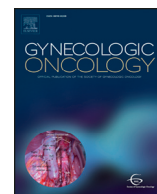




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POLE mutation combined with microcystic, elongated and fragmented (MELF) pattern invasion in endometrial carcinomas might be associated with poor survival in Chinese women

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HIGHLIGHTS

- *POLE* mutation was associated with favorable overall survival in this cohort.
- However, MELF invasion and higher staging were associated with increased progression risk in patients with *POLE* mutation.
- Thus, integrating *POLE* mutation with established clinicopathologic factors in the risk assessment of EC is safer.

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ABSTRACT

Objective. *POLE* mutation is a prognostic marker associated with excellent outcome in endometrial carcinoma (EC). However, these EC tumors frequently have aggressive histology. The aim of this study was to determine how to integrate the implications of *POLE* mutations into existing risk assessment strategies and further stratify patients.

Methods. We detected *POLE* mutations in a cohort of 426 ECs from Chinese women and observed their prognostic significance in terms of survival and recurrence outcomes in combination with histological and other molecular characteristics, including microcystic, elongated and fragmented (MELF) pattern invasion, histologic subtype, tumor grade, myometrial invasion and p53 protein and mismatch repair protein expression status.

Results. *POLE* mutations were identified in 38 of 426 ECs (8.9%). The most common mutations were P286R (31.6%), V411L (15.8%) and Q453R (15.8%). We confirmed that *POLE* mutation was associated with improved overall survival ($P = .047$), although it did not show a statistically significant relationship with progression-free survival ($P = .45$). Interestingly, further analyses indicated that in *POLE*-mutant tumors, MELF pattern invasion was associated with a 15.1-fold increase in tumor recurrence or progression risk ($HR = 15.1$, 95%CI = 1.57–145.3, $P = .018$), whereas this phenomenon was not present in the *POLE*-wild-type subgroup ($HR = 0.90$, 95%CI = 0.39–2.08, $P = .80$). Furthermore, higher staging and deeper myometrial invasion also showed much higher risk in patients harboring *POLE* mutations compared with those without *POLE* mutations.

Conclusions. Although *POLE* mutation was associated with favorable overall survival, the combined consideration of *POLE* mutation status and established clinicopathologic factors in the risk assessment of endometrial cancer is more accurate than the consideration of clinicopathologic factors alone and might lead to precise and individualized therapeutic strategies.

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1. Introduction

Endometrial carcinoma (EC) is the most common malignant disease of the female genital system and comprises several histologic types with distinct clinical features. Over the last decade, it has become increasingly apparent that endometrial cancers are a heterogeneous group of

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tumors not only in terms of biology, histology, and clinical features but also concerning their genetic make-up [1].

Recently, The Cancer Genome Atlas (TCGA) project stratified endometrial carcinomas into four prognostic groups based on genomic features: an ultramutated phenotype caused by *POLE* mutations, a hypermutated phenotype caused by DNA mismatch repair deficiency (dMMR) and the resultant microsatellite instability (MSI), a copy number-low phenotype, and a copy number-high phenotype [2]. A novel subgroup, of the “ultramutated” group harboring *POLE* exonuclease domain mutations included 7–12% of endometrial cancers. This subtype is characterized by somatic mutations in the exonuclease domain of *POLE*. *POLE* encodes the catalytic subunit of DNA polymerase [3,4]. Mutations in the exonuclease domain of *POLE* reduce proofreading activity during DNA replication and thereby contribute to a very high mutational burden. On the basis of the current and previous studies, *POLE*-mutant endometrial cancer will present at a relatively young age as a low-stage high-grade endometrioid-type tumor with a striking immune infiltrate. Despite the aggressive histologic appearance of these tumors, multiple independent studies have shown that *POLE* mutations in endometrial cancers are associated with markedly favorable survival [5–8]. *POLE* mutations are considered prognostic markers associated with excellent outcomes for endometrial carcinoma patients. Given that these tumors are frequently of high grade but have a very favorable prognosis, the question is raised of how to integrate the implications of *POLE* mutation into existing risk assessment strategies for EC.

In existing risk assessment strategies for EC, pathologic characteristics including high grade, lymph node involvement, lymphovascular space invasion (LVSI) and deep myometrial invasion (50%) are conventional poor prognostic indicators [9,10]. As molecular typing should be closely combined with histological characteristics in risk assessment strategies for EC, an integrated pathological and molecular indicator may be needed to refine the clinical risk prediction of EC patients and to provide more tailored treatment recommendations. In addition to conventional prognostic indicators, during 2003, Murray et al. first described a new invasion pattern in patients with EC, which was defined as the microcystic, elongated and fragmented (MELF) pattern. This pattern is histologically characterized by microcystic glands invading the myometrium that are elongated or clustered and lined by flattened epithelium [11]. Although it was initially thought to represent a simple degenerative process, subsequent studies suggested that the MELF pattern represented an active cellular process and epithelial-mesenchymal transition (EMT)-like process based on immunohistochemical findings such as loss of E-cadherin, membranous beta-catenin, hormone receptors, galectin-3, CD147, and matrix metalloproteinase-2 and upregulation of cyclin D1, fascin, cytokeratin 7, cytokeratin 19, and p16 [12–18]. Despite the documented association of the MELF pattern with lymphovascular space invasion (LVSI) and lymph node metastasis in patients with EC, it remains uncertain whether the presence of MELF pattern invasion has clinical prognostic significance in EC [15–18]. Our study therefore included MELF pattern invasion as one of the histological characteristics to be studied and explored its clinical prognostic significance.

In the present study, we investigated how to integrate *POLE* mutational status and histological characteristics into risk assessment strategies for EC to select patients who are considered high risk and tailor the management of the disease. We detected *POLE* mutations in a cohort of 426 ECs from Chinese women and observed their prognostic significance in terms of survival and recurrence outcomes when used in combination with histological characteristics and other molecular typing, including MELF pattern invasion, histologic subtype, tumor grade, myometrial invasion and p53 expression status. The aim of this study was to determine how to integrate the implications of *POLE* mutations into the existing risk assessment strategies and further stratify patients. In other words, the goal was to determine what kind of patients will fare poorly.

2. Materials and methods

2.1. Patients and samples

Formalin-fixed, paraffin-embedded (FFPE) tissue samples of 426 endometrial carcinomas collected between 2011 and 2016 were obtained from the Surgical Pathology files of the First Hospital, Peking University, China. The endometrial carcinoma cases were reviewed and classified using the 2014 World Health Organization criteria [19]. Tumors were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) guidelines [20]. The study was approved by the institutional ethics committee.

2.2. *POLE* polymerase chain reaction, sequencing, and mutational analysis

The hematoxylin and eosin-stained slides were checked by a pathologist to identify the tumor region. Genomic DNA was then isolated from the corresponding FFPE tissues for *POLE* mutation screening, which was performed fully automated with the Tissue Preparation System (MagCore Automated Nucleic Acid). The primer sequences used for the *POLE* mutation analyses are shown in Table S1.

By bidirectional Sanger sequencing, *POLE* was assessed for mutations in regions of the exonuclease domain (exons 9, 13, and 14) that harbor the majority of deleterious mutations, as described [21–23]. The observed mutational status was confirmed by 2 independent amplification and sequencing reactions.

2.3. Assessment of MELF pattern invasion

Two pathologists reviewed the cases for MELF pattern invasion, histologic subtype, tumor grade, lymphovascular space invasion, myometrial invasion and lymph node metastasis. MELF pattern invasion was defined as described by Murray et al. [11]. MELF pattern invasion is histologically characterized as neoplastic glands with outpouchings, which can show detachment, lined by a flattened epithelium, giving a “microcystic” appearance. These glands can appear “elongated” or “fragmented” into small clusters or single cells in a fibromyxoid stroma [24]. Representative photographs of MELF pattern invasion are presented in Fig. 1. We also investigated cytokeratin 19 (CK19) by immunohistochemical staining, since it has been reported that MELF-type tumor epithelium is consistently and strongly CK19 positive [15]. Immunohistochemical staining was performed using the Dako EnVision system (Dako, Herndon, VA, USA) on 3.5 μ m sections of FFPE tissue with primary antibodies against CK19 (ES05, 1:50, Abcam, Cambridge, UK).

2.4. Statistical analyses

The associations between biomarker alterations and clinicopathological features were calculated by Fisher's exact test and the χ^2 test. Overall survival (OS) was defined as the interval between the date of histological diagnosis of EC and either the date of death or the last known date on which the patient was still alive. Progression-free survival (PFS) was defined as the interval between the date of histological diagnosis and the first confirmed sign of recurrent disease or progression of the tumor; if there was no recurrence, the end date used was the date of the last follow-up. Survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. We used Cox proportional hazards models to calculate hazard ratios (HRs) for PFS and OS of *POLE*-mutant ECs relative to *POLE*-wild-type tumors by univariate analyses. A *P* value ≤ 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

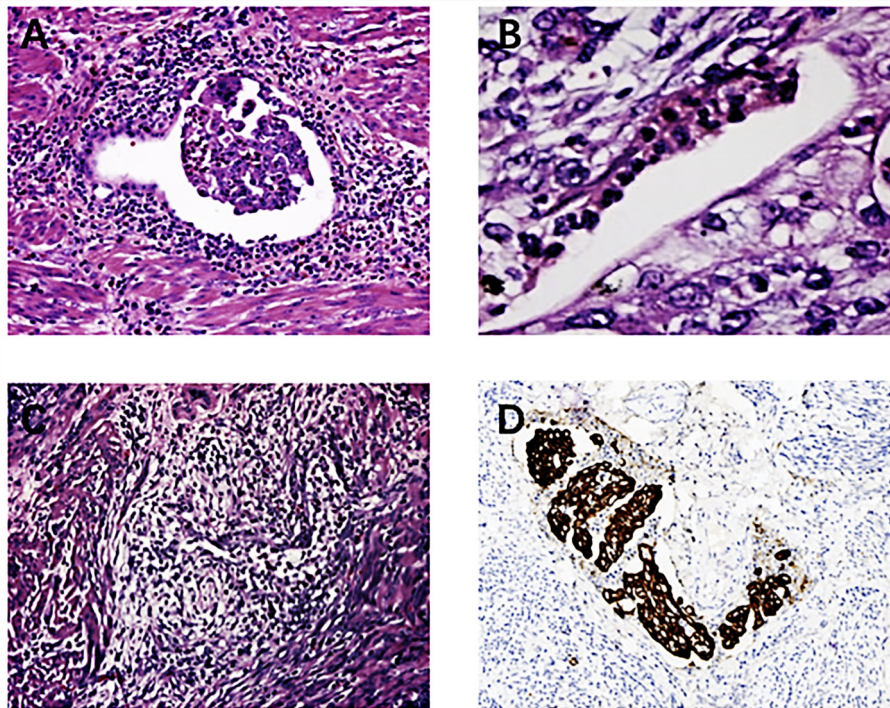


Fig. 1. Representative images of microcystic (A), elongated (B) and fragmented (C) (MELF) pattern invasion areas in endometrial carcinoma showing obvious cytokeratin 19 staining at 20 \times magnification. The majority of the tumors showed weakly positive cytokeratin 19 expression. In contrast, the areas of MELF pattern invasion were strongly positive, showing obvious staining at 20 \times magnification (D).

3. Results

3.1. Clinical and pathological findings

All 426 patients underwent surgical resection with staging and had received no prior treatment for their disease, including chemotherapy or radiotherapy. The median age of the patients was 54 years (range, 26–83 years). The majority presented at an early stage (FIGO IA; 72.5%), whereas the remainder presented at an advance stage (FIGO stage IB–IV; 27.5%). Among the 426 tumors, 364 were endometrioid carcinomas (4 of them containing a second component of mucinous carcinoma), 35 were serous carcinomas, 6 were clear cell carcinomas, 11 were mixed carcinomas, 6 were undifferentiated carcinomas and 4 were mixed serous and clear cell carcinomas. MELF pattern invasion was identified in 10.1% (43/426) of patients with endometrial carcinoma (Fig. 1). Eighty-five (19.9%) patients received adjuvant therapy (chemotherapy) after surgical resection. The median follow-up time was 55 months (range, 17–85 months). During the follow-up period, 48 patients died. With regard to the long-term outcomes, tumors recurred in 74 (17.4%, 74/426) patients. All the clinicopathological characteristics of the patients are shown in Table S2.

3.2. *POLE* mutations in 426 cases of endometrial carcinoma

A total of 38 cases (8.9%, 38/426) harbored missense mutations of *POLE* in the regions analyzed. Each case contained one missense mutation. Twenty mutations occurred in exon 9, seventeen mutations were in exon 13, and one was in exon 14. All of the mutations were missense mutations. The most common mutations were P286R (31.6%, 12/38; in exon 9), V411L (15.8%, 6/38; in exon 13) and Q453R (15.8%, 6/38; in exon 13) (Fig. 2A). In addition, we found 3 novel mutations (F274L, G420D, and V460A) that had not been reported in previous studies (Fig. 2B). They had not been reported in the literature or in the Catalog of Somatic Mutations in Cancer (<http://www.Sanger.ac.uk/genetics/>

CGP/cosmic). The frequency and distribution of the mutations are shown in Fig. 2C.

According to clinicopathological features, patients with *POLE* mutations were significantly more likely to have nonendometrioid tumors (34.2%, 13/38) than those without *POLE* mutations (12.6%, 49/388; $P = .001$). Regardless of histology, 18 of 38 (47.4%) patients with *POLE* mutations had high-grade carcinomas, whereas only 23.2% (90/388) patients without *POLE* mutations had high grade carcinomas, and the difference was statistically significant ($P = .003$). No significant differences were found between *POLE* mutation and other clinicopathological features, including stage, myometrial invasion, LVSI, lymph node metastasis and MELF pattern invasion (Table 1).

3.3. Clinical prognosis of EC patients with respect to stratification by *POLE* mutation

All patients with *POLE* mutations were alive with a median follow-up of 55 months. *POLE*-mutant tumors were found to be associated with improved OS ($P = .047$; Fig. 3A). The PFS curve is shown in Fig. 3B. Among the 38 patients with *POLE* mutations, four patients suffered from tumor recurrence or progression (11.3%, 4/38), while 70 patients with a wild-type tumors experienced recurrence (18.0%, 70/318), although the difference did not reach statistical significance ($P = .45$; Fig. 3B).

In addition, all other histological variables, including high stage, nonendometrioid histology, high grade, deep myometrial invasion, positive LVSI and positive lymph node metastases, were statistically significantly associated with increased tumor recurrence or progression ($P < .05$; Table 2). However, we did not find any associations of MELF pattern invasion with tumor recurrence or progression in the whole cohort.

Next, we assessed whether these associations were modified by *POLE* mutation status. In this study, we conducted stratified analysis by *POLE* mutation status. Interestingly, the association between MELF

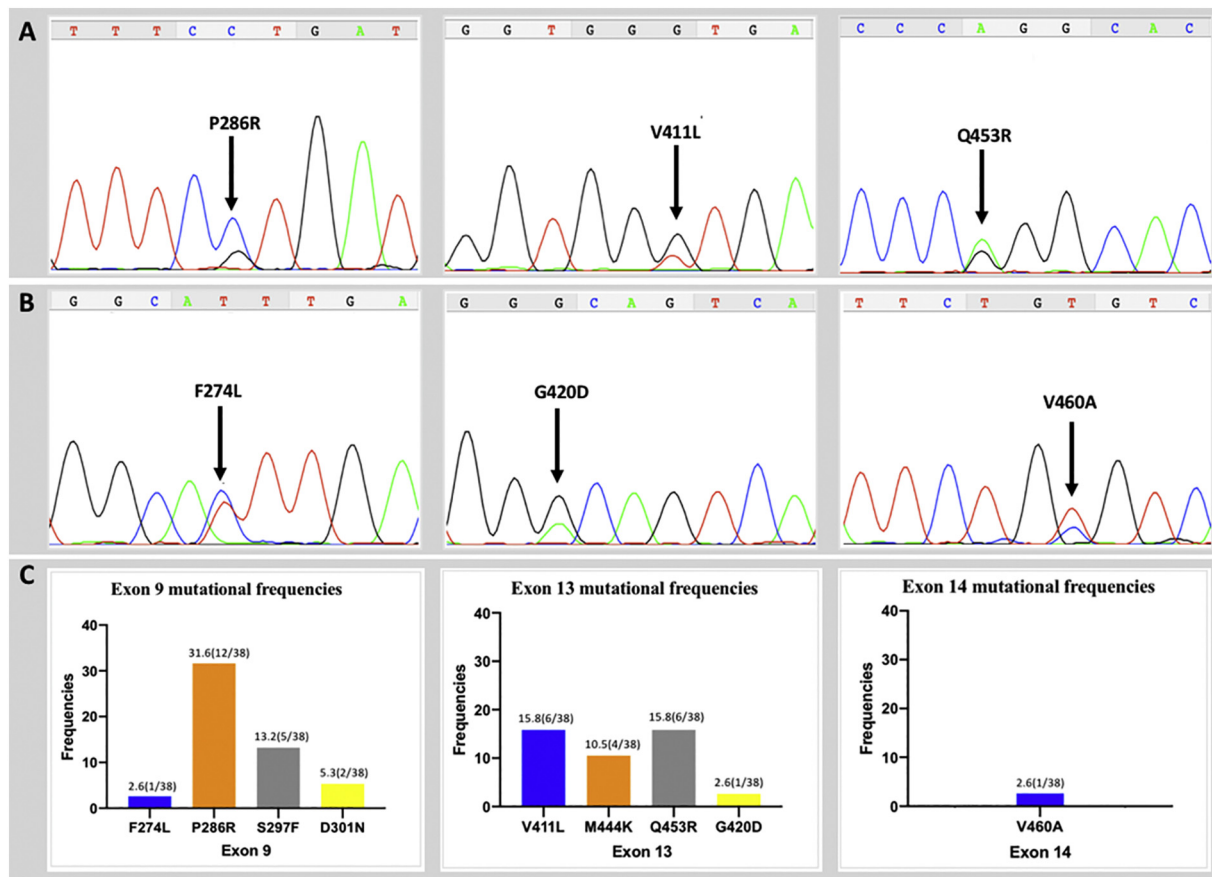


Fig. 2. Representative sequencing electropherograms of *POLE* mutations; the arrow refers to the locations of the mutations (A). Three novel mutations, p. Phe274Leu, p. Gly420Asp and p. Val460Ala, were each observed once (B). *POLE* mutational frequencies of 38 endometrial carcinomas (C).

pattern invasion and the risk of tumor recurrence or progression was especially significant among patients with *POLE* mutations (Table 2), in which MELF pattern invasion was associated with a 15.1-fold increase in tumor recurrence or progression risk among women with *POLE* mutations compared with those without *POLE* mutations (HR = 15.1, 95% CI = 1.57–145.3, $P = .018$). Furthermore, although high stage and deep myometrial invasion were significantly associated with an increased risk of tumor recurrence or progression regardless of *POLE* mutation status, these two histological factors showed a much higher risk in patients harboring *POLE* mutations than in those without *POLE* mutations (HR = 18.5, 95% CI = 1.90–180.9, $P = .012$ for both) (Table 2).

Similar analyses on other histological variables showed that nonendometrioid histology, high grade, positive LVSI and positive lymph node metastasis were risk factors for tumor recurrence or progression only in patients without *POLE* mutations. In other words, in patients with *POLE* mutations, the histological variables listed above, which are conventional poor prognostic indicators used in risk assessment for EC, were no longer associated with an increased risk of tumor recurrence or progression (Table 2).

3.4. Impact of adjuvant treatment on *POLE*-mutated cases

Next, we evaluated the impact of adjuvant treatment on the prognosis of patients with *POLE* mutations. Ninety-five patients received chemotherapy, and no patient received radiotherapy. The clinicopathologic characteristics of patients stratified by *POLE* mutation status and adjuvant treatment can be found in Table S3. In 38 patients with *POLE* mutations, 17 (44.7%, 17/38) received adjuvant chemotherapy. The majority (90.5%, 19/21) of *POLE*-mutated patients who did

not receive treatment had tumors that were stage IA, with 90.5% (19/21) of tumors being grade 1 or grade 2. For the *POLE*-mutated patients who received treatment, 58.9% (10/17) had stage IA tumors and 94.1% (16/17) had grade 3 tumors.

In the multivariate Cox hazard analysis, after adjusting for chemotherapy, *POLE* mutation was identified as an independent protective factor for tumor recurrence or progression with borderline significance (HR = 0.39, 95% CI = 0.14–1.08, $P = .07$). To further assess whether the effect of *POLE* mutational status on tumor recurrence or progression was altered for those receiving chemotherapy, an interaction term between chemotherapy and *POLE* mutational status was added into the Cox proportional hazards model. However, the effect of the interaction term was not statistically significant. This could be attributed to either a true lack of interaction between chemotherapy and *POLE* mutation status or a lack of power to detect the interaction. Given the current available data, we were unable to reach a definite conclusion regarding whether *POLE*-mutated tumors require adjuvant treatment.

3.5. Interactions between *POLE* mutation and MMR or p53 expression status

Finally, we assessed the association between *POLE* mutation and MMR or p53 expression status (Table 1). All patients with *POLE* mutations were MMR proficient, whereas the frequency of p53 expression was much higher in patients with *POLE* mutations (13/38, 34.2%) than in those without *POLE* mutations (64/388, 16.5%). We then tested whether MMR or p53 expression status was associated with tumor recurrence or progression in patients stratified according to *POLE* mutation status. MMR deficiency was significantly associated with an

Table 1
Association of *POLE* mutation with clinicopathologic features and MMR or p53 status.

	<i>POLE</i> mutation (N = 38)	<i>POLE</i> wide-type (N = 388)	P
Age			0.062
<45	9 (23.7%)	50 (12.9%)	
≥45	29 (76.3%)	338 (87.1%)	
Menopause			0.191
Yes	19 (50%)	228 (58.7%)	
No	19 (50%)	160 (41.3%)	
BMI			0.105
High (>30)	1 (2.6%)	39 (10.1%)	
Low (≤30)	37 (97.4%)	349 (89.9%)	
Staging			0.253
IA	31 (81.6%)	278 (71.6%)	
IB-IV	7 (18.4%)	110 (28.4%)	
Histology			0.001
Endometrioid	25 (65.8%)	339 (87.4%)	
Nonendometrioid	13 (34.2%)	49 (12.6%)	
Grade			0.003
1–2	20 (52.6%)	298 (76.8%)	
3	18 (47.4%)	90 (23.2%)	
Myometrial invasion			0.253
<1/2	31 (81.6%)	278 (71.6%)	
≥1/2	7 (18.4%)	110 (28.4%)	
LVSI			0.173
Yes	7 (18.4%)	41 (10.6%)	
No	31 (81.6%)	347 (89.4%)	
Lymph node metastases ^a			0.266
Yes	4 (10.5%)	18 (5.5%)	
No	34 (89.5%)	311 (94.5%)	
MELF			0.253
Yes	6 (15.8%)	37 (9.5%)	
No	32 (84.2%)	351 (90.5%)	
MMR			0.001
dMMR	0 (0)	94 (24.2%)	
pMMR	38 (100%)	294 (75.8%)	
p53			0.01
p53 abn	13 (34.2%)	64 (16.5%)	
p53 wt	25 (65.8%)	324 (83.5%)	

BMI, body mass index; LVSI, lymphovascular space invasion; ^a367 patients received lymphadenectomy; MELF, microcystic, elongated, fragmented; dMMR, MMR-deficient; pMMR, MMR-proficient; p53 abn, p53 abnormal; p53 wt, p53 wide-type; Bold values indicated that statistically significant differences ($P < 0.05$).

Bold values indicated that statistically significant differences ($P < 0.05$).

increased risk of tumor recurrence or progression regardless of *POLE* mutation status. However, p53 expression was only linked with increased risk among patients without *POLE* mutations. These data suggest that the association between p53 and tumor recurrence or progression risk was modified by *POLE* mutation status (Table 3).

4. Discussion

This is the first comprehensive study of the association of *POLE* mutation combined with histological characteristics with EC prognosis in a cohort of Chinese women. Our study shows that *POLE* mutation in

endometrial cancers was not always associated with favorable progression-free survival, especially when it was combined with MELF pattern invasion. Molecular typing should be closely combined with histological characteristics in risk assessment strategies for EC.

In our cohort, the prevalence of *POLE* mutations was 8.9%, which is within the range described in previous studies (5.6%–12%) [2,5–9]. The most common mutations were P286R, located in exon 9, and V411L and Q453R, located in exon 13, which was consistent with previous studies. Notably, we also identified three novel somatic missense mutations, F274L, G420D and V460A. All of the mutations identified were missense mutations. The novel F274L missense mutation is immediately within the Exo I motif required for the exonuclease function of *POLE* (residues 271–285) [25]. The two other novel mutations (G420D and V460A) were predicted to affect splice site changes and potentially affect protein features using two in silico pathogenicity prediction tools: PolyPhen-2 and Mutation Taster. However, these preliminary observations need further detailed structural and functional studies to determine the underlying mechanisms.

The survival advantage of *POLE*-mutant endometrioid carcinomas has been observed in multiple studies, but not in all of them [21]. In this cohort, we confirmed that *POLE*-mutant endometrial carcinomas have an excellent overall survival prognosis; however, no significant association between progression-free survival and *POLE* status was found. The reason may be that the percentage of cases with nonendometrioid histology with *POLE* mutations in this study was relatively high (34.2%, 13/38) compared to that in previous studies (1%–17.9%). It is accepted that cases with nonendometrioid histology, including serous cell, clear cell and undifferentiated carcinomas, generally have poor prognoses. Regardless of histology, *POLE*-mutant EC had an improved OS compared with *POLE*-wild-type EC. It has been shown that the favorable outcome of *POLE*-mutant endometrial carcinomas with their striking mutation burden may be a result of increased immunogenicity. It is clear that in many cancers, the immune system retains a degree of control over tumor growth, as illustrated by the association between an increased density of tumor-infiltrating lymphocytes (TILs) and favorable prognosis [2,26,27]. Indeed, *POLE*-mutant ECs are characterized by a robust intratumoral T cell response, which correlates with, and may be caused by, an enrichment of antigenic neopeptides.

Even though patients with *POLE*-mutant EC have an excellent prognosis, when we considered *POLE* mutation together with MELF pattern invasion, we found that MELF pattern invasion was associated with a significant increase in tumor recurrence or progression risk among patients with *POLE* mutation, whereas such an association was not present in *POLE*-wild-type patients. Many studies have indicated that the MELF pattern of myometrial invasion plays a critical role in lymphovascular space invasion and lymph node metastasis, however, its implication in survival and recurrence is ill defined [28–30]. Thus, according to our results, MELF pattern invasion might be a possible predictor of tumor recurrence or progression risk in the *POLE*-mutant subtype. However, the biological mechanism linking MELF and *POLE* mutation is

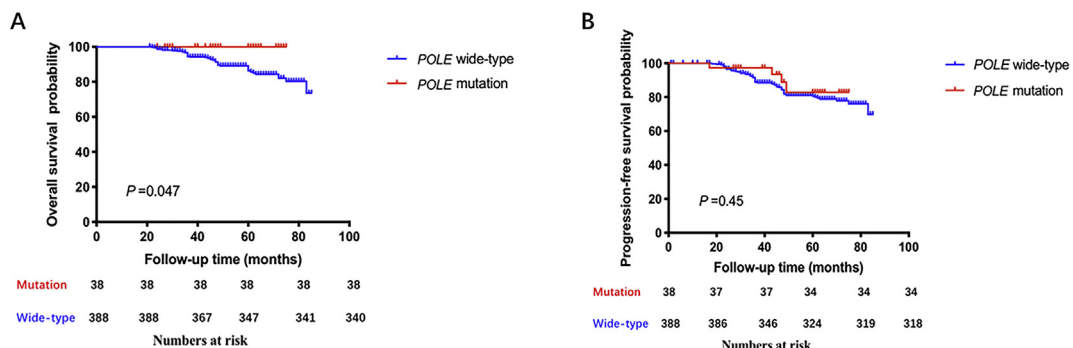


Fig. 3. The Kaplan-Meier survival curves (OS: A and PFS: B) for *POLE*-mutant and *POLE*-wild-type endometrial carcinomas.

Table 2
Correlation analysis between histological variables and tumor recurrence or progression stratified by *POLE* mutation in EC.

	All		<i>POLE</i> mutation		<i>POLE</i> wide-type	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
Staging						
IA	Reference		Reference		Reference	
IB-IV	2.00 (1.24–3.22)	0.005	18.5 (1.90–180.9)	0.012	1.75 (1.07–2.89)	0.027
Histology						
Endometrioid	Reference		Reference		Reference	
Nonendometrioid	6.03 (3.79–9.59)	<0.001	1.48 (0.50–4.72)	0.54	8.26 (5.12–13.3)	<0.001
Grade						
1–2	Reference		Reference		Reference	
3	1.28 (1.14–1.43)	<0.001	3.25 (0.34–31.3)	0.31	1.27 (1.14–1.42)	<0.001
Myometrial invasion						
<1/2	Reference		Reference		Reference	
≥1/2	4.18 (2.63–6.65)	<0.001	18.5 (1.90–180.9)	0.012	3.85 (2.39–6.19)	<0.001
LVSI						
No	Reference		Reference		Reference	
Yes	4.05 (2.45–6.68)	<0.001	446.1 (0–674.3)	0.557	3.47 (2.02–5.95)	<0.001
Lymph node metastases						
No	Reference		Reference		Reference	
Yes	6.52 (3.58–11.89)	<0.001	NA (0–∞)	0.932	5.54 (2.87–10.7)	<0.001
MELF						
No	Reference		Reference		Reference	
Yes	1.27 (0.63–2.55)	0.50	15.1 (1.57–145.3)	0.018	0.90 (0.39–2.08)	0.80

Bold values indicated that statistically significant differences ($P < 0.05$)

Table 3
The association of MMR or p53 expression status with tumor recurrence or progression in *POLE*-mutant or -wild-type subgroups.

	All		<i>POLE</i> mutation		<i>POLE</i> wide-type	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
dMMR						
No	Reference		Reference		Reference	
Yes	1.67 (1.02–2.71)	0.04	1.63 (0.99–2.67)	0.053	–	–
p53 abnormal						
No	Reference		Reference		Reference	
Yes	4.29 (2.70–6.83)	<0.001	1.87 (0.26–13.3)	0.53	4.85 (3.01–7.81)	<0.001

Bold values indicated that statistically significant differences ($P < 0.05$)

still unknown. Zinovkin DA et al. found that tumor-associated T-lymphocytes and macrophages are decreased in EC with MELF pattern invasion, and a low level of T lymphocytes and macrophages indicated an increased risk of MELF pattern invasion presence and poor survival [31]. Since *POLE*-mutated EC patients are characterized by high tumor infiltration of both CD4+ and CD8+ T cells and the superior prognosis of *POLE* patients is most likely linked to enhanced immunogenicity [32], we supposed that the enhanced immunogenicity of *POLE* patients may be suppressed by MELF pattern invasion presence. This hypothesis needs further experimental validation. In addition, high stage and deep myometrial invasion, two traditional histological factors, also showed increased predictive value in patients harboring *POLE* mutations compared to those without *POLE* mutations. Therefore, integrating *POLE* mutations with established clinicopathologic factors, including MELF pattern invasion, in the risk assessment of endometrial cancer will further stratify patients and lead to individualized therapy.

Studies have also indicated that up to one-third of *POLE*-ultramutated ECs harbor *TP53* mutations [33,34]. We had similar findings. In this cohort, 34.2% of *POLE*-mutant tumors (13/38) exhibited a p53-abnormal phenotype. However, survival analysis indicated that the prognostic impact of the p53-abnormal phenotype was different in the *POLE*-mutant and *POLE*-wild-type subgroups. In *POLE*-mutant tumors, p53 did not have any prognostic significance; that is, the clinical

outcomes of EC patients with both *TP53* and *POLE* abnormalities are strikingly different from what would be expected in EC patients with only *TP53* mutation [2,35,36]. In terms of clinical management, this means that the presence of *TP53* mutations in the context of *POLE*-mutant EC should not prompt intensified treatment. According to Alicia León-Castillo et al., the effects of mutational changes and somatic copy number alterations in ECs with both *TP53* and *POLE* mutations were similar to those in ECs with a single *POLE* mutation. This strongly suggests that p53 variants occurring in the context of *POLE*-mutant EC are likely passenger events and thus do not affect the molecular landscape of the tumor. In addition, the unusual high frequency (47–60%) of subclonal abnormal p53 staining in ECs with both *TP53* and *POLE* mutations strongly supports the interpretation that the *TP53* mutation is a later event during tumor progression that does not affect the molecular landscape or the phenotype.

Our study has some limitations. First, it was performed in a single medical center and has a retrospective design. Further studies with ethnically diverse populations are needed to verify our findings. Second, although our sample size was relatively large, the number of individuals was relatively small when the data were stratified. Therefore, we will accumulate more data to validate the findings of this study. Third, the biological mechanism responsible for the prognosis being so different for patients with different *POLE* status with MELF pattern invasion remains to be dissected.

In summary, in this cohort of Chinese women, we found that *POLE* exonuclease domain mutations in endometrial cancer combined with histological characteristics, including high stage, deep myometrial invasion, and especially MELF pattern invasion, predicted poor progression-free survival. Thus, integrating *POLE* mutation status with established clinicopathologic factors, including stage, myometrial invasion and MELF pattern invasion, in the risk assessment of endometrial cancer is more effective and might lead to a precise and individualized therapeutic strategy.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgyno.2020.07.102>.

Author contributions

Dan He and Ying Dong contributed to conception and design of study. Data and statistical analysis were performed by Dan He and Hui

Wang. Interpretation of results was done by Dan He and Hui Wang. The manuscript writing and editing were done by Dan He, Hui Wang and Ying Dong. Ying Dong and Ting Li contributed to final review of manuscript. Data interpretation and manuscript preparation by all other authors.

Declaration of Competing Interest

None declared.

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