Original Article

Polymerase ε (*POLE*) Mutations in Endometrial Cancer: Clinical Outcomes and Implications for Lynch Syndrome Testing

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BACKGROUND: DNA polymerase ε (*POLE*) exonuclease domain mutations characterize a subtype of endometrial cancer (EC) with a markedly increased somatic mutational burden. *POLE*-mutant tumors were described as a molecular subtype with improved progression-free survival by The Cancer Genome Atlas. In this study, the frequency, spectrum, prognostic significance, and potential clinical application of *POLE* mutations were investigated in patients with endometrioid EC. **METHODS:** Polymerase chain reaction amplification and Sanger sequencing were used to test for *POLE* mutations in 544 tumors. Correlations between demographic, survival, clinicopathologic, and molecular features were investigated. Statistical tests were 2-sided. **RESULTS:** Thirty *POLE* mutations (5.6%) were identified. Mutations were associated with younger age (<60 years; P=.001). *POLE* mutations were detected in tumors with microsatellite stability (MSS) and microsatellite instability (MSI) at similar frequencies (5.9% and 5.2%, respectively) and were most common in tumors with MSI that lacked mutL homolog 1 (*MLH1*) methylation (P<.001). There was no association with progression-free survival (hazard ratio, 0.22; P=.127). **CONCLUSIONS:** The discovery that mutations occur with equal frequency in MSS and MSI tumors and are most frequent in MSI tumors lacking *MLH1* methylation has implications for Lynch syndrome screening and mutation testing. The current results indicate that *POLE* mutations are associated with somatic mutation in DNA mismatch repair genes in a subset of tumors. The absence of an association between *POLE* mutation and progression-free survival indicates that *POLE* mutation status is unlikely to be a clinically useful prognostic marker. However, *POLE* testing in MSI ECs could serve as a marker of somatic disease origin. Therefore, *POLE* tumor testing may be a valuable exclusionary criterion for Lynch syndrome gene testing. ***Cancer*** **2014;000:000-000.** © *2014 American Cancer Society.*

KEYWORDS: endometrial cancer, DNA mismatch repair, Lynch syndrome, mutation.

INTRODUCTION

Cancers have a mutator phenotype.[1] An elevated mutation rate is central to tumorigenesis in human malignancies and significantly contributes to the disruption of regulatory processes essential to genomic stability. Endometrial cancers (ECs) are frequently defective in DNA mismatch repair (MMR). Reduced postreplication surveillance and repair result in a 100-fold increase in somatic mutations in human tumor cell lines.[2] Recently, it was demonstrated that loss of DNA proofreading function in the DNA polymerase ε (POLE) was similarly important for tumorigenesis in EC. Approximately 7% of ECs harbor mutations in the exonuclease domain of *POLE*.[3,4]

POLE encodes the major catalytic and proofreading subunits of the Polε DNA polymerase enzyme complex,[5] and the Polε enzyme complex synthesizes the leading strand.[5-8] The proofreading (exonuclease) function locates and replaces erroneous bases in the daughter strand through failed complementary pairing with the parental strand. High-fidelity incorporation of bases by *POLE*, coupled with its exonuclease proofreading function, ensures a low mutation rate. It has been demonstrated that POLE exonuclease domain mutations (EDMs) increase spontaneous mutation rates, contributing to tumorigenesis in yeast and mouse models.[9-14]

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See related Editorial on pages 000-000, this issue.

We thank the study team and patients treated at Washington University School of Medicine for making this work possible, and we recognize Dr. Li Ding (The Genome Institute at Washington University) for her valuable input.

Additional Supporting Information may be found in the online version of this article.

DOI: 10.1002/cncr.29046, **Received:** May 5, 2014; **Revised:** June 6, 2014; **Accepted:** June 11, 2014, **Published online** Month 00, 2014 in Wiley Online Library (wileyonlinelibrary.com)

The Cancer Genome Atlas (TCGA) reported a *POLE* mutant subtype of EC.[3] Tumors with *POLE* EDMs are referred to as "POLE ultramutated." ECs in this molecularly defined group are of endometrioid histology, predominantly have normal DNA MMR (microsatellite stable [MSS]), and have thousands of somatic mutations. Clinically, patients in the POLE ultramutated group reportedly had improved progression-free survival (PFS).[3] For the current study, we undertook an analysis of *POLE* mutations in a large cohort of endometrioid ECs to better understand the clinicopathologic significance of POLE EDMs.

MATERIALS AND METHODS

Study Population

Matched EC and normal tissues were prospectively collected at the time of hysterectomy by the Division of Gynecology Oncology at Washington University School of Medicine (St. Louis, Mo). All research participants consented to molecular analyses and follow-up (Washington University Human Research Protection Office protocols 91-507 and 93-0828). The analyses performed at The Ohio State University in Columbus, Ohio were undertaken with institutional review approval (2012C0117).

High-molecular-weight genomic DNA for 544 surgically staged endometrioid ECs was analyzed for *POLE* mutation. The tumor neoplastic cellularity and results from MSI and mutL homolog 1 (*MLH1*) methylation analyses have been described previously for the majority of patients.[15,16] Microsatellite analysis was performed using 5 National Cancer Institute consensus microsatellite markers (*BAT25*, *BAT26*, *D2S123*, *D5S346*, and *D17S250*).[17] The COBRA method[18] was used to evaluate methylation of the *MLH1* promoter. Polymerase chain reaction (PCR) primers and conditions have been previously published.[19] Extensive data are available for all patients, and the cohort has been described in previous publications.[15,16,20]

Mutation Testing

The exonuclease domain of POLE (residues 268-471) was assessed for mutations using PCR amplification (AmpliTaq Gold DNA Polymerase; Applied Biosystems Inc., Foster City, Calif) and Sanger sequencing. Primers and conditions are provided (Supporting Table 1; see online supporting information). PCR products (AmpliTaq Gold DNA Polymerase; Applied Biosystems Inc.) were treated with ExoSAP-IT (Affymetrix, Santa Clara, Calif) and sequenced (ABI Prism BigDye Terminator Cycle Sequencing Kit version 3.1; Applied Biosystems Inc.) in the Nucleic Acid Shared Resource Laboratory at the Ohio State University in Columbus, Ohio (available at: http://cancer.osu.edu/research/cancerresearch/sharedresources/na/services/dna_sequencing/pages/index.aspx; accessed June 1, 2014). Sequences were analyzed in Sequencher (GeneCodes, Ann Arbor, Mich), and all variants were tested in matched normal DNA to determine whether they were somatic or germline alterations.

Statistical Analyses

All analyses were based on available clinical and molecular data (as of February 1, 2014). The statistical software packages SAS Version 9.2 (SAS Institute Inc., Cary, NC) and STATASE 10 (StataCorp, College Station, Tex) were used for statistical analyses; *P* values were 2-sided. Demographic and clinicopathologic features were compared between *POLE* mutation and wild-type *POLE* using the chi-square test or the Fisher exact test for categorical or dichotomized variables or using a 2-sample *t* test for continuous variables. Because of the numerous tests performed and to control for type I errors, we considered *P* values $\leq$.01 significant.

The median times to death and recurrence were calculated using Kaplan-Meier estimates. The Kaplan-Meier curves were compared using a log-rank test. Overall survival (OS) was defined as the time from surgery to death from any cause. Patients were censored if they were alive (with or without disease) at the time of last follow-up, if they had a perioperative death, or if no outcome data were available. PFS was defined as the time from surgery to first recurrence or death from disease. For the PFS analysis, patients were censored if they were alive without disease at the time of last follow-up, if they were disease free and died of causes unrelated to their EC, if they had a perioperative death, or if no outcome data were available.

Multivariable Cox proportional hazards models were used to estimate survival hazard ratios (HRs) according to tumor *POLE* mutational status and progression HRs for all other clinicopathologic features. For OS, we used a stepwise modeling procedure starting with *POLE* mutation in the model and all significant univariate predictors at the 0.1 level. For PFS, we included all significant univariate predictors at the 0.1 level. For both outcomes, predictors with the highest *P* values were systematically removed from the model until the final model with all significant *P* values remained. All removed variables were added back in to verify whether they should be in the model.

RESULTS

POLE EDMs in Endometrioid EC

Mutations were identified in 30 of 535 (5.6%) successfully analyzed endometrioid tumors. Of the 8

TABLE 1. Polymerase ε Exonuclease Domain Variants Identified in Endometrioid Endometrial Cancers[a]

Variant	No. of Observations	Predicted Functional Impact		
		SIFT Score/Impact	MASS PFIS	PPH v2 Score/Impact
Missense mutations				
P286R c.857C>G	10	0/Damaging	Medium	1/Probably damaging
V411L c.1231G>C[b]	10	0/ Damaging	Medium	1/Probably damaging
S297F c.890C>T[b]	3	0/Damaging	Medium	1/Probably damaging
A456P c.1366G>C	3	0/Damaging	High	1/Probably damaging
P436R c.1307C>G	1	0.01/Damaging	High	1/Probably damaging
A465V c.1394C>T	1	0/Damaging	High	1/Probably damaging
A426V c.1277C>T[c]	1	0/Damaging	Medium	1/Probably damaging
H342R c.1025A>G[c]	1	0.26/Tolerated	Low	0.04/Benign
Polymorphisms				
rs139075637 D287E	2	0/Damaging	Medium	0.997/Probably damaging
rs5744760 N336S	2	0/Damaging	Medium	1/Probably damaging
rs200403177 R446W	1	0/Damaging	Medium	0.998/Probably damaging
rs5744777 D490D	8	—	—	—
rs75135381 (intronic)	2	—	—	—
I300I (unassigned)	2	—	—	—

Abbreviations: MASS PFIS, MutationAssessor predicted functional impact score (Reva 2011[23]); PPH v2, Polymorphism Phenotyping v2 score (Adzhubei 2010[22]); rs, reference single nucleotide polymorphism; SIFT, Sorting Intolerant From Tolerant score (Ng & Henikof 2003[21]).

[a] Among the 10 known Lynch syndrome mutation carries in 535 patients, none had polymerase ε mutations.

[b] One patient each with V411L and S297F mutations harbored 2 somatic *MSH6* mutations and lacked germline mutations.

[c] These were novel mutations.

different mutations identified, 6 have previously been described (Table 1). Representative examples of the somatic mutations are illustrated in Figure 1. The proline to arginine mutation at position 286 (p.Pro286Arg) and the valine to leucine mutation at position 411 (p.Val411Leu) (Fig. 1a,b) were each present in 10 tumors. Two novel mutations, p.Ala426Val and histidine to arginine at position 342 (p.His342Arg), were each observed once (Fig. 1c,d). The p.Ala426Val variant was reported as a rare single nucleotide polymorphism (rs374920539) but was clearly absent from the normal patient DNA. The p.Pro436Arg mutation (previously reported in colon cancer) was observed in 1 patient (Fig. 1e). Six germline polymorphisms were observed in 17 tumors (Table 1). Overall, *POLE* mutations were more common than polymorphisms (5.6% and 3.2%, respectively). Germline variants observed are rare (minor allele frequencies $\leq$0.01), and it is predicted that the 3 germline missense changes will have a deleterious impact on protein function (Table 1).

The predicted functional impact of *POLE* EDMs was assessed using a mutation assessment prediction program (Table 1). The majority of mutations reportedly had a damaging effect[21,22] and a medium or high impact score.[23] The novel mutation p.H342R was predicted to have a tolerated impact, whereas the other novel mutation, p.A426V, was predicted to have a damaging impact on function (Table 1).

POLE Mutations Are Distributed Similarly Between MSI and MSS Tumors and Are Most Common in MSI Tumors Lacking MLH1 Methylation

Eighteen of 306 tumors with MSS (5.9%) and 12 of 229 tumors with MSI (5.2%) harbored *POLE* EDMs. It is noteworthy that, among the tumors with MSI, mutations were significantly more frequent in those that lacked *MLH1* methylation (18% vs 2.4%; $P<.001$; Fisher exact test). Women who have tumors with MSI that lack MLH1 methylation are considered to have a high risk of germline mutation in DNA MMR genes (Lynch syndrome [LS]). Tissues for immunohistochemical (IHC) analysis were available for 3 of the 8 MSI, *MLH1*-unmethylated tumors with *POLE* EDMs. IHC for mutS homolog 2 (*MSH2*), *MSH6*, *MLH1*, and postmeiotic segregation increased 2 (*PMS2*) revealed 1 tumor with normal expression of all markers, 1 tumor that did not express *MSH6*, and 1 tumor that did not express *MSH2* or *MSH6*. MMR proficiency in 1 tumor was somewhat unexpected but may reflect an epitope stable mutation in 1 of the MMR genes. Loss of *MSH6* alone or loss *MSH6* and *MSH2* are characteristic of *MSH6* and *MSH2* mutations, respectively.[24]

POLE EDMs Are Associated With Younger Age in Women With EC

POLE mutations were associated with younger age at EC diagnosis. Seventy percent of women whose tumors harbored *POLE* EDMs were aged <60 years at diagnosis

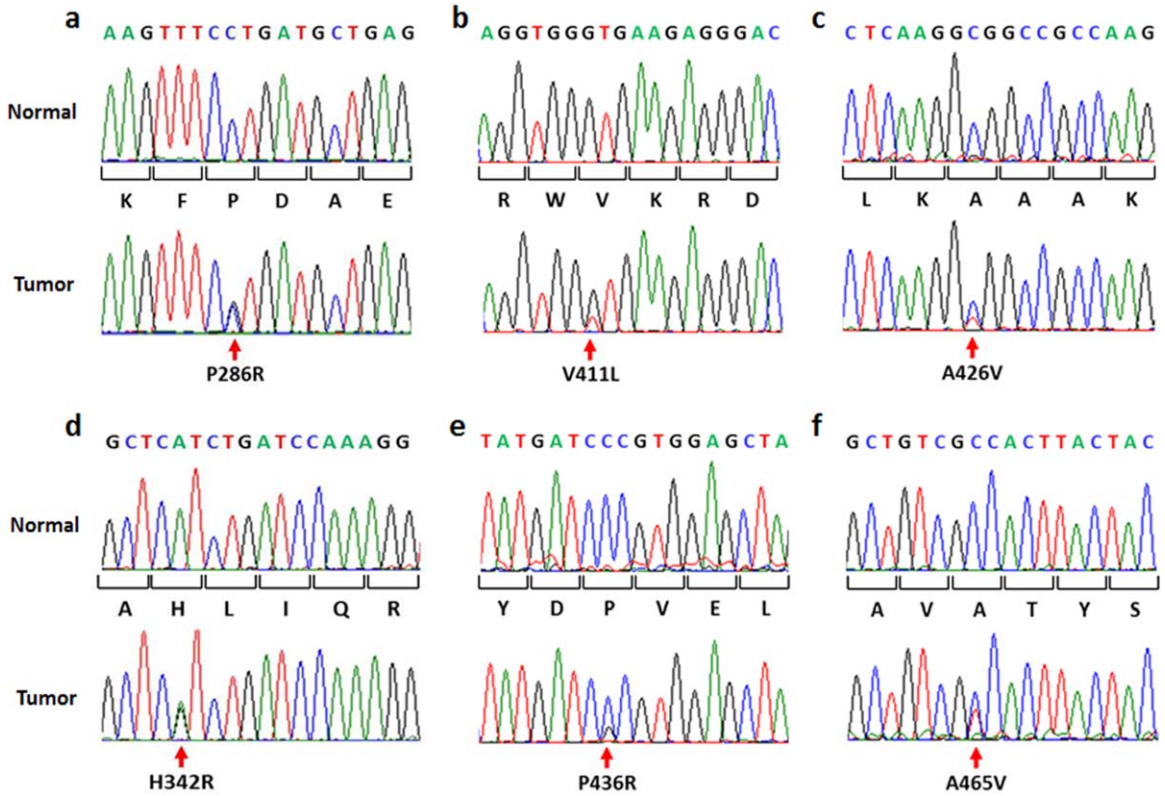

Figure 1. Polymerase ε (*POLE*) exonuclease domain mutations in patients with endometrioid endometrial cancer are illustrated, including (a,b) hotspot *POLE* mutations, (c,d) novel mutations, (e) mutation previously identified in a single colon cancer, and (f) an infrequent but known mutation. P286R indicates proline to arginine at position 286; V411L, valine to leucine at position 411; A426V, alanine to valine at position 426; H342R, histidine to arginine at position 342; P436R, proline to arginine at position 436; A465V, alanine to valine at position 465.

compared with 30% of those whose tumors had no mutation (P=.001; chi-square test). There were no statistically significant relations between mutation and the other clinicopathologic factors assessed (Table 2). Although patients with mutations tended to present at an earlier stage (stages I and II vs stages III and IV) and had higher grade tumors (grade 2 or 3), these associations did not reach the P value set for significance.

POLE Mutation Is Not Associated With Survival

TCGA reported improved PFS for patients in the ultramutated POLE subgroup.[3] Univariate analysis of our cohort revealed that advanced stage (stages III/IV), higher grade (grade 2 vs grade 1 and grade 3 vs grade 1), the presence of lymphovascular space invasion (LVSI), deep myometrial invasion, and receipt of adjuvant therapy were associated with significantly higher HRs for PFS (Tables 3,4). However, *POLE* mutations were not associated with PFS (Table 3). Kaplan-Meier curves similarly demonstrated no difference (P=.093) (Fig. 2A). There was 1 recurrence among the 30 patients (3.4%) whose tumors had a *POLE* EDM (median follow-up. 68.4 months). The recurrence rate for patients with wild-type *POLE* was 17% (median follow-up, 70.6 months). A multivariable model that included 6 factors with P values <.10 in univariate analysis (excluding *POLE* status and body mass index) identified stage, grade, and the presence of LVSI as ere significant variables (Table 4).

POLE mutation trended toward a lower HR for OS (P=.023) (Table 3), and Kaplan-Meier curves indicated a significant association with a lower HR for OS (P=.014) (Fig. 2B). However, in multivariable analysis, no significant association was observed between *POLE* mutation status and OS (Table 4).

DISCUSSION

Our analysis of a large cohort of endometrioid ECs confirms previous reports that *POLE* EDMs are present in 5% to 8% of sporadic ECs.[3,4] In the POLE ultramutated group described by TCGA, 17 endometrioid ECs with *POLE* EDMs were reported (overall rate, 6.9%). One

serous tumor (TCGA-AP-A1DQ) had a *POLE* EDM. Church and colleagues[4] described 13 *POLE* EDMs among 173 tumors tested (overall rate, 7.5%). Our observation that *POLE* EDMs occur with equal frequency in tumors with MSS and MSI was unexpected. Previous studies (including TCGA) in colorectal cancers and ECs have pointed to a *POLE*-mutant, hypermutated state occurring predominantly in MSS tumors.[3,4,25-28] Our data clearly indicate that *POLE* EDMs are observed in ECs with MSI. Furthermore, *POLE* EDMs are more common in MSI tumors that lack *MLH1* methylation compared with *MLH1*-methylated tumors (epigenetic silencing of *MLH1*; 8 of 44 unmethylated tumors vs 4 of 167 methylated tumors; P=.001; Fisher exact test). Although it has been reported that *POLE* mutations occur predominantly in tumors with MSS, a detailed analysis of TCGA mutation data revealed 15 MSI, *MLH1*-unmethylated tumors, of which 7 had *POLE* EDMs. Six of those 7 tumors were included in the 17 reported by TCGA (Table 5). Combining our data with TCGA data, we estimate that 25% of endometrioid tumors with MSI that lack *MLH1* methylation have *POLE* EDMs.

The high rate of somatic *POLE* EDMs in ECs with defective DNA MMR has implications for LS screening and mutation testing. MMR IHC and/or MSI analysis of ECs is used to screen for patients at who are increased risk for LS (germline mutation in *MLH1*, *MSH2*, *MSH6*, and *PMS2*). Most tumors with defective DNA MMR are caused by somatic methylation of the *MLH1* promoter region and loss of *MLH1* expression.[29-31] *MLH1* methylation can be used to triage IHC results and exclude patients from germline MMR gene mutation testing.[32,33] Alternatively, gene testing is indicated for women with ECs that have MSI or defective DNA MMR but lack *MLH1* methylation.[34] Our analysis and review of TCGA data suggest that 25% of tumors with MSI that lack

TABLE 2. Demographic and Clinicopathologic Features by Polymerase ε Mutation Status

Clinicopathologic Factor[a]	No. of Patients (%)		
	POLE Mutant	*POLE* Wild Type	P[b]
Age, y			
<60	21 (70)	200 (39.6)	.001
≥60	9 (30)	305 (60.4)	
Stage			
Early: I &II	29 (96.7)	408 (81)	.027
Advanced: III & IV	1 (3.3)	96 (19)	
Grade			
1	9 (30)	258 (51.2)	.024
2-3	21 (70)	246 (48.8)	
LVSI			
Present	9 (30)	172 (34.8)	.59
Absent	21 (70)	322 (65.2)	
Depth of invasion, %			
≥50	9 (33.3)	148 (31.8)	.865
<50	18 (66.7)	318 (68.2)	
Adjuvant therapy			
Any adjuvant therapy	23 (76.7)	347 (69.1)	.383
No further treatment	7 (23.3)	155 (30.9)	
BMI, kg/m^2			
<30	12 (48)	159 (35.3)	.199
≥30	13 (52)	291 (64.7)	
Race			
White	28 (93.3)	440 (87.5)	.609
African American	2 (6.7)	61 (12.1)	
Other: Asian, Native American	0 (0)	2 (0.4)	

Abbreviations: BMI, body mass index; LVSI, lymphovascular space involvement; *POLE*, polymerase ε gene.
[a] Missing data include grade for 1 patient, stage for 1 patient, LVSI for 11 patients, depth of invasion for 39 patients, adjuvant therapy for 3 patients, BMI for 55 patients, and race for 2 patients.
[b] P values ≤.01 were considered significant; the chi-square test or the Fisher exact test was used for categorical variables.

TABLE 3. Univariate Analyses of Progression-Free and Overall Survival in Endometrioid Endometrial Carcinoma

Variable	PFS		OS	
	HR (95% CI)	P	HR (95% CI)	P[a]
Advanced age: ≥60 y	1.53 (0.93-2.52)	.09[b]	2.32 (1.64-3.26)	<.001
Advanced stage: I/II vs III/IV	4.98 (3.11-7.97)	<.001	3.03 (2.18-4.23)	<.001
Grade 1 vs 2	2.81 (1.52-5.18)	.001	1.75 (1.24-2.46)	.001
Grade 1 vs 3	7.49 (4.01-13.98)	<.001	4.17 (2.84-6.12)	<.001
Presence of LVSI	3.94 (2.42-6.42)	<.001	2.61 (1.94-3.53)	<.001
Deep myometrial invasion, ≥50%	3.41 (2.09-5.55)	<.001	2.01 (1.48-2.72)	<.001
Adjuvant therapy, any kind	3.13 (1.95-5.01)	<.001	1.68 (1.25-2.27)	.001
BMI ≥30 kg/m^2	0.99 (0.97-1.02)	.574[b]	0.98 (0.97-1.00)	.085[b]
POLE mutation	0.22 (0.03-1.55)	.127[b]	0.27 (0.08-0.83)	.023[b]

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; LVSI, lymphovascular space involvement; OS, overall survival; PFS, progression-free survival; *POLE*, polymerase ε gene.
[a] To correct for multiple comparisons, a P value ≤0.01 was considered significant.
[b] Nonsignificant.

MLH1 methylation have *POLE* defects. Somatic *POLE* EDMs could phenocopy defective DNA MMR (by giving rise to strand slippage mutation and MSI) or could lead to the somatic inactivation of MMR genes (Fig. 3). Of the 3 *POLE*-mutant, MSI, *MLH1*-unmethylated tumors investigated for DNA MMR protein expression, 2 lacked 1 or more MMR proteins. It was demonstrated previously that 2 tumors with *POLE* EDMs (tumors 1442 and 1269) each had 2 somatic *MSH6* mutations and lacked germline mutations.[35] Somatic *MSH6* mutations in these tumors probably are secondary to the hypermutator state conferred by *POLE* EDMs. Of the 7 MSI, *MLH1*-unmethylated, *POLE*-mutant tumors in TCGA data (1 of which was excluded in POLE cluster by TCGA and classified as MSI), 4 had clear loss-of-function somatic mutations in the DNA MMR genes (*MLH1*, *MSH2*, and *MSH6* frameshift or nonsense mutations), and 2 additional tumors had deleterious missense changes (Table 5) (cBio Portal for Cancer Genomics[36]; available at: http://www.cbioportal.org/; accessed June 1, 2014). Among the 10 patients with known LS in our cohort, none had *POLE* mutations. Combined tumor and germline MMR and somatic *POLE* mutation testing should shed light on whether MMR defects secondary to *POLE* mutation are common. *POLE* mutation testing in IHC-abnormal/MSI/*MLH1*-unmethylated tumors may be important in clinical decision making for MMR gene mutation testing in patients with EC.

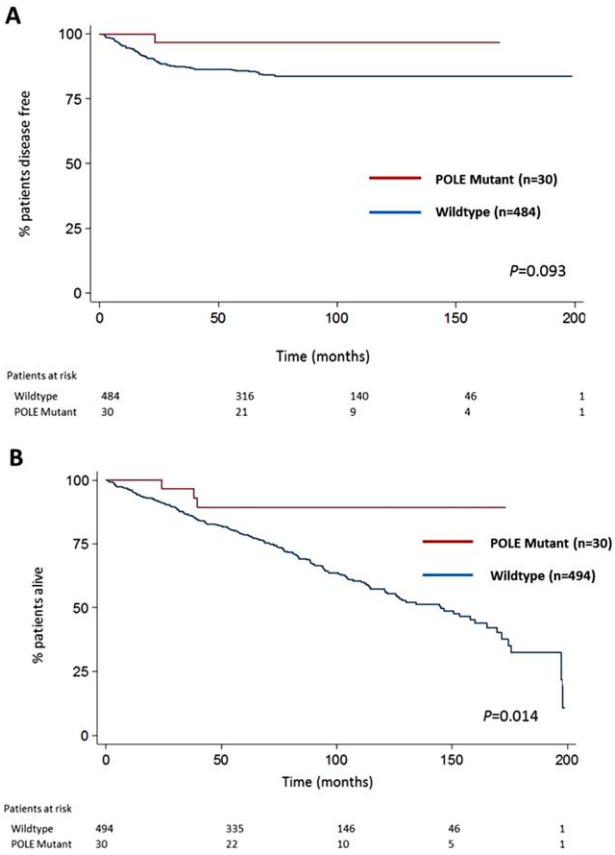

Figure 2. Kaplan-Meier estimates were calculated according to polymerase ε (*POLE*) mutational status and illustrate (A) progression-free survival and (B) overall survival. *P* values were calculated using the log-rank test (2-sided).

POLE Mutation and Patient Outcomes

POLE mutations were not associated with survival outcomes. This was unexpected, because TCGA reported a subtype of ECs with *POLE* EDMs with improved PFS. By focusing our outcome analyses on endometrioid tumors, the histologic subtype in which *POLE* defects are most common, we have provided an important clinical context for the POLE ultramutated subtype. A recent publication from Meng and colleagues[37] described improved PFS for patients with grade 3 endometrioid

TABLE 4. Multivariable Analysis of Progression-Free and Overall Survival in Endometrioid Endometrial Cancer[a]

	PFS				OS			
Variable	HR (95% CI)	SE	z	*P*	HR (95% CI)	SE	z	*P*
Advanced age: >60 y	1.21 (0.72-2.06)	.326	0.72	.472	2.02 (1.35-3.01)	0.413	3.42	**.001**
Advanced stage: I/II vs III/IV	2.65 (1.39-5.07)	.876	2.96	**.003**	2.95 (1.74-5.00)	0.795	4.01	**<.001**
High grade: 1 vs 2-3	2.51 (1.36-4.62)	.782	2.96	**.003**	1.51 (1.06-2.16)	0.275	2.28	**.023**
Presence of LVSI	1.80 (1.02-3.18)	.523	2.03	**.042**	1.60 (1.10-2.33)	0.308	2.45	**.014**
Deep myometrial invasion: ≥50%	1.59 (0.88-2.87)	.478	1.55	.122	1.38 (0.92-2.07)	0.285	1.57	.117
Adjuvant therapy: Any kind	0.99 (0.52-1.88)	.324	−0.05	.964	0.65 (0.40-1.05)	0.16	−1.76	.079
BMI ≥30 kg/m^2	—	—	—	—	1.00 (0.98-1.02)	0.01	−0.17	.863
POLE mutation	—	—	—	—	0.37 (0.09-1.54)	0.27	−1.37	.172

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; LVSI, lymphovascular space involvement; OS, overall survival; PFS, progression-free survival; *POLE*, polymerase ε gene; SE, standard error.
[a] Variables with *P* values <.1 in univariate analysis were included in the multivariate model.

TABLE 5. Polymerase ε and Somatic Mismatch Repair Gene Mutations in Microsatellite Unstable, Unmethylated Tumors in The Cancer Genome Atlas Data Set

			Gene/Mutation Type			
Tumor ID	*POLE*	Cluster	*MLH1*	*MSH2*	*MSH6*	*PMS2*
TCGA-D1-A17Q	P286R	POLE	**E34/nonsense**[a]	Q76H/neutral	D390N/neutral **E908/nonsense**[a]	
TCGA-BS-A0UV	P286R	POLE	K241N/neutral	K871N/deleterious N566H/neutral **E483/nonsense**[a]	K1013N/neutral	
TCGA-AP-A059	S297F	POLE			G529C/neutral **E1234/nonsense**[a] R178H/neutral	
TCGA-AX-A0J0	P286R	POLE			G1299D/deleterious K1101N/neutral	
TCGA-D1-A16Y	V411L	POLE				
TCGA-AP-A056	V411L	POLE		R929Q/neutral	R482Q/deleterious N960T/neutral	R628Q/neutral
TCGA-B5-A11H	Q453R	MSI	**S677fs/FS del** G67W/deleterious			
TCGA-A5-A0VP	—	MSI			**N335fs/FS del**	
TCGA-D1-A174	—	MSI				
TCGA-B5-A11G	—	MSI				A702D/deleterious
TCGA-BS-A0UJ	—	MSI				Q288R/deleterious
TCGA-B5-A11X	—	NA				
TCGA-B5-A11Y	—	CN LOW				
TCGA-BG-A187	—	CN LOW				
TCGA-D1-A15Z	—	CN LOW				

Abbreviations: CN LOW, copy-number low cluster; FS del, frameshift deletion; ID, identifier; *MLH1*, mutL homolog 1; *MSH2*, mutS homolog 2; *MSH6*, mutS homolog 6; MSI, microsatellite instability; NA, not assigned to a cluster by The Cancer Genome Atlas; *PMS2*, postmeiotic segregation increased 2; *POLE*, polymerase ε; TCGA, The Cancer Genome Atlas.

[a] Mutation was prediction using the consensus deleteriousness (CONDEL) score (MutationAssessor; Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY).

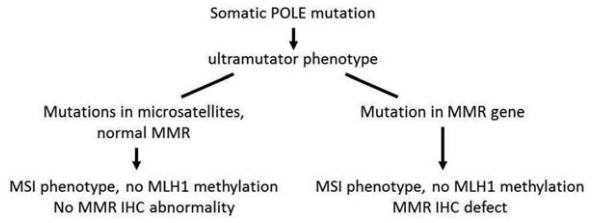

Figure 3. This chart illustrates the relation of polymerase ε (*POLE*) mutations, tumor microsatellite instability (MSI), and DNA mismatch repair (MMR) defects. Somatic POLE exonuclease domain mutations either could phenocopy defective DNA MMR (normal MMR but strand slippage mutations) or could lead to somatic inactivation of MMR genes with associated MSI and/or immunohistochemical (IHC) defects in tumors lacking mutL homolog 1 (*MLH1*) promoter methylation.

POLE mutant tumors in an analysis that combined TCGA data and findings from their own patient cohort. None of the 16 women who had *POLE* mutations had a recurrence (8 of those tumors were from TCGA). The significance of *POLE* mutations in grade 3 tumors remains uncertain, particularly because 1 of our patients had a POLE-mutant grade 3 tumor that recurred.

The reported improved survival for patients with *POLE* mutations from TCGA was based on a comparison of 4 molecularly defined subgroups.[3] POLE (ultramutated), MSI (hypermutated), copy-number low (endometrioid), and copy-number high (serous like) subgroups were compared, and the greatest difference in PFS was for the copy-number high and POLE subgroups. Outcomes for women with serous ECs are worse than outcomes for those with endometrioid tumors.[38-40] The poor outcome associated with serous histology, coupled with the finding that *POLE* mutations are infrequent in serous cancers,[3,4] may explain the differences in survival observed for the subgroups.

We recognize that our study is based on *POLE* mutation status and is not an integrated genomic analysis classification like that performed by TCGA. We do not have the whole-exome mutation burden that, in part, defines the TCGA POLE ultramutated subgroup. However, because all TCGA tumors that had *POLE* EDMs predicted to affect function were endometrioid carcinomas, we believe our approach to assessing the clinical significance of *POLE* EDMs is appropriately focused on endometrioid tumors.

Among the 30 patients with *POLE* mutations, there was 1 recurrence in a patient aged 55 years with a stage IB, grade 3 endometrioid tumor who had a pelvic recurrence

23 months after surgery. In the TCGA series, there were no recurrences among 17 patients. Together, our studies suggest an overall low rate of recurrence among patients with endometrioid EC (1 in 47 patients combined). If any difference in outcome does exist, then it is unlikely to be clinically useful in planning treatments for endometrioid tumors, given the traditionally impactful clinicopathologic features that would be considered.

POLE mutation status was associated with improved OS in univariate analysis in our cohort. The HR of death for patients with *POLE* EDMs was 0.27 (95% confidence interval, 0.08-0.83) (Table 3, Fig. 2B). In multivariable analysis, however, *POLE* mutation was no longer statistically significant. This is not surprising, because *POLE* EDMs were more common among women who were diagnosed at a younger age (mean age, 58.8 years vs 63.7 years for non-EDM patients) and trended toward being more common in early stage tumors ($P=.027$) (Table 2). These factors are expected to contribute to improved OS. The univariate and multivariable analyses for these and other factors revealed the importance of advanced disease stage, higher grade tumors, and the presence of LVSI for the risk of recurrence and, coupled with advanced age, for OS (Tables 3 and 4). The final multivariable models, following a step-wise modeling procedure (see Materials and Methods) for PFS and OS, illustrate these conclusions with adjusted HRs (Supporting Table 2; see online supporting information).

Clinical Implications for POLE EDMs

Our data do not support survival advantages for *POLE* EDMs in patients with endometrioid EC; and, as such, *POLE* mutations is unlikely to be a useful prognostic marker. We recognize that the low prevalence of the mutation and the low recurrence rate limit the power of our study. A much larger study could prove that a survival advantage does exist for women with *POLE*-mutant tumors. However, 2 factors make it unlikely that *POLE* mutation will have an impact on therapeutic decision making, even where a survival advantage is demonstrated. The first factor is the relatively low frequency of *POLE* EDMs (range, 5%-8%), and the second relates to the importance of tumor stage and grade in the use of adjuvant therapies for patients with EC. Only with a validated and large effect on improvement in the survival of patients with *POLE*-mutant EC (large effect size) would *POLE* mutation likely be part of a nomogram for patients with EC.

Although *POLE* mutation is unlikely to serve as a prognostic marker, the vast resource of genomic information provided by TCGA will lead the way to the discovery of other prognostic markers and molecular targets to serve as potential predictive factors. Markers that are clinically useful in determining treatment choices and improving outcomes require extensive validation[41] and should be universally available and cost-effective.

In summary, somatic *POLE* EDMs are common in endometrioid EC, are observed with equal frequency in tumors with MSS and those with MSI, and are not associated with survival. The majority of tumors with MSI that had *POLE* EDMs lacked *MLH1* methylation. *POLE* EDMs may provide an alternative pathway for MSI in these tumors; and, combining our results with TCGA data, we estimate that up to 25% of MSI, unmethylated tumors will harbor a *POLE* EDM. After future studies assessing somatic and germline defects in the MMR genes of *POLE*-mutant tumors, a positive tumor *POLE* mutation may serve as a marker for somatic origin of disease and act as an exclusionary criterion for LS testing in these patients.

FUNDING SUPPORT

This work was supported by funding from the National Institutes of Health (P30 CA016058) and by additional funding from the James Comprehensive Cancer Center and the Department of Obstetrics and Gynecology at The Ohio State University College of Medicine.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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