Title:

Endometrial carcinomas with *POLE* exonuclease domain mutations have a favorable prognosis

Running Title:

POLE mutations in endometrial cancer

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Conflicts of Interest:

DGH is founder and shareholder of Contextual genomics Inc. All other authors have no conflicts of interest.

Word Count:

3140

Manuscript type:

Research Article – Human Cancer Biology

Keywords: (up to 5)

POLE, endometrial cancer, prognosis, biomarker, treatment

Financial Support:

This study was supported by the BC Cancer Foundation Sarabjit Gill Fund. MKM was supported by the Canadian Institute of Health Research (CIHR) Doctoral Fellowship. JNM is supported by the Canadian Institute of Health Research (CIHR) New Investigators award.

Translational relevance:

In this study, we confirm that endometrial carcinoma (EC) patients with *POLE* exonuclease mutations (EDMs) have an improved progression-free survival (PFS) in a large cohort (n=406) of women with EC. Meta-analysis encompassing eight studies also confirms improved PFS and disease-specific survival for *POLE* mutated EC patients. Excellent outcomes were observed despite the presence of what are considered high-risk pathologic features (high-grade (62%), deep myometrial invasion (Stage1B) (37%), lymphovascular space invasion (49%)) in *POLE* mutated tumours, 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 ECs 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 EC management.

Abstract:

Purpose:

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

Experimental design:

A retrospective patient cohort of 496 EC patients was identified for targeted sequencing of the *POLE* exonuclease domain, yielding 406 evaluable tumours. 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 hazard ratios (HR) for PFS, DSS, and OS.

Results:

POLE EDMs were identified in 39 of 406 (9.6%) ECs. Women with *POLE* mutated ECs were younger, with Stage 1 (92%) tumors, grade 3 (62%), endometrioid histology (82%) and frequent (49%) lymphovascular invasion. In univariable analysis, *POLE* mutated ECs had significantly improved outcomes compared to patients with no EDMs for PFS, DSS and OS. In multivariable analysis, *POLE* EDMs 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* EDMs and improved PFS

and DSS with pooled HRs 0.34 (95% 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 EC patients. Further investigation is needed to conclusively determine if treatment is necessary for this group of women.

Introduction

Endometrial carcinoma (EC) is the most common gyneacological cancer diagnosed in the developed world, and the incidence is rising(1, 2). EC 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 clinicopathological features (stage, tumour 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 tumours, yielding inconsistent classification of ECs(8, 9). Recently, The Cancer Genome Atlas (TCGA) project stratified ECs 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 it's 3'-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 ECs, *POLE* exonuclease domain mutations (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 ultra-mutator phenotype. This phenotype is associated with C>A transversion, high-grade, endometrioid histology and microsatellite stability (MSS)(10, 13, 23).

In this study, we determined the frequency of *POLE* EDMs, and the prognostic impact in a large independent cohort of ECs. We analyzed the effect of *POLE* mutations on progression-free survival (PFS), disease-specific survival (DSS) 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 tumours 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 radiation would conserve resources, but more importantly, would spare these women from overtreatment and unnecessary toxic side effects. Lastly, we updated the survival meta-analysis(11) of *POLE* mutated ECs 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 ECs, and will change how we manage women with this disease.

Materials and Methods

Patient cohort

A retrospective patient cohort (n=496) from 1983-2013 was identified from five previously created endometrial tumour tissue microarrays (TMA). The original pathological 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) tumour blocks were obtained from the Vancouver General Hospital (VGH) archives and fresh frozen tumour samples, when available, from the OvCaRe Tumour Biobank. For banked specimens, all patients were approached for written informed consent to donate specimens for use in a research ethics board (REB) 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 tumours (n=150) and buffy coat were extracted using the Qiagen Gentra Puregene kit (Qiagen) as per manufacturers protocols. FFPE tumour 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 representative normal FFPE blocks.

Targeted Sequencing and Analysis

Targeted primers were designed to cover the *POLE* exonuclease domain exons 9-14. PCR products (150-200bp) were amplified using the Fluidigm 48X48 Access Arrays, as per manufacturers protocol, with input of 100ng FFPE derived DNA, and 50ng high-quality DNA from buffy coat or frozen tumour DNA. DNA barcodes (10bp) with Illumina cluster-generating adapters were added to the libraries, and 96 samples pooled. The library pools were sequenced using 300 cycle Illumina MiSeq kits for ultra-deep sequencing with >1000X coverage. All validated *POLE* mutations were bi-directionally sequenced twice at minimum, and once in the normal to validate somatic or germline status using either ultra-deep MiSeq sequencing or Sanger sequencing. Additional details can be found in the **Supplemental Appendix: Methods**.

Statistical Analysis

Univariable associations between the *POLE* marker and clinicopathological variables were tested using non-parametric tests. Multi-way 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 clinicopathological 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 clinicopathological parameters on survival outcomes (Progression-Free Survival (PFS), Disease-Specific Survival (DSS) and Overall Survival (OS)). A Firth bias reducing correction was applied as needed to obtain estimates, and the profile penalized likelihood was used to generate

confidence intervals. Additional details can be found in the **Supplemental Appendix: Methods and Tables S7-S13.**

Results

Mutation analysis

In this series, 496 endometrial tumours were identified, however after study exclusions (n=89) (**Methods**), 407 tumour 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 39/406 (9.6%) endometrial tumors with somatic *POLE* mutations (**Table 1**). The pathological features of these *POLE* mutated tumours have been previously described in detail(28); 32/39 (82%) were endometrioid histology with 7/39 (18%) grade 1, 6/39 (15%) grade 2, and 19/39 (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, 25/210 (12%) harbored somatic *POLE* EDMs. The most frequent mutations were found in *POLE* hotspot regions; 13/ 39 (33%) mutations encoded P286R/S, and 13/39 (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 (**Table1**).

Clinicopathological characteristics of *POLE* mutated carcinomas

We next determined the association of *POLE* somatic mutations with patient clinicopathological characteristics (**Supplemental Tables S1-S2**). The median follow-up time was 5.2 years (reverse KM), and the median age of *POLE* mutated patients (58 years) was statistically different ($p<0.001$) from patients with *POLE* wild-type tumours (66 years). The majority of *POLE* positive tumours were Stage I (95%), two Stage II-III, and no Stage IV tumours; compared to tumors without *POLE* mutations in which 248 (68%) were Stage I, and 116 (32%) Stage II-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 one patient with a *POLE* EDM was node positive ($p<0.05$). Although the majority of *POLE* tumours were endometrioid histology and grade 3, tumour grade was not statistically different between *POLE* mutated and *POLE* wild-type tumours in this series. Additionally, we used a logistic regression model with variable selection to show that the odds of having a *POLE* mutation were decreased with age (OR (odds ratio) 0.94 (95% CI 0.9-0.99) per year), 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 tumours 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, $p=0.006$), (**Figure 1 and Supplemental Figure S1**). Similarly, all other clinicopathological variables except BMI were statistically significant for PFS, DSS and OS

($p<0.05$) (**Supplemental 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 Supplemental Table S4**).

Previous studies have demonstrated that *POLE* mutated cases are mostly grade 3 ECs (11, 12, 29). To compare to these studies, we also assessed the outcome of all *POLE* mutated grade 3 tumour histologies. Women with these tumours harboring *POLE* mutations demonstrated significantly improved univariable PFS (HR=0.14, 95% CI=0.02-0.49, 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 tumours (**Figure 1, Supplemental Figure 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, Supplemental 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, 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) **(Figure 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 1B, 52 year old woman with endometrioid carcinoma and a P286R *POLE* mutation, who was treated with adjuvant pelvic EBRT (External Beam Radiation Therapy) post surgical 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 1B, 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. Clinicopathological 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 1A, with 44% as grade 1 and 44% grade 3. For patients that received treatment, their tumors were 48% as stage 1A, 43% stage 1B, 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 radiation) and *POLE* mutational status was added in a Cox proportional hazards 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 tumours require adjuvant treatment (**Supplemental Table S6).** A subgroup analysis of patients who received or did not receive adjuvant treatment, *POLE* cases consistently had a favorable prognosis compared to EC tumours without *POLE* EDMs (**Figure 2 and Supplemental Figure S2).** Of note, there were no PFS or DSS events in the *POLE* mutated group that did not receive adjuvant treatment. This included two patients with Stage 1B, 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 ECs account for a small percentage (8-12%) of all EC patients. Therefore, even relatively large studies lack power to measure the prognostic effects of *POLE* mutations, particularly since these cases tend to have fewer outcome events. Church *et al.*(11) presented a meta-analysis of *POLE* mutated EC 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 determines the aggregate prognostic effect of *POLE* on PFS, DSS, and OS were computed (**Supplemental Table S7, S9-S11)**. The effect on PFS was determined using six studies, resulting in a pooled HR=0.34, 95% CI=0.15-0.73, and DSS combined six studies showing an overall HR=0.34, 95% CI=0.13-0.91 (**Figure 3A-3B**). This shows that EC patients with *POLE* EDMs have an excellent prognosis with a three-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 (**Supplemental Figure S3A**). Lastly, we analyzed pooled HR's for grade 3 ECs from five studies to find a significant three-fold improvement of PFS (HR=0.32, 95% CI=0.11-0.97) (**Figure 3C**). DSS and OS pooled HR's can be found in **Supplemental Figure 3B-C, Supplemental Table S8, S12-S13**).

Discussion

In this series, we identified the presence of somatic *POLE* EDMs in 9.6% of a large independent cohort of 406 ECs. We confirmed the prognostic impact of *POLE* mutations in ECs, demonstrating favorable outcomes even when tumours 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 tumours is different from the previous literature. Church et al. did not report any *POLE* cases with LVSI and only 1% non-endometrioid histology in the combined 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 tumours 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 three-fold improved PFS compared to patients with tumours 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 ECs have an excellent prognosis. This study again reliably confirms *POLE* EDMs as a significant prognostic biomarker for endometrial cancers. Our cohort of grade 3 ECs 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 ECs is well known(8, 30). *POLE* mutations have been identified in serous carcinomas(13, 31, 32), however upon re-review these are generally tumours with ambiguous features that cause significant interobserver variability even between expert pathologists(12). Regardless, the few patients with *POLE* mutated tumours 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 pathological high-risk features such as high grade (62%), deep myometrial invasion (Stage 1B) (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 to 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 1A/B) tumours. We did observe two recurrences and deaths secondary to EC in the *POLE* wild-type group of similar early stage low-grade ECs, which 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 ECs, 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 EC risk assessment(33) for enhanced patient management. The ideal cohort to determine the relative effect of treatment and *POLE* status on EC outcomes would be from clinical trial(s) (archival material available for determination of *POLE* mutation status) where one arm received no additional therapy post-surgery. Additionally, a collaborative pooled analysis with known *POLE* mutation status, treatment details, and outcomes would enable us to determine if women with *POLE* mutated EC truly need adjuvant treatment.

Defects in proofreading DNA polymerases to produce a mutator phenotype has been established in model systems such as yeast, bacteria, and mice(34), and these defects cause genomic instability(35) that can promote tumourigenesis. *POLE* EDMs cause complete disruption of exonuclease activity (P286S, M295R, S297A)(23) or reduction in proofreading ability (F367C/L/S)(23). Through conservation studies with T4 DNA polymerase it is likely that mutations at the residues L424P, P436R, P441L, 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 tenfold increase in the frequency of mutagenesis and these mice develop tumours(37). Evidence shows that *POLE* EDMs cause increased nonsense mutation rates in key tumour suppressor genes (*TP53, PIK3R1, ATM, ATR*), which likely aid in tumour initiation(23). However, we can hypothesize that there may be a threshold of mutational burden that tumour cells can tolerate(38). There is also evidence that *POLE* ultramutated tumours are associated with peritumoral lymphocytes and tumour infiltrating lymphocytes (28), which exhibit an enhanced T-cell antitumour response to antigenic neo-epitope expression(39, 40). This immune response may play a mechanistic role in the observed favorable prognosis, as an increased immune response in these tumours may decrease metastatic potential leading to a less aggressive tumour (28). An increase in tumour infiltrating T-cells has also been observed in microsatellite unstable endometrial and colorectal tumours, where the immune response may also contribute to anti-tumour 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 is, as yet, no data on response to immune checkpoint blockade/anti PD-1 therapy in *POLE* mutated ECs. It is interesting to note that in contrast to ECs, *POLE* EDMs in patients with colorectal cancer (CRC) had impaired survival but was not significant. Alternatively, in a subgroup analysis of CRC patients with *POLE* EDMs, high stage disease, and received adjuvant therapy had 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

tumour characteristics or tumour response to therapeutics are tissue and context-dependent(44). While these tumours exhibit prominent host immune response in most cases, given the overall excellent outcomes in these women it is not yet warranted to consider expensive immuno-therapy regimens. It is as yet unclear if in the women receiving adjuvant therapy, whether there is a synthetic lethality effect to reduce tumour 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 EC management and provide important prognostic information to the women with this disease(45). However, our preliminary data suggests that continuing our current standard of care is advisable.

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Tables and Figure Legends

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 tumours		367	39 (9.6%)	

Indicated percentages in *POLE* mutated column are: percentage of total *POLE* mutated cases (n=39), and percentage of total EC cases (n=406), respectively.

Table 2: Clinical outcome of *POLE* mutated compared to *POLE* wild-type endometrial carcinomas determined by univariable and multivariable analysis

	Univariable Survival			Multivariable survival		
Outcome	**# of events/n**	**HR (95% CI)**	**LRT p-value**	**# 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

Legend: PFS = Progression-Free Survival, DSS = Disease Specific Survival, OS = Overall Survival, HR = Hazard Ratio, LRT = Likelihood Ratio Test, [F] = Firth correction

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				
1A	149 (73%)	12 (75%)	40 (25%)	10 (48%)
1B	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%)
Histological 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%)
Histological 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%)
Lymphovascular Invasion (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%)

Legend: IQR=Interquartile range, EBRT=External Beam Radiation Therapy, Vag. Brachy= Vaginal brachytherapy; high dose radiation to the vagina. Percentages are based on columns, and percentages do not include unknowns.

Figure Legends

Figure 1. Kaplan-Meier survival curves for *POLE* mutated and *POLE* wild-type endometrial carcinomas.

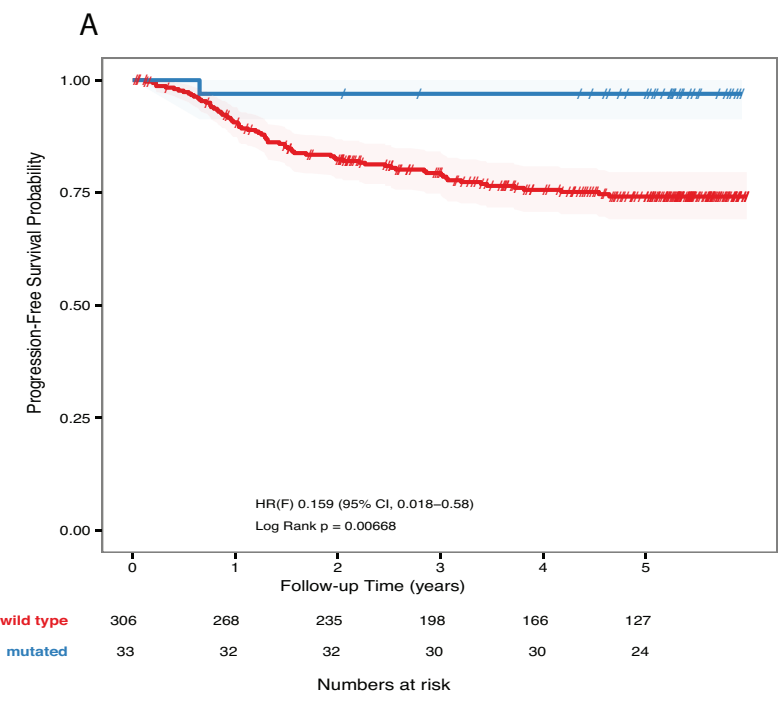
A. Progression-free survival (PFS) for the full endometrial carcinoma cohort. **B.** Disease-specific survival (DSS) for the whole endometrial carcinoma cohort. **C.** Progression-free survival for grade 3 endometrial carcinomas only. **D.** Disease-specific survival (DSS) for grade 3 endometrial carcinoma cohort only. Blue lines = *POLE* mutated cases; Red lines = *POLE* wild-type cases. P-values were obtained by a two-sided log-rank test. HR = hazard ratio, CI = confidence interval, F = Firth correction.

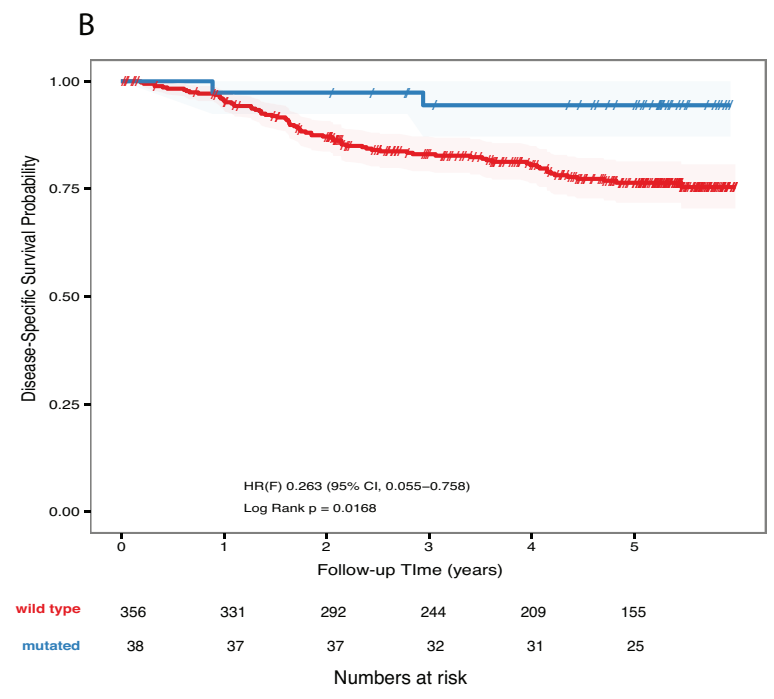
Figure 2. Kaplan-Meier survival curves of *POLE* mutated and *POLE* wild-type endometrial carcinomas that received and did not receive adjuvant treatment. **A.** Progression-free survival (PFS). **B.** Disease-specific survival. P-values were obtained by a two-sided log-rank test. Red lines = *POLE* 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. HR = hazard ratio, CI = confidence interval, ^{F} = Firth correction.

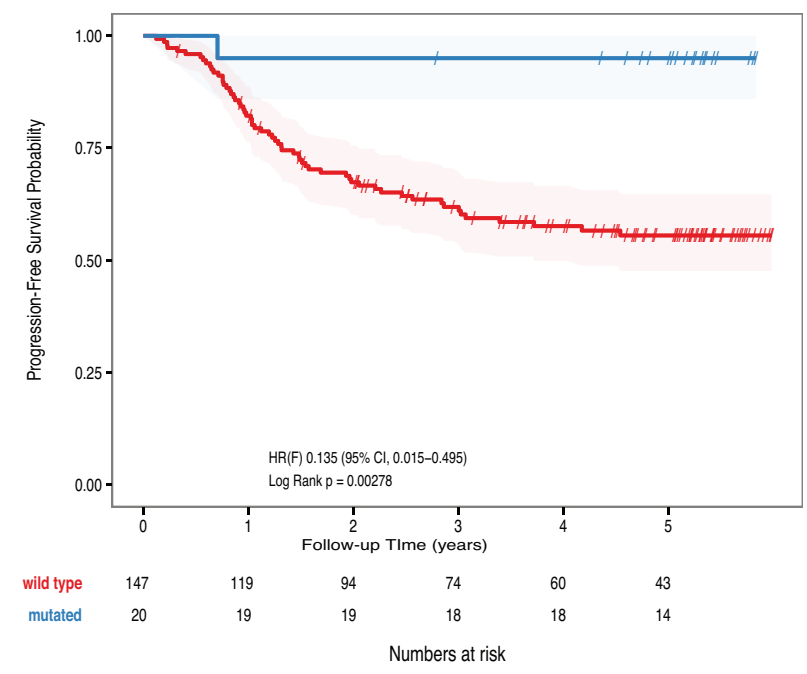
Figure 3. Pooled multivariable hazard ratio plots **A.** Progression-free survival of *POLE* mutated endometrial carcinomas including six combined studies. **B.** Disease-specific survival of *POLE* mutated endometrial carcinomas encompassing six combined studies. **C.** Progression-free survival of *POLE* mutated grade 3 endometrial carcinomas including five

combined studies. Combined pooled multivariable meta-analysis with weights computed using the inverse variance method, hazard ratios (HR), and 95% confidence intervals (CI). The overall HR (grey diamond) was generated by using a fixed effect model. ^{F} = Firth correction.

Figure 1







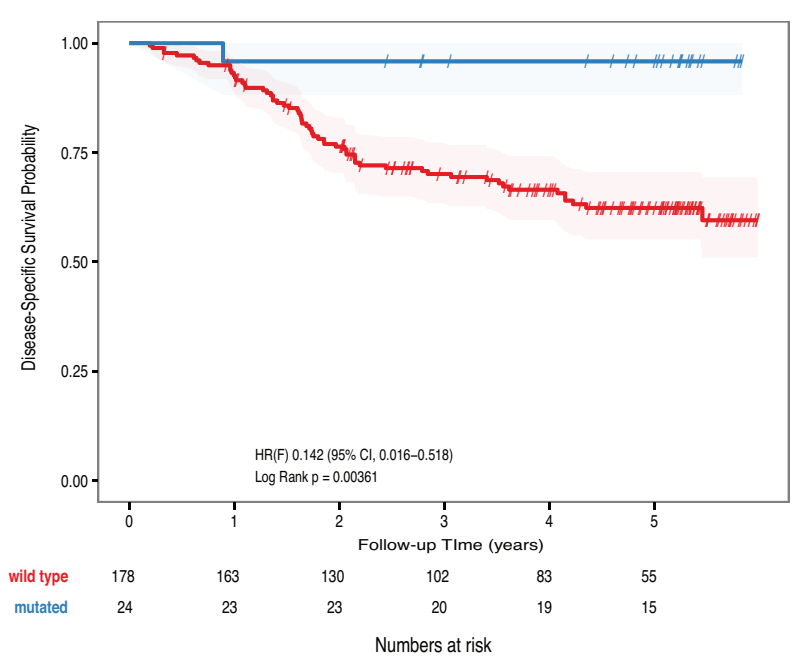
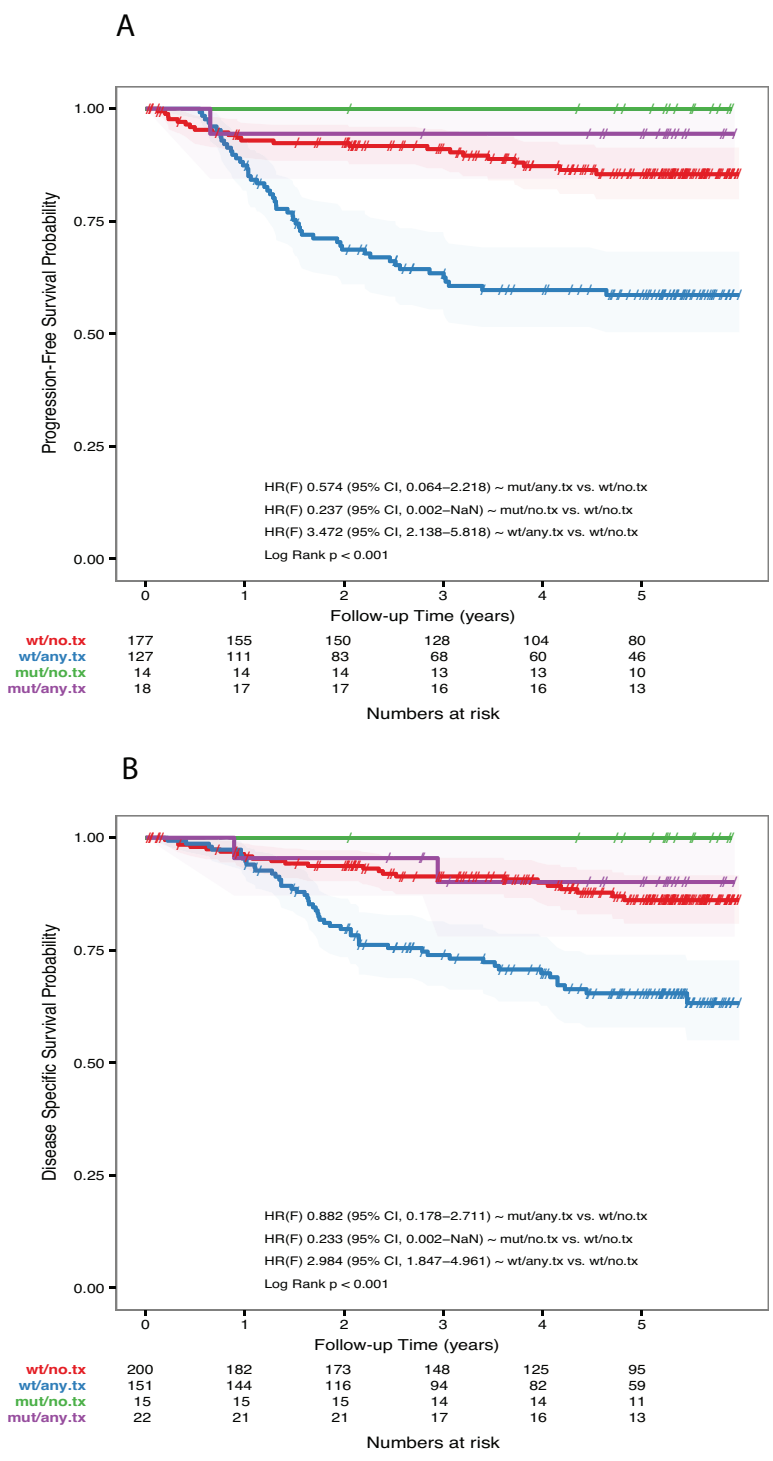
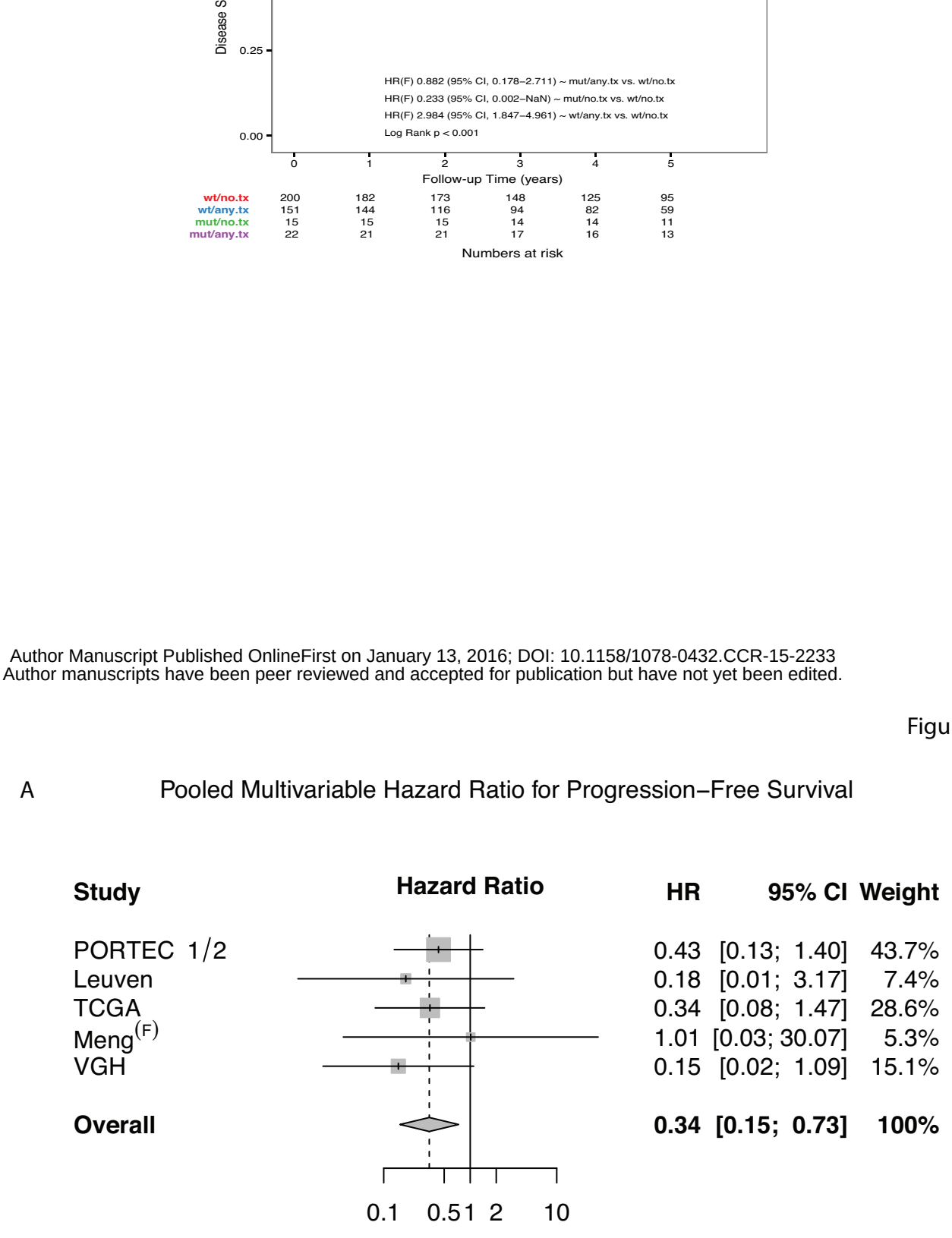


Figure 2



Author Manuscript Published OnlineFirst on January 13, 2016; DOI: 10.1158/1078-0432.CCR-15-2233
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 3



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Clin Cancer Res Published OnlineFirst January 13, 2016.