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Comprehensive molecular characterization of early stage grade 3 endometrioid endometrial adenocarcinoma

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HIGHLIGHTS

- Treatment of stage IB grade 3 endometrioid endometrial adenocarcinoma has varying practice patterns and outcomes.
- Refining the classification of endometrial cancer can provide more precise patient stratification and treatment approaches.
- This cohort was molecularly diverse, despite the same histology, stage and grade.
- Despite this, a mutational signature, SBS5, was noted to be correlated with a high risk of recurrence.

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ABSTRACT

Objective. The treatment for stage IB grade 3 endometrioid endometrial adenocarcinoma is challenging with variable practice. Molecular characterization may help identify adjuvant therapy strategies beyond stage. We aimed to better understand the molecular features of these tumors by characterizing them by ProMisE classification, mutational signature, and commonly mutated genes.

Methods. Patients with stage IB grade 3 EEC at two institutions were included. Immunohistochemistry and whole exome sequencing were performed on archival FFPE tissue sections to determine ProMisE classification. Personal Cancer Genome Reporter was used for somatic variant annotation, and mutational signatures were generated based on COSMIC single base substitution mutational signatures.

Results. 46 patients were included with variable adjuvant treatment. Nine patients recurred (19.6%), most with extra-abdominal disease ($n = 5$, or 55.6%). 10 had POLE mutations (21.7%), 18 were MMR deficient (39.1%), 6 had abnormal p53 (13.0%), and 12 were p53 wildtype (26.1%). There were no recurrences in the POLE subgroup.
A dominant mutational signature was identified in 38 patients: 17 SBS5 signature (44.7%), 10 SBS15 or SBS44 signature (26.3%), 7 SBS10a or SBS10b signature (18.4%), 3 SBS14 signature (7.9%), and 1 SBS40 signature (2.6%). The six patients that recurred had a SBS5 signature.
Frequently mutated genes included ARID1A ($n = 30$, 65%), PTEN ($n = 28$, 61%), MUC16 ($n = 27$, 59%), and PIK3CA ($n = 25$, 54%).

Conclusions. This comprehensive evaluation found a molecularly diverse cohort of tumors, despite the same histology, stage and grade. Mutational signature SBS5 correlated with a high risk of recurrence. Further refining of endometrial cancer classification may enable more precise patient stratification and personalized treatment approaches.

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1. Introduction

Endometrial cancer is the most common gynecologic malignancy in the developed world; yet it is managed with significant variability. Specifically, the treatment of stage IB grade 3 endometrioid endometrial adenocarcinoma (EEC) is challenging because of varying practice patterns and outcomes [1–3]. Although patients with deeply invasive grade 3 EEC have a higher risk for extra-pelvic recurrences [3], the role of adjuvant therapy remains controversial with strategies ranging from observation to chemotherapy with external beam radiation [3]. Because of the heterogenous nature of this disease, classifying endometrial malignancies by histology alone is insufficient for risk stratification and treatment. The use of molecular characteristics has recently been incorporated into FIGO2023 staging [4]. Further clarity of the influence of these molecular characteristics on prognosis can guide appropriate surgical, radiation and systemic therapies.

The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) is a classification system used to identify four prognostic groups of endometrial cancer based on immunohistochemistry (IHC) and mutation analysis [5,6]. These subgroups are: polymerase epsilon exonuclease domain mutated (POLE EDM), mismatch repair deficient (MMRd), p53-mutated (p53 abn) and p53 wildtype (p53 wt) subgroups. They each have distinct clinical and pathologic characteristics, reflecting the diverse molecular landscape and pathogenesis of endometrial cancer that transcend histology, stage, and grade. For example, POLE EDM is characterized by somatic mutations in the polymerase epsilon DNA polymerase (POLE) exonuclease domain and high mutation rate (232 × 10–6 mutations per Mb) [7–9]. This group has an excellent prognosis with low risk of recurrence, irrespective of tumor grade [7–9]. On the other hand, p53 abn group has a poor prognosis with low mutational burden and high TP53 mutation rate. In this way, molecular classification may better stratify patients for appropriate adjuvant treatment and prevent significant under- or overtreatment [10].

To better understand the development of endometrial cancers, whole exome sequencing has revealed numerous genomic alterations associated with pathogenesis. For instance, endometrioid carcinomas are often characterized by alterations in the PI3K-PTEN-AKT-mTOR, RAS-MEK-ERK, and canonical WNT-β-catenin pathways. MMR deficiency leading to genomic instability is also commonly associated with endometrioid adenocarcinomas with genetic and epigenetic alterations. The landscape of such genomic alterations provides a deeper understanding of the role of driver mutations, pathogenesis of endometrial malignancies and actionable mutations to target.

Genome-wide analyses also shed light into patterns of mutations termed mutational signatures. Mutational processes often involve an inciting component such as DNA damage, environmental or endogenous mutagen exposures that impact the development of human cancers [11]. Single-base substitution (SBS) signatures are defined by characteristic patterns of single nucleotide base changes. Thus far, 96 SBS signatures have been identified [11]. Other mutational signatures may be based on doublet base substitutions (DBS), small insertion and deletions (ID), or copy number variations (CN). These signatures are molecular footprints that may delineate molecular pathways implicated in cancer development and ultimately help guide treatment. Additionally, mutational signatures have associated prognostic significance in some cancers including colorectal and multiple myeloma [12,13].

Only 11 SBS signatures are relevant for uterine cancers within the COSMIC SBS mutational signatures. These can be associated with a proposed etiology, but many are unknown. SBS5 and SBS14 signatures are associated with aging. SBS10a and SBS10b signatures are associated with POLE mutations, generating a large number of somatic mutations and termed hypermutators. SBS15 and SBS44 signatures are associated with microsatellite instability. SBS14 signature is associated with concurrent POLE mutation and defective DNA mismatch repair. Many signatures may be associated with one another and can be commonly found in the same samples. These signatures are understudied in endometrial malignancies, and investigation of their clinical significance within gynecologic cancers is warranted.

With the increasing advances in genomic and molecular medicine, our group sought to better characterize a poorly stratified cohort of uterine cancers to improve future prognostic indicators and treatment regimens for patients. This study aimed to describe stage IB grade 3 EEC by molecular characteristics, including use of ProMisE classification, mutational signatures, and review of common mutations amongst endometrial carcinomas.

2. Methods

2.1. Patients and tissue selection

Institutional Review Board approval was obtained at two respective institutions: Oslo University Hospital (OUH) and University of Texas MD Anderson Cancer Center (MDACC). All patients surgically staged between January 2005 and December of 2017 and diagnosed with 2009 Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) grade 3 stage 1B EEC were identified. Only patients with available archival tissue were included. For these specimens, independent pathology review was performed, and histologic diagnosis confirmed.

Demographic, clinical, and pathologic data including age at time of surgery, body mass index (BMI), race, type of surgery completed, depth of myometrial invasion and presence of lymphovascular space invasion were retrospectively collected for each patient. Adjuvant treatment, recurrence, mortality, and subsequent follow-up were also determined for each patient. Patient identity was protected.

Archival formalin fixed paraffin embedded (FFPE) tissue sections were obtained from the primary tumor of each patient, and for some, normal tissue also obtained. Further molecular testing and exome sequencing was performed at each respective institution with similar approaches. See Supplemental Methodology for details of the different approaches.

2.2. Whole exome sequencing and variant calling

DNA was extracted from two to five 10-20 μm FFPE-sections per tumor and/or normal tissue FFPE sections from the same patient using the Qiagen AllPrep DNA/RNA FFPE protocol (see Supplementary Methods). Whole exome sequencing was performed at each respective institution using the Twist Library Preparation Enzymatic fragmentation Kit 2.0 and Human Comprehensive Exome panel for OUH samples, and the Twist Universal Adapter System and Twist Human Exome Core Kit for MDACC samples. Libraries were sequenced paired-end (2 × 150 bp) on the NovaSeq6000 system (Illumina).

Variant calling was performed by the Bioinformatics Core Facility at OUH, with Illumina Dragen Bio-IT v. 3.7 for the OUH data and v. 3.9 for the MDACC data. Sequences were mapped and aligned to the human reference genome GRCh38 (patch p12).

2.3. Variant annotation and filtration

The open-source software package Personal Cancer Genome Reporter (PCGR) [14] was used for somatic variant annotation. For each individual sample, a report was generated that summarized the detected single nucleotide variant (SNV) and insertion/deletion polymorphism (Indels), along with computed tumor mutational burden (TMB), microsatellite instability (MSI) status and mutational signatures. Variants relevant for endometrial cancer based on mutations noted from Bianco et al. were extracted [15] (Supplemental Table 1), non-coding mutations removed and filtered for read depth < 30 and allele frequency < 0.05. In the OUH tumor-only data, potential germ line

mutations with allele frequencies close to 0.5 or 1 were removed manually.

A ranked list of each tumor's correlation to COSMIC SBS mutational signatures was generated by the PCGR workflow when the total number of SNVs in the tumor exceeded 200. Only the 11 SBS signatures relevant for uterine cancers were included to generate the estimates. For the OUH tumor-only data, variants in the Single Polymorphism Database (dbSNP) were removed prior to the comparison to SBS signatures (Supplementary Table 2, Supplementary Methods).

2.4. Immunohistochemistry and scoring

IHC staining was performed for MMR proteins and p53. Unstained FFPE tumor tissue sections from OUH were stained with Dako Flex+ protocol for: MLH (Mouse Clone G168–728, (1:300); Cell Marque, Sigma-Aldrich, Rocklin, CA), MSH2 (Mouse Clone FE11, (1:100); Calbiochem, San Diego, CA), MSH6 (Dako, rabbit clone EP49, (1:50); Dako, Carpinteria, CA), PMS2 (rabbit clone EP51, (1:40), Dako, Carpinteria, Ca), and p53 (Mouse clone DO-1, (1:500), Santa Cruz Biotechnology, Dalla, TX). At MDACC, unstained FFPE tumor tissue sections were stained for MLH1 (Mouse Clone G168–728, (1:300); Cell Marque, Sigma-Aldrich, Rocklin, CA), MSH2 (Mouse Clone FE11, (1:100); Calbiochem, San Diego, CA), MSH6 (Mouse Clone 44, (1:300); BD Biosciences, San Jose, CA), PMS2 (Mouse Clone A16–4, (1:125); BD Biosciences, San Jose, CA) and p53 (Mouse Clone DO-7, (RTU: 1:1); Leica Microsystems, Wetzlar, Germany).

A deficiency of a MMR marker by IHC was defined as loss of nuclear expression of the immunohistochemical stain, with intact expression of adjacent stromal cells. Rare cases of sub-clonal loss can be observed, which was defined as loss of expression in at least 10% of the tumor cells in a convincing and distinct tumor subpopulation, with intact expression of adjacent stromal cells. Aberrant p53 expression was defined as overexpression (>75% of tumor) with strong intensity or when completely negative (null phenotype), with wild-type expression of background stromal cells as internal control.

2.5. ProMisE classification

Molecular classification using ProMisE was performed for our cohort of patients [5]. First, presence of POLE mutations was identified by whole exome sequencing. Only pathologic POLE variants were included as previously described by Leon-Castillo et al. [16] Those with one of these 11 pathologic mutations was grouped into the POLE mutation subgroup. Loss of MMR expression and aberrant p53 expression, described as p53 abnormal, were determined by IHC staining. Multiple-classifier carcinomas, which harbor more than one molecular classifying feature, were segregated first by the presence of a pathogenic POLE mutation, then by MMR status if POLE was not mutated, followed by the p53 subtype [5,17] (Fig. 1).

2.6. Statistical analysis

Summary statistics were used to describe the demographic and clinical characteristics of the study population and by institution. *t*-test or rank-sum test were used to compare continuous variables by institution and chi-squared or Fisher's exact test for categorical variables. Recurrence-free survival (RFS) and overall survival (OS) were estimated using the methods of Kaplan and Meier and modeled via Cox proportional hazards regression. RFS was calculated from date of surgery to earliest date of recurrence or death due to any cause. RFS time was censored at date of last contact for patients alive and recurrence free. OS was calculated from date of surgery to earliest date of death due to any cause or last contact. All statistical analysis were performed using Stata/MP v17.0 (College Station, TX) and R (R Core Team, 2022).

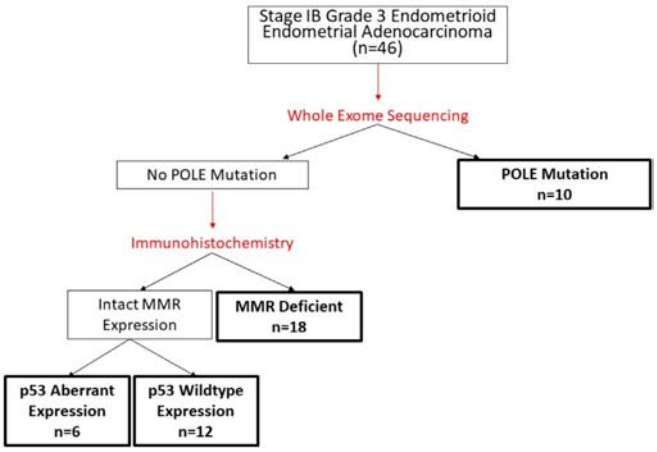

Fig. 1. Cohort ProMisE Classification.
ProMisE algorithm was used to classify our cohort of patients. This was performed by first assessing POLE mutation by whole exome sequencing, then for the presence of mismatch repair (MMR) proteins by IHC, and finally by aberrant expression of p53 by IHC. Subgroups included: POLE (n = 10), MMR deficient (n = 18), abnormal p53 (n = 6), and p53 wildtype (n = 12).

3. Results

3.1. Total patient population

A total of 46 patients were included in the study: 30 from OUH and 16 from MDACC. The patients at both institutions were similar in age (median 67.6 vs 63.4, $p = 0.196$), but differed in BMI (median 27.95 vs 36, $p = 0.026$) and race (White 100% vs 81%, $p = 0.037$). All patients had deep myometrial invasion, while 80% ($n = 35$) had lymphovascular space invasion present. Most had a pelvic lymph node dissection performed ($n = 37$, 80%), and of these, all were negative for metastasis. Twenty-seven patients (58.7%) had a para-aortic lymph node dissection performed, and all were negative (Table 1).

Most patients received adjuvant therapy after surgery ($n = 34$, 74%), including vaginal brachytherapy ($n = 3$, 6.5%), external beam radiotherapy with or without brachytherapy ($n = 6$, 13%), systemic chemotherapy with or without brachytherapy ($n = 22$, 47.8%), or chemotherapy in combination with external beam radiotherapy with or without brachytherapy (n = 3, 6.5%). The two institutions differed in their distribution of adjuvant treatment: OUH only used chemotherapy with or without brachytherapy for their patients who received adjuvant therapy ($p < 0.001$).

Median follow up time was 4.76 years (range, 0.19 to 15.12 years). Median RFS was not reached. Nearly 20% of patients recurred ($n = 9$), most with extra-pelvic disease ($n = 5$, or 55.6%) including lung ($n = 2$), bone ($n = 1$), para-aortic lymph node (n = 1), and para-anal region (n = 1) (Supplemental Table 3). Four of these 5 patients received adjuvant chemotherapy. Only one had a vaginal cuff recurrence, despite receiving vaginal brachytherapy. One patient had a multi-site recurrence with pulmonary nodules as well as a pelvic mass; she did not receive any adjuvant treatment. Two patients had peritoneal disease and ascites; both received at least systemic chemotherapy. Of note, three patients amongst those with a recurrence ($n = 9$) did not have lymph node assessment at the time of surgery. Those recurrences included disease to the lung ($n = 2$) and para-aortic lymph node ($n = 1$). All recurrences that occurred at OUH ($n = 4$) had extra-pelvic disease despite receiving adjuvant chemotherapy. The median time to recurrence was 21.3 months (range, 4 to 36.7 months).

At data cut-off time, 8 of the 9 patients who recurred were deceased, and one was alive with no evidence of disease. Median OS was not reached. In the entire cohort, fifteen patients (32.6%) had passed including: 6 (40%) who died of their disease, 7 (46.7%) who died of other

Table 1
Clinicopathologic features by two institutions.

	All Patients $n = 46$	OUH Patients $n = 30$	MDACC Patients $n = 16$	p-value
Median Age (years)	67.0	67.6	63.7	0.20
Median BMI (kg/m^2)	29.9	28.0	36.0	0.03
Race (n, %)				0.04
White	43 (93.5%)	30 (100%)	13 (81.3%)	
Hispanic	3 (6.5%)	0	3 (18.8%)	
Lymphovascular Invasion (n, %)				0.12
Not Present	9 (19.6%)	8 (26.7%)	1 (6.3%)	
Present	35 (76.1%)	20 (66.7%)	15 (93.8%)	
Unknown	2 (4.4%)	2 (6.7%)	0	
Lymph Node Dissection (n, %)				
Pelvic	37 (80.4%)	22 (73.3%)	15 (93.8%)	0.13
Para-aortic	27 (58.7%)	17 (56.7%)	10 (62.5%)	0.70
Adjuvant Therapy (n, %)				<0.001
No Adjuvant therapy	12 (26.1%)	10 (33.3%)	2 (12.5%)	
Vaginal Brachytherapy only	3 (6.5%)	0	3 (18.8%)	
External beam Radiation +/− VBT	6 (13.0%)	0	6 (37.5%)	
Chemotherapy +/− VBT	22 (47.8%)	20 (66.7%)	2 (12.5%)	
Chemo with EBRT +/− VBT	3 (6.5%)	0	3 (18.8%)	
Recurrence (n, %)	9 (19.6%)			0.127
Vaginal	1 (11.1%)	0	1 (20%)	
Peritoneal	2 (22.2%)	0	2 (40%)	
Extra-abdominal	5 (55.6%)	4 (100%)	1 (20%)	
Multiple sites	1 (11.1%)	0	1 (20%)	
Mean Time to recurrence (months)	20.8	30.0	13.4	

known causes, and 2 (13.3%) who died of unknown causes. Median time to death was 38.1 months (range, 9.3 to 118.2 months).

3.2. ProMisE subclassification

Tumors were subclassified by ProMisE molecular characteristics (Table 2). Within our cohort, 21.7% ($n = 10$) had POLE mutations, 39.1% ($n = 18$) were MMR deficient, 13.0% ($n = 6$) had abnormal p53 and 26.1% ($n = 12$) were p53 wildtype (Fig. 1). The subgroups were similar in age ($p = 0.44$), BMI ($p = 0.13$) and race ($p = 0.23$). The subgroups had a statistical difference amongst their mean TMB: POLE EDM was 486.2 mut/Mb, MMRd was 100.9 mut/Mb, p53 abn was 46.0 mut/Mb, and p53 wt subgroup was 52.8 mut/Mb ($p < 0.001$). There was no difference in adjuvant treatment use amongst the subgroups ($p = 0.94$).

There was no observed difference in median RFS amongst the subgroups ($p = 0.22$, Fig. 2A). There were no recurrences in the POLE EDM subgroup. In the MMRd subgroup, 22% ($n = 4$) recurred, and all recurrences were extra-abdominal (lung $n = 2$, bone $n = 1$ and para-aortic lymph node n = 1). Three of these four patients received adjuvant chemotherapy as part of their primary treatment. Within the p53 abn subgroup ($n = 6$), 33% recurred (n = 2): one patient had multifocal disease (pulmonary nodules and pelvic mass) and did not receive any adjuvant therapy while the other patient had para-anal disease and received adjuvant chemotherapy. Within the p53 wt subgroup, 25% recurred ($n = 3$). One patient had a vaginal recurrence and received

Table 2
Clinicopathologic Features by ProMisE Classification.

	POLE Mutation $n = 10$	MMR deficient $n = 18$	p53 abnormal $n = 6$	p53 wildtype $n = 12$	p-value
Median Age (years)	60.7	67.5	66.7	68.7	0.44
Median BMI (kg/m^2)	26.8	30.5	33.6	32.6	0.13
Race (n, %)					0.23
White	9 (90%	18 (100%)	5 (83.3%)	11 (91.7%)	
Hispanic	1 (10%)	0 (0%)	1 (16.7%)	1 (8.3%)	
Lymphovascular Invasion (n, %)					0.49
Not Present	3 (30%)	3 (16.7%)	0 (0%)	3 (25%)	
Present	6 (60%)	14 (77.8%)	6 (100%)	9 (75%)	
Unknown	1 (10%)	1 (5.6%)	0 (0%)	0 (0%)	
Lymph Node Dissection (n, %)					
Pelvic	10 (100%)	14 (77.8%)	4 (66.7%)	9 (75%)	0.28
Para-aortic	8 (80%)	11 (61.1%)	4 (66.7%)	4 (33.3%)	0.18
Adjuvant Therapy (n, %)					0.94
No Adjuvant therapy	2 (20%	4 (22.2%)	2 (33.3%)	4 (33.3%)	
Vaginal Brachytherapy only	1 (10%)	1 (5.6%)	0 (0%)	1 (8.3%)	
External beam Radiation +/− VBT	1 (10%)	2 (11.1%)	1 (16.7%)	2 (16.7%)	
Chemotherapy +/− VBT	6 (60%)	10 (55.6%)	3 (50%)	3 (25%)	
Chemo with EBRT +/− VBT	0 (0%)	1 (5.6%)	0 (0%)	2 (16.7%)	
Recurrence (n, %)	0 (0%)	4 (22.2%)	2 (33.3%)	3 (25%)	0.016
Vaginal	0 (0%)	0 (0%)	0 (0%)	1 (33.3%)	
Peritoneal	0 (0%)	0 (0%)	1 (50%)	2 (66.7%)	
Extra-abdominal	0 (0%)	4 (100%)	1 (50%)	0 (0%)	
Multiple sites	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Mean Time to recurrence (months)	n/a	25.7	15.8	17.6	

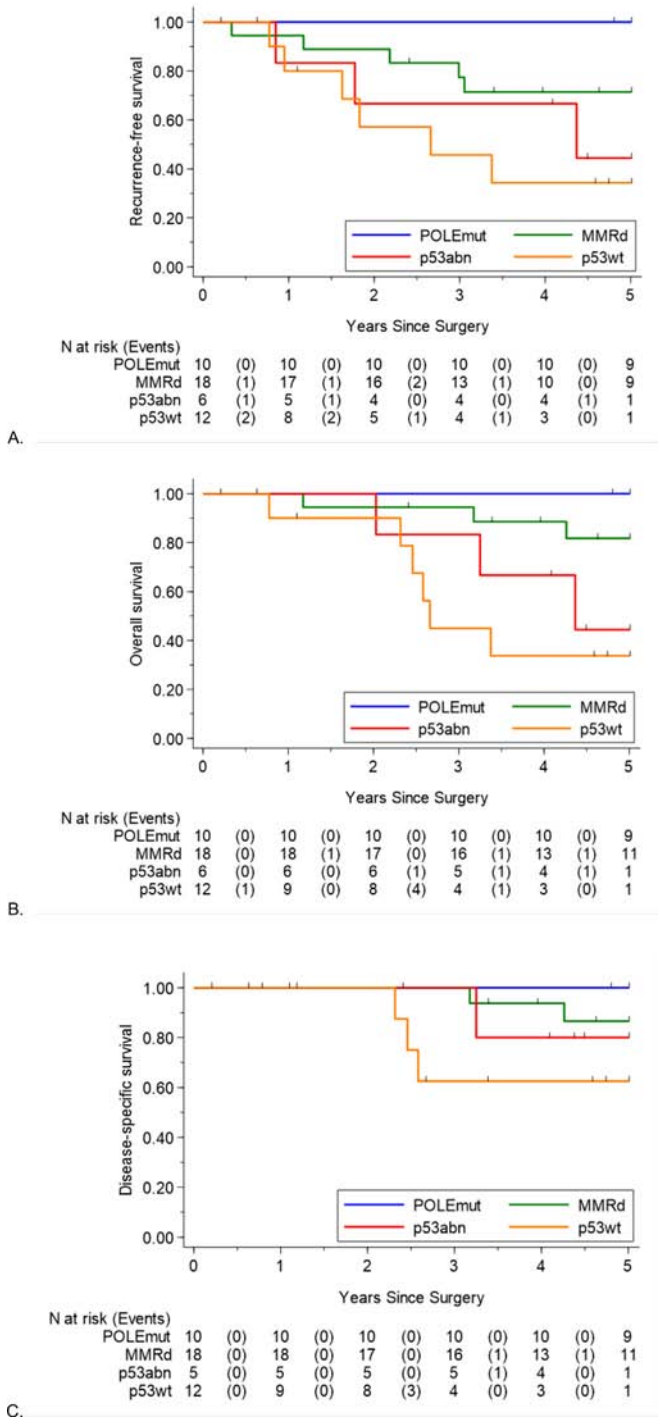

Fig. 2. Survival curves by ProMisE molecular subgroup.
Kaplan-Meier survival curves for Stage IB Grade 3 Endometrioid Endometrial Adenocarcinomas by ProMisE Classification. POLE-mutated (blue), MMR-deficient (green), p53 abnormal (red), and p53 wildtype (orange). **(A) Recurrence free survival.** There is no observed difference in RFS amongst the four subgroups (p = 0.22). **(B) Overall Survival.** OS was statistically different amongst the subgroups (p = 0.017). The median OS was not reached for the POLE or MMR deficient group but was 52.4 months for the abnormal p53 group and 40.5 months for the p53 wildtype group. **(C) Disease-Specific Survival.** Within the POLE mutation subgroup ($n = 10$), one patient (10%) died but not of her cancer. Within the MMR deficient group ($n = 18$), five patients (27.8%) died including two of cancer, two of causes other than cancer, and one of an unknown cause. Within the abnormal p53 subgroup ($n = 6$), three patients died (50%): one of cancer, one from cause other than cancer, and one of unknown cause. Within the p53 wildtype subgroup ($n = 12$), 6 patients died (50%): three from their cancer and three from a cause other than their cancer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

vaginal brachytherapy, while two patients had peritoneal recurrences, including one who received chemotherapy and another who received chemotherapy with EBRT.

The difference in OS was statistically significant amongst the subgroups ($p = 0.017$) (Fig. 2B); however, not all patients died of their disease (Fig. 2C). Within the POLE EDM subgroup ($n = 10$), one patient (10%) died but not of cancer. Within the MMRd subgroup ($n = 18$), five patients (27.8%) died including two of cancer, two of other causes, and one of an unknown cause. Within the p53 abn subgroup ($n = 6$), three patients died (50%): one of cancer, one from other cause, and one of unknown cause. Within the p53 wt subgroup ($n = 12$), 6 patients died (50%): three from cancer and three from other causes. POLE EDM and MMRd were associated with a significant improvement in overall survival, despite no difference in treatment. Overall, median OS was not reached in patients with POLE EDM or MMRd tumors.

3.3. Mutational signatures

Using the whole exome sequencing data, characteristic mutational signatures based on single base-pair substitutions were estimated for each tumor. For 8 patients (17.3%), the total number of SNVs was too limited to determine a dominant mutational signature. In the 38 patients where a dominant mutational signature was identified, the majority had a SBS5 signature (44.7%, $n = 17$) as their most dominant signature, 10 patients had a SBS15 or SBS44 signature (26.3%), 7 patients had a SBS10a or SBS10b signature (18.4%), 3 patients had a SBS14 signature (7.9%), and one patient had a SBS40 signature (2.6%) (Table 3). These subgroups were similar in age ($p = 0.069$), BMI ($p = 0.167$) and race ($p = 0.289$). There was a significant difference in mean TMB amongst the mutational signatures: SBS5 was 166.3 mut/Mb, SBS10a and SBS10b was 329.9 mut/Mb, SBS14 was 426.2 mut/Mb, SBS15 and SBS44 was 102.1 mut/Mb, SBS40 was 82.5 mut/Mb ($p < 0.017$). There was no difference in the adjuvant therapy given amongst subgroups ($p = 0.927$).

For tumors characterized by the SBS5 signature ($n = 17$), two tumors were part of the POLE EDM ProMisE subgroup (11.8%), 6 were MMRd (35.3%), four were p53 abn (23.5%), and five were p53 wt (29.4%) (Supplemental Fig. 1). Six patients (35%) recurred including: one recurrence at the vaginal cuff, one peritoneal recurrence, and four extra-abdominal recurrences (lung n = 1, bone n = 1, para-aortic lymph node n = 1, and para-anal region n = 1). Of the six patients that recurred, three (50%) were MMRd, one (16.7%) was p53 abn, and two (33.3%) were p53 wt. Eight patients died (47%): four of their cancer, three of other causes, and one of an unknown cause.

Within the SBS10a or SBS10b signature group ($n = 7$), 6 patients were categorized as POLE EDM (85.7%) and one was p53 wt (14.3%) (Supplemental Fig. 1). None of these patients recurred and only one patient died of a cause other than cancer.

Within the SBS15 or SBS44 signature group ($n = 10$), all were MMRd (Supplemental Fig. 1). None of these patients recurred and two died of causes other than cancer.

In the SBS14 signature subgroup ($n = 3$), two patients were POLE EDM (66.7%) and one was MMRd (33.3%) (Supplemental Fig. 1). There were no recurrences and no deaths in this subgroup.

Finally, the patient with an SBS40 signature was p53 wt (Supplemental Fig. 1), did not recur, and died of a cause other than cancer.

3.4. Common gene mutations

Within our cohort of grade 3 EEC, the most frequently mutated gene was ARID1A ($n = 30$, 65%). Other commonly mutated genes included PTEN ($n = 28$, 61%), MUC16 ($n = 27$, 59%), and PIK3CA ($n = 25$, 54%).

Commonly mutated genes within the ProMisE Classification subgroups were assessed (Table 4a). Within POLE EDM ($n = 10$), all patients had a POLE mutation as well as a FAT4 mutation. Within the

Table 3
Clinicopathologic Features by Mutational Signature.

	SBS 5 $n = 17$	SBS10a and SB10b $n = 7$	SBS15 and SBS44 $n = 10$	SBS14 $n = 3$	SBS40 $n = 1$	p = value
Median Age (years)	67.1	58.5	67.2	67.6	89.1	0.07
Median BMI (kg/m^2)	31.1	28.1	33.3	22.2	34.0	0.17
Race (n, %)						0.29
White	17(100%)	6 (85.7%)	10 (100%)	3 (100%)	1 (100%)	
Hispanic	0	1 (14.3%)	0 (0%)	0	0 (0%)	
Lymphovascular Invasion (n, %)						0.31
Not Present	2 (11.8%)	3 (42.9%)	3 (30%)	1 (33.3%)	0 (0%)	
Present	15 (88.2%)	4 (57.1%)	6 (60%)	1 (33.3%)	1 (100%)	
Unknown	0 (0%)	0 (0%)	1 (10%)	1 (33.3%)	0 (0%)	
Lymph Node Dissection (n, %)						
Pelvic	12 (70.6%)	7 (100%)	9 (90%)	3 (100%)	0 (0%)	0.12
Para-aortic	10 (58.8%)	5 (71.4%)	7 (70%)	2 (66.7%)	0 (0%)	0.78
ProMisE Classification						<0.001
POLEmut	2 (11.8%)	6 (85.7%)	0 (0%)	2 (66.7%)	0 (0%)	
MMRd	6 (35.3%)	0 (0%)	10 (100%)	1 (33.3%)	0 (0%)	
p53abn	4 (23.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
p53wt	5 (29.4%)	1 (14.3%)	0 (0%)	0 (0%)	1 (100%)	
Adjuvant Therapy (n, %)						0.93
No Adjuvant therapy	3 (17.7%)	2 (28.6%)	4 (40%)	1 (33.3%)	1 (100%)	
Vaginal Brachytherapy only	2 (11.8%)	1 (14.3%)	0 (0%)	0 (0%)	0 (0%)	
External beam Radiation +/− VBT	1 (5.9%)	1 (14.3%)	1 (10%)	0 (0%)	0 (0%)	
Chemotherapy +/− VBT	10 (58.8%)	3 (42.9%)	5 (50%)	2 (66.7%)	0 (0%)	
Chemo with EBRT +/− VBT	1 (5.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Recurrence (n, %)	6 (35.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	n/a
Vaginal	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Peritoneal	1 (16.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Extra-abdominal	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Multiple sites	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Mean Time to recurrence (months)	19.9	n/a	n/a	n/a	n/a	

MMRd subgroup ($n = 18$), only 9 patients (50%) had a MLH1, MLH3, MSH2, MSH6, PMS2, TGFBR2, and/or EPCAM mutation. In the p53 abn subgroup ($n = 6$), 4 patients (66.7%) had a mutated TP53 gene. There were no commonly shared mutated genes noted within the p53 wildtype subgroup.

Similar analysis was performed within the mutational signature subgroups (Table 4b). In the SBS5 signature, commonly mutated genes included: ARID1A ($n = 12$, 70.6%), MUC16 ($n = 9$, 53.9%), and PIK3CA (n = 9, 53.9%). In the SBS14 signature, all patients had the following mutations: ARID1A, FAT4, FBXW7, MUC16, PTEN, PIK3CA, PIK3R1, JAK1, RNF43, ALK, CTNNB1, and MSH6. Two (66.6%) had a POLE mutation. Within the SBS10a or SBS10b signature, all patients had a FAT4 mutation while 6 (85.7%) had a POLE mutation. Within the SBS15 and SBS44 signatures, only 5 patients (50%) had a MLH1, MLH3, MSH2, MSH6, PMS2, TGFBR2, and/or EPCAM mutation. For the one tumor with a SBS40 signature, only 4 gene mutations were detected: MYC, ALK, FAT1 and MUC16.

In our total cohort of patients, the majority had at least one mutation within the PI3K-PTEN-AKT-mTor pathway ($n = 42$, 91.3%). Fewer patients had a mutation in the RAS-MEK-ERK pathway ($n = 10$, 21.7%) and the canonical WNT-β-catenin pathway ($n = 19$, 41.3%).

Table 4a
Commonly Mutated Genes within ProMisE Subgroups.

POLE Mutation $n = 10$		MMR deficient $n = 18$		p53 abnormal $n = 6$		p53 wildtype $n = 12$	
FAT4	10	MUC16	14	PIK3CA	5	PTEN	6
POLE	10	ARID1A	13	ARID1A	4	ARID1A	4
ARID1A	9	PTEN	13	PTEN	4	PIK3CA	4
PIK3CA	9	RPL22	11	TP53	4	MUC16	3
MSH6	9	ZFHX3	10	MUC16	2	PIK3R1	3
BRCA2	8	JAK1	10			ZFHX3	3
FAT1	8	PIK3CA	7				
MUC16	8						
MTOR	8						

4. Discussion

In this multi-institutional translational study, we characterized a cohort of stage IB grade 3 EEC by ProMisE classification, mutational signature, and commonly mutated genes. This comprehensive evaluation found a molecularly diverse cohort of tumors, despite having the same histology, stage, and grade. Our molecular evaluation provided more precise prognostic insight into our patient cohort and revealed significant characteristic findings.

Although there were no differences in RFS amongst the ProMisE subgroups, survival trends were noted. For instance, there were no recurrences in the POLE EDM subgroup; additionally, median OS could not be reached in the patients with POLE EDM or MMRd tumors. The poor outcome of the p53 wt group, as estimated on the Kaplan-Meier curve, likely reflects the many patients lost to follow-up, as the recurrence rate for this group was 25%, similar to the MMRd group and consistent with published literature [18–20]. Ultimately, none of these

Table 4b
Commonly Mutated Genes within Mutational Signature Subgroups.

SBS 5 $n = 17$		SBS10a and SBS10b $n = 7$		SBS15 and SBS44 $n = 10$		SBS14 $n = 3$		SBS40 $n = 1$	
ARID1A	12	FAT4	7	PTEN	10	ARID1A	3	FAT1	1
MUC16	9	ARID1A	6	ARID1A	8	CTNNB1	3	MUC16	1
PIK3CA	9	POLE	6	MUC16	8	FAT4	3	MYC	1
PTEN	7	MSH6	6	RPL22	7	FBXW7	3	ALK	1
ZFHX3	7	BRCA2	5	JAK1	7	MUC16	3		
BRCA2	5	ERBB2	5	ARID5B	5	PTEN	3		
FAT1	5	MUC16	5	ZFHX3	5	PIK3CA	3		
FAT4	5	PIK3CA	5	CTNNB1	4	PIK3R1	3		
TP53	5	ZFHX3	5	FAT1	4	JAK1	3		
MTOR	5	MET	5	PIK3CA	4	RNF43	3		
		MTOR	5	PIK3R1	4	ALK	3		
				MSH6	4	MSH6	3		

trends reached statistical significance likely due to the small number of patients within each subgroup analysis.

As expected, TMB was high in the POLE EDM and MMRd subgroups, but low in the p53 abn and p53 wt subgroups. The TMB was much more variable within mutational signatures, but still statistically different amongst these groups. For instance, the TMB of the SBS5 group ranged from 2.9 mut/MB to 1062.4 mut/Mb. Survival analysis based on TMB was not performed.

All endometrioid adenocarcinomas within our cohort fit within only 7 of the 11 previously described uterus-relevant mutational signatures. Not all tumors had enough SNVs to determine a dominant mutational signature. Overall, 38 tumors had a dominant mutational signature identified. Due to the similarities between signatures, they were paired for our analysis: SBS10a with SB10b because of their common POLE mutation etiology, SBS15 with SBS44 because of their common defective mismatch repair mechanism.

For the 38 patients with an identified dominant mutational signature, all recurrences occurred within the SBS5 group, despite a range of adjuvant treatments. Furthermore, of the patients who died of their disease, all had a dominant SBS5 signature. This mutational signature has an unknown etiology, but is hypothesized to be related to aging, tobacco smoking, and nucleotide excision repair (NER) deficiency The number of mutations in most cancer and normal cells correlates with the age of the individual. In our cohort, this group was poorly defined by the ProMisE molecular classification: all four subgroups (POLE EDM, MMRd, p53 abn, and p53 wt) were noted within the SBS5 group, suggesting that ProMisE classification and mutational signatures may not have overlapping molecular markers. Instead, this study suggests that perhaps mutational signatures provide further precisional subclassification – given that all recurrences were categorized within one mutational signature compared to the use of ProMisE classification. While mutational signatures are still an understudied concept in endometrial cancer, Ashley et al. have previously reported an association between copy-number high and copy-number low endometrial tumors with aging-related signatures, in their case SBS1 [21]. Our findings generate additional interest in understanding the correlation between mutational signatures, patient outcomes, and response to therapy.

As for specific genetic mutations, a review of the literature by Blanco et al. found that the most frequently mutated genes in endometrioid carcinomas are: PTEN (>77%), PIK3CA (53%), PIK3R1 (37%), CTNNB1 (36%), ARID1A (35%), K-RAS (24%), CTCF (20%), RPL22 (12%) TP53 (11%), FGFR2 (11%), and ARID5B (11%) [14]. Our cohort differed with an alarming number of ARID1A (65%), known to be associated with the initiation and progression of endometrial cancer, and MUC16 (59%), whose serum levels are often associated with prognosis of endometrial cancer. MUC16 encodes for the protein Ca125. Although expression of Ca125 was not assessed in this study, this could provide insight into the molecular implications of Ca125 in endometrial adenocarcinoma, as a prognostic marker and possible therapeutic target. Other identified mutations within our cohort that had therapeutic implications included those with BRCA1 (21.7%), BRCA2 (28.3%), and ERBB2 (28.3%) mutations. Furthermore, most patients had a mutation within the PI3K-PTEN-AKT-mTor pathway, providing actionable targets for this cohort. Understanding the mutational landscape of stage 1B grade 3 EEC can facilitate the development of targeted therapies that exploit the vulnerabilities conferred by these mutations.

The strength of this study lies in the new approach to study molecular markers. By broadening our molecular classification strategy, we identified a mutational signature with potential prognostic implications. Mutational signatures have yet to be fully described for grade 3 EEC; hence these results add to current literature on the molecular diversity of this highly heterogenous group of tumors.

This study has limitations. The availability of archival tissue restricted the size to only 47 patients. The small sample size limits the generalizability of the findings, and the heterogeneity in our patient population may introduce clinical variables impacting patient outcomes. For example, although most of our patients underwent a lymph node assessment, not all were fully surgically staged and may have been understaged. The type of adjuvant treatment received was center-dependent. Additionally, inherent tumor heterogeneity can introduce variability in mutational signatures, necessitating larger cohorts for robust conclusions. While the identification of mutational signatures is hypothesis generating, functional validation through in vitro and in vivo experiments is crucial to establish their biological relevance on tumor behavior and response to therapy. Finally, technical limitations of sequencing are also important to consider: the detection and characterization of mutations can be technically challenging due to sequencing artifacts, low variant allele frequencies, and complexity of mutational processes. The presence of concurrent somatic mutations, germline variants, and environmental exposures can confound mutational signature analysis.

5. Conclusion

In this study, we identified a mutational signature, SBS5, correlated with a high risk of recurrence. While ProMisE classification is a useful subtyping tool, we strive to refine the classification for endometrial cancer, with the goal to enable more precise patient stratification and personalized treatment approaches. Future studies should address limitations by incorporating larger, well-characterized patient cohorts, rigorous functional validation, and comprehensive analysis of confounding factors. This will enhance our understanding of the biology underlying this aggressive cancer subtype and pave the way for improved diagnostic, prognostic, and therapeutic strategies.

Disclosures

Dr. Ben Davidson is a consultant and speaker at Merck & Co. But all other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

During the preparation of this work, the authors did not use any generative AI or AI-assisted technologies in the writing process.

Credit authorship contribution statement

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Appendix A. Supplementary data

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