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REVIEW

# The Clinical and Pathological Characteristics of POLE-Mutated Endometrial Cancer: A Comprehensive Review

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**Abstract:** Endometrial cancer shows high histological and molecular heterogeneity. The *POLE* mutation is a significant molecular alteration in endometrial cancer, leading to the identification of a specific subtype known as *POLE*-mutated endometrial cancer. This subtype exhibits a high tumor mutation burden, abundant lymphocyte infiltration, and a favorable prognosis, making it a promising candidate for immune checkpoint inhibitor therapy. This paper presents a comprehensive review of the clinical and pathological characteristics, outcomes, treatment advancements, pathogenic *POLE* gene detection, and alternative testing methods for *POLE*-mutated endometrial cancer.

**Keywords:** endometrial cancer, molecular subtyping, *POLE*, *POLE*-mutated endometrial cancer, immunotherapy

## Background

In the year 2020, the global incidence of endometrial cancer (EC) reached 417,367 new cases, resulting in 16,607 reported deaths.[1] This concerning trend can be attributed to the increasing prevalence of high-risk populations, including individuals with obesity, diabetes, and hypertension, thereby elevating the annual rates of both incidence and mortality for endometrial cancer (EC). Furthermore, this rise in cases has notably affected younger individuals.[2] It is worth noting that a significant proportion of EC patients are fortunate enough to receive an early diagnosis, leading to a commendable five-year survival rate of 81%.[3] However, in cases of local spread or distant metastasis, the five-year survival rates decline to 68% and 17%, respectively.[4–6] The conventional Bokhman classification[7] and the WHO (World Health Organization)classification based on histological characteristics, have been pivotal in the diagnosis and treatment EC. Nevertheless, the substantial molecular heterogeneity present in EC poses a challenge to the effectiveness of these traditional classifications in genetic syndrome screening, prognosis assessment, personalized treatment guidance, and treatment outcome prediction.

In 2013, The Cancer Genome Atlas (TCGA) project conducted an analysis of a multi-omics dataset consisting of 373 cases of EC, disregarding histopathological diagnoses. As a result, EC was classified into four molecular subtypes: POLE mutation (POLE-mut), Microsatellite Instability (MSI), Copy Number-Low (CN-L), and Copy Number-High (CN-H).[8] This pioneering study offered an extensive examination of the molecular genetic landscape of EC, marking the onset of a novel era of comprehension via molecular subtyping. The advent of molecular subtyping has provided valuable insights into significant biological and clinical distinctions among these subtypes, underscoring the need for improved prognostic classifications. Notably, POLE-mutated endometrial cancers (ECs) have been linked to a favorable prognosis. This paper aims to offer a comprehensive review of the clinical and pathological characteristics of POLE-mutated ECs, recent therapeutic advancements, and the methodologies employed for identifying pathogenic mutations in the POLE gene.

## Traditional EC Classification and Limitations

The conventional categorization of endometrial cancer (EC) is predominantly grounded on the Bokhman classification and the WHO histological classification.Bokhman (1983)[7] delineated two types of EC, taking into account histological attributes,



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clinical characteristics, and hormone receptor expression. Type I EC is reliant on estrogen and arises from prolonged estrogen stimulation, resulting in endometrial epithelial hyperplasia and endometrial intraepithelial neoplasia, ultimately advancing to invasive EC. This subtype is frequently detected at an early stage, exhibiting a favorable prognosis with a five-year survival rate of 85%.[9] On the contrary, Type II endometrial cancer (EC) is not reliant on estrogen and originates from atrophic endometrium. It encompasses non-endometrioid tumors, including serous and clear cell carcinomas. Type II EC is typically detected at a later stage, resulting in a less favorable prognosis with a five-year survival rate of 55%. However, there is a significant molecular overlap between the two types. Type I EC frequently exhibits alterations in the PI3K pathway, with a prevalence of over 90%. These alterations include PTEN mutations (75–85%), PIK3CA mutations (50–60%), and PIK3R1 mutations (40–50%). Interestingly, PIK3CA mutations are also observed in 42% of serous cancers.Type II (serous) cancers predominantly exhibit HER-2 amplification and TP53 mutations, with TP53 mutations also being present in 12% of endometrioid cancers.[5,6,8] High-grade (Grade 3, G3) EC encompasses both endometrioid and serous types at the histological level, resulting in limited agreement among observers and an ambiguous binary classification. Regarding treatment and prognosis, approximately 20% of endometrioid cancers (Type I) experience recurrence, whereas 50% of non-endometrioid cancers (Type II) do not recur.Furthermore, it should be noted that the Bokhman classification fails to include endometrioid cancers in individuals with Lynch syndrome, characterized by a low Body Mass Index (BMI) and absence of endometrial hyperplasia.[10] Moreover, specific Type II endometrial cancers have been linked to obesity.[10] Although these conventional classifications continue to be extensively employed in routine clinical settings, their shortcomings have become more evident with the advancement of personalized precision medicine.

## Molecular Subtyping

In contrast to conventional classifications, the TCGA classification, which utilizes molecular features, has presented promising prospects for targeted therapy, immunotherapy, and combination therapy among patients. Nevertheless, the implementation of the TCGA classification is hindered by its reliance on high-throughput sequencing, which incurs substantial costs, involves a complex process, and encounters challenges in achieving widespread adoption in clinical practice.[8] Consequently, various research groups have devised modified classifications, primarily employing Sanger sequencing and immunohistochemistry, to categorize EC into four subtypes: POLE-mut, MMR-d (MMR deficiency), and p53-abn (p53 Abnormality), and non-specific molecular profile (NSMP).[11] This classification strategy has been found to substantially decrease testing expenses, possess user-friendly features, and exhibit strong concordance with the TCGA classification. Consequently, it has been regarded as a viable substitute for the TCGA molecular classification and is progressively being incorporated into clinical practice.

## POLE-Mutated Endometrial Cancer

### POLE Gene and POLE-Mutated Tumors

The POLE gene is responsible for encoding the catalytic subunit of DNA polymerase epsilon (ε), which exhibits DNA polymerase activity and 3'-5' exonuclease proofreading activity. These activities are crucial for DNA chain elongation and the correction of mismatched bases during DNA replication, thereby ensuring accurate replication. However, when pathological mutations arise in the exonuclease domain of the POLE gene, the mismatch repair function is compromised, resulting in an aberrant accumulation of genomic mutations and the subsequent development of tumors.The POLE gene plays a crucial role in the regulation of cell cycle checkpoints and chromatin modifications, and as such, mutations in this gene can lead to a range of cellular abnormalities.[12,13] Additionally, research has shown that pathogenic somatic mutations in the POLE gene are often early occurrences, serving as the initiating events in the development of endometrial and colorectal cancers.[14] These mutations can even be detected in precancerous lesions. It is worth noting that POLE mutations predominantly manifest as somatic mutations, with germline mutations being relatively rare.[15,16] Pathogenic mutations have been identified in exons 9–14, specifically in the exonuclease domain of the POLE gene. These mutations, including P286R, V411L, S297F, A456P, and S459F, are collectively referred to as "hotspot POLE mutations", with P286R and V411L being the most prevalent.[17,18] However, it is important to note that aside from these pathogenic mutations, there exist numerous mutations whose significance remains unknown. A recent study involving 458 late-stage tumors with POLE mutations revealed that only

15% of these mutations were confirmed as pathogenic, while the majority, approximately 69.1%, lacked clear significance.[18] In order to enhance the evaluation of the pathogenicity of POLE mutations in endometrial cancer (EC), Castillo et al,[19] devised a practical scoring system that relies on the examination of characteristic gene sequences in POLE hotspot mutations. This scoring system encompasses several criteria, including the presence of C>A mutations surpassing 20% (score of 1), T>G mutations exceeding 4% (score of 1), insertion mutations below 5% (score of 1), C>G mutations lower than 0.6% (score of 1), a Tumor Mutational Burden (TMB) surpassing 100 mutations per megabase (mut/Mb) (score of 1), and the occurrence of recurring mutations in EC (score of 1). Mutations with a score of ≥4 are classified as pathogenic, those with a score of ≤2 are considered non-pathogenic, and mutations with a score ranging from 2 to 4 are categorized as indeterminate. However, the current body of research on tumors related to POLE mutations is limited, particularly in regards to mutations with uncertain significance and recently identified mutation sites. Extensive case studies are still required to elucidate the implications of these mutations and their clinical-pathological characteristics.

## Clinical and Pathological Features of POLE-Mutated Endometrial Cancer

Despite being the smallest subgroup within molecular subtyping, POLE-mutant EC exhibits the highest mutation rate of the POLE gene, ranging from 7–12%.[20] In three studies encompassing 119 patients with POLE-mut ECs (Table 1),[21–23] the majority of cases were classified as endometrioid carcinoma, while non-endometrioid carcinomas such as serous, clear cell, and mixed types were also observed. YR Hussein's report on 99 cases of high-grade endometrial cancer, encompassing various subtypes such as endometrioid, serous, clear cell, and undifferentiated types, revealed the identification of 8 cases as POLE-mutated endometrioid carcinomas.[20,22] Among these cases, 7 exhibited notable intratumoral heterogeneity. Furthermore, 3 cases exhibited serous carcinoma components, while 4 cases displayed ambiguous and uncertain morphological characteristics. Accurate differentiation of this particular group holds substantial significance in terms of prognosis and treatment. Four recent meta-analyses[18,24,25] conducted within the past three years have demonstrated that the majority of patients with POLE-mutant endometrial cancer (EC) display early characteristics typically associated with low risk, such as myometrial invasion below 50% and a lower incidence of lymph node metastases. However, these patients also exhibit high-grade features that are indicative of a heightened risk of recurrence and metastasis. Despite these adverse prognostic indicators, POLE-mutant ECs exhibit more favorable clinical outcomes in comparison to POLE-wild EC, including improved overall survival (OS), progression-free survival (PFS), and disease-specific survival (DSS).This favorable prognosis can be attributed to the notably elevated tumor mutational burden (TMB) and a significant infiltration of tumor-infiltrating lymphocytes (TILs).[26] Furthermore, within POLE-mutated ECs, there is an upregulation of immune checkpoint molecules and regulatory T cell markers, including LAG3, TIM-3, TIGIT, PD-1, CTLA-4, and FOXP3, potentially as a result of immune evasion caused by prolonged exposure to antigens.[27] This implies that immune checkpoint inhibitors hold potential for a subset of patients with recurrent or metastatic POLE-mut ECs.[27–31] Nevertheless, the factors contributing to the absence of heightened immune responses in certain POLE-mut patients remain unclear, including whether it is attributed to a low mutation burden or other immune evasion mechanisms. A study examining early-stage low-grade EC identified five distinct immunotypes that exhibited superior sensitivity and specificity in predicting recurrence. Notably, this predictive capacity is not reliant on the presence of POLE-mut ECs.[32] Multiple studies have demonstrated a correlation between molecular subtyping and immune subtyping in EC, specifically in relation to immune cell infiltration and immune genes associated with EC.[33–35] Investigating the immune microenvironment of EC has the potential to enhance the accuracy of prognosis prediction, inform clinical treatment strategies, and facilitate the implementation of precision medicine.

## "Multiple-Classifier" Endometrial Cancer with Diverse Characteristics

Another significant aspect in molecular subtyping is the observation that a small percentage of EC patients, ranging from 3% to 5%, exhibit alterations in multiple molecular features, which we refer to as "Multiple-classifier" EC.[36] In the most extensive cohort of such cases documented thus far,[37] 107 tumors (3%) demonstrated two or more molecular alterations. These included 64 cases with MMRd - p53abn, 31 cases with POLE mutation - p53abn, and 12 cases with all three alterations (MMRd - POLE mutation - p53abn). The study revealed that patients with both MMRd and p53 abnormalities experienced contrasting clinical outcomes compared to those with only one mutation. However, it was observed that MMRd - p53abn EC and POLE mutation - p53abn EC displayed similar profiles of single-nucleotide variants (SNVs), patterns of somatic copy-number

**Table 1** Clinical and Pathologic Characteristics of the Study Cohort

| Type | Patient Age, Mean, y | Tumor Histotype | | | | FIGO Grade | | FIGO Stage | | Lympho vascular Space Invasion (LVSI), No.(%) | Tumor-Infiltrating Lymphocytes (TILs) | Peritumoral Lymphocyte Infiltration | Tumor Giant Cells | Adjuvant Therapy | | Recurrence | p53-abn | MMRd | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Endometrioid | Serous | Clear Cell | Mixed | Grade 1–2 | Grade3 | I–II | III–IV | | | | | Radiotherapy | Chemotherapy | | | | |
| POLE-mut (n=25) | 55 | 18/25 (72%) | 0 | 0 | 7/25 (28%) | 10/25 (40%) | 15/25 (60%) | 20/25 (80%) | 5/25 (20%) | | 19/25(76%) | 19/25(76%) | 10/25(40%) | 6/24 (25%) | 3/24 (7%) | 0 | | | [22] |
| POLE-mut (n=43) | 58 | 34/43 (79%) | 2/43 (5%) | 1/34 (2%) | 6/34 (14%) | 15/34 (45%) | 19/34 (56%) | 41/43 (98%) | 1/43 (2%) | 20/43(47%) | 18/43(42%) | 34/43(79%) | | 80% | 40% | | 5/43 | 3/43 | [21] |
| POLE-mut (n=51) | 62.1 | 51/51 (100%) | 0 | 0 | 0 | 35/51 (69%) | 16/52 (31%) | 49/51 (96%) | 2/52 (4%) | 7/51(14%) | 30/51(59%) | 41/51(80%) | 17/51(33%) | | | | 8/51 (16%) | 5/51 (10%) | [20] |
| POLE-wild (n=67) | 65 | 66/67 (99%) | 0 | 0 | 1/67 (1%) | 53/67 (79%) | 14/67 (21%) | 61/67 (91%) | 6/67 (9%) | 9/67(13%) | 19/67(28%) | 29/67(43%) | 7/67(10%) | | | | 6/67 (6%) | 31/67 (46%) | [20] |

alterations (SCNAs), clinical outcomes, and characteristics associated with MMRd or POLE mutation EC. These characteristics included tumor-infiltrating lymphocytes, peritumoral lymphocytes, and squamous differentiation, rather than features related to p53 abnormalities.[38] These findings provide evidence for classifying MMRd - p53abn EC as MMRd type and POLE mutation - p53abn EC as POLE mutation type, indicating that p53 mutations are incidental occurrences in the context of MMRd and POLE mutation.[39] In a recent study conducted by Hwang et al,[40] it was observed that two patients diagnosed with non-pathogenic POLE mutations and MMRd colorectal cancer displayed mutation patterns akin to those found in POLE-mutated tumors, specifically a higher occurrence of missense mutations and a lower frequency of insertions/deletions. These findings suggest that the presence of non-pathogenic POLE mutations in MMRd cases may impact the functionality of the POLE gene, resulting in a significantly elevated tumor mutational burden (TMB) that closely resembles that of POLE-mutated tumors. Determining the driver mutation between POLE mutation and MMRd presents a challenge in cases of triple mutations (MMRd - POLE mutation - p53abn) in EC.[36] León-Castillo et al recommend classifying patients with all three mutations based on the presence or absence of pathogenic POLE mutations. Specifically, if Whole Exome Sequencing (WES) data is available, individuals with pathogenic POLE mutations should be classified as POLE mutation type EC. Conversely, if WES data is not available but one of the 11 pathogenic POLE mutations is detected, patients should be classified as MMRd type EC. Those without pathogenic POLE mutations should be classified as MMRd type EC.[19,37]

## Latest Advances in the Treatment of Pathogenic POLE Mutations in Endometrial Cancer

The determination of adjuvant therapy in patients relies on adverse prognostic factors, including depth of myometrial invasion, grade, histological type, and the presence of LVSI. Nevertheless, there exists a dearth of high-quality evidence supporting the utilization of adjuvant radiation/chemotherapy. The TCGA molecular subtypes offer novel evidence to enhance comprehension of the biological characteristics of endometrial cancer.The European Society of Gynaecological Cancer (ESGO), European Society for Radiotherapy and Oncology (ESTRO), and European Society of Pathology (ESP)[41] have put forth a risk stratification model for endometrial cancer (EC) that incorporates both molecular and clinical characteristics. This model has the potential to provide more accurate recommendations for the treatment of EC. The findings from the PORTEC-4a trial will shed light on the efficacy of adjuvant therapy when considering molecular profiling alongside standard clinical-pathological factors. Additionally, this trial will offer insights into the potential reduction of adjuvant therapy for ECs with POLE mutations that fall into the intermediate risk category.[42]

Approximately half of the POLE-mutated endometrial cancers (ECs) are classified as high-risk and receive adjuvant therapy.[43] The favorable prognosis of these therapies may be attributed to their exceptional sensitivity. However, it is worth noting that POLE-mutated embryonic stem cells do not exhibit heightened sensitivity to radiation when compared to POLE wild-type stem cells. Furthermore, POLE-mutated EC cells demonstrate resistance to chemotherapy following treatment with chemotherapeutic drugs such as carboplatin and paclitaxel.[44] In their study, León-Castillo et al discovered that among a cohort of 264 high-grade endometrial cancer (EC) patients who did not undergo adjuvant therapy, those with POLE mutations (n=26) did not experience recurrence.[45] Furthermore, a systematic review conducted by McAlpine et al on the therapeutic efficacy of POLE-mutated EC revealed that patients with pathogenic POLE mutations did not derive any benefits from adjuvant therapy. Additionally, the review found that early-stage POLE-mutated EC patients who received adjuvant therapy had worse clinical outcomes compared to those who did not receive adjuvant therapy.[46] The findings of this study indicate that POLE-mutated EC possesses inherent traits that contribute to improved survival, regardless of its sensitivity to adjuvant therapy. Specifically, the increased mutational burden and extensive infiltration of T lymphocytes enhance the immunogenicity of POLE-mut EC.[46] Consequently, patients with this subtype of EC may derive therapeutic benefits from immune checkpoint inhibitors (ICIs). Nevertheless, it is important to note that the response to immunotherapy is intricate, with sustained advantages observed only in a limited subgroup of patients. Thus, the identification of appropriate predictive biomarkers to optimize patient selection for ICI therapy remains a significant challenge.[47] Benjamin Garmezy et al,[48] identified a significant association between pathogenic POLE mutations and favorable outcomes in patients undergoing ICI treatment. This finding suggests that pathogenic POLE mutations hold promise as potential biomarkers for ICI therapy. However, a separate study involving a cohort of 453 patients with POLE-mutant tumors revealed that not only patients with pathogenic POLE mutations, but also

a substantial proportion (38%) of those with POLE mutations of unknown significance, exhibited positive responses to ICI therapy.[48,49] Increased PD1 and PDL1 expression is specifically observed in MMRd-EC, while it is not significantly associated with POLE-mut EC. This suggests that the PD1 and PDL1 pathways are only partially involved in immune suppression in POLE-mut EC, with other pathways playing supplementary roles. Consequently, additional investigation is warranted to elucidate the immune microenvironment and specific immune suppression pathways in POLE-mut EC, which could potentially lead to novel therapeutic interventions for advanced-stage POLE-mutant tumors.[50–52]

## Detection and Alternative Testing Methods for Pathogenic POLE Mutations

In a thorough examination of 530 endometrial cancer (EC) cases using Whole-genome sequencing within the Cancer Genome Atlas (TCGA), the predominant occurrence of POLE mutations was observed within the exonuclease domain, specifically concentrated in exons 9 to 14. Nevertheless, as further investigation of cases with POLE mutations was conducted, additional mutation sites beyond the exonuclease domain were identified (Table 2). The characterization of these mutations continues to pose a significant challenge. The specific mutations that result in favorable outcomes remain uncertain, and the included POLE mutations vary across different studies.[8,35,39,45,53] In the analysis conducted by Alicia León-Castillo et al, which incorporated all POLE mutations and utilized the Kaplan Meier curve, no substantial enhancement in clinical outcomes was observed when comparing cases of EC with POLE mutations to those without.[12] To evaluate the pathogenicity of POLE mutations, the authors devised an innovative scoring system that takes into account established genomic alterations linked to pathogenic POLE mutations. Through this scoring system, they identified a total of 11 pathogenic POLE mutations, thereby enabling the assessment of the pathogenic nature of POLE mutations.[19]

The identification of POLE mutations is predominantly conducted through Sanger sequencing, which is characterized by its high cost and limited applicability in clinical settings. However, emerging techniques such as SNaPshot have successfully addressed the need for a more balanced approach in terms of sensitivity, cost, and efficiency for POLE mutation testing. SNaPshot specifically detects mutations within 15 nucleotides of POLE exons 9, 11, 13, and 14, exhibiting a sensitivity range of 90–95% for hotspot mutations.[53] In a meta-analysis comprising 1,169 patients with endometrial cancer (EC) from 10 studies, it was discovered that TIL-H can serve as an alternative marker for identifying POLE mutations in cases where sequencing is challenging, after excluding MMRd EC.[54] A histological and immunohistochemical examination of 51 POLE-mutated EC, 67 POLE-wild EC, and 15 POLE-wild serous EC with unknown molecular features revealed several distinguishing characteristics.

**Table 2** Different Detection Methods for Pathogenic POLE Genes in Various Studies

| Cohort | Incorporation of Mutation in POLE-mut EC | Sequencing Method | Ref. |
|---|---|---|---|
| TCGA 2013 | P286R,V411L,S297F,A456P,M444K,L424I | POLE Whole genome sequencing | [7] |
| Tomlinson et al 2013 | P286R,S297P,V411L,A456P,A275V | Codon 268–471 sequencing | [44] |
| Boosse et al 2015 | P286R,S297F,V411L,M299V,S297T | POLE exon 9 and 13 sequencing | [54] |
| McAlpine et al 2015 | P286R,S297P,V411L,A456P,M295R,F367S/C, P436R, L424P,P441L,F367L,E396G | POLE exon 9–14 sequencing | [43] |
| Ngeow et al 2016<br>Billingsley et al 2015 | P286R,V411L,A456P,S459F,A465F,M444K,S459P | Next-generation sequencing, Sanger sequencing confirmation | [55] |
| Goodfellow et al 2017 | P286R,S297P,V411L,A456P,P436R,A465F,A426V | Codon 268–471 sequencing | [20] |
| Inge C Van Gool et al 2017 | P286R,S297P,V411L,A456P | POLE exon 9,13,14 Sanger sequencing | [25] |
| Sara Imboden et al 2019 | P286R,S297P,V411L,A456P,S459F | POLE exon 9–14 sequencing | [56] |
| Alicia Leon-Castillo et al | P286R,S297P,V411L,A456P,S459F,F367S,L424I, M295R, P436R,M444K,D368Y | POLE Whole genome sequencing and 6 silicone tools | [35] |
| Tian,Wenjuan et al 2022 | P286R,V411L,R375Q,P452L | POLE Whole genome sequencing and Bioinformatics tools | [49] |

POLE-mutated EC demonstrated significantly higher levels of peritumoral lymphocytes and TIL ($p < 0.01$) compared to POLE-wild EC. Additionally, they exhibited a greater presence of giant cells. Furthermore, it was observed that the majority of EC with POLE mutations exhibited a wild-type p53 and focal positive/negative p16 status, along with normal expression of mismatch repair proteins. The combination of these histological and immunohistochemical characteristics, including tumor type, grade, peritumoral lymphocytes, MLH1 expression, and p53 expression, significantly enhanced the likelihood of identifying POLE mutations, increasing the detection rate from 7% to 33%.[21] A recent study has proposed that the combination of BMI and the expression levels of cyclin B1, caspase 8, and XBP1 could potentially serve as alternative markers for distinguishing between POLE-mut and CN-L EC. It is worth noting that immunohistochemistry (IHC) for cyclin B1 has the potential to replace POLE gene sequencing.[55] Despite the availability of various detection and alternative detection methods for pathogenic POLE mutations, the current clinical acceptance lies in the Alicia Leon-Castillo scoring system, which identifies 11 pathogenic mutations. In the future, there is a need to explore more accurate and cost-effective methods for detecting POLE mutations and alternative markers. In subsequent research, it is imperative to investigate more precise and economically viable approaches for identifying POLE mutations and alternative indicators. This is of utmost significance for the pragmatic implementation of molecular subtyping.

## Conclusion

The investigation of the clinical and pathological attributes of POLE-mutated endometrial cancer (EC) has revealed a notable frequency of mutations, distinctive mutational patterns, significant tumor-infiltrating lymphocytes (TIL), and a favorable prognosis. The primary factor contributing to this positive prognosis is believed to be the substantial presence of tumor antigens in POLE-mutated EC, which elicit a robust anti-tumor immune response. Furthermore, the observed overexpression of PD1/PDL1 indicates that POLE-mutated EC holds considerable potential as a candidate for PD1/PDL1 immunotherapy. However, the identification of efficacious biomarkers for immunotherapy and the incorporation of immunotherapy into other supplementary treatments continue to present significant challenges.

Although five hotspot mutations of the POLE gene have been recognized, the pathogenicity of additional mutations remains indeterminate. The criteria for including pathogenic POLE mutations differ across various studies, underscoring the necessity for further investigation and the utilization of bioinformatics tools to elucidate the pathogenic POLE mutations.

## Abbreviations

EC, Endometrial cancer; WHO, World Health Organization; TCGA, The Cancer Genome Atlas; MSI-H, microsatellite instability-high; CN-H, copy number high; CN-L, copy number low; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TMB, tumor mutation burden; TILs, tumor-infiltrating lymphocytes; CRC, colorectal cancer; LVSI, lymphovascular space invasion; MMRd, mismatch repair-deficient; p53abn, p53 abnormality; NSMP, non-specific molecular profile; SNV, single-nucleotide variant; SCNA, somatic copy-number alteration; WES, whole exome sequencing; PFS, progression-free survival; DFS, disease-free survival; OS, overall survival; MSS, microsatellite-stable; GOG, Gynecologic Oncology Group; ESGO, The European Society of Gynaecological Cancer; ESTRO, European Society for Radiotherapy and Oncology; ESP, European Society of Pathology; PORTEC-4a, Post-operative Radiation Therapy in Endometrial Cancer; ICI, immune checkpoint inhibitors; Indel, insertion/deletion; LAG3, lymphocyte-activation gene 3; Tim-3, mucin-domain-containing molecules 3; TIGIT, T cell immunoglobulin and ITIM domain; PD-1, Programmed Death-1; CTLA-4, Cytotoxic T lymphocyte-associated antigen-4; FOXP3, Fork head box P3.

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## References


1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med*. 2003;348(17):1625–1638. doi:10.1056/NEJMoa021423
3. Lu KH, Broaddus RR. Endometrial cancer. *N Engl J Med*. 2020;383(21):2053–2064.
4. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
5. Makker V, MacKay H, Ray-Coquard I, et al. Endometrial cancer. *Nat Rev Dis Primers*. 2021;7(1):88. doi:10.1038/s41572-021-00324-8
6. Crosbie EJ, Kitson SJ, McAlpine JN, et al. Endometrial cancer. *Lancet*. 2022;399(10333):1412–1428.
7. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol*. 1983;15(1):10–17. doi:10.1016/0090-8258(83)90111-7
8. Kandoth C. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67–73. doi:10.1038/nature12113
9. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet*. 2016;387(10023):1094–1108.
10. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. *Lancet Oncol*. 2014;15(7):e268–78. doi:10.1016/S1470-2045(13)70591-6
11. Talhouk A, McConechy MK, Leung S, et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. *Cancer*. 2017;123(5):802–813. doi:10.1002/cncr.30496
12. Rayner E, van Gool IC, Palles C, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat Rev Cancer*. 2016;16(2):71–81. doi:10.1038/nrc.2015.12
13. 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):1740–1750. doi:10.1101/gr.174789.114
14. Temko D, Van Gool IC, Rayner E, et al. Somatic POLE exonuclease domain mutations are early events in sporadic endometrial and colorectal carcinogenesis, determining driver mutational landscape, clonal neoantigen burden and immune response. *J Pathol*. 2018;245(3):283–296. doi:10.1002/path.5081
15. Forgó E, Gomez AJ, Steiner D, et al. Morphological, immunophenotypical and molecular features of hypermutation in colorectal carcinomas with mutations in DNA polymerase ε (POLE). *Histopathology*. 2020;76(3):366–374. doi:10.1111/his.13984
16. Jiang M, Jia Y, Han J, et al. Distinct clinical pattern of colorectal cancer patients with POLE mutations: a retrospective study on real-world data. *Front Genet*. 2022;13:963964. doi:10.3389/fgene.2022.963964
17. Tian W, Ji Z, Wang J, et al. Characterization of hotspot exonuclease domain mutations in the DNA polymerase ϵ gene in endometrial cancer. *Front Oncol*. 2022;12:1018034. doi:10.3389/fonc.2022.1018034
18. Jumaah AS, Salim MM, Al-Haddad HS, et al. The frequency of POLE-mutation in endometrial carcinoma and prognostic implications: a systemic review and meta-analysis. *J Pathol Transl Med*. 2020;54(6):471–479. doi:10.4132/jptm.2020.07.23
19. León-Castillo A, Britton H, McConechy MK, et al. Interpretation of somatic POLE mutations in endometrial carcinoma. *J Pathol*. 2020;250(3):323–335. doi:10.1002/path.5372
20. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. *Cell*. 2017;171(5):1042–1056.e10. doi:10.1016/j.cell.2017.09.048
21. Van Gool IC, Ubachs JEH, Stelloo E, et al. Blinded histopathological characterisation ofPOLEexonuclease domain - mutant endometrial cancers: sheep in wolf's clothing. *Histopathology*. 2018;72(2):248–258. doi:10.1111/his.13338
22. Hussein YR, Weigelt B, Levine DA, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. *Mod Pathol*. 2015;28(4):505–514. doi:10.1038/modpathol.2014.143
23. Bakhsh S, Kinloch M, Hoang LN, et al. Histopathological features of endometrial carcinomas associated withPOLEmutations: implications for decisions about adjuvant therapy. *Histopathology*. 2016;68(6):916–924. doi:10.1111/his.12878
24. Wu Q, Zhang N, Xie X. The clinicopathological characteristics of POLE-mutated/ultramutated endometrial carcinoma and prognostic value of POLE status: a meta-analysis based on 49 articles incorporating 12,120 patients. *BMC Cancer*. 2022;22(1):1157. doi:10.1186/s12885-022-10267-2
25. He Y, Wang T, Li N, et al. Clinicopathological characteristics and prognostic value of POLE mutations in endometrial cancer: a systematic review and meta-analysis. *Medicine*. 2020;99(8):e19281. doi:10.1097/MD.0000000000019281
26. Dong D, Lei H, Liu D, et al. POLE and mismatch repair status, checkpoint proteins and tumor-infiltrating lymphocytes in combination, and tumor differentiation: identify endometrial cancers for immunotherapy. *Front Oncol*. 2021;11:640018. doi:10.3389/fonc.2021.640018
27. Wang T, Yu D, Wang J, et al. Immune signatures of the POLE mutation in endometrial carcinomas: a systematic study based on TCGA data and clinical cohort validation. *Front Oncol*. 2023;13:1250558. doi:10.3389/fonc.2023.1250558
28. Cao W, Ma X, Fischer JV, et al. Immunotherapy in endometrial cancer: rationale, practice and perspectives. *Biomark Res*. 2021;9(1):49. doi:10.1186/s40364-021-00301-z
29. Ma X, Dong L, Liu X, et al. POLE/POLD1 mutation and tumor immunotherapy. *J Exp Clin Cancer Res*. 2022;41(1):216. doi:10.1186/s13046-022-02422-1
30. Howitt BE, Shukla SA, Sholl LM, et al. Association of polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. *JAMA Oncol*. 2015;1(9):1319–1323. doi:10.1001/jamaoncol.2015.2151
31. Mehnert JM, Panda A, Zhong H, et al. Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer. *J Clin Invest*. 2016;126(6):2334–2340. doi:10.1172/JCI84940
32. López-Janeiro Á, Villalba-Esparza M, Brizzi ME, et al. The association between the tumor immune microenvironments and clinical outcome in low-grade, early-stage endometrial cancer patients. *J Pathol*. 2022;258(4):426–436. doi:10.1002/path.6012
33. Bagaev A, Kotlov N, Nomie K, et al. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. *Cancer Cell*. 2021;39(6):845–865.e7.
34. Li BL, Wan XP. Prognostic significance of immune landscape in tumour microenvironment of endometrial cancer. *J Cell Mol Med*. 2020;24(14):7767–7777. doi:10.1111/jcmm.15408

35. Cai Y, Chang Y, Liu Y. Multi-omics profiling reveals distinct microenvironment characterization of endometrial cancer. *Biomed Pharmacother*. 2019;118:109244. doi:10.1016/j.biopha.2019.109244
36. Bogani G, Betella I, Multinu F, et al. Characteristics and outcomes of surgically staged multiple classifier endometrial cancer. *Eur J Surg Oncol*. 2024;50(1):107269. doi:10.1016/j.ejso.2023.107269
37. León-Castillo A, Gilvazquez E, Nout R, et al. Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas. *J Pathol*. 2020;250(3):312–322. doi:10.1002/path.5373
38. Devereaux KA, Weiel JJ, Pors J, et al. Prospective molecular classification of endometrial carcinomas: institutional implementation, practice, and clinical experience. *Mod Pathol*. 2022;35(5):688–696.
39. Fang H, Barbour JA, Poulos RC, et al. Mutational processes of distinct POLE exonuclease domain mutants drive an enrichment of a specific TP53 mutation in colorectal cancer. *PLoS Genet*. 2020;16(2):e1008572. doi:10.1371/journal.pgen.1008572
40. Hwang HS, Kim D, Choi J. Distinct mutational profile and immune microenvironment in microsatellite-unstable and POLE -mutated tumors. *J Immunother Cancer*. 2021;9(10):e002797. doi:10.1136/jitc-2021-002797
41. Concin N, Matias-Guiu X, Vergote I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer*. 2021;31(1):12–39.
42. van den Heerik A, Horeweg N, Nout RA, et al. 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):2002–2007. doi:10.1136/ijgc-2020-001929
43. Paulino E, de Melo AC. Adjuvant treatment of endometrial cancer in molecular era: are we ready to move on? *Crit Rev Oncol Hematol*. 2020;153:103016. doi:10.1016/j.critrevonc.2020.103016
44. Bellone S, Bignotti E, Lonardi S, et al. 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):146–152. doi:10.1016/j.ygyno.2016.11.023
45. Leon-Castillo A, Horeweg N, Peters EEM, et al. Prognostic relevance of the molecular classification in high-grade endometrial cancer for patients staged by lymphadenectomy and without adjuvant treatment. *Gynecol Oncol*. 2022;164(3):577–586. doi:10.1016/j.ygyno.2022.01.007
46. 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):2409–2422. doi:10.1002/cncr.33516
47. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350–1355. doi:10.1126/science.aar4060
48. Garmezy B, Gheeya J, Lin HY, et al. Clinical and molecular characterization of POLE mutations as predictive biomarkers of response to immune checkpoint inhibitors in advanced cancers. *JCO Precis Oncol*. 2022;6(6):e2100267. doi:10.1200/PO.21.00267
49. Wang F, Zhao Q, Wang Y-N, et al. Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. *JAMA Oncol*. 2019;5(10):1504–1506. doi:10.1001/jamaoncol.2019.2963
50. Zhang C, Wang M, Wu Y. Features of the immunosuppressive tumor microenvironment in endometrial cancer based on molecular subtype. *Front Oncol*. 2023;13:1278863. doi:10.3389/fonc.2023.1278863
51. van der Woude H, Hally KE, Currie MJ, et al. Importance of the endometrial immune environment in endometrial cancer and associated therapies. *Front Oncol*. 2022;12:975201. doi:10.3389/fonc.2022.975201
52. Mamat @ Yusof MN, Chew KT, Kampan NC, et al. Expression of PD-1 and PD-L1 in endometrial cancer: molecular and clinical significance. *Int J Mol Sci*. 2023;24(20):15233. doi:10.3390/ijms242015233
53. Devereaux KA, Steiner DF, Ho C, et al. A multiplex SNaPshot assay is a rapid and cost-effective method for detecting POLE exonuclease domain mutations in endometrial carcinoma. *Int J Gynecol Pathol*. 2022;41(6):541–551. doi:10.1097/PGP.0000000000000841
54. Raffone A, Travaglino A, Raimondo D, et al. Tumor-infiltrating lymphocytes and POLE mutation in endometrial carcinoma. *Gynecol Oncol*. 2021;161(2):621–628.
55. Kim K, Hwangbo S, Kim H, et al. Clinicopathologic and protein markers distinguishing the "polymerase epsilon exonuclease" from the "copy number low" subtype of endometrial cancer. *J Gynecol Oncol*. 2022;33(3):e27. doi:10.3802/jgo.2022.33.e27
56. Imboden S, Nastic D, Ghaderi M, et al. Phenotype of POLE-mutated endometrial cancer. *PloS One*. 2019;14(3):e0214318. doi:10.1371/journal.pone.0214318