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An NRG Oncology/GOG study of molecular classification for risk prediction in endometrioid endometrial cancer

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Abstract

Objectives—The purpose of this study was to assess the prognostic significance of a simplified, clinically accessible classification system for endometrioid endometrial cancers combining Lynch syndrome screening and molecular risk stratification.

Methods—Tumors from NRG/GOG GOG210 were evaluated for mismatch repair defects (MSI, MMR IHC, and *MLH1* methylation), *POLE* mutations, and loss of heterozygosity. *TP53* was evaluated in a subset of cases. Tumors were assigned to four molecular classes. Relationships

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between molecular classes and clinicopathologic variables were assessed using contingency tests and Cox proportional methods.

Results—Molecular classification was successful for 982 tumors. Based on the NCI consensus MSI panel assessing MSI and loss of heterozygosity combined with *POLE* testing, 49% of tumors were classified copy number stable (CNS), 39% MMR deficient, 8% copy number altered (CNA) and 4% POLE mutant. Cancer-specific mortality occurred in 5% of patients with CNS tumors; 2.6% with POLE tumors; 7.6% with MMR deficient tumors and 19% with CNA tumors. The CNA group had worse progression-free (HR 2.31, 95%CI 1.53–3.49) and cancer-specific survival (HR 3.95; 95%CI 2.10–7.44). The POLE group had improved outcomes, but the differences were not statistically significant. CNA class remained significant for cancer-specific survival (HR 2.11; 95%CI 1.04–4.26) in multivariable analysis. The CNA molecular class was associated with *TP53* mutation and expression status.

Conclusions—A simple molecular classification for endometrioid endometrial cancers that can be easily combined with Lynch syndrome screening provides important prognostic information. These findings support prospective clinical validation and further studies on the predictive value of a simplified molecular classification system.

Keywords

endometrioid endometrial cancer; molecular classification; prognosis; combined Lynch syndrome screening and molecular classification

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in developed nations, with growing incidence [1]. Most cases are sporadic. An estimated 3–5% of women, however, develop EC because of an inherited mutation in DNA mismatch repair and thus have Lynch syndrome (LS). Tumor-based universal screening for LS syndrome has been recommended and adopted by many centers [2].

For most EC patients, surgery is curative. Adjuvant radiation, chemotherapy, or combined chemo-radiation therapy is used to reduce risk of recurrence in patients with clinicopathologic features that have been associated with poor outcomes. Unfortunately, currently available risk prediction algorithms for the endometrioid histologic subtype (EEC) that represents ~ 85% of cases are suboptimal.

Molecular classification systems to complement current approaches to risk stratification have been proposed but have not been fully validated and molecular classification for risk prediction is yet to become a standard of care. A combined tumor-based LS screening and molecular classification of risk for recurrence system could be a cost-effective approach to reducing EC disease burden. The landmark molecular profiling study of EC performed by The Cancer Genome Atlas (TCGA) included molecular classification based on whole exome sequencing, microsatellite instability (MSI) analysis, and assessment of copy number alterations across the entire genome [3]. The four molecular subtypes developed by TCGA have largely non-overlapping overall mutational burdens, distinct spectra of mutations, differences in mismatch repair (MMR as evidenced by MSI), and differences in the fraction

of genome exhibiting copy number alterations. TCGA molecular subtypes are copy number high (serous-like), copy number low (endometrioid-like), POLE (ultramutated), and MSI (hypermutated).

The extensive genomic characterization undertaken by TCGA cannot be readily accomplished in most clinical settings and translation to clinical application is impractical. In 2015, Talhouk and colleagues proposed a clinically-applicable molecular-based classification system for EC [4]. A key strength of the proposed system, subsequently confirmed in a second cohort and referred to as ProMisE [5], is that analyses can be undertaken with routinely prepared pathology specimens. The p53 and MMR protein immunohistochemistry (IHC) and *POLE* mutation analyses were applied to all histologic subtypes [6]. The prognostic significance of the ProMisE in EEC, the most common subtype and the group for which decision making for use of adjuvant therapies is most challenging, however has not been fully established [7]. We sought to develop a clinically applicable molecular classification system that incorporates MMR IHC, *POLE* mutation analysis, and combined MSI and loss of heterozygosity (LOH) analysis for EEC in a large cohort developed by the Gynecologic Oncology/NRG group. The prognostic significance of molecular classification in this subtype of EC was determined and molecular class:adjuvant treatment interactions were explored.

Methods

Study design and participants

1040 EECs from the NRG/Gynecologic Oncology Group Study GOG210, *A Molecular Staging of Endometrial Cancer NCT00340808*, were evaluated. Subjects were accrued 2003–2007 with appropriate written consent [8]. Laboratory investigators are blinded to clinical data. Two cases studied here were also included in TCGA.

Procedures

MSI testing, IHC, and *MLH1* methylation analysis were used to classify MMR status as reported [9]. *POLE* was assessed for mutation in regions of the exonuclease domain (exons 9, 13, and 14) that harbor the majority of deleterious mutations, as described [10]. For some tumors shorter amplicons capturing the key amino acids were evaluated. Primers and conditions are provided in Table S1.

Tumors were assigned hierarchically to four molecular classes that parallel those described by TCGA [3]: copy number altered (CNA), copy number stable (CNS), POLE mutant, and MMR deficient (Fig. S1). Tumors were classified MMR deficient based on MSI and/or IHC defects. For cases with normal MMR, determination of copy number status was based on LOH at three highly informative microsatellite repeats included in the 5-plex MSI analysis [11]. Fragment sizes and peak heights were used to determine informativity and if there was evidence of loss of heterozygosity (LOH), no loss (NL), or not informative (homozygous). LOH at one or more marker was used to classify a tumor as CNA. Tumors informative for at least one marker and retained heterozygosity were classified as CNS. The

POLE class was assigned to CNS cases (CNA and MMR deficient tumors with *POLE* mutations were not classified as POLE).

Analysis of *TP53*—*TP53* mutation testing was performed for 20 tumors from each molecular class. Laboratory investigators are blinded to the clinicopathologic data and there was no selection for any feature other than molecular class. The entire coding region (exons 2–11) was evaluated using PCR amplification and Sanger sequencing (Table S1). For all *TP53* mutations, the relative peak heights for somatic variants were used to determine if there was loss of the wild-type allele (LOH). Single nucleotide polymorphisms (SNPs) were also used to assess LOH. For *TP53* variants not previously reported as mutations or when peak height for the normal and variant bases were similar, corresponding normal DNAs were tested to confirm mutations were somatic.

p53 Immunohistochemistry—IHC for p53 was performed on formalin-fixed paraffin-embedded tissue sections using a mouse monoclonal anti-human antibody (Novocastra™ Clone DO-7) at a dilution of 1:800. Staining was performed on a representative whole section using the Leica Bond RX autostainer (Leica Biosystems, Buffalo Grove, IL, USA). Heat induced epitope retrieval was performed using ER2 antigen retrieval solution. Stained slides were examined by an experienced gynecologic pathologist [AAS] who was blinded to molecular data. Results were reported as “wild-type” or “mutant” pattern. A “mutant” pattern was defined as strong nuclear staining in >60% of neoplastic cells with adequate internal controls.

Outcomes

The primary endpoints were progression-free and endometrial cancer-specific survival for the four molecular classes of EEC with hazard ratios and confidence intervals.

Statistical analysis

The relationship between molecular class and clinical and demographic features was assessed using Chi-square and analysis of deviance tests. Endometrial cancer-specific survival (ECS), progression-free survival (PFS), and overall survival (OS) analyses were performed as previously described [12]. Cox proportional hazard models were fitted to assess the effects of known covariates and molecular class on outcomes [13]. All analyses were two-sided and significance was set at $P=0.05$ using R [14].

Results

Molecular classification was successful for 982 of 1040 (94%) EECs evaluated. The distribution of the four molecular classes, copy number stable (CNS), mismatch repair deficient (MMR deficient), copy number altered (CNA) or *POLE* mutant (POLE) is shown in Fig. 1A. Non-informativity for the markers used to assess LOH accounted for 51 instances in which molecular classification was not possible. The overall rate of informativity was 92% (one or more marker heterozygous) among the tumors with intact MMR. Among the 84 CNA cases, eight had LOH at all three loci, 22 at two loci, and 54 at a single marker. *D17S250* was the most frequently lost marker (51-of-76 informative cases)

(Fig. S2). Analysis of TCGA data assessing copy number status for the D17S250, D5S346 and D2S123 regions in EEC revealed that tumors that would be classified as copy number altered based on the three regions overlapped substantially with the with the TCGA copy number high (serous-like) group. Among the 191 EECs for which integrated molecular classification was reported [3], 16 tumors had copy number alterations in the D17S250, D5S346 and D2S123 regions (8.4%). Fourteen of those were in TCGA copy number high (serous-like) group (a total of 19 EECs were classified as copy number high). One case was seen in each of the POLE and MSI groups (Fig. S3).

POLE mutations were seen in 18 MMR deficient tumors (5%) and three of the CNA tumors (3%) as shown in Fig. 1B. Thirty-nine of the 60 *POLE* mutations were the p.P286R or p.V411L changes associated with the ultramutated state [3,15], with both hotspots being more common in tumors classified as POLE or CNA than in MMR deficient (34-of-42 vs. 5-of-18, 80% vs. 27%, $P<0.001$, Fisher's exact test). Two of the three CNA cases with *POLE* mutations were hotspots. The majority of the MMR deficient tumors with *POLE* mutations lacked *MLH1* methylation (11-of-18) and thus are likely to have germline or somatic MMR gene mutations [10,16].

Age, body mass index (BMI), grade, stage, and lymphovascular space invasion (LVSI) were all significantly associated with tumor molecular class as shown in Table 1 and summarized in Fig. 1C. Age at surgery for women with tumors classified as MMR deficient (63.4 and 63.1, mean and median) and CNA (65.2 and 63.2, mean and median) was higher than for either the CNS or POLE groups. POLE patients were significantly younger than all three other groups (57.4 and 56.9, mean and median) consistent with previous reports [10,15]. Women in the CNS group had primarily low grade tumors (50% G1, 42% G2) and higher BMIs as previously reported for type I EECs [17]. Overall, patients whose tumors were classified as MMR deficient, POLE or CNA had clinicopathologic features associated with poor outcomes [18].

Univariate analysis demonstrated molecular class was associated with PFS, ECS, and OS, as were stage, grade, depth of invasion, and LVSI (Table 2). Median follow-up time for the cohort was 60.62 months (95% CI 59.96–61.21). Age was associated with PFS but not with ECS. Women whose tumors were classified as CNA had increased hazard ratios for PFS, ECS, and OS (2.31, 95% CI 1.53–3.49; 3.95, 95% CI 2.10–7.44 and 2.46, 95% CI 1.50–4.05, respectively). CNA cases have much poorer outcomes than the rest of the cohort, MMR deficient and CNS cases have similar outcomes, and the POLE group has the best survival (Fig. 2). Among the 84 CNA cases there were 31 recurrences (37%) and 16 ECS deaths (19%). There were two recurrences and one EC death among the 39 POLE cases.

In multivariable analyses that included clinical factors associated with outcomes, molecular class remained significant for PFS ($P=0.0148$) and OS ($P=0.0295$) with a P value of 0.0789 for ECS (likelihood ratio tests). The hazard ratio (HR) for ECS for the CNA group was 2.11 (95% CI 1.04–4.26, $P=0.0347$) with advanced stage, tumor G3 and presence of LVSI significantly associated with ECS (Table 3).

In light of the fact that the MMR deficient and CNA molecular subtypes (so-called MIN and CIN groups [19]) are predictive in CRC [20,21,22,23], we assessed molecular class:treatment interactions to determine if there were differences in outcomes for the molecular groups, focusing on PFS. We realized that power was limited by the small numbers of patients in the CNA and POLE groups who received adjuvant therapy. The global test (Cox regression analysis) for molecular class, adjuvant treatment exposure, and molecular class:adjuvant treatment revealed that only molecular class was significant ($P<0.0001$) (Table S2). When the covariates stage, grade, LVSI, and age were included in Cox regression for PFS age, stage, grade, and CNA remained significant. Although the overall molecular class:adjuvant therapy interaction was not significant, the specific MMR deficient:adjuvant therapy effect was significant ($P=0.04$) (Table S3). The HR for age >60 , stage III/IV, and tumor G3 ranged from 1.70 ($P=0.008$) to 2.49 ($P=0.012$) (Table S3). MMR deficient:adjuvant treatment interaction was the only significant interaction effect. The adjuvant:no adjuvant treatment HR among the MMR patients is 0.45 the size of the adjuvant:no adjuvant treatment HR of the CNS patients (95% CI, 0.21–0.96). However, in light of the limited power for the interaction studies, caution in interpreting non-significant p-values is warranted. For ECS, Cox regression analysis showed a similar association with molecular class. When the covariates were considered, stage III, tumor G3, presence of LVSI, and the CNA class remained significant (data not shown).

We tested *TP53* mutation and expression in a subset of tumors to determine the relationship between molecular class and *TP53* status. Twenty-three tumors had mutations (29%). The mutation frequency varied across the four classes ($P<0.001$, Chi-squared test) with the CNA group having the highest mutation frequency (55%) and the CNS group having the lowest rate of mutation (5%) (Table S4). IHC was successful for 77 of 80 tumors. Again, IHC findings were associated with molecular class ($P=0.007$). Eight tumors (10%) had abnormal IHC findings (“mutant” staining), seven of which were in the CNA group (37% of the CNA tumors). Fig. S4 shows representative IHC staining results. All eight tumors that showed abnormal IHC staining (classified as mutant) carried missense alleles and had two mutational events (LOH or two *TP53* mutations). None of the POLE tumors had abnormal staining (nine tumors with mutations) nor did either of the two MMR deficient tumors with *TP53* mutations. The tumors with stop mutations were less frequently IHC positive, consistent with previously reported findings [24]. The *TP53* mutational spectrum was strikingly different in the POLE tumors. Five POLE tumors had the p.R213* mutation (TCG>TCT) and in one case, both copies of the gene carried the mutation.

There were six recurrences among the 20 CNA cases assessed for *TP53* mutations (30%, consistent with the event rate for the entire 84 CNA cases as shown in Fig. 2A). The CNA cases studied for *TP53* were primarily early stage (80% stage I or II) and higher grade (25% G1, 50% G2, and 25% G3). Only two cases had adjuvant treatment after surgery (one stage I/G1 case received radiation therapy and one stage III/G3 case received chemotherapy) (Table S5). Three of the six recurrences had *TP53* mutations and two of those also had abnormal IHC staining. Eight of the CNA cases with *TP53* mutation did not recur (73%) and five of the seven cases with abnormal *TP53* IHC (71%) did not recur.

Discussion

Women with tumors classified as CNA (8% of cases) had the worst outcomes among the four molecular classes studied in this large EEC cohort. PFS, ECS, and OS were all significantly reduced for the CNA subjects in univariate analysis (Table 2) and in multivariable analysis (Table 3) molecular group remained a significant factor for PFS and OS. The POLE group had the best outcomes, but the differences were not statistically significant.

Among the 39 POLE cases, nine (24%) received adjuvant therapy (data not shown) which was the highest rate of adjuvant treatment among the four molecular subtypes. The high rate of adjuvant therapy is not unexpected given the fact the 23% of women in the POLE group had advanced stage disease and that the POLE tumors were frequently G3 (27%). At present, it is unclear whether the improved survival seen for women with POLE mutations [3,15] is attributable to the ultramutator phenotype and biologic consequences of the high mutational burden these tumors acquire or to the combined effects of adjuvant therapy and the tumor biology.

Our data show that among EECs, CNA tumors have increased biologic aggressiveness. The CNA classification was based on analysis of only three highly informative markers, *D17S250*, *D5S346*, and *D2S123*, which are part of the NCI consensus panel for determining MSI [11]. It is appealing to suggest that CNA tumors have a genomic scarring phenotype [25,26,27], but with only three loci investigated, we are unable to speculate as to whether there is genome-wide LOH and/or whether it is sub-chromosomal LOH, as is often used to define homologous recombination deficiency [25,28]. Regardless, the substantially higher HR for recurrence for the CNA group, coupled with the fact that 37% of women with CNA tumors recurred, points to the prognostic value of the classification system. The therapeutic implications for the CNA classification we used in this study remain to be determined. Nonetheless, it is noteworthy that among the 31 women with CNA tumors who recurred, there were 16 ECS deaths. The salvage rate for this group of patients was less than 50%, whereas the MMR deficient and CNS groups had proportionally fewer ECS deaths (34% and 29%, respectively of the women who recurred) (Fig. 2). The ECS HRs for CNA cases of 3.95 (95% CI, 2.10–7.44) in univariate analysis and 2.11 (95% CI, 1.04–4.26) for multivariable analysis strongly suggests that upfront adjuvant and/or alternate approaches to salvage therapies should be explored in this group of patients.

Although TCGA pointed to EC cases with copy number alterations as having worse outcomes, the analyses included EEC, serous, and mixed histologies with over-sampling for high grade EEC [3]. The serous-like group included only 16 EECs and it thus follows that the poorer outcomes for patients in the copy number high group reflected the large number of serous carcinoma (N=41) and mixed histology (N=3) cases. At present, adjuvant therapy is recommended for most women with non-endometrioid ECs given the extensive literature that outcomes are worse for women with these tumors [1,29]. Our focus on the EEC subtype allowed us to determine the prognostic significance of a molecular classification system that parallels the one put forth by TCGA in the most common histologic subtype of EC and the

one for which determining risk for recurrence and planning adjuvant treatments is most challenging, particularly for women with early stage disease.

Although *TP53* mutation status was not a key feature in TCGA's molecular classification system, it was noted that the copy number high (serous-like) tumors have frequent *TP53* mutation [3]. Among the 188 TCGA endometrioid tumors, 27 had *TP53* mutations (26%) and of those 7 were stop or frameshift mutations (26%). Most of the stop and frameshift mutations were in the *POLE* (ultramutated) tumors (4 of 17 cases). A more clinically applicable molecular classification system (ProMisE) was developed by Talhouk and colleagues [6] that relies largely on IHC analysis of ECs (IHC for MMR proteins and p53) coupled with *POLE* mutation testing. A strength of the ProMisE [5] classification system is that those analyses can be easily undertaken with routine pathology specimens. The p53 and MMR protein IHC and *POLE* mutation testing [5] were, however, applied to all histologic subtypes. Strong positive p53 staining is an established feature of serous ECs [3,30,31]. The ProMisE system relies on a three class p53 staining system with absent (abnormal) staining associated with *TP53* stop, splice, or frameshift mutations. The study by Talhouk and colleagues [4,32] included 20 endometrioid cases with *TP53* mutations and IHC staining data, of which 12 had normal MMR and lacked *POLE* mutations. Two of the 12 cases had frameshift *TP53* mutations, one of which had a normal IHC staining pattern and the other had absent staining (score 0, considered mutant). None of the 61 MSS tumors lacking *TP53* mutations were classified as having absent staining and one was classified as having abnormal p53 staining (score 2). A study by Stelloo and colleagues [33] also showed that abnormal p53 staining was associated with poor outcomes in a large series of EEC cases from the PORTEC-1 and -2 cohorts. The PORTEC studies were undertaken to determine the benefit of radiotherapies in management of EC [34,35].

The vast majority of the subjects studied by Stelloo et al. [33] had stage I disease and adjuvant therapy and thus represent a different patient group than the one we have studied here. The LOH-based CNA class we propose does not rely on IHC interpretation, and is correlated with *TP53* status (both IHC and mutation) in EEC. It is noteworthy that none of the MMR deficient or *POLE* tumors in our cohort had abnormal p53 IHC. At present, it is unknown whether p53 IHC classification is a better predictor of outcomes than the LOH-based method we describe.

Our work supports earlier studies suggesting that molecular classification of EC may improve risk prediction and aid in planning adjuvant therapies. We believe that the molecular analyses we performed for this large EEC cohort could be easily implemented at most regional reference centers using tests that are already in clinical use, or could be developed rapidly. MMR tumor studies are performed routinely using IHC, MSI, or combined IHC/MSI analyses as part of LS screening. Our approach for combined MSI and LOH analysis using the NCI consensus 5-plex panel could be accepted as the standard for LS screening, and provide additional prognostic information. *TP53* IHC remains a promising approach to molecular classification but will require further validation for EEC. Prospective validation of the increased HR for ECS we observed for the CNA group in the GOG210 cohort and further assessing molecular class:adjuvant therapy interactions will be an important step to personalized medicine and improved outcomes for women with EEC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Molecular classes and association with clinicopathologic and demographic variables for 982 endometrioid endometrial cancers

Frequency distribution of the four molecular classes. **(A)** Overlap of POLE mutations with MMR deficient and copy number altered molecular classes. The POLE classification was restricted to those tumors with normal DNA mismatch repair with no evidence of copy number alteration/LOH **(B)** and patterns seen across the four classes for significantly associated variables. Pearson's Chi-squared tests P values **(C)**.

Figure 2. Survival analysis for the four molecular classes of endometrioid endometrial cancer Shown are the Kaplan-Meier plots of patient (A) progression-free survival, (B) endometrial cancer-specific, and (C) overall survival by molecular group. Nominal P values are presented with no adjustment for multiple testing. HR=hazard ratio.

Table 1

Association between tumor molecular class and clinicopathologic and demographic variables.

Clinicopathologic Factor	Copy number stable		MMR deficient		Copy number altered		POLE	P value*
	N (%)		N (%)		N (%)		N (%)	
Age								
<60 years	233 (48.5)		151 (39.8)		33 (39.3)		27 (69.2)	0.001
60 years	247 (51.5)		228 (60.2)		51 (60.7)		12 (30.8)	
BMI								
<25 (normal or underweight)	51 (10.7)		64 (16.9)		10 (11.9)		7 (17.9)	0.008
25–30 (overweight)	86 (18.0)		74 (19.6)		23 (27.4)		9 (23.1)	
30–35 (obese class I)	105 (22.0)		97 (25.7)		12 (14.3)		10 (25.6)	
35 (severe or super obese)	236 (49.4)		143 (37.8)		39 (46.4)		13 (33.3)	
Race								
White	428 (89.2)		351 (92.6)		74 (88.1)		36 (92.3)	0.339
African American	27 (5.6)		19 (5.0)		7 (8.3)		1 (2.6)	
Other (Asian, Native American, Unknown)	25 (5.2)		9 (2.4)		3 (3.6)		2 (5.1)	
Grade								
1	241 (50.2)		136 (35.9)		16 (19.0)		14 (35.9)	<0.001
2	203 (42.3)		175 (46.2)		30 (35.7)		15 (38.5)	
3	36 (7.5)		68 (17.9)		38 (45.2)		10 (25.6)	
Stage								
I	366 (76.3)		276 (72.8)		60 (71.4)		30 (76.9)	0.006
II	50 (10.4)		31 (8.2)		10 (11.9)		0 (0)	
III	56 (11.7)		67 (17.7)		9 (10.7)		9 (23.1)	
IV	8 (1.7)		5 (1.3)		5 (6.0)		0 (0)	
LVSI								
Present	74 (15.7)		120 (32.2)		21 (25.9)		12 (31.6)	<0.001
Absent	398 (84.3)		253 (67.8)		60 (74.1)		26 (68.4)	
Depth of invasion								
None	87 (18.6)		53 (14.5)		11 (13.3)		6 (15.8)	0.713
Inner half	260 (55.7)		207 (56.6)		48 (57.8)		22 (57.9)	

Clinicopathologic Factor	Copy number stable N (%)	MMR deficient N (%)	Copy number altered N (%)	POLE N (%)	P value*
Outer half or serosal Adjuvant therapy	120 (25.7)	106 (29.0)	24 (28.9)	10 (26.3)	
Any adjuvant therapy	86 (18.0)	89 (23.5)	16 (19.0)	9 (23.7)	0.221
No further treatment	393 (82.0)	289 (76.5)	68 (81.0)	29 (76.3)	

* Pearson's Chi-squared tests; Missing data: LVSI for 18 patients, depth of invasion for 28 patients, adjuvant therapy for 3 patients and BMI for 3 patients.

Table 2

Univariate outcome analysis of all EC subjects (n=982)

	Progression-free survival (PFS)			EC survival (ECS)			Overall survival (OS)		
	HR	95% CI	P*	HR	95% CI	P*	HR	95% CI	P*
Age (older vs < 60)	1.82	1.35–2.45	<0.001	1.17	0.73–1.89	0.510	1.86	1.29–2.68	0.001
Race (ref = white)			0.243			0.759			0.224
Black	1.47	0.87–2.49	0.151	1.42	0.57–3.53	0.452	1.61	0.87–2.99	0.130
Other	0.68	0.28–1.64	0.387	1.15	0.36–3.67	0.810	0.61	0.19–1.91	0.391
BMI (ref <25)			0.446			0.542			0.682
25–30	1.29	0.78–2.14	0.328	0.62	0.27–1.40	0.249	1.08	0.57–2.07	0.807
>30–35	1.38	0.84–2.25	0.204	0.97	0.47–2.00	0.937	1.35	0.74–2.49	0.330
>35	1.09	0.68–1.73	0.722	0.75	0.38–1.47	0.401	1.29	0.73–2.27	0.383
Stage (ref = 1)			<0.001			<0.001			<0.001
2	1.06	0.64–1.76	0.819	0.99	0.35–2.81	0.989	0.99	0.51–1.91	0.973
3	1.86	1.31–2.64	0.001	4.94	2.97–8.20	<0.001	2.42	1.63–3.59	<0.001
4	4.53	2.45–8.40	<0.001	9.25	3.87–22.12	<0.001	4.08	1.88–8.83	<0.001
Grade (ref = 1)			<0.001			<0.001			<0.001
2	1.36	0.97–1.90	0.076	1.49	0.76–2.91	0.246	1.29	0.84–1.98	0.245
3	3.02	2.09–4.34	<0.001	7.02	3.77–13.08	<0.001	3.82	2.48–5.89	<0.001
LVSI (present or absent)	1.89	1.41–2.55	<0.001	3.81	2.38–6.09	<0.001	2.44	1.72–3.46	<0.001
Depth of invasion (ref = none)			<0.001			<0.001			<0.001
inner half	1.51	0.91–2.51	0.108	1.15	0.50–2.64	0.735	1.24	0.68–2.26	0.488
outer half or serosal	2.93	1.76–4.88	<0.001	3.08	1.36–6.95	0.007	2.99	1.64–5.45	<0.001
Adjuvant Therapy (Yes or No)	1.01	0.72–1.43	0.941	1.62	0.97–2.70	0.077	0.99	0.65–1.51	0.975
Molecular class (ref=CopY number stable)			<0.001			<0.001			<0.001
CopY number altered	2.31	1.53–3.49	<0.001	3.95	2.10–7.44	<0.001	2.46	1.50–4.05	<0.001
MMR deficient	1.38	1.01–1.87	0.041	1.58	0.92–2.72	0.096	1.41	0.97–2.05	0.070
POLE	0.27	0.07–1.10	0.068	0.48	0.06–3.51	0.466	0.22	0.03–1.57	0.129

* Analysis of deviance for global tests, Wald test for individual parameters

Table 3

Multivariable analysis for all 982 subjects.

	Progression-free survival (PFS)				Endometrial cancer-specific survival (ECS)				Overall survival (OS)			
	HR	95% CI	P*		HR	95% CI	P*		HR	95% CI	P*	
Age												
> 60	1.79	1.32–2.42	<0.001		1.29	0.80–2.11	0.295		1.87	1.28–2.72	0.001	
Stage			0.009				<0.001				0.014	
II	0.98	0.58–1.66	0.932		0.87	0.31–2.50	0.801		0.87	0.43–1.75	0.701	
III	1.72	1.16–2.56	0.007		3.43	1.91–6.15	<0.001		2.04	1.30–3.22	0.002	
IV	2.60	1.28–5.30	0.008		2.70	0.96–7.63	0.061		1.81	0.73–4.49	0.201	
Grade			<0.001				<0.001				<0.001	
2	1.17	0.82–1.66	0.376		1.10	0.55–2.20	0.794		1.05	0.67–1.66	0.823	
3	2.25	1.46–3.47	<0.001		3.54	1.71–7.33	<0.001		2.76	1.65–4.60	<0.001	
LVSI												
Present	1.39	0.98–1.98	0.067		1.80	1.02–3.20	0.044		1.64	1.07–2.50	0.022	
Molecular group			0.015				0.079				0.029	
MMR deficient	1.08	0.78–1.50	0.642		1.03	0.58–1.84	0.908		1.04	0.70–1.56	0.835	
Copy number altered	1.56	0.99–2.48	0.057		2.11	1.04–4.26	0.037		1.61	0.93–2.78	0.088	
POLE	0.26	0.06–1.05	0.059		0.36	0.05–2.71	0.323		0.19	0.03–1.35	0.096	
Adjuvant therapy												
Yes	0.66	0.45–0.95	0.027		0.70	0.40–1.22	0.207		0.57	0.36–0.90	0.016	

* Analysis of deviance for global tests, Wald test for individual parameters