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Kramer, P.; Talhouk, A.; Brett, M.A.; Chiu, D.S.; Cairns, E.S.; Scheunhage, D.A.; ... ; Anglesio, M.S.

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Pauline Krämer[1,2], Aline Talhouk[2,3], Mary Anne Brett[4], Derek S. Chiu[3,5], Evan S. Cairns[2], Daniëlla A. Scheunhage[6], Rory F.L. Hammond[7], David Farnell[3,8], Tayyebeh M. Nazeran[3,8], Marcel Grube[1], Zhouchunyang Xia[3,8], Janine Senz[3,8], Samuel Leung[3,8], Lukas Feil[1,2], Jana Pasternak[1], Katherine Dixon[9], Andreas Hartkopf[1], Bernhard Krämer[1], Sara Brucker[1], Florian Heitz[10,11], Andreas du Bois[10], Philipp Harter[10], Felix K.F. Kommoss[12], Hans-Peter Sinn[12], Sabine Heublein[13], Friedrich Kommoss[14], Hans-Walter Vollert[15], Ranjit Manchanda[16,17], Cornelis D. de Kroon[18], Hans W. Nijman[19], Marco de Bruyn[19], Emily F. Thompson[3,8], Ali Bashashati[3,5,8,20], Jessica N. McAlpine[2,3], Naveena Singh[7], Anna V. Tinker[3,21], Annette Staebler[22], Tjalling Bosse[6], Stefan Kommoss[1], Martin Köbel[23], and Michael S. Anglesio[2,3]

ABSTRACT

Purpose: Endometrioid ovarian carcinoma (ENOC) is generally associated with a more favorable prognosis compared with 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 The Cancer Genome Atlas–inspired endometrial carcinoma (EC) molecular subtyping in a cohort of ENOC.

Experimental Design: IHC 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), and moderate-risk with no specific molecular profile (NSMP). Survival analysis with established clinicopathologic and subtype-specific features was performed.

Results: A total of 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 multivariate analysis.

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

Introduction

Today, the scientific community widely agrees that ovarian carcinoma is a heterogeneous disease and that different histologic types are best considered as different disease entities (1). The next step toward 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

[1]Department of Women's Health, Tuebingen University Hospital, Tuebingen, Germany. [2]Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada. [3]BC Cancer, Vancouver General Hospital, and University of British Columbia, British Columbia's Gynecological Cancer Research Team (OVCARE), Vancouver, British Columbia, Canada. [4]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada. [5]Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, British Columbia, Canada. [6]Department of Pathology, Leiden University Medical Centre (LUMC), Leiden, the Netherlands. [7]Barts Health National Health Service Trust, Department of Pathology, London, United Kingdom. [8]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada. [9]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada. [10]Kliniken Essen Mitte, Department of Gynecology and Gynecologic Oncology, Essen, Germany. [11]Charité Campus Virchow-Klinikum, Department for Gynecology with the Center for Oncologic Surgery, Berlin, Germany. [12]Heidelberg University Hospital, Institute of Pathology, Heidelberg, Germany. [13]Department of Obstetrics and Gynecology, Heidelberg University Hospital, Heidelberg and National Center for Tumor Diseases, Heidelberg, Germany. [14]Medizin Campus Bodensee, Institute of Pathology, Friedrichshafen, Germany. [15]Department of Obstetrics and Gynecology, Medizin Campus Bodensee, Friedrichshafen, Germany. [16]Barts CRUK Cancer Centre, Wolfson Institute of Preventative Medicine, Queen Mary University of London, Charterhouse Square, London, United Kingdom. [17]Department of Gynaecological Oncology, St. Bartholomew's Hospital, London, United Kingdom. [18]Department of Gynecology, Leiden University Medical Centre (LUMC), Leiden, the Netherlands. [19]Department of Obstetrics and Gynecology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands. [20]School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada. [21]Department of Medical Oncology, BC Cancer, Vancouver, British Columbia, Canada. [22]University Hospital Tuebingen, Institute of Pathology and Neuropathology, Tuebingen, Germany. [23]Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada.

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P. Krämer and A. Talhouk contributed equally as co-first authors of this article.

Corresponding Author: Michael S. Anglesio, University of British Columbia, 2660 Oak Street, OVCARE, Vancouver, British Columbia V6H 3Z6, Canada. Phone: 1-604-875-4111; E-mail: m.anglesio@ubc.ca

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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 histologic similarity with endometrial carcinomas (EC), in particular endometrioid EC, 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 clinicopathologic 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.

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 approximately 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 histopathologic presentation (7–12). Compared with 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 (OS) rates (~80% 5-year survival; ref. 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 was 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 were split by fraction of their genomes involved in copy-number alterations: copy-number low and copy-number high (18). Following TCGA study, two groups simultaneously derived near-identical minimal biomarker-based surrogates for TCGA EC molecular subtypes (19–21). The end result, an algorithm referred to as the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), uses IHC markers and targeted sequencing of *POLE* to identify three molecular subtypes: (i) *POLE* mutant "POLEmut": defined by pathogenic *POLE* exonuclease domain mutations that identify a group with favorable outcome and an ultramutation phenotype, (ii) mismatch repair deficient "MMRd": defined by IHC markers for DNA mismatch repair complex (MLH1, PMS2, MSH2, and MSH6) and identifies cases with microsatellite instability and a corresponding hypermutation phenotype, and (iii) 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 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 for patients with EC (21).

Similarities between EC and ENOC have led several groups to hypothesize that ENOC could be stratified into the same four molecular subtypes as seen in EC. Parra-Herran and colleagues showed the general feasibility of this approach, with their conclusions hindered by a small cohort (22). Cybulska and colleagues 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 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 this 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.

Materials and 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 nine centers across four countries ($n = 604$). Tissue samples were provided from Canadian and European centers: Department of Women's Health, Tuebingen University Hospital (Tuebingen, Germany); Department of Gynecology and Gynecologic Oncology, Kliniken Essen Mitte (Essen, Germany); Department of Obstetrics and Gynecology, Heidelberg University Hospital (Heidelberg, Germany); Department of Gynecology and Obstetrics, Medizin Campus Bodensee (Friedrichshafen, Germany); Department of Gynecological Oncology, Barts Health National Health Service Trust (London, United Kingdom); Department of Gynecology, Leiden University Medical Centre (Leiden, the Netherlands); Department of Obstetrics and Gynecology, University Medical Center Groningen (Groningen, the Netherlands); the OVCARE Gynecological Tissue Bank (Vancouver, British Columbia, Canada); and the Canadian Ovarian Experimental Unified Resource (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 the Netherland and United Kingdom used tissue

microarrays (TMA) for IHC markers. United Kingdom and the Netherland studies used full section IHC (Supplementary Fig. S1A).

All samples were subjected to WT1 and p53 IHC. 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 (Supplementary Fig. S1B).

We analyzed the cohort as a whole as well as considering a subset of low-stage [Federation Internationale des Gynaecologistes et Obstetristes (FIGO) I–IIA; ref. 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 understaged ($n = 9$) 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 NOS ($n = 17$). A single case was also reported to have dedifferentiated features, this case was omitted from grade-specific analyses.

Molecular subtype assignment

IHC

Because of contributions from various centers, there are slight differences in assays performed on different cohorts as outlined below (see also Supplementary Fig. S1; Supplementary Table S1). MMRd was assigned by IHC using four mismatch repair pathway markers. Staining was performed on a Dako Omni Platform with 30-minute heat-induced pretreatment 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 nontumor cells on each evaluated core (internal positive control). Cases were considered 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-minute heat-induced pretreatment 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 normal/wild-type p53 represented by variable intensity and distribution of staining in tumor cell nuclei (29).

POLE sequencing

DNA was extracted from formalin-fixed, paraffin-embedded (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 Supplementary Table S2.

For all studies, except United Kingdom, the Netherlands, 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 >1,700× (per amplicon; Supplementary 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 the United Kingdom, the Netherlands, and Heidelberg, Sanger sequencing was performed over POLE hotspots noted above. Primer sets for the United Kingdom and Netherlands cohorts also included pathogenic variants p.P436R and p.M444K. Technical repeat was performed for any observed variants (see also Supplementary Fig. S1A). Note that neither NGS nor Sanger sequencing strategies provided coverage for rare pathogenic variants in exon 11 (p.F367S and p.D368Y; ref. 31).

POLE variants were classified as (i) germline based on reference to dbSNP and consistent allele frequency, (ii) pathogenic somatic variants based on presence in COSMIC and corroborating data from genomic studies with evidence of an ultramutated phenotype (18, 23), (iii) nonpathogenic somatic variants that are observed in other genomic studies but without an ultramutated phenotype, and (iv) 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 (Supplementary Table S3; ref. 31).

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

Pearson's χ^2 test was used to evaluate univariate associations for categorical variables and Welch one-way test for continuous variables. Differences in univariate survival outcomes were analyzed using the log-rank test. To evaluate the independent prognostic significance of molecular classification, the Cox proportional hazards model was used, adjusting for known clinicopathologic risk variables. A Firth bias reducing correction was applied in the calculation of HR estimates, when more than 80% censoring was present in any one category of the variable of interest. The profile likelihood was 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 was 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 patients with ENOC with full subtype information available ($n = 511$). The cohort was first examined in the context of clinicopathologic 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 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 at December 31 of the 10th year postdiagnosis to minimize ascertainment bias and ensure noninformative censoring. Molecular subtyping was then analyzed alone and in context with noted features. Finally, ENOC data were compared with data from EC studies (19, 30, 36).

Results

Cohort description

Patients were diagnosed between 1985 and 2018 (Supplementary 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 grade 1, 34% grade 2, and 20% grade 3. Clinicopathologic variables were not significantly different between European and Canadian cohorts (Supplementary 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, and PFS) were all more favorable in patients with low-stage disease and no residual tumor ($P < 0.001$ for all; **Fig. 1A** and **B**; Supplementary Fig. S2). Prognostic value of grade was significant in OS, DSS, and PFS analyses for the full cohort ($P < 0.001$ for all; **Fig. 1C**; Supplementary Fig. S2), with grade 3 cases performing worse. However, when restricted to low stage, grade was no longer prognostically significant ($P = 0.538$; **Fig. 1D**; Supplementary Fig. S2).

Multivariate analysis of established risk factors is shown in Supplementary Table S5. With a DSS HR of 3.5, stage was the strongest prognosticator across patients with ENOC 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 (Supplementary 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 eight cases, and a single case was disqualified from classification due to uninterpretable p53 staining. A total of 511 cases were fully subtyped, and all results are restricted to this set (**Fig. 2A**; Supplementary Fig. S1B). Eighteen cases (3.5%) harbored pathogenic *POLE* mutations (POLEmut; **Fig. 2A** and **B**; Supplementary Fig. S3; Supplementary 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 eight cases that were also p53abn by IHC and six cases with heterogeneity in MMR marker scores across multiple replicate TMA cores. A total of 49 cases were assigned to p53abn (9.6%; not including eight assigned to MMRd), two of which showed heterogeneity in p53 IHC between TMA cores suggesting potential subclonality of *TP53* mutation. The remaining 374 cases (73.2%) were NSMP (**Fig. 2A** and **B**; Supplementary Fig. S3).

In a subset of 15 cases, whole-genome sequencing data were available from a previously published study (37). This enabled us to verify the expected genomic profiles (Supplementary Fig. S4).

Clinicopathologic associations of molecular subtypes

Significant univariate association was observed between age, stage, grade, residual disease, and postsurgical chemotherapy and molecular subtype (**Table 1**; see also **Fig. 2B**; Supplementary Fig. S3). Patients with POLEmut ENOC were generally younger (median, 45 years) and 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 postsurgical treatment compared with 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 showed distinct survival outcomes in all three endpoints (**Fig. 2C–E**; $P < 0.001$ for all). No disease-specific deaths were observed in POLEmut patients, one POLEmut patient died of a nondisease-related cause. p53abn patients had a disease-specific 10-year survival rate of only 39% (**Table 2**). For POLEmut, median OS time was not reached, in NSMP group it was 18.1 years, in MMRd group 12.3, and in p53abn group 4.7 years.

After adjusting for currently used clinical risk factors (stage, grade, age, and residual disease) and postsurgical chemotherapy in multivariate analysis, molecular subtypes were still statistically significant for OS, DSS, and PFS ($P < 0.001$; **Table 2**). Among clinicopathologic features, multivariate analysis showed age to be significant only for OS, while postsurgical chemotherapy retained significance only for OS and PFS (**Table 2**).

Stratified analysis of low-stage ENOC

In univariate analysis of low-stage ENOC, molecular subtypes were associated with substages ($P = 0.032$) but not with age, grade, or postsurgical chemotherapy (Supplementary Table S7). Outcomes of molecular subtypes (OS, DSS, and PFS) were still statistically different (**Fig. 2F–H**). In multivariate analysis, correcting for age, stage, grade, and postsurgical chemotherapy, subtypes retained significance for OS ($P = 0.004$), DSS ($P = 0.034$), and PFS ($P = 0.048$). However, among other variables, only grade was significant in OS (Supplementary Table S8).

We further stratified outcomes of patients with low-stage ENOC (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 and IC/IIA) according to the current international guidelines (**Table 3**; refs. 16, 17). Patients with POLEmut ENOC had neither disease-specific deaths nor progression. In the subset more likely to be recommended for adjuvant chemotherapy, patients with p53abn and MMRd ENOC had proportionally more disease-specific deaths, if they did not receive adjuvant treatment. Among low-stage NSMP, there were three (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 (χ^2 test; both $P < 0.001$).

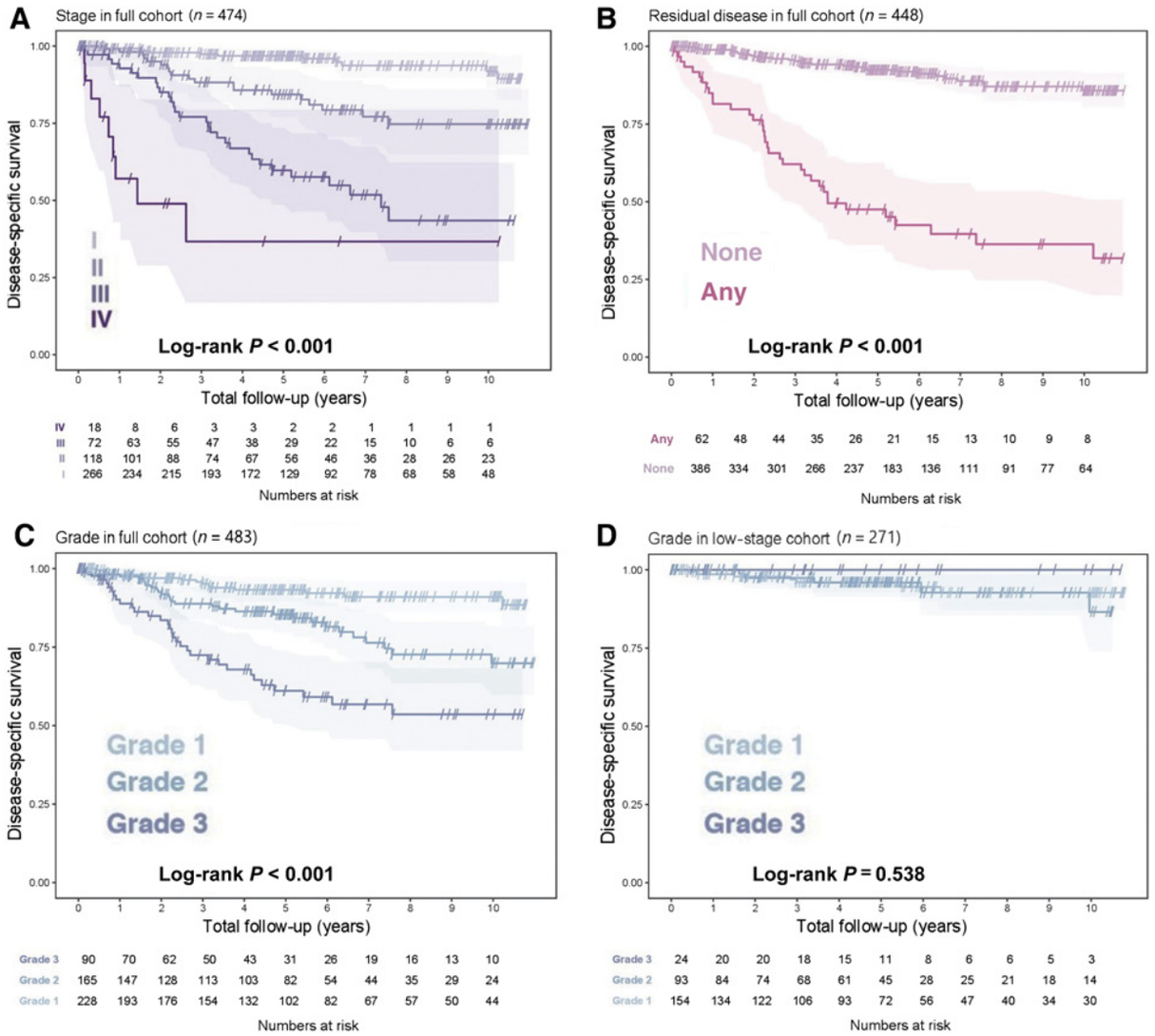


Figure 1.

Kaplan–Meier survival curves for DSS in ENOC showing log-rank *P* values 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, and 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, and 3. **D,** DSS in low-stage (IIA or less) ENOC with categories split by grade 1, 2, and 3. Similar results, including detail on OS and PFS can be found in Supplementary Fig. S2.

Compared with all EC, POLEmut, MMRd, and p53abn cases were all less frequent in ENOC, while the NSMP group was substantially larger (**Fig. 3A**). However, when we restricted to EEC, the number of p53abn cases (5.6%) dropped in comparison with ENOCs (9.6%), trends in the other subtypes remained unchanged (**Fig. 3A**).

Patients with EC, or EEC, were consistently older than those with ENOC across all molecular subtypes (Supplementary Tables S9 and S10). Patients with ENOC POLEmut, MMRd, and NSMP presented at a higher stage compared with EC or EEC. Stage was not significantly different in p53abn cases when comparing ENOC with all EC, but was higher in ENOC compared with EEC (Supplementary Tables S9 and 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 (Supplementary Tables S9 and S10).

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

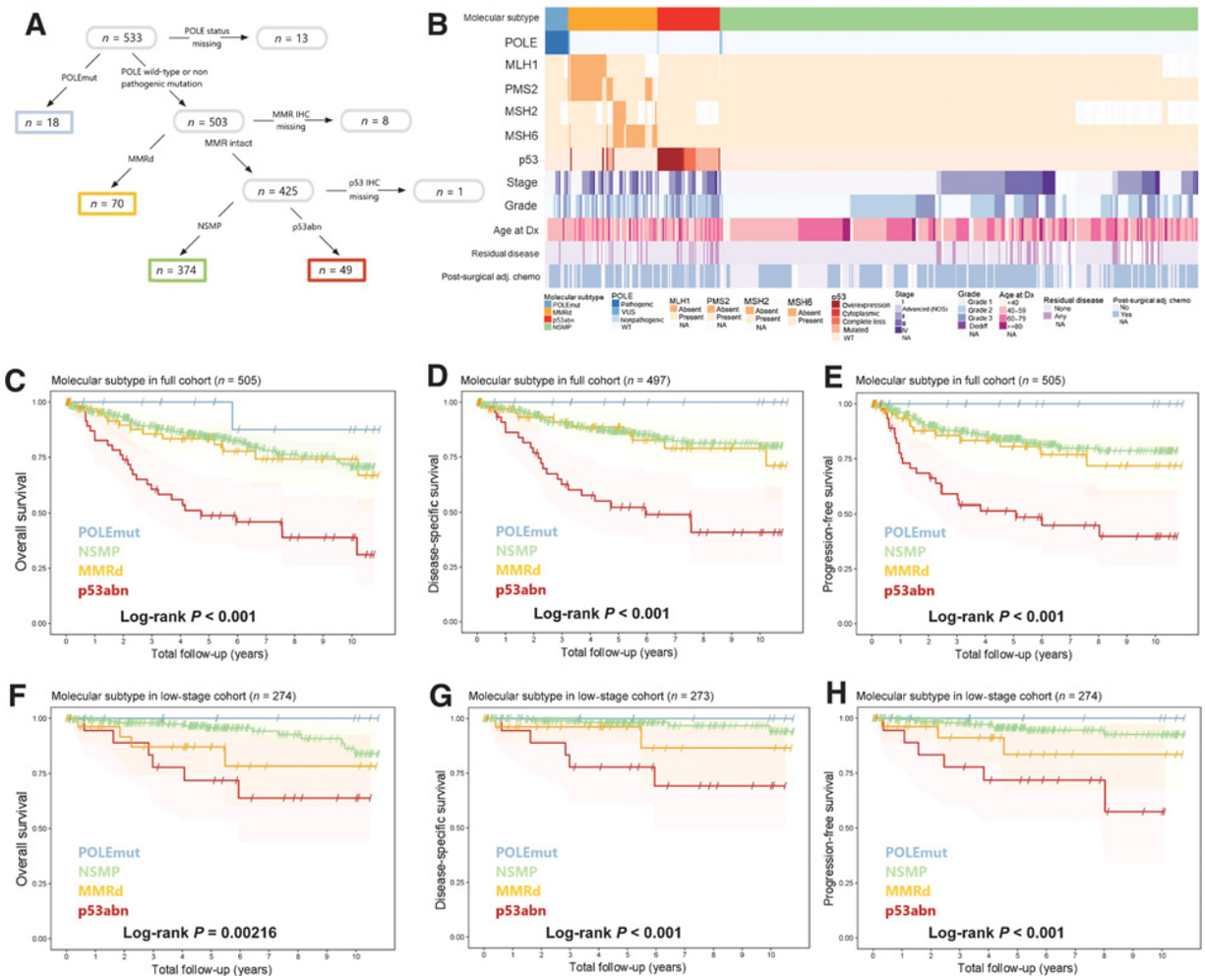


Figure 2.

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 (21) 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 clinicopathologic features. A full-size version of the oncoplot can be found in Supplementary Fig. S3. For MMR data, a subset of cohort 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 Supplementary Fig. S1. **C–E,** Illustration of OS and PFS [POLEmut ($n = 18$), NSMP ($n = 370$), MMRd ($n = 69$), and p53abn ($n = 48$)], and DSS [POLEmut ($n = 17$), NSMP ($n = 365$), MMRd ($n = 69$), and p53abn ($n = 46$)] Kaplan–Meier survival curves, respectively, by molecular subtype for the entire cohort. **F–H,** Similarly, illustration of OS and PFS [POLEmut ($n = 11$), NSMP ($n = 216$), MMRd ($n = 28$), and p53abn ($n = 19$)], and DSS [POLEmut ($n = 11$), NSMP ($n = 215$), MMRd ($n = 28$), and p53abn ($n = 19$)] Kaplan-Meier survival curves, respectively, restricted to low-stage (FIGO I–IIA) ENOC cases. Numbers at risk for **C–H** can be found in Supplementary Table S6.

remain significant in multivariate analysis independent of age, stage, grade, residual disease, and postsurgical 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 deescalation or escalation of adjuvant therapy in subsets of 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 with MMRd cases with noticeable differences in OS versus 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 with 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 (nonendometrioid) EC, which appears to have aggressive spread regardless of anatomic borders. Patients with ENOC also tended to be younger than EC regardless of molecular subtype. While patients with nonendometrioid EC are known to be

Table 1. Univariate associations between molecular subtypes and clinicopathologic variables in ENOC.

Variable	Levels	NSMP	p53abn	POLEmut	MMRd	Total	*P*
Total	*N* (%)	374 (73%)	49 (10%)	18 (4%)	70 (14%)	511 (100%)	One-way test
Age at diagnosis	Mean (SD)	58 (13)	57 (13)	47 (10)	55 (13)	57 (13)	<0.001
	Median (IQR)	56 (49–67)	57 (48–67)	45 (42–48)	53 (46–62)	55 (48–66)	
	Missing	2	0	0	0	2	
							χ^2 test
Stage	Low	219 (62%)	20 (43%)	11 (61%)	28 (42%)	278 (57%)	0.003
	Advanced	136 (38%)	27 (57%)	7 (39%)	39 (58%)	209 (43%)	
	Missing	19	2	0	3	24	
FIGO stage	I	216 (61%)	16 (34%)	11 (61%)	28 (42%)	271 (56%)	0.002
	II	82 (23%)	13 (28%)	5 (28%)	21 (31%)	121 (25%)	
	III	42 (12%)	16 (34%)	2 (11%)	15 (22%)	75 (15%)	
	IV	15 (4%)	2 (4%)	0 (0%)	3 (4%)	20 (4%)	
	Missing	19	2	0	3	24	
Grade	Grade 1	191 (52%)	7 (15%)	10 (56%)	23 (34%)	231 (47%)	<0.001
	Grade 2	116 (32%)	19 (41%)	5 (28%)	28 (41%)	168 (34%)	
	Grade 3	57 (16%)	20 (43%)	3 (17%)	17 (25%)	97 (20%)	
	Missing	10	3	0	2	15	
Residual disease	None	290 (88%)	34 (71%)	17 (94%)	54 (86%)	395 (86%)	0.010
	Any	40 (12%)	14 (29%)	1 (6%)	9 (14%)	64 (14%)	
	Missing	44	1	0	7	52	
Postsurgical chemotherapy	No	105 (31%)	8 (17%)	6 (33%)	11 (17%)	130 (28%)	0.040
	Yes	234 (69%)	39 (83%)	12 (67%)	53 (83%)	338 (72%)	
	Missing	35	2	0	6	43	
Neoadjuvant therapy	No	274 (98%)	37 (100%)	13 (100%)	46 (94%)	370 (98%)	0.236
	Yes	6 (2%)	0 (0%)	0 (0%)	3 (6%)	9 (2%)	
	Missing	94	12	5	21	132	

Abbreviation: IQR, interquartile range.

older at diagnosis than EEC (38), the age difference was still substantial when comparing ENOC with EEC. Major epidemiologic 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/noninvasive 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 nonpathogenic *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 (Supplementary Table S3). It should also be noted that our sequencing strategy may have missed a subset of less common pathogenic *POLE* mutations (see Materials and 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 deescalation 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 are still needed for NSMP.

MMRd ENOCs were also less common than observed in EC, and in particular in EEC. The difference appears, in part, because of reduced proportion of MLH1/PMS2-deficient cases in ENOC, suggesting somatic hypermethylation of the MLH1 promoter may be more prevalent in EEC compared with ENOC. Nonetheless, our results corroborate universal MMR biomarker testing in ENOC to screen for Lynch syndrome (44, 45). Patients with MMRd ENOC may be eligible for immune checkpoint inhibitor therapy, either on trial or as part standard of care (FDA, HC; refs. 46, 47). In following the EC subtyping guidelines, we also chose to retain eight 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 eight cases (seven with follow-up, two of which were censored prior to 2 years) is insufficient to address this rare but curious group.

p53abn ENOCs were substantially less common than p53abn ECs, which appear to be entirely due to nonendometrioid p53abn EC, as the frequency of p53abn EEC was much lower than p53abn ENOC. We

Table 2. Ten-year survival rates, HRs, and multivariate survival of molecular subtypes in ENOC.

10-year survival rates		**OS**		**DSS**		**PFS**	
NSMP		(*n* = 370) 0.707		(*n* = 364) 0.798		(*n* = 357) 0.784	
p53abn		(*n* = 48) 0.389		(*n* = 45) 0.39		(*n* = 46) 0.393	
POLEmut		(*n* = 18) 0.875		(*n* = 17) 1		(*n* = 18) 1	
MMRd		(*n* = 69) 0.742		(*n* = 67) 0.777		(*n* = 68) 0.716	
Full cohort		(*n* = 505) 0.68		(*n* = 493) 0.754		(*n* = 489) 0.739	
HRs		**OS**	LRT	**DSS**	LRT	**PFS**	LRT
Number of events		104/505	*P*	80/497	*P*	90/505	*P*
Reference group: NSMP							
p53abn		3.56 (2.24–5.5)[F]		4.2 (2.52–6.81)[F]		3.98 (2.43–6.33)[F]	
POLEmut		0.45 (0.05–1.66)[F]	<0.001	0.22 (0-NA)[F]	<0.001	0.17 (0-NA)[F]	<0.001
MMRd		1.2 (0.64–2.09)[F]		1.25 (0.6–2.34)[F]		1.35 (0.7–2.41)[F]	
Multivariate survival		**OS**		**DSS**		**PFS**	
Number of events		79/411		57/405		72/411	
Variable	Levels	HR (95% CI)	LRT *P*	HR (95% CI)	LRT *P*	HR (95% CI)	LRT *P*
Molecular subtype (reference group: NSMP)	p53abn	3.75 (2.17–6.34)[F]	0.001	5.32 (2.85–9.78)[F]	<0.001	3.51 (1.95–6.13)[F]	<0.001
	POLEmut	0.68 (0.08–2.54)[F]		0.63 (0–4.71)[F]		0.16 (0–1.14)[F]	
	MMRd	1.1 (0.53–2.09)[F]		1.07 (0.44–2.28)[F]		0.95 (0.44–1.87)[F]	
Age at diagnosis		1.02 (1–1.04)[F]	0.036	1.02 (1–1.04)[F]	0.088	1 (0.98–1.01)[F]	0.720
Stage (reference group: low)	Advanced	3.14 (1.62–6.17)[F]	0.001	3.91 (1.71–9.35)[F]	0.001	4.47 (2.16–9.55)[F]	<0.001
Grade (reference group: grade 1/2)	Grade 3	1.73 (1.04–2.88)[F]	0.037	1.95 (1.1–3.45)[F]	0.023	1.79 (1.05–3.02)[F]	0.036
Residual disease (reference group: none)	Any	3.06 (1.8–5.3)[F]	<0.001	3.94 (2.14–7.5)[F]	<0.001	4.09 (2.38–7.16)[F]	<0.001
Postsurgical chemotherapy (reference group: no)	Yes	0.48 (0.25–0.94)[F]	0.038	0.53 (0.23–1.28)[F]	0.174	0.45 (0.22–0.98)[F]	0.050

Note: F, indicates that the Firth penalized maximum likelihood bias reduction method was used to estimate the HR.

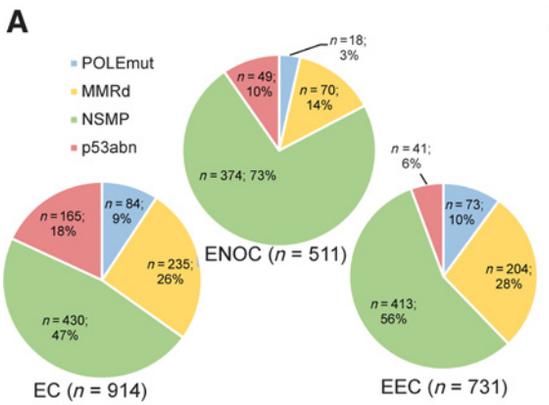
also observed subclonality in p53 status (2/49 ENOC) which may indicate this alteration is not an obligate truncal/tumor-initiating event. Because of our use of IHC (combination of WT1/p53 to identify HGSOC), we can exclude that misclassification of HGSOC as ENOC caused the higher frequency in ENOC than EEC. However, we cannot exclude that misclassification of p53abn EEC as nonendometrioid 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 ENOCs have generally a favorable prognosis, some cases in the low-stage/low-risk setting, which did not

Table 3. Number (and percent) of disease-specific events in patients with low-stage (FIGO I–IIA) ENOC compared with their respective actual treatment profile, whether or not they received adjuvant chemotherapy.

		Actual treatment received			
		Received adjuvant treatment		**Received no adjuvant treatment**	
		DSS event	**No DSS event**	**DSS event**	**No DSS event**
Treatment recommended by current guidelines	POLEmut				
	FIGO IA/B G1/2	0 (0%)	1 (100%)	0 (0%)	3 (100%)
	FIGO IA/B G3, IC, IIA	0 (0%)	4 (100%)	0 (0%)	3 (100%)
	MMRd				
	FIGO IA/B G1/2	0 (0%)	6 (100%)	0 (0%)	3 (100%)
	FIGO IA/B G3, IC, IIA	1 (10%)	9 (90%)	2 (33,3%)	4 (66,7%)
	NSMP				
	FIGO IA/B G1/2	0 (0%)	25 (100%)	3 (4.7%)	61 (95.3%)
	FIGO IA/B G3, IC, IIA	1 (1.3%)	76 (98.7%)	1 (4.6%)	21 (95.4%)
	p53abn				
	FIGO IA/B G1/2	0 (0%)	0 (0%)	0 (0%)	3 (100%)
	FIGO IA/B G3, IC, IIA	3 (25%)	9 (75%)	1 (50%)	1 (50%)

Note: For each of the four molecular subtypes, we further display rows defined by current guidelines (Colombo and colleagues, 2019; ref. 17 and National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology – Ovarian Cancer v1.2020; ref. 16) for treatment with adjuvant chemotherapy, that is, 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).



B

5-year survival rates

ENOC	OS	DSS	PFS
NSMP	(*n* = 370) 0.843	(*n* = 364) 0.866	(*n* = 357) 0.839
p53abn	(*n* = 48) 0.488	(*n* = 45) 0.511	(*n* = 46) 0.507
POLEmut	(*n* = 18) 1	(*n* = 17) 1	(*n* = 18) 1
MMRd	(*n* = 69) 0.834	(*n* = 67) 0.882	(*n* = 68) 0.804
Full cohort	(*n* = 505) 0.808	(*n* = 493) 0.834	(*n* = 489) 0.806
EC	**OS**	**DSS**	**PFS**
NSMP	(*n* = 430) 0.832	(*n* = 427) 0.908	(*n* = 393) 0.887
p53abn	(*n* = 165) 0.464	(*n* = 159) 0.551	(*n* = 135) 0.472
POLEmut	(*n* = 84) 0.885	(*n* = 83) 0.962	(*n* = 80) 0.975
MMRd	(*n* = 235) 0.719	(*n* = 231) 0.812	(*n* = 212) 0.811
EEC	**OS**	**DSS**	**PFS**
NSMP	(*n* = 413) 0.84	(*n* = 410) 0.92	(*n* = 380) 0.902
p53abn	(*n* = 41) 0.487	(*n* = 40) 0.704	(*n* = 33) 0.694
POLEmut	(*n* = 73) 0.883	(*n* = 72) 0.97	(*n* = 70) 0.971
MMRd	(*n* = 204) 0.711	(*n* = 200) 0.804	(*n* = 185) 0.809

Figure 3.

Comparison of ENOC and EC/EEC molecular subtypes, EC/EEC data are combined from Talhouk and colleagues, 2015 (19); Talhouk and colleagues, 2017 (36); and Kommoss and colleagues, 2018 (30). **A,** Proportions of surrogate biomarker–defined molecular subtypes in ENOC, EC, and EEC. **B,** Five-year survival rates for ENOC, EC, and EEC.

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 patients with HGSOC [mean, 60–62 years (2, 14, 15) or older; ref. 51]. Similarly, our cohort of ENOC was 81% stage I/II, in contrast to HGSOC where stage I/II cases were relatively rare (mean, 18%–19.5%; refs. 14, 15). We confirmed the prognostic relevance of clinically established factors (stage, residual disease, and grade) in patients with ENOC. 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 multivariate analysis, it is important to note that we were unable to replicate this association within the important low-stage subset. However, molecular subtype was 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 ENOCs 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 clinicopathologic 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- versus overtreatment.

Finally, the conclusions above generally follow the assumption that both EEC and ENOC are etiologically the same disease, presenting at different anatomic 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.

Disclosure of Potential Conflicts of Interest

P. Krämer reports non-financial support from AstraZeneca and MSD outside the submitted work. A. Hartkopf reports personal fees from GlaxoSmithKline, AstraZeneca, Roche, and Clovis outside the submitted work. B. Krämer reports personal fees from AstraZeneca, Medtronic, and Pantec Medical outside the submitted work. F. Heitz reports personal fees and non-financial support from Roche, PharmaMar, GlaxoSmithKline, and Clovis, grants, personal fees, and non-financial support from AstraZeneca, and grants from New Oncology outside the submitted work. A. du Bois reports personal fees from Astra Zeneca, Roche, Clovis, GlaxoSmithKline/Tesaro, Pfizer, BIOCAD, and Genmab outside the submitted work. P. Harter reports grants and personal fees from AstraZeneca, Roche, and Tesaro/GlaxoSmithKline, personal fees from Sotio, Stryker, Zai Lab, MSD, Lilly, Clovis, and Immunogen, and grants from Genmab outside the submitted work. S. Heublein reports grants from Heuer Stiftung, Novartis Oncology, and Deutsche Forschungsgemeinschaft within the funding programme Open Access Publishing, Baden-Württemberg Ministry of Science, Research and the Arts, and Ruprecht-Karls-Universität Heidelberg outside the submitted work. R. Manchanda reports grants from Barts Charity during the conduct of the study, and grants from Eve Appeal, Rosetrees Trust, and AstraZeneca/MSD (honorarium for advisory board meeting) outside the submitted work. H.W. Nijman reports grants from Dutch Cancer Society, DCPrime, and Aduro and non-financial support from Merck and BioNovion outside the submitted work. M. de Bruyn reports grants from Dutch Cancer Society, Health-Holland, and ERC, non-financial support from BioNTech, AIMM Therapeutics, and ViciniVax outside, and grants and non-financial support from DCPrime and Aduro Biotech outside the submitted work, and has a patent for antibodies targeting CD103, with a focus on potential imaging

applications (de Bruyn and colleagues no. 62/704,258), filed by Aduro Biotech with inventors from Aduro Biotech and the University Medical Center Groningen, which has not been licensed and is unrelated to the submitted work. N. Singh reports personal fees from AstraZeneca/MSD (advisory board member on 1-day meeting regarding BRCA testing within the National Health Service in the United Kingdom, December 5, 2019) outside the submitted work. A.V. Tinker reports grants from AstraZeneca outside the submitted work. S. Kommoss reports personal fees from Tesaro/GlaxoSmithKline, AstraZeneca, Roche/Genentech, Clovis, and MSD outside the submitted work. M.S. Anglesio reports grants from Terry Fox Research Institute [Canadian Ovarian Experimental Unified Resource (COEUR; funded project)] and other from Janet D. Cottrelle Foundation [Scholars Program (salary support)] during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

P. Krämer: Resources, data curation, formal analysis, investigation, visualization, writing-original draft, writing-review and editing. **A. Talhouk:** Conceptualization, data curation, formal analysis, supervision, investigation, methodology, writing-original draft, writing-review and editing. **M.A. Brett:** Resources, data curation, investigation. **D.S. Chiu:** Data curation, software, formal analysis, visualization. **E.S. Cairns:** Data curation, investigation, methodology. **D.A. Scheunhage:** Resources, data curation, investigation, writing-review and editing. **R.F.L. Hammond:** Resources, data curation, investigation. **D. Farnell:** Resources, data curation, investigation. **T.M. Nazeran:** Resources, data curation, investigation. **M. Grube:** Resources, data curation. **Z. Xia:** Data curation, software, investigation. **J. Senz:** Data curation, supervision, investigation, methodology. **S. Leung:** Resources, data curation, software. **L. Feil:** Resources, data curation, investigation. **J. Pasternak:** Resources, data curation. **K. Dixon:** Resources, software. **A. Hartkopf:** Resources, data curation, writing-review and editing. **B. Krämer:** Resources, data curation. **S. Brucker:** Resources, funding acquisition, writing-review and editing. **F. Heitz:** Resources, data curation, writing-review and editing. **A. du Bois:** Resources, data curation. **P. Harter:** Resources, data curation, writing-review and editing. **F.K.F. Kommoss:** Resources, data curation, investigation. **H.-P. Sinn:** Resources, data curation. **S. Heublein:** Resources, data curation, writing-review and editing. **F. Kommoss:** Resources, data curation, writing-review and editing. **H.-W. Vollert:** Resources, data curation. **R. Manchanda:** Resources, data curation. **C.D. de Kroon:** Resources, data curation. **H.W. Nijman:** Resources, data curation. **M. de Bruyn:** Resources, data curation. **E.F. Thompson:** Resources, data curation, investigation. **A. Bashashati:** Resources, software, supervision, writing-review and editing. **J.N. McAlpine:** Resources, data curation, writing-review and editing. **N. Singh:** Resources, data curation, investigation, writing-review and editing. **A.V. Tinker:** Conceptualization, resources, writing-review and editing. **A. Staebler:** Resources, data curation, investigation. **T. Bosse:** Conceptualization, resources, data curation, supervision, investigation, methodology, writing-review and editing. **S. Kommoss:** Resources, data curation, supervision, investigation, writing-review and editing. **M. Köbel:** Conceptualization, resources, funding acquisition, investigation, methodology, writing-review and editing. **M.S. Anglesio:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, visualization, methodology, writing-original draft, project administration, writing-review and editing.

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References

1. Köbel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med 2008;5:e232.
2. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609–15.
3. Köbel M, Kalloger SE, Huntsman DG, Santos JL, Swenerton KD, Seidman JD, et al. Differences in tumor type in low-stage versus high-stage ovarian carcinomas. Int J Gynecol Pathol 2010;29:203–11.
4. National Academies of Sciences, Engineering and Medicine. Ovarian cancers: evolving paradigms in research and care. Washington, D.C: The National Academies Press. 2016.
5. Anglesio MS, Yong PJ. Endometriosis-associated Ovarian Cancers. Clin Obstet Gynecol 2017;60:711–27.
6. Matias-Guiu X, Stewart CJR. Endometriosis-associated ovarian neoplasia. Pathology 2018;50:190–204.
7. Anglesio MS, Wang YiK, Maassen M, Horlings HM, Bashashati A, Senz J, et al. Synchronous endometrial and ovarian carcinomas: evidence of clonality. J Natl Cancer Inst 2016;108:djv428.
8. Schultheis AM, Ng CKY, De Filippo MR, Piscuoglio S, Macedo GS, Gatius S, et al. Massively parallel sequencing-based clonality analysis of synchronous endometrioid endometrial and ovarian carcinomas. J Natl Cancer Inst 2016;108:djv427.
9. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon, France: IARC-WHO Press. 2014.
10. McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, et al. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. Mod Pathol 2014;27:128–34.
11. McConechy MK, Talhouk A, Leung S, Chiu D, Yang W, Senz J, et al. Endometrial carcinomas with POLE exonuclease domain mutations have a favorable prognosis. Clin Cancer Res 2016;22:2865–73.
12. Hoang LN, McConechy MK, Köbel M, Anglesio M, Senz J, Maassen M, et al. Polymerase epsilon exonuclease domain mutations in ovarian endometrioid carcinoma. Int J Gynecol Cancer 2015;25:1187–93.
13. Peres LC, Cushing-Haugen KL, Köbel M, Harris HR, Berchuck A, Rossing MA, et al. Invasive epithelial ovarian cancer survival by histotype and disease stage. J Natl Cancer Inst 2019;111:60–8.
14. Rambau PF, Vierkant RA, Intermaggio MP, Kelemen LE, Goodman MT, Herpel E, 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;4:250–61.
15. Le Page C, Rahimi K, Köbel M, Tonin PN, Meunier L, Portelance L, et al. Characteristics and outcome of the COEUR Canadian validation cohort for ovarian cancer biomarkers. BMC Cancer 2018;18:347.

16. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology - Ovarian Cancer (including fallopian tube cancer and primary peritoneal cancer) v1.2020. Available from: https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf.
17. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO–ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. Ann Oncol 2019;30:672–705.
18. Cancer Genome Atlas Research Network, C K, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67–3.
19. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 2015;113:299–310.
20. Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, 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;22:4215–24.
21. McAlpine J, Leon-Castillo A, Bosse T. The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses. J Pathol 2018;244: 538–49.
22. Parra-Herran C, Lerner-Ellis J, Xu B, Khalouei S, Bassiouny D, Cesari M, et al. Molecular-based classification algorithm for endometrial carcinoma categorizes ovarian endometrioid carcinoma into prognostically significant groups. Mod Pathol 2017;30:1748–59.
23. Cybulska P, Paula ADaC, Tseng J, Leitao MM Jr, Bashashati A, Huntsman DG, et al. Molecular profiling and molecular classification of endometrioid ovarian carcinomas. Gynecol Oncol 2019;154:516–23.
24. Köbel M, Rahimi K, Rambau PF, Naugler C, Le Page C, Meunier L, et al. An immunohistochemical algorithm for ovarian carcinoma typing. Int J Gynecol Pathol 2016;35:430–41.
25. Dieters-Castator DZ, Rambau PF, Kelemen LE, Siegers GM, Lajoie GA, Postovit L-M, et al. Proteomics-derived biomarker panel improves diagnostic precision to classify endometrioid and high-grade serous ovarian carcinoma. Clin Cancer Res 2019;25:4309–19.
26. McConechy MK, Talhouk A, Li-Chang HH, Leung S, Huntsman DG, Gilks CB, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. Gynecol Oncol 2015;137:306–10.
27. Roth RM, Haraldsdottir S, Hampel H, Arnold CA, Frankel WL. Discordant mismatch repair protein immunoreactivity in lynch syndrome-associated neoplasms: a recommendation for screening synchronous/metachronous neoplasms. Am J Clin Pathol 2016;146:50–6.
28. Köbel M, Piskorz AM, Lee S, Lui S, LePage C, Marass F, et al. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. J Pathol Clin Res 2016;2:247–58.
29. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. Int J Gynecol Pathol 2019;38:S123–S131.
30. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol 2018;29:1180–88.
31. León-Castillo A, Britton H, McConechy MK, McAlpine JN, Nout R, Kommoss S, et al. Interpretation of somatic POLE mutations in endometrial carcinoma. J Pathol 2020;250:323–35.
32. Singh N, Faruqi A, Kommoss F, McCluggage WG, Trevisan G, Senz J, 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 2018;31:652–9.
33. Do H, Dobrovic A. Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization. Clin Chem 2015;61:64–71.
34. León-Castillo A, Gilvazquez E, Nout R, Smit VT, McAlpine JN, McConechy M, et al. Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas. J Pathol 2020;250:312–22.
35. Balduzzi S, Rucker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. Evid Based Ment Health 2019;22:153–60.
36. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. Cancer 2017;123:802–13.
37. Wang YiK, Bashashati A, Anglesio MS, Cochrane DR, Grewal DS, Ha G, et al. Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes. Nat Genet 2017;49:856–65.
38. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang Y-B, et al. Type I and II endometrial cancers: have they different risk factors? J Clin Oncol 2013;31: 2607–18.
39. Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel AV, et al. Ovarian cancer risk factors by histologic subtype: an analysis from the ovarian cancer cohort consortium. J Clin Oncol 2016;34:2888–98.
40. Clarke MA, Long BJ, Sherman ME, Lemens MA, Podratz KC, Hopkins MR, et al. A prospective clinical cohort study of women at increased risk for endometrial cancer. Gynecol Oncol 2020;156:169–77.
41. Soong TR, Howitt BE, Horowitz N, Nucci MR, Crum CP. The fallopian tube, "precursor escape" and narrowing the knowledge gap to the origins of high-grade serous carcinoma. Gynecol Oncol 2019;152: 426–33.
42. Temko D, Van Gool IC, Rayner E, Glaire M, Makino S, Brown M, 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; 245:283–96.
43. McAlpine JN NR, Kommoss S, Church D, Bellone S, Clarke B, DeLair D, et al. Survival benefit in women with endometrial cancers harboring pole mutations may be independent of adjuvant therapy. In: Proceedings of the 17th Biennial Meeting of the International Gynecologic Cancer Society; 2018 Sept 14–16; Kyoto, Japan. p. 42.
44. Nakonechny QB, Gilks CB. Ovarian cancer in hereditary cancer susceptibility syndromes. Surg Pathol Clin 2016;9:189–99.
45. Rambau PF, Duggan MA, Ghatage P, Warfa K, Steed H, Perrier R, et al. Significant frequency of MSH2/MSH6 abnormality in ovarian endometrioid carcinoma supports histotype-specific Lynch syndrome screening in ovarian carcinomas. Histopathology 2016;69:288–97.
46. Merck Sharp & Dohme Corp. (KEYTRUDA prescribing information). U.S. Food & Drug Administration. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/125514s040lbl.pdf.
47. Regulatory Decision Summary for Keytruda. Health Canada. Available from: https://hpr-rps.hres.ca/reg-content/regulatory-decision-summary-detail.php?lang=en&linkID=RDS00514.
48. Rambau P, Kelemen L, Steed H, Quan M, Ghatage P, Köbel M. Association of hormone receptor expression with survival in ovarian endometrioid carcinoma: biological validation and clinical implications. Int J Mol Sci 2017;18: 515.
49. Wang L, Rambau PF, Kelemen LE, Anglesio MS, Leung S, Talhouk A, et al. Nuclear beta-catenin and CDX2 expression in ovarian endometrioid carcinoma identify patients with favourable outcome. Histopathology 2019;74: 452–62.
50. Hollis RL, Stanley B, Iida Y, Thomson J, Churchman M, Rye T, et al. Hormone receptor expression patterns define clinically meaningful subgroups of endometrioid ovarian carcinoma. Gynecol Oncol 2019;155:318–23.
51. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin 2018;68:284–96.
52. Parra-Herran C, Bassiouny D, Vicus D, Olkhov-Mitsel E, Cesari M, Ismiil N, et al. FIGO versus silverberg grading systems in ovarian endometrioid carcinoma: a comparative prognostic analysis. Am J Surg Pathol 2019;43: 161–7.
53. Singh N, Piskorz AM, Bosse T, Jimenez-Linan M, Rous B, Brenton JD, et al. p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies. J Pathol 2020;250:336–45.
54. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng 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;143:46–53.
55. Gilks CB, Ionescu DN, Kalloger SE, Köbel M, Irving J, Clarke 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;39:1239–51.
56. Werner HMJ, Trovik J, Marcickiewicz J, Tingulstad S, Staff AC, Engh ME, 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;49:625–32.