Endometrial Cancer Molecular Risk Stratification is Equally Prognostic for Endometrioid Ovarian Carcinoma

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ABSTRACT

Purpose: Endometrioid ovarian carcinoma (ENOC) is generally associated with a more favorable prognosis compared to other ovarian carcinomas. Nonetheless, current patient treatment continues to follow a “one-size-fits-all” approach. Even though tumor staging offers stratification, personalized treatments remain elusive. As ENOC shares many clinical and molecular features with its endometrial counterpart, we sought to investigate TCGA-inspired endometrial cancer (EC) molecular subtyping in a cohort of ENOC.

Experimental Design: Immunohistochemistry and mutation biomarkers were used to segregate 511 ENOC tumors into four EC-inspired molecular subtypes: low-risk *POLE* mutant (POLEmut); moderate-risk mismatch repair deficient (MMRd); high-risk p53 abnormal (p53abn); moderate-risk with no specific molecular profile (NSMP). Survival analysis with established clinicopathological and subtypes specific features was performed.

Results: 3.5% of cases were POLEmut, 13.7% MMRd, 9.6% p53abn and 73.2% NSMP, each showing distinct outcomes ($p < 0.001$) and survival similar to observations in EC. Median OS was 18.1 years in NSMP, 12.3 years in MMRd; 4.7 years in p53abn and not reached for POLEmut cases. Subtypes were independent of stage, grade, and residual disease in multivariable analysis.

Conclusions: EC-inspired molecular classification provides independent prognostic information in ENOC. Our findings support investigating molecular-subtype specific management recommendations for ENOC patients; for example, subtypes may provide guidance when fertility-sparing treatment is desired. Similarities between ENOC and EC suggest that ENOC patients may benefit from management strategies applied to EC and the opportunity to study those in umbrella trials.

Statement of translational relevance:

The translation and implementation of molecular research findings in cancer management remains a challenge. This is especially ambitious in uncommon cancers, such as endometrioid ovarian carcinoma (ENOC). Compounded by historical inaccuracies in diagnosis, ENOC is one of the least well studied histotypes of ovarian carcinoma, with little evidence available to guide treatments or identify patients likely to experience excellent outcomes versus those with aggressive disease. ENOC shares considerable molecular and histological similarity with endometrial carcinomas (EC), in particular endometrioid EC (EEC), with endometrial tissue well accepted to be the origin of both diseases. The similarity between ENOC and EC suggests that clinical developments from the much larger EC patient cohort could be rapidly translated to the less common ENOC population. In this context, we provide direct evidence that molecular subtypes defined in EC also exist in ENOC with equivalent features and clinicopathological behavior. Our data provide a basis to

investigate molecularly stratified management strategies in ENOC and suggest collective research and subtype-specific trials across EC and ENOC may provide advantages to both cancers.

Introduction

Today, the scientific community widely agrees that ovarian carcinoma is a heterogeneous disease and that different histological types are best considered as different disease entities [1]. The next step towards type-specific treatment approaches is to further stratify each ovarian carcinoma histotype. The Cancer Genome Atlas research network (TCGA) study on ovarian carcinomas in 2011 brought considerable insights into the most common tubo-ovarian high-grade serous carcinoma (HGSOC) histotype [2]. In-depth genomic studies of the other histotypes are few and far between.

Endometriosis-associated ovarian carcinomas, specifically endometrioid and clear cell histotypes, are collectively the second most common forms of ovarian carcinoma and account for a combined ~20% of ovarian carcinomas [3, 4]. Both are believed to originate from endometrial cells and most frequently thought to develop via an endometriosis intermediate [5, 6]. Endometrioid ovarian carcinoma (ENOC), is near identical to its endometrial endometrioid carcinoma (EEC) counterpart with respect to theory on origin, common synchronous occurrence, similar genotype, phenotype, risk factors, and near-indistinguishable histopathological presentation [7-12]. Compared to HGSOC, women with ENOC are on average 6 years younger (more often premenopausal), diagnosed at earlier stage (stage I/II in 80% of cases) and show higher overall survival rates (~ 80% 5-year survival) compared to most other histotypes of ovarian carcinomas [13-15].

Despite these differences between dominantly poor outcome and dominantly favorable outcome entities, consensus guidelines for patient management and chemotherapy still parallel those of the more aggressive HGSOC [16, 17]. The similarities between endometrial carcinoma (EC), specifically EEC, and ENOC suggest EC/EEC may provide more reliable benchmarks for the management of ENOC. TCGA study on EC recently proposed a prognostic molecular stratification scheme for EC based on unique genomic phenotypes. One group is defined by pathogenic mutations in the exonuclease domain of DNA polymerase epsilon (POLE) and an ultramutated genome, another by deficiency in the DNA mismatch repair pathway and a microsatellite unstable/hypermutated genome, the final two groups are split by fraction of their genomes involved in copy number alterations: copy-number low and copy-number high [18]. Following the TCGA study, two groups simultaneously derived near-identical minimal-biomarker based surrogates for the TCGA EC molecular subtypes [19-21]. The end result, an algorithm referred to as the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), uses immunohistochemistry markers and targeted sequencing of *POLE* to identify three molecular subtypes: (1) *POLE* mutant “POLEmut”: defined by pathogenic *POLE*

exonuclease domain mutations that identify a group with favorable outcome and an ultramutation phenotype; (2) mismatch repair deficient “MMRd”: defined by IHC markers for DNA mismatch repair complex (MSH1, PMS2, MSH2, and MSH6) and identifies cases with microsatellite instability and a corresponding hypermutation phenotype; and (3) p53 abnormal subtype “p53abn” (also referred to as p53-aberrant or p53-mutant): defined by abnormal p53 IHC staining pattern and correlating with copy-number high genomic phenotype. A fourth group without any of these three characteristics is correlated with the copy-number low class from the TCGA EC classification, also known as no specific molecular profile class (NSMP). In EC, ProMisE classification is proven to be not only prognostic but also predictive and is expected to bring advantageous changes into clinical practice of EC patients [21].

Similarities between EC and ENOC have led several groups to hypothesize that ENOC could be stratified into the same four molecular subtypes seen in EC. Parra-Herran et al. showed the general feasibility of this approach, with their conclusions hindered by a small cohort [22]. Cybulska et al. demonstrated that the genomic phenotypes of EC subtypes could be captured in a small cohort of ENOC [23]. Together these observations strongly support a need for validating a molecular stratification scheme in ENOC. Opportunities may exist both to reduce overtreatment (unnecessary chemotherapy), in patients with expected excellent outcomes, while still identifying patients in need of more aggressive treatment (avoid undertreatment). The aim of the current study was to validate the frequency of biomarker-defined EC molecular subtypes, and their prognostic patterns, to the current set of ENOC clinical risk factors such as stage, grade and residual tumor burden.

Methods

Study cohorts

Available cases with clinically identified primary ovarian carcinoma and a diagnosis of endometrioid histotype were identified from clinical and/or research databases from 9 centers across 4 countries (n=604). Tissue samples were provided from Canadian and European centers: Department of Women’s Health, Tuebingen University Hospital, Germany (GER); Department of Gynecology and Gynecologic Oncology, Kliniken Essen Mitte, GER; Department of Obstetrics and Gynecology, Heidelberg University Hospital, GER; Department of Gynecology and Obstetrics, Medizin Campus Bodensee, Friedrichshafen, GER; Department of Gynecological Oncology, Barts Health National Health Service Trust, London, United Kingdom (UK); Department of Gynecology University Medical Centre, Leiden, the Netherlands (NL); Department of Obstetrics and Gynecology, University Medical Center Groningen, NL; the OVCARE gynaecological tissue bank, Vancouver, CA; and the Canadian Ovarian Experimental Unified Resource (COEUR)[15]. All contributing institutions approved collection and use of materials and associated clinical data through local research ethics boards. The project was conducted in compliance with the Canadian Tri-Council Policy Statement on Ethical Conduct for Research

Involving Humans (TCPS2, 2018) and Declaration of Helsinki. Study samples from Canada, the United Kingdom, and Germany were obtained with written informed consent. A subset of German study samples, where consent was not reasonably achievable (e.g. deceased), and specimens from The Netherlands were obtained with institutional ethics board approved waiver of consent. Studies except for those from NL and UK used tissue microarrays (TMAs) for immunohistochemical (IHC) markers. UK and NL studies used full section IHC (Figure S1A).

All samples were subjected to WT1 and p53 immunohistochemistry. Cases showing both WT1 expression and p53 abnormal/mutant staining pattern (n=36) or uninterpretable/missing (n=35) results were considered likely HGSOC [24, 25] and excluded. Finally, after accounting for any biomarker assessment failures, complete subtyping was available on 511 cases and all results are restricted to this series (Figure S1B).

We analyzed the cohort as a whole as well as considering a subset of low-stage (FIGO I-IIA [17]) cases. In the latter group we assumed no residual disease if debulking status was not reported (n=73), whereas if residual disease was reported after primary debulking surgery, we assumed cases were under-staged (n=9) and reclassified these as advanced-stage, not otherwise specified (NOS). Reclassification was done as the presence of residual tumor is generally incompatible with low-stage. We were unable to resolve discrepancies in stage IIC (FIGO 2009; n=51) which may have resulted in a subset of these cases restaged to stage IIA (FIGO 2014; low-stage for our analysis); these were retained as advanced-stage as were stage II not otherwise specified (NOS; n=17). A single case was also reported to have dedifferentiated features, this case was omitted from grade-specific analyses.

Molecular subtype assignment

Immunohistochemistry

Due to contributions from various centres there are slight differences in assays performed on different cohort as outlined below (see also Figure S1 and Table S1). MMRd was assigned by IHC using 4 mismatch repair pathway markers. Staining was performed on a Dako Omni platform with 30 min heat-induced pre-treatment using high retrieval buffer pH using the Omnis protocol H30-10M-30 with the ready-to-use clone ES05 (DAKO) for MLH1; H20-10R-20 with the ready-to-use clone EP51 (DAKO) for PMS2; H30-10M-30 with the ready-to-use clone FE11 (DAKO) for MSH2; and H30-10R-30 with the ready-to-use clone EP49 (or EPR3945) (DAKO) for MSH6. Interpretation of mismatch repair staining was dependent on retained nuclear staining in non-tumor cells on each evaluated core (internal positive control). Cases were considered mismatch repair deficient (MMRd) if absence of nuclear staining in tumor cells with retained internal control was observed for any individual core on any of the following markers: MLH1, PMS2, MSH2, or MSH6. [26, 27]

p53abn was assigned by surrogate p53 IHC, which has been established as an accurate predictor of the TP53 mutation status [28]. IHC for p53 was performed on a Dako Omni platform with 30 min heat-induced pre-treatment using high pH retrieval buffer and Omnis protocol H30-10M-30 with the ready-to-use clone DO-7 (GA61661-2; DAKO). Results were interpreted according to guidelines of the International Society of Gynecological Pathologists, with three abnormal patterns (overexpression, complete absence in tumor cells but retaining internal control, and cytoplasmic with unequivocal cytoplasmic and variable nuclear staining) and wild type p53 represented by variable intensity and distribution of staining in tumor cell nuclei [29].

POLE sequencing

DNA was extracted from FFPE tumor tissue sections using a modified procedure with the Qiagen QIAamp FFPE DNA extraction kit as described previously [11, 30]. Primer sets for Sanger sequencing and next-generation sequencing (NGS) strategies can be found in Table S2.

For all studies except UK, NL, and Heidelberg: three redundant sets of primers were designed to cover common *POLE* exonuclease domain hotspots in exons 9, 13 and 14 (p.P286R, p.M295R, p.S297F, p.V411L, p.L424I, p.A456P, and p.S459F) described to be pathogenic [31] in a tailed-amplicon sequencing strategy [32]. Sequencing of overlapping redundant amplicons was used to mitigate fixation errors common to FFPE-derived tissues [33]. PCR products were amplified using QuantStudio 6 Flex Real-Time PCR System with 2.5 ng of DNA. Amplicons were pooled on a per-sample basis and each sample pool was barcoded with unique indexes. Following indexing, all samples were pooled equimolar for sequencing on a MiSeq instrument (Illumina) using a 300 cycle v2 sequencing kit. Median coverage was >1,700X (per amplicon) (Table S3) over hotspots of interest. Mutations were called across primer sets and manually verified in bam files to ensure at least two (of three) amplicons contained the variant of interest.

For studies from UK, NL, and Heidelberg Sanger sequencing was performed over *POLE* hotspots noted above and additionally p.P436R and p.M444K. Technical repeat was performed for any observed variants (see also Figure S1A). Note that neither NGS, nor Sanger, strategies provided coverage for rare pathogenic variants in Exon 11 (p.F367S, p.D368Y) [31].

POLE variants were classified as (1) germline based on reference to dbSNP and consistent allele frequency; (2) pathogenic somatic variants based on presence in COSMIC and corroborating data from genomic studies with evidence of an ultramutated phenotype [18, 23]; (3) non-pathogenic somatic variants that are observed in other genomic studies but without an ultramutated phenotype; and (4) somatic variants of unknown significance (VUS) for other somatic variants that have not previously been reported with corroborating genomic data. Only pathogenic alterations were considered for assignment to the POLEmut class [31] (Table S3).

Classification Algorithm

We followed a classification schema proposed for EC in TCGA EC study, considering POLEmut first followed by MMRd, p53abn and finally NSMP. In rare cases with multiple possible classes we prioritized features in the order presented by TCGA EC study [18] and recommended for ProMisE using surrogate biomarkers [21, 34].

Statistical Analysis

Chi-squared test was used to evaluate univariable associations for categorical variables and Welch's one-way test for continuous variables. Differences in univariable survival outcomes were analyzed using the log-rank test. To evaluate the independent prognostic significance of molecular classification, the Cox proportional hazards model is used, adjusting for known clinicopathological risk variables. A Firth bias reducing correction is applied in the calculation of hazard ratio estimates, when more than 80% censoring is present in any one category of the variable of interest. The profile likelihood is used to calculate confidence intervals. Statistical significance was evaluated by the omnibus likelihood ratio test in the Cox models. Only observations with complete cases (by list-wise deletion) were used in modeling.

Statistical significance level is set to 0.05. P-values are two-sided, not corrected for multiple comparisons, and truncated to an inequality if less than 0.001. All statistical analyses were performed using the statistical software R (R Core Team, 2019), R version 3.5.3 (2019-03-11), and relevant R Packages [35].

All analyses were done only on ENOC patients with full subtype information available (n=511). The cohort was first examined in the context of clinicopathological features: age, stage (FIGO), grade (1/2 vs 3), residual disease (no visible macroscopic vs any) and adjuvant chemotherapy (none vs any). Outcome data included overall survival (OS), disease-specific survival (DSS) and progression free survival (PFS), where progression was determined by the treating physician. In all cases, the variable was calculated as time from diagnosis to time of event (death/progression) or censoring. Follow up that exceeded 10 years (or 5 years as noted below) was right censored as at December 31st of the 10th year post-diagnosis to minimize ascertainment bias and ensure non-informative censoring. Molecular subtyping was then analyzed alone and in context with noted features. Finally, ENOC data was compared to data from EC studies [19, 30, 36].

Results

Cohort Description

Patients were diagnosed between 1985 and 2018 (Table S3) Median follow-up time (OS) was 5.34 years (reverse Kaplan-Meier). Median age was 55 years, 57% of patients presented at low-stage (FIGO I-IIA), 47% were G1, 34% G2 and 20% G3.

Clinicopathological variables were not significantly different between European and Canadian cohorts (Table S4).

Survival analysis using established risk factors

OS, DSS, and PFS were all significantly different between FIGO stages and patients with or without residual disease. Outcomes (OS, DSS, PFS) were all more favorable in patients with low-stage disease and no residual tumor respectively ($p<0.001$ for all; Figure 1A, B; Figure S2). Prognostic value of grade was significant in OS, DSS and PFS analyses for the full cohort ($p<0.001$ for all; Figure 1C, Figure S2), with grade 3 cases performing worse. However, when restricted to low-stage, grade was no longer prognostically significant ($p=0.538$) (Figure 1D, Figure S2).

Multivariable Analysis of established risk factors is shown in Table S5. With a DSS hazard ratio (HR) of 3.5, stage was the strongest prognosticator across ENOC patients in this cohort ($p=0.001$) while residual disease ($HR=3.1$, $p<0.001$) and grade ($HR=2.17$, $p=0.006$) were also significant. Similarly, stage, grade and residual disease retained significance for OS and PFS. Age at diagnosis was of borderline significance for OS ($HR=1.02$, $p=0.042$), but was not significant for DSS or PFS (Table S5).

Molecular Subtype Assignment

Of 533 cases with sufficient tissue for molecular assignment, *POLE* sequencing failed or was uninterpretable in 13 cases, MMR IHC was uninterpretable due to lack of internal control in 8 cases, and a single case was disqualified from classification due to uninterpretable p53 staining. 511 cases were fully subtyped and all results are restricted to this set (Figure 2A, Figure S1B). 18 cases (3.5%) harbored pathogenic *POLE* mutations (POLEmut; Figure 2A-B; Figure S3; Table S3). All 18 POLEmut cases were p53 wild type and MMR proficient by IHC surrogates. A total of 70 cases (13.7%) were assigned to MMRd, including 8 cases that were also p53 abnormal by IHC and 6 cases with heterogeneity in MMR marker scores across multiple replicate TMA cores. 49 cases were assigned to p53abn (9.6%; not including 8 assigned to MMRd), two of which showed heterogeneity in p53 IHC between TMA cores suggesting potential subclonality of p53 mutation. The remaining 374 cases (73.2%) were NSMP (Figure 2A-B; Figure S3).

In a subset of 15 cases, whole genome sequencing data was available from a previously published study [37]. This enabled us to verify the expected genomic profiles (Figure S4).

Clinicopathological associations of molecular subtypes

Significant univariable association is observed between age, stage, grade, residual disease and post-surgical chemotherapy and molecular subtype (Table 1; see also Figure 2B and S3). Patients with POLEmut ENOC were generally younger (median:

45 years), diagnosed at lower stage and grade. The oldest subset of patients fell into the p53abn class (median: 57 years) and was typically diagnosed at higher stage and grade. Accordingly, 33% of patients with POLEmut ENOC received no post-surgical treatment compared to 17% with p53abn ENOC.

Survival Associations of Molecular subtypes

OS and PFS data were available for 505 cases, DSS data for 497 cases. Kaplan-Meier curves show distinct survival outcomes in all three endpoints (Figure 2C-D-E, $p<0.001$ for all). No disease-specific deaths were observed in POLEmut patients, one POLEmut patient died of a non-disease related cause. p53abn patients had a disease-specific 10-year-survival-rate of only 39% (Table 2). For POLEmut, median overall survival time is not reached, in NSMP group it is 18.1 years, in MMRd group 12.3, in p53abn group 4.7 years.

After adjusting for currently used clinical risk factors (stage, grade, age, residual disease) and post-surgical chemotherapy in multivariable analysis, molecular subtypes were still statistically significant for OS, DSS and PFS ($p<0.001$) (Table 2). Amongst clinicopathological features, multivariable analysis showed age to be significant only for OS, while post-surgical chemotherapy retained significance only for OS and PFS (Table 2).

Stratified analysis of low-stage ENOC

In univariable analysis of low-stage ENOC, molecular subtypes were associated with substages ($p=0.032$) but not with age, grade, or post-surgical chemotherapy (Table S7). Outcomes of molecular subtypes (OS, DSS and PFS) were still statistically different (Figure 2F,G,H). In multivariable analysis, correcting for age, stage, grade, and post-surgical chemotherapy, subtypes retained significance for OS ($p=0.004$), DSS ($p=0.034$) and PFS ($p=0.048$). However, amongst other variables only grade was significant in OS (Table S8).

We further stratified outcomes of low-stage ENOC patients (FIGO IIA or less) across molecular subtypes in two subsets: one less likely to be recommended for adjuvant chemotherapy (stage IA/B G1/2) and the other more likely to be so (FIGO IA/B G3, IC/IIA) according to current international guidelines [16, 17] (Table 3). POLEmut ENOC patients had neither disease specific deaths nor progression. In the subset more likely to be recommended for adjuvant chemotherapy, p53abn and MMRd ENOC patients had proportionally more disease specific deaths, if they did not receive adjuvant treatment. Amongst low-stage NSMP there were 3 (4.7%) DSS events (vs. 0%) in the group that did not receive chemotherapy, and would generally not have been referred (Table 3).

Comparison of molecular subtyping in ENOC and EC

We pooled EC data from previous studies [19, 30, 36] and further separated EEC because of similarities to ENOC. Subtype distribution differed between ENOC and both EC and EEC (chi-square, both $p<0.001$). Compared to all EC, POLEmut, MMRd, and p53abn cases were all less frequent in ENOC, while the NSMP group was substantially larger (Figure 3A). However, when we restricted to EEC, the number of p53abn cases (5.6%) dropped in comparison to ENOCs (9.6%), trends in the other subtypes remained unchanged (Figure 3A).

Patients with EC, or EEC, were consistently older than those with ENOC across all molecular subtypes (Table S9, S10). ENOC POLEmut, MMRd and NSMP patients presented at a higher stage compared to EC or EEC. Stage was not significantly different in p53abn cases when comparing ENOC to all EC but was higher in ENOC compared to EEC (Table S9, S10). Both p53abn and MMRd EC were of higher grade than the corresponding subtypes of ENOC. Grade was not significantly different in NSMP and of borderline significance in POLEmut subtype (Table S9, S10).

We further compared 5-year-censored outcomes between ENOC (Table S11) and EC/EEC (Figure 3B). The proportion of surviving patients across all molecular subtypes was generally similar in ENOC compared to EC/EEC, with the exception of p53abn ENOC performing worse than p53abn EEC (5-year-DSS: 51% vs 70%; Figure 3B).

Discussion

This is the largest study to report molecular stratification of ENOC by translating a classification tool previously validated in EC [19-21, 30, 36]. We show that analogous molecular subtypes are prognostic in ENOC in all 3 critical endpoints (OS, DSS, and PFS). These findings validate and improve upon previous smaller studies [22, 23], specifically: we show that subtype to outcome associations remain significant in multivariable analysis independent of age, stage, grade, residual disease and post-surgical treatment. Furthermore, our stratified analysis suggests molecular subtype may provide particularly valuable information for low-stage patients where we were unable to show a significant impact for grade. Thus, molecular subtypes have the potential for immediate clinical translation, informing clinical trials that seek to test de-escalation or escalation of adjuvant therapy in specific low-stage patients.

Within molecular subtypes POLEmut cases showed an excellent outcome while patients with p53abn ENOC had the lowest survival rates even at low stage. NSMP and MMRd patients had largely equivalent, intermediate outcomes when the entire cohort was considered. However, in low-stage cases the outcome of NSMP patients tends to be more favorable compared to MMRd cases with noticeable differences in OS vs DSS/PFS. The generally more favorable outcomes in ENOC, in contrast with the more common HGSOC, require monitoring of both DSS and OS.

In comparison to EC, the Kaplan-Meier curves and 5-year-DSS-rates of molecular subtypes in ENOC were similar to those in EC [18-21, 30, 36]. ENOC were generally diagnosed at higher stage. Possible explanations include a less restricted access to the peritoneal cavity and obscured symptoms (e.g. lack of abnormal uterine bleeding). An exception is p53abn (non-endometrioid) EC, which appears to have aggressive spread regardless of anatomical borders. ENOC also tended to be younger than EC regardless of molecular subtype. While non-endometrioid EC are known to be older at diagnosis than EEC [38], the age difference was still substantial when comparing ENOC with EEC. Major epidemiological risk factors such as obesity and hormonal exposures are common to both EEC and ENOC [39, 40]. A plausible explanation may be the opportunity of occult/non-invasive EC (and hyperplastic) lesions to be shed during menstruation along with the functionalis of the endometrium in premenopausal women, thus contributing in part to the delayed onset of EC. In contrast, ovarian ENOC precursor lesions, such as endometriosis, would not be shed and may allow persistence of (pre)neoplastic cells. Such events are similar to the paradigm described as precursor escape in HGSOC [41]. Alternatively, as-yet undefined characteristics of a distinct, younger, population may also contribute to greater risk of ovarian disease.

POLEmut patients had excellent outcomes even at advanced-stage, and were less frequently observed than in EC/EEC but consistent with a previous study in ENOC [12]. Some early reports in EC may have overestimated the frequency of the POLEmut subtype by reporting non-pathogenic *POLE* mutations without evidence of ultramutator genotype, but regardless our ENOC POLEmut frequency is still lower (3.5% vs. range 6-9.4%). In EC it has also been suggested that pathogenic *POLE* mutations are quite early events [42]. While our design precludes a conclusive statement on whether *POLE* mutations are truncal in the context of ENOC, the variant frequency from informative cases would generally not favor emergence of *POLE* mutations in rare subclonal populations (Table S3). It should also be noted that our sequencing strategy may have missed a subset of less common pathogenic *POLE* mutations (see methods). In EC, retrospective data suggest no additional value of adjuvant chemotherapy in POLEmut EC cases [43]. A prospective clinical trial PORTEC4a (NCT03469674) is currently underway to investigate treatment de-escalation in EC. Results are equally relevant for ENOC. Subtype may be useful if fertility-preserving procedures are considered, POLEmut and p53abn currently stand at extremes, whereas additional data is still needed for NSMP.

MMRd ENOC were also less common than observed in EC, and in particular in EEC. The difference appears in part due to reduced proportion of MLH1/PMS2 deficient cases in ENOC suggesting somatic hypermethylation of the MLH1 promoter may be more prevalent in EEC compared to ENOC. Nonetheless, our results corroborate universal MMR biomarker testing in ENOC to screen for Lynch syndrome [44, 45]. MMRd ENOC patients may be eligible for immune checkpoint inhibitor therapy, either on trial or as part standard of care (FDA, HC) [46, 47]. In following the EC

subtyping guidelines we also chose to retain 8 so-called “multiple classifier” specimens with abnormal p53 IHC and MMRd in the MMRd subtype [34]. Within EC such dual-class cases have outcomes similar to MMRd. Unfortunately, our small cohort of 8 cases (7 with follow up, 2 of which were censored prior to 2 years) is insufficient to address this rare but curious group.

p53abn ENOC were substantially less common than p53abn EC, which appears to be entirely due to non-endometrioid p53abn EC, as the frequency of p53abn EEC was much lower than p53abn ENOC. We also observed subclonality in p53 mutation (2/49 ENOC) which may indicate this alteration is not an obligate truncal/tumor initiating event. Because of our use of IHC (combination of WT1/p53 to identify HGSOC) we can exclude that misclassification of HGSOC as ENOC caused the higher frequency in ENOC than EEC. However, we cannot exclude that misclassification of p53abn EEC as non-endometrioid EC may have contributed to the low p53abn EEC frequency. Objective, biomarker-integrated histotype diagnosis across EC may be needed prior to further validation.

Finally, the substantially higher frequency of tumors with NSMP may also suggest additional, yet to be identified, features within this subtype provide a particular advantage in colonizing the ovarian microenvironment. While NSMP ENOC have generally a favorable prognosis, some cases in the low-stage/low-risk setting which did not receive adjuvant therapy did succumb to the disease suggesting there is in fact a broad spectrum of outcomes within this subtype. As the NSMP is considerable (73.2% of cases), with many having no progression or disease-specific death events, additional biomarkers are needed to identify specific patients within this group that may have no additional benefit from chemotherapy (potential overtreatment) versus those in need of more aggressive management [14, 48-50].

Our larger cohort also allowed us a unique opportunity to evaluate the current standard of ovarian carcinoma clinical/prognostic risk factors within the ENOC histotype. As expected, patients were generally younger (mean: 57 years) than expected for HGSOC patients (mean: 60-62 years[2, 14, 15] or older [51]). Similarly, our cohort of ENOC were 81% stage I/II, in contrast to HGSOC where stage I/II cases are relatively rare (mean:18-19.5%) [14, 15]. We confirmed the prognostic relevance of clinically established factors (stage, residual disease and grade) in ENOC patients. This provides validation to the WHO’s endorsement of FIGO grading for ENOC based on extrapolation of the same schema used for EEC, without previous studies showing independent prognostic significance [52]. Despite being the first to show significance of grade in multivariable analysis, it is important to note that we were unable to replicate this association within the important low-stage subset. However, molecular subtype is prognostic at low-stage and may have the potential to better inform treatment guidelines in this group by supplementing or replacing grade. For example, 15% of p53abn ENOC were assigned to grade 1 and some of these cases did not receive adjuvant therapy. Molecular subtype may have

stronger prognostic association by virtue of identifying key drivers of oncogenic pathways in ENOC. Objective precision and reproducibility has been demonstrated for the key subtype biomarker used in our study, something that has been lacking for grade [53-56].

Particular strengths of our study are its size, totality in clinicopathological annotation, relatively long follow up time, biomarker-integrated review for inclusion of ENOC histotype, and use of validated biomarkers for molecular subtype classification. However, even with a long window for clinical follow-up data (almost 35 years for one cohort) a large fraction of our series was eventually lost to follow up. We also lacked substantial overlapping whole genome data to support genomic phenotypes; in particular, we lacked functional data on two observed *POLE* VUS (p.S421N and p.D462E) leading to them being omitted from POLEmut. Still, all cases with overlapping genomic data were concordant with predicted phenotypes. Despite a large cohort we did not have sufficient number or heterogeneity in management to properly address concerns around under- vs over-treatment.

Finally, the conclusions above generally follow the assumption that both EEC and ENOC are etiologically the same disease - presenting at different anatomical sites - a theory supported by substantial, albeit circumstantial, evidence [7-12]. While molecular classification clearly brings valuable prognostic data, further investigation of the broad range of ENOC covered by the NSMP class is still needed, as is a validation of our classification results with particular emphasis on low-stage disease and potential to modify treatment guidelines. Such studies stand to bring considerable precision to cancer management decisions by both healthcare professionals and women diagnosed with ENOC.

Figure Legends

Figure 1: Kaplan-Meier survival curves for disease-specific survival (DSS) in endometrioid ovarian carcinoma (ENOC) showing log-rank p-values and numbers and numbers at risk. Cross hatches represent censoring and shaded areas represent the 95% confidence bands. **(A)** DSS for the entire cohort by FIGO Stage I, II, III, IV. **(B)** DSS for entire cohort by presence of residual disease, where "none" is defined as no visible macroscopic disease after primary debulking surgery. **(C)** DSS for the entire cohort by Grade 1, 2, 3 **(D)** DSS in low-stage (IIA or less) ENOC with categories split by Grade 1, 2, 3. Similar results, including detail on overall and progression-free survival can be found in supplemental Figure S2.

Figure 2: Results from molecular classification of ENOC, note that all included samples had interpretable IHC data for MMR markers and p53, as well as *POLE* sequencing. **(A)** Molecular subtype assignment using surrogate biomarkers described for endometrial carcinoma and classification scheme following the current recommendations for endometrial carcinoma (McAlpine et al., 2018) prioritizing POLEmut, then MMRd, then p53abn and finally NSMP molecular subtypes. **(B)** Oncoplot outlining our full cohort of cases (in columns) along with molecular class, individual biomarkers that define each class, as well as clinicopathological features. A full-size version of the oncoplot can be found in Figure S3. For MMR data, a subset of cohorts used a two-marker IHC strategy on full section slides, whereas the majority of specimens were subject to four-markers IHC in TMA format. See also supplemental Figure S1. **(C-D-E)** Illustrate OS and PFS (POLEmut (n=18), NSMP (n=370), MMRd (n=69), p53abn (n=48)), and DSS (POLEmut (n=17), NSMP (n=365), MMRd (n=69), p53abn (n=46)) Kaplan-Meier survival curves, respectively, by molecular subtype for the entire cohort. **(F-G-H)** similarly illustrate OS and PFS (POLEmut (n=11), NSMP (n=216), MMRd (n=28), p53abn (n=19)), and DSS (POLEmut (n=11), NSMP (n=215), MMRd (n=28), p53abn (n=19)) Kaplan-Meier survival curves, respectively, restricted to low-stage (FIGO I – IIA) ENOC cases. Numbers at risk for C to H can be found in Table S6.

Figure 3: Comparison of ENOC and EC/EEC molecular subtypes, EC/EEC data are combined from Talhouk et al., 2015, Talhouk et al., 2017, and Kommoss et al., 2018. **(A)** Proportions of surrogate-biomarker defined molecular subtypes in ENOC, EC and EEC. **(B)** 5-year survival rates for ENOC, EC and EEC.

Table 1: Univariable associations between molecular subtypes and clinicopathological variables in ENOC.

Table 2: 10-year-survival-rates, Hazard Ratios and multivariable survival of molecular subtypes in ENOC.

Table 3: Number (and percent) of disease-specific events in low-stage (FIGO I – IIA) ENOC patients compared to their respective actual treatment profile, whether or not

they received adjuvant chemotherapy. For each of the four molecular subtypes we further display rows defined by current guidelines (Colombo et al., 2019, Armstrong et al., 2019) for treatment with adjuvant chemotherapy, i.e. group less likely to be referred for adjuvant chemotherapy (FIGO IA/B and Grade 1 or 2) versus group more likely to be referred for adjuvant chemotherapy (FIGO IA/B and G3, FIGO IC/IIA any grade).

Supplemental Figure Legends

Figure S1: Workflow of **(A)** biomarker analyses and **(B)** case numbers for each contributing center. *London performed whole section IHC for PMS2, MSH6, WT1, p53; Groningen and Leiden performed whole section IHC for MLH1, PMS2, MSH6, WT1, p53.

Figure S2: (A) OS and **(E)** PFS of ENOC patients by FIGO Stage I, II, III, IV. **(B)** OS and **(F)** PFS of ENOC patients by any or no residual disease after surgery. **(C)** OS and **(G)** PFS of ENOC patients by grade in cases of all stages and **(D, H)** in low-stage (FIGO IIA and less) cases.

Figure S3: Enlarged version of the oncoplot from Figure 2B outlining our full cohort of cases (in columns) along with molecular class, individual biomarkers that define each class, as well as clinicopathological features. Note that for MMR IHC markers, a subset of cohorts used a two-marker IHC strategy on full section slides, whereas the majority of specimens were subject to four-markers IHC in TMA format. See also supplemental Figure S1.

Figure S4: Comparison of whole genome sequencing (WGS) data and molecular subtype derived from surrogate IHC and *POLE* sequencing biomarkers. Overlap of surrogate biomarker and WGS was available for 1 POLEmut, 1 p53abn, 5 MMRd (including 3 that also had abnormal p53 staining), and 8 NSMP. WGS data, COSMIC signature probabilities, and copy number data as reported in supplemental materials in Wang, Bashashati, et al. Nat Genet. 2017 Jun;49(6):856-865. While our data are anecdotal due to small numbers they are consistent with reports from endometrial cancer. **(A)** Shows the contribution of a dysfunctional POLE DNA damage profile (S.POLE) COSMIC signature #10 across samples with surrogate biomarker based molecular subtypes. **(B)** Shows the contribution of a mismatch repair deficient DNA damage profile (S.MMR) COSMIC signature #6 across samples with surrogate biomarker based molecular subtypes. **(C)** Shows the fraction of the genome involved in copy number changes as a sum of amplifications (AMP; with copy number ≥ 4), deletions (Del), and Loss of heterozygosity events (LOH; including copy-neutral and allelic imbalance).

Table S1: Number of cases, study design, year of diagnosis, month/year of last follow-up, constitutional reviewer (Initials), IHC based review (Initials) and TMA format per center. *9 cases from 2014-2016 were diagnosed only by the local gyn-

path specialist). **Some cases were represented on more than one TMA. All scores from all available TMA cores of a case where considered for IHC results.

Table S2: Primer sequences for Sanger sequencing (NL/UK and Heidelberg cohort) and NGS strategy (CS1 and CS2 tailed).

Table S3: List of all observed *POLE* variants.

Table S4: Univariable associations between Cohort and clinicopathological features comparing Canadian and European cohorts.

Table S5: Multivariate analysis of established risk factors (age, FIGO stage, grade, residual disease) in endometrioid ovarian carcinoma.

Table S6: Numbers at risk of OS, PFS and DSS KM plots of the molecular subtypes in the cohort of all stages as well as low-stage cases (FIGO Stage I-IIA) in Figure 2 C to H.

Table S7: Univariable survival of endometrioid ovarian carcinoma molecular subtypes and clinicopathological variables in low-stage (FIGO I-IIA).

Table S8: Multivariable survival of endometrioid ovarian carcinoma molecular subtypes and clinicopathological variables in low-stage (FIGO I-IIA).

Table S9: Univariable Associations between Molecular subtypes in EC and ENOC.

Table S10: Univariable Associations between Molecular subtypes in EEC and ENOC.

Table S11: ENOC models using 5-year censoring on survival data, for comparison with previously published 5-year censored data from EC. See also Figure 3.

Abbreviations used in this manuscript

COEUR - Canadian Ovarian Experimental Unified Resource
COSMIC - Catalogue of Somatic Mutations in Cancer
dbSNP - Database of Single Nucleotide Polymorphisms
DSS - Disease-specific survival
EC - Endometrial carcinoma
EEC - Endometrioid endometrial carcinoma
ENOC - Endometrioid ovarian carcinoma
FFPE - Formalin-Fixed, Paraffin-Embedded
FIGO - Fédération Internationale de Gynécologie et d'Obstétrique
GER - Germany
G - Grade
HGSOC - High-grade serous ovarian carcinoma
HR - Hazard Ratio
IHC - Immunohistochemistry

MMRd - Mismatch repair deficient (subtype designation)
NGS - Next Generation Sequencing
NL - Netherlands
NSMP - No specific molecular profile (subtype designation)
NOS - Not otherwise specified
OS - Overall survival
OVCARE - British Columbia's Gynecological Cancer Research team
p53abn - p53 abnormal (subtype designation showing abnormal p53 IHC staining pattern)
PFS - Progression-free survival
POLE - DNA Polymerase Epsilon
POLEmut - pathogenic POLE mutant (subtype designation)
ProMisE - Proactive Molecular Risk Classifier for Endometrial Cancer
TCGA - The Cancer Genome Atlas network
TMA - Tissue Microarray
TP53 - Tumor suppressor Protein 53
UK - United Kingdom
VAF - Variant Allele Frequency
VUS - (Somatic) Variants of Unknown Significance
WGS - Whole genome sequencing

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References:

1. Kobel, M., et al., *Ovarian carcinoma subtypes are different diseases: implications for biomarker studies.* PLoS Med, 2008. **5**(12): p. e232.
2. Cancer Genome Atlas Research Network, *Integrated genomic analyses of ovarian carcinoma.* Nature, 2011. **474**(7353): p. 609-15.
3. Kobel, M., et al., *Differences in tumor type in low-stage versus high-stage ovarian carcinomas.* Int J Gynecol Pathol, 2010. **29**(3): p. 203-11.
4. Committee on the State of the Science in Ovarian Cancer Research, et al., eds. *Ovarian Cancers: Evolving Paradigms in Research and Care*. 1 ed. Institute of Medicine, ed. E. National Academies of Sciences, and Medicine. 2016, The National Academies Press: Washington, D.C.
5. Anglesio, M.S. and P.J. Yong, *Endometriosis-associated Ovarian Cancers.* Clin Obstet Gynecol, 2017. **60**(4): p. 711-727.
6. Matias-Guiu, X. and C.J.R. Stewart, *Endometriosis-associated ovarian neoplasia.* Pathology, 2018. **50**(2): p. 190-204.
7. Anglesio, M.S., et al., *Synchronous Endometrial and Ovarian Carcinomas: Evidence of Clonality.* J Natl Cancer Inst, 2016. **108**(6): p. djv428.
8. Schultheis, A.M., et al., *Massively Parallel Sequencing-Based Clonality Analysis of Synchronous Endometrioid Endometrial and Ovarian Carcinomas.* J Natl Cancer Inst, 2016. **108**(6): p. djv427.
9. Kurman, R.J., et al., eds. *WHO Classification of Tumours of Female Reproductive Organs*. 4 ed. WHO Classification of Tumours. Vol. 6. 2014, IARC-WHO Press: Lyon, France. 316.
10. McConechy, M.K., et al., *Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles.* Mod Pathol, 2014. **27**(1): p. 128-34.
11. McConechy, M.K., et al., *Endometrial Carcinomas with POLE Exonuclease Domain Mutations Have a Favorable Prognosis.* Clin Cancer Res, 2016. **22**(12): p. 2865-73.
12. Hoang, L.N., et al., *Polymerase Epsilon Exonuclease Domain Mutations in Ovarian Endometrioid Carcinoma.* Int J Gynecol Cancer, 2015. **25**(7): p. 1187-93.
13. Peres, L.C., et al., *Invasive Epithelial Ovarian Cancer Survival by Histotype and Disease Stage.* J Natl Cancer Inst, 2019. **111**(1): p. 60-68.
14. Rambau, P.F., et al., *Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an Ovarian Tumor Tissue Analysis consortium study.* J Pathol Clin Res, 2018. **4**(4): p. 250-261.
15. Le Page, C., et al., *Characteristics and outcome of the COEUR Canadian validation cohort for ovarian cancer biomarkers.* BMC Cancer, 2018. **18**(1): p. 347.
16. Armstrong, D.K., et al. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Ovarian Cancer Including Fallopian Tube Cancerand Primary Peritoneal Cancer*. 2019. *Version* **3.**2019.
17. Colombo, N., et al., *ESMO–ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†.* Annals of Oncology, 2019. **30**(5): p. 672-705.
18. Cancer Genome Atlas Research Network, K.C., Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding

L, Zhang W, Mills GB, Kucherlapati R, Mardis ER, Levine DA, *Integrated genomic characterization of endometrial carcinoma.* Nature, 2013. **497(7447)**: p. 67-73.
19. Talhouk, A., et al., *A clinically applicable molecular-based classification for endometrial cancers.* Br J Cancer, 2015. **113**(2): p. 299-310.
20. Stelloo, E., et al., *Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts.* Clin Cancer Res, 2016. **22**(16): p. 4215-24.
21. McAlpine, J., A. Leon-Castillo, and T. Bosse, *The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses.* J Pathol, 2018. **244**(5): p. 538-549.
22. Parra-Herran, C., et al., *Molecular-based classification algorithm for endometrial carcinoma categorizes ovarian endometrioid carcinoma into prognostically significant groups.* Mod Pathol, 2017. **30**(12): p. 1748-1759.
23. Cybulska, P., et al., *Molecular profiling and molecular classification of endometrioid ovarian carcinomas.* Gynecol Oncol, 2019. **154**(3): p. 516-523.
24. Kobel, M., et al., *An Immunohistochemical Algorithm for Ovarian Carcinoma Typing.* Int J Gynecol Pathol, 2016. **35**(5): p. 430-41.
25. Dieters-Castator, D.Z., et al., *Proteomics-Derived Biomarker Panel Improves Diagnostic Precision to Classify Endometrioid and High-grade Serous Ovarian Carcinoma.* Clin Cancer Res, 2019. **25**(14): p. 4309-4319.
26. McConechy, M.K., et al., *Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas.* Gynecol Oncol, 2015. **137**(2): p. 306-10.
27. Roth, R.M., et al., *Discordant Mismatch Repair Protein Immunoreactivity in Lynch Syndrome-Associated Neoplasms: A Recommendation for Screening Synchronous/Metachronous Neoplasms.* Am J Clin Pathol, 2016. **146**(1): p. 50-6.
28. Kobel, M., et al., *Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma.* J Pathol Clin Res, 2016. **2**(4): p. 247-258.
29. Kobel, M., et al., *Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility.* Int J Gynecol Pathol, 2019. **38 Suppl 1**: p. S123-S131.
30. Kommoss, S., et al., *Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series.* Ann Oncol, 2018. **29**(5): p. 1180-1188.
31. Leon-Castillo, A., et al., *Interpretation of somatic POLE mutations in endometrial carcinoma.* J Pathol, 2020. **250**(3): p. 323-335.
32. Singh, N., et al., *Extrauterine high-grade serous carcinomas with bilateral adnexal involvement as the only two disease sites are clonal based on tp53 sequencing results: implications for biology, classification, and staging.* Mod Pathol, 2017.
33. Do, H. and A. Dobrovic, *Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization.* Clin Chem, 2015. **61**(1): p. 64-71.
34. Leon-Castillo, A., et al., *Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas.* J Pathol, 2020. **250**(3): p. 312-322.
35. Balduzzi, S., G. Rucker, and G. Schwarzer, *How to perform a meta-analysis with R: a practical tutorial.* Evid Based Ment Health, 2019. **22**(4): p. 153-160.
36. Talhouk, A., et al., *Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer.* Cancer, 2017. **123**(5): p. 802-813.
37. Wang, Y.K., et al., *Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes.* Nat Genet, 2017. **49**(6): p. 856-865.
38. Setiawan, V.W., et al., *Type I and II endometrial cancers: have they different risk factors?* J Clin Oncol, 2013. **31**(20): p. 2607-18.
39. Wentzensen, N., et al., *Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium.* J Clin Oncol, 2016. **34**(24): p. 2888-98.

40. Clarke, M.A., et al., *A prospective clinical cohort study of women at increased risk for endometrial cancer.* Gynecol Oncol, 2020. **156**(1): p. 169-177.
41. Soong, T.R., et al., *The fallopian tube, "precursor escape" and narrowing the knowledge gap to the origins of high-grade serous carcinoma.* Gynecol Oncol, 2019. **152**(2): p. 426-433.
42. Temko, D., et al., *Somatic POLE exonuclease domain mutations are early events in sporadic endometrial and colorectal carcinogenesis, determining driver mutational landscape, clonal neoantigen burden and immune response.* J Pathol, 2018. **245**(3): p. 283-296.
43. McAlpine J.N., N.R., Kommoss S., Church D., Bellone S., Clarke B., DeLair D., Leung S., Britton H., Bernardini M., Lee C.H., Ngeow J.Y., Soslow R., Brucker S., Koebel M., Santin A., Gilks C.B., Goodfellow P., Bosse T., Talhouk A., *Survival benefit in women with endometrial cancers harboring pole mutations may be independent of adjuvant therapy*, in *17th Biennial Meeting of the International Gynecologic Cancer Society*. 2018, Int J Gynecol Cancer: Kyoto, Japan. p. 42.
44. Nakonechny, Q.B. and C.B. Gilks, *Ovarian Cancer in Hereditary Cancer Susceptibility Syndromes.* Surg Pathol Clin, 2016. **9**(2): p. 189-99.
45. Rambau, P.F., et al., *Significant frequency of MSH2/MSH6 abnormality in ovarian endometrioid carcinoma supports histotype-specific Lynch syndrome screening in ovarian carcinomas.* Histopathology, 2016. **69**(2): p. 288-97.
46. Merck Sharp & Dohme Corp. *(KEYTRUDA Prescribing information).* U.S. Food & Drug Administration website. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/125514s040lbl.pdf, Revised 2019. **Access date** 03/27/2020.
47. *Regulatory Decision Summary for Keytruda.* Health Canada. https://hpr-rps.hres.ca/reg-content/regulatory-decision-summary-detail.php?lang=en&linkID=RDS00514, 2019. **Access date 04/04/2020**.
48. Rambau, P., et al., *Association of Hormone Receptor Expression with Survival in Ovarian Endometrioid Carcinoma: Biological Validation and Clinical Implications.* Int J Mol Sci, 2017. **18**(3).
49. Wang, L., et al., *Nuclear beta-catenin and CDX2 expression in ovarian endometrioid carcinoma identify patients with favourable outcome.* Histopathology, 2019. **74**(3): p. 452-462.
50. Hollis, R.L., et al., *Hormone receptor expression patterns define clinically meaningful subgroups of endometrioid ovarian carcinoma.* Gynecol Oncol, 2019. **155**(2): p. 318-323.
51. Torre, L.A., et al., *Ovarian cancer statistics, 2018.* CA Cancer J Clin, 2018. **68**(4): p. 284-296.
52. Parra-Herran, C., et al., *FIGO Versus Silverberg Grading Systems in Ovarian Endometrioid Carcinoma: A Comparative Prognostic Analysis.* Am J Surg Pathol, 2019. **43**(2): p. 161-167.
53. Singh, N., et al., *p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies.* J Pathol, 2020. **250**(3): p. 336-345.
54. Talhouk, A., et al., *Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: Earlier prognostic information to guide treatment.* Gynecol Oncol, 2016. **143**(1): p. 46-53.
55. Gilks, C.B., et al., *Tumor cell type can be reproducibly diagnosed and is of independent prognostic significance in patients with maximally debulked ovarian carcinoma.* Hum Pathol, 2008. **39**(8): p. 1239-51.
56. Werner, H.M., et al., *A discordant histological risk classification in preoperative and operative biopsy in endometrial cancer is reflected in metastatic risk and prognosis.* Eur J Cancer, 2013. **49**(3): p. 625-32.