lorenzo L, Núñez-Córdoba JM, Gonzalez Martin A, Minguez JA, Chiva L, SENECA Working Group. SENECA study: staging endometrial cancer based on molecular classification. Int J Gynecol Cancer. 2024;34(9):1313–21.

Colombo N, Biagioli E, Harano K, Galli F, Hudson E, Antill Y, et al. Atezolizumab and chemotherapy for advanced or recurrent endometrial cancer (AtTEnd): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2024;25(9):1135–46.

Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, Ledermann J, Bosse T, Chargari C, Fagotti A, Fotopoulou C, Gonzalez Martin A, Lax S, Lorusso D, Marth C, Morice P, Nout RA, O'Donnell D, Querleu D, Raspollini MR, Sehouli J, Sturdza A, Taylor A, Westermann A, Wimberger P, Colombo N, Planchamp F, Creutzberg CL. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer. 2021;31(1):12–39.

Creutzberg CL, Koper PCM, van Putten WLJ, Dercksen R, van Lent M, Beerman H, et al. Molecular Classification Predicts Response to Radiotherapy in the Randomized PORTEC-1 and PORTEC-2 Trials for Early-Stage Endometrioid Endometrial Cancer. J Clin Oncol. 2023; Available from: https://ascopubs.org/doi/10.1200/JCO.23.00062. Cited 2024 Sep 9

Darbandsari A, Farahani H, Asadi M, Wiens M, Cochrane D, Khajegili Mirabadi A, Jamieson A, Farnell D, Ahmadvand P, Douglas M, Leung S, Abolmaesumi P, Jones SJM, Talhouk A, Kommoss S, Gilks CB, Huntsman DG, Singh N, McAlpine JN, Bashashati A. AI-based histopathology image analysis reveals a distinct subset of endometrial cancers. Nat Commun. 2024;15(1):4973.

De Boer SM, Powell ME, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, Ottevanger PB, Ledermann JA, Khaw P, Colombo A, Fyles A, Baron MH, Jürgenliemk-Schulz IM, Kitchener HC, Nijman HW, Wilson G, Brooks S, Carinelli S, Provencher D, Hanzen C, Lutgens LCHW, Smit VTHBM, Singh N, Do V, D'Amico R, Nout RA, Feeney A, Verhoeven-Adema KW, Putter H, Creutzberg CL, PORTEC study group. Adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): final results of an international, open-label, multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19(3):295–309.

De Vitis LA, Schivardi G, Caruso G, Fumagalli C, Vacirca D, Achilarre MT, Aloisi A, Garbi A, Zanagnolo V, Aletti G, Guerini-Rocco E, Mariani A, Maggioni A, Barberis M, Bogani G, Colombo N, Multinu F, Betella I. Clinicopathological characteristics of multiple-classifier endometrial cancers: a cohort study and systematic review. Int J Gynecol Cancer. 2024;34(2):229–38.

Endris V, Buchhalter I, Allgäuer M, Rempel E, Lier A, Volckmar AL, et al. Measurement of tumor mutational burden (TMB) in routine molecular diagnostics: in silico and real-life analysis of three larger gene panels. Int J Cancer. 2019;144:2303–12.

Eskander RN, Sill MW, Beffa L, Moore RG, Hope JM, Musa FB, et al. Pembrolizumab plus chemotherapy in advanced endometrial cancer. N Engl J Med. 2023;388(23):2159–70.

Eskander RN. The epigenetic landscape in the treatment of gynecologic malignancies. Am Soc Clin Oncol Educ Book. 2018;23(38):480–7.

Fader AN, Roque DM, Siegel E, Buza N, Hui P, Abdelghany O, Chambers S, Secord AA, Havrilesky L, O'Malley DM, Backes FJ, Nevadunsky N, Edraki B, Pikaart D, Lowery W, ElSahwi K, Celano P, Bellone S, Azodi M, Litkouhi B, Ratner E, Silasi D, Schwartz PE, Santin AD. Randomized phase II trial of carboplatin-paclitaxel compared with carboplatin-paclitaxel-trastuzumab in advanced (stage III-IV) or recurrent uterine serous carcinomas that overexpress Her2/Neu (NCT01367002): updated overall survival analysis. Clin Cancer Res. 2020;26(15):3928–35.

Fan R, Lin W, Zhao R, Li L, Xin R, Zhang Y, Liu Y, Ma Y, Wang Y, Wang Y, Zheng W. Morules and β-catenin predict POLE mutation status in endometrial cancer: A pathway to more cost-effective diagnostic procedures. Am J Clin Pathol. 2024;162(2):141–50.

Ferriss JS, Erickson BK, Shih IM, Fader NA. Uterine serous carcinoma: key advances and novel treatment approaches. Int J Gynecol Cancer. 2021;31(8):1165–74.

Fremond S, Andani S, Barkey Wolf J, Dijkstra J, Melsbach S, Jobsen JJ, Brinkhuis M, Roothaan S, Jurgenliemk-Schulz I, Lutgens LCHW, Nout RA, van der Steen-Banasik EM, de Boer SM, Powell ME, Singh N, Mileshkin LR, Mackay HJ, Leary A, Nijman HW, Smit VTHBM, Creutzberg CL, Horeweg N, Koelzer VH, Bosse T. Interpretable deep learning model to predict the molecular classification of endometrial cancer from haematoxylin and eosin-stained whole-slide images: a combined analysis of the PORTEC randomised trials and clinical cohorts. Lancet Digit Health. 2023;5(2):e71–82. https://doi.org/10.1016/S2589-7500(22)00210-2. Epub 2022 Dec 7. PMID: 36496303.

Heitzer E, Tomlinson I. Replicative DNA polymerase mutations in cancer. Curr Opin Genet Dev. 2014;24(100):107–13.

Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, Stover E, Strickland KC, D'Andrea AD, Wu CJ, et al. Association of polymerase e-mutated and microsatellite-instable 409 endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and 410 expression of PD-1 and PD-L1. JAMA Oncol. 2015;1:1319–23.

Inoue F, Sone K, Toyohara Y, Takahashi Y, Kukita A, Hara A, Taguchi A, Tanikawa M, Tsuruga T, Osuga Y. Targeting epigenetic regulators for endometrial cancer therapy: its molecular biology and potential clinical applications. Int J Mol Sci. 2021;22(5):2305.

Integrated genomic characterization of endometrial carcinoma, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding L, Zhang W, Mills GB, Kucherlapati R, Mardis ER, Levine DA. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67–73.

Jamieson A, de Sobral BJ, Cochrane DR, et al. Targeted and shallow whole-genome sequencing identifies therapeutic opportunities in p53abn endometrial cancers. Clin Cancer Res. 2024;30(11):2461–74. https://doi.org/10.1158/1078-0432.CCR-23-3689.

Jamieson A, Huvila J, Chiu D, Thompson EF, Scott S, Salvador S, Vicus D, Helpman L, Gotlieb W, Kean S, Samouelian V, Kobel M, Kinloch M, Parra-Harran C, Offman S, Grondin K, Irving J, Lum A, Senz J, Leung S, McConechy MK, Plante M, Kommoss S, Huntsman DG, Talhouk A, Gilks CB, McAlpine JN. Grade and estrogen receptor expression identify a subset of no specific molecular profile endometrial carcinomas at a very low risk of disease-specific death. Mod Pathol. 2023;36(4):100085.

Jamieson A, Thompson EF, Huvila J, Gilks CB, McAlpine JN. p53abn Endometrial Cancer: understanding the most aggressive endometrial cancers in the era of molecular classification. Int J Gynecol Cancer. 2021;31(6):907–13.

Joehlin-Price AS, Komforti MK, Ladwig NR, Devine P, Hoyle C, McCoy L, Sprague C, Astbury C, Hoda R, Chen Y, Garg K. HER2 IHC expression and gene amplification in p53-aberrant high-grade endometrial endometrioid carcinoma suggests that this population may benefit from HER2 testing and targeted therapy. Am J Surg Pathol. 2023;47(5):580–8.

Karpel H, Slomovitz B, Coleman RL, Pothuri B. Biomarker-driven therapy in endometrial cancer. Int J Gynecol Cancer. 2023;33(3):343–50.

Kaya M, Post CCB, Tops CM, Nielsen M, Crosbie EJ, Leary A, Mileshkin LR, Han K, Bessette P, de Boer SM, Jürgenliemk-Schulz IM, Lutgens L, Jobsen JJ, Haverkort MAD, Nout RA, Kroep J, Creutzberg CL, Smit VTHBM, Horeweg N, van Wezel T, Bosse T. Molecular and clinicopathologic characterization of mismatch repair-deficient endometrial carcinoma not related to MLH1 promoter hypermethylation. Mod Pathol. 2024;37(3):100423.

Keyhanian K, Han L, Howitt BE, Longacre T. Specific pathology features enrich selection of endometrial carcinomas for POLE testing. Am J Surg Pathol. 2024;48(3):292–301.

Köbel M, Kang EY. The many uses of p53 immunohistochemistry in gynecological pathology: proceedings of the ISGyP companion society session at the 2020 USCAP annual9 meeting. Int J Gynecol Pathol. 2021;40(1):32–40.

Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. Int J Gynecol Pathol. 2019;38 Suppl 1(Iss 1 Suppl 1):S123–31.

Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, Britton H, Kommoss F, Grevenkamp F, Karnezis A, Yang W, Lum A, Krämer B, Taran F, Staebler A, Lax S, Brucker SY, Huntsman DG, Gilks CB, McAlpine JN, Talhouk A. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol. 2018;29(5):1180–8.

León-Castillo A, de Boer SM, Powell ME, Mileshkin LR, Mackay HJ, Leary A, Nijman HW, Singh N, Pollock PM, Bessette P, Fyles A, Haie-Meder C, Smit VTHBM, Edmondson RJ, Putter H, Kitchener HC, Crosbie EJ, de Bruyn M, Nout RA, Horeweg N, Creutzberg CL, Bosse T, TransPORTEC consortium. Molecular classification of the PORTEC-3 trial for high-risk endometrial cancer: impact on prognosis and benefit from adjuvant therapy. J Clin Oncol. 2020;38(29):3388–97.

León-Castillo A, Gilvazquez E, Nout R, Smit VT, McAlpine JN, McConechy M, Kommoss S, Brucker SY, Carlson JW, Epstein E, Rau TT, Soslow RA, Ganesan R, Matias-Guiu X, Oliva E, Harrison BT, Church DN, Gilks CB, Bosse T. Clinicopathological and molecular characterisation of "multiple-classifier" endometrial carcinomas. J Pathol. 2020;250(3):312–22.

Léon-Castillo A. Update in the molecular classification of endometrial carcinoma. Int J Gynecol Cancer. 2023;33(3):333–42.

Longacre TA, Broaddus R, Chuang LT, Cohen MB, Jarboe EA, Mutter GL, Otis CN, Zaino RJ, Members of the Cancer Biomarker Reporting Committee, College of American Pathologists. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the endometrium. Arch Pathol Lab Med. 2017;141(11):1508–12.

Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International patterns and trends in endometrial cancer incidence, 1978–2013. J Natl Cancer Inst. 2018;110(4):354–61.

Makker V, Colombo N, Herráez AC, Monk BJ, Mackay H, Santin AD, et al. Lenvatinib Plus Pembrolizumab in Previously Treated Advanced Endometrial Cancer: Updated Efficacy and Safety From the Randomized Phase III Study 309/KEYNOTE-775. J Clin Oncol. 2023; Available from: https://ascopubs.org/doi/10.1200/JCO.22.02152. Cited 2024 Sep 9

Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the Phase II KEYNOTE-158 study. J Clin Oncol. 2020;38(1):1–10.

Ma X, Dong L, Liu X, Ou K, Yang L. POLE/POLD1 mutation and tumor immunotherapy. J Exp Clin Cancer Res. 2022;41(1):216.

McAlpine JN, Chiu DS, Nout RA, et al. Evaluation of treatment effects in patients with endometrial cancer and POLE mutations: an individual patient data meta-analysis. Cancer. 2021;127(14):2409–22. https://doi.org/10.1002/cncr.33516.

McNamara B, Mutlu L, Greenman M, Harold J, Santin A. HER2 oncogene as molecular target in uterine serous carcinoma and uterine carcinosarcoma. Cancers (Basel). 2023;15(16):4085.

Mendiola M, Heredia-Soto V, Ruz-Caracuel I, Baillo A, Ramon-Patino JL, Escudero FJ, Miguel M, Pelaez-Garcia A, Hernandez A, Feliu J, Hardisson D, Redondo A. Comparison of methods for testing mismatch repair status in endometrial cancer. Int J Mol Sci. 2023;24(19):14468.

Meric-Bernstam F, Makker V, Oaknin A, Oh D-Y, Banerjee S, González-Martín A, Jung KH, Ługowska I, Manso L, Manzano A, Melichar B, Siena S, Stroyakovskiy D, Fielding A, Ma Y, Puvvada S, Shire N, Lee J-Y. Efficacy and safety of Trastuzumab Deruxtecan in patients with HER2-expressing solid tumors: primary results from the DESTINY-PanTumor02 phase II trial. J Clin Oncol. 2024;42(1):47–58.

Mirza MR, Chase DM, Slomovitz BM, dePont CR, Novák Z, Black D, et al. Dostarlimab for primary advanced or recurrent endometrial cancer. N Engl J Med. 2023;388(23):2145–58.

Oaknin A, Bosse TJ, Creutzberg CL, Giornelli G, Harter P, Joly F, Lorusso D, Marth C, Makker V, Mirza MR, Ledermann JA, Colombo N, ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Endometrial cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2022;33(9):860–77.

O'Malley DM, Bariani GM, Cassier PA, Marabelle A, Hansen AR, De Jesus Acosta A, et al. Pembrolizumab in Patients With Microsatellite Instability–High Advanced Endometrial Cancer: Results From the KEYNOTE-158 Study. J Clin Oncol. 2022; Available from:https://ascopubs.org/doi/10.1200/JCO.21.01874. Cited 2024 Sep 9

Oaknin A, Gilbert L, Tinker AV, Brown J, Mathews C, Press J, et al. Safety and antitumor activity of dostarlimab in patients with advanced or recurrent DNA mismatch repair deficient/microsatellite instability-high (dMMR/MSI-H) or proficient/stable (MMRp/MSS) endometrial cancer: interim results from GARNET-a phase I, single-arm study. J Immunother Cancer. 2022;10(1):e003777. https://doi.org/10.1136/jitc-2021-003777.

Pina PS, Lheureux S. Novel molecular targets in endometrial cancer: mechanisms and perspectives for therapy. Biologics. 2024;21(18):79–93.

RAINBO Research Consortium. Refining adjuvant treatment in endometrial cancer based on molecular features: the RAINBO clinical trial program. Int J Gynecol Cancer. 2022;33(1):109–17.

Rosa RCA, Yurchenko AA, Chahud F, Ribeiro-Silva A, Brunaldi MO, Silva WA Jr, Kannouche PL, Nikolaev S, Ferraz VEF. First description of ultramutated endometrial cancer caused by germline loss-of-function and somatic exonuclease domain mutations in POLE gene. Genet Mol Biol. 2020;43(4):e20200100.

Ross DS, Devereaux KA, Jin C, Lin DY, Zhang Y, Marra A, Makker V, Weigelt B, Ellenson LH, Chui MH. Histopathologic features and molecular genetic landscape of HER2-amplified endometrial carcinomas. Mod Pathol. 2022;35(7):962–71.

Rottmann D, Assem H, Matsumoto N, Wong S, Hui P, Buza N. Does specimen type have an impact on HER2 status in endometrial serous carcinoma? Discordant HER2 status of paired endometrial biopsy and hysterectomy specimens in the presence of frequent intratumoral heterogeneity. Int J Gynecol Pathol. 2021;40(3):263–71.

Shinbrot E, Henninger EE, Weinhold N, et al. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. Genome Res. 2014;24(11):1740–50. https://doi.org/10.1101/gr.174789.114.

Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E, Parkash V, Carlson J, McCluggage WG, Gilks CB. Endometrial carcinoma diagnosis: use of FIGO grading and genomic subcategories in clinical practice: recommendations of the international society of gynecological pathologists. Int J Gynecol Pathol. 2019;38 Suppl 1(Iss 1 Suppl 1):S64–74.
Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, van der Steen-Banasik EM, Nijman HW, Putter H, Bosse T, Creutzberg CL, Smit VT. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC Cohorts. Clin Cancer Res. 2016;22(16):4215–24.
Talhouk A, Derocher H, Schmidt P, Leung S, Milne K, Gilks CB, Anglesio MS, Nelson BH, McAlpine JN. Molecular subtype not immune response drives outcomes in endometrial carcinoma. Clin Cancer Res. 2019;25(8):2537–48.
Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, Yang W, Senz J, Boyd N, Karnezis AN, Huntsman DG, Gilks CB, McAlpine JN. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer. 2015;113(2):299–310.
Travaglino A, Raffone A, Mascolo M, Guida M, Insabato L, Zannoni GF, Zullo F. TCGA molecular subgroups in endometrial undifferentiated/Dedifferentiated carcinoma. Pathol Oncol Res. 2020;26(3):1411–6.
Travaglino A, Raffone A, Mascolo M, Guida M, Insabato L, Zannoni GF, Zullo F. Clear cell endometrial carcinoma and the TCGA classification. Histopathology. 2020;76(2):336–8.
Travaglino A, Raffone A, Gencarelli A, Mollo A, Guida M, Insabato L, Santoro A, Zannoni GF, Zullo F. TCGA classification of endometrial cancer: the place of carcinosarcoma. Pathol Oncol Res. 2020;26(4):2067–73.
Travaglino A, Raffone A, Mollo A, Borrelli G, Alfano P, Zannoni GF, Insabato L, Zullo F. TCGA molecular subgroups and FIGO grade in endometrial endometrioid carcinoma. Arch Gynecol Obstet. 2020;301(5):1117–25.
Van den Heerik ASVM, Horeweg N, Nout RA, Lutgens LCHW, van der Steen-Banasik EM, Westerveld GH, van den Berg HA, Slot A, Koppe FLA, Kommoss S, Mens JWM, Nowee ME, Bijmolt S, Cibula D, Stam TC, Jurgenliemk-Schulz IM, Snyers A, Hamann M, Zwanenburg AG, Coen VLMA, Vandecasteele K, Gillham C, Chargari C, Verhoeven-Adema KW, Putter H, van den Hout WB, Wortman BG, Nijman HW, Bosse T, Creutzberg CL. PORTEC-4a: international randomized trial of molecular profile-based adjuvant treatment for women with high-intermediate risk endometrial cancer. Int J Gynecol Cancer. 2020;30(12):2002–7.
Van Hoeck A, Tjoonk NH, van Boxtel R, Cuppen E. Portrait of a cancer: mutational signature analyses for cancer diagnostics. BMC Cancer. 2019;19(1):457. https://doi.org/10.1186/s12885-019-5677-2. Published 2019 May 15.
van Gool IC, Eggink FA, Freeman-Mills L, Stelloo E, Marchi E, de Bruyn M, Palles C, Nout RA, de Kroon CD, Osse EM, Klenerman P, Creutzberg CL, Tomlinson IP, Smit VT, Nijman HW, Church DN. *POLE* proofreading mutations elicit an antitumor immune response in endometrial cancer. Clin Cancer Res. 2015;21(14):3347–55.
Vermij L, Horeweg N, Leon-Castillo A, Rutten TA, Mileshkin LR, Mackay HJ, Leary A, Powell ME, Singh N, Crosbie EJ, Smit VTHBM, Creutzberg CL, Bosse T. Her2 status in high-risk endometrial cancers (PORTEC-3): relationship with histotype, molecular classification, and clinical outcomes. Cancers (Basel). 2020;13(1):44.
Vermij L, Léon-Castillo A, Singh N, Powell ME, Edmondson RJ, Genestie C, et al. p53 immunohistochemistry in endometrial cancer: clinical and molecular correlates in the PORTEC-3 trial. Mod Pathol. 2022;35(10):1475–83. https://doi.org/10.1038/s41379-022-01102-x.
Vermij L, Smit V, Nout R, Bosse T. Incorporation of molecular characteristics into endometrial cancer management. Histopathology. 2020;76(1):52–63.
Walsh CS, Hacker KE, Secord AA, DeLair DF, McCourt C, Urban R. Molecular testing for endometrial cancer: An SGO clinical practice statement. Gynecol Oncol. 2023;168:48–55.
Westin SN, Moore K, Chon HS, Lee JY, Thomes Pepin J, Sundborg M, et al. Durvalumab plus Carboplatin/Paclitaxel followed by maintenance Durvalumab with or without Olaparib as first-line treatment for advanced endometrial cancer: the phase III DUO-E trial. J Clin Oncol. 2024;42(3):283–99.
WHO Classification of Tumours Editorial Board. Female genital tumours. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 4). https://publications.iarc.fr/592.
Yao X, Feng M, Wang W. The clinical and pathological characteristics of POLE-mutated endometrial cancer: a comprehensive review. Cancer Manag Res. 2024;16:117–25 Published 2024 Mar 4.
Yasuda M. New clinicopathological concept of endometrial carcinoma with integration of histological features and molecular profiles. Pathol Int. 2024;74:557.
Zhou Y, Tao L, Qiu J, Xu J, Yang X, Zhang Y, Tian X, Guan X, Cen X, Zhao Y. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Signal Transduct Target Ther. 2024;9(1):132.

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