

Molecular-based classification algorithm for endometrial carcinoma categorizes ovarian endometrioid carcinoma into prognostically significant groups

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The Cancer Genome Atlas classification divides endometrial carcinoma in biologically distinct groups, and testing for p53, mismatch repair proteins (MMR), and polymerase ϵ (*POLE*) exonuclease domain mutations has been shown to predict the molecular subgroup and clinical outcome. While abnormalities in these markers have been described in ovarian endometrioid carcinoma, their role in predicting its molecular profile and prognosis is still not fully explored. Patients with ovarian endometrioid carcinomas treated surgically in a 14-year period were selected. Only tumors with confirmation of endometrioid histology and negative WT1 and Napsin-A were included. *POLE* mutational analysis and immunohistochemistry for p53, MLH1, MSH2, MSH6, and PMS2 was performed in formalin-fixed, paraffin-embedded tissue. Following the molecular classifier proposed for endometrial carcinoma (*Br J Cancer* 2015;113:299–310), cases were classified as *POLE* mutated, MMR abnormal, p53 abnormal, and p53 wild type. Clinicopathologic information was recorded, including patient outcome. In all, 72 cases were included, distributed as follows: 7 (10%) *POLE* mutated; 6 (8%) MMR abnormal; 17 (24%) p53 abnormal; and 42 (58%) p53 wild type. The molecular classification correlated with disease-free survival in multivariate analysis ($P=0.003$), independently of tumor grade and stage. Correlation with overall survival approached statistical significance ($P=0.051$). *POLE*-mutated and MMR-abnormal tumors had excellent survival, whereas p53-abnormal tumors had significantly higher rates of recurrence and death. Ovarian endometrioid carcinoma can be classified in clinically meaningful subgroups by testing for molecular surrogates, akin to endometrial cancer. MMR and *POLE* alterations seem to identify a subset of ovarian endometrioid carcinomas with excellent outcome; conversely, abnormal p53 carries a worse prognosis. In the era of personalized medicine, the use of these markers in the routine evaluation of ovarian endometrioid tumors should be considered.

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In 2013, The Cancer Genome Atlas (TCGA) identified four genomic groups of endometrial carcinoma that correlate with clinical outcome.¹ The proposed classification has since then provided important insight into the biology of this prevalent gynecologic

disease and tools to better assess the prognosis and therapeutic options for endometrial cancer patients. The classification describes a novel subset of tumors with mutations in the polymerase ϵ (*POLE*) exonuclease domain and a consequent ‘ultramutated’ phenotype, which carry a favorable prognosis regardless of the tumor grade or histology. In contrast, tumors classified as ‘copy number high’ are associated with poor prognosis. The ‘microsatellite instability’ and ‘copy number low’ groups have intermediate disease-free survival.

On the basis of the above associations, determining the molecular group in patients diagnosed with

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endometrial carcinoma is clinically relevant. To achieve this goal cost-effectively, ancillary testing using formalin-fixed, paraffin-embedded tumoral tissue can serve as a surrogate of the genomic profile, and thus the molecular group.² A molecular-based classifier was recently proposed and validated by Talhouk *et al*,^{3,4} based on the TCGA classification using *POLE* mutational analysis as well as immunohistochemistry for p53 and mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2). This algorithm not only correlated with clinical outcomes similarly to the TCGA classification in univariate and multivariate analysis but also improved patient prognostic stratification when combined with established clinicopathologic risk factors. The main advantage of using such a tool is its potential feasibility, as immunohistochemical testing for p53 and mismatch repair proteins is already widely available in clinical laboratories.

Ovarian endometrioid carcinoma is a frequent primary epithelial malignancy of the ovary, closely related to endometrioid endometrial carcinoma. The architectural and cytologic morphology and the range of variant differentiation (squamous, mucinous, and secretory) largely overlap in both tumors. In addition, endometriosis is frequently found in patients with ovarian endometrioid carcinoma and is regarded as a non-obligatory precursor lesion.

The morphologic and biologic similarities between ovarian and endometrial endometrioid carcinomas suggest similarities in the genomic landscape. While abnormalities in p53, MMR, and *POLE* have been described in ovarian endometrioid carcinoma, the role of these markers in predicting the molecular profile and thus the prognosis is still not fully explored. We hypothesize that ovarian endometrioid carcinomas can be classified in clinically meaningful subgroups by testing for these markers as molecular surrogates, akin to endometrial cancer.

Materials and methods

This study was approved by the Research Ethics Boards at Sunnybrook Health Sciences Centre and Mount Sinai Hospital in Toronto, ON, Canada.

Case Selection and Review

In-house ovarian cancer patients treated surgically at Sunnybrook Health Sciences Centre between 2000 and 2013 were retrieved from the Anatomic Pathology database. After exclusion of metastatic tumors, cases with controversial site of origin, and cases with minimal microscopic disease post neoadjuvant chemotherapy, 702 patients with primary carcinoma were available. Tumors diagnosed as endometrioid type per World Health Organization criteria were further selected; cases with synchronous myoinvasive endometrial carcinoma or endometrioid metastases to ovary from endometrium on report

were excluded. Follow-up data were collected from the electronic patient record, including time to recurrence, last date of follow-up, and status at follow-up. Histologic diagnosis of endometrioid carcinoma was confirmed in all cases by independent review of all available hematoxylin-and-eosin-stained slides by one fellow and at least two gynecologic pathologists; only cases with full consensus on histotype were included. In addition, presence of at least one confirmatory endometrioid feature was required for inclusion: background of endometriosis and/or endometrioid adenofibroma or metaplasia (squamous, mucinous, and/or secretory). Further confirmation of the diagnosis involved the use of immunohistochemistry (see section below). Histologic grade and pathologic staging were recorded according to the international Federation of Gynecology and Obstetrics (FIGO) grading and staging systems.

Immunohistochemistry

Two 1 mm cores representative of the tumor were extracted from paraffin blocks and arrayed using a tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue microarray sections with monoclonal antibodies against p53 (DO7; Ventana), MLH1 (G168-15; Bio Care Medical), MSH2 FE11; Calbiochem), MSH6 (BC/44; Becton Dickinson), PMS2 (A16-4; Becton Dickinson), WT1 (6 F-H2; Ventana), Napsin-A (MRQ-60, Ventana), ARID1A (HPA005456; dilution 1:100; Sigma Aldrich), and PTEN (9559; dilution 1:25; Cell Signaling). A positive control was included in every run. All stains were reviewed independently by at least two observers. P53 was interpreted as abnormal (overexpressed with strong nuclear staining in more than 75% of tumor cells, or null with absent staining) or normal (nuclear staining with variable intensity and patchy distribution). When the two cores from any given tumor were discordant, a whole slide section was re-stained. Mismatch protein stains were scored as normal (retained nuclear staining) or abnormal (absent staining or very weak nuclear staining in < 5% of tumor cells). Cases without immunoreactive stromal cells in the microarray were further tested on whole tissue sections. WT1 and Napsin-A stains were interpreted as negative (absent staining) or positive (any intensity). ARID1A and PTEN stains were interpreted as retained (any intensity) or lost expression (absent staining).

POLE Mutational Analysis

DNA was isolated from microdissected tumor tissue (curls) obtained from block areas with high tumor cellularity (80% or more) marked by a pathologist. DNA extraction was carried out using the QIA-symphony DSP DNA Mini kit 192 Version-1 and

included a ProK digest buffer ATL (Qiagen, Hilden, Germany). DNA was amplified by PCR for exons 9, 11, 13, and 14 of the *POLE* gene on chromosome 12 (hg19 refGene: NM_006231). DNA dideoxy-capillary-based sequencing was performed on an ABI 3730 DNA Analyzer. Primer sequences are available on request. Analysis included the coding regions and splice sites. Data analysis was carried out using Mutation Surveyor software (Softgenetics, PA, USA). Variant classification was based on the 2015 American College of Medical Genetics and Genomics guidelines for interpreting sequence variants and followed assessment procedures described by the Canadian Open Genetics Repository.^{5,6}

DNA Molecular Classification Model

A model for molecular classification of endometrial carcinoma was used as proposed by Talhouk et al based on survival analyses, C-index, anticipated clinical benefit of testing, and cost/accessibility of methods.³ Tumors were divided into four groups based on *POLE*, MMR, and p53 results as follows (Figure 1):

- *POLE*-mutated group: any case with a pathogenic or likely pathogenic *POLE* mutation.
- MMR-deficient group: any remaining case with abnormal expression of any MMR marker.
- P53 abnormal: any remaining case with an abnormal p53 expression.
- P53 wild type: all remaining cases with absence of *POLE*, MMR, or p53 abnormalities as described above.

Statistical Analysis

For analysis purposes, time of recurrence and time of death were used to estimate disease-free and overall survival, respectively. The time to recurrence was defined from the date of primary surgery to the date of first recorded recurrence by imaging or tissue diagnosis. Likewise, time of death was defined from the date of primary surgery. Prognostic value of the molecular classifier categories and other variables was determined using univariate log rank test against overall, disease-free and disease-specific survival. Multivariate Cox proportional hazard regression model analysis was subsequently conducted to weight the molecular classifier, and other clinical and pathologic variables that were significant on univariate analysis (ie, FIGO stage, tumor grade, lymph node status and adjuvant therapy). The correlation between molecular groups and various clinicopathologic characteristics was calculated using appropriate statistical tests, ie, χ^2 -test for categorical variables and one-way ANOVA for continuous variables. All statistical analyses were performed using the SPSS software 24.0 (IBM Corporation, New York, NY, USA). *P*-values < 0.05 were considered statistically significant.

Results

A total of 97 cases with initial diagnosis of ovarian endometrioid carcinoma were identified. Of these, 16 were excluded after secondary review and immunohistochemistry: 15 tumors were re-classified as serous based on morphologic features and WT1 positivity, and 1 was re-classified as clear cell based on morphology and Napsin-A positivity. Of the remaining 81 cases, 9 were further excluded due to lack of clinical follow-up (6 patients) or failure to extract DNA for molecular testing (3 patients). The remaining 72 cases comprise the final cohort of the study. Median patient age was 53 years (mean 55, range 27–91). A total of 6 patients (8%) underwent salpingo-oophorectomy only; the remaining 66 patients (92%) underwent bilateral salpingo-oophorectomy plus hysterectomy, omentectomy, and/or peritoneal biopsies. Upon second review, 3 patients were found to have synchronous endometrial endometrioid carcinoma without myometrial invasion. In 1, the ovarian carcinoma showed normal p53 and absence of MLH1/PMS2 expression, whereas the synchronous endometrial tumor had normal p53 and retained MMR. The other two cases had normal p53 and MMR expression in both tumors; however, the ovarian carcinoma was FIGO grade 2 and the endometrial carcinoma was FIGO grade 1.

Five patients (7%) received neoadjuvant chemotherapy. Twenty-nine patients (40%) received adjuvant platinum-based chemotherapy, either after initial surgery or after first recurrence; three patients (4%) received adjuvant pelvic radiation. On the basis of clinical notes, none of the patients had family or personal history suggestive of *BRCA* mutation carrier status. None of the patients in our cohort had undergone counseling or genetic testing for *BRCA* or Lynch syndrome at the time of last follow-up.

Distribution by FIGO histologic grade was as follows: 33 (46%) grade 1; 24 (33%) grade 2; and 15 (21%) grade 3. All cases had at least one confirmatory endometrioid feature; ovarian endometriosis was identified in 65/72 (90%) cases. Endometrioid glandular morphology was consistently seen in all cases. Non-morular solid growth was present in varying proportions. Predominant transitional-like, papillary or micropapillary growth patterns were not observed in any of the cases included. The morphologic spectrum of ovarian endometrioid carcinoma in our series is depicted in Figures 1 and 2.

Figure 3 shows the case distribution following the molecular classifier. Among the 17 cases in p53-abnormal group, 9 (53%) tumors showed a strong and diffuse p53 staining pattern, whereas 8 tumors (47%) showed complete absence of staining. Most tumors in this category were low grade (12/17, 71%). None of the cases showed concurrent p53/MMR abnormality or p53/*POLE* abnormality.

Among the six carcinomas in the MMR-abnormal group, four were MLH1-/PMS2-deficient, one was

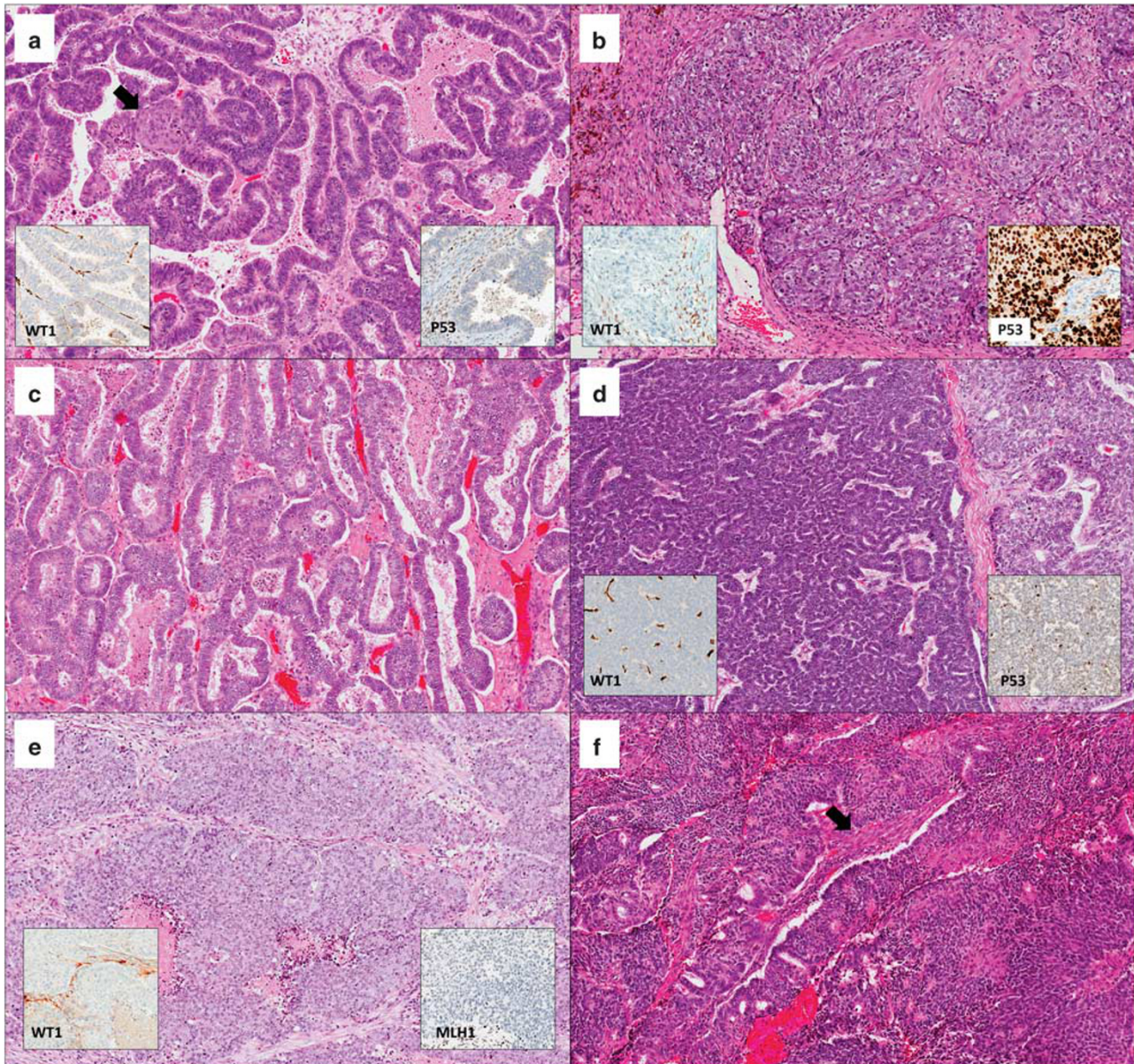


Figure 1 (a, b) Low-grade (a) and high-grade (b) ovarian endometrioid carcinomas, with negative WT1 and abnormal p53 expression (absent in a, overexpressed in b); both patients developed tumor recurrence. (c, d) Low-grade (c) and high-grade (d) ovarian endometrioid carcinomas with negative WT1 and wild-type p53 expression. (e) High-grade ovarian endometrioid carcinoma with MLH1 loss. (f) High-grade ovarian endometrioid carcinoma harboring a pathogenic *POLE* mutation. Arrows in (a and f) indicate foci of squamous differentiation.

MSH2-/MSH6-deficient, and one was MSH6-deficient only. A prominent intratumoral lymphocytic infiltrate was noted in two MMR-deficient tumors (one grade 2 and one grade 3).

The pathogenic or likely pathogenic variant mutations identified in the *POLE* gene are listed in Table 1. Of these, six constitute novel variants not previously described in ovarian endometrioid carcinoma. One tumor in the *POLE*-mutated group also showed loss of MLH1 and PMS2. A prominent intratumoral inflammatory component was observed in only one *POLE*-mutated case (grade 3).

Table 2 and Figure 4 show the distribution of clinical and pathologic variables by molecular group and grade. Compared to p53 wild-type and *POLE*-mutated tumors, the p53-abnormal and MMR-abnormal groups showed higher rates of ovarian surface involvement and advanced stage at presentation (FIGO stage II or more). The molecular subgroups did not show significant associations with FIGO grade, laterality, tumor size, or lymphovascular space invasion.

In all, 66 tumors were successfully tested for PTEN and ARID1A immunohistochemistry: 29 (44%)

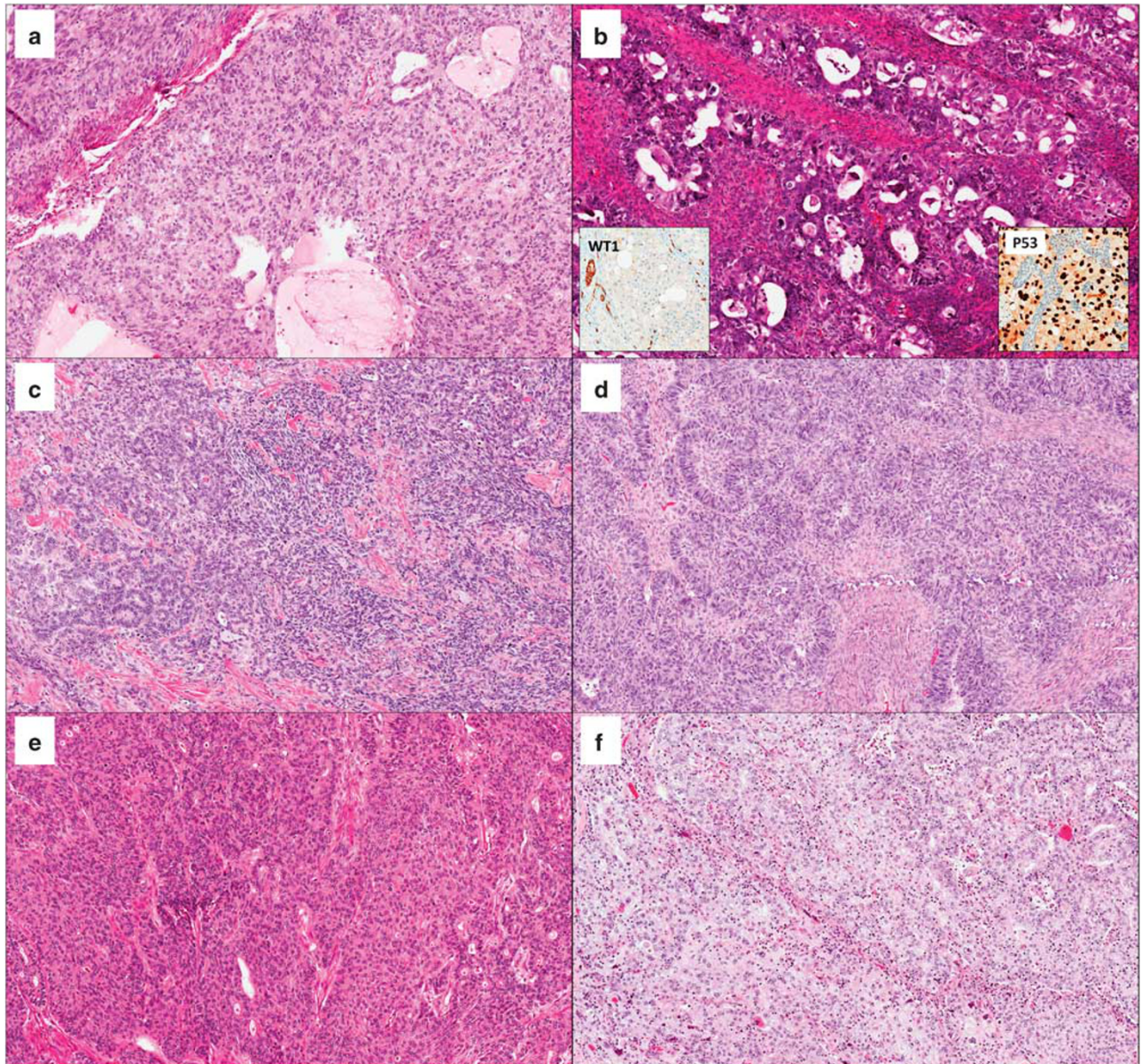


Figure 2 High-grade (FIGO 3) ovarian endometrioid carcinomas. (a–c) Tumors with abnormal p53 expression; these tumors are characterized by a predominant solid growth, admixed with endometrioid-type glands and exceeding 50% of the tumor volume; severe nuclear pleomorphism is noted in (a and b); inserts in (b) depict negative WT1 and p53 overexpression. (d, e) Tumors with wild-type p53; predominant non-morular solid growth; tumor in (d) had areas with sex cord appearance. (f) MMR-deficient tumor with glandular and solid architecture.

showed PTEN loss and 11 (17%) showed ARID1A loss. Distribution among cases divided by molecular group and FIGO grade is depicted in Table 3. Most (86%) *POLE*-mutated tumors showed PTEN loss, compared to 60%, 36%, and 40% in the MMR-abnormal, p53 wild-type, and p53-abnormal groups, respectively ($P=0.004$). Similarly, 60% of MMR-deficient cases showed ARID1A loss, compared to 17, 13 and 7% of *POLE*-mutated, p53 wild-type and p53-abnormal tumors, respectively ($P=0.02$).

The median follow-up period was 62 months (mean 70 months, range 1–179). Univariate survival

analysis results using log rank test are depicted in Table 4 and Figure 5. The four molecular categories obtained with the classifier correlated significantly with disease-free survival. P53-abnormal tumors carried a poor prognosis with a 5-year disease-free survival of 42%. P53 wild-type cases had intermediate prognosis with a 5-year disease-free survival of 82%. *POLE*-mutated and MMR-abnormal groups carried excellent prognosis with no recurrences or deaths reported (5-year disease-free survival = 100%). The 5-year overall survival rate for p53-abnormal, p53 wild-type, MMR-abnormal, and

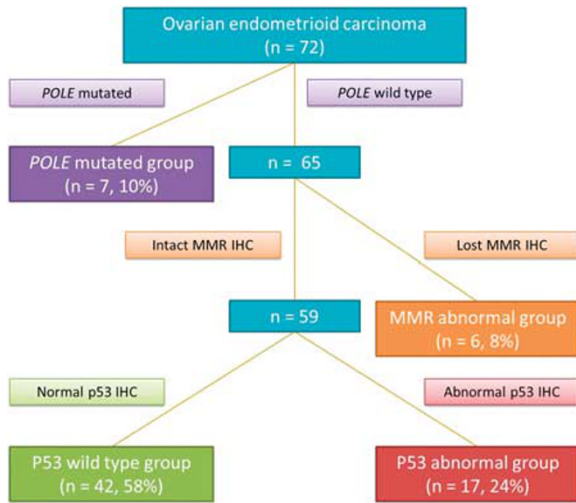


Figure 3 Diagram outlining patient distribution according to the molecular algorithm.

POLE-mutated groups was 76%, 86%, 100%, and 100% respectively. Bilaterality, tumor grade, advanced stage, and lymphovascular space invasion all were associated with worse disease-free, overall, and disease-specific survival. Ovarian surface involvement correlated with disease-free and disease-specific survival.

Table 5 shows multivariate analysis results. The molecular classification and stage were the only significant independent predictors of disease-free survival ($P=0.003$ and 0.026 , respectively, hazard ratio = 0.194 and 5.432 , respectively). In addition, the molecular classification was a near-significant predictor for overall survival ($P=0.051$, hazard ratio = 0.375 , 95% confidence interval $0.140-1.002$).

Discussion

Endometrioid carcinomas account for ~10–25% of all ovarian carcinomas.⁷ It is predominantly seen in perimenopausal women in the context of endometriosis, which appears to act as a precursor lesion.⁸ Endometrioid carcinomas frequently harbor AT-rich interactive domain 1A (*ARID1A*) mutations leading to loss of ARID1A protein expression,⁹ as well as B-catenin (*CTNNB1*) somatic mutations, *PTEN* mutations, and microsatellite instability.^{10,11}

Our aim was to apply the endometrial carcinoma molecular classification to ovarian endometrioid carcinoma using a validated classifier algorithm. The molecular classification for carcinoma of the endometrium represents a promising approach to stratify endometrial cancer patients into prognostically significant groups and therefore improve management. This positive impact in personalized care is particularly important in patients with high-grade endometrial carcinomas, a group with significant genomic heterogeneity and overlapping features with serous carcinoma^{12,13} and significant

Table 1 Pathogenic polymerase ϵ (*POLE*) exonuclease domain mutations identified in patients with ovarian endometrioid carcinoma

Case code	Exon	Mutation	Zygosity	Classification	Patient age	Tumor grade	Previously reported
OEC6	9	c.857C>T	Heterozygous	Likely pathogenic	44	1	Colon ³⁷
	9	c.857C>G	Heterozygous	Pathogenic			Ovary, endometrium, colon, brain ^{3,34,35,38-47}
OEC21	13	*p.P286R	Heterozygous	Likely pathogenic	46	1	Endometrium, colon ^{38,44,48}
OEC28	14	p.P436S	Heterozygous	Likely pathogenic	42	1	Endometrium, colon ^{3,37,44,49-51}
OEC76	13	p.A456P	Heterozygous	Likely pathogenic	53	2	Endometrium ^{34,39,44,47}
OEC79	13	*p.V411L	Heterozygous	Likely pathogenic	51	3	N/A
OEC88	13	c.1229G>A	Heterozygous	Likely pathogenic	41	2	Endometrium ^{34,39,44,47}
OEC90	9	*p.V411L	Heterozygous	Likely pathogenic	46	2	Colon, melanoma ^{37,52}
		p.V270M	Heterozygous	Likely pathogenic			

POLE gene reference sequence (refGene: NM_006231).

Table 2 Clinical and pathologic variables of patients with ovarian endometrioid carcinoma distributed by molecular group

	Group				P-value ^a
	<i>p53</i> abn	<i>p53</i> wt	MMR	POLE	
Number of cases	17 (24%)	42 (58%)	6 (8%)	7 (10%)	
Age (years)					
Median (mean)	55 (60)	55 (55)	55 (53)	46 (46)	0.147
Range	27–91	35–80	33–65	41–53	
Tumor size (cm)					
Median (mean)	10 (12.1)	14 (12.9)	12 (15.2)	16 (15.1)	0.642
Range	3.8–31	1.9–27	7.5–30	8–20.5	
Laterality					
Unilateral	12 (71%)	38 (90%)	5 (83%)	6 (86%)	0.294
Bilateral	5 (29%)	4 (10%)	1 (17%)	1 (14%)	
FIGO grade					
1	4 (24%)	24 (57%)	2 (33%)	3 (43%)	0.362
2	8 (47%)	11 (26%)	2 (33%)	3 (43%)	
3	5 (29%)	7 (17%)	2 (33%)	1 (14%)	
LVI					
Absent	13 (77%)	38 (90%)	3 (50%)	6 (86%)	0.074
Present	4 (23%)	4 (10%)	3 (50%)	1 (14%)	
EMS					
Absent	2 (12%)	5 (12%)	0	1 (14%)	0.836
Present	15 (88%)	37 (88%)	6 (100%)	6 (86%)	
Surface involvement					
Absent	8 (47%)	31 (74%)	1 (17%)	5 (72%)	0.029
Present	8 (47%)	11 (26%)	5 (83%)	2 (28%)	
Unknown	1 (6%)	0	0	0	
FIGO stage					
I	8 (47%)	31 (74%)	1 (17%)	6 (86%)	0.015
II–IV	8 (47%)	11 (26%)	5 (83%)	1 (14%)	
Unknown	1 (6%)	0	0	0	
Follow-up period (months)					
Median (mean)	35 (48)	65 (78)	84 (75)	63 (73)	0.133
Range	1–128	3–179	37–98	32–168	
Disease status at last follow-up					
ANED	6 (35%)	35 (83%)	6 (100%)	7 (100%)	Tables 3 and 4
AWD	6 (35%)	1 (2.5%)	0	0	
DOD	4 (24%)	5 (12%)	0	0	
DOC	1 (6%)	1 (2.5%)	0	0	

Abbreviations: Abn, abnormal; ANED, alive with no evidence of disease; AWD, alive with disease; DOC, dead of other causes; DOD, dead of disease; EMS, endometriosis; FIGO, International Federation of Gynecology and Obstetrics; LVI, lymphovascular space invasion; MMR, mismatch repair; wt, wild type.

^aP-values were obtained using χ^2 -test for categorical variables and one-way ANOVA for continuous variables.

interobserver variation in its diagnosis.^{14,15} In contrast to endometrial carcinoma, ovarian carcinoma subtypes have more distinct biologic and clinical differences, including stage, survival, and

management.^{16,17} Moreover, ovarian carcinoma subtyping has been demonstrated to have high reproducibility,^{7,18} which greatly improves with the use of a targeted immunohistochemistry panel.^{19–21} Although the existence of a high-grade ovarian endometrioid type has been debated, the evidence shows that a small, but significant subset of ovarian endometrioid carcinomas are indeed high grade.^{22,23} Moreover, low-grade endometrial tumors have potential for adverse outcome, as seen in our study. For these reasons, ancillary testing has a role in the work-up of ovarian endometrioid carcinomas beyond tumor type and grade, akin to endometrial carcinoma.

While histopathology and protein expression optimally separate ovarian carcinoma into clinically different types, further proteomic and genomic characterization may provide useful prognostic information. This is important for the ovarian endometrioid carcinoma subtype, which, similar to its uterine counterpart, represents a heterogeneous group of tumors. Although most ovarian endometrioid carcinomas are low risk and early stage, a subset has high-risk features and behaves aggressively, as seen in our study. We demonstrate that *p53*, MMR, and *POLE* analysis can be used to assess prognosis in ovarian endometrioid carcinoma as surrogates of the molecular profile, similar to endometrial endometrioid carcinoma.

Misclassification of other types of ovarian carcinoma, especially serous, as endometrioid is documented; in fact, it has been postulated that the majority of high-grade endometrioid carcinomas represent serous carcinomas with variant (solid, endometrioid, and transitional—SET) morphology.²³ To rule out mimics of endometrioid morphology, expert pathologist review was carried to confirm an endometrioid phenotype and exclude serous carcinomas with SET morphology. Histopathologic analysis showed a spectrum akin to that observed in endometrial tumors, characterized by endometrioid-type glands with smooth luminal borders and columnar cells. High-grade cases had non-morular solid growth exceeding 50% of the tumor volume; nuclear pleomorphism ranged from mild to severe. Moreover, all carcinomas in our study had at least one confirmatory endometrioid feature as an inclusion criterion (endometriosis, endometrioid adenofibroma background, and/or squamous, mucinous, or secretory metaplasia). In addition, we only included tumors that were negative for WT1 and Napsin-A by immunohistochemistry. WT1-positive ovarian endometrioid carcinomas have a gene expression profile similar to serous carcinomas, and likely represent serous tumors misclassified as or mimicking endometrioid morphology.^{22,23} Thus, under current immunohistochemical algorithms ovarian endometrioid carcinoma is defined as hormone receptor-positive, Napsin-A-negative and WT1-negative.^{19,20,24} Some authors have reported WT1 expression in up to 30% of endometrioid carcinomas,^{25,26} although misclassification of

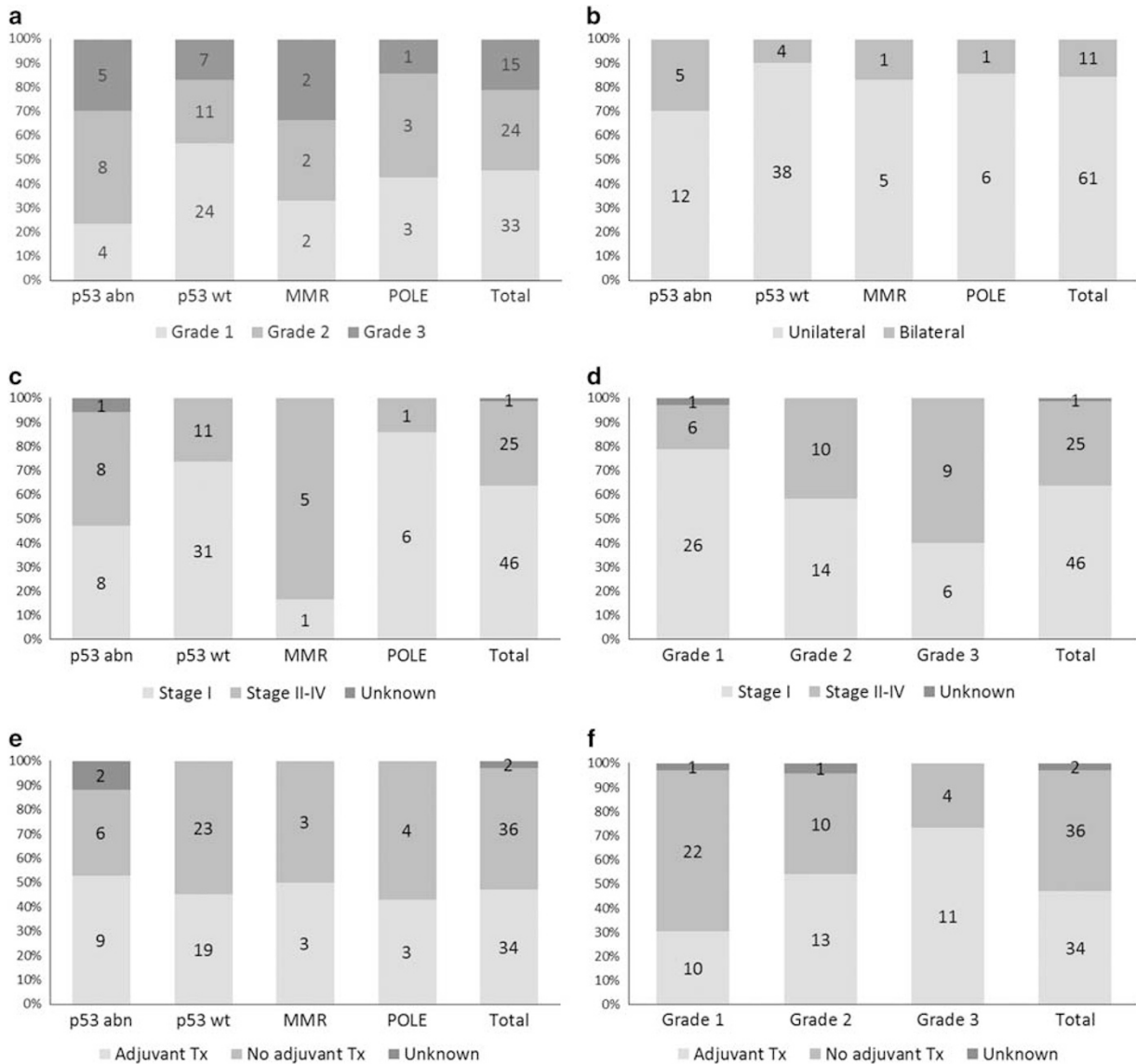


Figure 4 Case distribution according to molecular group *versus* histologic grade (a), molecular group *versus* tumor laterality (b), molecular group *versus* stage (c), grade *versus* stage (d), molecular group *versus* adjuvant treatment (e), and grade *versus* adjuvant treatment (f). Numbers in each bar represent absolute case numbers.

Table 3 ARID1A and PTEN loss by immunohistochemistry in ovarian endometrioid carcinomas classified by molecular group and grade

	ARID1A	PTEN
p53 abn (n = 15)	1 (7%)	6 (40%)
p53 wt (n = 39)	5 (13%)	14 (36%)
MMR (n = 5)	3 (60%)	3 (60%)
POLE (n = 7)	2 (29%)	6 (86%)
Grade 1 (n = 29)	5 (17%)	12 (41%)
Grade 2 (n = 23)	4 (17%)	10 (43%)
Grade 3 (n = 14)	2 (14%)	7 (50%)
Total (n = 66)	11 (17%)	29 (44%)

high-grade serous carcinomas as endometrioid in such studies is probable.²² On the basis of the criteria outlined above, tumor type misclassification in our cohort is considered unlikely.

The classifier used in this study allowed us to divide ovarian endometrioid carcinomas into four groups with distinct overall and disease-free survival. Among them, the ‘p53-abnormal’ group carried the worst prognosis. Abnormal p53 expression has been previously reported in 13–39% of ovarian endometrioid carcinomas, usually associated with grade 3 and advanced-stage tumors.^{22,27,28} In one cohort of 29 ovarian endometrioid carcinomas, p53 overexpression was present in 6 tumors (21%), most of them high grade.²⁹ We found a similar proportion of carcinomas with abnormal p53 expression (24%). Remarkably, in our study 4 out of the 17 tumors in the p53-abnormal group were grade 1; of these, 2 patients developed tumor recurrence. Moreover, 8 subjects (47%) had grade 2 tumors, of which two recurred and 2 died of disease. These observations

Table 4 Univariate analysis of survival in patients with ovarian endometrioid carcinoma

	DFS				OS			DSS		
	Total	Event	% of event	P	Event	% of event	P	Event	% of event	P
<i>Molecular group</i>										
p53 abn	17	10	58.8	< 0.001	5	29.4	0.094	4	23.5	0.214
p53 wt	42	6	14.3		6	14.3		5	11.9	
MMR	6	0	0.0		0	0.0		0	0.0	
POLE	7	0	0.0		0	0.0		0	0.0	
<i>Laterality</i>										
Unilateral	61	11	18.0	0.010	7	11.5	0.003	6	9.8	0.028
Bilateral	11	5	45.5		4	36.4		3	27.3	
<i>Surface involvement</i>										
Absent	45	5	11.1	0.001	5	11.1	0.077	3	6.7	0.024
Present	26	11	42.3		6	23.1		6	23.1	
<i>FIGO grade</i>										
1	33	3	9.1	0.006	2	6.1	0.009	1	3.0	0.003
2	24	6	25.0		4	16.7		3	12.5	
3	15	7	46.7		5	33.3		5	33.3	
<i>LVI</i>										
0	60	10	16.7	0.004	7	11.7	0.019	5	8.3	0.007
1	12	6	50.0		4	33.3		4	33.3	
<i>FIGO stage</i>										
I	46	4	8.7	< 0.001	4	8.7	0.011	2	4.3	0.002
II–IV	25	12	48.0		7	28.0		7	28.0	

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival; LVI, lymphovascular space invasion; OS, overall survival.

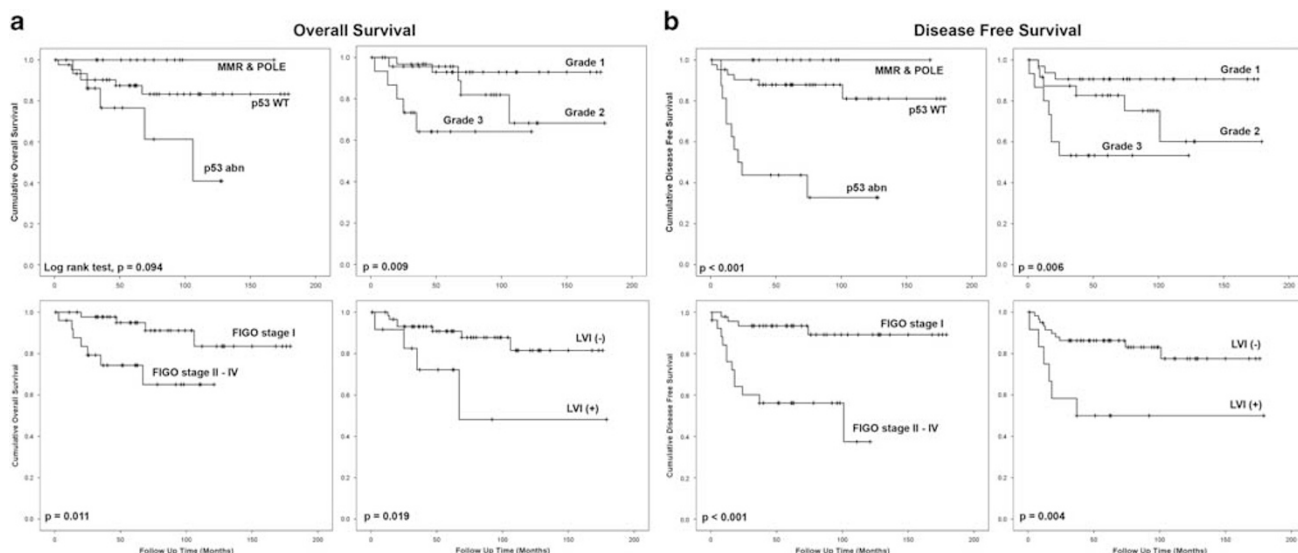


Figure 5 Kaplan–Meier survival analysis curves for disease-free survival (a) and overall survival (b). Each composite displays survival according to molecular subgroup (upper left), tumor grade (upper right), stage (lower left), and lymphovascular space invasion (LVI, lower right).

highlight the potential role of p53 as a marker of adverse behavior, independent of traditional parameters such as tumor grade.

Defective mismatch repair protein immunohistochemistry has been reported in 10–14% of ovarian endometrioid carcinomas,^{30–32} similar to the prevalence seen in our cohort (6/72 cases, 8%). Over

50% of MMR-deficient ovarian endometrioid carcinomas have loss of MSH2 and/or MSH6.^{31,33} In addition, a significant subset of these tumors has been related to Lynch syndrome.³² Previous studies have failed to demonstrate a significant correlation between MMR status and survival, possibly due to the low prevalence of MMR deficiency in

Table 5 Multivariate analysis of survival using Cox proportional hazards models in patients with ovarian endometrioid carcinoma

	P-value	HR	95.0% CI for HR	
			Lower	Upper
DFS				
Molecular group	0.003	0.194	0.066	0.572
Laterality	0.367	1.850	0.486	7.038
Surface	0.773	1.265	0.257	6.215
Grade	0.572	1.271	0.554	2.917
LVI	0.739	1.256	0.327	4.824
Stage	0.026	5.432	1.227	24.051
OS				
Molecular group	0.051	0.375	0.140	1.002
Laterality	0.081	3.960	0.842	18.628
Grade	0.215	1.857	0.698	4.940
LVI	0.976	1.026	0.188	5.590
Stage	0.084	3.718	0.839	16.482
DSS				
Molecular group	0.154	0.485	0.180	1.310
Laterality	0.251	2.796	0.482	16.207
Surface	0.487	0.464	0.053	4.053
Grade	0.110	2.665	0.800	8.878
LVI	0.833	1.237	0.171	8.961
Stage	0.043	9.630	1.079	85.953

Abbreviations: CI, confidence interval; DFS, disease-free survival; DSS, disease-specific survival; HR, hazard ratio; LVI, lymphovascular space invasion; OS, overall survival.

endometrioid ovarian carcinoma. Nonetheless, one study reported death in only 2/14 (14%) of patients with MMR-deficient tumors.³⁰ Our results show a good prognosis in the MMR-deficient group with no recurrences or death, suggesting that MMR status may also have value in patient prognostication. Interestingly, morphologic characteristics of MMR-deficient endometrial carcinomas such as a peri- and intratumoral dense lymphoplasmacytic infiltrate and syncytial-like appearance were not prevalent in our cohort, being observed in only 2 of 6 MMR-deficient ovarian tumors (1 FIGO grade 2 and 1 FIGO grade 3).

Mutations in the exonuclease domain of *POLE* have been found in 7–8% of endometrial carcinomas.^{1,3} Tumors with this alteration are described as ‘ultramutated’, as *POLE* mutations lead to an increased C to A transversion frequency in multiple genes. This group of carcinomas is characterized by an improved progression-free survival compared to other genomic groups, regardless of tumor grade.^{1,34} *POLE* exonuclease domain mutations were reported in 4 out of 89 (4.5%) ovarian endometrioid carcinomas in one study, all missense point mutations affecting previously known hotspots (P286R and V411L).³⁵ Of note, three of these tumors were FIGO grade 1 and one was FIGO grade 2; all four followed an indolent clinical course. The *POLE*-mutated group comprised 10% of our study cohort (7 out of 72 patients). Remarkably, all patients in the *POLE*-mutated group were alive with no evidence of

disease at time of follow-up, indicating that *POLE* has an important prognostic role in ovarian endometrioid carcinomas similar to endometrial cancer. Importantly, one tumor in this group was grade 3 and three were grade 2, suggesting that *POLE* mutations are not exclusive of low-grade (FIGO grade 1) tumors in the ovary. Indeed, *POLE* mutations (somatic and germline) have been reported in up to 29% FIGO grade 3 endometrial endometrioid carcinomas.³⁴ Similarly, in a separate study 63% of *POLE*-mutated endometrial carcinomas were FIGO grade 3.³⁶ This study also noted a prominent lymphoplasmacytic infiltrate in *POLE*-mutated endometrioid carcinomas; we observed such finding in only one FIGO grade 3 *POLE*-mutated ovarian endometrioid carcinoma. Loss of PTEN expression was seen in most *POLE*-mutated cases, at a higher rate compared to the other groups. This is consistent with previous literature showing frequent concurrent *POLE* and *PTEN* mutations in endometrial cancer.^{1,3}

Six *POLE* mutation variants were classified as either likely pathogenic or pathogenic. All pathogenic/likely pathogenic variants except one have been previously reported in the literature, mostly in endometrial and colorectal carcinomas (Table 1). Of note, only one of the variants found in our cohort has been previously reported in ovarian endometrioid carcinoma (c.857C>G, p.P286R).³⁵ The c.1229G>A variant, previously not reported, produces a nonsense mutation near the 5'-end of the exonuclease domain leading to a premature stop codon at position 410 (p.W410X), which predictably causes protein truncation and loss of function. Typically, missense mutations have been reported in the exonuclease domain as the type of variant expected to cause tumorigenesis. Thus, this variant is best classified as likely pathogenic. Loss of heterozygosity or allelic heterogeneity may be responsible for the clinical manifestations in this case.

In addition to the pathogenic/likely pathogenic variants described above, 20 variants of uncertain significance were identified in the *POLE* gene in this cohort (Supplementary Table 1). Although several in silico prediction software (AlighGVGD, SIFT, PolyPhen, Mutation TASTER, etc) suggest these variants may be deleterious, there was limited evidence either from the literature or other database sources to determine whether or not these variants are clinically significant according to the ACMG 2015 guidelines. Thus, the proportion of mutations in the *POLE* gene in patients with endometrioid carcinomas may be higher than what we report here. Further investigation into the mutational spectrum of the *POLE* gene is required to evaluate the clinical significance of these variants.

In summary, we demonstrate that ovarian endometrioid carcinoma is a heterogeneous group of tumors, harboring alterations in p53, MMR, and *POLE*. Such abnormalities are associated with patient outcome, and their detection can be useful

to subclassify ovarian endometrioid tumors into prognostically significant groups analogous to endometrial cancer. The value and feasibility of the proposed molecular algorithm seem promising, as the proposed markers are either already available in clinical laboratories or on the verge to enter into routine practice.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)