Clinical Cancer Research

Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer—Combined Analysis of the PORTEC Cohorts

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Abstract

Purpose: Recommendations for adjuvant treatment for women with early-stage endometrial carcinoma are based on clinicopathologic features. Comprehensive genomic characterization defined four subgroups: p53-mutant, microsatellite instability (MSI), *POLE*-mutant, and no specific molecular profile (NSMP). We aimed to confirm the prognostic capacity of these subgroups in large randomized trial populations, investigate potential other prognostic classifiers, and integrate these into an integrated molecular risk assessment guiding adjuvant therapy.

Experimental Design: Analysis of MSI, hotspot mutations in 14 genes including *POLE*, protein expression of p53, ARID1a, β-catenin, L1CAM, PTEN, ER, and PR was undertaken on 947 available early-stage endometrioid endometrial carcinomas from the PORTEC-1 and -2 trials, mostly high-intermediate risk (n = 614). Prognostic value was determined using univariable and multivariable Cox proportional hazard models. AUCs of different risk stratification models were compared.

Results: Molecular analyses were feasible in >96% of the patients and confirmed the four molecular subgroups: p53-mutant (9%), MSI (26%), *POLE*-mutant (6%), and NSMP (59%). Integration of prognostic molecular alterations with established clinicopathologic factors resulted in a stronger model with improved risk prognostication. Approximately 15% of high-intermediate risk patients had unfavorable features (substantial lymphovascular space invasion, p53-mutant, and/or >10% L1CAM), 50% favorable features (*POLE*-mutant, NSMP being microsatellite stable, and *CTNNB1* wild-type), and 35% intermediate features (MSI or *CTNNB1*-mutant).

Conclusions: Integrating clinicopathologic and molecular factors improves the risk assessment of patients with early-stage endometrial carcinoma. Assessment of this integrated risk profile is feasible in daily practice, and holds promise to reduce both overtreatment and undertreatment. *Clin Cancer Res; 22(16); 4215–24.*

Introduction

Endometrial cancer is the most common gynecologic cancer in developed countries (1). Over 50% of women with endometrial carcinoma present with early-stage, low-risk disease, and are treated with surgery alone (2). Adjuvant therapy recommendations are based on the individual patient's risk of disease recurrence using clinicopathologic factors such as age, stage, histologic subtype, tumor grade, and lymphovascular space invasion (LVSI; ref. 3). Endometrial carcinoma patients are generally stratified in three risk groups; however, various definitions exist (4–6). The PORTEC-1 and -2 (postoperative radiation therapy for endometrial carcinoma) clinical trials have contributed evidence that adjuvant radiotherapy can be safely omitted in patients with low-intermediate risk features, and that endometrial carcinoma patients with high-intermediate risk features can effectively be treated with vaginal brachytherapy (4, 7), Despite this clinicopathologic risk stratification considerable overtreatment and undertreatment remains: 7 patients with stage I high-intermediate risk endometrial carcinoma need to receive vaginal brachytherapy to prevent one recurrence, whereas 8% of patients develop distant metastases that may have been prevented or delayed with adjuvant chemotherapy. We hypothesized that the clinicopathologic risk assessment might be improved by integration of molecular biomarkers predictive of individual tumor behavior.

Many studies addressing the prognostic significance of molecular alterations in endometrial carcinoma have focused on one or two biomarkers (8, 9). Integrated genomic characterization by

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Translational Relevance

Current adjuvant treatment strategies for endometrial cancer patients are based on clinicopathologic risk assessment. However, overusage and underusage of adjuvant therapy remains a clinical problem. In this work, an integrated analysis is presented of The Cancer Genome Atlas proposed molecular subgroups, a multigene mutation analysis and established biomarkers such as L1CAM, ER/PR, and lymphovascular space invasion in two large early-stage endometrial cancer trial populations. In keeping with previous observations, we show that endometrioid endometrial carcinoma is a molecular heterogeneous disease, and that endometrial carcinoma comprises four molecular subgroups with distinct outcome. Integrating clinicopathologic and molecular risk factors in a risk assessment stratified patients more accurately in risk groups. The analysis is easily applicable in clinical practice and may be used in new prospective trials to reduce overtreatment and undertreatment of patients with endometrial cancer.

The Cancer Genome Atlas (TCGA) defined four distinct endometrial carcinoma subgroups with possible prognostic value (10). Using methods broadly available in clinical practice, these four subgroups can be easily determined by their surrogate markers: p53, microsatellite instability (MSI), and *POLE* resulting in a practically and clinically useful molecular classification tool (11, 12). In relatively small series of unselected endometrial carcinomas, the combination of both the clinicopathologic and molecular classification improved the clinicopathologic risk assessment (12). At present, it is unclear how other potential molecular prognosticators, such as mutations in *CTNNB1*, *PIK3CA*, and L1CAM overexpression should be integrated in the suggested TCGA subgroups.

The aims of this study were to confirm and validate the prognostic significance of the proposed molecular classification tool in early-stage endometrioid endometrial carcinomas (EEC), mainly high-intermediate risk, from two large randomized trials (PORTEC-1 and -2) with mature long-term follow-up data and to investigate whether incorporation of other molecular alterations and established clinicopathologic risk factors will result in an refined improved risk assessment.

Materials and Methods

Patients and study design

For both PORTEC-1 and -2 trials, central pathology review was undertaken, during which formalin-fixed paraffin-embedded (FFPE) tumor material was collected. All tumor samples with confirmed endometrioid histology were included in the current analysis. The design and clinical results of both randomized trials have been published previously (4, 7). In brief, PORTEC-1 (1990–1997) included 714 patients with stage I endometrial carcinoma, grade 1 or 2 with deep myometrial invasion, or grade 2 or 3 with superficial invasion. PORTEC-2 (2000–2006) included 427 endometrial carcinoma patients with high-intermediate risk features: stage I, age >60 years, grade 1–2 with deep invasion, or grade 3 with superficial invasion and stage IIA disease (except grade 3 with deep invasion). The PORTEC study protocols were approved by the Dutch Cancer Society and the medical ethics committees at participating centers. All patients provided informed consent. Data on patient and tumor characteristics, including results of pathology review and outcome, were obtained from the trial databases. The presence of substantial LVSI, diffuse or multifocal LVSI around the tumor, was evaluated and reported previously (13). The REMARK criteria were followed, wherever possible, throughout this study (14).

Procedures

For immunohistochemical analyses, all slides were evaluated by two investigators and a gyneco-pathologist, blinded for patient characteristics and outcome. Evaluations were done independently with discrepancies resolved at simultaneous viewing. For DNA analyses, tumor DNA was isolated as reported previously (11).

p53 expression, MSI, and *POLE* exonuclease domain mutation status were assessed, as described previously, to identify the four molecular endometrial carcinoma subgroups (11). In short, immunohistochemical expression of p53 (clone DO-7, 1:2,000; Neomarkers) was scored positive if >50% of the tumor cells showed a strong positive nuclear staining, or when discrete geographical patterns showed >50% tumor cell positivity. Tumors in which no p53 staining of the tumor was observed and cases with only DNA present ($n = 119$) were sequenced for exon 5–8 *TP53* mutations (15). The MSI status was determined using the Promega MSI analysis system (version 1.2). Tumors with instability in at least two markers were defined as being MSI, whereas those showing no instability were classified as being stable (MSS). Tumors in which instability at one repeat was observed or MSI status could not be determined because of poor DNA quality ($n = 121$) were stained manually for the mismatch repair proteins MLH1 (clone ES05, 1:100; DAKO), MSH2 (clone FE11, 1:200, DAKO), MSH6 (clone EPR3945, 1:800, Genetex), and PMS2 (clone EP51, 1:75, DAKO; ref. 11). Both methodologies, MSI assay and mismatch repair protein expression, are highly sensitive methods for the identification of a defective DNA mismatch repair system (16). Tumors were then considered MSI if tumor cells showed loss of nuclear staining of at least one of the mismatch repair proteins, and MSS if tumor cells showed nuclear positivity for all mismatch repair proteins. *POLE* exonuclease domain hotspot mutations (named *POLE* mutations throughout this article) were detected by Sanger sequencing of exon 9 and 13. KASPar competitive allele-specific PCR (LGC Genomics) assays were used to screen for *POLE* variants at codons 286, 297, and 411 in tumors with poor DNA quality ($n = 98$, primer sequences are available upon request). Parts of these results were published previously (17).

To assess mutations in other frequently altered genes in endometrial carcinoma, we used the Sequenom MassARRAY system and the GynCarta multigene analysis 2.0 (Sequenom) to test for 159 hotspot mutations in *BRAF*, *CDKNA2*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, *PTEN* as described previously (15).

Further immunohistochemical analyses were performed for estrogen receptor (ER), progesterone receptor (PR; clone PGR636, 1:200; DAKO), PTEN (clone 6H2.1, 1:200; DAKO), β-catenin (clone 14, 1:1,600; BD transduction), and ARID1a (clone PSG-3, 1:800; Santa Cruz Biotechnology) expression. Immunohistochemical procedures were as described previously except for ER expression analysis (clone EP1, DAKO, 1:100, Tris-EDTA pH 9.0, 3,3′-*diaminobenzidine+; refs.*11,15). ER and PR were scored positive when at least >10% of tumor cells showed nuclear

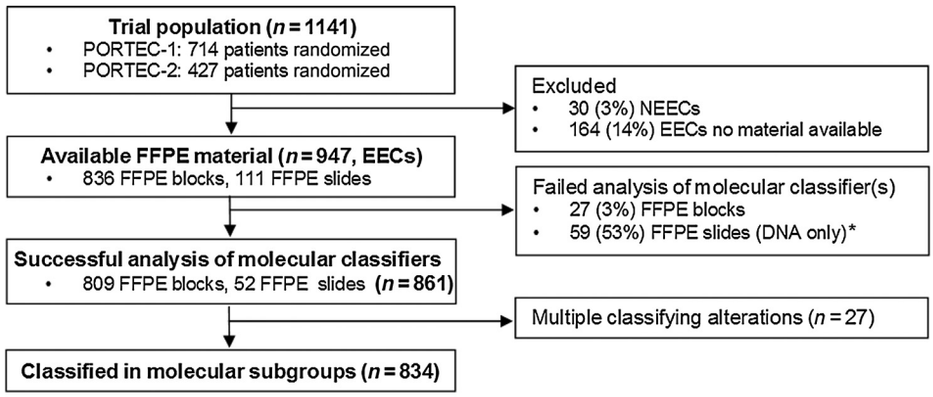

Figure 1.
Flow chart of sample analyses.

* The majority of cases with incomplete analysis were PORTEC-1 cases from which only FFPE tumor slides were available
EEC, Endometrioid endometrial cancer, NEEC, nonendometrioid endometrial cancer

expression. PTEN, β-catenin, and ARID1a staining were evaluated as described previously (11, 15). In short, PTEN staining was evaluated in three categories as negative, positive, and heterogeneous. Activated Wnt signaling was defined as nuclear staining of β-catenin. ARID1a was scored as negative, weak positive, or strong positive nuclear staining or as "clonal loss." Previously published results of immunohistochemical L1CAM expression (clone 14.10, 1:500; Covance Inc.) on the same patients in this study were integrated for analysis (18). Tumors with >10% positive tumor cells were considered L1CAM positive.

Statistical analysis

Associations between clinicopathologic features and molecular alterations were tested using χ^2 statistics or Fisher exact test in case of categorical and *t* test or ANOVA for continuous variables. Time-to-event analyses were calculated from the date of randomization to date of recurrence (vaginal and/or pelvic for locoregional recurrence, and distant metastases for distant recurrence) or to date of endometrial cancer death (disease-specific survival) or to date of death (overall survival) or to date of any recurrence or death (recurrence-free survival); patients who were alive and without recurrence were censored at the date of last follow-up. Survival curves were calculated using the Kaplan–Meier method with log-rank test. Cox proportional hazards models were used to evaluate the prognostic value of each factor. Factors with $P < 0.10$ were included in a multivariable Cox model with a stepwise method to include in the final model. In the last step, significant factors from the forward selection model ($P < 0.05$) were included in a final Cox model together with established clinicopathologic prognostic factors: age as continuous variable, grade (1–2 vs. 3), LVSI (substantial vs. none or mild), and adjuvant treatment (vaginal brachytherapy, external beam radiotherapy, or no additional therapy). Discrimination between the risk stratification models was quantified using the area under the receiver operating curve with 95% confidence intervals (CI). All reported *P* values were based on two-sided tests with $P < 0.05$ considered statistically significant (IBM SPSS 20.0).

Results

In total, 947 (83% of randomized patients) EECs from PORTEC-1 and -2 were available (Fig. 1). Analysis of classifying alterations (p53, MSI, *POLE*) was successful in 809 of 836 (97%) cases for which sufficient material was available. For 111 PORTEC-1 cases, only FFPE slides were available for DNA isolation, which provided 52 (47%) additional successfully analyzed cases. Patient, tumor, and treatment characteristics did not differ between included, excluded, and failed cases (Supplementary Table S1). The median follow-up was 131 months (range 0.2–219.2 months).

The four molecular subgroups displayed marked differences in clinicopathologic characteristics, alterations in potential other classifiers, and clinical outcome (Fig. 2; Table 1 and Supplementary Table S2). In total, 834 EECs could be classified in one of the four subgroups: 74 (9%) p53-mutant, 219 (26%) MSI, 49 (6%) *POLE*-mutant, and 492 (59%) NSMP. Twenty-seven (3%) tumors were found to have more than one classifying alteration (p53, MSI, or *POLE*). p53-mutant tumors were significantly associated with grade 3, loss of hormone receptors, >10% L1CAM expression, *PPP2R1a*, and *FBXW7* mutations. MSI tumors presented more frequently with substantial LVSI, and abnormal ARID1a expression. *POLE* mutations occurred more frequently in younger women, grade 3, and often co-occurred with *PTEN* mutations. In contrast, the NSMP tumors were more frequently grade 1, and *CTNNB1*-mutant. The prognosis was unfavorable in the p53-mutant group, intermediate in the MSI and NSMP group, and the *POLE*-mutant group had a favorable prognosis with no local and only two distant recurrences (Fig. 2). In addition, women with a *POLE*- and p53-mutant tumor developed, no recurrences (0/7), whereas some of the women with MSI tumors with *POLE* (2/6) or p53 mutation (2/13), or both (1/1) developed recurrences (Supplementary Table S3). Within the four subgroups, distant recurrence and endometrial cancer-related death rates were similar.

The prognostic value of the molecular subgroups and additional molecular alterations was evaluated in univariable analysis and multivariable analysis with the clinicopathologic factors (age, grade, depth of myometrial invasion, LVSI) and treatment, both in the whole population (Supplementary Table S4) and in an analysis restricted to cases with high-intermediate risk features (Table 2, univariable analysis; Table 3, multivariable analysis). In both analyses, p53-mutant and substantial LVSI were the strongest prognostic factors for locoregional, distant recurrence, and overall survival, whereas >10% L1CAM expression was prognostic for distant recurrence and overall survival. After excluding cases with favorable (*POLE*-mutant) and unfavorable factors (substantial LVSI, p53-mutant, and >10% L1CAM), a final analysis found MSI prognostic for distant recurrence and overall survival, and

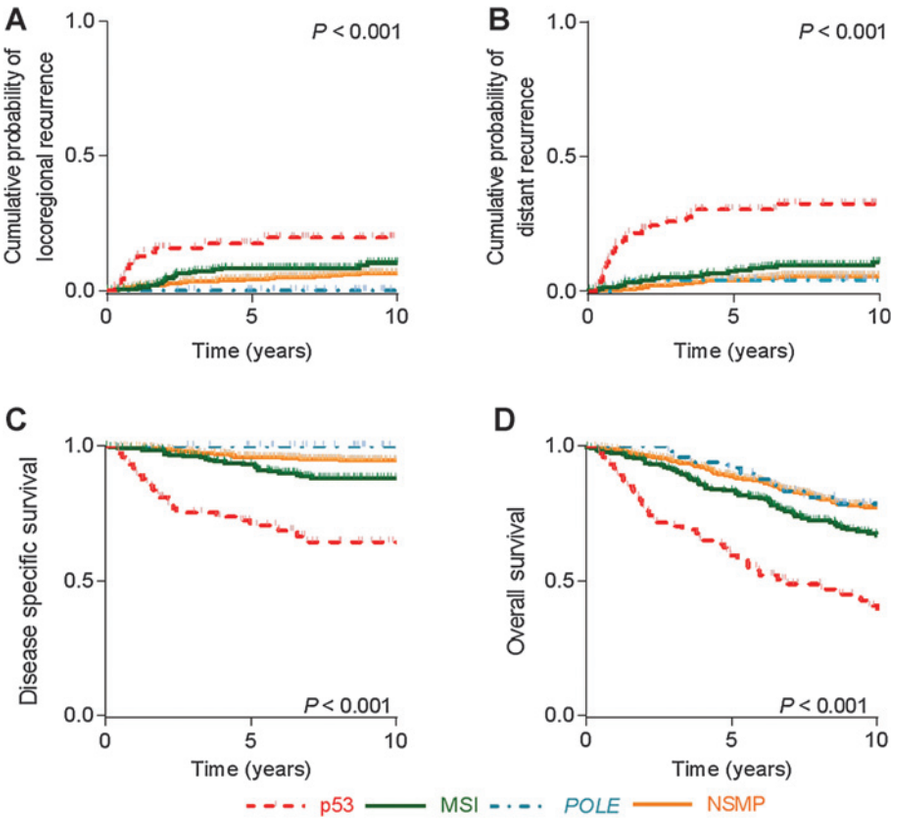

Figure 2.
Survival analyses of molecular subgroups in early-stage endometrial cancer (n = 834). Rate of locoregional recurrences (**A**), rate of distant recurrences (**B**), disease-specific survival (**C**), and overall survival (**D**).

CTNNB1 exon 3 mutation status prognostic for distant recurrence (Table 3, Supplementary Table S4). Univariable prognostic factors, *FGFR2* mutation and loss of hormone receptor expression, lost its significance in multivariable analysis in the presence of other (un)favorable prognostic factors. Univariable analysis in 242 endometrial carcinomas with low-risk features showed a higher rate of locoregional and distant recurrences and lower overall survival in the 8 patients with >10% L1CAM, and a trend for p53-mutant patients (Supplementary Table S5).

On the basis of the outcomes of multivariable analysis, a molecular integrated risk assessment was defined that combines clinicopathologic and molecular risk factors (Fig. 3A). Substantial LVSI, p53-mutant, and >10% L1CAM tumors were designated unfavorable, whereas in the remaining cases, both MSI and *CTNNB1*-mutant were distinguished from the favorable group of *POLE*-mutant tumors and NSMP tumors being MSS and *CTNNB1* wild-type (Fig. 3A). As PORTEC-1 included patients that are currently considered low risk, and central pathology review in both trials identified additional low- and high-risk cases, the AUC was estimated for the molecular integrated risk assessment taking these different starting points into account (Fig. 3B and C). Compared with the original pathology reports, central pathology review, or molecular classification improved the AUC. However, AUCs of the integrated molecular risk assessment showed a substantial improvement, without additional improvement when using findings of central pathology review. Approximately 15% of the high-intermediate risk patients had unfavorable features, and 50% had favorable features leaving 35% intermediate (Fig. 3D and E). In tumors with unfavorable features, targetable alterations were found: 65% PI3K/AKT alterations, 9% *FBXW7* mutations, 7% *FGFR2* mutations, 28% L1CAM positivity 78% ER positivity, and 61% PR positivity.

Discussion

In 834 early-stage EECs from two randomized trials (PORTEC-1 and -2) with mature long-term follow-up, the prognostic impact of the four molecular subgroups, originally proposed by the TCGA, was confirmed (10). Clinically applicable molecular analysis methods for surrogate markers were used and proved feasible in >96% of EEC patients. Integration of prognostic molecular alterations with established clinicopathologic factors results in a stronger risk assessment. As a consequence, within the high-intermediate risk population, who are currently thought to be relatively homogenous with regard to clinical outcomes, approximately 15% patients with a marked unfavorable and 50% with a favorable prognosis could be identified.

L1CAM, p53, and LVSI were consistent independent prognostic factors for distant recurrence, overall, and disease-specific survival. p53-mutant tumors exhibit a high degree of genomic instability linked to tumor progression, and invasion by upregulation of p53-mutant target genes, and *TP53* mutation is well known for its prognostic impact in endometrial carcinoma (8, 10). LVSI, especially when quantified as substantial, and L1CAM have similar strong negative prognostic value. LVSI strongly increases the risk of tumor spread via lymphatics and capillaries. L1CAM is known to enhance motility and migration of tumor cells. Both were recently published in this same population as single risk factors (13, 18) and by Zeimet and colleagues (19), but were now confirmed to be independent prognostic factors in an integrated

Table 1. Clinicopathologic characteristics and alterations in potential other molecular classifiers according to the four molecular subgroups in early-stage endometrial cancer ($n = 834$)

	Total $n = 834$	**p53-mutant** $n = 74$ (8.9%)	**MSI** $n = 219$ (26.3%)	***POLE*-mutant** $n = 49$ (5.9%)	**NSMP** $n = 492$ (59.0%)	***P***
Age, years						
Mean (range)	68 (41–90)	69 (51–86)	69 (43–89)	62 (46–81)	68 (41–90)	0.000
<60	138 (16.5)	7 (9.5)	35 (16.0)	19 (38.8)	77 (15.7)	0.001
60–70	360 (43.2)	32 (43.2)	89 (40.6)	18 (36.7)	221 (44.9)	
>70	336 (40.3)	35 (47.3)	95 (42.4)	12 (24.5)	194 (39.4)	
Grade						
1–2	724 (86.8)	48 (64.9)	135 (83.6)	33 (73.4)	457 (92.9)	0.000
3	110 (13.2)	26 (35.1)	36 (16.4)	13 (26.6)	35 (7.1)	
Myometrial invasion						
<50%	251 (30.1)	35 (47.3)	71 (32.4)	25 (51.0)	120 (24.4)	0.000
>50%	583 (69.9)	39 (52.7)	148 (67.6)	24 (49.0)	372 (75.6)	
LVSI[a]						
Absent/focal	784 (95.5)	70 (94.6)	194 (91.1)	47 (100)	473 (97.1)	0.002
Substantial	37 (4.5)	4 (5.4)	19 (8.9)	0	14 (2.9)	
Risk group						
Low	242 (29.0)	22 (29.7)	62 (28.3)	24 (49.0)	134 (27.2)	0.013
High-intermediate	546 (65.5)	44 (59.5)	143 (65.3)	23 (46.9)	336 (68.3)	
High	46 (5.5)	8 (10.8)	14 (6.4)	2 (4.1)	22 (4.5)	
Treatment						
NAT	241 (28.9)	17 (23.0)	63 (28.8)	16 (32.7)	145 (29.5)	0.688
EBRT	409 (49.0)	38 (51.3)	113 (51.6)	25 (51.0)	233 (47.4)	
VBT	184 (22.1)	19 (25.7)	43 (19.6)	8 (16.3)	114 (23.1)	
Mutations[b]						
CDKN2A	2 (0.2)	0	0	0	2 (0.4)	0.707
CTNNB1	157 (19.5)	5 (7.0)	19 (9.0)	8 (17.0)	125 (26.3)	0.000
FBXW7	40 (5.0)	8 (11.3)	13 (6.1)	1 (2.1)	18 (3.8)	0.032
FGFR2	80 (9.9)	2 (2.8)	20 (9.4)	0	58 (12.2)	0.007
KRAS	139 (17.3)	7 (9.9)	43 (20.3)	3 (6.4)	86 (18.1)	0.042
NRAS	25 (3.1)	1 (1.4)	8 (3.8)	0	16 (3.4)	0.456
PIK3CA	261 (32.4)	17 (23.9)	70 (33.0)	24 (51.1)	150 (31.6)	0.019
PPP2R1a	39 (4.8)	12 (16.9)	6 (2.8)	1 (2.1)	20 (4.2)	0.000
PTEN	349 (43.4)	15 (21.1)	106 (50.0)	34 (72.3)	194 (40.8)	0.000
Altered protein expression[c]						
>10% L1CAM	44 (5.6)	27 (39.7)	5 (2.4)	1 (2.6)	16 (3.4)	0.000
<10% ER	38 (5.0)	16 (24.2)	5 (2.5)	4 (10.5)	13 (2.8)	0.000
<10% PR	81 (10.6)	25 (39.1)	19 (9.4)	9 (23.7)	28 (6.1)	0.000
Loss/clonal ARID1a	329 (45.4)	17 (27)	123 (63.7)	13 (35.1)	176 (40.8)	0.000
Loss/heterogeneous PTEN	395 (51.5)	28 (43.1)	130 (64.4)	19 (48.7)	218 (47.3)	0.000
Nuclear β-catenin	184 (23.6)	7 (10.6)	34 (16.3)	3 (7.7)	140 (30.1)	0.000

Abbreviations: EBRT, external beam radiotherapy; LVSI, lymphovascular space invasion; NAT, no additional treatment; VBT, vaginal brachytherapy.
[a]Degree of LVSI unknown for 13 (1.6%) cases.
[b]Mutation analysis failed for 29 (3.5%) cases.
[c]Immunohistochemical analysis failed, or no available FFPE slides for 111 (13%) ARID1a, 56 (6.7%) β-catenin, 73 (9%) ER, 68 (8%) PTEN, and 73 (9%) PR.

analysis. In contrast, patients with *POLE*-mutant or MSS and *CTNNB1* wild-type tumors displayed a more favorable prognosis. The favorable outcome of *POLE*-mutant endometrial carcinomas with their striking mutation burden may be explained by an increased immunogenicity, and became evident in endometrial carcinoma recently (20, 21). *CTNNB1* mutations result in activation of Wnt signaling contributing to tumor progression, abnormal expression of cell proliferation, and progression genes. Similarly to our results, a previous report showed that endometrial carcinomas carrying a *CTNNB1* mutation characterize a more aggressive subset within low-grade early-stage EEC (10, 22). The prognostic importance of MSI has been controversial, although the strongest association with poor clinical outcome has been observed in early-stage endometrial carcinoma similar to our observation (23). This report integrates a large number of single prognostic factors in the context of clinical trial material resulting in a comprehensive overview.

In this large cohort, only few (3%) tumors had multiple classifying alterations (e.g., *POLE* and MSI). Classification of this small subset would require further analyses, such as mutational load and copy-number status. Supek and colleagues reported that colorectal and stomach tumors with both MSI and *POLE* mutation had an overall mutational load similar to MSI tumors, whereas 2 of 3 MSI/*POLE* endometrial tumors had a much higher mutational load and different mutational signature (24). Furthermore, Shinbrot and colleagues showed that the *TP53* gene is frequently affected by *POLE* mutation induced strand-specific mutations (25). These data support that mutational load, mutation signature, and pattern may be useful for molecular classification of rare tumors that present with combinations of MSI, *POLE*, or *TP53* mutations. With the advent of next-generation sequencing technologies, these can be easily analyzed.

Several molecular alterations, such as hormone receptor expression, *CTNNB1* and *FGFR2* mutations, have been previously reported as having prognostic potential in single biomarker studies (8, 9, 13, 19, 22, 26, 27). Some univariable factors, *FGFR2* mutation and hormone receptor status, lost significance in multivariable analysis. This may be due to the fact that *FGFR2*

Table 2. Univariable analysis of clinicopathologic characteristics, molecular subgroups, and potential other molecular classifiers in high-intermediate risk early-stage endometrial cancer ($n = 546$)

		Locoregional recurrence 42 events		Distant recurrence 50 events		Overall survival 182 events	
	Total *n*	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (continuous)	546	1.035 (0.988–1.085)	0.145	1.015 (0.972–1.060)	0.508	1.085 (1.062–1.110)	0.000
Grade							
1–2	492	1		1		1	
3	54	1.784 (0.751–4.239)	0.190	3.038 (1.552–5.945)	0.001	1.741 (1.149–2.639)	0.009
Myometrial invasion							
<50	62	1		1		1	
>50	484	0.539 (0.239–1.216)	0.137	0.304 (0.161–0.574)	0.000	0.586 (0.392–0.877)	0.009
LVSI							
Absent/mild	507	1		1		1	
Substantial	28	3.733 (1.567–8.891)	0.003	4.895 (2.368–10.121)	0.000	2.791 (1.668–4.432)	0.000
Given treatment							
NAT	113	1		1		1	
EBRT	276	0.311 (0.150–0.642)	0.002	0.873 (0.425–1.796)	0.713	0.833 (0.595–1.167)	0.289
VBT	157	0.546 (0.257–1.157)	0.114	1.108 (0.511–2.401)	0.796	0.745 (0.480–1.157)	0.190
Molecular subgroup							
NSMP	336	1		1		1	
p53	44	6.787 (3.069–15.012)	0.000	11.083 (5.629–21.821)	0.000	4.861 (3.098–7.073)	0.000
MSI	143	2.476 (1.182–4.776)	0.015	2.220 (1.180–4.447)	0.025	1.853 (1.329–2.584)	0.000
POLE	23	—	0.970	0.869 (0.116–6.532)	0.891	0.907 (0.367–2.237)	0.832
CTNNB1							
No mutation	433	1		1		1	
Mutation	101	0.575 (0.225–1.467)	0.247	0.934 (0.453–1.929)	0.854	0.669 (0.438–1.023)	0.063
FBXW7							
No mutation	512	1		1		1	
Mutation	22	0.666 (0.091–4.848)	0.688	0.530 (0.073–3.847)	0.531	1.569 (0.827–2.977)	0.168
FGFR2							
No mutation	468	1		1		1	
Mutation	66	0.746 (0.256–2.095)	0.578	0.296 (0.072–1.219)	0.092	0.556 (0.316–0.979)	0.042
KRAS							
No mutation	453	1		1		1	
Mutation	81	0.998 (0.419–2.379)	0.997	1.322 (0.639–2.734)	0.452	1.035 (0.686–1.561)	0.871
NRAS							
No mutation	519	1		1		1	
Mutation	15	—	0.430	—	0.398	0.635 (0.231–1.690)	0.354
PIK3CA							
No mutation	358	1		1		1	
Mutation	176	0.572 (0.272–1.201)	0.140	0.814 (0.436–1.516)	0.516	0.921 (0.668–1.271)	0.618
PPP2R1A							
No mutation	504	1		1		1	
Mutation	30	1.599 (0.492–5.203)	0.435	2.128 (0.842–5.375)	0.110	1.640 (0.932–2.888)	0.086
PTEN							
No mutation	305	1		1		1	
Mutation	229	0.908 (0.484–1.702)	0.763	0.517 (0.277–0.965)	0.038	0.797 (0.588–1.080)	0.144
L1CAM							
<10%	496	1		1		1	
>10%	30	3.283 (1.283–8.404)	0.013	7.718 (3.993–14.917)	0.000	3.763 (2.379–5.953)	0.000
ER							
>10%	499	1		1		1	
<10%	21	3.547 (1.259–9.993)	0.017	6.194 (2.882–13.310)	0.000	2.139 (1.183–3.865)	0.012
PR							
>10%	465	1		1		1	
<10%	51	2.828 (1.297–6.165)	0.009	5.684 (3.042–10.622)	0.000	2.096 (1.379–3.188)	0.001
ARID1a							
Positive	249	1		1		1	
Loss/clonal	228	0.792 (0.423–1.483)	0.467	0.827 (0.455–1.503)	0.533	0.878 (0.643–1.200)	0.415
PTEN							
Positive	232	1		1		1	
Loss/heterogeneous	283	0.979 (0.529–1.812)	0.946	0.988 (0.553–1.765)	0.967	1.043 (0.769–1.414)	0.788
β-catenin							
Membrane	399	1		1		1	
Nuclear	126	0.758 (0.350–1.643)	0.483	0.846 (0.420–1.704)	0.640	0.704 (0.471–1.051)	0.086

mutations were equally frequent in MSI and NSMP endometrial carcinomas, and that hormone receptor loss was mainly found in p53-mutant and L1CAM-positive endometrial carcinomas but was also frequently observed in *POLE*-mutant endometrial carcinomas (10, 26–28). MSI, p53, and L1CAM proved stronger independent prognosticators in this analysis. *CTNNB1* mutation

Table 3. Multivariable analysis on the prognostic role of the clinicopathologic characteristics, molecular subgroups, and potential other molecular classifiers in high-intermediate risk endometrial cancers ($n = 546$) and in a subset of HIR EC without substantial LVSI, >10% L1CAM, p53 and *POLE* mutation ($n = 443$)

	All cases of HIR EC ($n = 546$)					
	Locoregional recurrence 41 events		Distant recurrence 46 events		Overall survival 170 events	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (continuous)	1.032 (0.984–1.081)	0.197	1.016 (0.972–1.062)	0.469	1.076 (1.051–1.101)	0.000
Grade						
1–2	1		1		1	
3	0.203 (0.021–1.946)	0.167	0.162 (0.029–0.904)	0.038	0.262 (0.057–1.204)	0.085
Myometrial invasion						
<50%	1		1		1	
>50%	0.201 (0.024–1.678)	0.138	0.126 (0.026–0.624)	0.011	0.254 (0.058–1.101)	0.067
LVSI						
Absent/mild	1		1		1	
Substantial	3.190 (1.301–7.821)	0.011	4.303 (1.833–10.099)	0.001	2.637 (1.542–4.509)	0.000
Treatment						
NAT	1		1		1	
EBRT	0.277 (0.133–0.574)	0.001	1.154 (0.498–2.677)	0.738	0.897 (0.623–1.292)	0.559
VBT	0.466 (0.212–1.027)	0.058	1.134 (0.465–2.769)	0.782	0.707 (0.445–1.123)	0.142
Molecular subgroup						
NSMP	1		1		1	
p53	7.340 (3.168–17.009)	0.000	5.766 (2.400–13.854)	0.000	3.777 (2.364–6.037)	0.000
MSI	2.319 (1.105–4.866)	0.026	2.154 (1.022–4.540)	0.044	1.879 (1.307–2.700)	0.001
POLE	—	0.973	0.883 (0.113–6.890)	0.906	1.105 (0.394–3.101)	0.850
L1CAM						
<10%			1		1	
>10%			4.303 (1.833–10.099)	0.001	2.462 (1.453–4.170)	0.001
	HIR EC without substantial LVSI, >10% L1CAM, p53 and *POLE* mutation ($n = 443$)					
	Locoregional recurrence 27 events		Distant recurrence 23 events		Overall survival 127 events	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (continuous)	1.063 (1.000–1.130)	0.052	1.007 (0.944–1.074)	0.837	1.102 (1.070–1.134)	0.000
Grade						
1–2	1		1		1	
3	0.060 (0.004–0.842)	0.037	0.350 (0.033–3.765)	0.387	0.409 (0.050–3.381)	0.407
Myometrial invasion						
<50%	1		1		1	
>50%	0.076 (0.009–0.668)	0.020	0.099 (0.011–0.859)	0.036	0.277 (0.037–2.070)	0.211
Treatment						
NAT	1		1		1	
EBRT	0.249 (0.106–0.585)	0.001	0.862 (0.329–2.262)	0.764	0.806 (0.541–1.201)	0.289
VBT	0.181 (0.054–0.605)	0.005	0.511 (0.139–1.877)	0.312	0.559 (0.312–1.003)	0.051
Molecular subgroup						
NSMP	1		1		1	
MSI	1.816 (0.815–4.048)	0.145	2.520 (1.049–6.051)	0.039	1.672 (1.146–2.438)	0.008
CTNNB1						
No mutation			1			
Mutation			2.959 (1.234–7.098)	0.015		

Abbreviations: EC, endometrial cancers; HIR, high-intermediate risk.

status was sufficiently strong to emerge in multivariable analysis, stressing its independent prognostic significance. Using this combined approach, an improved risk assessment resulted in which *POLE*, L1CAM, MSI, and *CTNNB1* are integrated with histopathologic factors.

Previous studies have shown improved risk stratification obtained by central pathology review (29, 30). The reviewed pathology in our analyses had the advantage to exclude prototypical nonendometrioid cancers. With regard to grading, lack of prognostic relevance of grade 2 was shown, advocating the use of a two-tiered grading system, as was also proposed by others (31–33). The increased AUC of the model based on central pathology review as compared with the original inclusion pathology confirms these findings. The molecular integrated risk model showed an even higher increase in AUC; however, central pathology review did not add any additional value to the molecular integrated risk model. The molecular integrated risk model has three major advantages. First, it is based on more objective variables, such as mutational status of *POLE*. Second, the molecular integrated risk model identifies significantly more patients with favorable features that would otherwise be classified as high-intermediate risk with central pathology review alone. Finally, this approach has also the advantage to facilitate prescreening for Lynch syndrome.

Despite the strength of a randomized trial population, mature long-term follow-up, large group of early-stage EEC, and straightforward molecular analysis, this study has some limitations. Our focused and practical approach provides analyses that can easily be implemented in prospective studies and clinical practice. Most common hotspot mutations were analyzed but this does not rule out the possibility that other clinical relevant alterations may have been missed. Although, molecular alterations were highly

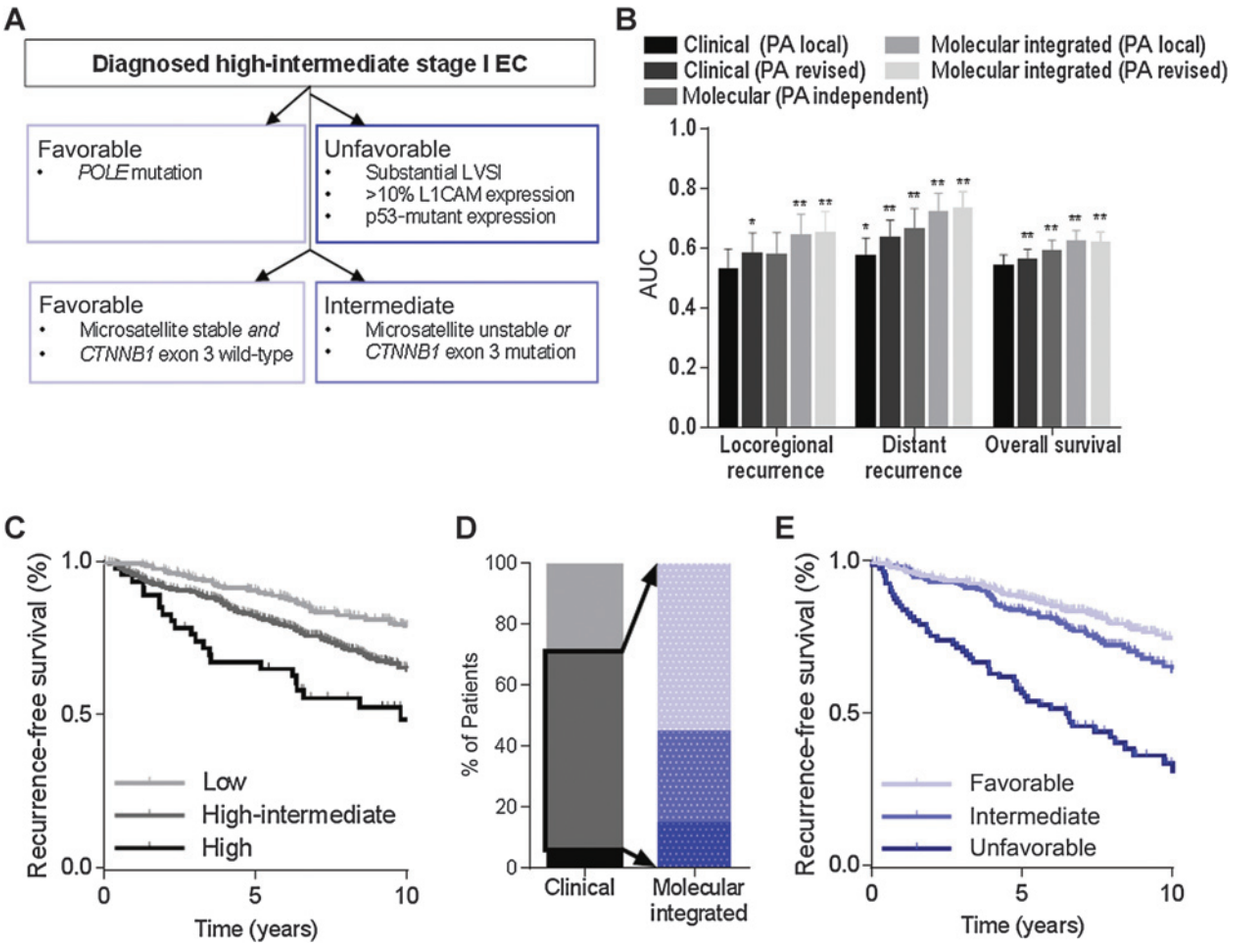

Figure 3.
Molecular integrated risk assessment. **A,** flow chart of the molecular integrated risk model. **B,** AUC for the clinical and molecular, and molecular integrated risk assessment, with and without central pathology review (*, $P < 0.05$; **, $P < 0.01$). **C,** recurrence-free survival of clinical risk assessment in early-stage endometrial cancer ($n = 834$, $P < 0.001$). **D,** bar chart of the proportion of clinically low-, high-intermediate-, and high-risk patients based on central pathology review (left) and the proportion of clinically high-intermediate risk patients reclassified into favorable, intermediate, and unfavorable molecular integrated risk groups. E, recurrence-free survival of molecular integrated risk assessment in early-stage high-intermediate risk endometrial cancer ($n = 546$, $P < 0.001$).

concordant between curettage and hysterectomy specimen (15, 17, 18), intratumor heterogeneity may interfere with prediction of the patient's prognosis and requires further study. LVSI and the classic histopathology, included in the integrated risk model, cannot be evaluated on preoperative specimen, therefore, it is recommended not to rely on preoperative specimens. No automated immunohistochemical protocols were used, whereas it is likely that robust, standardized automated staining procedures are the preferred method in diagnostic pathology. Molecular alterations in our integrated risk model have been proven in single biomarker studies; however, this integrated risk model needs to be validated or prospectively analyzed. As the majority of our patient cohort has received adjuvant radiotherapy, the decision to omit adjuvant radiotherapy especially in the favorable subgroup remains to be elucidated in a prospective study. There is also need to further investigate whether certain molecular defined subgroups of endometrial carcinoma may be more sensitive to radiotherapy. Nevertheless, we believe our data is unique and informative for patient's outcome, and may guide molecular-based trials and therapies for endometrial carcinoma.

The proposed molecular integrated risk model outperforms the current clinicopathologic approach; therefore, the question arises whether this integrated model can be used for new clinical studies and guide treatment decisions. Especially in high-intermediate endometrial carcinoma, this risk model may substantially reduce overtreatment of favorable cases, and select unfavorable cases that might need more intensive treatment. The clinical utility for tailoring adjuvant therapy, the feasibility of determining the molecular integrated profile within tight time limits and the cost-effective aspects of this approach (e.g., costs of molecular testing vs. saving costs of adjuvant radiotherapy) will be prospectively established in a planned prospective trial PORTEC-4. Within approximately 10% of low-risk patients, p53 and L1CAM seem prognostic indicators for high recurrence rate and impaired survival, which is in line with Talhouk and colleagues (12). However, the small number of events in this subgroup limits these findings. Factors that are associated with favorable outcome or predict

chemotherapy response in high-risk endometrial carcinoma remain to be elucidated in future studies.

In conclusion, integration of molecular risk factors with clinicopathologic factors in early-stage endometrial carcinoma leads to improved risk stratification with potential clinical utility. This molecular integrated risk prediction holds promise to reduce both overtreatment and undertreatment and should form the basis for future prospective clinical studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The sponsor of the study (Dutch Cancer Society) had no role in study design, data collection, data analysis, data interpretation, or with writing the report.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.
2. Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006;95Suppl 1:S105–43.
3. Kong A, Johnson N, Kitchener HC, Lawrie TA. Adjuvant radiotherapy for stage I endometrial cancer: an updated Cochrane systematic review and meta-analysis. J Natl Cancer Inst 2012;104:1625–34.
4. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet 2000;355:1404–11.
5. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a Gynecologic Oncology Group study. Gynecol Oncol 2004;92:744–51.
6. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24Suppl 6:vi33–8.
7. Nout RA, Smit VT, Putter H, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. Lancet 2010;375:816–23.
8. Salvesen HB, Haldorsen IS, Trovik J. Markers for individualised therapy in endometrial carcinoma. Lancet Oncol 2012;13:e353–61.
9. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol 2014;15:e268–78.
10. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67–73.
11. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. Mod Pathol 2015;28:836–44.
12. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 2015;113:299–310.
13. Bosse T, Peters EE, Creutzberg CL, Jurgenliemk-Schulz IM, Jobsen JJ, Mens JW, et al. Substantial lymph-vascular space invasion (LVSI) is a significant risk factor for recurrence in endometrial cancer - a pooled analysis of PORTEC 1 and 2 trials. Eur J Cancer 2015;51:1742–50.
14. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387–91.
15. Stelloo E, Nout RA, Naves LC, Ter Haar NT, Creutzberg CL, Smit VT, et al. High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens in patients with endometrial carcinoma. Gynecol Oncol 2014;133:197–204.
16. McConechy MK, Talhouk A, Li-Chang HH, Leung S, Huntsman DG, Gilks CB, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. Gynecol Oncol 2015;137:306–10.
17. Church DN, Stelloo E, Nout RA, Valtcheva N, Depreeuw J, ter HN, et al. Prognostic significance of POLE proofreading mutations in endometrial cancer. J Natl Cancer Inst 2015;107:402.
18. Bosse T, Nout RA, Stelloo E, Dreef E, Nijman HW, Jurgenliemk-Schulz IM, et al. L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results. Eur J Cancer 2014;50:2602–10.
19. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Azim SA, et al. L1CAM in early-stage type I endometrial cancer: results of a large multicenter evaluation. J Natl Cancer Inst 2013;105:1142–50.
20. van Gool IC, Eggink FA, Freeman-Mills L, Stelloo E, Marchi E, de BM, et al. POLE proofreading mutations elicit an antitumor immune response in endometrial cancer. Clin Cancer Res 2015;21:3347–55.
21. Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, et al. Association of polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. JAMA Oncol 2015;1:1319–23.

22. Liu Y, Patel L, Mills GB, Lu KH, Sood AK, Ding L, et al. Clinical significance of CTNNB1 mutation and Wnt pathway activation in endometrioid endometrial carcinoma. J Natl Cancer Inst 2014;106.
23. Diaz-Padilla I, Romero N, Amir E, Matias-Guiu X, Vilar E, Muggia F, et al. Mismatch repair status and clinical outcome in endometrial cancer: a systematic review and meta-analysis. Crit Rev Oncol Hematol 2013;88:154–67.
24. Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. Nature 2015;521:81–4.
25. Shinbrot E, Henninger EE, Weinhold N, Covington KR, Goksenin AY, Schultz N, et al. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. Genome Res 2014;24:1740–50.
26. Byron SA, Gartside M, Powell MA, Wellens CL, Gao F, Mutch DG, et al. FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features. PLoS One 2012;7:e30801.
27. Trovik J, Wik E, Werner HM, Krakstad C, Helland H, Vandenput I, et al. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. Eur J Cancer 2013;49:3431–41.
28. Huszar M, Pfeifer M, Schirmer U, Kiefel H, Konecny GE, Ben-Arie A, et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. J Pathol 2010;220:551–61.
29. Scholten AN, van Putten WL, Beerman H, Smit VT, Koper PC, Lybeert ML, et al. Postoperative radiotherapy for Stage 1 endometrial carcinoma: long-term outcome of the randomized PORTEC trial with central pathology review. Int J Radiat Oncol Biol Phys 2005;63:834–8.
30. Khalifa MA, Dodge J, Covens A, Osborne R, Ackerman I. Slide review in gynecologic oncology ensures completeness of reporting and diagnostic accuracy. Gynecol Oncol 2003;90:425–30.
31. Lax SF, Kurman RJ, Pizer ES, Wu L, Ronnett BM. A binary architectural grading system for uterine endometrial endometrioid carcinoma has superior reproducibility compared with FIGO grading and identifies subsets of advance-stage tumors with favorable and unfavorable prognosis. Am J Surg Pathol 2000;24:1201–8.
32. Conlon N, Leitao MMJr, Abu-Rustum NR, Soslow RA. Grading uterine endometrioid carcinoma: a proposal that binary is best. Am J Surg Pathol 2014;38:1583–7.
33. Alkushi A, Abdul-Rahman ZH, Lim P, Schulzer M, Coldman A, Kalloger SE, et al. Description of a novel system for grading of endometrial carcinoma and comparison with existing grading systems. Am J Surg Pathol 2005;29:295–304.