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Molecular Classification Outperforms Histologic Classification in Prognostication of High-grade Endometrial Carcinomas With Undifferentiated and Sarcomatous Components

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Abstract: Since the establishment of 4 molecular subgroups of endometrial carcinoma (EC), there has been significant interest in understanding molecular classification in the context of histologic features and diagnoses. ECs with undifferentiated, spindle, and/or sarcomatous components represent a diagnostically challenging subset of tumors with overlapping clinical and histologic features. We examined the clinicopathologic, morphologic, immunohistochemical, and molecular features of these

tumors identified in our institutions' pathology databases using immunohistochemistry and targeted sequencing. Disease-specific survival (DSS) and progression-free survival (PFS) were analyzed using Kaplan-Meier curves and log-rank tests. One hundred sixty-two ECs were included: carcinosarcomas (UCS; n=96), dedifferentiated/undifferentiated EC (DDEC/UDEC; n=49), and grade 3 endometrioid EC with spindled growth (GR3spEEC) (n=17). All molecular subgroups were represented in all histologic subtypes and included 12 (7%) *POLE*-mutated (*POLE*mut), 43 (27%) mismatch repair-deficient (MMRd), 77 (48%) p53-abnormal (p53abn), and 30 (19%) no specific molecular profile (NSMP) tumors. However, the molecular classification (irrespective of histologic diagnosis) was a significant predictor for both DSS ($P=0.008$) and $P\leq 0.0001$). *POLE*mut EC showed an excellent prognosis with no recurrences or deaths from the disease. MMRd tumors also showed better outcomes relative to NSMP and p53abn tumors. In conclusion, molecular classification provides better prognostic information than histologic diagnosis for high-grade EC with undifferentiated and sarcomatous components. Our study strongly supports routine molecular classification of these tumors, with emphasis on molecular group, rather than histologic subtyping, in providing prognostication.

Key Words: Endometrial carcinoma, molecular classification, carcinosarcoma, undifferentiated carcinoma, dedifferentiated carcinoma

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Endometrial carcinoma (EC) is the most common cancer of the female reproductive tract in the Western world and is increasing in incidence.¹ In 2013, The Cancer Genome Atlas (TCGA) established 4 molecular subgroups for endometrial carcinoma that have prognostic significance: *POLE* mutated (*POLE*mut), microsatellite instability-high (mismatch repair deficient, MMRd), copy-number high/serous-like (p53-abnormal, p53abn), and copy-number low (no specific molecular profile, NSMP).²

Since then, molecular classification has gradually been integrated into the pathologic assessment of EC to better reflect their complex and varied nature as well as their underlying biological behavior.^{3–7} Of note, the landmark TCGA² and subsequent studies^{4–7} included primarily endometrioid and serous histotypes. Less common forms of EC have also been shown to have prognostic stratification based on TCGA subgroup, but these studies have been limited by small numbers and usually focused on a single histologic entity.

High-grade EC constitutes nearly 25% of EC⁸ and overall is associated with a poor prognosis but with some degree of heterogeneity and unpredictable clinical behavior based on morphologic classification alone.⁹ Furthermore, high-grade EC is often diagnostically challenging for pathologists because of overlapping clinical features, morphology, and immunophenotypes, as well as non-uniform diagnostic criteria and sampling issues. Even among gynecologic pathology subspecialists, interobserver agreement is suboptimal, with kappa values ranging from 0.55 to 0.68.^{9–13} Many histologic subtypes are considered in high-grade EC including; serous endometrial carcinoma (USC), clear cell carcinoma (CCC), FIGO grade 3 endometrioid endometrial carcinoma (GR3EEC), uterine carcinosarcoma (UCS) and undifferentiated/dedifferentiated (DDEC). The latter 2 histologic entities (UCS and DDEC) can be particularly challenging to differentiate pathologists, especially when they present with only focal spindled or heterologous components.¹⁴ In addition, other high-grade ECs may show non-squamous spindled morphology without meeting the criteria for sarcomatous transformation.

Molecularly, high-grade ECs are also heterogeneous. Although *TP53* mutations are common in UCS (reported range 62%–91%^{15–18}), they are not ubiquitous. Furthermore, there exists a subset of *POLE*mut and MMRd UCS with distinct prognostic and morphologic differences^{16,19,20} from the more aggressive p53abn and NSMP UCS.¹⁷ All molecular classes are represented in the histologic entity of DDEC/UDEC, with a predominance of the MMRd group.^{21,22}

The utility in distinguishing UCS from DDEC/UDEC or other high-grade ECs with sarcomatous or spindled components has not been established, particularly in the context of TCGA molecular classification. Considering the poor reproducibility of these histologic diagnoses and the challenges encountered by pathologists, we aimed to address this question and elucidate the prognostic significance of molecular classification versus histologic classification among high-grade ECs with spindled, sarcomatous, or undifferentiated components.

METHODS

Study Cohort

Endometrial carcinomas (EC) from Stanford University, Medisch Spectrum Twente (MST), and the University of Virginia (UVA) were included in the study (Fig. 1). Both institutional cases and those reviewed in the

consultation were examined. In addition, we included a cohort of tumors prospectively collected for the PORTEC-3 international clinical trial (which, per protocol, included a central pathology review), previously published by De Boer et al.²³ (n = 7). Cases from Stanford were enriched for p53 wild-type UCS as well as those with non-serous carcinomatous components to capture the less common forms of UCS with molecular classifications other than p53abn. Cases included from UVA were all p53 wild-type UCS, previously published by Jenkins et al.²⁰ (n = 6). Cases from MST were previously published by Vermij et al.²⁴ (n = 33). We retrieved relevant patient information from our respective institutions' electronic medical records or from trial databases^{5,23,24} including patient age, body mass index (BMI), menopause status, clinical stage at presentation, and clinical outcome. Patients without follow-up after hysterectomy were excluded from the study.

Histologic Diagnosis

Tumors diagnosed as UCS, DDEC/UDEC, and grade 3 endometrioid EC with spindled growth (GR3spEEC) were retrospectively identified from our institutions' surgical pathology databases (Table 1, Fig. 1). In all cases, pathology was re-reviewed by one or more of the authors to confirm the diagnosis. Histologic sections from the MST²⁴ and PORTEC-3²³ cohorts were confirmed previously by TB, although slides were not available for re-review at the time of this study. Stanford and UVA cases were reviewed by PMH and BEH. EEC with spindled components were identified by retrospectively reviewing the histopathology (PMH and BEH). EEC that were defined as grade 3 based on >50% nonsquamous/non-spindled solid growth pattern or 5% to 50% with the presence of severe cytological atypia in the majority of cells (>50%).²⁵ Grade 3 EEC that showed at least focal spindling that raised the possibility of UCS, but did not meet criteria for mesenchymal differentiation by morphology or IHC, were included as G3spEEC (Fig. 2C, F, I, L). Morphologic features that supported G3spEEC over UCS included high-grade glandular pattern blending/merging into a spindled pattern of any grade, lack of sharp juxtaposition of spindled and carcinomatous components, lack of heterologous differentiation, and lack of unequivocal high-grade mesenchymal component. Low-grade EEC with spindle cell features but without a high-grade epithelial component, such as grade 1 to 2 corded, hyalinized, and spindled carcinomas (CHEC)-pattern EEC, were excluded. Spindled areas of squamous/morular metaplasia were not considered sufficient for inclusion. UCS were defined as tumors with unequivocal high-grade malignant epithelial and mesenchymal components (Fig. 2A, D, G, J).²⁵ All tumors demonstrated at least focal high-grade carcinoma histology with undifferentiated, sarcomatous, or spindled components. PAX7, myogenin, and desmin were reviewed when available to confirm heterologous rhabdomyosarcomatous differentiation. In addition, immunohistochemical (IHC) studies performed at the time of the diagnostic workup were reviewed. These included PAX8, cytokeratins, EMA, p53, estrogen receptor (ER),

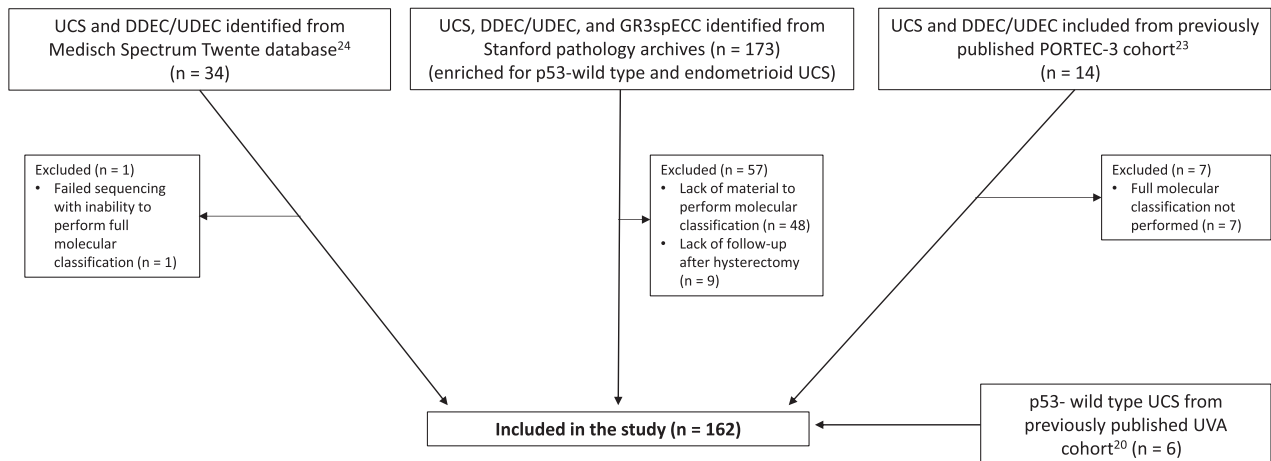


FIGURE 1. Consort diagram of patients included in the study cohort.

progesterone receptor (PR), E-cadherin, SMARCA4 (BRG1), SMARCB1 (INI), PTEN, p16, SATB2, and/or SALL4.^{9,26–29} A full list of IHC reagents and conditions used for cases from Stanford University is provided in supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/PAS/B824>. DDEC and UDEC were confirmed based on morphology and IHC profile. Because of diagnostic overlap, the possibility of sampling bias, and limited slide review in some cases, tumors diagnosed as DDEC or UDEC were combined into 1 histotype (DDEC/UDEC). Uterine sarcomas (leiomyosarcoma, endometrial stromal sarcoma, and adenosarcoma) were excluded.

Histopathologic Features

Histopathologic features, including tumor size, lymph node status, lymphovascular space invasion (LVSI), depth of myometrial invasion, and FIGO stage (based on the 2009 staging system)³⁰ were recorded. The morphologic features of all cases with available material, with respect to nuclear grade, epithelial component morphology, and presence or absence of heterologous differentiation with specific type of differentiation (rhabdomyosarcomatous, chondrosarcomatous, liposarcomatous, or osteosarcomatous) were reviewed, when possible. The predominant epithelial component of each tumor was classified as endometrioid, serous, clear cell, or ambiguous.

Tissue Microarray

Tissue microarray blocks were constructed from Stanford and UVA cases with available materials. For each case, representative tumor areas were selected from formalin-fixed paraffin-embedded (FFPE) blocks based on examination of corresponding H&E-stained slides (slide review for tumor circling performed by PMH and BEH). Each tumor was sampled at least in duplicate for the TMA construction, using either 1 mm or 1.5 mm diameter core punches.

Immunohistochemistry

For molecular classification, IHC for p53, MMR proteins PMS2 and MSH6 were evaluated on all cases with available material. For cases from Stanford University and UVA, a combination of tissue microarrays (TMAs) and whole sections were used depending on tissue block availability and if stains were performed at the time of clinical diagnosis. IHC for cases from MST and the PORTEC-3 cohort were performed retrospectively for research purposes on whole slide sections. Abnormal p53 expression was defined as either > 90% of tumor cells with strong nuclear staining, no expression in tumor cell nuclei in the presence of appropriate internal controls (“null” pattern), or diffuse cytoplasmic expression.^{31,32} With appropriate staining of internal controls, PMS2 and MSH6 were considered lost (abnormal) in the complete absence of staining in tumor cells and were considered subclonal if > 10% of tumor cells showed loss of staining in a discrete/zonal area. In addition, ER and PR status and extent of positivity of the high-grade carcinomatous component were recorded from pathology reports. We then simplified ER and PR status as either negative or positive, using cutoffs of both $\geq 1\%$ and $\geq 10\%$ to establish positivity.^{24,33–35}

POLE Testing

Cases with available material from Stanford and UVA underwent *POLE* mutational analysis using a hot-spot SNaPshot assay performed on DNA extracted from FFPE blocks. This assay was designed to cover the most common hotspot positions in the published literature as of 2018.³⁶ The detailed description of the assay methods, variant coverage, and interpretation of pathogenicity is reported by Devereaux et al.³⁶ For 35 cases from PORTEC-3 and MST, *POLE* status was determined previously using an NGS targeted pan-cancer hotspot panel covering the complete exonuclease domain of *POLE*, with detailed methods reported by Leon-Castillo et al.⁵ and Vermij et al.²⁴ For a subset of cases (n=4) from PORTEC-3/MST with failed NGS sequencing KASPAR competitive

TABLE 1. Summary of Clinical, Histologic, and Molecular Features in the Cohort

Clinical and molecular features	Carcinosarcoma (n = 96)		DDEC/UDEC (n = 49)		GR3spEEC (n = 17)	
Molecular subtype	POLE = 4 (4.2%) MMRd = 11 (11.6%) P53abn = 63 (65.6%) NSMP = 18 (18.8%)		POLE = 7 (14.3%) MMRd = 27 (55.1%) P53abn = 8 (16.3%) NSMP = 7 (14.3%)		POLE = 1 (5.9%) MMRd = 5 (29.4%) P53abn = 6 (35.3%) NSMP = 5 (29.4%)	
Predominant epithelial component	(n=69 evaluated) Endometrioid = 27 (39.1%) Serous = 20 (29%) Ambiguous/undifferentiated = 22 (31.9%) Unknown = 27		(n=32 evaluated) Endometrioid = 18 (56.3%) Ambiguous/Undifferentiated = 13 (40.6%) Clear Cell = 1 (3.1%) Unknown = 17		(n=17 evaluated) Endometrioid = 17	
Heterologous differentiation	Total=28/71 (39%) Rhabdomyosarcomatous = 17 (60.7%) Chondrosarcomatous = 7 (25%) Osteosarcomatous = 1 (3.6%) Liposarcomatous = 1 (3.6%) Mixed = 2 (7.1%)		Total=1/35 (2.9%) Chondrosarcomatous = 1 (100%)		Total=0/17 (0%)	
FIGO stage	Stage I-II (n = 53)	Stage III-IV (n = 43)	Stage I-II (n = 27)	Stage III-IV (n = 22)	Stage I-II (n = 9)	Stage III-IV (n = 8)
Age at surgery (y), median	67 (25–85)	68.5 (47–91)	68 (35–81)	60 (28–79)	64 (43–84)	61.5 (34–78)
BMI, Median	29.3	25.4	33.6	30.3	29.9	34.6
Tumor size (cm), median	5.3 (1–17)	5.4 (1.2–17.2)	4.8 (3.3–10)	6.4 (1.1–15)	4.0 (3.5–7.2)	9.1 (3.5–14)
Lymph node involvement	0% (0/39)	72% (18/25)	0% (0/19)	55% (6/11)	0% (0/7)	75% (3/4)

DDEC/UDEC indicates Dedifferentiated/undifferentiated carcinoma; GR3spEEC, grade 3 endometrioid endometrial carcinoma with a spindled component.

allele-specific polymerase chain reactions (LGC Genomics, Berlin, Germany) was used to identify hotspot *POLE* variants in codons 286, 297, 411, 456, and 459.⁵

Molecular Classification

All cases were molecularly classified as either *POLE*mut, MMRd, p53abn, or NSMP according to the WHO 2020 diagnostic algorithm.^{25,37} Classification was determined by IHC^{38,39} and single gene *POLE* sequencing in the majority of cases (n=108), and by targeted DNA sequencing panels in a subset. NGS methods to determine TCGA subtype included TEMPUS xT⁴⁰ (n=7), FoundationOne CDx⁴¹ (n=12), Cancer Hotspot Panel v5 (n=6),^{5,24} and Cancer Hotspot Panel v.6 (n=29).^{5,24} Instances in which both IHC and NGS were performed were classified based on sequencing results. Cases that could not be assigned to any of the above molecular categories due to lack of available material or failed assays were excluded from this study.

Statistical Analysis

Progression-free survival (PFS) and disease-specific survival (DSS) by either molecular subtype or histologic diagnosis were calculated using the Kaplan-Meier method and the log-rank (Mantel-Cox) test at a cutoff of 3 years of follow-up. PFS was defined as the time from diagnosis to the time of first recurrence or death. DSS was defined as the time from diagnosis to the time of cause-specific death. Chi-square tests were used in the analysis of group differences. Kaplan-Meier plots were created using Graph-Pad Prism 10.0. (La Jolla, CA, USA) Cox’s proportional hazards models were used for survival analysis of additional clinical and histologic factors associated with DSS and PFS⁴² and identified using R (version 4.2.3) statistical software. When performing multivariate analysis, known

risk factors for progression, including tumor stage⁴² and patient age,⁴³ in addition to TCGA molecular subtype and ER expression ($\geq 10\%$) were fit to a multivariate model. *P* value of less than 0.05 was considered significant.

RESULTS

Cohort Composition

A total of 162 high-grade EC with sarcomatous/spindled or undifferentiated components were identified from Stanford University (n = 117), MST (n = 33), PORTEC-3 cohort (n = 14), and UVA (n = 6) (Fig. 1). They included cases diagnosed between 1987 and 2022. Patient ages ranged from 25 to 91 years (median, 67 y). Mean and median follow-up were 3.7 and 1.9 years, respectively (range, 1 mo to 26.3 y). The majority of patients (97 of 115 with known menopausal status) were postmenopausal at the time of diagnosis. Clinical, molecular, and histopathologic features by histotype are summarized in Table 1.

Histopathologic Features by Histotype

Cases included those diagnosed as UCS (n=96), DDEC/UDEC (n=49), and GR3spEEC with spindled growth (n = 17). When divided by histotype, there were no significant differences in age, menopausal status, BMI, histologic grade, tumor size, FIGO stage, or presence of lymph node involvement among the UCS, DDEC/UDEC, and GR3spEEC (Supplemental Digital Content 2, Table 2, <http://links.lww.com/PAS/B825>).

Heterologous differentiation was evaluated in 122 tumors and found to be present in 28 of 72 (39%) UCS and 1 of 33 (3%) DDEC/UDEC (Table 1). No tumors diagnosed as GR3spEEC demonstrated heterologous differentiation (0/17). One DDEC demonstrated focal

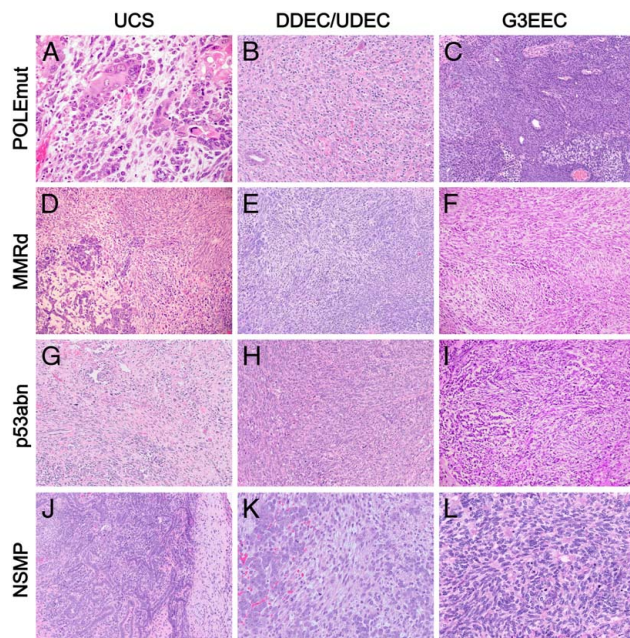


FIGURE 2. Uterine carcinosarcoma (UCS), dedifferentiated/undifferentiated carcinoma (DDEC/UDEC), and grade 3 endometrioid endometrial carcinoma (GR3spEEC) may all show spindled morphology and morphologic overlap. In our cohort, all molecular subtypes were represented in each histotype: (A–C) *POLE*-mutated (*POLE*mut), (D–F) mismatch repair deficient (MMRd), (G–I) p53 abnormal (p53abn), (J–L) and no specific molecular profile (NSMP).

chondrosarcomatous differentiation. This tumor demonstrated loss of MSH6 throughout and loss of BRG1 in the undifferentiated component. Heterologous differentiation was usually associated with serous (12/28, 43%) or ambiguous/undifferentiated (10/28, 36%) carcinoma morphology, although it was also seen in association with high-grade endometrioid morphology (6/28, 21%).

Immunohistochemical features by histotype are summarized in Table 2. Using $\geq 10\%$ staining as a cutoff, 70 of 141 (50%) of tumors at least focally expressed ER. Within the carcinomatous components, ER was expressed in 12 of 16 (75%) GR3spEEC, 39 of 80 (49%) UCS, and 19 of 45 (42%) DDEC/UDEC. Using a cutoff of $\geq 1\%$ expression, 23 additional tumors were considered positive for ER: 13 of 16 (81%) GR3spEEC, 58 of 80 (73%) UCS, and 22 of 45 (49%) DDEC/UDEC. Using a cutoff of $\geq 10\%$, 66 of 142 (46%) cases were positive for PR, including 10 of 16 (63%) GR3spEEC, 37 of 80 (46%) UCS, and 19 of 46 (43%) DDEC/UDEC. Using a cutoff of $\geq 1\%$ expression, 17 additional tumors were considered positive for PR (12/16 [75%] GR3spEEC, 48/80 [60%] UCS, and 23/46 [50%] DDEC/UDEC).

Histopathologic Features by Molecular Classification

The 162 EC were classified into one of the 4 molecular subgroups: 12 (7%) *POLE*mut, 43 (27%) MMRd,

77 (48%) p53abn, and 30 (19%) NSMP. Histopathologic features by molecular subtype are summarized in Table 3. All molecular groups were represented in all histotypes included (Table 1, Fig. 2). There were no significant clinicopathologic differences in patient age, BMI, menopausal status, FIGO stage, tumor size, or lymph node involvement among the molecular groups (Supplemental Digital Content 3, Table 3, <http://links.lww.com/PAS/B826>). Although there were no statistically significant differences in FIGO stage and TCGA group, some trends by histotype were noted: 49% of p53abn cases were diagnosed as stage 3 or higher, compared with only 30% of MMRd cases diagnosed as stage 3 or higher. Detailed staging information by histotype and TCGA group is presented in Supplemental Digital Content 4, Table 4 (<http://links.lww.com/PAS/B827>).

Twelve *POLE*mut tumors were identified in our cohort: 7 DDEC/UDEC, 4 UCS, and 1 GR3spEEC (Fig. 2A–C). The majority of *POLE*mut tumors showed wild-type expression of p53 ($n = 10/12$, 83%), and 2 cases demonstrated abnormal expression of p53 (17%), including a UCS with a serous carcinomatous component. Two *POLE*mut cases (17%) had a loss of expression of at least 1 MMR protein. A complete list of the morphologic and immunohistochemical features and specific *POLE* mutations identified in our cohort are presented in Supplemental Digital Content 5, Table 5 (<http://links.lww.com/PAS/B828>).

Forty-three (27%) MMRd tumors were identified: 11 UCS, 27 DDEC/UDEC, and 5 GR3spEEC (Fig. 2D–F). Within the MMRd group, 5 of 11 (45%) MMRd UCS (Fig. 3) and 4 of 7 (15%) MMRd DDEC/UDEC also demonstrated abnormal expression of p53 by IHC.

Seventy-seven (48%) tumors were molecularly classified as p53abn: 63 UCS, 8 DDEC/UDEC, and 6 GR3spEEC (Fig. 2G–I). Seventy-six were identified or confirmed by p53 IHC, and 1 was identified by sequencing only.

The remaining 30 (19%) tumors were classified as NSMP, including 18 UCS, 7 DDEC/UDEC, and 5 GR3spEEC (Fig. 2J–L). One was classified based on sequencing alone, and the rest were identified or confirmed by IHC demonstrating intact MMR protein expression and wild-type p53 staining in the absence of *POLE* mutation. Using a $\geq 10\%$ cutoff, 18/27 (67%) NSMP tumors expressed ER and 18/27 (67%) expressed PR in the carcinomatous component.

Prognostic Stratification

When plotted by histotype, there were no significant differences in DSS ($P = 0.58$) or PFS ($P = 0.96$) at 3 years (Fig. 4A, B). Three-year survival rates were 58% for UCS, 67% for DDEC/UDEC, and 76% for GR3spEEC. There were no significant differences in DSS or PFS by histologic diagnosis by institution.

When plotted by molecular subtype, there were significant differences in both DSS ($P = 0.001$) and PFS ($P \leq 0.0001$) across the entire cohort at 3 years (Fig. 4C–D). When divided by histotype, stratification by molecular

TABLE 2. Immunohistochemical Features by Histotype

IHC	Carcinosarcoma		DDEC/UDEC		GR3pEEC	Total
Lost or subclonal PMS2 and/ or MSH6	12/88 (14%)	DDEC 13/22 (59%)	UDEC 4/9 (44%) 28/49 (56%)	DDEC/UDEC 11/18 (67%)	5/16 (31%)	45/153 (29%)
Abnormal p53 expression*	66/90 (73%)	DDEC 3/22 (14%)	UDEC 6/9 (67%) 13/49 (27%)	DDEC/UDEC 4/18 (22%)	6/13 (46%)	85/152 (56%)
ER positive† cutoff $\geq 10\%$	39/80 (49%)	DDEC 14/21 (67%)	UDEC 0/8 (0%) 19/45 (42%)	DDEC/UDEC 5/16 (32%)	12/16 (75%)	70/141 (50%)
cutoff $\geq 1\%$	58/80 (73%)	DDEC 16/21 (76%)	UDEC 1/8 (13%) 22/45 (49%)	DDEC/UDEC 5/16 (31%)	13/16 (81%)	93/141 (67%)
PR positive† cutoff $\geq 10\%$	37/80 (46%)	DDEC 12/21 (57%)	UDEC 1/8 (13%) 19/46 (43%)	DDEC/UDEC 6/17 (35%)	10/16 (63%)	66/142 (46%)
cutoff $\geq 1\%$	48/80 (60%)	DDEC 13/21 (62%)	UDEC 3/8 (38%) 23/46 (50%)	DDEC/UDEC 7/17 (41%)	12/16 (75%)	83/142 (58%)

*Diffuse or subclonal overexpression, total loss ("null" pattern), or cytoplasmic expression of p53.

†Carcinomatous components in heterologous tumors.

DDEC/UDEC indicates dedifferentiated /undifferentiated carcinoma; GR3pEEC, grade 3 endometrioid endometrial carcinoma with a spindled component.

classification also showed significant differences in DSS (DDEC/UDEC) and PFS (UCS and DDEC/UDEC) (Fig. 5). There were no differences in DSS or PFS when *POLE*mut, MMRd and NSMP tumors were separated by histologic diagnosis into UCS, DDEC/UDEC, and G3spEEC. However, p53abn tumors showed a significant difference in PFS at 3 years ($P=0.005$) when separated by histologic diagnosis, with p53abn DDEC/UDEC and G3spEEC showing worse PFS than p53abn UCS (Fig. 6). There was no difference in DSS in the p53abn group.

Within the UCS group, there were no significant differences in DSS ($P=0.37$) or PFS ($P=0.40$) between tumors with and without heterologous differentiation. *POLE*mut tumors were associated with an excellent prognosis (100% survival at 3 yr), with no cases having disease recurrence or death from disease. Outcomes were poor in both p53abn and NSMP tumors, with 3-year survival rates of 50% and 48%, respectively. There were no differences in DSS or PFS between these 2 groups. MMRd tumors were associated with a better prognosis than p53 and NSMP tumors in both DSS and PFS, with a 3-year survival rate of 83%.

The results of univariate and multivariate analyses across the entire cohort are summarized in Table 4. In multivariable analysis, FIGO stage remained the most significant independent clinical risk factor for progression and death from disease across the entire cohort ($P\leq 0.001$). In addition, there were no significant differences in outcomes based on ER expression by IHC (using cutoffs of $\geq 10\%$ expression), including when stratified by molecular subtype. In the UCS cohort, older age and higher stage were both independent clinical risk factors for death from disease. Refer to Supplemental Digital Content 6, Tables 6, <http://links.lww.com/PAS/B829> and Supplemental Digital Content 7, Tables 7, <http://links.lww.com/PAS/B830> for detailed univariate and multivariate analysis of PFS and DSS by molecular subtype.

Eleven of 19 (58%) patients with rhabdomyosarcomatous differentiation died of disease, whereas only 1 patient (11%) with chondrosarcomatous differentiation died of disease. The patient with osteosarcomatous differentiation died of disease at 10 months, and the patient with liposarcomatous differentiation experienced a recurrence at 2.7 years and was alive with no evidence of disease at last follow-up.

DISCUSSION

In this study, we evaluated the prognostic relevance of molecular classification compared to histologic classification among a large cohort of diagnostically challenging high-grade ECs: those with spindled, sarcomatous or undifferentiated components. Importantly, our study reveals that the molecular classification, compared to histologic classification, has greater prognostic significance in both disease recurrence and death from disease in UCS and DDEC/UDEC. Moreover, we found that there is no significant difference in DSS or PFS between UCS, DDEC/UDEC, and G3spEEC based on histologic diagnosis alone. The lack of outcome differences by histotype is not entirely surprising given the known interobserver variability in classification, as well as the often aggressive nature of these tumors, however when UCS and DDEC/UDEC are stratified by molecular subtype alone, the prognostic differences are pronounced.

Similar to that reported in TCGA and subsequent studies,^{2,44–46} *POLE*mut tumors in our study demonstrated an excellent prognosis across all histotypes, despite

TABLE 3. Histopathologic Features by Molecular Subtype				
	<i>POLE</i> mut (n = 12)	MMRd (n = 43)	p53abn (n = 77)	NSMP (n = 30)
Histotype	UCS = 4 (33%) DDEC/UDEC = 7 (58%) GR3spEEC = 1 (8%) (n=6 evaluated)	UCS = 11 (26%) DDEC/UDEC = 27 (63%) GR3spEEC = 5 (12%) (n=10 evaluated)	UCS = 63 (82%) DDEC/UDEC = 8 (10%) GR3spEEC = 6 (8%) (n=57 evaluated)	UCS = 18 (60%) DDEC/UDEC = 7 (23%) GR3spEEC = 5 (17%) (n=23 evaluated)
Predominant epithelial component	Endometrioid = 3 (50%) Serous = 1 (17%) Ambiguous/undifferentiated = 2 (33%)	Endometrioid = 8 (80%) Serous = 0 (0%) Ambiguous/undifferentiated = 2 (20%)	Endometrioid = 17 (30%) Serous = 16 (28%) Ambiguous/undifferentiated = 23 (40%) Clear cell = 1 (2%)	Endometrioid = 18 (78%) Serous = 2 (9%) Ambiguous/undifferentiated = 3 (13%) Total = 3/26 (12%)
Heterologous differentiation	Total = 0 (0%)	Chondrosarcomatous = 2 (100%)	Rhabdomyosarcomatous = 16 (67%) Chondrosarcomatous = 4 (17%) Osteosarcomatous = 1 (4%) Liposarcomatous = 1 (4%) Mixed = 2 (8%)	Rhabdomyosarcomatous = 1 (33%) Chondrosarcomatous = 2 (66%)

DDEC/UDEC indicates dedifferentiated/undifferentiated carcinoma; GR3spEEC, grade 3 endometrioid endometrial carcinoma with a spindled component; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal; *POLE*mut, *POLE*-mutated; UCS, uterine carcinosarcoma.

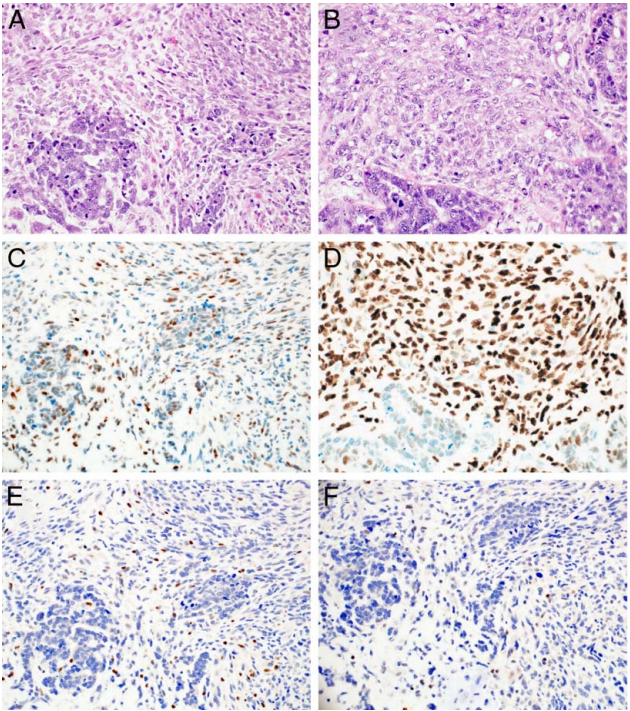


FIGURE 3. (A and B, H&E) Example of a mismatch repair deficient uterine carcinosarcoma. (C) p53 demonstrated both wild-type patterns and (D) areas of abnormal overexpression. Both (E) PMS2 and (F) MSH6 showed complete loss of expression throughout the tumor.

known unfavorable histopathologic features. MMRd was associated with intermediate outcomes, whereas the NSMP and p53abn groups were associated with poorest clinical outcomes. Studies assessing the molecular classification in UCS have demonstrated similar findings with the identification of poor prognosis in the NSMP group, which was found to be worse than p53abn endometrioid and serous carcinomas.^{16–18,47–49} This is concordant with prior literature identifying the wide variability in prognosis in NSMP EC, from good to intermediate in low-grade EC to poor in non-endometrioid EC, and emphasizing the importance of histotyping and grading within this molecular subgroup.^{2,50,51} Recently, ER status has also demonstrated prognostic refinement in high-risk NSMP ECs.²⁴ Additional evaluation of the sarcomatous and undifferentiated components in these tumors may help clarify the prognostic features of the NSMP subgroup.

The 12 *POLE*mut tumors in our cohort occurred in all histologic subtypes but were most commonly in the DDEC/UDEC group (n = 7). *POLE*mut UCS were rare in our cohort (n = 4, 4.1%), but confirm that they do occur, as has been reported previously.^{18,47} Despite high FIGO stage in about half of the cases, there were no recurrences or death from disease. Most *POLE*mut tumors were associated with ambiguous/undifferentiated or endometrioid morphology, although a single *POLE*mut UCS appeared to be composed of a serous carcinomatous component.

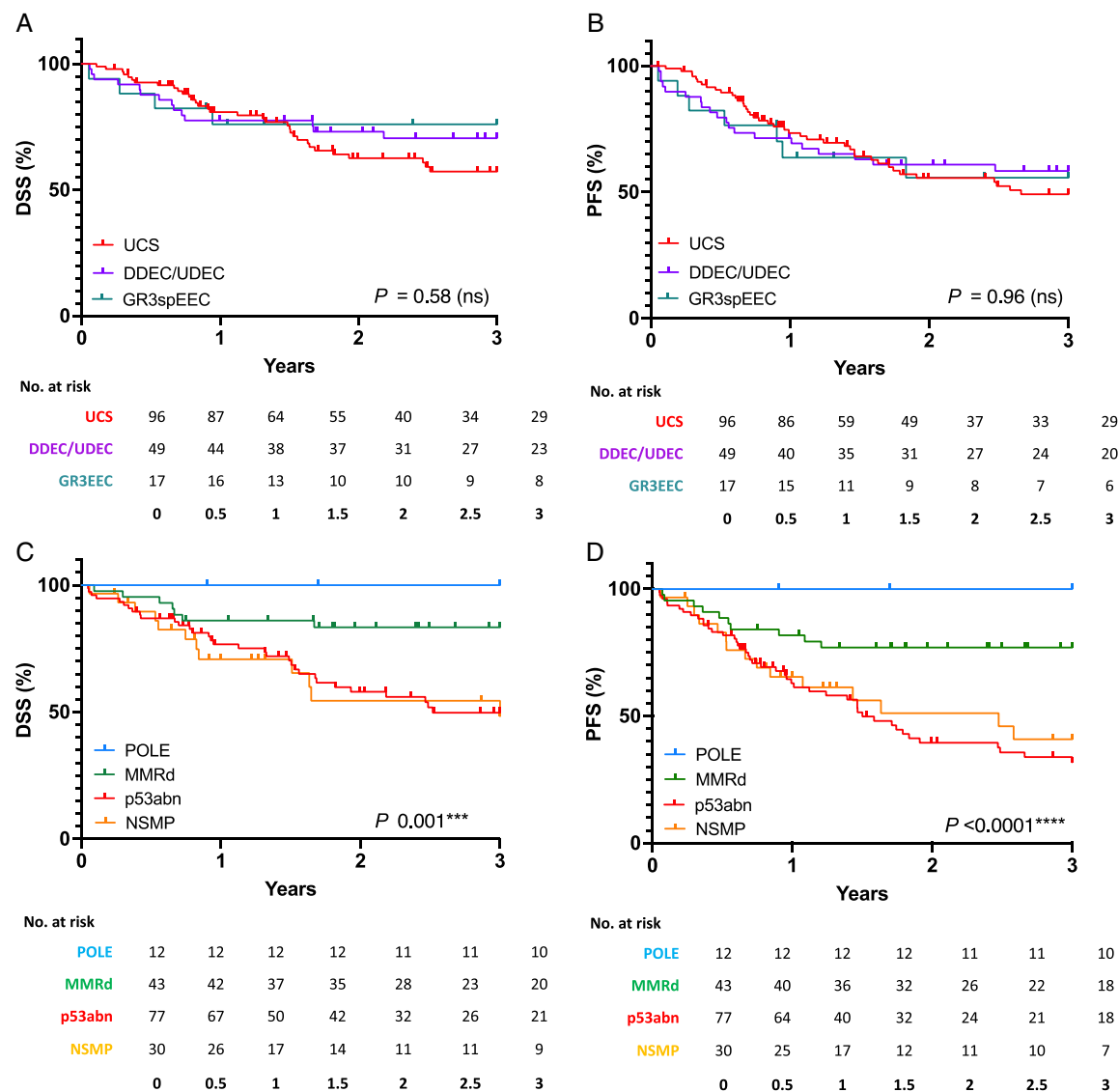


FIGURE 4. Disease-specific survival (DSS) and progression-free survival (PFS) by (A-B) histotype (uterine carcinosarcoma (UCS), dedifferentiated/undifferentiated carcinoma (DDEC/UDEC), and grade 3 endometrioid endometrial carcinoma (GR3spEEC) and (C-D) molecular classification (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and no specific molecular profile (NSMP).

Loss of expression of at least 1 MMR protein occurred in 20% of assessed *POLE*mut tumors. Notably, 2 *POLE*mut tumors (1 UCS and 1 UDEC) demonstrated abnormal expression of p53 by IHC, emphasizing a potential pitfall for inaccurate classification as p53abn without concurrent molecular testing for *POLE* status. Neither of the *POLE*mut-p53abn double classifier tumors demonstrated disease progression or death from disease, and although numbers are small and based on retrospective data, our study findings would support the 2021 European Society of Gynaecological Oncology/European Society for Therapeutic Radiotherapy and Oncology/European Society of Pathology (ESGO/ESTRO/ESP) guidance suggesting that *POLE*mut EC, regardless of histotype/grade, should be considered low risk.⁵² Our study demonstrated some

histologic clues for *POLE*mut status that have been reported previously, including ambiguous histomorphology, loss or subclonal expression of expression of MMR proteins, and subclonal p53 expression.⁵³ However, larger studies of *POLE*mut UCS, DDEC, and UDEC are needed to draw conclusions. Given the overall lack of specific histopathologic diagnostic features but consistently excellent prognosis, *POLE* testing should be performed on all EC, or at the minimum, and possibly most importantly in histologic types such as UCS, DDEC, and UDEC in which the result may alter adjuvant treatment recommendations.

Although all molecular subgroups were identified in all histologic subtypes, some associations between histotype and molecular classification could be observed

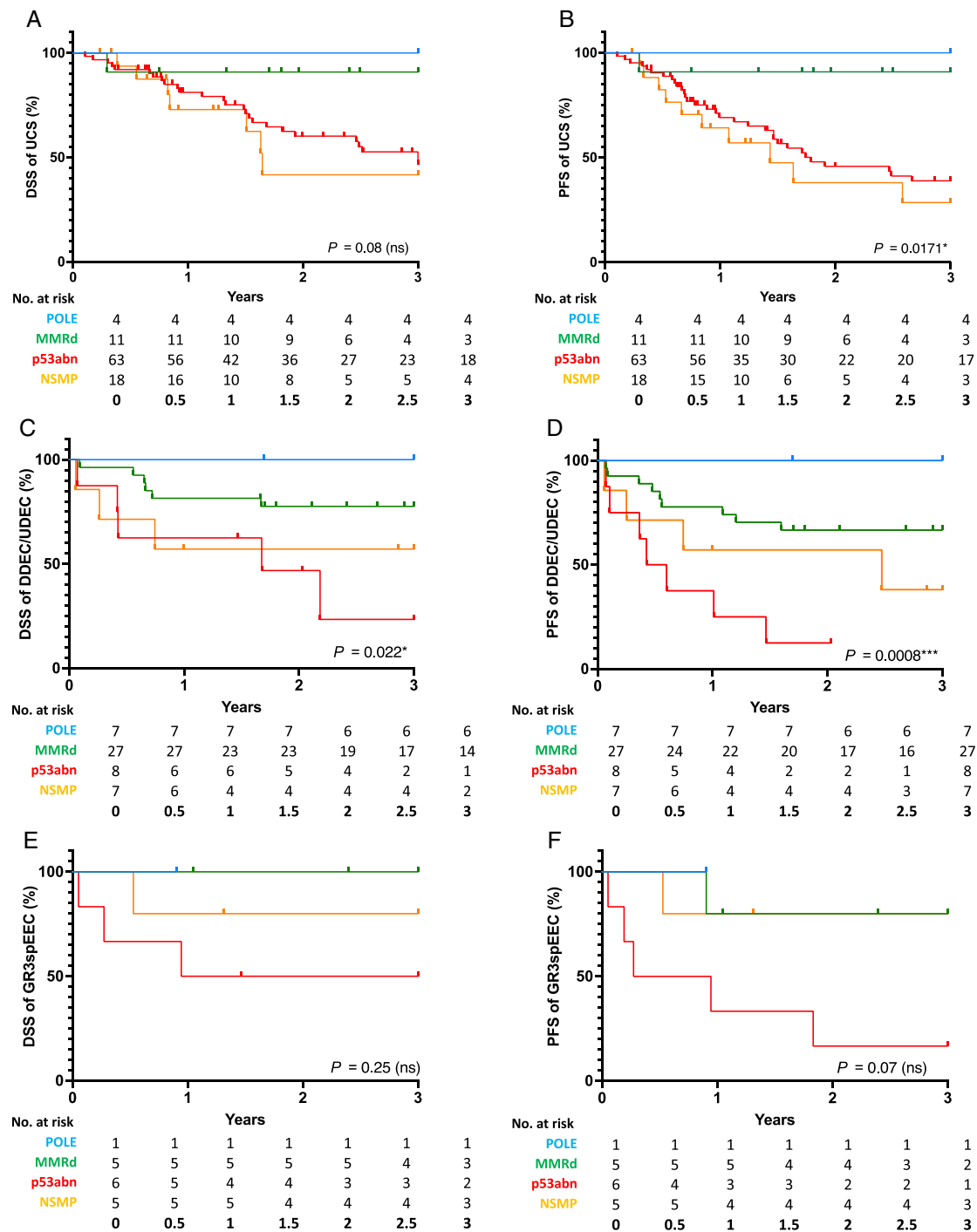


FIGURE 5. Disease-specific survival (DSS) and progression-free survival (PFS) by molecular classification when divided by histologic diagnosis as (A, B) uterine carcinosarcoma (UCS), (C, D) dedifferentiated/undifferentiated carcinoma (DDEC/UDEC), and (E, F) grade 3 endometrioid endometrial carcinoma with a spindled component (GR3spEEC).

(Table 1). Our study supports previous literature showing that hypermutated/ultramutated tumors constitute the majority of DDEC/UDEC.^{21,22,46,54} In a meta-analysis of 73 DDEC/UDEC carcinomas, the pooled prevalence of each molecular subgroup was: 12.4% *POLE*mut, 44% MMRd, 18.6% p53abn, and 25% NSMP.²¹ In

comparison, our DDEC/UDEC cohort demonstrated a higher prevalence of MMRd (55%) and *POLE*mut (14%) tumors, with a lower proportion of p53 (16%) and NSMP (14%) tumors. Although not a distinct entity, our GR3spEEC cohort with spindled features showed molecular breakdown similar to GR3spEEC reported by

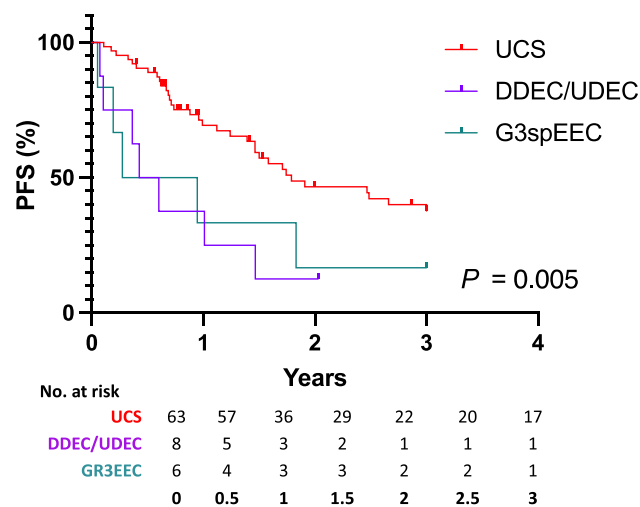


FIGURE 6. Progression-free survival (PFS) of p53abn tumors stratified by histotype.

Bosse et al,⁶ which included 12.9% *POLE*mut, 36.2% MMRd, 20.7% p53abn, and 30.2% NSMP. Our findings are concordant with prior studies^{18,47} suggesting that most, but not all, UCS are p53abn. Sixty-three percent of UCS were p53abn, although our study underrepresents the true incidence of p53 abnormalities in UCS due to our study design with enrichment for UCS with endometrioid morphology and p53 wild-type status. As expected, serous morphology in the carcinomatous component was highly associated with the p53abn group.² Recent literature has suggested that UCS are almost exclusively p53abn molecular subtype after mimics, such as CHEC pattern EEC, G3spEEC, and DDEC/UDEC, are excluded.⁵⁵ Although we agree that the finding of any other molecular subtype in UCS warrants careful pathology review to exclude mimics, unequivocal cases of *POLE*mut, MMRd, and NSMP UCS were identified. This

includes a *POLE*mut UCS with serous morphology, heterologous differentiation, and abnormal p53 expression, 5 MMRd UCS with abnormal p53 expression, and 2 NSMP UCS with serous morphology. Given the marked differences in prognosis, the identification of non-p53abn UCS is of great clinical significance, and the identification of aberrant p53 IHC expression in the absence of MMRd and *POLE* testing is insufficient for classification as p53abn. By enriching for UCS with endometrioid morphology, we identified a group of MMRd UCS with unique morphologic, molecular, and prognostic features, including 3 cases previously reported by Jenkins et al.²⁰ MMRd UCS generally represents <5% of UCS,^{18,20,47} although rates up to 41%⁵⁶ and 44%⁴⁸ have been reported. Even in our enriched cohort, MMRd tumors represented only 13% of UCS. Within our MMRd UCS cohort, p53 expression was wild-type or subclonal except a single MMRd UCS. Although endometrioid and ambiguous morphology, lack of heterologous differentiation, and lack of *TP53* mutations have been previously demonstrated in MMRd UCS,^{20,57} prognostic differences have been unclear.^{20,57} In our cohort, only the MMRd with diffuse abnormal p53 expression demonstrated disease progression, whereas none of the 9 MMRd, p53 wild-type or subclonal abnormal p53 UCS showed disease progression. Dual classified MMRd-p53abn ECs are generally thought to have the same prognosis as MMRd-p53 wild-type EC,⁵⁸ and this one case in isolation is insufficient to make broad claims on MMRd-p53abn UCS. However, MMRd UCS may represent a biologically unique group of UCS with a much better prognosis than conventional UCS and warrant further study. Although uncommon, the findings provide further support for full molecular classification for all EC, including UCS. Endometrioid carcinoma is known for its diverse morphology and may pose a diagnostic dilemma when it contains a spindle component. Although not a distinct

TABLE 4. Univariate and Multivariate Analysis of Risk Factors for Disease Progression (PFS) and Death from Disease (DOD) Across Entire Cohort

	Univariate analysis				Multivariate analysis*			
	PFS HR (CI)	P	DOD HR (CI)	P	PFS HR (CI)	P	DOD HR (CI)	P
Age	1.01 (0.99–1.03)	0.366	1.03 (1.01–1.06)	0.022	1.01 (0.99–1.03)	0.489	1.03 (1.00–1.06)	0.051
ER positive	1.02 (0.62–1.66)	0.954	0.74 (0.40–1.36)	0.333	1.07 (0.64–1.79)	0.8	0.76 (0.41–1.43)	0.4
FIGO Stage								
I	Reference		Reference		Reference		Reference	
II	2.81 (1.36–5.85)	0.006	2.68 (0.99–7.25)	0.052	3.21 (1.36–7.60)	0.008	3.44 (1.01–11.64)	0.047
III	2.47 (1.40–4.34)	0.002	4.05 (1.99–8.25)	<0.001	3.20 (1.71–6.00)	<0.001	5.46 (2.44–12.20)	<0.001
IV	4.34 (2.15–8.77)	<0.001	6.48 (2.79–15.03)	<0.001	4.56 (2.07–10.05)	<0.001	6.88 (2.62–18.07)	<0.001
Molecular Subtype								
NSMP	Reference		Reference		Reference		Reference	
p53abn	1.11 (0.64–1.93)	0.717	1.04 (0.54–2.01)	0.905	1.39 (0.73–2.66)	0.314	0.98 (0.47–2.03)	0.956
MMRd	0.38 (0.18–0.80)	0.011	0.30 (0.12–0.77)	0.012	0.52 (0.23–1.19)	0.122	0.38 (0.13–1.08)	0.068
POLEmut	0†	N/A	0†	N/A	0†	N/A	0†	N/A

*Multivariate analysis on TCG, adjusted by age, FIGO stage (based on 2009 staging system), and ER status.
†HR not estimable due to no events in the POLE group.
CI indicates confidence interval; HR, Hazard Ratio; MMRd, Mismatch repair deficient; NSMP, No specific molecular profile; p53abn, p53 abnormal; POLEmut, POLE-mutated.

diagnostic entity, spindle cell morphology is a recognized histologic pattern of EEC and an important consideration in the differential diagnosis of spindled endometrial lesions. Therefore, we included a group of G3spEEC with spindled morphology that did not meet the criteria for high-grade mesenchymal transformation to warrant classification as UCS. G3spEEC was an uncommon classification, with only 17 cases identified on review, precluding the ability to perform multivariate analysis. There were no survival differences in UCS and G3spEEC by histopathologic diagnosis, highlighting the aggressive nature of G3spEEC. Although there were similar trends toward differences in PFS at 3 years based on the TCGA molecular subgroup, with more recurrences in the p53abn group, the results did not reach statistical significance ($P=0.07$), possibly due to underpowering. However, we noted that 1 p53abn G3spEEC recurred at 5.6 years, and additional studies with long-term follow-up and larger sample size may identify the prognostic differences.

The multicenter nature of this study leads to some limitations in uniformity in diagnostic approaches and methods based on different institutional practices. For example, material for full histologic assessment and IHC review was not available in all cases. In addition, we acknowledge that this distinction between UCS, DDEC/UDEC, and G3spEEC shows significant morphologic and IHC overlap and analysis of interobserver agreement between authors was not performed. Because G3spEEC is not a distinct entity, the classification of this group of tumors may be particularly subjective and challenging to identify. However, our study provides insight into the importance of molecular classification over morphologic and histologic nuances. Because of enrichment for endometrioid UCS, there is some bias introduced in molecular class representation and morphologic features within this cohort, and this study should not be used to make larger generalizations about the prevalence of molecular subgroups or carcinomatous morphology within UCS.

In conclusion, although there were no differences in outcomes among UCS and DDEC/UDEC when stratified by histologic classification, we found striking prognostic differences when stratified by molecular group. These findings reinforce the prognostic power of molecular classification in endometrial carcinoma in previously under-investigated tumor types and highlight the heterogeneity of clinical behavior across “aggressive” endometrial carcinoma histotypes that can be largely accounted for with molecular characterization. Our study therefore supports full molecular classification for all high-grade EC with undifferentiated and sarcomatous components, with emphasis on molecular group, rather than histologic diagnosis, in guiding treatment decisions.

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