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Article

Clinicopathological Features and Risk Stratification of Multiple-Classifer Endometrial Cancers: A Multicenter Study from Poland

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Simple Summary

Endometrial cancers with more than one molecular alteration—so-called multiple-classifier tumors, such as MMRd with p53 abnormalities or POLEmut with p53abn—are identified in 3–11% of patients. According to current classification, these tumors are assigned to a single dominant molecular group, but emerging evidence suggests that co-existing p53 abnormalities may influence their clinical behavior. In this multicenter study involving over 1000 patients, we found that multiple-classifier tumors—especially MMRd-p53abn and POLEmut-p53abn—were more frequently classified as high-intermediate or high-risk according to ESGO/ESTRO/ESP criteria. Moreover, lymph node metastases were more common in POLEmut-p53abn and POLEmut-MMRd-p53abn cases. These findings highlight the need for careful interpretation of tumors with overlapping molecular features and support further research to refine risk stratification and optimize treatment strategies in this unique subgroup of endometrial cancer patients.

Abstract

Rationale: The ProMisE molecular classification improves risk assessment in endometrial cancer (EC), but 3–11% of cases exhibit overlapping molecular features, complicating clinical decisions. We analyzed the prevalence and clinicopathological profiles of multiple-classifier ECs in a large Polish cohort. **Methods:** In this retrospective study (2022–2025), 1075 ECs from four institutions were classified by MMR and p53 immunohistochemistry and POLE exon sequencing. Tumors showing ≥ 2 molecular features (e.g., MMRd-p53abn, POLEmut-p53abn) were categorized as multiple-classifier ECs. **Results:** Multiple-classifier ECs comprised 6.9% (74/1075), with MMRd-p53abn (3.9%) being most common. These tumors exhibited more aggressive features vs MMRd-only: G3 (28.57% vs 11.79%, $p=0.002$), non-endometrioid histology (11.9% vs 2.85%, $p=0.018$), and higher-risk stratification (59.52% vs 37.80%, $p=0.001$). POLEmut-p53abn (N=4) and POLEmut-MMRd-p53abn (N=10) tumors showed advanced stages (75% and 40% FIGO III–IV, respectively), in contrast to classical POLEmut tumors (6.7% FIGO III–IV), and higher rates of nodal metastases. **Conclusion:** Co-occurrence of molecular classifiers, including triple-classifier tumors, correlates with more adverse profiles and may undermine current stratification paradigms. This study emphasizes the need to further investigate and refine molecular risk models to account for overlapping profiles.

Keywords: endometrial carcinoma; molecular classification; multiple-classifier; POLEmut; MMRd; p53abn; triple classifier; ProMisE; ESGO

1. Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in developed countries, with over 417,000 new cases and nearly 100,000 deaths reported worldwide in 2020 [1]. Its incidence continues to rise, driven by aging populations and the increasing prevalence of obesity and metabolic disorders [2,3]. While early-stage EC has a favorable prognosis, advanced disease remains a major therapeutic challenge, with a five-year survival rate below 20% [4].

The molecular classification proposed by The Cancer Genome Atlas (TCGA) divides EC into four prognostically relevant subtypes: POLE ultramutated (POLEmut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and no specific molecular profile (NSMP) [5]. These categories, translated into clinical practice through the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), now incorporated into international guidelines, including those from the European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology (ESGO/ESTRO/ESP, 2021), the European Society for Medical Oncology (ESMO), and the International Federation of Gynecology and Obstetrics (FIGO, 2023) [6,7].

A growing subset of ECs demonstrates features of more than one molecular subtype. These so-called **multiple-classifier tumors** (e.g., MMRd–p53abn, POLEmut–p53abn) raise questions about risk classification and prognosis, particularly when conflicting prognostic signals coexist [8,9]. The MMRd–p53abn subgroup, in particular, remains under-investigated and lacks clear recommendations regarding risk assignment and clinical behavior.

Moreover, molecular EC studies are still limited in Central and Eastern Europe, despite growing efforts to standardize molecular diagnostics. To address this gap, we conducted a multicenter study across four Polish oncology centers to evaluate the prevalence and clinicopathological features of multiple-classifier ECs, with a particular focus on MMRd–p53abn tumors. The findings aim to support future refinements in risk stratification and clinical decision-making.

2. Materials and Methods

2.1. Study Design and Population

This multicenter retrospective study included 1075 patients with newly diagnosed endometrial cancer (EC) who underwent surgical treatment between April 2022 and March 2025 in four Polish gynecologic oncology centers (Silesian, Lesser Poland, Subcarpathian, and Lublin Voivodeships). Histotype and tumor grade were assigned according to the 2020 WHO classification [10]. Lymphovascular space invasion (LVSI) was classified as absent (no vessels), focal (1–4 vessels), or substantial (≥ 5 vessels). Histopathological assessment was conducted independently at each center according to WHO criteria, without central pathology review.

Lymph node status was evaluated in 794 of 1075 patients (73.9%). Among these, 552 (69.5%) underwent sentinel lymph node mapping and 242 (30.5%) underwent systematic pelvic lymphadenectomy. In the remaining 281 patients (26.1%), lymph node evaluation was omitted based on low-risk criteria or clinical judgment.

High-intermediate risk (HIR) and high-risk (HR) groups were defined per ESGO/ESTRO/ESP 2021 guidelines, based exclusively on clinicopathological parameters (e.g., FIGO stage, histotype, grade, LVSI, lymph node involvement), independent of molecular classification to avoid bias from multiple-classifier overlap [7].

2.2. Molecular Classification and Immunohistochemistry (IHC)

Tumor tissue was subjected to immunohistochemical evaluation of mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, PMS2) and p53 using the Ventana BenchMark Ultra platform (Roche Diagnostics, Indianapolis, IN, USA). Standardized staining protocols were applied across institutions. MMR deficiency (MMRd) was defined as the loss of at least one MMR protein. Abnormal p53 expression (p53abn) included diffuse overexpression (>80% of tumor cells), complete absence, or subclonal staining, according to ProMisE criteria [11].

2.3. DNA Extraction and POLE Mutation Analysis

DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue using either the QIAamp DSP DNA FFPE Tissue Kit (Qiagen) or Maxwell RSC DNA FFPE Kit (Promega). POLE exon 9, 11, 13, and 14 sequencing was performed using Sanger sequencing (BigDye Terminator v3.1, Applied Biosystems). In selected cases with discordant or inconclusive findings, next-generation sequencing (NGS) was conducted on the IonTorrent platform using AmpliSeq panels covering POLE, TP53, and MMR genes. Only pathogenic or likely pathogenic variants (per ClinVar, OncoKB, Varsome) were included in molecular classification.[12–14] The same molecular testing protocols and variant interpretation standards were previously applied in a national analysis of EC classification practices in Poland [15].

2.4. Statistical Analysis

Statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using Fisher's exact test (for expected cell counts <5) or chi-square test (for ≥5). Continuous variables were assessed using Student's t-test. Logistic regression was used to estimate odds ratios (OR) with 95% confidence intervals (CI) for binary outcomes. Statistical significance was set at $p < 0.05$.

2.5. Ethical Considerations

The study was approved by the Institutional Review Board of the Maria Skłodowska-Curie National Research Institute of Oncology, Krakow Branch (Approval No. 6/2025), and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants. Data collection complied with EU General Data Protection Regulation (GDPR) standards.

3. Results

3.1. Prevalence of Multiple-Classier Tumors

Among 1075 patients, multiple-classifier endometrial cancers (ECs) accounted for 6.9% (74/1075), including MMRd-p53abn (3.9%, 42/1075), POLEmut-p53abn (0.4%, 4/1075), POLEmut-MMRd (1.7%, 18/1075), and POLEmut-MMRd-p53abn (0.9%, 10/1075).

3.2. MMRd, p53abn, and MMRd-p53abn Comparison

Table 1 presents the clinicopathological characteristics of patients with endometrial cancer by molecular subtype. Compared to classical MMRd tumors (N = 246), MMRd-p53abn tumors (N = 42) had higher rates of non-endometrioid histology (11.90% vs 2.85%, $p = 0.018$; OR = 3.78, 95% CI: 1.19–12.02), more frequent high-grade (G3) tumors (28.57% vs 11.79%, $p = 0.002$; OR = 3.26, 95% CI: 1.55–6.86), and a greater proportion assigned to high-intermediate/high-risk (HIR/HR) groups (59.52% vs 37.80%, $p = 0.001$; OR = 2.81, 95% CI: 1.50–5.25). These comparisons are visually represented in Figure 1 (Histotype comparison), Figure 2 (Grade comparison), and Figure 3 (Risk group comparison).

Compared to p53abn tumors (N = 156), MMRd-p53abn tumors had a lower proportion of non-endometrioid histology (11.90% vs 35.26%, $p = 0.001$). MMRd-p53abn tumors also trended toward

more frequent advanced FIGO III–IV stages (23.81% vs 13.82%, $p = 0.192$), although this did not reach statistical significance.

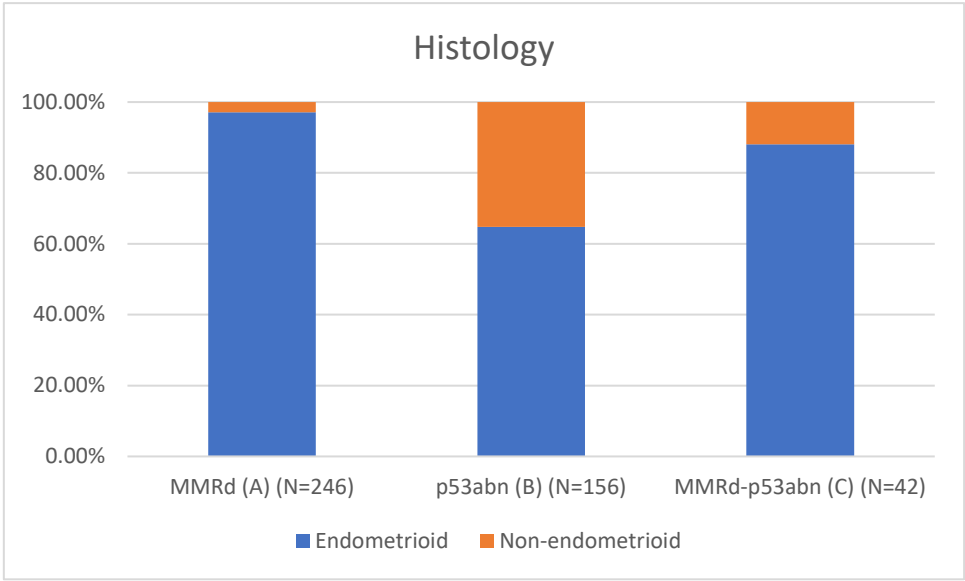


Figure 1. Histotype comparison.

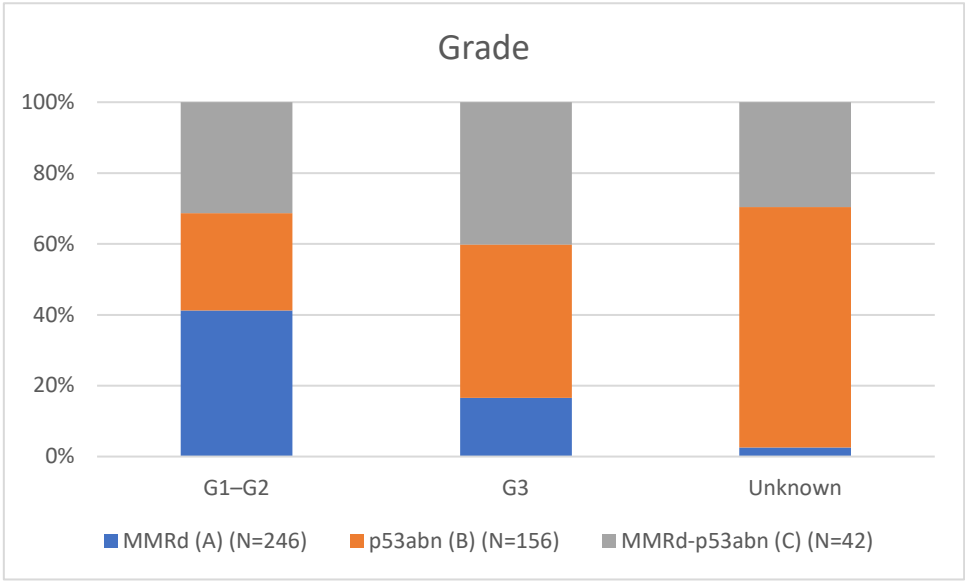


Figure 2. Grade comparison.

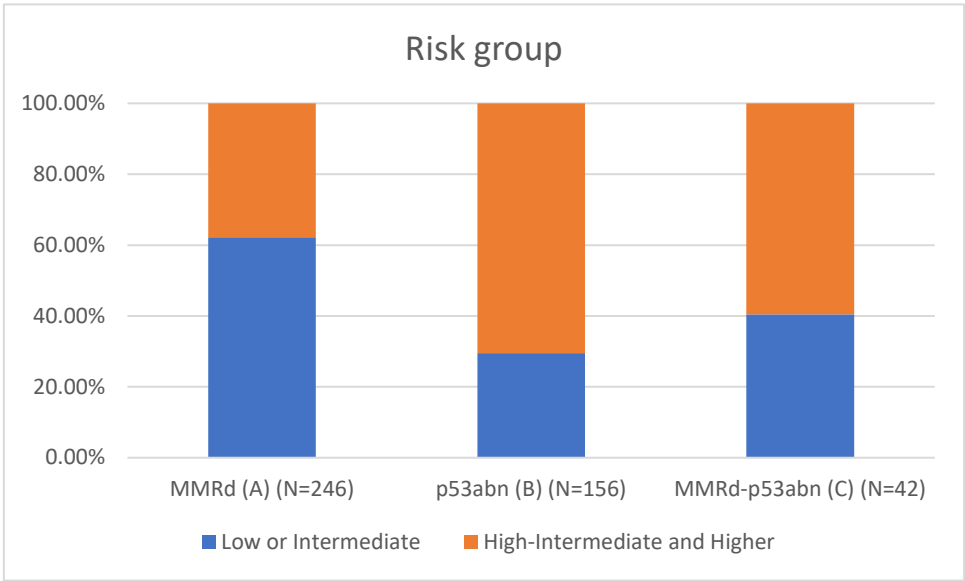


Figure 3. Risk group comparison.

Table 1. Clinicopathological Characteristics of Patients with Endometrial Cancer by Molecular Subtype.

Variable	Group	MMRd (A) (N=246)	p53abn (B) (N=156)	MMRd- p53abn (C) (N=42)	p-value (A vs C)	OR (95% CI, A vs C)	p-value (B vs C)	MMRd + MMRd- p53abn (N=288)
Age at surgery (years)	Mean (SD)	66.05 (9.62)	68.19 (9.90)	67.81 (11.85)	0.291	-	0.718	66.32 (9.99%)
	< 60 years	72 (29.27%)	24 (15.38%)	11 (26.19%)	0.300	-	0.098	83 (28.82%)
	60–70 years	80 (32.52%)	51 (32.69%)	9 (21.43%)				89 (30.90%)
	> 70 years	93 (37.80%)	77 (49.36%)	22 (52.38%)				115 (39.93%)
	Unknown	1 (0.41%)	0 (0.00%)	0 (0.00%)				1 (0.35%)
Histology	Endometrio id	239 (97.15%)	101 (64.74%)	37 (88.10%)	0.018	3.78 (1.19– 12.02)	0.002	276 (95.83%)
	Non- endometrio id	7 (2.85%)	55 (35.26%)	5 (11.90%)				12 (4.17%)
Grade	G1–G2	216 (87.80%)	91 (58.33%)	28 (66.67%)	0.002	3.26 (1.55– 6.86)	0.170	244 (84.72%)
	G3	29 (11.79%)	48 (30.77%)	12 (28.57%)				41 (14.24%)
	Unknown	1 (0.41%)	17 (10.90%)	2 (4.76%)				3 (1.04%)
LVSI	Absent or focal	181 (73.58%)	102 (65.38%)	25 (59.52%)	0.054	1.88 (0.96– 3.66)	0.818	206 (71.53%)
	Substantial	64 (26.02%)	51 (32.69%)	17 (40.48%)				81 (28.13%)
	Unknown	1 (0.41%)	2 (1.28%)	0 (0.00%)				1 (0.35%)
Myometrial invasion	< ½	140 (56.91%)	81 (51.92%)	18 (42.86%)	0.091	1.81 (0.94– 3.49)	0.277	158 (54.86%)
	≥ ½	105 (42.68%)	75 (48.08%)	23 (54.76%)				128 (44.44%)

Cervical involvement	Unknown	1 (0.41%)	0 (0.00%)	1 (2.38%)	0.359	1.35 (0.67–2.71)	1.000	2 (0.69%)
	No	177 (71.95%)	101 (64.74%)	27 (64.29%)				204 (70.83%)
	Yes	68 (27.64%)	55 (35.26%)	14 (33.33%)				82 (28.47%)
Lymph node metastases	Unknown	1 (0.41%)	0 (0.00%)	1 (2.38%)	0.067	3.02 (1.08–8.45)	0.721	2 (0.69%)
	No	208 (84.55%)	124 (79.49%)	31 (73.81%)				239 (82.99%)
	Yes	13 (5.28%)	16 (10.26%)	6 (14.29%)				19 (6.60%)
Distant metastases	Unknown	25 (10.16%)	16 (10.26%)	5 (11.90%)	1.000	-	0.208	30 (10.42%)
	No	236 (95.93%)	145 (92.95%)	41 (97.62%)				277 (96.18%)
	Yes	3 (1.22%)	8 (5.13%)	0 (0.00%)				3 (1.04%)
FIGO stage	Unknown	7 (2.85%)	3 (1.92%)	1 (2.38%)	0.192	2.13 (0.96–4.72)	0.854	8 (2.78%)
	Early (I–II)	205 (83.33%)	111 (71.15%)	31 (73.81%)				236 (81.94%)
	Advanced (III–IV)	34 (13.82%)	42 (26.92%)	10 (23.81%)				44 (15.28%)
Risk group	Unknown	7 (2.85%)	3 (1.92%)	1 (2.38%)	0.001	2.81 (1.50–5.25)	0.319	8 (2.78%)
	Low or Intermediate	153 (62.20%)	46 (29.49%)	17 (40.48%)				170 (59.03%)
	High-Intermediate and Higher	93 (37.80%)	110 (70.51%)	25 (59.52%)				118 (40.97%)

Notes: Abbreviations: MMRd = mismatch repair deficient; p53abn = p53 abnormal; LVSI = lymphovascular space invasion; FIGO = International Federation of Gynecology and Obstetrics; SD = standard deviation; OR = odds ratio; CI = confidence interval. Statistical significance: *p < 0.05. Columns: MMRd (A): Classical MMRd tumors; p53abn (B): Classical p53abn tumors; MMRd-p53abn (C): Multiple-classifier MMRd-p53abn tumors; MMRd + MMRd-p53abn: Combined MMRd and MMRd-p53abn cases. p-values: Calculated for comparisons between MMRd vs MMRd-p53abn and p53abn vs MMRd-p53abn using Fisher’s exact test for small cell counts or chi-square test for larger counts. OR (95% CI): Calculated for comparisons with p<0.05 (MMRd vs MMRd-p53abn) using logistic regression for binary outcomes. Risk group assigned according to ESGO/ESTRO/ESP 2020 guidelines-molecular classification unknown. LVSI definitions: absent (0 vessels), focal (1–4 vessels), substantial (≥5 vessels).

3.3. POLEmut and Multiple-Classifier POLEmut Comparison

Table 2 presents the clinicopathological comparison of classical POLEmut tumors with multiple-classifier subgroups involving POLE mutations. Compared to classical POLEmut tumors (N=30), the POLEmut-p53abn subgroup (N=4) demonstrated significantly higher rates of G3 tumors (75.0% vs 6.7%, p=0.005; OR = 42.00, 95% CI: 2.87–614.8), lymph node metastases (50.0% vs 3.3%, p=0.013; OR = 29.00, 95% CI: 1.77–475.3), and FIGO stage III–IV disease (75.0% vs 6.7%, p=0.005; OR = 42.00, 95% CI: 2.87–614.8). However, interpretation is limited by the small sample size and absence of outcome data.

The POLEmut-MMRd subgroup (N=18) also showed higher, though not statistically significant, rates of G3 tumors (16.7% vs 6.7%, p=0.198; OR = 2.80, 95% CI: 0.47–16.62), lymph node metastases (5.6% vs 3.3%, p=1.000; OR = 1.71, 95% CI: 0.11–27.48), and advanced FIGO stages (11.1% vs 6.7%, p=1.000; OR = 1.75, 95% CI: 0.23–13.26) relative to classical POLEmut.

Lastly, POLEmut–MMRd–p53abn tumors (N=10) exhibited more adverse features, including higher rates of G3 tumors (20.0% vs 6.7%, $p=0.247$; OR = 3.50, 95% CI: 0.46–26.46), lymph node involvement (30.0% vs 3.3%, $p=0.192$; OR = 12.44, 95% CI: 1.05–147.88), and FIGO III–IV stage (40.0% vs 6.7%, $p=0.033$; OR = 9.33, 95% CI: 1.23–70.66).

Although these trends suggest a deviation from the favorable prognosis associated with classical POLEmut tumors, the small numbers and lack of survival data preclude definitive conclusions.

Table 2. Comparison of POLEmut and Multiple-Classifier POLEmut Tumors.

Variable	Group	POLEmut (N=30)	POLEmut- MMRd (N=18)	POLEmut- p53abn (N=4)	POLEmut- MMRd- p53abn (N=10)	p-value (POLEmut vs POLEmut- MMRd)	p-value (POLEmut vs POLEmut- p53abn)	OR (95% CI, POLEmut vs POLEmut- p53abn)	p-value (POLEmut vs POLEmut- MMRd- p53abn)	Total (N=62)
Grade	G3	2 (6.67%)	3 (16.67%)	3 (75.00%)	2 (20.00%)	0.198	0.005*	42.00 (2.87– 614.8)	0.247	10 (16.13%)
Lymph node metastases	Yes	1 (3.33%)	1 (5.56%)	2 (50.00%)	3 (30.00%)	1.000	0.013	29.00 (1.77– 475.3)	0.192	7 (11.29%)
FIGO stage	Advanced (III–IV)	2 (6.67%)	2 (11.11%)	3 (75.00%)	4 (40.00%)	1.000	0.005*	42.00 (2.87– 614.8)	0.033*	11 (17.74%)

Notes: Abbreviations: POLEmut = POLE ultramutated; MMRd = mismatch repair deficient; p53abn = p53 abnormal; FIGO = International Federation of Gynecology and Obstetrics; OR = odds ratio; CI = confidence interval. Statistical significance: * $p < 0.05$. Data presentation: Values are presented as n (%) unless otherwise specified. Statistical tests: Fisher’s exact test for categorical variables (small cell counts). Unknown categories excluded from statistical tests. p-values: Calculated for comparisons between POLEmut and each multiple-classifier subgroup. OR and 95% CI: Calculated for POLEmut vs POLEmut-p53abn. Risk group assigned according to ESGO/ESTRO/ESP 2021 guidelines, incorporating molecular classification per ProMisE.

4. Discussion

This is the first multicenter study from Central-Eastern Europe analyzing multiple-classifier endometrial cancers (ECs), with a 6.9% prevalence, consistent with the 3–11% range reported globally [8,9,16]. Our higher rate compared to De Vitis et al. (4.8%) [9] may reflect the large cohort size (N=1075), regional genetic variations, or differences in molecular testing protocols, underscoring the need for standardized diagnostics.

In contrast to León-Castillo et al. [8], who suggested that MMRd-p53abn ECs behave similarly to MMRd-only tumors (recurrence-free survival: 92.2% vs. 70.8% for p53abn; $p = 0.024$), our MMRd-p53abn cases (3.9%, N=42) exhibited more aggressive features. These included higher rates of:

- Non-endometrioid histology: 11.9% vs. 2.85% in MMRd (OR = 3.78; $p = 0.018$),
- Grade 3 tumors: 28.6% vs. 11.8% (OR = 3.26; $p = 0.002$),
- High-intermediate/high-risk status per ESGO/ESTRO/ESP: 59.5% vs. 37.8% (OR = 2.81; $p = 0.001$).

These findings suggest that MMRd-p53abn tumors may be biologically closer to p53abn than to MMRd, challenging current ESGO/ESTRO/ESP guidelines [7] that classify them as MMRd.

Compared to p53abn tumors, MMRd-p53abn ECs showed fewer non-endometrioid types (11.9% vs. 35.3%; $p = 0.001$), suggesting a distinct molecular profile. Bogani et al. [17] similarly reported an increased recurrence risk in MMRd-p53abn tumors, further supporting the need for reevaluation of this subgroup.

Similarly, both POLEmut-p53abn (N=4) and POLEmut-MMRd-p53abn (N=10) tumors demonstrated aggressive features, particularly regarding advanced stage:

- FIGO III–IV in 75% (POLEmut-p53abn) and 40% (POLEmut-MMRd-p53abn), compared to 6.7% in classical POLEmut ECs [18],
- Grade 3 tumors in 75% vs. 6.7% (OR = 42.00; $p = 0.005$),
- Lymph node metastases in 50% vs. 3.3% (OR = 29.00; $p = 0.013$).

These data raise concern that coexisting p53 abnormalities may negate the typically favorable prognosis of POLEmut tumors. This is consistent with Jamieson et al. [19], who reported 14% nodal involvement in POLEmut ECs, aligning with our findings.

The aggressive features observed in MMRd-p53abn tumors also have potential implications for immunotherapy. Owing to their MMRd status, these tumors may respond to immune checkpoint inhibitors such as dostarlimab, as demonstrated by Mirza et al. in the GARNET trial [20]. However, the coexistent p53abn component may compromise efficacy, emphasizing the need for prospective trials tailored to multiple-classifier ECs.

Importantly, additional evidence supports the notion that p53 abnormalities may exert a dominant negative prognostic influence even within MMRd tumors. Kato et al. [18] reported that in non-Lynch MMRd tumors, the presence of p53abn was associated with significantly worse 5-year OS (53.6% vs. 93.9%; $p = 0.0016$). Michalova et al. [21] similarly observed that among five MMRd/TP53mut patients with follow-up, three developed metastases, and one patient died, supporting the notion of biological aggressiveness. De Vitis et al. [9] also noted a trend toward higher recurrence in MMRd-p53abn tumors, although the finding did not reach statistical significance.

Taken together, these observations underscore that multiple-classifier ECs, especially those involving p53 abnormalities, may not fit neatly into existing risk stratification schemes. Future studies with larger patient cohorts and long-term survival data are essential to refine classification and optimize individualized treatment strategies.

5. Limitations

This study has several limitations. First, the absence of overall survival (OS) and progression-free survival (PFS) data precludes direct prognostic evaluation of multiple-classifier endometrial cancers (ECs). Second, the relatively small size of some molecular subgroups—particularly POLEmut-p53abn and POLEmut-MMRd-p53abn—limits statistical power and the generalizability of their clinicopathological features. Third, the lack of central pathology review may have introduced interobserver variability in histotype, grade, and LVSI assessment, although standardized WHO 2020 criteria were applied at all sites. Fourth, detailed characterization of MMR protein loss (e.g., isolated vs. paired MLH1/PMS2 or MSH2/MSH6 loss) was not performed, which may limit interpretation of MMRd subtypes. Molecular testing was performed using harmonized protocols, but variation in the use of NGS (only in selected cases) may have impacted the detection of rare multiple-classifier combinations.

6. Conclusions

Multiple-classifier ECs, particularly MMRd-p53abn, POLEmut-p53abn, and POLEmut-MMRd-p53abn, appear to exhibit distinct clinicopathological features compared to single-classifier tumors. The presence of p53 abnormalities—even in tumors harboring POLEmut or MMRd—may be associated with more aggressive phenotypes, including high-grade histology, advanced FIGO stage, and lymph node metastases. These observations underscore the complexity of interpreting co-existing molecular alterations and suggest that multiple-classifier tumors may not be adequately captured by current risk stratification schemes. Although our study does not allow for prognostic conclusions, the observed patterns support further investigation into whether these tumors warrant distinct consideration in future classification and treatment frameworks.

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J, P.B.; supervision,P.B.; project administration, W.Sz, M.N-J.; All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: At the start of the treatment, written consent was obtained from all 1075 subjects involved in the study for the purpose of retrospective analysis of their medical data.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author(s).

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Abbreviations

The following abbreviations are used in this manuscript:

EC	Endometrial cancer
POLEmut	POLE ultramutated
MMRd	Mismatch repair deficient
p53abn	p53 abnormal
NSMP	No specific molecular profile
HIR/HR	High-intermediate/high-risk
G3	High-grade
TCGA	To Cancer Genome Atlas
LVSI	Lymphovascular space invasion
IHC	Immunohistochemistry for
NGS	Next-generation sequencing
VUS	Variants of unknown significance
OS	Overall survival rate
PFS	Progression-free survival
ESGO/ES	European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/Europe Society of Pathology
TRO/ESP	
FIGO	International Federation of Gynecology and Obstetrics
ProMISE	Proactive Molecular Risk Classifier for Endometrial Cancer

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