Clinical Cancer Research

Endometrial Carcinomas with *POLE* Exonuclease Domain Mutations Have a Favorable Prognosis

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Abstract

Purpose: The aim of this study was to confirm the prognostic significance of *POLE* exonuclease domain mutations (EDM) in endometrial carcinoma patients. In addition, the effect of treatment on *POLE*-mutated tumors was assessed.

Experimental Design: A retrospective patient cohort of 496 endometrial carcinoma patients was identified for targeted sequencing of the *POLE* exonuclease domain, yielding 406 evaluable tumors. Univariable and multivariable analyses were performed to determine the effect of *POLE* mutation status on progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS). Combining results from eight studies in a meta-analysis, we computed pooled HR for PFS, DSS, and OS.

Results: *POLE* EDMs were identified in 39 of 406 (9.6%) endometrial carcinomas. Women with *POLE*-mutated endometrial carcinomas were younger, with stage I (92%) tumors, grade 3 (62%), endometrioid histology (82%), and frequent (49%) lymphovascular invasion. In univariable analysis, *POLE*-mutated endometrial carcinomas had significantly improved outcomes compared with patients with no EDMs for PFS, DSS, and OS. In multivariable analysis, *POLE* EDM*s* were only significantly associated with improved PFS. The effect of adjuvant treatment on *POLE*-mutated cases could not be determined conclusively; however, both treated and untreated patients with *POLE* EDMs had good outcomes. Meta-analysis revealed an association between *POLE* EDM*s* and improved PFS and DSS with pooled HRs 0.34 [95% confidence interval (CI), 0.15–0.73] and 0.35 (95% CI, 0.13–0.92), respectively.

Conclusions: *POLE* EDMs are prognostic markers associated with excellent outcomes for endometrial carcinoma patients. Further investigation is needed to conclusively determine if treatment is necessary for this group of women. *Clin Cancer Res; 22(12); 2865–73. ©2016 AACR.*

Introduction

Endometrial carcinoma is the most common gynecological cancer diagnosed in the developed world, and the incidence is rising (1, 2). Endometrial carcinoma is not just one disease, but encompasses several different histologies with the most common being endometrioid (70%–80%), serous, and clear cell carcinomas (10%–20%). Histologic subtype and other clinicopathologic features [stage, tumor grade, presence of lymphovascular space invasion (LVSI)] are associated with prognosis; these variables are used to direct surgery and adjuvant treatment (3–7). However, the determination of histotype and grade is unreliable, particularly in high-grade tumors, yielding inconsistent classification of endometrial carcinomas (8, 9). Recently, The Cancer Genome Atlas (TCGA) project stratified endometrial carcinomas into four prognostic groups based on genomic features (10). A novel subgroup, termed "ultramutated" harboring *POLE* exonuclease domain mutations (EDM), was found to be associated with markedly favorable progression-free survival (10). This association of *POLE* EDMs with improved outcomes has subsequently been validated in other studies (11, 12).

Somatic and germline *POLE* mutations have been identified in a number of different cancers, including endometrial, colorectal, and giant cell high-grade glioma (10, 13–16). *POLE* encodes the DNA polymerase epsilon, which is responsible for leading strand DNA replication (17, 18). POLE high replication fidelity depends, in part, on its 3′ to 5′ proofreading abilities (19, 20). Early studies of substitutions in DNA polymerases were shown to inactivate or suppress proofreading abilities, thus causing increased replicative error rates (21, 22). In endometrial carcinomas, POLE EDMs are mostly found in hotspot regions (V411L and P286R). These amino acid substitutions lead to an accumulation of a high number of mutations, resulting in an ultramutator phenotype. This phenotype is associated with C>A transversion, high-grade, endometrioid histology, and microsatellite stability (10, 13, 23).

In this study, we determined the frequency of *POLE* EDMs and the prognostic impact in a large independent cohort of endometrial carcinomas. We analyzed the effect of *POLE* mutations on progression-free survival (PFS), disease-specific survival (DSS),

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Translational Relevance

In this study, we confirm that endometrial carcinoma patients with *POLE* exonuclease domain mutations (EDM) have an improved progression-free survival (PFS) in a large cohort ($n = 406$) of women with endometrial carcinoma. Meta-analysis encompassing eight studies also confirms improved PFS and disease-specific survival for *POLE*-mutated endometrial carcinoma patients. Excellent outcomes were observed despite the presence of what are considered high-risk pathologic features [high-grade (62%), deep myometrial invasion (stage IB; 37%), lymphovascular space invasion (49%)] in *POLE*-mutated tumors, suggesting that this feature will have independent and profound clinical value. From this cohort, patients with *POLE* EDMs had very good outcomes irrespective of treatment; however, we were not sufficiently powered to resolve the key question of whether *POLE*-mutated cancers respond well to standard therapies or do not require adjuvant therapy. Future studies in endometrial carcinomas should include *POLE* mutation testing in order to provide important prognostic information for women, and enable stratification of clinical trials for molecular subtype-specific approaches to endometrial carcinoma management.

and overall survival (OS). We also attempted to determine whether the effect of *POLE* mutational status on outcomes differed for patients who received adjuvant treatment. Given that many tumors with *POLE* mutations are high-grade, adjuvant therapy is often administered, and it is unclear whether favorable outcomes are dependent on treatment. The ability to identify patients with excellent prognosis who may not require chemotherapy or radiotherapy would conserve resources, but, more importantly, would spare these women from overtreatment and unnecessary toxic side effects. Last, we updated the survival meta-analysis (11) of *POLE*-mutated endometrial carcinomas to include results from eight different studies (6, 10–12, 24–27) including our own results. The overall findings significantly strengthen the growing body of evidence that *POLE* mutations are highly favorable prognostic markers in endometrial carcinomas, and will change how we manage women with this disease.

Materials and Methods

Patient cohort

A retrospective patient cohort ($n = 496$) from 1983 to 2013 was identified from five previously created endometrial tumor tissue microarrays (TMA). The original pathologic histotype diagnosis, as rendered in the surgical pathology report, was used for this series as being representative of clinical practice. Pathology was reviewed for all cases only to assign FIGO 2009 stage criteria assignment and to identify suitable blocks for molecular analysis. The associated formalin-fixed paraffin-embedded (FFPE) tumor blocks were obtained from the Vancouver General Hospital archives and fresh-frozen tumor samples, when available, from the OvCaRe Tumor Biobank. For banked specimens, all patients were approached for written informed consent to donate specimens for use in a research ethics board–approved research protocol. Inclusion criteria included primary tissue availability, hysterectomy ($n = 494$), or endometrial biopsy ($n = 2$), and surgery dates prior to January 1, 2012, ensuring minimum 2-year follow-up. Exclusion criteria included lost to follow-up ($n = 2$), neoadjuvant therapy ($n = 10$), surgery after January 1, 2012 ($n = 44$), inadequate quantity/quality of DNA ($n = 33$), and germline *POLE* mutation (H422Y; $n = 1$), which left 406 unique patients for *POLE* somatic mutation testing.

DNA extractions

DNA from frozen tumors ($n = 150$) and buffy coat were extracted using the Qiagen Gentra Puregene Kit (Qiagen) as per the manufacturer's protocols. FFPE tumor blocks ($n = 258$) were extracted using the Qiagen FFPE tissue Kit, and all DNA was quantified using the Qubit fluorometer Kit (Life Technologies). To determine somatic status, normal DNA was either extracted from available buffy coat or from representative normal FFPE blocks.

Targeted sequencing and analysis

Targeted primers were designed to cover the *POLE* exonuclease domain exons 9 to 14. PCR products (150–200 bp) were amplified using the Fluidigm 48 × 48 Access Arrays, as per the manufacturer's protocol, with input of 100 ng FFPE-derived DNA, and 50 ng high-quality DNA from buffy coat or frozen tumor DNA. DNA barcodes (10 bp) with Illumina cluster-generating adapters were added to the libraries, and 96 samples were pooled. The library pools were sequenced using 300 cycle Illumina MiSeq kits for ultradeep sequencing with >1,000× coverage. All validated *POLE* mutations were bidirectionally sequenced twice at minimum, and once in the normal to validate somatic or germline status using either ultradeep MiSeq sequencing or Sanger sequencing. Additional details can be found in the Supplementary Appendix: Methods.

Statistical analysis

Univariable associations between the *POLE* marker and clinicopathologic variables were tested using nonparametric tests. Multiway associations were estimated with a multivariable Firth penalized likelihood logistic regression model with *POLE* status as a dependent variable. A backward selection procedure was used to determine the most relevant clinicopathologic features associated with *POLE* mutations. Log-rank tests for univariable Kaplan–Meier's (KM) survival analysis and multivariable Cox proportional hazards models were used to determine the effect of *POLE* mutational status and clinicopathologic parameters on survival outcomes (PFS, DSS, and OS). A Firth bias reducing correction was applied as needed to obtain estimates, and the profile-penalized likelihood was used to generate confidence intervals (CI). Additional details can be found in Supplementary Appendix: Methods and Supplementary Tables S7 to S13.

Results

Mutation analysis

In this series, 496 endometrial tumors were identified; however after study exclusions ($n = 89$; Materials and Methods), 407 tumor samples were sequenced for *POLE* EDMs. One case was excluded for the presence of a germline mutation encoded by the amino acid substitution H422Y in a serous carcinoma. Therefore, we identified 39 of 406 (9.6%) endometrial tumors with somatic *POLE* mutations (Table 1). The pathologic features of these *POLE*-mutated tumors have been previously described in detail (28); 32

Table 1. *POLE* EDM endometrial carcinomas

Histology	Grade	*POLE* wild-type	*POLE* mutated (%)	*POLE* mutation (#)
Endometrioid	1	117	7 (18%, 1.7%)	P286R/S (4), V411L (2), M295R (1)
	2	62	6 (15%, 1.5%)	P286R (2), A456P (2), V411L (1) S279A/V411L (1)
	3	104	19 (49%, 4.7%)	V411L (7), P286R (5), F367S/C (2), P436R (2), A456P (2), L424P (1)
Serous	3	77	3 (8%, 0.7%)	V411L (1), P441L (1), F367L (1)
Clear cell	3	0	1 (3%, 0.2%)	P286R (1)
Undifferentiated	3	0	1 (3%, 0.2%)	V411L (1)
Mixed carcinomas	1, 3	7	2 (5%, 0.5%)	P286R (1), E396G (1)
Total 406 tumors		367	39 (9.6%)	

NOTE: Indicated percentages in *POLE*-mutated column are as follows: percentage of total *POLE*-mutated cases ($n = 39$) and percentage of total endometrial carcinoma cases ($n = 406$), respectively.

of 39 (82%) were endometrioid histology with 7 of 39 (18%) grade 1, 6 of 39 (15%) grade 2, and 19 of 39 (49%) grade 3 endometrioid carcinoma. *POLE* EDMs were also present in non-endometrioid histologies; 3 serous carcinoma, 1 clear cell carcinoma, 2 mixed histology, and 1 undifferentiated carcinoma. Of all the grade 3 carcinomas, regardless of histology, 25 of 210 (12%) harbored somatic *POLE* EDMs. The most frequent mutations were found in *POLE* hotspot regions; 13 of 39 (33%) mutations encoded P286R/S, and 13 of 39 (33%) encoded V411L. Low-frequency mutations outside of these two hotspot amino acids were also identified in the exonuclease domain: A465P, E396G, F367C/L/S, L424P, M295R, P436R, and S297A (Table 1).

Clinicopathologic characteristics of *POLE*-mutated carcinomas

We next determined the association of *POLE* somatic mutations with patient clinicopathologic characteristics (Supplementary Tables S1 and S2). The median follow-up time was 5.2 years (reverse Kaplan–Meier), and the median age of *POLE*-mutated patients (58 years) was statistically different ($P < 0.001$) from patients with *POLE* wild-type tumors (66 years). The majority of *POLE*-positive tumors were stage I (95%), two stage II to III, and no stage IV tumors; compared with tumors without *POLE* mutations in which 248 (68%) were stage I and 116 (32%) were stage II to IV ($P < 0.001$). LVSI was present in 49% of *POLE*-mutated carcinomas, yet out of 262 patients who had a lymph node dissection, not 1 patient with a *POLE* EDM was node positive ($P < 0.05$). Although the majority of *POLE* tumors were endometrioid histology and grade 3, tumor grade was not statistically different between *POLE*-mutated and *POLE* wild-type tumors in this series. In addition, we used a logistic regression model with variable selection to show that the odds of having a *POLE* mutation were decreased with age (OR, 0.94; 95% CI, 0.9–0.99 per year), body mass index (BMI; OR, 0.92; 95% CI, 0.84–0.98 per unit increase), and advanced stage (OR, 0.04; 95% CI, 0–0.39 relative to stage I).

Clinical outcome of *POLE*-mutated endometrial carcinomas

The prognostic effect of *POLE* mutations on clinical outcomes was analyzed with univariable and multivariable survival models. Using univariable analyses, *POLE*-mutated tumors were found to be significantly associated with improved PFS (HR, 0.16; 95% CI, 0.02–0.58; $P < 0.001$), DSS (HR, 0.26; 95% CI, 0.05–0.76; $P = 0.005$), and OS (HR, 0.35; 95% CI, 0.12–0.81; $P = 0.006$; Fig. 1 and Supplementary Fig. S1). Similarly, all other clinicopathologic variables except BMI were statistically significant for PFS, DSS, and OS ($P < 0.05$; Supplementary Table S3). In multivariable analysis, *POLE* was associated with improved PFS (HR, 0.22; 95% CI, 0.02–0.83; $P = 0.010$), but we did not find a statistically significant association with DSS (HR, 0.48; 95% CI, 0.1–1.48; $P = 0.1452$) or OS (HR, 0.69; 95% CI, 0.22–1.67; $P = 0.332$; Table 2 and Supplementary Table S4).

Previous studies have demonstrated that *POLE*-mutated cases are mostly grade 3 endometrial carcinomas (11, 12, 29). To compare with these studies, we also assessed the outcome of all *POLE*-mutated grade 3 tumor histologies. Women with these tumors harboring *POLE* mutations demonstrated significantly improved univariable PFS [HR, 0.14; 95% CI, 0.02–0.49; Likelihood Ratio Test (LRT) $P < 0.001$], DSS (HR, 0.14; 95% CI, 0.02–0.52; LRT $P < 0.001$), and OS (HR, 0.29; 95% CI, 0.08–0.74; LRT $P = 0.003$) compared with *POLE* wild-type tumors (Fig. 1; Supplementary Fig. S1). In a multivariable analysis, the mutational status of *POLE* was associated with an improved DSS (HR, 0.34; 95% CI, 0.04–1.33; LRT $P = 0.073$) and PFS (HR, 0.26; 95% CI, 0.03–1; LRT $P = 0.025$; Table 2; Supplementary Table S5), though the sample size was too small to properly assess the significance of this association. There were no disease-specific deaths observed in the grade 3 *POLE*-mutated patients, and survival analyses demonstrated that patients with *POLE* EDMs had a significantly improved disease-specific survival relative to those with no *POLE* EDMs ($P = 0.001$;Fig. 1D). In this series, there were three survival events associated with POLE-mutated patients. A single disease recurrence was observed in a grade 3, stage IB, 52-year-old woman with endometrioid carcinoma and a P286R POLE mutation, who was treated with adjuvant pelvic EBRT (external beam radiation therapy) postsurgical staging. Two deaths secondary to disease occurred in women who also had the P286R POLE substitution. Both patients were older (73 and 75 years), with one diagnosed as stage IB, grade 2 endometrioid carcinoma given adjuvant pelvic EBRT and vaginal brachytherapy, and the other with stage IIIC1, grade 3, mixed serous/endometrioid histology, treated with both carboplatin and pelvic EBRT.

Impact of adjuvant treatment on *POLE*-mutated cases

We next evaluated the role of adjuvant treatment on the prognosis of patients with *POLE* EDMs. Clinicopathologic characteristics of patients by *POLE* mutation status and adjuvant treatment can be found in Table 3. The majority (75%) of *POLE* patients who did not receive treatment had tumors that were stage IA, with 44% as grade 1 and 44% grade 3. For patients that received treatment, their tumors were 48% as stage IA, 43% stage IB, and 77% as grade 3. To assess whether the effect of *POLE* mutational status on survival outcomes was altered for those receiving adjuvant treatment, an interaction term between any adjuvant treatment (chemotherapy or radiotherapy) and *POLE* mutational status was added in a Cox proportional hazards

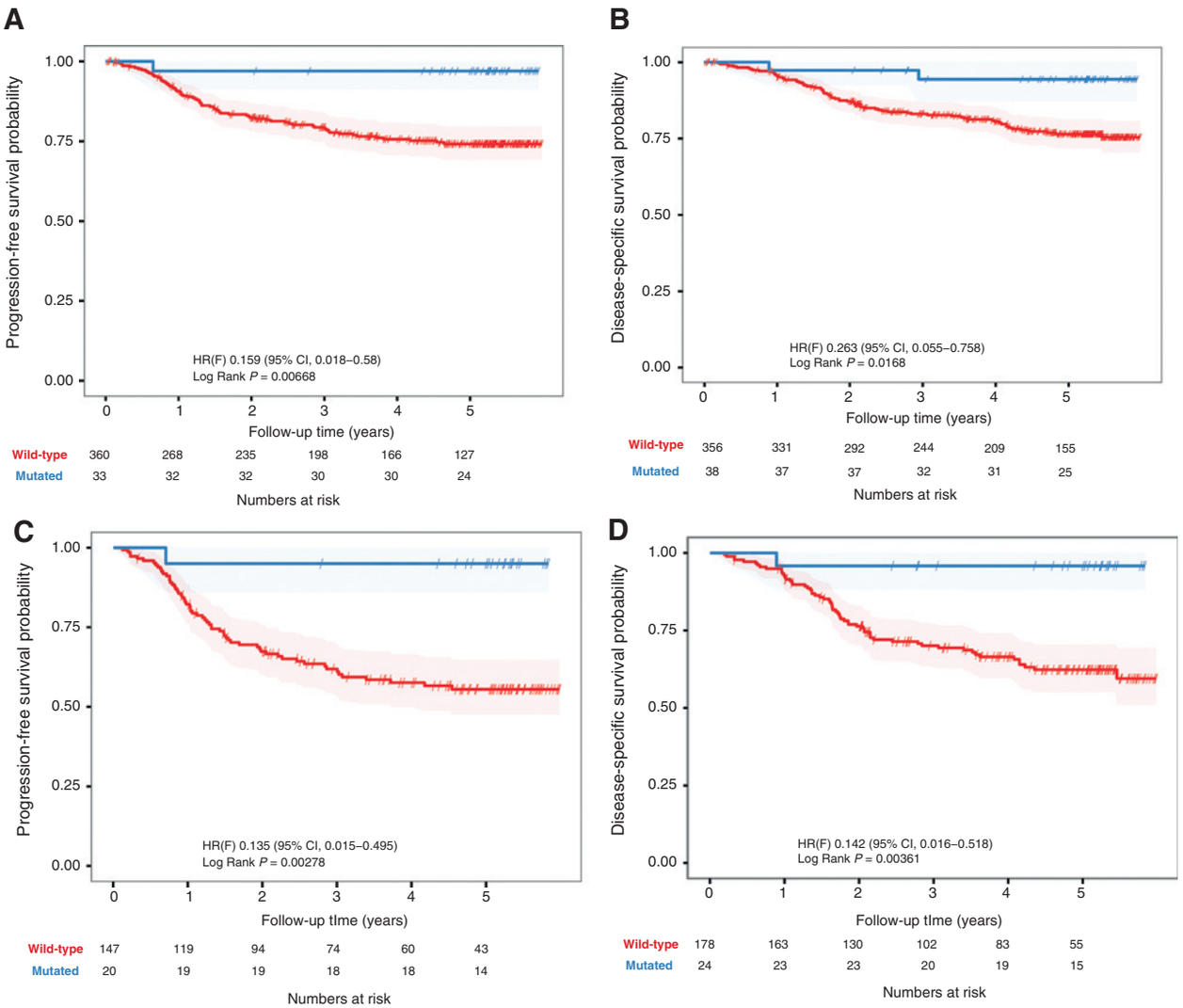

Figure 1.
Kaplan–Meier survival curves for *POLE*-mutated and *POLE* wild-type endometrial carcinomas. A, PFS for the full endometrial carcinoma cohort. B, DSS for the whole endometrial carcinoma cohort. C, PFS for grade 3 endometrial carcinomas only. D, DSS for grade 3 endometrial carcinoma cohort only. Blue lines, *POLE*-mutated cases; red lines, *POLE* wild-type cases. *P* values were obtained by a two-sided log-rank test. F, Firth correction.

model; the effect of the interaction term was not statistically significant. This could be attributed to either a true lack of interaction between treatment and *POLE* mutation status or to a lack of power to detect the interaction. Given the current available data, we were unable to conclude whether *POLE*-mutated tumors require adjuvant treatment (Supplementary Table S6). In a subgroup analysis of patients who received or did not receive adjuvant treatment, *POLE* cases consistently had a favorable prognosis compared with endometrial carcinoma tumors without *POLE* EDMs (Fig. 2 and Supplementary Fig. S2). Of note, there

Table 2. Clinical outcome of *POLE*-mutated compared with *POLE* wild-type endometrial carcinomas determined by univariable and multivariable analyses

	Univariable survival			Multivariable survival		
Outcome	**Number of events/*n***	**HR (95% CI)**	**LRT *P* value**	**Number of events/*n***	**HR (95% CI)**	**LRT *P* value**
All cases						
PFS	73/339	0.16 (0.02–0.58)^F	<0.001	68/320	0.22 (0.02–0.83)^F	0.010
DSS	77/394	0.26 (0.05–0.76)^F	0.005	68/366	0.48 (0.10–1.48)^F	0.145
OS	105/406	0.35 (0.12–0.81) ^{F}	0.006	94/377	0.69 (0.22–1.67)^F	0.332
Only grade 3 cases						
PFS	62/167	0.14 (0.02–0.49)^F	<0.001	58/156	0.26 (0.03–1.00)^F	0.025
DSS	63/202	0.14 (0.02–0.52)^F	<0.001	55/183	0.34 (0.04–1.33)^F	0.073
OS	74/211	0.29 (0.08–0.74)^F	0.003	65/191	0.69 (0.18–1.90)^F	0.3899

Abbreviations: DSS, disease specific survival; ^{F}, Firth correction; HR, hazard ratio; LRT, likelihood ratio test; OS, overall survival; PFS, progression-free survival.

Table 3. Description of clinical characteristics of *POLE*-mutated and *POLE* wild-type patients who did and did not receive adjuvant treatment

	No adjuvant treatment		With adjuvant treatment	
	***POLE* wild-type**	***POLE* mutated**	***POLE* wild-type**	***POLE* mutated**
Total	204	16	158	22
Age at surgery				
Median (IQR)	67 (57–76)	58 (55–64)	67 (59–74)	56 (48–62)
BMI				
Median (IQR)	31 (27–41)	24 (20–28)	29 (23–36)	28 (25–32)
Stage				
IA	149 (73%)	12 (75%)	40 (25%)	10 (48%)
IB	36 (18%)	4 (25%)	22 (14%)	9 (43%)
II	8 (4%)	0	20 (13%)	0
III	9 (4%)	0	53 (34%)	2 (10%)
IV	2 (1%)	0	23 (15%)	0
Unknown	0	0	0	1
Grade				
1	106 (52%)	7 (44%)	11 (7.0%)	1 (5%)
2	40 (20%)	2 (13%)	24 (15%)	4 (18%)
3	58 (28%)	7 (44%)	123 (78%)	17 (77%)
Histologic subtype				
Endometrioid	185 (90.7%)	15 (93.8%)	94 (60%)	16 (73%)
Serous	18 (8.8%)	1 (6.2%)	58 (37%)	2 (9%)
Clear cell	0	0	0	1 (5%)
Mixed*	1 (0.5%)	0	6 (4%)	2 (9%)
Undifferentiated	0	0	0	1 (5%)
Histologic subtype and grade				
G1 or G2 endometrioid	145 (71%)	9 (56%)	34 (22%)	4 (18%)
G1 or G2 non-endometrioid	1 (0.5%)	0	1 (1%)	1 (5%)
G3 endometrioid	40 (20%)	6 (38%)	60 (38%)	12 (55%)
G3 non-endometrioid	18 (9%)	1 (6%)	63 (40%)	5 (23%)
LVSI				
Yes	36 (19%)	6 (40%)	91 (61%)	12 (57%)
No	156 (81%)	9 (60%)	59 (39%)	9 (43%)
Unknown	12	1	8	1
Nodal status				
Positive	2 (1%)	0	34 (22%)	0
Negative	91 (45%)	11 (69%)	37 (24%)	5 (23%)
Not tested	111 (54%)	5 (31%)	83 (54%)	17 (77%)
Unknown	0	0	4	0
Adjuvant treatment				
No treatment	204	16	0	0
With treatment	0	0	158	22
Both	0	0	66 (42%)	10 (46%)
Chemo only	0	0	30 (19%)	3 (14%)
Pelvic EBRT only	0	0	57 (36%)	7 (32%)
Vag. Brachy only	0	0	5 (3%)	2 (9%)

NOTE: Percentages are based on columns, and percentages do not include unknowns.

Abbreviations: EBRT, external beam radiation therapy; IQR, interquartile range; Vag. Brachy, vaginal brachytherapy, high-dose radiation to the vagina.

*, indicates mixed endometrioid and clear cell, mixed endometrioid with undifferentiated, mixed serous and carcinosarcoma, mixed serous and clear cell, mixed serous and endometrioid.

were no PFS or DSS events in the *POLE*-mutated group that did not receive adjuvant treatment. This included 2 patients with stage IB, grade 3 endometrioid *POLE*-mutated carcinomas. Even though the separation of survival curves appears larger in the treated group, we could only determine that the *POLE* mutation was prognostic of outcome, and could not be adequately statistically assessed as a predictive marker for the effect of treatment.

Meta-analysis of survival outcome

POLE-mutated endometrial carcinomas account for a small percentage (8%–12%) of all endometrial carcinoma patients. Therefore, even relatively large studies lack power to measure the prognostic effects of *POLE* mutations, particularly because these cases tend to have fewer outcome events. Church and colleagues (11) presented a meta-analysis of *POLE*-mutated endometrial carcinoma using five independent studies (6, 10, 25–27), and we have updated these results to include eight studies (6, 10–12, 24–27), including our own cohort. Pooled HRs that determine the aggregate prognostic effect of *POLE* on PFS, DSS, and OS were computed (Supplementary Tables S7, S9–S11). The effect on PFS was determined using six studies, resulting in a pooled HR of 0.34 (95% CI, 0.15–0.73), and DSS combined six studies showing an overall HR of 0.34 (95% CI, 0.13–0.91; Fig. 3A and B). This shows that endometrial carcinoma patients with *POLE* EDMs have an excellent prognosis with a 3-fold improvement of PFS and DSS. Pooled OS analysis using eight cohorts was also performed to show that *POLE* status is not significantly prognostic of OS (Supplementary Fig. S3A). Last, we analyzed pooled HRs for grade 3 endometrial carcinomas from five studies to find a significant 3-fold improvement of PFS (HR, 0.32; 95% CI, 0.11–0.97; Fig. 3C). DSS and OS pooled HRs can be found in Supplementary Fig. S3B–S3C and Supplementary Tables S8, S12, and S13).

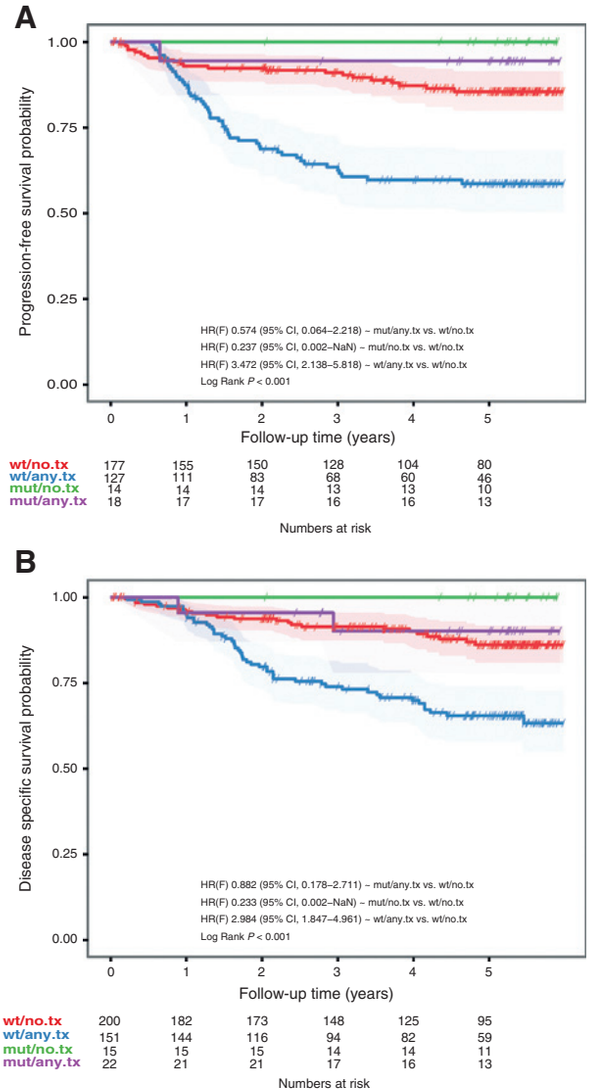

Figure 2.
Kaplan–Meier survival curves of *POLE*-mutated and *POLE* wild-type endometrial carcinomas that received and did not receive adjuvant treatment. A, PFS. B, DSS. *P* values were obtained by a two-sided log-rank test. Red lines, *POLE* wild-type, no adjuvant treatment; blue lines, *POLE* wild-type, any adjuvant treatment; green lines, *POLE* mutant, no adjuvant treatment; purple lines, *POLE* mutant, any adjuvant treatment; wt, wild-type; mut, *POLE* mutant; any.tx, any treatment; no.tx, no treatment. F, Firth correction.

Discussion

In this series, we identified the presence of somatic *POLE* EDMs in 9.6% of a large independent cohort of 406 endometrial carcinomas. We confirmed the prognostic impact of *POLE* mutations in endometrial carcinomas, demonstrating favorable outcomes even when tumors demonstrate high-risk features, such as high-grade (62%), LVSI (49%), or non-endometrioid histology (18%). The presence of LVSI and non-endometrioid histology in *POLE*-mutated tumors is different from the previous literature. Church and colleagues did not report any *POLE* cases with LVSI and only 1% of non-endometrioid histology in the combined Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) case series. This difference is of potential interest; however, it should be noted that LVSI and histotype are features that are generally subjective with interobserver variability and may not be reproducible between series. Univariable analysis revealed that *POLE*-mutated tumors were associated with improved PFS, DSS, and OS. In a multivariable analysis of the compete cohort, *POLE* mutations were shown to have more than a 3-fold improved PFS compared with patients with tumors that do not harbor *POLE* EDMs. Meta-analysis results that included data from several independent studies reporting *POLE* mutation status and clinical outcomes confirmed a favorable prognosis for cases with *POLE* mutations for both PFS and DSS. We were not able to show a significant effect of *POLE* mutation on pooled OS; this may reflect the relatively small number of patients with *POLE* mutations and censoring at 5 years. Longer follow-up time with more patients will be needed to address the significance of *POLE* mutations on OS.

The survival advantage of *POLE*-mutated high-grade endometrioid carcinomas has been observed in multiple studies (10–12, 29) but not all (24). We did not show a significant association between grade, histology, and *POLE* status; however, we did confirm that *POLE*-mutated grade 3 endometrial carcinomas have an excellent prognosis. This study again reliably confirms *POLE* EDMs as a significant prognostic biomarker for endometrial cancers. Our cohort of grade 3 endometrial carcinomas also included serous and clear cell carcinomas that are generally assumed to have poor prognoses. The presence of non-endometrioid histologies in the *POLE*-mutated cohort may be seen as a potential weakness in this series; however, the irreproducibility of histotype assignment in high-grade endometrial carcinomas is well known (8, 30). *POLE* mutations have been identified in serous carcinomas (13, 31, 32); however upon re-review, these are generally tumors with ambiguous features that cause significant interobserver variability even between expert pathologists (12). Regardless, the few patients with *POLE*-mutated tumors and non-endometrioid histotype had an improved outcome, and determination of POLE mutation status appears to be as important than other "traditional" pathologic parameters of known prognostic significance, e.g., histotype, and the presence of LVSI. Subjectivity and inconsistent assignment of these parameters support the incorporation of reproducible molecular features such as *POLE* mutation status in risk stratification.

One novel objective of this study was to determine if favorable outcomes in patients harboring somatic *POLE* EDMs were independent of adjuvant therapy. However, we were not powered to answer this definitively. A high proportion of our *POLE*-mutated cases are considered high-risk and thus were given adjuvant treatment, due to the presence of pathologic high-risk features, such as high-grade (62%), deep myometrial invasion (stage IB; 37%) or disease beyond the uterus (5%), or lymphovascular space invasion (49%). Thus, the number of comparative cases with *POLE* EDMs that did not receive adjuvant treatment was small (42%). Overall, we observed excellent outcomes in both adjuvant-treated and untreated *POLE*-mutated patients compared with patients without *POLE* EDMs. Outcome events were rare overall in *POLE*-mutated cases, and completely absent in the untreated *POLE*-mutated cohort, which were primarily early-stage (stage IA/B) tumors. We did observe two recurrences and deaths secondary to endometrial carcinoma in the *POLE* wild-type group of similar early-stage low-grade endometrial carcinomas, which

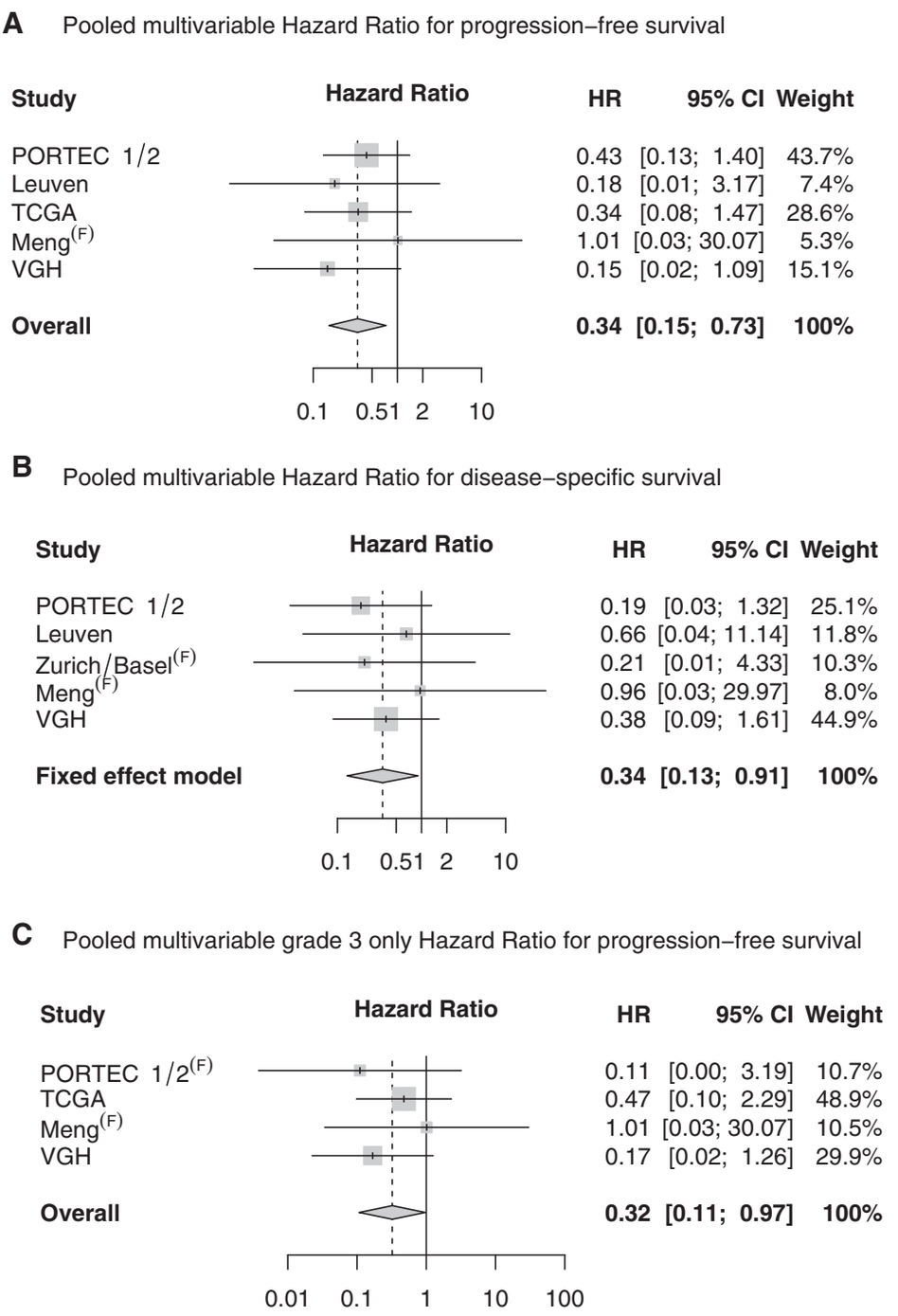

Figure 3.
Pooled multivariable HR plots. A, PFS of *POLE*-mutated endometrial carcinomas, including six combined studies. B, DSS of *POLE*-mutated endometrial carcinomas encompassing six combined studies. C, PFS of *POLE*-mutated grade 3 endometrial carcinomas, including five combined studies. Combined pooled multivariable meta-analysis with weights computed using the inverse variance method, HR, and 95% CI. The overall HR (gray diamond) was generated by using a fixed effect model. F, Firth correction.

would have been anticipated to have a good prognosis and were not given adjuvant therapy. This raises the possibility of a subset of low-grade endometrial carcinomas, where a biomarker is yet to be identified, that may need additional treatment to prevent recurrence. We may be missing an opportunity for curative therapy, underscoring the need to improve the current systems of endometrial carcinoma risk assessment (33) for enhanced patient management. The ideal cohort to determine the relative effect of treatment and *POLE* status on endometrial carcinoma outcomes would be from clinical trial(s) (archival material available for determination of *POLE* mutation status) where one arm received no additional therapy after surgery. In addition, a collaborative pooled analysis with known *POLE* mutation status, treatment details, and outcomes would enable us to determine if women with *POLE*-mutated endometrial carcinoma truly need adjuvant treatment.

Defects in proofreading DNA polymerases to produce a mutator phenotype have been established in model systems, such as yeast, bacteria, and mice (34), and these defects cause genomic instability (35) that can promote tumorigenesis. *POLE* EDMs cause complete disruption of exonuclease activity (P286S, M295R, S297A; ref. 23) or reduction in proofreading ability (F367C/L/S; ref. 23). Through conservation studies with T4 DNA polymerase, it is likely that mutations at the residues L424P, P436R, P441L, and A456P near the exonuclease III domain also reduce proofreading resulting in a mutator phenotype (23, 34, 36). In Polε exonuclease-deficient mice, there is a 10-fold increase in the frequency of mutagenesis, and these mice develop tumors (37). Evidence shows that *POLE* EDMs cause increased nonsense mutation rates in key tumor suppressor genes (*TP53*, *PIK3R1*, *ATM*, *ATR*), which likely aid in tumor initiation (23). However, we can hypothesize that there may be a threshold of mutational burden that tumor cells can tolerate (38). There is also evidence that *POLE* ultramutated tumors are associated with peritumoral lymphocytes and tumor-infiltrating lymphocytes (28), which exhibit an enhanced T-cell antitumor response to antigenic neoepitope expression (39, 40). This immune response may play a mechanistic role in the observed favorable prognosis, as an increased immune response in these tumors may decrease metastatic potential leading to a less-aggressive tumor (28). An increase in tumor-infiltrating T cells has also been observed in microsatellite unstable endometrial and colorectal tumors, where the immune response may also contribute to antitumor effects and in the case of colorectal cancers, improved prognosis (39, 41). Response to PD-1 blockade has been demonstrated in progressive metastatic carcinomas with mismatch repair deficiency (42); there are, as yet, no data on response to immune checkpoint blockade/anti–PD-1 therapy in *POLE*-mutated endometrial carcinomas. It is interesting to note that in contrast with endometrial carcinomas, *POLE* EDMs in patients with colorectal cancer had impaired survival but was not significant (43). Alternatively, in a subgroup analysis of colorectal cancer patients with *POLE* EDMs, with high stage disease, and received adjuvant therapy showed a statistically significant increased mortality (43). It is unclear as to why there is such a drastic difference in survival of *POLE*-mutated cancers arising at different primary sites, i.e., colorectal versus endometrial. These observations strengthen the view that effects of mutations on tumor characteristics or tumor response to therapeutics are tissue and context-dependent (44). While these tumors exhibit prominent host immune response in most cases, given the overall excellent outcomes in these women, it is not yet warranted to consider expensive immunotherapy regimens. It is, as yet, unclear if in the women receiving adjuvant therapy, whether there is a synthetic lethality effect to reduce tumor viability or an increased stimulation of an immune response.

Future studies should focus on determining if patients with *POLE*-mutated tumors require any adjuvant therapy to achieve favorable outcomes. *POLE* mutation status could be used for stratification of patients and clinical trials to enable evaluation within this unique subgroup. The integration of testing for *POLE* mutation status into endometrial classification and risk assessment will ultimately help guide endometrial carcinoma management and provide important prognostic information to the women with this disease (45). However, our preliminary data suggest that continuing our current standard of care is advisable.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Other (helped with the comparisons of amino acid changes in DNA polymerase epsilon in tumor cells to amino acid changes in model organisms (phage T4 and Yeast) that decrease exonuclease "proofreading" activity and also helped to write the section of the manuscript that discusses the comparisons): L.J. Reha-Krantz

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