

ORIGINAL RESEARCH

Clinicopathological significance of immunohistochemical expression of p53 and PTEN in endometrial carcinoma

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(Chavdar Tsvetkov)**Abstract**

Background: Endometrial carcinoma (EC) is prognostically heterogeneous; p53 immunohistochemistry (IHC) is an established marker for risk stratification, while phosphatase and tensin homolog (PTEN) has also been investigated as a complementary biomarker alongside p53. This study evaluated the relationship between p53 and PTEN IHC expression and clinicopathologic characteristics in EC. **Methods:** Seventy-eight patients with EC who underwent primary surgical staging between 2019 and 2020 were retrospectively analysed. Clinicopathological variables, IHC expression of p53 and PTEN, and survival outcomes were assessed. Overall survival (OS) and disease-specific survival (DSS) were analysed using Kaplan-Meier estimates and Cox proportional hazards models. **Results:** Of the 78 cases, 70 (89.7%) were endometrioid, 5 (6.4%) serous, 2 (2.6%) clear cell, and 1 (1.3%) carcinosarcoma. Immunohistochemically, 16 (20.5%) tumours showed abnormal p53 expression, and 62 (79.5%) showed a p53 wild-type pattern. Compared with p53 wild-type group, p53 abnormal expression was associated with significantly higher frequencies of lymphovascular space invasion (LVSI) ($p = 0.028$), myometrial invasion (MI) $>50\%$ ($p = 0.044$), and grade 3 (G3) tumours ($p = 0.014$ and $p = 0.004$). Low/negative PTEN expression correlated with LVSI ($p = 0.023$). Combined analysis of p53 and PTEN showed significant associations with LVSI ($p = 0.015$) and histologic grade ($p = 0.038$ and $p = 0.011$). Significant differences in OS and DSS were observed according to p53 expression ($p = 0.004$; $p = 0.001$). Multivariate Cox regression showed that abnormal p53 expression remained an independent prognostic factor for both OS and DSS in EC. **Conclusions:** Aberrant p53 expression was associated with adverse clinicopathological features and poorer survival in EC. PTEN loss was associated with LVSI but did not demonstrate independent prognostic value. Prospective studies are needed to validate these findings.

Keywords

Endometrial carcinoma (EC); p53; PTEN; Lymphovascular space invasion (LVSI); Myometrial invasion (MI); Histologic grade (G)

1. Introduction

Endometrial carcinoma is a heterogeneous group of neoplasms whose initiation and progression are driven by the accumulation of genetic and epigenetic alterations that affect signalling pathways, proto-oncogene activation, and inactivation of tumour suppressor genes [1–4]. Although surgical, radiation, and pharmacological treatments are well-established, the identification and validation of biomarkers remain key to improving our understanding of molecular pathogenesis, enabling more accurate prognostic assessment, and facilitating the development of molecularly targeted therapies, especially in cases of recurrent disease and unfavourable clinical outcomes [1–4]. Over the past decade, molecular classification has become the gold standard for stratifying EC,

offering a biologically meaningful framework beyond traditional histopathologic parameters. The Cancer Genome Atlas (TCGA) project identified four molecular subtypes with distinct prognostic implications: DNA Polymerase Epsilon (POLE)-mutated, Mismatch Repair (MMR)-deficient, no specific molecular profile, and p53-abnormal [5]. These groups differ markedly in molecular alterations and clinical outcomes, with p53-abnormal tumours showing the poorest prognosis and frequent serous-like morphology. The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) provides a clinically applicable surrogate of the TCGA taxonomy using immunohistochemistry for p53 and MMR proteins, combined with targeted POLE sequencing [6]. Within this framework, aberrant expression of p53 and PTEN reflects critical oncogenic events and remains central to prognostic stratification in

EC [7].

The proapoptotic gene *p53* is a tumour suppressor gene and expresses a nuclear 53-kDa phosphoprotein called p53. The p53 protein is a transcription factor that induces cell cycle arrest at the G1 checkpoint, promotes DNA repair when damaged, and initiates apoptosis when this repair fails [8, 9]. Dysfunction of *p53* promotes uncontrolled cellular proliferation and tumorigenesis. Immunohistochemically, p53 expression stratifies EC into a wild-type and a mutant staining pattern (p53abnormal EC) (p53abn). Although p53abn accounts for about 15% of all ECs, this subgroup is associated with a disproportionately high mortality rate (50–70%) and poor clinical outcomes, particularly in poorly differentiated (G3) and serous carcinomas [10]. Increasing evidence suggests that the addition of systemic chemotherapy to radiotherapy improves oncological outcomes in p53-abnormal EC. Furthermore, targeted therapeutic strategies are currently being explored [10]. PTEN (phosphatase and tensin homolog) is a tumour suppressor gene that suppresses cell proliferation and differentiation and participates in the insulin signalling pathway. This gene plays a key role in the phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT) pathway by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the cell membrane [11–14]. Under normal physiological conditions, PTEN exerts inhibitory effects on intracellular signalling pathways, cellular proliferation, cell migration, and cell adhesion, while also promoting apoptosis in damaged cells [15, 16]. The absence of functional PTEN protein leads to unregulated activation of the PI3K signalling pathway [17]. One of the main factors in the pathogenesis of many tumours, particularly ECs, is the loss of *PTEN* function through somatic mutations, transcriptional/post-transcriptional mechanisms, and microRNA dysregulation [18].

Despite the well-established individual roles of p53 and PTEN, their combined IHC assessment and its association with key clinicopathological characteristics in surgically staged cohorts remain insufficiently explored in the literature. This study investigates the associations between p53 and PTEN expression, both individually and in combination, and clinicopathological parameters in EC. We hypothesise that p53-abnormal expression and low or negative PTEN expression are associated with adverse clinicopathological features.

2. Materials and methods

2.1 Study design and population

In a retrospective cohort study at our institution, we identified all the archived histological specimens from patients with endometrial carcinoma who underwent primary surgery at the gynecologic oncology clinic between 2019 and 2020. We reviewed the clinical and histopathological data in detail, including FIGO (International Federation of Obstetrics and Gynecology) stage (2009), degree of differentiation (G1–G3), histological subtype [19], lymphovascular space invasion (LVSI), depth of myometrial invasion (MI), and lymph node metastases. Total hysterectomy with bilateral salpingo-oophorectomy (TH-BSO) alone was performed in 4 patients, whereas 3 underwent class II radical hysterectomy, and 1

underwent class III radical hysterectomy. In the remaining 70 patients, TH-BSO was combined with lymph node dissection of different extent, as demonstrated in Table 1. Regarding adjuvant treatment, 7 patients did not receive any postoperative therapy, 17 patients received brachytherapy alone, 35 patients received postoperative external beam radiotherapy, and 19 patients underwent adjuvant combined chemoradiotherapy. Decisions on adjuvant treatment were based on established clinicopathologic risk factors, including FIGO stage, histologic grade, LVSI, lymph node status, and tumour size assessed on the surgical specimen. All treatments were administered in accordance with the contemporary international treatment guidelines. Histologic grading (G1–G3) was applied to tumors with endometrioid differentiation, whereas non-endometrioid histotypes, including serous carcinoma, clear cell carcinoma, and carcinosarcoma, were considered high-grade by definition and were not assigned a conventional histologic grade. Although molecular classification systems such as TCGA have refined the biological understanding of endometrial carcinoma, the traditional dualistic model remains clinically relevant and is widely used. For additional statistical stratification, tumors were grouped according to this model as Type I (G1–G2 endometrioid carcinomas) and Type II (serous, clear cell, carcinosarcoma, and high-grade (G3) endometrioid carcinomas).

2.2 Inclusion and exclusion criteria

Only cases with histologically confirmed EC and primary surgical staging were included. Cases with incomplete medical documentation, the presence of other concomitant malignancies, or receipt of neoadjuvant radiotherapy or chemotherapy administered before surgery were excluded.

2.3 Data sources and ethics

We collected data from the clinic's medical records, the National Cancer Registry, and the Civil Registration and Administrative Services General Directorate (CRASGD). This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Medical University–Pleven (approval No. 733-KENID, dated 05 June 2023). Because this was a retrospective study, the requirement for individual informed consent was waived by the ethics committee in accordance with institutional and ethical guidelines.

2.4 Immunohistochemical examination

p53 and PTEN status were assessed by IHC. Aberrant p53 staining patterns (overexpression or complete absence of staining) were interpreted as p53 abnormality, whereas PTEN loss or reduced expression was interpreted as functional inactivation rather than a confirmed genetic mutation. We selected tissue samples from archived hysterectomy specimens of patients with EC, fixed in 10% buffered formalin for 24 to 36 hours at room temperature, sectioned, and embedded in paraffin. IHC examination was performed on 5 µm sections of representative invasive tumour tissue and, where possible, on available normal mucosa as a reference control. Selection and verification were made by a pathologist on hematoxylin and eosin (H&E)-stained sections. Epitope retrieval was per-

TABLE 1. Clinicopathologic characteristics and expression profiles of p53 and PTEN proteins in patients with endometrial cancer (N = 78).

Variables	Number (%)
Age group at diagnosis	
≤65	29 (37.2)
>65	49 (62.8)
Tumor size	
<2 cm	20 (25.6)
>2 cm	58 (74.4)
Type of lymph node dissection	
Pelvic (para-aortic lymphadenectomy performed in 2 cases)	48 (61.5)
Selective pelvic	23 (29.5)
Pelvic lymph node biopsy only	3 (3.9)
No lymph node dissection performed	4 (5.1)
Histological diagnosis	
Endometrial Endometrioid Carcinoma (EEC)	70 (89.7)
Serous endometrial carcinoma (SEC)	5 (6.4)
Clear cell endometrial carcinoma (CCEC)	2 (2.6)
Endometrial Carcinosarcoma	1 (1.3)
Endometrial carcinoma (EC) type	
Type I	58 (74.4)
Type II	20 (25.6)
Histologic grade in 71 patients ¹	
G1	12 (16.9)
G2	46 (64.8)
G3	13 (18.3)
Histologic grade (two-tier classification: G1–G2 vs. G3)	
G1 + G2	58 (81.7)
G3	13 (18.3)
FIGO (2009) stage of EC	
Stage I	52 (66.7)
Stage II	6 (7.7)
Stage III	15 (19.2)
Stage IV	5 (6.4)
FIGO (2009) stage of EC after pairing	
I + II	58 (74.4)
III + IV	20 (25.6)

TABLE 1. Continued.

Variables	Number (%)
MI	
<50%	37 (47.4)
>50%	41 (52.6)
Lymphovascular space invasion (LVSI)	
Yes	18 (23.1)
No	60 (76.9)
LN meta ²	
Yes	12 (16)
No	62 (84)
p53 status	
Wild-type	62 (79.5)
Mutant	16 (20.5)
PTEN status	
Negative	30 (38.5)
Low	17 (21.8)
Moderate	20 (25.6)
High	11 (14.1)
PTEN status in pairs	
Negative + Low	47 (60.3)
Moderate + High	31 (39.7)
Co-expression of p53 and PTEN	
p53 wild-type + PTEN negative/low	38 (48.7)
p53 wild-type + PTEN moderate/high	24 (30.8)
p53 mutant + PTEN negative/low	9 (11.5)
p53 mutant + PTEN moderate/high	7 (9.0)

LN meta: Lymph node metastases; PTEN: phosphatase and tensin homolog; p53: p53 protein encoded by the TP53 gene; G: Histologic grade; FIGO: International Federation of Gynecology and Obstetrics; MI: Depth of myometrial invasion. ¹Histologic grade was not determined in 7 cases; ²Lymph node dissection was not performed in 4 patients. The label “p53 mutant” used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

formed for 20 minutes at 97 °C using DAKO PT Link (code PT100/PT101; Agilent Technologies, Santa Clara, CA, USA). Tumour sections were labelled with antibodies (DAKO Denmark©, Glostrup, Denmark), as follows:

- PTEN: 6H2.1 (Monoclonal mouse Anti-PTEN Antibody (ready to use); Ref. No. PDM374).
- p53: DO-7 (Monoclonal Mouse Anti-Human p53, ready-to-use; Ref. No. IR61661-2).

Visualisation was performed using the EnVision FLEX system, High pH (K800021-2, Agilent Technologies, Santa Clara,

CA, USA).

The incubation time for all antibodies was 20 minutes at room temperature. Dako Autostainer Link 48 (automated immunostaining system; Agilent Technologies, Santa Clara, CA, USA) was used for staining. The manufacturer's kit was used for external negative controls. According to the manufacturer's instructions, depending on the antibody, the following were used for external positive controls:

- p53: colon tissue (including negative control, positive control, and weak expressor) and high-grade serous carcinoma with p53 overexpression (overexpressor) were used as external positive controls.
- PTEN: ovarian carcinoma tissues with mutation status confirmed by next-generation sequencing (NGS).

2.5 Analysis of results

Two experienced pathologists analysed, categorised, and reported all histopathological results independently, following standardized diagnostic algorithms and protocols of the College of American Pathologists (CAP), the British Gynaecological Cancer Society (BGCS), and the British Association of Gynaecological Pathologists (BAGP) [20, 21].

2.5.1 p53 immunohistochemical assessment

The p53 expression pattern was evaluated according to established interpretation criteria. Normal (wild-type) expression was defined by variable nuclear staining intensity corresponding to that of internal non-neoplastic controls such as fibroblasts and lymphocytes. Abnormal (mutant-type) expression included three distinct patterns:

(1) Overexpression, characterised by intense and diffuse nuclear staining in more than 80% of tumor cells; (2) Complete absence of expression, defined as total loss of both nuclear and cytoplasmic staining in tumor cells, with preserved internal positive controls; and (3) Cytoplasmic staining, with or without accompanying nuclear positivity, involving more than 80% of tumor cells.

2.5.2 PTEN immunohistochemical assessment

Our interpretation of PTEN immunostaining followed the Immunoreactivity Scoring System (IRS) [14, 22, 23]. Positive PTEN expression was defined by cytoplasmic and/or nuclear immunoreactivity, assessed semiquantitatively using two parameters evaluated simultaneously: staining intensity and the percentage of positive tumour cells. The final IRS score represented the product of these two parameters, each measured on a four-point scale. Vascular endothelial and stromal cells served as internal positive controls to validate staining adequacy.

For PTEN evaluation in EC, staining intensity was graded as follows: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The percentage of positive cells was categorized as 0 = 0%, 1 = <10%, 2 = 10–50%, 3 = 51–80%, and 4 = >80%. The final IRS score was derived as the product of these two parameters and interpreted as: 0 = negative, 1–4 = low expression, 5–8 = moderate expression, and 9–12 = high expression.

2.6 Endpoints

Demographic data, pathological variables, and oncological outcome information were collected. Survival was analysed as overall survival (OS) and disease-specific survival (DSS). Censoring rules were based on the date of last follow-up from available registries and documentation.

2.7 Statistical analysis

The statistical analysis was performed using descriptive statistics and hypothesis-testing methods. Quantitative variables were summarized using the mean, standard deviation, standard error of the mean, 95% confidence interval for the mean, and range (minimum–maximum). Qualitative variables were described using absolute frequencies, relative frequencies (%), and cumulative relative frequencies (%). Appropriate graphical methods were used for data visualization.

Associations between categorical variables were evaluated using non-parametric hypothesis-testing methods, using the chi-square (χ^2) test or Fisher's exact test, as appropriate. Survival outcomes were analyzed using life-table methods and the Kaplan-Meier estimator to illustrate unadjusted survival differences. Differences in survival distributions between groups were assessed using the log-rank (Mantel-Cox) test and, where appropriate, the Gehan-Wilcoxon test. Cox proportional hazards regression analysis was applied to assess the independent prognostic impact of p53 and PTEN after adjustment for relevant clinicopathological covariates, with hazard ratios estimated in both univariate and multivariate models. The strength of association between categorical variables was additionally assessed using Cramer's *V*. All statistical tests were two-sided, and a *p*-value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016 for Windows (Microsoft Corporation, Redmond, WA, USA).

3. Results

The analysis included 78 consecutive patients with histologically verified EC (Table 1).

Lymph node assessment was performed in 74 patients (94.8%). Pelvic lymphadenectomy was carried out in 48 patients, including 2 cases in which para-aortic lymphadenectomy was additionally performed. Selective pelvic lymphadenectomy was performed in 23 patients, and only 3 patients underwent pelvic lymph node biopsy. Four patients did not undergo lymph node dissection. Regional lymph node metastases were identified in 12 patients (16%); 10 had pelvic nodal metastases and 2 had combined pelvic and para-aortic metastases.

Histologic grade (G1–G3) was not assigned in 7 patients (5 serous carcinomas, 1 pure clear cell carcinoma, and 1 carcinosarcoma), as these histotypes are generally regarded as high-grade by definition. The second clear cell carcinoma was of mixed type with a defined endometrioid component, and the reported grade (G3) referred to the epithelial (endometrioid) component.

One carcinosarcoma case was included in the cohort. The

patient was 67 years old at the time of surgery and had FIGO stage IB disease. She underwent total hysterectomy with pelvic lymphadenectomy (19 lymph nodes were removed), followed by adjuvant chemoradiotherapy, and was alive at the end of follow-up. Immunohistochemically, the tumor demonstrated a p53 wild-type expression pattern and high PTEN expression. As carcinosarcoma is considered a high-grade tumor by definition, no conventional histologic grading (G1–G3) was assigned. Accordingly, this case was included in the high-grade (Type II) category within the dualistic classification used in the study. Given that only a single carcinosarcoma case was included in the cohort, no separate statistical analysis was performed for this subtype.

Of all the analysed cases, 58 were classified as Type I EC (G1–G2 endometrioid carcinomas), whereas 20 were classified as Type II EC, including serous, clear cell, carcinosarcoma, and high-grade (G3) endometrioid carcinomas. The surgical interventions were performed in 2019 ($n = 35$; 44.9%) and 2020 ($n = 43$; 55.1%). Throughout the follow-up (until 31 December 2024), 17 (21.8%) patients died from the disease, 14 (17.9%) died from other causes, and 47 (60.3%) survived with no evidence of recurrence.

Immunohistochemical assessment of p53 and PTEN was performed retrospectively for the study and did not influence

therapeutic decisions.

In terms of IHC expression categories, 62 (79.5%) tumours were classified as p53 wild-type (p53wt), and 16 (20.5%) showed aberrant p53 expression (p53mut). Based on the IRS, 30 (38.5%) cases showed negative expression for PTEN, 17 (21.8%) low expression, 20 (25.6%) moderate expression, and 11 (14.1%) high expression. For analytical purposes, cases were grouped into two categories: PTEN negative + low ($n = 47$; 60.3%) versus PTEN moderate + high ($n = 31$; 39.7%) (Table 1). Representative histologic and immunohistochemical findings are illustrated in Fig. 1.

A comparison of the clinical and pathological characteristics between tumors with p53 wild-type expression (p53wt) and aberrant p53 expression (p53mut) revealed a more adverse clinicopathological profile in the p53mut group: the frequency of LVSI was higher (43.7% vs. 17.7%; $p = 0.028$), deep myometrial invasion ($>50\%$) was more common (75.0% vs. 46.8%; $p = 0.044$), and the proportion of poorly differentiated tumors (G3) was significantly higher in the p53mut group (46.2% vs. 12.07%) both in the three-grade comparison (G1 vs. G2 vs. G3; $p = 0.014$) and in the binary comparison (G1 + G2 vs. G3; $p = 0.004$) (Table 2).

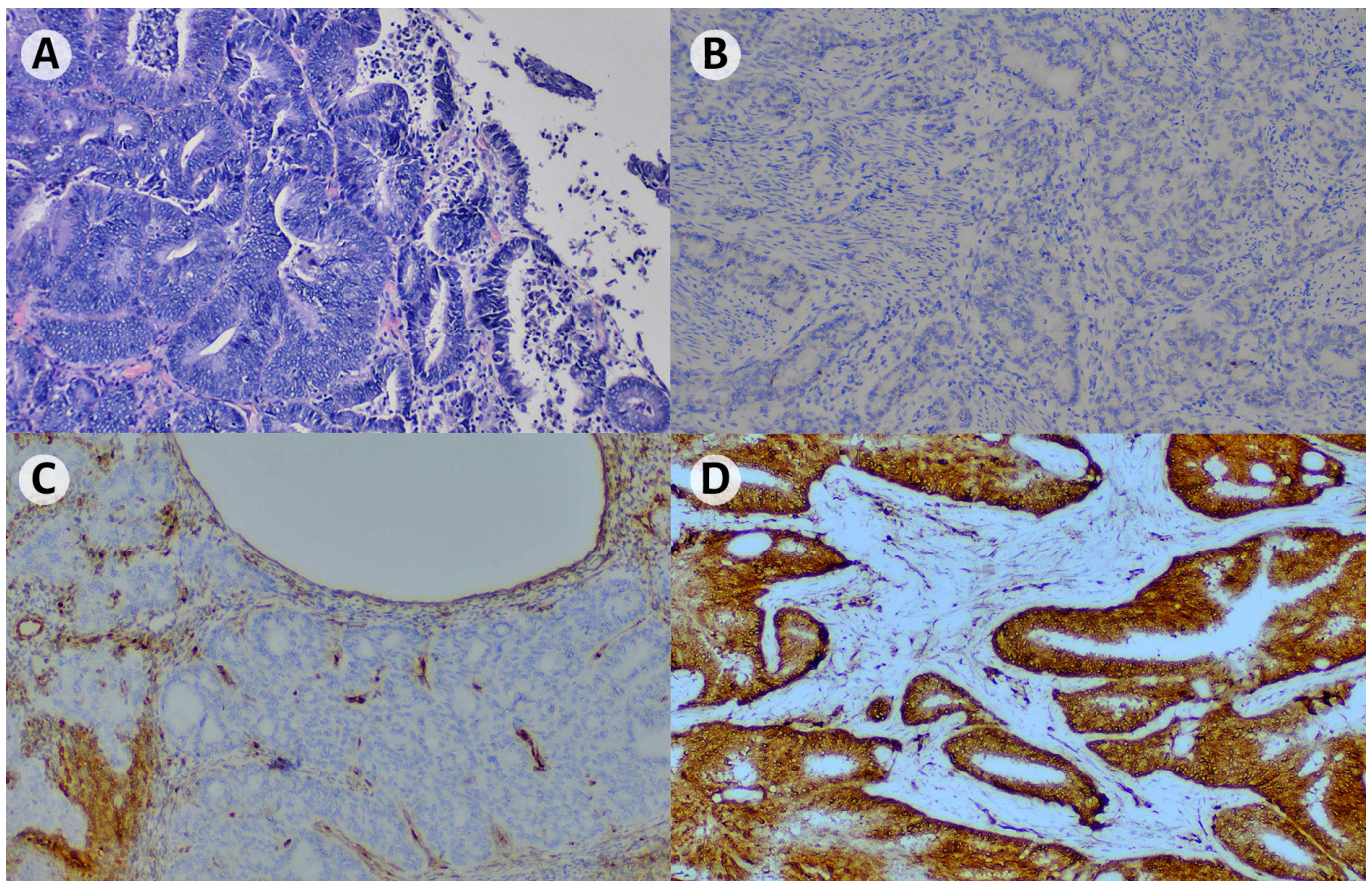


FIGURE 1. Representative histologic and immunohistochemical findings in endometrial carcinoma. (A) Hematoxylin-eosin staining showing endometrioid carcinoma G2 $\times 10$. (B) Aberrant p53 expression (null pattern) G2 $\times 10$. (C) Loss of PTEN expression in tumor cells G2 (score 0) $\times 10$. (D) Strong PTEN expression showing intense cytoplasmic staining in tumor cells G2 (score 12) $\times 10$. Panels A–C are from the same tumour, whereas panel D represents a different tumour. All tumours correspond to endometrioid carcinoma, grade 2.

TABLE 2. Individual p53 and PTEN expression profiles in relation to clinicopathological variables.

Variables	p53wt (n = 62)	p53mut (n = 16)	χ^2	Cramer's <i>V</i>	<i>p</i> -value	PTEN Negative + Low (n = 47)	PTEN Moderate + High (n = 31)	χ^2	Cramer's <i>V</i>	<i>p</i> -value
Grade ¹										
- G1	11 (18.96%)	1 (7.6%)				5 (11.6%)	7 (25%)			
- G2	40 (68.97%)	6 (46.2%)	8.592	0.345	0.014	29 (67.5%)	17 (60.71%)	2.192	0.174	0.334
- G3	7 (12.07%)	6 (46.2%)				9 (20.9%)	4 (14.29%)			
Grade ²										
- G1 + G2	51 (87.93%)	7 (53.8%)	8.466	0.343	0.004	34 (79.1%)	24 (85.71%)	0.596	0.091	0.440
- G3	7 (12.07%)	6 (46.2%)				9 (20.9%)	4 (14.29%)			
EC type										
- Type I	48 (77.4%)	10 (62.5%)	1.485	0.138	0.223	34 (72.3%)	24 (77.4%)	0.253	0.057	0.615
- Type II	14 (22.6%)	6 (37.5%)				13 (27.7%)	7 (22.6%)			
MI										
- <50%	33 (53.2%)	4 (25.0%)	4.064	0.228	0.044	22 (46.8%)	15 (48.4%)	0.019	0.015	0.891
- >50%	29 (46.8%)	12 (75.0%)				25 (53.2%)	16 (51.6%)			
LVSI										
- Yes	11 (17.7%)	7 (43.7%)	4.846	0.249	0.028	15 (31.9%)	3 (9.7%)	5.204	0.258	0.023
- No	51 (82.3%)	9 (56.3%)				32 (68.1%)	28 (90.3%)			

Associations between biomarker expression and clinicopathologic variables were assessed using Pearson's chi-square test (χ^2) or Fisher's exact test, where appropriate. Effect size is reported as Cramer's *V*. ¹G: Histologic grade (not determined in 7 cases; percentages for grade-related variables were calculated based on cases with available histologic grade within each biomarker subgroup); ²Grade (2-tier): G1–2 vs. G3; MI: Myometrial Invasion; LVSI: Lymphovascular space invasion; PTEN: phosphatase and tensin homolog; p53: p53 protein encoded by the TP53 gene; EC: Endometrial carcinoma; p53wt: p53 Wild-type; p53mut: p53 Mutant. The label "p53mut" used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

Among low-grade tumours (G1–2), seven cases (9.0% of the total cohort) demonstrated aberrant p53 expression. The median age in this subgroup was 66 years (range 61–83). The disease stage was heterogeneous, including early-stage disease (IA–IB) in five patients, locally advanced disease (IIIB) in one patient, and advanced metastatic disease (IVB) in one patient. LVSI was present in 3 of 7 cases. Regarding adjuvant treatment, two patients received combined chemoradiotherapy, three received external beam radiotherapy alone, and two received brachytherapy. One patient died one month after surgery due to an intercurrent disease, before an exhaustive oncologic assessment could be performed. During follow-up, two patients died of endometrial carcinoma at 30 and 55 months, respectively; two patients died of causes unrelated to the malignancy (at 45 months and one month postoperatively, respectively), and three patients remain alive without evidence of disease. Despite their favourable histologic grade, this subgroup demonstrated heterogeneous but overall, clinically aggressive behaviour, including advanced stage at diagnosis, frequent LVSI, and disease-related mortality.

The assessment of PTEN in relation to LVSI showed that the PTEN negative + low group was associated with a higher frequency of LVSI compared with the PTEN moderate + high group (31.9% vs. 9.7%). This combined PTEN negative + low expression group was significantly associated only with LVSI ($p = 0.023$), whereas no statistically significant associations were observed with histologic grade, myometrial invasion, or histologic subtype (Type I vs. Type II) (Table 2). The combined analysis of p53 and PTEN demonstrated statistically significant differences in grade distribution between the molecular subgroups (Table 3). A higher proportion of G3 tumors was observed in the p53 abnormal + PTEN negative/low group compared to the p53 wild-type + PTEN moderate/high group (37.5% vs. 4.2%; $p = 0.038$ and $p = 0.011$). However, given that p53 status alone was significantly associated with grade (Table 2), whereas PTEN expression was not, this enrichment of G3 cases is most likely attributable to p53 abnormality rather than to an independent effect of PTEN expression.

Of the 24 cases with p53wt + PTEN moderate/high, only one had a G3 tumour. The frequency of LVSI was also higher in the p53mut + PTEN negative/low group compared to the p53wt + PTEN moderate/high group (55.6% vs. 4.2%; $p = 0.015$) (Table 3). The survival rate analysis supports the prognostic relevance of p53: OS is lower in the p53mut group compared with the p53wt group (log-rank $\chi^2 = 6.706$; $p = 0.01$), with a median OS of 55.23 months (95% confidence interval (CI): 49.236–61.223) for p53wt versus 34.30 months (95% CI: 20.396–48.204) for p53mut (Table 4; Fig. 2).

The analysis of DSS showed that of the 17 patients who died from the disease, 9 (52.94%) had a p53 abnormality. The DSS was lower in the p53mut group compared with the p53wt group (log-rank $\chi^2 = 16.841$; $p = 0.001$), with a mean DSS of 63.715 months (95% CI: 58.926–68.504) for p53wt versus 38.040 months (95% CI: 23.328–52.752) for p53mut (Table 4; Fig. 3).

A significant difference was also found in one-, three-, and five-year OS and DSS (Gehan-Breslow-Wilcoxon = 7.695; $p = 0.006$ and 13.451; $p = 0.001$, respectively), with lower values observed in the p53mut group (50%, 38%, and 29% for OS;

54%, 48% and 37% for DSS, respectively) (Table 5).

Univariate and multivariate Cox proportional hazards regression analyses were performed to evaluate prognostic factors for OS and DSS, including p53 status and relevant clinicopathologic covariates (Tables 6,7). In the multivariate model for OS (Table 6), Type II EC (Hazard Ratio (HR) 2.544, $p = 0.021$), regional lymph node metastases (HR 3.697, $p = 0.001$), and p53 abnormal expression (IHC) (HR 2.703, $p = 0.019$) remained independent prognostic factors. In the multivariate model for DSS (Table 7), age >65 years (HR 10.975, $p = 0.029$), G3 histology (HR 6.757, $p = 0.027$), lymph node metastases (HR 8.843, $p = 0.003$), and p53 abnormal expression (IHC) (HR 4.651, $p = 0.047$) were independently associated with poorer DSS.

We included the subgroup of low-grade (G1–2) tumours with aberrant p53 expression in the multivariate Cox regression model, and aberrant p53 status remained independently associated with survival after adjustment for stage, grade, LVSI, lymph node status, and adjuvant treatment.

4. Discussion

In the present study, we investigated the relationship between clinicopathological characteristics and IHC expression of p53 and PTEN in a surgically staged cohort of patients with EC (2019–2020). The proportion of tumours showing aberrant p53 expression (p53mut) was 20.5%, which is consistent with previously reported frequencies of approximately 15–20% in EC [10, 24]. In our study, p53 abnormality was associated with LVSI, deep myometrial invasion, and a higher histologic grade, while PTEN negative/low expression was significantly associated only with LVSI. Although co-expression analysis revealed differences in grade distribution among subgroups, these findings appear to be largely attributable to p53 status, as PTEN alone showed no significant association with grade.

Our cohort-specific distribution of p53mut tumours by histotype and grade (serous: 3/5, 60%; G3: 5/13, 38.5%; G1–G2: 7/58, 12.07%; clear-cell: 1/2, 50%) partially differs from aggregated molecularly classified cohort studies, which report approximate percentages: serous ~93%, carcinosarcoma ~85%, clear cell ~38%, G3 endometrioid ~22%, G1–2 endometrioid ~5% [7, 10, 25–27]. Such a distribution probably reflects differences in cohort composition, subgroup sizes, and methodologies, although our overall frequency remains within the ranges reported in the literature [10, 24]. It is noteworthy that certain endometrial carcinomas may harbour *TP53* mutations while retaining a normal, wild-type p53 immunostaining pattern. These non-disruptive mutations result in functionally abnormal, yet immunohistochemically undetectable, protein alterations. Because our study relied solely on IHC evaluation, such cases may have been misclassified as p53 wild-type despite an underlying *TP53* mutation. This potential discrepancy could contribute to the biological and prognostic heterogeneity found within the p53-wild-type group. Nevertheless, immunohistochemistry remains a practical and widely accepted surrogate marker of *TP53* mutation status in both diagnostic and research practice.

The presence of aberrant p53 expression in a subset of morphologically low-grade (G1–2) tumours represents a bio-

TABLE 3. Co-expression of p53 and PTEN in relation to key clinicopathological variables.

Variables	p53 wild-type + PTEN negative/low (n = 38) %	p53 wild-type + PTEN moderate/high (n = 24) %	p53 mutant + PTEN negative/low (n = 9) %	p53 mutant + PTEN moderate/high (n = 7) %	p-value (χ^2)
Grade ¹					
G1	5 (14.3)	6 (26.08)	0	1 (20.0)	0.038
G2	24 (68.6)	16 (69.57)	5 (62.5)	1 (20.0)	
G3	6 (17.1)	1 (4.35)	3 (37.5)	3 (60.0)	
Grade ²					
G1 + G2	29 (82.9)	22 (95.65)	5 (62.5)	2 (40.0)	0.011
G3	6 (17.1)	1 (4.35)	3 (37.5)	3 (60.0)	
MI					
<50%	19 (50.0)	14 (58.3)	3 (33.3)	1 (14.3)	0.168
>50%	19 (50.0)	10 (41.7)	6 (66.7)	6 (85.7)	
LVSI					
Yes	10 (26.3)	1 (4.2)	5 (55.6)	2 (28.6)	0.015
No	28 (73.7)	23 (95.8)	4 (44.4)	5 (71.4)	
Age (yr)					
≤65	16 (42.1)	8 (33.3)	4 (44.4)	1 (14.3)	0.509
>65	22 (57.9)	16 (66.7)	5 (55.6)	6 (85.7)	
LN meta ³					
Yes	7 (20.0)	3 (12.5)	1 (11.1)	1 (16.7)	0.853
No	28 (80.0)	21 (87.5)	8 (88.9)	5 (83.3)	

Group differences were assessed using Pearson's chi-square test χ^2 or Fisher's exact test, as appropriate; effect size was expressed as Cramer's V; ¹G: Histologic grade (not determined in 7 cases; percentages for grade-related variables were calculated based on cases with available histologic grade within each co-expression subgroup); ²G: Histologic grade (2-tier): G1–2 vs. G3; ³Lymph node dissection was not performed in 4 cases; LN meta: Lymph node metastases; MI: Myometrial Invasion; LVSI: Lymphovascular space invasion; PTEN: phosphatase and tensin homolog; p53: p53 protein encoded by the TP53 gene. The label "p53mutant" used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

TABLE 4. OS and DSS according to p53 status estimated by the Kaplan-Meier method.

Variable	Mean	Std. Error	95% CI	Median	Std. Error	95% CI	log-rank test (Mantel-Cox)	
							χ^2	p-value
p53 status	Overall Survival							
Wild-type	55.230	3.058	49.236–61.223	NR		-		
Mutant	34.300	7.094	20.396–48.204	23.000	19.000	0.00–60.240	6.706	0.010
p53 status	Disease-Specific Survival							
Wild-type	63.715	2.443	58.926–68.504	NR		-		
Mutant	38.040	7.506	23.328–52.752	30.000	25.005	0.00–79.009	16.841	0.001

Survival was estimated using the Kaplan-Meier method and compared using the log-rank (Mantel-Cox) test; Median survival was not reached (NR) in the p53wt group during follow-up; p53: p53 protein encoded by the TP53 gene; OS: Overall survival; DSS: Disease-specific survival; Std. Error: Standard Error; CI: Confidence Interval. The label "p53mutant" used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

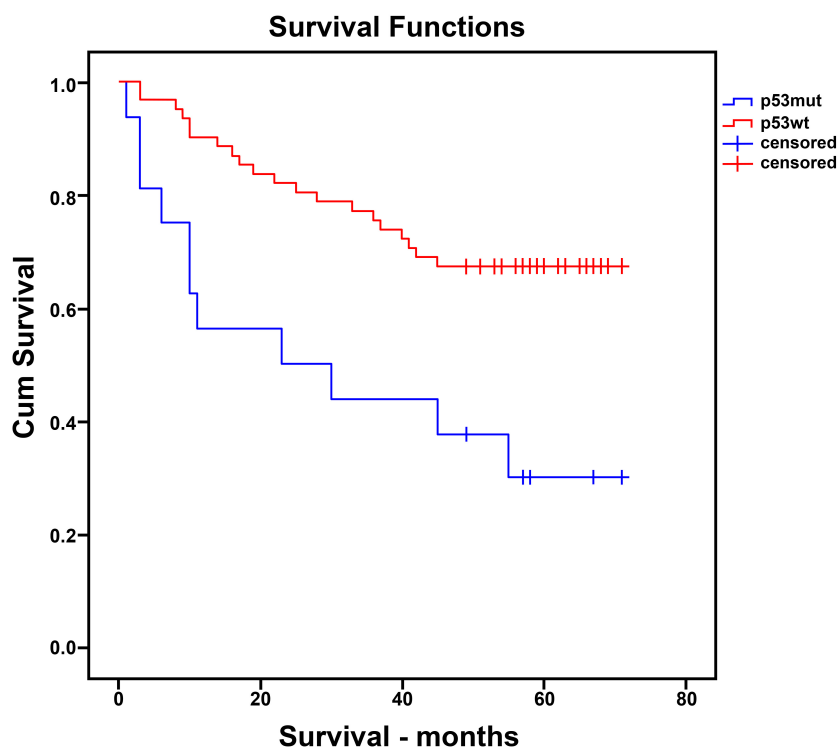


FIGURE 2. Overall survival of patients with endometrial carcinoma according to p53 status (p53wt vs. p53mut). Kaplan-Meier analysis demonstrated significantly reduced OS in the p53mut group compared with the p53wt group (log-rank $\chi^2 = 6.706$, $p = 0.01$). Censored observations are indicated by tick marks. The label “p53mut” used in the figure corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern). Detailed survival estimates are presented in Table 4. p53wt: p53 Wild-type; p53mut: p53 Mutant.

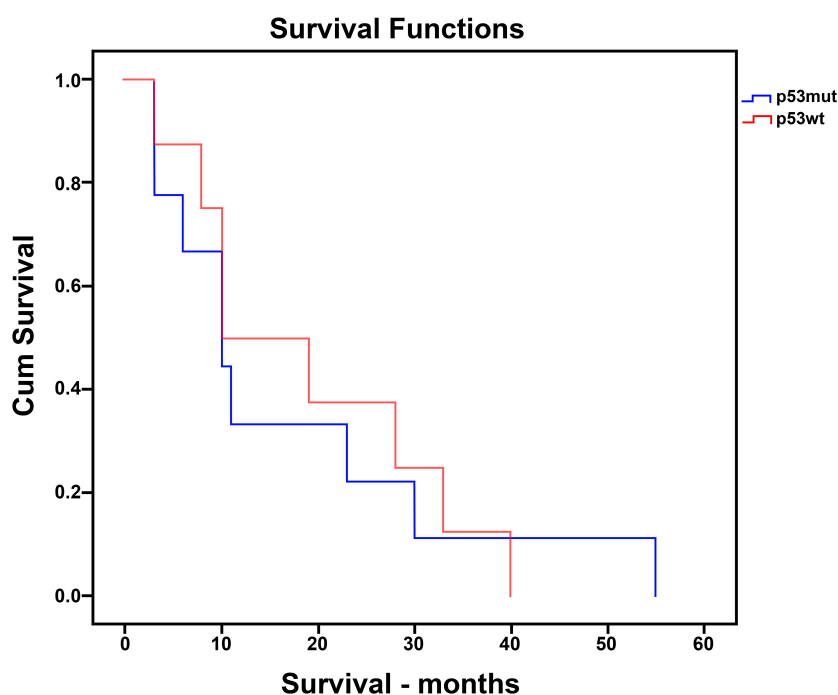


FIGURE 3. Disease-specific survival of patients with endometrial carcinoma according to p53 status (p53wt vs. p53mut). Kaplan-Meier analysis demonstrated significantly reduced DSS in the p53mut group compared with the p53wt group (log-rank $\chi^2 = 16.841$, $p = 0.001$). The label “p53mut” used in the figure corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern). Detailed survival estimates are presented in Table 4. p53wt: p53 Wild-type; p53mut: p53 Mutant.

TABLE 5. One-, three-, and five-year OS and DSS by p53 status (Life Table analysis).

Variable	Estimated survival (%) at:			Overall comparisons	
	1st year	3rd year	5th year	Gehan-Wilcoxon (χ^2)	p-value
p53 status			Overall Survival		
- Wild-type	82%	67%	67%		
- Mutant	50%	38%	29%	7.695	0.006
p53 status			Disease-Specific Survival		
- Wild-type	92%	86%	86%		
- Mutant	54%	48%	37%	13.451	0.001

Survival rates were estimated using life table analysis; group differences were assessed using the Gehan-Breslow-Wilcoxon test (χ^2 , $df = 1$); p53: p53 protein encoded by the TP53 gene; OS: Overall survival; DSS: Disease-specific survival. The label "p53mutant" used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

TABLE 6. Univariate and multivariate Cox PH regression analysis (overall survival).

Variables	N (%)	Univariate		Multivariate	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Age (yr)					
≤65	29 (37.2)	1.00			
>65	49 (62.8)	2.540 (1.096–5.921)	0.030		
EC Type					
Type I	58 (74.4)	1.00			
Type II	20 (25.6)	3.593 (1.743–7.407)	0.001	2.544 (1.150–5.631)	0.021
FIGO Stage (2009)					
I	52 (66.7)	1.00			
II	6 (7.7)	4.110 (1.166–14.488)	0.028		
III	15 (19.2)	4.162 (1.852–9.350)	0.001		
IV	5 (6.4)	4.139 (1.347–12.719)	0.013		
FIGO Stage (2009)					
I + II	58 (74.4)	1.00			
III + IV	20 (25.6)	3.554 (1.748–7.227)	0.001		
Grade ¹					
G1 + G2	58 (81.69)	1.00			
G3	13 (18.31)	4.276 (1.890–9.673)	0.001		
MI					
<50%	37 (47.4)	1.00			
>50%	41 (52.6)	4.251 (1.826–9.899)	0.001		
LVSII					
No	60 (76.9)	1.00			
Yes	18 (23.1)	2.947 (1.424–6.097)	0.004		
Tumor Size					
<2 cm	20 (25.6)	1.00			
>2 cm	58 (74.4)	2.245 (0.861–3.852)	0.098		
LN meta ²					
No	62 (84)	1.00			
Yes	12 (16)	3.240 (1.541–6.812)	0.002	3.697 (1.696–8.058)	0.001
p53					
wt	62 (79.5)	1.00		1.00	
mut	16 (20.5)	2.939 (1.405–6.149)	0.004	2.703 (1.177–6.211)	0.019

HR = 1.00 indicates the reference category. Variables significant in univariate Cox regression were entered into the multivariable model together with clinically relevant covariates. HR: Hazard ratio; CI: Confidence interval; G: Histologic grade (2-Tier)—G1–2 vs. G3; MI: Myometrial Invasion; LVSII: Lymphovascular space invasion; EC: Endometrial carcinoma; LN meta: Lymph node metastases; p53: p53 protein encoded by the TP53 gene; FIGO: International Federation of Gynaecology and Obstetrics; ¹Histologic grade was not determined in 7 cases. ²Lymph node dissection was not performed in 4 cases. The label "p53mut" used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

TABLE 7. Univariate and multivariate Cox PH regression analysis (disease-specific survival).

Variables	N (%)	Univariate		Multivariate	
		HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Age (yr)					
≤65	29 (37.2)	1.00		1.00	
>65	49 (62.8)	5.435 (1.241–23.804)	0.025	10.975 (1.283–93.871)	0.029
Grade ¹					
G1 + G2	58 (81.69)	1.00		1.00	
G3	13 (18.31)	10.110 (3.276–31.198)	0.001	6.757 (1.246–36.643)	0.027
p53					
wt	62 (79.5)	1.00		1.00	
mut	16 (20.5)	5.775 (2.222–15.012)	0.001	4.651 (1.022–21.176)	0.047
LN meta ²					
No	62 (84)	1.00		1.00	
Yes	12 (16)	3.828 (1.445–10.144)	0.007	8.843 (2.152–36.333)	0.003

HR = 1.00 indicates the reference category. Variables significant in univariate Cox regression were entered into the multivariable model. HR: Hazard ratio; CI: Confidence interval; G: Histologic grade (2-Tier)—G1–2 vs. G3; LN meta: Lymph node metastases; p53: p53 protein encoded by the TP53 gene; wt: Wild-type; mut: Mutant. ¹Histologic grade was not determined in 7 cases; ²Lymph node dissection was not performed in 4 cases. The label “p53mut” used in the table corresponds to aberrant p53 expression detected by immunohistochemistry (p53 abnormal pattern).

logically discordant yet clinically highly relevant phenotype. In our cohort, these tumours demonstrated a heterogeneous stage distribution, frequent LVSI, and disease-related mortality, underscoring the fact that favourable histologic grade did not necessarily translate into indolent clinical behaviour. Disease-related deaths occurred despite the use of intensified adjuvant treatment, including combined chemoradiotherapy, highlighting the dominant role of tumour biology over conventional histopathologic risk factors. These findings support the concept that p53 status may override histologic grade in defining tumour aggressiveness and should be incorporated into risk stratification even in tumours classified as low-grade by conventional morphology. The adverse prognostic impact of aberrant p53 expression persisted in multivariate analysis even when low-grade tumours were included, further supporting the concept that molecular alterations may supersede histologic grade in defining tumour aggressiveness. In the endometrioid-only subgroup (n = 70; 12 disease-specific events), multivariable survival modelling would have been underpowered and prone to overfitting. Larger cohort studies are needed to evaluate histotype-restricted prognostic effects.

Regarding PTEN, our data (IRS: negative 38.5%, low: 21.8%, moderate: 25.6%, high: 14.1%; analytically combined as negative + low 60.3% versus moderate + high 39.7%) showed an association of negative/low expression with LVSI (31.9% versus 9.7%; *p* = 0.023) (Table 2). For cases with a negative score, PTEN alteration or functional loss is more likely, whereas cases with a high score are more likely to retain PTEN function. The remaining cases with low and moderate scores form a heterogeneous group: some cells harbour mutations to varying degrees, while others do not. For this reason, we grouped the PTEN scores into two pairs: negative + low and moderate + high. The literature data are

heterogeneous, with some studies reporting no significant association between PTEN expression and standard prognostic factors [16]. Limitations of immunohistochemical assessment may partly explain this variability, as recent evidence suggests that certain dominant-negative PTEN alterations may retain protein expression despite functional inactivation, potentially masking associations with clinicopathological outcomes [28]. However, Stavropoulos *et al.* [29] reported a negative correlation between the percentage of PTEN-positive cells and the depth of myometrial invasion. Similarly, Daniilidou *et al.* [30] found that PTEN negativity correlates with G3. In contrast, in our series, 9/13 G3 cases were PTEN-negative and low, versus 4/13 moderate and high, without statistical significance. We also observed an age-related association: of the 9 patients with a p53mut + PTEN-negative/low profile, 8 were over 60. However, given the small number of cases, this observation should be interpreted cautiously and requires confirmation in larger cohorts.

Co-expression analysis reinforces the stratification value of the biomarkers studied. The combination of p53mut + PTEN negative/low is associated with higher frequencies of LVSI and G3. At the same time, we did not find any significance for the MI (Table 3). Similar observations of adverse outcomes with simultaneous PTEN and p53 pathway abnormalities in EC have been reported [31]. In addition, Terakawa *et al.* [32] reported that PTEN overexpression is a favourable prognostic marker for OS in advanced EC treated with adjuvant chemotherapy, possibly due to increased chemosensitivity. Based on our findings, we support the view that concomitant assessment of p53 and PTEN expression may contribute to the characterisation of tumour behaviour in EC [33–38].

Our findings are consistent with the molecular framework defined by the TCGA and ProMisE classifiers, which recog-

nise p53-abnormal tumours as a subgroup associated with aggressive morphology and poor prognosis [5, 6]. Large molecularly classified cohort studies have reported TP53 mutation rates approaching 90–95% in endometrial serous carcinoma. However, pooled analyses of whole-exome sequencing studies have demonstrated variability, with an average TP53 mutation rate of approximately 75% (range 60–88%) [39]. In addition, several immunohistochemical series have reported rates of aberrant p53 expression below 80%, indicating that variability exists across studies and may reflect differences in methodology, case mix, and the use of IHC versus molecular confirmation. In our cohort, p53 abnormal (IHC) expression was observed in 3 of 5 serous carcinoma cases (60%). Given the small number of serous cases included and the absence of molecular confirmation, this finding should be interpreted cautiously.

Similar to previous reports, our study confirmed the independent prognostic impact of aberrant p53 expression. As the role of p53 is already well established as a surrogate marker of the copy-number-high molecular subtype, attention has shifted to additional biomarkers. In this context, PTEN emerges as a potentially valuable complementary biomarker alongside p53. In our cohort, loss of PTEN expression was associated with adverse pathological features such as LVSI, and this loss was more frequently observed in high histologic grade tumours, suggesting a link between PTEN inactivation and more aggressive tumour morphology. This observation aligns with evidence that PTEN alterations represent one of the most frequent and earliest molecular events in endometrioid endometrial carcinogenesis and can be reliably identified by immunohistochemistry, which has been shown to perform robustly for assessing PTEN status [13, 40]. Furthermore, studies evaluating the combined expression of p53 and PTEN have shown that their concomitant immunohistochemical profiles correlate with histologic differentiation and other clinicopathologic features, supporting the notion that PTEN may further refine risk assessment when interpreted together with p53 status [29].

The clinical implications of our results are twofold. Among the investigated biomarkers, p53 stands out as an easily accessible prognostic marker. If p53 is not tested, patients with apparently low-risk (FIGO stage IA and IB) tumours and p53 abnormalities may not receive adequate adjuvant treatment and follow-up. Even with limited resources, routine IHC assessment can be useful in preoperative planning (by identifying the expected risk for LVSI and G3 tumours) and justify a more intensive adjuvant strategy in borderline cases. PTEN, both as an indicator of LVSI and, when assessed in combination with p53, as a marker of an enhanced risk profile, adds valuable information to standard morphology. This approach is particularly relevant given the limited implementation of molecular classification in Bulgaria. Testing for POLE/DNA Polymerase Delta 1 (*POLD1*) mutational status by Polymerase Chain Reaction (PCR) or POLE NGS, along with systematic immunohistochemical evaluation of mismatch repair (MMR) proteins and p53, is not yet available at all centres. Even a partial panel assessment (MMR + p53) would improve risk stratification, and p53 is key, given the disproportionately high risk in the p53 abnormal subgroup. Although the tra-

ditional histopathologic grouping was retained for analytic consistency, the molecular classification proposed by TCGA provides a more accurate biological framework and supersedes the former type I/II dichotomy.

Taken together, our findings reinforce the central prognostic role of aberrant p53 expression in endometrial carcinoma and support its value as a robust surrogate marker of aggressive tumour biology. Our data also suggest that PTEN loss, although not independently prognostic, is associated with adverse pathologic features and may provide additional biological stratification within the heterogeneous p53-wild-type subgroup. Despite the heterogeneity in adjuvant treatment modalities across our cohort, aberrant p53 expression remained an independent prognostic factor for survival in multivariate analysis, underscoring its prognostic relevance beyond treatment-related effects. In line with contemporary TCGA-based and Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC)-based integrated risk models, these findings support the concept that combining molecular markers with established clinicopathologic parameters yields a more refined, clinically meaningful risk stratification in EC.

The strengths of the study are the uniform surgically staged cohort, the standardised IHC protocol, and the long follow-up period (until 31 December 2024). Limitations include the retrospective design, the moderate subgroup sizes (especially the p53mut group), the use of IHC for assessment, and the lack of information on IHC expression of p53 and PTEN during patient treatment.

5. Conclusions

Aberrant p53 expression was significantly associated with adverse clinicopathological features and poorer survival outcomes. PTEN loss (negative/low IHC expression) was correlated with LVSI but did not demonstrate independent prognostic value in multivariate analysis. Although combined expression patterns revealed differences in risk distribution, these findings appeared to be largely attributable to p53 status. Further prospective studies are needed to clarify the potential clinical relevance of PTEN in endometrial carcinoma.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

CT—conceptualisation, methodology, formal analysis and investigation, data collection, writing—original draft preparation, writing—review and editing, validation. SP—conceptualisation, methodology, formal analysis and investigation, writing—review and editing, validation. KP—methodology, formal analysis and investigation, data collection, validation. All authors contributed to critical editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Medical University–Pleven, Bulgaria (approval No. 733-KENID, dated 05 June 2023). Because this was a retrospective study, the requirement for individual informed consent was waived by the ethics committee, in accordance with institutional and ethical guidelines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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