

CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence

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Although the majority of low grade, early stage endometrial cancer patients will have good survival outcomes with surgery alone, those patients who do recur tend to do poorly. Optimal identification of the subset of patients who are at high risk of recurrence and would benefit from adjuvant treatment has been difficult. The purpose of this study was to evaluate the impact of somatic tumor mutation on survival outcomes in this patient population. For this study, low grade was defined as endometrioid FIGO grades 1 or 2, while early stage was defined as endometrioid stages I or II (disease confined to the uterus). Next-generation sequencing was performed using panels comprised of 46–200 genes. Recurrence-free and overall survival was compared across gene mutational status in both univariate and multivariate analyses. In all, 342 patients were identified, 245 of which had endometrioid histology. For grades 1–2, stages I–II endometrioid endometrial cancer patients, age (HR 1.07, 95% CI 1.03–1.10), *CTNNB1* mutation (HR 5.97, 95% CI 2.69–13.21), and *TP53* mutation (HR 4.07, 95% CI 1.57–10.54) were associated with worse recurrence-free survival on multivariate analysis. When considering endometrioid tumors of all grades and stages, *CTNNB1* mutant tumors were associated with significantly higher rates of grades 1–2 disease, lower rates of deep myometrial invasion, and lower rates of lymphatic/vascular space invasion. When both *TP53* and *CTNNB1* mutations were considered, presence of either *TP53* mutation or *CTNNB1* mutation remained a statistically significant predictor of recurrence-free survival on multivariate analysis and was associated with a more precise confidence interval (HR 4.69, 95% CI 2.38–9.24). Thus, mutational analysis of a 2 gene panel of *CTNNB1* and *TP53* can help to identify a subset of low grade, early stage endometrial cancer patients who are at high risk of recurrence.

Modern Pathology (2017) 30, 1032–1041; doi:10.1038/modpathol.2017.15; published online 10 March 2017

Endometrial cancer is the most common gynecologic malignancy, with an estimated 60 050 new cases in 2016.¹ The vast majority of endometrial cancers have endometrioid histology and are diagnosed at an early stage.² Treatment primarily consists of surgical management, and 5 year survival is 69–88% for FIGO stages I–II disease.² However, a subset of these

patients will have poor outcomes, and determining which patients are at highest risk for a recurrence of their disease—and would, therefore, benefit most from adjuvant treatment or more extensive surgical staging—has been challenging.

Prior research has sought to identify clinical and/or pathological risk factors that place patients with seemingly lower risk endometrial cancer at higher risk of recurrence. In the Gynecologic Oncology Group (GOG) 99 trial, the GOG considered the ‘high-intermediate risk’ group to be based on deep myometrial invasion, histologic grades 2 or 3 disease, or lymphatic/vascular space invasion.³ Depending on the patient’s age, the presence of either one, two, or three

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Received 8 December 2016; revised 27 January 2017; accepted 27 January 2017; published online 10 March 2017

of those factors dictated whether adjuvant therapy was recommended. Similarly, in the Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trials, the 'high-intermediate risk' group was considered to be those patients with at least two of the following characteristics: deep myometrial invasion, age > 60, and grade 3 histology.^{4,5} Both criteria have been implemented in subsequent clinical trials and are used in clinical practice. However, despite these and other proposed algorithms,^{6–9} the appropriate criteria for allocating early endometrial cancer patients to adjuvant treatment remains uncertain. Balancing concerns about over-treatment with the reality that recurrences still occur in this population and are largely incurable reinforces the need for better risk-prediction strategies.

With the advent of the Cancer Genome Atlas (TCGA) and the greater clinical laboratory incorporation of next-generation sequencing, one proposed strategy for improved risk-stratification has been through the use of molecular biomarkers. The purpose of this study was to evaluate the impact of somatic tumor mutation on recurrence-free survival in this patient population.

Materials and methods

Patient Selection

We performed a retrospective analysis of endometrial cancer patients at the University of Texas MD Anderson Cancer Center who had genomic profiling of their endometrial cancer performed since the year 2000. Patients were included if they had endometrial cancer that was histologically confirmed by pathologists at MD Anderson Cancer Center and had undergone molecular assessment of their tumor at our institution. Molecular evaluation of mutational status was performed using either a next-generation sequencing panel of 46 or 50 genes¹⁰ in a clinical molecular diagnostics lab or a next-generation sequencing panel of 200 genes¹¹ in a research setting.

Patients who had neoadjuvant treatment were excluded from analyses of tumor grade, myometrial invasion, lymphatic/vascular invasion, and tumor size. Patients were excluded from survival analyses if they did not have surgical treatment of their endometrial cancer, had a concurrent cancer diagnosis requiring adjuvant treatment, or had a prior cancer diagnosis with a recurrence of disease after treatment for endometrial cancer was initiated. Patients with progressive disease were excluded from recurrence-free survival analyses, but included in other analyses.

Data Collection

Demographic information was obtained by a review of the electronic medical record. Operative reports, clinic notes, and pathology reports were reviewed for

acquisition of clinical information. Patients who received neoadjuvant chemotherapy were considered to be inevaluable for tumor grade, myometrial invasion, lymphatic/vascular space invasion, or tumor size. Tumor stage was determined using the FIGO Staging System, and stage was determined using surgical reports, imaging studies, pathology reports, and clinical findings when appropriate. Date of recurrence was defined as the first clinical identification of recurrent disease, either by imaging or tissue diagnosis. Due to the large number of patients who sought at least part of their care at an outside institution, outside records were used when available. When outside records were not available for review, patient-reported information was used. When exact dates were unavailable, dates were estimated based on available records and patient report, with the default date designated to be the first day of the reported month or year. Data were censored at 28 August 2015. Study data were collected and managed using the REDCap electronic database.¹² The study was approved by the University of Texas MD Anderson Cancer Center's institutional review board (Protocol LAB01-718).

Immunohistochemistry

When tissue was available for tumors in which a *CTNNB1* mutation was detected, immunohistochemistry to detect localization of β -catenin protein was performed using formalin-fixed, paraffin-embedded sections as previously detailed (clone 14, dilution 1:500; BD Biosciences, San Jose, CA, USA).¹³ When possible, the same paraffin block/mirror image block that was used for sequencing was also used for immunohistochemistry. Presence or absence of nuclear staining was evaluated and percentage of tumor demonstrating nuclear staining was recorded. Presence of membrane staining in other epithelial cells served as an internal positive control. Immunohistochemistry assessment was performed by a trained gynecologic pathologist (RRB).

Statistical Analysis

Summary statistics were used to describe the demographic and clinical characteristics of the patients. Fisher's exact, χ^2 , Kruskal–Wallis, and Wilcoxon rank-sum tests were used comparing demographic and clinical characteristics of the patients between *CTNNB1* and *TP53* mutation statuses. Recurrence-free survival and overall survival were estimated using the Kaplan–Meier product-limit estimator, and then a log-rank test was conducted to compare *CTNNB1* and *TP53* mutation status. Overall survival was measured from the date of surgery to the date of last contact or death. Patients alive were censored at the date of last contact or clinic visit. Recurrence-free survival was measured from

Table 1 Clinical and pathology characteristics for the entire cohort and for the endometrioid tumors only

Characteristic	All endometrial cancer (n = 342)	Endometrioid histology (n = 245)	Mixed endometrioid & non-endometrioid histology (n = 50)	Non-endometrioid histology (n = 47)
Age in years, mean (s.d.)	60.6 (11.7)	59.2 (11.7)	63.1 (10.3)	65.3 (12.1)
Body mass index in kg/m ² , mean (s.d.) ^a	33.8 (10.5)	35.0 (10.7)	32.6 (9.5)	28.9 (8.8)
<i>Race, n (%)</i>				
White	245 (72%)	174 (71%)	36 (72%)	35 (74%)
Black	28 (8%)	12 (5%)	8 (16%)	8 (17%)
Hispanic	54 (16%)	47 (19%)	4 (8%)	3 (6%)
Asian	14 (4%)	11 (5%)	2 (4%)	1 (2%)
Other	1 (0%)	1 (0%)	0 (0%)	0 (0%)
<i>Histology, n (%)</i>				
Endometrioid	245 (72%)	NA	NA	NA
Mixed endometrioid and non-endometrioid	50 (15%)			
Non-endometrioid	47 (14%)			
<i>Grade for pure endometrioid tumors, n (%)^b</i>				
1	NA	30 (13%)	NA	NA
2		161 (67%)		
3		48 (20%)		
<i>Myometrial invasion, n (%)^c</i>				
< 50%	183 (58%)	141 (60%)	25 (57%)	17 (44%)
≥ 50%	134 (42%)	93 (40%)	19 (43%)	22 (56%)
<i>LVSI, n (%)^d</i>				
No	138 (45%)	116 (50%)	13 (32%)	9 (24%)
Yes	170 (55%)	114 (50%)	28 (68%)	28 (76%)
<i>Tumor size in cm, mean (s.d.)^e</i>	4.8 (3.2)	4.5 (3.2)	5.2 (2.9)	6.2 (3.6)
<i>Stage, n (%)^f</i>				
I or II	210 (63%)	173 (72%)	24 (49%)	13 (28%)
III or IV	126 (38%)	68 (28%)	25 (51%)	33 (72%)
<i>Mutations, n (%)</i>				
PTEN	154 (45%)	132 (54%)	17 (34%)	5 (11%)
PIK3CA	135 (39%)	101 (41%)	22 (44%)	12 (26%)
ARID1A ^g	86 (38%)	68 (42%)	13 (36%)	5 (19%)
PIK3R1 ^g	53 (24%)	43 (27%)	9 (25%)	1 (4%)
TP53	73 (21%)	32 (13%)	19 (38%)	22 (47%)
KRAS	66 (19%)	52 (21%)	9 (18%)	5 (11%)
CTNNB1	60 (18%)	53 (22%)	6 (12%)	1 (2%)
FGFR2	32 (9%)	28 (11%)	4 (8%)	0 (0%)

^aIn total, 340 patients were included in BMI assessment for the overall cohort; 2 patients did not have either a height or weight recorded at the time of their initial evaluation at the University of Texas MD Anderson Cancer Center.

^bIn total, 239 patients were included in the endometrioid grade assessment; 6 received neoadjuvant chemotherapy.

^cIn total, 317 patients were included in the myometrial invasion assessment for the overall cohort; 16 received neoadjuvant, 9 did not have information available.

^dIn total, 308 patients were included in the lymphatic/vascular space invasion assessment for the overall cohort; 16 received neoadjuvant chemotherapy, 18 patients did not have information available.

^eIn total, 300 patients were included in the tumor size assessment for the overall cohort; 16 received neoadjuvant chemotherapy, 26 did not have accurate tumor size information available.

^fIn total, 336 patients were included in the stage assessment for the overall cohort; 6 patients did not have clinical, pathology, or radiological information available for stage assessment.

^gIn total, 225 patients were included in the analyses for the overall cohort for both ARID1A and PIK3R1.

the date of surgery to the earliest date of the last clinic visit, date of first recurrence, or date of death. Patients alive and recurrence-free were censored at the date of last contact or clinic visit. Stata v14.2 (College Station, TX, USA) was used to conduct statistical analysis.

Results

Patient Characteristics

In total, 342 endometrial cancer patients met our criteria for evaluation. Clinical and pathology characteristics are listed in Table 1. The mean age of our

endometrial cancer cohort was 60.6 years, and the majority of patients had tumors with endometrioid histology, grade 2, and FIGO stage I or II at diagnosis. *PTEN* was the most frequent mutation (45%), followed by *PIK3CA* (39%) and *ARID1A* (38%); all mutations that were present in at least 10% of the patients in our cohort are listed in Table 1.

Characteristics were then evaluated by each histology type (endometrioid, mixed histology with both endometrioid and non-endometrioid components, and non-endometrioid). For the endometrioid cohort ($n=245$), the mean age was slightly younger at 59.2 years, and a larger proportion of tumors were grades 1 or 2 (designated as low grade). The endometrioid cohort remained predominantly stages I or II, which we designated early stage in this analysis as these are patients with tumors that are confined to the uterus and cervix and who therefore are less likely to receive adjuvant systemic treatment. *PTEN* remained the most frequent mutation (54%), followed by *ARID1A* (42%) and *PIK3CA* (41%). For all subsequent analyses, the cohort was limited to only those patients with endometrioid histology, as patients with non-endometrioid or mixed endometrioid/non-endometrioid endometrial carcinomas typically receive more aggressive systemic therapy due to worsened outcomes in these subsets.

Survival Analyses Of Low Grade, Early Stage Tumors

Because high grade (grade 3) and advanced stage (stages III–IV) are both known to be associated with recurrence of disease and worse survival outcomes in general, these patients often receive more aggressive adjuvant therapy, frequently including systemic treatment in the setting of advanced stage disease.² For this reason, the survival analyses were limited to patients with low grade and early stage tumors in order to better identify patients who would be higher risk within an otherwise lower risk cohort. On univariate analyses of common clinical and pathology characteristics, as well as the most common somatic mutations in our cohort, only age at diagnosis (HR 1.03, 95% CI 1.01–1.06, $P=0.005$), *CTNNB1* exon 3 mutation (HR 2.06, 95% CI 1.15–3.69, $P=0.02$), and *TP53* mutation (2.49, 95% CI 1.05–5.90, $P=0.04$) were associated with significantly worse recurrence-free survival. Kaplan–Meier curves for *CTNNB1* and *TP53* mutation are shown in Figure 1a and b. BMI, tumor size, the receipt of adjuvant therapy, the presence of lymphatic/vascular space invasion, the presence of deep myometrial invasion, or any of the other mutations listed in Table 1 were not significantly associated with recurrence-free survival (data not shown). Univariate analyses for overall survival showed only age at diagnosis to have a statistically significant impact (HR 1.08, 95% CI 1.04–1.11, $P<0.001$).

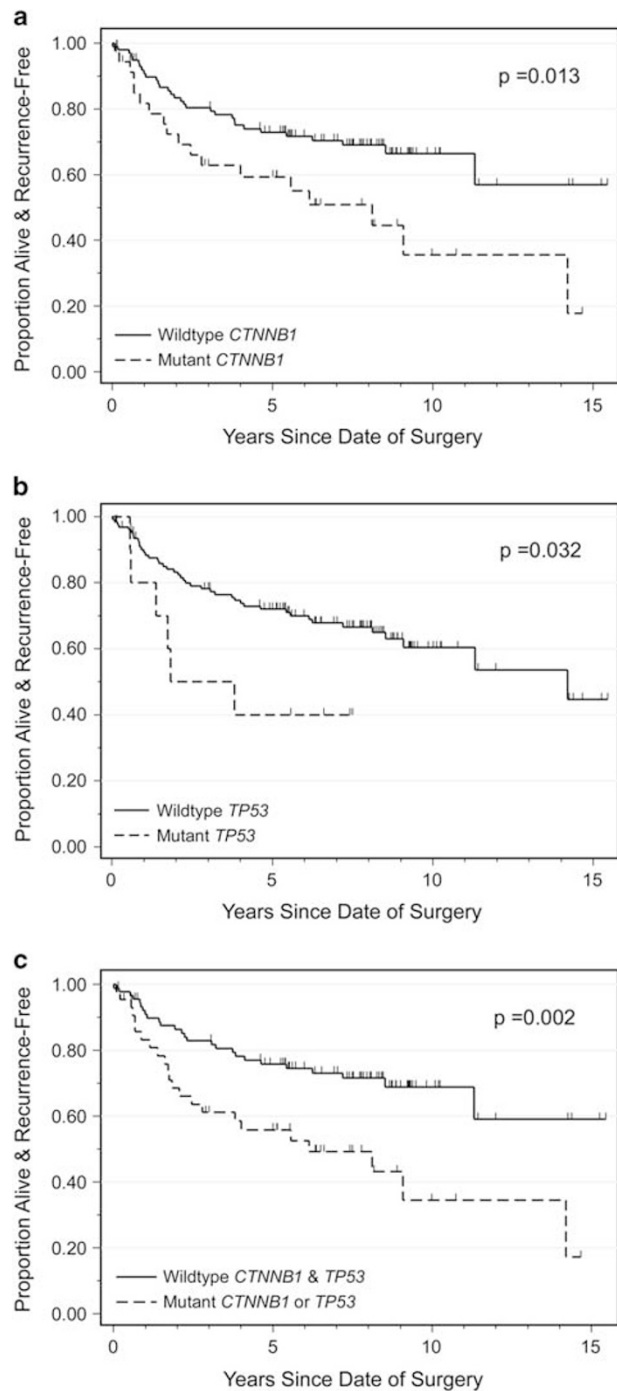


Figure 1 Recurrence-free survival for endometrioid endometrial cancer patients, grades 1–2 and stages I–II, by *CTNNB1* mutation status (a), *TP53* mutation status (b), and combined *CTNNB1* and *TP53* mutation (c). Presence of mutation in *CTNNB1* or *TP53* is associated with worse recurrence-free survival in this subset of endometrial cancer patients.

Multivariate analysis for recurrence-free survival for this low grade, early stage endometrioid cohort was next performed. The multivariate analysis included clinical and pathology characteristics which have previously been shown to be associated

Table 2 Multivariate analysis for recurrence-free survival in patients with grades 1–2, stages I–II endometrioid endometrial cancer ($n = 125$)^a

Variable	Hazard ratio	95% CI	P-value
Age at diagnosis	1.07	1.03–1.10	< 0.001
Body mass index	1.00	0.96–1.03	0.83
Myometrial invasion $\geq 50\%$	0.80	0.35–1.83	0.59
Lymphatic/vascular space invasion	1.84	0.84–4.03	0.13
Tumor size	0.95	0.80–1.11	0.50
Adjuvant treatment ^b	0.80	0.37–1.72	0.80
TP53 mutation	4.07	1.57–10.54	0.004
CTNNB1 mutation	5.97	2.69–13.21	< 0.001

^aIn total, 125 patients had the above information available and were included in the analysis.

^bAdjuvant treatment was treated as a time-dependent covariate.

with survival outcomes and somatic mutations with $P < 0.2$ on the univariate survival analyses (CTNNB1 and TP53). Variables ultimately included were age at diagnosis, BMI, myometrial invasion, lymphatic/vascular space invasion, tumor size, adjuvant treatment, CTNNB1 mutation, and TP53 mutation (Table 2). CTNNB1 was found to have the highest hazard ratio in this multivariable analysis, with a hazard ratio of 5.97 (95% CI 2.69–13.21). Other statistically significant variables included TP53 mutation with an HR 4.07 (95% CI 1.57–10.54), and age at diagnosis with an HR 1.07 (95% CI 1.03–1.10). Of note, only 13 out of 148 patients with grades 1 or 2 and stages I or II tumors had a mutation in TP53, and thus the utility of TP53 mutation as biomarker of recurrence may be limited. In comparison, CTNNB1 mutation is present in 26% of these same patients.

Characterization of CTNNB1 Mutant Patient Cohort

In addition to significantly worse recurrence-free survival, patients with CTNNB1 somatic mutations have other unique characteristics compared to patients with wild-type tumors (Table 3). Patients with tumors harboring CTNNB1 mutation were younger (age 61 vs 53, $P < 0.001$). Despite the worse prognosis, endometrial carcinomas with CTNNB1 mutation showed higher rates of low-grade tumors (76 vs 92%, $P < 0.001$), lower rates of lymphatic/vascular space invasion (54 vs 33%, $P = 0.003$), and lower rates of deep myometrial invasion (43 vs 27%, $P = 0.04$). There were no significant differences in race, BMI, or tumor size between the mutant and wild-type group. Tumors with CTNNB1 mutation were also significantly less likely to have KRAS mutation, TP53 mutation, and FGFR2 mutation. Despite the fact that CTNNB1 mutation co-segregates with factors that otherwise would be expected to be associated with good outcomes (younger age, lower tumor grade, less myometrial invasion, lower incidence of lymphatic/vascular space invasion, and lower frequency of co-TP53 mutation), presence of

Table 3 Clinical and pathology characteristics of patients with endometrioid endometrial cancer, stratified by CTNNB1 mutation status

Characteristic	CTNNB1 wild type (n = 192)	CTNNB1 mutant (n = 53)	P-value
Age in years, mean (s.d.)	60.9 (11.5)	52.9 (10.2)	< 0.001
Body mass index in kg/m ² , mean (s.d.) ^a	34.6 (10.7)	36.3 (10.7)	0.19
Race, n (%)			0.60
White	139 (72%)	35 (66%)	
Black	10 (5%)	2 (4%)	
Hispanic	35 (18%)	12 (23%)	
Asian	7 (4%)	4 (8%)	
Other	1 (1%)	0 (0%)	
Grade, n (%) ^b			< 0.001
1	15 (8%)	15 (29%)	
2	128 (68%)	33 (63%)	
3	44 (24%)	4 (8%)	
Myometrial invasion, n (%) ^c			0.04
< 50%	106 (57%)	35 (73%)	
$\geq 50\%$	80 (43%)	13 (27%)	
Lymphatic/vascular space invasion, n (%) ^d			0.01
No	85 (46%)	31 (67%)	
Yes	99 (54%)	15 (33%)	
Tumor size in cm, mean (s.d.) ^e	4.6 (3.0)	4.1 (3.8)	0.18
Stage, n (%) ^f			0.56
I or II	134 (71%)	39 (75%)	
III or IV	55 (29%)	13 (25%)	
Mutations			
KRAS	47 (24%)	5 (9%)	0.02
PIK3CA	82 (43%)	19 (36%)	0.37
TP53	30 (16%)	2 (4%)	0.02
PTEN	98 (51%)	34 (64%)	0.09
FGFR2	26 (14%)	2 (4%)	0.05
ARID1A ^g	60 (44%)	8 (30%)	0.15
PIK3R1 ^g	37 (27%)	6 (22%)	0.58

^aIn total, 243 patients were included in BMI assessment for the endometrioid cohort; 2 patients did not have either a height or weight recorded at the time of their initial evaluation at the University of Texas MD Anderson Cancer Center.

^bIn total, 239 patients were included in the grade assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy.

^cIn total, 234 patients were included in the myometrial invasion assessment for the endometrioid cohort; 6 received neoadjuvant, 5 did not have invasion information available.

^dIn total, 230 patients were included in the lymphatic/vascular space invasion assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy, 9 patients did not have lymphatic/vascular space invasion information available.

^eIn total, 222 patients were included in the tumor size assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy, 17 did not have accurate tumor size information available.

^fIn total, 241 patients were included in the stage assessment for the endometrioid cohort; 4 patients did not have clinical, pathology, or radiological information available for stage assessment.

^gIn total, 162 patients were included in the analyses for the endometrioid cohort for both ARID1A and PIK3R1.

Table 4 Clinical and pathology characteristics of patients with endometrioid endometrial cancer, stratified by *TP53*

Characteristic	<i>TP53</i> wild type (n = 213)	<i>TP53</i> mutant (n = 32)	P-value
Age in years, mean (s.d.)	59.2 (11.4)	59.1 (13.7)	0.98
Body mass index in kg/m ² , mean (s.d.) ^a	34.9 (10.5)	35.8 (12.3)	0.99
Race, n (%)			> 0.99
White	150 (70%)	23 (75%)	
Black	11 (5%)	1 (3%)	
Hispanic	41 (19%)	6 (19%)	
Asian	10 (5%)	1 (3%)	
Other	1 (0%)	0 (0%)	
Grade, n (%)^b			< 0.001
1	27 (13%)	3 (9%)	
2	148 (71%)	13 (41%)	
3	32 (15%)	16 (50%)	
Myometrial invasion, n (%)^c			0.51
< 50%	124 (61%)	17 (55%)	
≥ 50%	79 (39%)	14 (45%)	
Lymphatic/vascular space invasion, n (%)^d			0.16
No	104 (52%)	12 (39%)	
Yes	95 (48%)	19 (61%)	
Tumor size in cm, mean (s.d.) ^e	4.4 (2.9)	5.1 (4.4)	0.89
Stage, n (%)^f			0.59
I or II	152 (72%)	21 (68%)	
III or IV	58 (28%)	10 (32%)	
Mutations			
<i>KRAS</i>	46 (22%)	6 (19%)	0.71
<i>PIK3CA</i>	87 (41%)	14 (44%)	0.76
<i>CTNNB1</i>	51 (24%)	2 (6%)	0.02
<i>PTEN</i>	120 (56%)	12 (38%)	< 0.05
<i>FGFR2</i>	23 (11%)	5 (16%)	0.42
<i>ARID1A</i> §	64 (44%)	4 (27%)	0.28
<i>PIK3R1</i> §	38 (26%)	5 (33%)	0.53

^aIn total, 243 patients were included in BMI assessment for the endometrioid cohort; 2 patients did not have either a height or weight recorded at the time of their initial evaluation at the University of Texas MD Anderson Cancer Center.

^bIn total, 239 patients were included in the grade assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy.

^cIn total, 234 patients were included in the myometrial invasion assessment for the endometrioid cohort; 6 received neoadjuvant, 5 did not have invasion information available.

^dIn total, 230 patients were included in the lymphatic/vascular space invasion assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy, 9 patients did not have lymphatic/vascular space invasion information available.

^eIn total, 222 patients were included in the tumor size assessment for the endometrioid cohort; 6 received neoadjuvant chemotherapy, 17 did not have accurate tumor size information available.

^fIn total, 241 patients were included in the stage assessment for the endometrioid cohort; 4 patients did not have clinical, pathology, or radiological information available for stage assessment.

§In total, 162 patients were included in the analyses for the endometrioid cohort for both *ARID1A* and *PIK3R1*.

this mutation is associated with significantly worse recurrence-free survival. *CTNNB1* mutation does not appear to be altering the location of metastasis/recurrence, as patients with mutant and wild-type tumors have comparable incidences of extra-vaginal spread when only patients who had a recurrence of their disease are considered (68% of recurrences for wild-type group vs 60% of recurrences for mutant group; $P=0.61$).

TP53 Mutation Characterization

In the univariate and multivariate recurrence-free survival analyses, presence of a *TP53* mutation was also associated with significantly worse survival. Therefore, we also stratified the baseline clinical and pathology characteristics for the endometrioid cohort by *TP53* mutation status (Table 4). Patients with tumors harboring *TP53* mutation were more likely to have grade 3 tumors (50 vs 15%, $P<0.001$), but there were no significant differences in age at diagnosis, BMI, race, deep myometrial invasion, lymphatic/vascular space invasion, tumor size, or stage at diagnosis. Endometrial carcinomas with *TP53* mutation were also less likely to have a *PTEN* co-mutation (38 vs 56%, $p<0.05$), but no other correlations with other frequent somatic mutations were seen.

Analyses Using Combination Of CTNNB1 And TP53 Mutation

TP53 mutation was present in only 9% of grades 1 or 2 and stages I or II endometrioid carcinomas, while *CTNNB1* mutation was present in 26% of this same subset. Thus, *TP53* has limited utility as a single biomarker in the low grade, early stage endometrial cancer patients. Of note, only one patient had a tumor with a mutation in both *CTNNB1* and *TP53*, suggesting that these mutations occur in relatively distinct subsets of patients. We therefore sought to evaluate the association of the presence of *CTNNB1* or *TP53* mutation for prediction of recurrence-free survival in order to potentially increase the number of patients captured by molecular evaluation. On a univariate recurrence-free survival evaluation, the presence of either a *TP53* mutation or a *CTNNB1* mutation was associated with a significantly worse recurrence-free survival ($P=0.002$; Figure 1c). We then evaluated a multivariate model which included the same variables as our previous multivariable model, with the exception of a new combination variable which encompassed the presence of a *CTNNB1* or *TP53* mutation compared with having neither mutation. In this model, the combination variable of *CTNNB1* or *TP53* mutation remained statistically significant, with an HR 4.69 (95% CI 2.38–9.24; Table 5). Age at diagnosis was the only other variable with a significant association with recurrence-free survival (HR 1.06, 95% CI 1.03–1.09).

Table 5 Multivariate analysis for recurrence-free survival in patients with grades 1–2, stages I–II endometrioid endometrial cancer evaluating a combination of *CTNNB1* and *TP53* mutations^a

Characteristic	Hazard ratio	95% CI	P-value
Age at diagnosis	1.06	1.03–1.09	< 0.001
BMI	1.00	0.96–1.03	0.87
Myometrial invasion ($\geq 50\%$)	0.86	0.39–1.90	0.72
Lymphatic/vascular space invasion	1.83	0.84–3.99	0.13
Tumor size	0.95	0.81–1.12	0.57
Adjuvant therapy ^b	0.78	0.37–1.65	0.51
<i>CTNNB1</i> or <i>TP53</i> mutation	4.69	2.38–9.24	< 0.001

^aIn total, 125 patients had the above information available and were included in the analysis.

^bAdjuvant treatment was treated as a time-dependent covariate.

Immunohistochemistry

Of the 60 tumors with *CTNNB1* mutation, 50 were able to be evaluated for β -catenin immunohistochemistry. Of these, 42 (84%) demonstrated nuclear expression. The proportion of the tumor with nuclear staining ranged from 5 to 60%. Eleven of 42 tumors (26%) had nuclear expression in at least 30% of the tumor. All tumors, even those with no nuclear expression, had cytoplasmic protein expression.

Discussion

Our findings demonstrate a significantly decreased recurrence-free survival for patients with low grade, early stage endometrioid endometrial cancers whose tumors harbored a *CTNNB1* or *TP53* mutation and showed that this reduction persisted on a multivariate analysis. The effect of these mutations on overall survival is more uncertain, likely due in part to the longer clinical course associated with this subset of endometrial cancer patients. *CTNNB1* mutation was associated with worse overall survival on multivariate but not univariate analysis, and *TP53* mutation had no effect on overall survival in either the univariate or multivariate analyses. From a practical standpoint, although both biomarkers were useful independently, incorporation of both *TP53* and *CTNNB1* mutation information led to more precise estimates of recurrence risk than either alone. Further, use of either individual or combination evaluation was associated with a higher hazard ratio than any other clinical or pathology finding in their respective multivariate analyses and specifically was higher than commonly relied upon histologic characteristics such as deep myometrial invasion and lymphatic/vascular space invasion.

Following the publication of TCGA endometrial cancer data, we have a more thorough understanding of the genomics of endometrial cancer. The TCGA analysis revealed high rates of PI3K/AKT pathway mutations, as well as *KRAS*, *CTNNB1*, and *ARID1A* mutations within endometrioid tumors.¹⁴ Tumors with *CTNNB1* mutation were predominantly

contained within the microsatellite-stable, copy-number low endometrioid cluster.¹⁴ A recent reanalysis of TCGA data limited to the 271 tumors with endometrioid histology, excluding the non-endometrioid serous carcinomas, found that those patients whose tumors had activation of the Wnt/ β -catenin pathway activation had worse overall survival even when compared to other low-grade cohorts.¹⁵ Higher expression of *Cyclin D1* and *Myc*, two genes known to be activated by Wnt/ β -catenin pathway activation, were associated with worse survival.¹⁵ *CTNNB1* mutation is one mechanism that can activate this pathway; the TCGA cluster with Wnt/ β -catenin pathway activation and *CTNNB1* mutation had the lowest number of other concurrent mutations. Similarly, in our patient cohort the group with *CTNNB1* mutation had a significantly lower incidence of concurrent *KRAS* and *TP53* mutations. In support of the idea that *CTNNB1* mutation is a driver, rather than passenger, in endometrial carcinogenesis, exon 3 deletion of the *CTNNB1* gene in a murine model led to upregulation of the Wnt/ β -catenin pathway and the development of endometrial hyperplasia, a precursor to endometrioid-type endometrial carcinoma.¹⁶ In a different mouse model, activation of uterine targeted β -catenin and loss of PTEN resulted in endometrial adenocarcinoma that was earlier in onset and more aggressive than in mice with PTEN loss alone.¹⁷ At this point, we do not know whether *CTNNB1* gene mutation or Wnt/ β -catenin pathway activation as measured by upregulation of pathway genes is a more powerful prognostic indicator. However, sequencing of hotspot mutations in *CTNNB1* is a less technically challenging assay for the clinical molecular diagnostics laboratory and as it is a dichotomous variable has less challenges with setting cut offs.

Several prior studies have evaluated the impact of *CTNNB1* mutation in endometrial cancer. A case-control study of 47 stage IA grade 1 endometrial cancer patients found a nine times higher odds of *CTNNB1* mutation in tumors of those patients who recurred compared to those who did not, with no differences in odds of *KRAS* or *PIK3CA* mutation.¹⁸ Alternatively, a 2012 study by Byron *et al.* evaluated disease-free survival and overall survival in 386 cases of stages I or II endometrioid endometrial cancer, and found no difference based on *CTNNB1* mutation status.¹⁹ In contrast to our study, however, these data included all grades of tumors in the early stage analysis. When our data included all grades of endometrioid tumors, we similarly did not find *CTNNB1* to be statistically significantly associated with recurrence-free survival. This observation further underscores the importance of considering *CTNNB1* mutation within the context of low grade, early stage tumors, as there is likely to be limited utility of this assessment within the very heterogeneous cohort of all endometrial cancers. Two other studies also evaluated a more heterogeneous endometrial cancer patient population, including high

grade and/or late stage tumors, and had conflicting results in terms of survival outcomes. Both of these studies used immunohistochemistry as a surrogate for mutation status.^{20,21} The prevalence of mutant tumors based on immunohistochemistry in the study by Athanassidou *et al.* was significantly higher than that previously described in the literature for endometrial carcinomas, suggesting that there may be a discordance in β -catenin immunohistochemical staining pattern and presence of mutation as defined by sequencing. Although the majority of tumors in our study demonstrated nuclear expression of β -catenin protein, 16% did not. Furthermore, only 24% of tumors had nuclear expression in 30% or more of the tumor cells. This pattern of nuclear expression is consistent with that reported previously.^{22–24} Therefore, for endometrial cancer, it is unclear if immunohistochemistry can act as an effective surrogate to *CTNNB1* gene sequencing.

The presence of miRNAs has also been shown to be associated with lymph node metastases.²⁵ As data have linked specific miRNAs to the Wnt/ β -catenin pathway,^{26,27} these miRNAs may represent another viable option for identifying higher risk tumors, or further elucidating mechanisms for the worse prognoses of these patients. A disadvantage of this approach is that currently there are no clinical-grade assays for miRNA assessment.

Less research has delineated the epidemiologic or clinical characteristics of low-grade tumors with *TP53* mutation. Prevalence rates of *TP53* mutation have been reported to be ~10–20% in endometrioid endometrial cancer,^{20,28,29} with the majority occurring in grade 3 endometrioid tumors.^{30–33} A 2012 reanalysis of a subset of the PORTEC-2 trial population found that 9 out of 48 patients with low-risk or high-intermediate risk EC (all with grades 1–2 and stage I disease) had a *TP53* mutation based on increased levels on immunohistochemistry analysis and demonstrated *TP53* to be the single most significant prognostic factor on multivariate disease-free survival analysis. Although the prevalence of *TP53* mutation was higher than in our current study, the overall association with disease recurrence mirrors the findings presented in our current research.

In our current study, neither myometrial invasion nor lymphatic/vascular space invasion were associated with recurrence-free survival in our multivariate analysis of low grade, early stage patients, which is contrary to the high-intermediate risk criteria currently in use.^{3,5} Interestingly, *CTNNB1* and *TP53* were not only both associated with recurrence-free survival, but had significantly elevated hazard ratios on par with those seen with tumor grade in prior studies of early stage endometrial cancers.^{5,9} We suspect the reason that myometrial invasion, lymphatic/vascular invasion, and tumor size were not independent predictors in this retrospective cohort may have been related to common adjuvant treatment decision-making

strategies within this retrospective cohort, as providers may have already taken these variables into account and thereby decreased these patients' risks for recurrence. However, this finding further highlights the importance of the patient population identified by *CTNNB1* and *TP53* assessment, as these patients are not being captured by current risk-prediction algorithms. In fact, patients with endometrial carcinomas with *CTNNB1* mutation are significantly more likely to have tumors with pathological characteristics commonly associated with lower clinical risk of recurrence (lower FIGO grade, less incidence of deep myometrial invasion, and less incidence of lymphatic/vascular space invasion). This seeming paradox highlights that pathological variables traditionally used to assess recurrence risk may not be optimal clinical benchmarks.

This study adds to the growing body of literature that suggests that molecular testing may be able to inform treatment decision making for endometrial cancer patients and highlights a subgroup of endometrial cancer patients whose optimal treatment strategies remain uncertain. Several strategies have been proposed,^{14,34,35} most recently using data from PORTEC. McAlpine *et al.* proposed an approach, using *POLE* sequencing, mismatch repair protein immunohistochemistry, and p53 immunohistochemistry, in which the two TCGA subgroups with higher grade endometrioid and serous carcinomas could be separated into two distinct groups based on survival differences, with the *POLE* mutant group having significantly better survival. Similarly, we propose that assessment of *CTNNB1* and *TP53* mutation status can help to stratify the two TCGA groups with the lower grade endometrioid carcinomas into prognostic groups. In the PORTEC study, an algorithm was proposed in which tumors were stratified using *TP53* mutation, microsatellite instability, and *POLE* mutation, and those that remained following the three prior evaluations.³⁴ Within this broad fourth category, *CTNNB1* mutation was found to be associated with increased risk of distant recurrence and thus the authors advocated for its evaluation in risk-stratification evaluation.³⁴ Including Myers *et al.*, PORTEC, and our current study, there are now three published studies in three distinct endometrial cancer patient populations that *CTNNB1* tumor mutation was associated with recurrence of disease. Thus, we believe that use of *CTNNB1* sequencing as a prognostic should be studied in prospective clinical trials. Furthermore, as our current study also found a significant rate of recurrences outside of the vagina, these findings suggest that vaginal brachytherapy alone in patients with *CTNNB1* mutant tumors may be insufficient for recurrence-prevention.

With the growing clinical availability of molecular testing, including molecular information along with the usual pathology and clinical data in treatment planning algorithms is becoming a more realistic

goal. Our current data suggest that even these patients with low grade, early stage disease may benefit from molecular profiling of their endometrial cancers. Prospective clinical trials are needed to better characterize the value of adjuvant treatment strategies in otherwise low-risk patients with high-risk mutations, with the ultimate goal of incorporating molecular information into routine endometrial cancer treatment algorithms.

Acknowledgments

NIH Research Training Grant (KCK) T32 CA101642; NIH SPORE in Uterine Cancer (RRB) NIH 2P50 CA098258; The Red and Charline McCombs Institute Center for Global Cancer Early Detection (RRB); and NIH through MD Anderson's Cancer Center Support Grant (BMF and DU) CA016672.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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