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Original Article

Clinicopathological and molecular characterization of high-grade endometrial carcinoma with POLE mutation: a single center study

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ABSTRACT

Objective: The molecular classification system of endometrial carcinoma (EC) in 'The Cancer Genome Atlas' is widely acknowledged for its prognostic utility. Subsequently, more simplified classification system that incorporate DNA polymerase epsilon (POLE) exonuclease domain mutations, mismatch repair deficiencies (MMRd), and abnormal p53 (P53abn) has also demonstrated its clinical utility. These classifications helped identifying a 'POLE ultramutated' (POLEmut) category of patients, most of whom show excellent prognoses despite having high-grade ECs. We aimed to investigate the clinicopathological and molecular characteristics of high-grade ECs with POLEmut.
Methods: We investigated 414 patients with high-grade ECs (including endometrioid carcinomas grade 3, serous carcinomas, clear cell carcinomas, mixed carcinomas, undifferentiated and dedifferentiated carcinomas, and carcinosarcomas) by sequencing and immunohistochemical staining.
Results: Forty-three tumors (10.4%) were classified as POLEmut, including 2 with new, possibly pathogenic POLE mutations at P286C and L424V. These patients had very good prognoses except for 1 with stage IV disease and residual tumor. Eleven patients in this group also had P53abn and 4 had MMRd; molecular analysis revealed that patients with synchronous POLE pathogenic mutation and other mutations had a POLEmut or MMRd phenotype; survival analysis found no difference in prognosis between these patient categories. The prognoses of patients in the POLEmut EC group were not significantly influenced by treatment or risk category.
Conclusions: Patients with high-grade EC exhibiting POLEmut have very good clinical outcomes, and should be identified urgently in daily work owing to their conflicting morphology. Our findings also provide guidance on subclassifying ECs with poor histological appearance.

Keywords: DNA Polymerase epsilon; Molecular; Endometrial Carcinoma; Endometrioid Carcinoma; p53; Mismatch Repair

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
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Synopsis
We investigated patients with high-grade endometrial carcinoma (EC) exhibiting POLEmut. We identified 2 new POLE mutations that are possibly pathogenic. All our 43 patients except for the one with stage IV disease had good prognoses. The prognoses of patients with POLEmut EC were not significantly influenced by treatment or risk level.

INTRODUCTION

Endometrial carcinoma (EC) was categorized into 4 molecular subtypes by The Cancer Genome Atlas (TCGA), of which classification has been shown to be a powerful prognostic guide of great clinical relevance [1,2]. The molecular subtyping of ECs has greatly enhanced the morphological classification of this disease most significantly because it accurately reclassified high-grade ECs exhibiting poor differentiation that nevertheless have good prognoses into the new category of DNA Polymerase epsilon (POLE) 'ultramutated' (POLEmut) [3]. The identification of patients with POLEmut is of high clinical significance [4]: on one hand, it provides more opportunities for women of childbearing age to complete their childbearing plan; on the other hand, it avoids the harm and waste of resources associated with overtreatment among women who are elderly or have no fertility requirements.

Many studies found that POLEmut commonly occurs with high-grade ECs [5,6]; however, the dynamics of this association is not fully clear. Therefore, it is necessary to identify patients with high-grade EC who exhibit POLEmut and further determine the ramifications of such mutations. Hence, we performed this study at our institution to investigate this very question.

MATERIALS AND METHODS

1. Patients and tissue selection

The archives of the Peking Union Medical College Hospital were searched using the terms "endometrioid carcinoma, grade 3", "serous carcinoma", "clear cell carcinoma", "mixed carcinoma", "undifferentiated and dedifferentiated carcinomas" and "carcinosarcoma"; patients who were treated between June 1, 2010 and October 31, 2018, were identified. A total of 414 cases with available formalin-fixed paraffin-embedded (FFPE) tumor tissues were analyzed. Samples with POLE exonuclease domain (exon 9–14) mutations were included, and those with pathogenic POLE mutations were deemed to be 'POLEmut'. Samples with abnormal P53 immunohistochemical staining were referred to as "P53abn" and those with mismatch repair (MMR) deficiencies were "MMRd". This study was approved by our Institutional Review Board (No. S-K688); informed consent was not required owing to the retrospective nature of the study.

2. Histopathological review

We retrieved the original diagnoses from the archived hysterectomy specimen data with matched curettage specimens if could, because sometimes tumor tissue was mostly in the curettage specimen but not hysterectomy specimen. Two additional gynecological pathologists then reviewed the hematoxylin and eosin-stained slides and reassigned the tumor histological type and grade. We limited our study to specimens with endometrioid carcinoma (grade 3), serous carcinoma, clear cell carcinoma, mixed carcinoma,

undifferentiated and dedifferentiated carcinoma, and carcinosarcoma. Each pathologist was blind to the original diagnosis and to the other pathologist's interpretation. Discrepant cases were examined under a multi-head microscope by more than 2 pathologists to arrive at a consensus.

3. DNA isolation, Sanger sequencing, and whole exome sequencing (WES)

Approximately 10 sections (10 μm) of every FFPE sample were prepared, and tumor tissue was collected by macro-dissection. After DNA isolation, sequencing of the POLE exonuclease domain (exon 9–14) was performed using the Sanger approach via both forward and reverse sequence tests as described previously [7]. Approximately 300 ng of high-quality genomic DNA was sheared and purified, after which libraries were prepared using the SureSelect Human All Exon V5 kit (Agilent Technologies, Santa Clara, CA, USA). WES was further performed using an Illumina NovaSeq6000 system following Illumina (San Diego, CA, USA)-provided protocols for 2×150 paired-end sequencing at Mingma Technologies Co., Ltd. (Shanghai, China). Somatic mutation on coding exons were used to perform mutational signature analysis, and R package deconstruct Sigs was used to decompose each tumor's mutation spectrum into 30 curated COSMIC signatures [8,9].

4. Immunohistochemical staining and interpretation of results

Immunohistochemistry for DNA MMR proteins and P53 was performed on representative whole FFPE slides (4 μm) using a BenchMark ULTRA autostainer, version 12.3 (Ventana Medical Systems, Oro Valley, AZ, USA). The samples were probed in accordance with the manufacturers' recommendations using primary antibodies for MLH1 (M1, 1 μg /mL; Roche, Basel, Switzerland), PMS2 (A16-4, 1 pg/mL; Roche), MSH2 (G219-1129, 20 μg/mL; Roche), MSH6 (SP93, 1 μg/mL; Roche), and P53 (MX008, ready to use; MX-BIO, Fuzhou Maixin Biotech, Ltd., Fuzhou, China). Two observers independently scored the entire slide and were blind to the patient's characteristics and clinical outcome. Discrepancies were resolved under a multi-head microscope by more than 2 pathologists to reach a consensus.

The MMR proteins MLH1, PMS2, MSH2, and MSH6 were deemed deficient if tumor nuclear staining was completely or partially absent in the presence of an intact internal control. Moreover, P53 protein expression was deemed aberrant if either ≥80% of tumor cells showed strong and diffuse nuclear staining, or a complete absence of nuclear staining was noted in the presence of an intact internal control, or significant cytoplasmic staining in the presence of variable nuclear staining was observed. Nuclear staining extents of 1% to 80%, with variable staining intensities, was considered normal P53 expression (wildtype). Immunostaining of a subclonal mutant P53 was defined as the presence of 1 of 3 aberrant patterns (when comprising 5–95% of the section), combined with a normal P53 expression pattern [10-12].

5. Statistical analysis

Clinical data were obtained by reviewing medical records, and follow-up data were available from the date of diagnosis to January 19, 2021. Progression-free survival (PFS) and disease-specific survival (DSS) were calculated using the Kaplan-Meier method and log-rank test. For analysis of PFS, any type of progression (e.g., recurrence or local/distant metastasis) was considered an event, while for DSS, only death caused by EC was considered an event. The SPSS software version 25.0 (IBM Corp., Armonk, NY) and Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA) were used for all statistical analyses, and statistical significance was set at a 2-tailed p-value <0.05.

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RESULTS

1. Mutational locus profiles and clinicopathological characteristics of 53 POLEmut high-grade ECs

We examined samples from 414 patients with high-grade EC, including 196 endometrioid carcinomas (grade 3 [G3]), 58 serous carcinomas, 39 clear cell carcinomas, 87 mixed carcinomas, 6 undifferentiated and dedifferentiated carcinomas, and 28 carcinosarcomas. The sequences of exons 9–14 of the POLE exonuclease domain were examined by Sanger sequencing, upon which 53 patients with POLE point mutations were identified. Among these patients, 41 had mutations that were known to be pathogenic [13], while the mutations in the remaining 12 were unreported and were of uncertain significance (i.e., it was unknown whether they were pathogenic or not). The most common pathogenic mutations in our cohort were P286R and V411L, which accounted for 76% (31/41) of all such mutations detected. Additional pathogenic mutations found included A456P (4 cases), M444K (3 cases), S459F (2 cases), and M295R (1 case) (**Fig. 1A**).

The highest proportion of POLE pathogenic mutation occurred in G3 endometrioid carcinoma (15.8%), followed by mixed cell carcinoma (8%), clear cell carcinoma (5.1%), carcinosarcoma (3.6%), and (the lowest) in serous carcinomas (1.7%). Moreover, a single POLE pathogenic mutation was detected among the 6 undifferentiated/dedifferentiated carcinomas (**Table 1**).

The mean age of the 43 patients with pathogenic POLE mutations (including 2 who were subsequently found to have a possible POLE mutant phenotype) was 54.6±10.6 years (range, 31–78 years). Most patients were of normal weight with a mean body mass index of 23.6±3.0 kg/m^2. Of those falling outside the normal range, 12 patients (27.9%) had a body mass index of over 25 kg/m^2 (overweight) and 1 (2.3%) was over 30 kg/m^2 (obese). Most patients had endometrioid carcinoma (31/43, 72.1%), International Federation of Gynaecological Oncology (FIGO) stage I (37/43, 86%), and no lymph node metastasis (38/43, 88.4%), whereas only 4.7% of the patients (2/43) presented with multiple areas of lymphovascular space invasion. Therapeutic approaches included surgery followed by observation, radiotherapy, chemotherapy, or chemoradiotherapy; radiotherapy was the most commonly used adjuvant treatment (17/43, 39.5%). The median follow-up time was 40 months (range, 16–106 months). The 5-year PFS and DSS of patients with POLE pathogenic mutations were 97.7% and 96.6%, respectively (**Fig. 1B** above the dotted line and **Table 2**).

According to European Society for Medical Oncology (ESMO), European Society for Radiotherapy and Oncology (ESTRO), and European Society of Gynaecological Oncology (ESGO) clinical practice guidelines of 2016 [14], 48.8% our patients were in the high-intermediate risk group and 46.5% were in the high-risk group. According to the ESMO-ESTRO-ESGO clinical practice guidelines of 2021 (molecular classification unknown) [15], 44.1% of our patients were in intermediate risk group, 30.2% were in the high-intermediate risk group (30.2%), and 20.9% were in the high-risk group (20.9%). When using the ESMO-ESTRO-ESGO clinical practice guidelines of 2021 (molecular classification known), most patients (93%) were in the low-risk group.

2. P286C or L424V may be pathogenic POLE mutations

WES analysis of 11 of our 12 patients with mutations of uncertain significance (1 patient had insufficient tumor tissue) revealed that 2 patient each with P286C or L424V had characteristics of a POLE pathogenic mutation.

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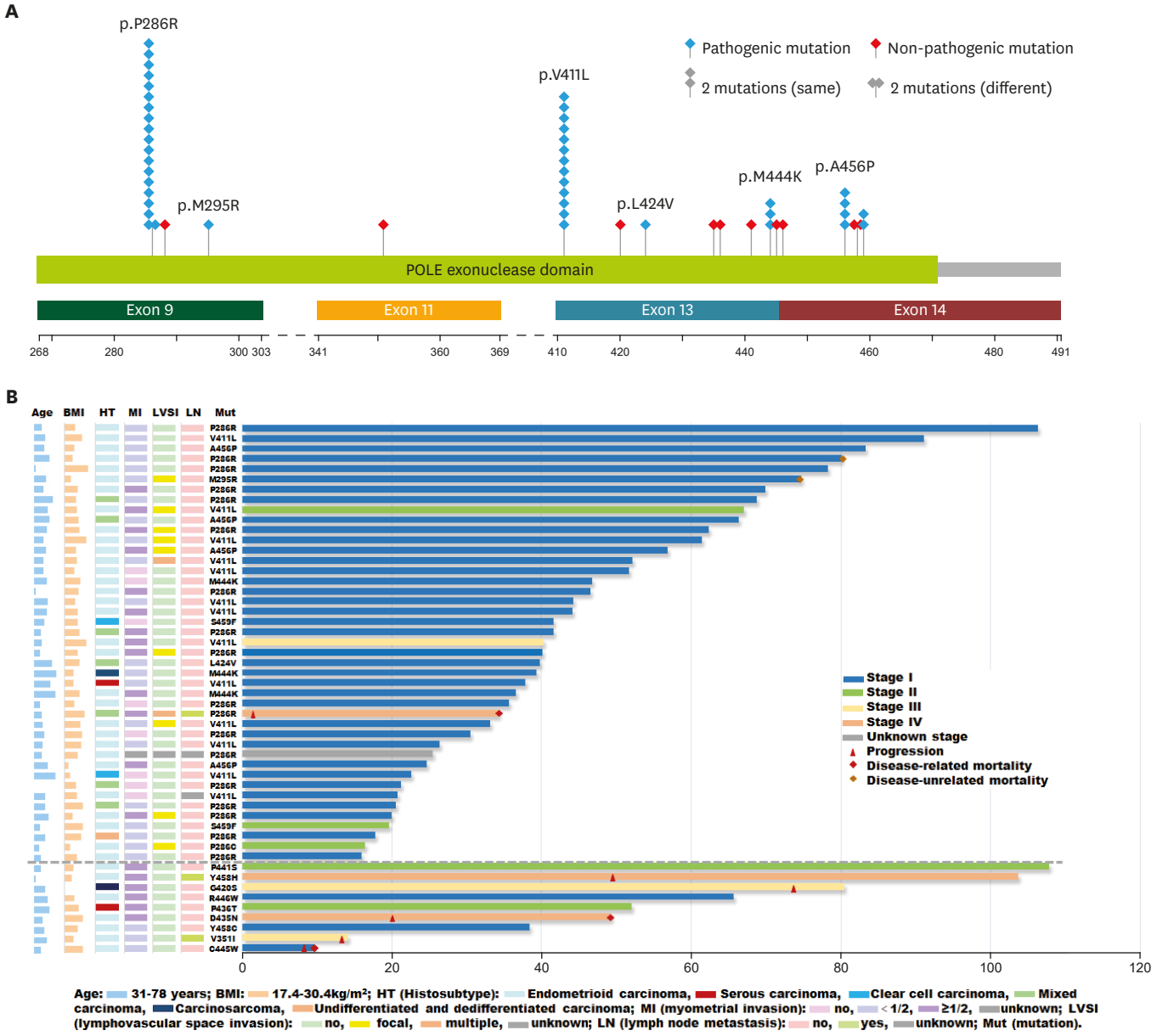

Fig. 1. The point mutation profile in POLE-mutated high-grade endometrial carcinomas (A). Clinicopathological features and survival data of POLE mutated patients (B).
BMI, body mass index; HT, histosubtype; LN, lymph node; LVSI, lymphovascular space invasion; MI, myometrial invasion; Mut, mutation; POLE, polymerase epsilon.

Table 1. POLE mutation profiles in patients with high-grade endometrial carcinoma (including carcinosarcoma) of the uterine corpus

Histosubtypes	No. of sequenced patients	No. of patients with exon 9–14 exonuclease domain mutation	No. of patients with pathogenic mutation	Proportion of patients with pathogenic mutation in different histosubtypes (%)
Endometrioid carcinoma, grade 3	196	38	31	15.8
Serious carcinoma	58	2	1	1.7
Clear cell carcinoma	39	2	2	5.1
Carcinosarcoma	28	2	1	3.6
Mixed carcinoma	87	7	7	8
Undifferentiated and dedifferentiated carcinoma	6	2	1	16.7
Total	414	53	43	10.4

POLE, DNA Polymerase epsilon.

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Table 2. Clinicopathological and molecular features of the 43 patients with endometrial carcinoma exhibiting pathogenic POLEmut

Variable	Total (n=43)	POLEmut-P53abn (n=11)	POLEmut-dMMR (n=4)	POLEmut-P53abn-dMMR (n=2)
Age				
Mean±SD	54.6±10.6	60.7±8.4	55.3±3.8	52±0
<60 yr	31 (72.1)	5 (45.4)	4 (100)	2 (100)
≥60 yr	12 (27.9)	6 (54.5)	0 (0)	0 (0)
Body mass index				
Mean±SD	23.6±3.0	24.4±3.7	25.1±3.0	26.3±4.7
<25.0 kg/m^2	31 (72.1)	6 (54.5)	3 (75)	1 (50)
≥25.0 kg/m^2	12 (27.9)	5 (45.4)	1 (25)	1 (50)
Histologic subtype				
Endometrioid carcinoma	31 (72.1)	5 (45.5)	4 (100)	2 (100)
Serous carcinoma	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Clear cell carcinoma	2 (4.7)	0 (0)	0 (0)	0 (0)
Carcinosarcoma	1 (2.3)	0 (0)	0 (0)	0 (0)
Mixed carcinoma	7 (16.3)	4 (36.4)	0 (0)	0 (0)
Undifferentiated and dedifferentiated carcinoma	1 (2.3)	1 (9.1)	0 (0)	0 (0)
FIGO stage				
Ia	28 (65.1)	9 (81.8)	2 (50)	2 (100)
Ib	9 (20.9)	1 (9.1)	2 (50)	0 (0)
II	3 (7.0)	0 (0)	0 (0)	0 (0)
III	1 (2.3)	0 (0)	0 (0)	0 (0)
IV	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Unknown	1 (2.3)	0 (0)	0 (0)	0 (0)
Lymphovascular space invasion				
Absent	31 (72.1)	7 (63.6)	1 (25)	0 (0)
Present (focal)	9 (20.9)	2 (18.2)	2 (50)	1 (50)
Present (multiple)	2 (4.7)	2 (18.2)	1 (25)	1 (50)
Unknown	1 (2.3)	0 (0)	0 (0)	0 (0)
Lymph node metastasis				
Absent	38 (88.4)	10 (90.1)	4 (100)	2 (100)
Present	3 (7.0)	1 (9.1)	0 (0)	0 (0)
Unknown	2 (4.7)	0 (0)	0 (0)	0 (0)
P53 staining				
Normal	32 (74.4)	0 (0)	2 (50)	0 (0)
Abnormal	11 (25.6)	11 (100)	2 (50)	2 (100)
Subclonal	2 (18.2)	2 (18.2)	0 (0)	0 (0)
Mismatch repair protein status				
Intact	39 (90.7)	9 (81.8)	0 (0)	0 (0)
Deficient	4 (9.3)	2 (18.2)	4 (100)	2 (100)
Adjuvant therapy				
Observation	6 (14.0)	0 (0)	0 (0)	0 (0)
Radiotherapy	17 (39.5)	1 (9.1)	1 (25)	0 (0)
Chemotherapy	6 (14.0)	4 (36.4)	0 (0)	0 (0)
Chemoradiotherapy	13 (30.2)	5 (45.5)	3 (75)	2 (100)
Unknown	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Risk classification (ESMO-ESTRO-ESGO clinical practice guidelines, 2016)				
Low risk	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate	0 (0)	0 (0)	0 (0)	0 (0)
High-intermediate	21 (48.8)	4 (3.4)	2 (50.0)	2 (100)
High	20 (46.5)	6 (54.5)	2 (50.0)	0 (0)
Advanced or metastatic	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Not assessable	1 (2.3)	0 (0)	0 (0)	0 (0)
Risk classification (ESGO/ESTRO/ESP clinical practice guidelines, 2021, molecular classification unknown)				
Low risk	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate	19 (44.1)	8 (72.7)	1 (25)	1 (50)
High-intermediate	13 (30.2)	2 (18.2)	3 (75)	1 (50)
High	9 (20.9)	0 (0)	0 (0)	0 (0)
Advanced or metastatic	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Not assessable	1 (2.3)	0 (0)	0 (0)	0 (0)

(continued to the next page)

Table 2. (Continued) Clinicopathological and molecular features of the 43 patients with endometrial carcinoma exhibiting pathogenic POLEmut

Variable	Total (n=43)	POLEmut-P53abn (n=11)	POLEmut-dMMR (n=4)	POLEmut-P53abn-dMMR (n=2)
Risk classification (ESGO/ESTRO/ESP clinical practice guidelines, 2021, molecular classification known)				
Low risk	40 (93.0)	10 (91.0)	4 (100)	2 (100)
Intermediate	0 (0)	0 (0)	0 (0)	0 (0)
High-intermediate	0 (0)	0 (0)	0 (0)	0 (0)
High	1 (2.3)	0 (0)	0 (0)	0 (0)
Advanced or metastatic	1 (2.3)	1 (9.1)	0 (0)	0 (0)
Not assessable	1 (2.3)	0 (0)	0 (0)	0 (0)

Values are presented as number (%) not otherwise specified.
dMMR, mismatch repair deficient; ESGO, European Society of Gynaecological Oncology; ESMO, European Society for Medical Oncology; ESP, European Society of Pathology; ESTRO, European Society for Radiotherapy and Oncology; FIGO, International Federation of Gynecological Oncology; P53abn, abnormal P53; POLEmut, polymerase epsilon ultramutation; SD, standard deviation.

The P286R (857C>G) mutation of the POLE gene is recognized as a pathogenic mutation. Aside from the patient with a C>G mutation at site 857, another had a C>T mutation at site 856, resulting in a shift of the coded protein (P286C). All parameters of this case, including tumor mutation burden (TMB), the POLE-score (a scoring system that assesses C>A substitutions >20%, T>G substitutions >4%, C>G substitutions <0.6%, indels <5%, and TMB >100 mut/Mb [13]) (**Table S1**), and COSMIC signature feature (**Fig. 2A**) suggested that the P286C mutation was consistent with a pathogenic mutation in POLE. And this patient shared a set of similar gene mutations with other POLEmut patients (**Fig. 2B**). Follow-up data showed that this patient had no recurrence and was alive 16 months after surgery. The histopathology of this tumor was high-grade endometrioid carcinoma (**Fig. 3A and B**) with cervical mesenchymal involvement (FIGO stage II), and postoperative radiotherapy and chemotherapy were performed given its high risk according to the 2016 ESMO-ESGO-ESTRO consensus risk assessment at diagnosis.

The L424V POLE mutation has been reported as pathogenic in colon cancer [16], but has not been confirmed as such in EC. The TMB, POLE score (**Table S1**), and COSMIC signature feature (**Fig. 2A**) of the single patient with this mutation clearly showed that the tumor had the characteristics of a POLE pathogenic mutation. Histopathological tissue analysis revealed a mixed endometrioid-serous carcinoma (**Fig. 3C and D**); the patient had experienced no recurrence or metastasis 40 months after surgery (**Table S2**, patient #9).

WES analysis of the 9 patients with mutations of uncertain significance showed that the POLE score values were all equal to or less than 3 (6 patients had scores of 0, while 1 patient each had a score of 1, 2, and 3). The 6 patients with POLE scores of 0 had low TMBs (<100) (**Table S1**), and none of them showed a COSMIC signature of 10 (POLEmut signature). The remaining 3 patients with POLE scores of 1/2/3 had a COSMIC signature 6/15 (i.e., an MMRd signature) (**Fig. 2A**).

On comparing the 43 patients with POLE pathogenic mutations (including the 2 with P286C and L424V) to the 10 patients with mutations of uncertain significance, the former showed significantly better prognoses than the latter (**Fig. 3E and F**).

3. Patients with synchronous POLE pathogenic and other molecular mutations exhibited the POLEmut or MMRd phenotype

We examined P53 and MMR protein expression in the 43 patients with EC who had POLE pathogenic mutations via immunohistochemistry and found that 13 of them had additional molecular alterations (11 had P53 mutations, 4 had MMRd, and 2 had both alterations) (**Fig. 4A**).

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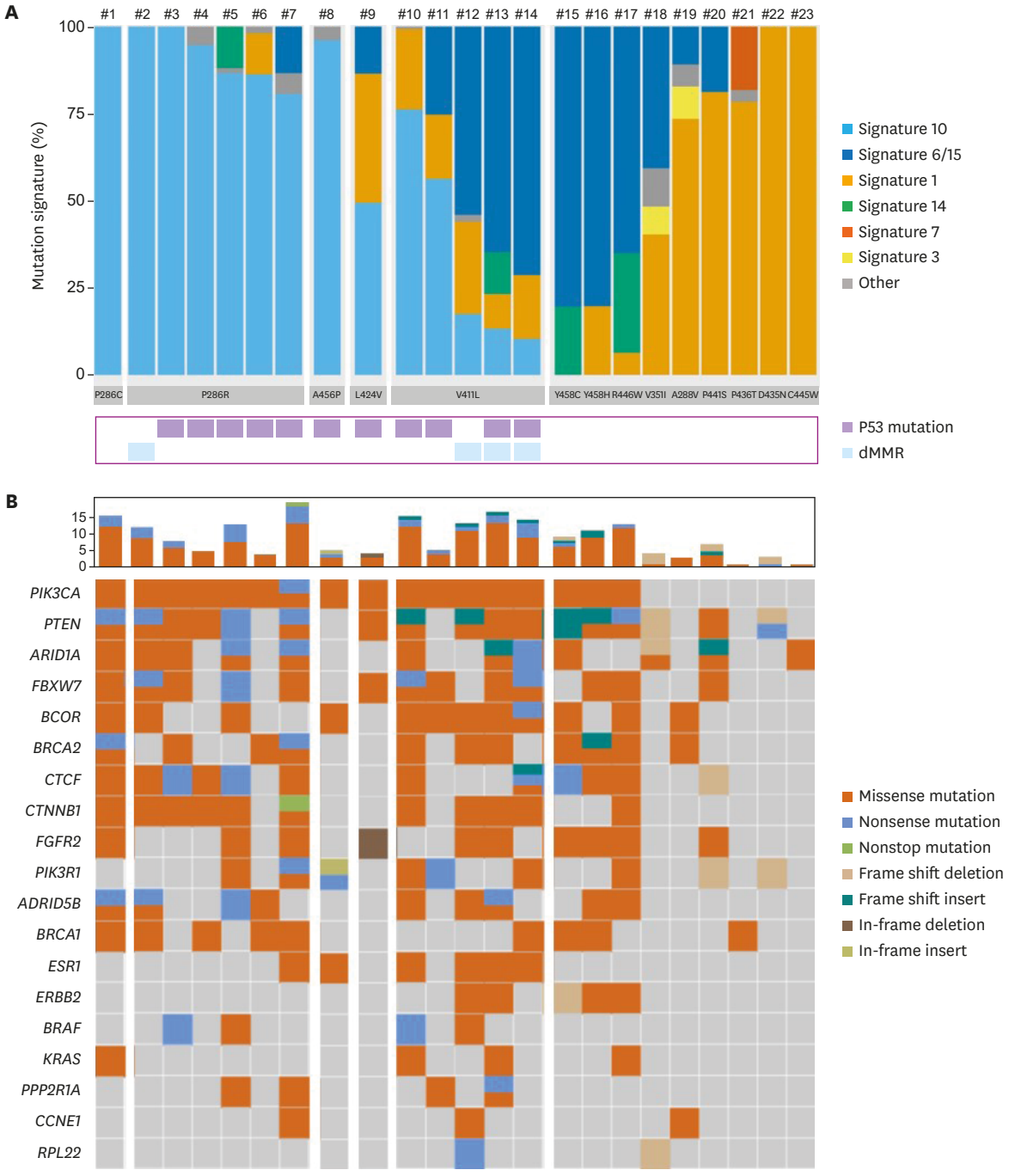

Fig. 2. The COSMIC signature feature of the patients with unreported POLE mutation (#1, 15–23) and the patients with multiple-molecular alterations (POLE mutation and MMR deficiency or P53 aberration) (#2–14) (A), and their main gene variations correspondingly (B).
MMR, mismatch repair; MMRd, mismatch repair deficiency; POLE, polymerase epsilon.

There were no differences in PFS and DSS between the 11 patients with P53 mutations and those with other (non-P53) POLE pathogenic mutations (**Fig. 4B and C**).

Furthermore, among the 13 patients with multiple molecular alterations, WES analysis of signature features revealed that 6 patients with P286R mutations displayed relative homogeneity (all showing COSMIC signature 10), while 5 with V411L mutations exhibited

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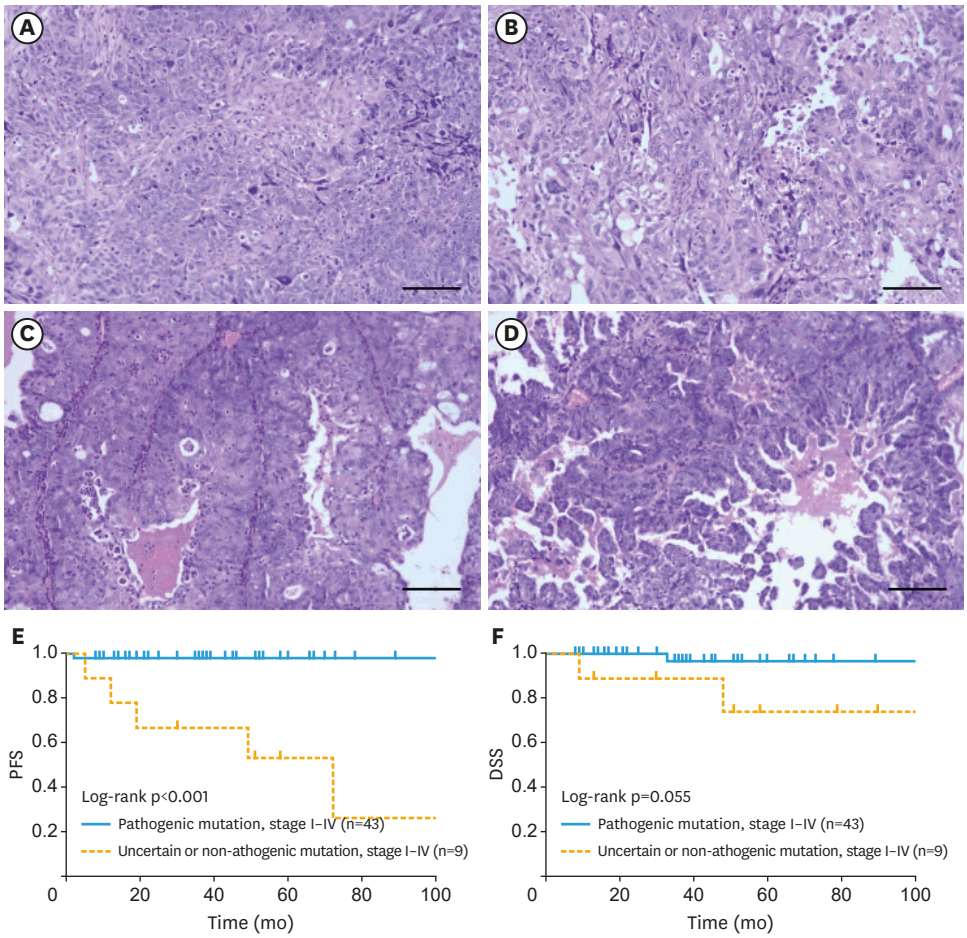

Fig. 3. The patients with P286C and L424V POLEmut showed high-grade endometrioid carcinoma (A and B) and mixed endometrioid-serous carcinoma (C and D) respectively (200 magnification). Forty-three patients with POLE pathogenic mutations showed significantly better prognoses than the patients with POLE mutations of uncertain significance in PFS (E) and DSS (F).
DSS, disease-specific survival; PFS, progression-free survival; POLE, polymerase epsilon; POLEmut, polymerase epsilon ultramutation.

heterogeneity (2 had COSMIC signature 10 and the remaining 3 had COSMIC signature 6/15; these latter 3 patients happened to have a deficiency in MMR protein expression) (**Fig. 2A**).

All 11 patients with synchronous pathogenic POLE and P53 mutations showed COSMIC signatures of 10 (9 patients) or 6/15 (2 patients), but their tumor signatures were not copy number-high. Meanwhile, 3 of the 4 patients with synchronous pathogenic POLE mutations and MMRd showed COSMIC signatures of 6/15. Moreover, 2 patients with triple molecular alterations also showed COSMIC signatures of 6/15 (**Fig. 2A**).

Patients with COSMIC signatures of 10 (**Fig. 2A**, patients #1–11) and those with COSMIC signatures of 6/15 (**Fig. 2A**, patients #12–17) shared a set of similar gene mutations from the WES, including in *PIK3CA*, *PTEN*, *ARID1A*, and other. However, the 'no specific molecular profile' group (**Fig. 2A**, patients #18–23 with no POLE pathogenic mutation, no MMRd, and no P53 mutation) showed few or no mutations in these genes (**Fig. 2B**).

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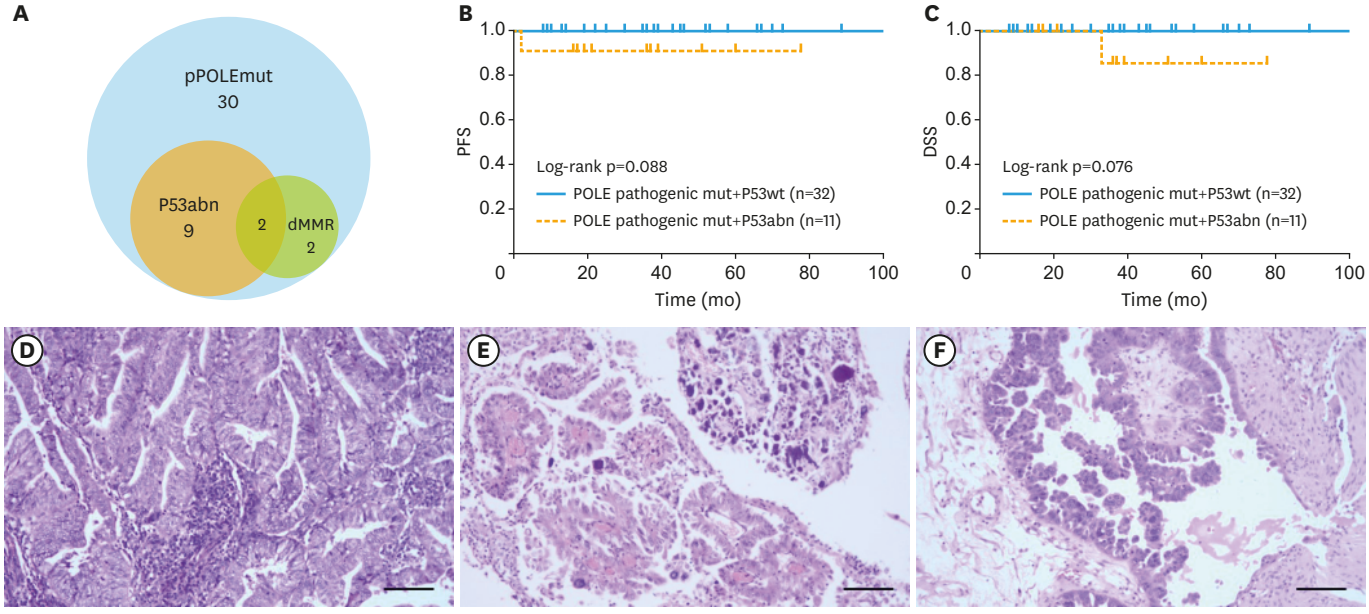

Fig. 4. Thirteen out of 43 POLEmut cases had additional molecular alterations (MMR deficiency or P53 aberration) (A). The comparison of the POLEmut patients with and without P53 aberration showed no significant differences in PFS (B) and DSS (C). The relapsed patient has a mixed endometrioid (D) -serous (E) carcinoma, with metastasis (F) (200 magnification).
DSS, disease-specific survival; PFS, progression-free survival; MMR, mismatch repair; POLEmut, polymerase epsilon ultramutation.

Of note, 1 patient in our cohort with dual molecular alterations (POLE pathogenic and P53 mutations) relapsed 3 months after surgery and died of the disease 34 months after. This was the only patient who experienced relapse and/or death in the pathogenic POLE mutation group (**Fig. 1B**). This individual had a pathogenic mutation at P286R of POLE, a POLE score of 6 (**Table S1**, patient #3), and COSMIC signature of 10 (**Fig. 2A**, patient #3); all molecular analyses demonstrated a clear POLE hypermutant phenotype. The histopathology of this tumor was mixed carcinoma (endometrioid-serous carcinoma) (**Fig. 4D and E**) with metastasis (**Fig. 4F**), and the clinical stage was FIGO stage IV with residual tumor. Although postoperative adjuvant radiotherapy and chemotherapy were administered, the patient ultimately died.

4. Lack of correlation in the prognoses of patients with POLEmut EC in different treatment or risk groups

Regardless of additional molecular alterations, patients with POLE pathogenic mutations were divided into 4 groups according to the adjuvant treatments they received: observation (6 patients), radiotherapy (17 patients), chemotherapy (6 patients), and chemoradiotherapy (13 patients). We found no differences in PFS and DFS between these 4 groups (**Fig. S1**).

Referring to the 2021 ESGO/ESTRO/European Society of Pathology (ESP) guidelines, patients with unknown molecular classifications were categorized into 4 groups according to their risk factors (including stage, grade, lymphovascular space invasion, histopathology, myometrial invasion, and residual disease). Nineteen patients were in the intermediate risk group, 13 were in the high-intermediate risk group, 9 were in the high-risk group, and 1 was in the advanced/metastatic group (this was the only patient with FIGO stage IV). There were no recurrences or deaths in the first 3 groups after a follow-up of 16–106 months, and our analysis revealed no differences between these 3 groups in terms of prognosis. When applying the 2021 ESGO/ESTRO/ESP guidelines for patients with known molecular classifications, the former 3 groups were all categorized into low-risk group (stage I–II

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POLEmut EC, no residual disease), while the patient with FIGO stage IV disease with residual tumors was classified into the advanced/metastatic group.

DISCUSSION

Molecular subclassification of EC cannot be performed without testing the POLE gene. On one hand, the pathogenic POLE mutation tremendously affects a patient's prognosis [17]; on the other hand, 3%–6% of patients with EC have multiple molecular alterations [12,18], including various combinations of POLE mutation, P53 mutation, and MMRd. Such patients usually have good prognoses as long as they possess the POLE pathogenic mutation, which are similar to patients with POLEmut-expressing ECs. Patients with unknown POLE gene statuses may be misclassified as having P53 mutations or MMRd, or else be deemed to have 'no specific molecular profile.' In our study, a high proportion of patients in the POLEmut group had P53 mutations (25.6%, 11/43), and had they not been tested for POLE gene status, they would likely be classified into the P53abn group based on the P53 protein analysis; this would have led to overtreatment and misjudging of prognosis.

Patients with POLEmut generally have early-stage disease [5]; in our study, 93% (40/43) of all POLEmut patients had stage I or II EC, which was consistent with previously reported data [19]. In terms of treatment strategy, our data suggest that the type of adjuvant therapy after surgery does not impact the survival of patients with POLEmut ECs who are FIGO stage I–II disease, which is consistent with the ESGO/ESTRO/ESP clinical practice guidelines [15]; as such, these data maybe support omitting additional adjuvant therapy for such patients, besides, the recent meta-analysis from a 294-patients group also give the similar conclusion [20]. However, FIGO stage III and IV patients with POLEmut are rare [5]; our cohort comprised only 1 patient each with stage III and stage IV (**Fig. 1B**). The individual with stage III had a very good prognosis (i.e., no recurrence or death 40 months after surgery), but the individual with stage IV had a poor prognosis (**Fig. 2**, **Tables S1** and **S2**, patient #3). The 2021 ESGO/ESTRO/ESP guidelines define patients with stage III–IV disease with residual tumor as advanced risk, regardless of molecular type [15]. However, no recommendations or instructions are provided for patients with stage III–IVA disease exhibiting POLEmut without residual tumors because of limited data.

The incidence of POLEmut in patients with mixed carcinomas was much higher than that in patients with non-endometrioid carcinomas in our study, accounting for 8% (7/87) of the mixed carcinomas; this proportion was consistent with a previously reported value (11%, 1/9) [21]. The 7 patients with POLEmut who had mixed carcinoma subtypes included 5 with mixed endometrioid-serous ECs and 2 with mixed endometrioid-clear cell ECs; these 2 histotypes are also the most common among mixed carcinomas [3]. In fact, the proportion of POLEmut in clear cell carcinoma or serous carcinoma is not high [22,23], which indicates that POLEmut is prone to appear in endometrioid carcinoma or carcinoma that involves the endometrioid component. The frequent presence of ambiguous morphology was observed in high-grade EC exhibiting POLEmut [24], and in our study poor inter-observers reproducibility in the diagnosis was relatively easy to occur in theses mixed carcinomas group. Therefore, if a patient in a real-life clinical setting with high-grade histology cannot be categorized based on morphology, the POLE gene status should be tested given that it is a powerful complement to the existing morphological classifications.

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Secondary P53abn occurred in 11 of 43 POLEmut ECs in our cohort (26%); separately, it has been reported in up to 42% of POLEmut ECs [1,12]. Molecular characterization analysis revealed that POLEmut-p53abn ECs are similar to POLEmut tumors [12], and our data showed no differences in prognosis and survival between these 2 subgroups, which further confirmed their similar clinical behaviors.

It was reported that the POLE mutation spectrum is shaped by several factors, including the mutant allele identity, its abundance, and MMR status [25]. In our cohort, 3 of 4 POLEmut-MMRd ECs presented with an MMRd signature but not a POLEmut signature, although these 3 patients carried the V411L POLE mutation.

Additionally, 2 patients with EC (0.48% of our cohort) had triple molecular alterations (concurrent POLEmut, P53abn, and MMRd); this is consistent with the reported frequency of 0.3% in TCGA and a pooled series study [12]. Complete details of the pathogenesis of multiple gene changes in EC remain unclear, although most studies support the notion that POLEmut is an initiating factor in pathogenic POLE mutation cases [12,26,27].

Our study exclusively focused on high-grade EC patients and was a relatively large sample size (n=414) over 8 years from a single center. However, several limitations existed in our study. Firstly, it was a retrospective study and was therefore subject to some inherent biases. Secondly, the proportion of mixed carcinoma was much higher in our cohort, which indirectly reflects the ambiguous or indistinct morphology features in these high-grade ECs and the molecular classification was eagerly needed to be introduced. Finally, small size of undifferentiated/dedifferentiated carcinoma patients was included because of its lowest incidence in population. Despite these limitations, our preliminary study demonstrated that POLE detection in molecular classification was vital and necessary among high grade ECs, and our data supported the integration of molecular typing into morphological classification.

In conclusion, our analysis of patients with high-grade ECs who have POLE mutations has provided a more detailed basis for their molecular subclassification, clinical treatment, and prediction of prognosis.

SUPPLEMENTARY MATERIALS

Table S1

Distinctive tumoral molecular alterations in patients with multiple pathogenic molecular mutations and those with POLE mutations of unknown significance

Click here to view

Table S2

POLE ultramutation-exhibiting EC with additional P53 mutations and/or MMR deficiency

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Fig. S1

No difference was found in PFS (A) and DFS (B) between 4 different treatment groups.

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