Identification of potentially actionable genetic variants in epithelial ovarian cancer: A retrospective cohort study

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Identification of potentially actionable genetic variants in epithelial ovarian cancer: A retrospective cohort study

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Ovarian cancer is the most lethal gynecological malignancy, mainly due to late-stage diagnosis, frequent recurrences, and eventually therapy resistance. To identify potentially actionable genetic variants, sequencing data of 351 Belgian ovarian cancer patients were retrospectively captured from electronic health records. The cohort included 286 (81%) patients with high-grade serous ovarian cancer, 17 (5%) with low-grade serous ovarian cancer, and 48 (14%) with other histotypes. Firstly, an overview of the prevalence and spectrum of the BRCA1/2 variants highlighted germline variants in 4% (11/250) and somatic variants in 11% (39/348) of patients. Secondly, application of a multi-gene panel in 168 tumors revealed a total of 214 variants in 28 genes beyond BRCA1/2 with a median of 1 (IQR, 1 to 2) genetic variant per patient. The ten most often altered genes were (in descending order): TP53, BRCA1, PIK3CA, BRCA2, KRAS, TERT promoter, RB1, PIK3R1, PTEN, and ERBB2 (HER2). Of note, the genetic landscape vastly differed between the studied histotypes. Finally, using ESCAT the clinical evidence of utility for every genetic variant was scored. Only BRCA1/2 pathogenic variants were classified as tier-I. Nearly all patients (151/168; 90%) had an ESCAT tier-II variant, most frequently in TP53 (74%), PIK3CA (9%) and KRAS (7%). In conclusion, our findings imply that although only a small proportion of genetic variants currently have direct impact on ovarian cancer treatment decisions, other variants could help to identify novel (personalized) treatment options to address the poor prognosis of ovarian cancer, particularly in rare histotypes.

INTRODUCTION

Epithelial ovarian cancer (OC) is an umbrella term for distinct malignancies affecting the ovaries, each characterized by its own histological and molecular profile. OC is the most lethal gynecological malignancy and the eighth most prevalent female cancer worldwide, with an annual abysmal mortality rate of two million fatalities worldwide1. The existence of late symptom onset and multiple overlapping symptoms with other gastrointestinal, genitourinary, and gynecological diseases often leads to late-stage diagnosis with already extensive extra-ovarian metastasis present, whereby the 5-year survival plummets to less than 30%2–5. Despite their inherent differences, most OCs are generally treated in the same manner: the cornerstones are cytoreductive surgery and platinum-based chemotherapy, with or without Poly (ADP-Ribose) Polymerase (PARP) inhibitors (PARPi) and/or bevacizumab6. Unfortunately, up to 70% of patients will experience recurrence7. Thus, it is well established that a “one-size-fits-all” treatment approach should no longer be applied. Tailored treatment options based on individualized molecular tumor data are warranted to enable practitioners to more effectively treat specific OC subtypes. For instance, EMA and FDA have approved PARPi targeting germline or somatic BRCA1/2 mutated advanced OCs. Given their efficacy, mutational screening of BRCA1/2 genes in OC has become of great importance8–11. Besides PARPi, targeted therapies are increasingly available for other gene alterations, including KRAS, BRAF and TRK inhibitors12. Therefore, implementation of companion diagnostic tests, which go beyond somatic and germline BRCA1/2 testing is highly warranted.

To help clinicians with interpreting genomic reports, the European Society for Medical Oncology (ESMO), Translational Research and Precision Medicine Working Group published a systemic framework in 2018 to rank molecular targets based on available evidence supporting their value as clinical targets13. Its main goal was to implement a harmonized vocabulary and to facilitate communication between academia, pharmaceutical industry, healthcare professionals and patients. The ESMO scale for Clinical Actionability of Molecular Targets (ESCAT) was previously applied across multiple cancer types, but OC has never been the focus of such studies and histopathologic subtypes were never distinguished14–17.

In the present retrospective cohort study, we aim to characterize (potentially targetable) genetic variants in patients with OC across various histotypes, using the ESCAT framework.
METHODS

Patients and samples
This retrospective cohort study included 358 patients diagnosed with OC between January 2014 and December 2022, who underwent molecular testing of blood and/or tumor samples at the Center for Medical Genetics Ghent (Ghent University Hospital, Belgium). The study was approved by the ethics committee of Ghent University Hospital (ONZ-2023-0389); the need for informed consent of patients was waived because of the retrospective nature of the study and anonymized data was transferred to the investigators. The study was conducted in accordance with the Declaration of Helsinki and followed the ’Strengthening the Reporting of Observational Studies in Epidemiology’ (STROBE) guideline. Histopathologic information and molecular sequencing data were extracted from the institutional electronic health records. A gynecological pathologist (KV) reviewed all available tumor slides (using both HE and immunohistochemistry) in a blind manner to classify them into distinct ovarian cancer histotypes: high-grade serous ovarian cancer (HGSOC), low-grade serous ovarian cancer (LGSOC), clear cell carcinoma of the ovaries (CCOC), endometrioid ovarian cancer (EOC), mucinous ovarian cancer (MOC), mixed malignant mullerian tumor (MMMT) and mesonephric-like adenocarcinoma of the ovary (MLAOC). All borderline or non-epithelial tumors were excluded for further analysis.

Molecular profiling
Tumor samples obtained before January 2019 were only examined for somatic genetic variants in BRCA1 and BRCA2 using the PCR-based BRCA Tumor Mastr Plus Dx kit (Multiplicom). In parallel, germline testing was performed for BRCA1, BRCA2, TP53 and PALB2 using an in-house developed amplicon-based target enrichment method, followed by next generation sequencing (Miseq, Illumina) as previously described. Starting from 2019, a capture-based approach was applied for target enrichment. A detailed description of the methods can be found as Supplementary Information (1). Both tumor and blood samples were examined using a customized cancer gene panel that has been updated over time (all panel versions can be found in Supplementary Information 2-4. Only variants that were classified as pathogenic or likely pathogenic were considered for further analysis.

Functional domains BRCA1 and BRCA2
Functional domains were defined as:

The Ovarian Cancer Cluster Regions (OCCRs) were defined for BRCA1: AA 460-1354; and for BRCA2: OCCR1 - AA 1083-1894 and OCCR2- AA 2215-2490.

Level of actionability
Genetic variants were ranked according to the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) into six levels of evidence ranging from ‘ready for routine use’ (tier-I) to ‘lack of evidence’ (tier-X), previously described by Mateo et al. To rank the specific variants, the results of clinical trials (ClinicalTrials.gov) were summarized and classified them based on several characteristics including prospectiveness, randomization, and availability of results. When no clinical trials in OC were available, basket trials or trials in other cancer types were examined. All listed drug-target matches were investigated for FDA or EMA approvals and for treatment recommendations by NCCN. OncoKB and publications which already describe ESCAT scoring systems in other cancer types were used to propose a score in case of doubt about drug-target matches. The proposed ranking was validated by the co-authoring medical oncologists, pathologists and molecular geneticist until consensus. Patients with multiple genetic variants were ranked by the highest level of evidence. A comprehensive report of all genetic variants and matched drugs accompanied with clinical evidence and ESCAT ranking is included in Supplementary Information (6).
Signaling pathways
For the most frequently mutated genes the canonical pathways in which they are playing a role, were determined. Five signaling pathways were previously curated by TCGA PanCancer Atlas: (1) cell cycle pathway (CCND1, CDKN2A, RB1), (2) RTK/RAS/MAPK pathway (KRAS, NRAS, Braf, FGFR1, FGFR2, FGFR3, MET), (3) p53 pathway (TP53), (4) PI3K/AKT/mTOR pathway (AKT1, PIK3CA, PIK3R1, PTEN), (5) WNT pathway (CTNNB1, RNF43). Two additional pathways included the HR pathway (BRCA1, BRCA2, BAP1, ATM) and ErbB kinases (ERBB2). Seven additional genes with at least one genetic variant could not be assigned to any of these seven pathways (SMARCA4, SMARCBI, GNAS, POLE, SPOP, TERT, VHL).

Statistical analysis
All analyses and visualizations were performed using Excel version 2307 and R Studio version R4.3.1 (R program for Statistical Computing). To summarize all actionable variants for the individual patients, an oncoprint was generated in R Studio using the Complex Heatmap package. Patients were grouped according to histotype; genes were grouped in their respective pathway. The lollipop plot for BRCA1 and BRCA2 variants was adapted from cBioportal (genome build GRCh38).

RESULTS
Patients and samples
HE slides from 358 OC patients who received surgery between 2014 and 2022 were reviewed by an expert pathologist; seven borderline or non-epithelial tumors were excluded for further analysis (n=7). From the final cohort of 351 patients, sequencing data were available from 348 tumor samples and 250 blood samples. Patient matched tumor and blood samples were available for 247 patients (Figure 1-A). The most common histotypes were HGSOC (n=286) and LGSOC (n=17), with other (rare) histotypes accounting for 48 patients: 15 CCOC, 14 EOC, 11 MMMT, 6 MOC and 2 MLAOC (Figure 1-B).

Genetic variants
BRCA1/2
Among the 351 OC patients analyzed, 49 (14%) patients harbored BRCA1 (9%) and/or BRCA2 (5%) variants. In one HGSOC patient, both a somatic BRCA1 and a germline BRCA2 variant were detected. Eleven variants were of germline origin (six in BRCA1 and five in BRCA2). BRCA1/2 variants were most prevalent in the HGSOC (14%) subgroup; notably, all germline variants were found in this histotype. The remaining somatic BRCA1/2 variants were found in CCOC (13%), MMMT (9%) and EOC (7%) histotypes. No BRCA1/2 variants were found in the LGSOC, MOC, and MLAOC histotypes (Table 1).
To evaluate if the variants cluster in specific regions, all (likely) pathogenic variants are depicted in a lollipop plot, illustrating that the variants were clearly spread over the entire coding sequence of \textit{BRCA1} and \textit{BRCA2} genes, without particular hotspot regions (Figure 2). Less than half of the variants were found in the ovarian cluster regions: sixteen in the \textit{BRCA1} OCCR (13/27 somatic, 3/6 germline), four in \textit{BRCA2} OCCR1 and one in \textit{BRCA2} OCCR2 (all somatic). Twenty-two (6\% of patients) and eleven (3\% of patients) variants occurred in a functional domain of \textit{BRCA1} and \textit{BRCA2}, respectively. In \textit{BRCA1}, this concerns seven variants in the RING domain, twelve in the DBD and three in the BRCT domain. In \textit{BRCA2}, four variants clustered in the RAD51-BD and seven in the DBD. In Supplementary Information (5), a detailed overview is provided of all germline and somatic \textit{BRCA1/2} variants identified in the different histotypes.

![Figure 2. Lollipop plots of variant distribution in BRCA1 and BRCA2.](image-url)

The diagrams linearly represent \textit{BRCA1/2} protein domains (x-axis). \textit{BRCA1} domains: green, C3HC4-type RING finger; red, DBD (DNA-binding domain of \textit{BRCA1}); blue, BRCT (\textit{BRCA1} C-terminal) domain. \textit{BRCA2} domains: orange, RAD51-BD (RAD51-binding domain); red, DBD (DNA-binding domain). Each variant is represented by a single lollipop; the stick heights indicate the mutation frequency (y-axis), and dots are color-coded according to histotype: pink dots, HGSOC; green dots, LGSOC; orange dots, CCOC; blue dots, MMMT; yellow dots, EOC. Germline variants are indicated by an arrow. The deletion of exons 5 & 6 in \textit{BRCA1} is marked by a horizontal lollipop under the plot. OCCR (ovarian cancer cluster regions) are indicated by a thick black line under the plot. The graphs are adapted from MutationMapper tool, cBioportal (http://www.cbioportal.org/mutation_mapper).
Beyond BRCA1/2

A multi-gene panel was introduced in 2019 for solid cancers and revealed a wide range of genetic variants in 168 OCs. Beyond BRCA1/2, 214 variants across 28 genes were detected in 134 patients (80%) with a median of 1 variants (interquartile range (IQR), 1 to 2) per patient. The majority of these variants were substitutions (164/214, 77%), small deletions (33/214, 15%), or duplications (9/214, 4%). In a limited number of samples gene amplifications were observed: PIK3CA (2%), ERBB2 (2%), FGFR2 (1%), FGFR3 (1%), MET (1%), and CCND1 (1%). Sixty-six tumors harbored more than one (likely) pathogenic variant in the same gene or multiple genes. Table 2 displays an overview of the most frequently altered genes per histotype. A complete list of all variants found in our cohort is provided in Supplementary Information (6).

Table 2. Overview of the frequency of altered genes according to histotypes. The table shows the number of tumors with at least one variant in the corresponding gene.

<table>
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<tr>
<th>Gene</th>
<th>HGSOC</th>
<th>CCOC</th>
<th>LGSOA</th>
<th>EOC</th>
<th>MMTT</th>
<th>MLAOC</th>
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Summarized, in HGSOC, the most frequently altered gene was TP53 in 94% of patients. Other altered genes include PIK3CA (4.8%), RB1 (3.2%), KRAS (2.4%), and PIK3R1 (2.4%). Sporadically altered genes (only 1 or 2 tumors) in this subtype are RNF43, FGFR2, ERBB2, MET, AKT1, PTEN, GNAS, FGFR1, FGFR3, SMARCA4, VHL, and SPOP. In fifteen CCOC tumors, variants were identified in eleven genes with PIK3CA being mutated in six (40%) and the TERT promoter in four (27%) tumors, respectively. Other genes (TP53, ATM, PIK3R1, PTEN, KRAS, CCND1, CDKN2A, SMARCB1 and POLE) were only sporadically affected in CCOC. Ten out of eleven LGSOC tumors had variants identified in NRAS (27%), ERBB2 (27%) and sporadically in BAP1, KRAS, CDKN2A and BRAF. Six out of seven EOC tumors harbored variants in TP53, AKT1, PIK3CA, PTEN, KRAS, CCND1 and CTNNB1. In three EOC tumors multiple variants were detected (one tumor with six variants, another had 3 variants and a third had two variants). Out of six carcinosarcomas, three had variants in TP53. Both mucinous and mesonephric-like adenocarcinomas tumors harboured the same KRAS p.(Gly12Asp) variant. Additionally, one mucinous tumor harbored a TP53 variant and another had a PIK3CA variant.

Affected signaling pathways

We investigated if specific molecular pathways were involved per histotype (Figure 3). The p53 signaling pathway was most frequently altered regardless of the histotype (124/168). Almost all HGSOC tumors (116/124) had genetic variants in the TP53 signaling pathway. Additionally, the HR (21/124), followed by PI3K/AKT/MTOR (11/124), RTK/RAS/MAPK (9/124), cell cycle (4/124), ERBB2 (2/124), and WNT (2/124) pathways were altered in this subtype. In contrast, nearly half of CCOC tumors (7/15) displayed an aberrant P13/AKT/MTOR pathway, while TP53 (2/15), HR (2/15), RTK/RAS/MAPK (2/15), and cell cycle (2/15) were less frequently altered. In LGSOC
tumors alterations in the RTK/RAS/MAPK (4/11) and ERBB2 (3/11) pathways were most prevalent. The HR and cell cycle pathways were each affected in only one LGSOC tumors each. Four out of seven EOC tumors displayed an aberrant PI3K/AKT/MTOR signaling pathway, besides the TP53 (3/7), WNT (2/7), and cell cycle (1/7) pathways. Four out of six adenocarcinomas had an altered TP53 pathway, and in one a pathogenic variant in the HR pathway was detected. The RTK/RAS/MAPK pathway was affected in both mucinous tumors that underwent multi-gene panel testing, in addition to the PI3K/AKT/MTOR pathway or TP53 pathway. Both mesonephric-like adenocarcinomas were altered in the RTK/RAS/MAPK pathway but no other pathways were affected. Although there are some trends, no significantly enriched pathways between the subgroups could be obtained, as the number of some histotypes is rather small.

Classification of genetic variants using ESCAT
To determine the clinical utility of the variants identified by the multi-gene cancer panel, ESCAT scores were assigned for all genetic variants detected in 168 patients who underwent multi-gene panel testing. A comprehensive list of all clinical evidence leading to ESCAT ranking is described in Supplementary Information (7). In 156/168 (93%) OC tumors, at least one actionable alteration was detected. Noteworthy, only 17% (28/168) were classified as ESCAT tier-I (BRCA1 and BRCA2). Nearly all patients (151/168) had an ESCAT tier-II classified variant, most frequently in TP53 (127/168), followed by PIK3CA (17/168) and KRAS (10/168). Rare tier-II variants, affecting <3% of the OC tumors include: ATM, AKT1, PIK3R1, CDKN2A, PTEN, ERBB2, NRAS, BRAF, SMARCA4, and SMARCB1. All other variants were scored as ESCAT tier-III-A, tier-III-B, tier-IV or tier-X. The oncoplot in Figure 3 provides a detailed overview of all genetic variants affecting individual patients across different histotypes.

DISCUSSION
This study unraveled the genomic landscape from a unique cohort of 351 Belgian OC patients across different histotypes using real-world data. Rapid evolution of next-generation sequencing currently allows the medical community to produce a myriad of genetic information; the clinical utility, however, is not always apparent. Here, we demonstrated that a cancer gene panel offers a focused approach allowing the medical community to capture the most relevant genetic variants and that scoring their clinical utility is feasible.

The first objective of this study was to give an overview of the BRCA1/2 mutational status in epithelial OC. In total, eleven (4%) patients were found to have a germline, and 39 (11%) had a somatic (likely) pathogenic BRCA1/2 variant. Compared to other reports from European populations (15-20%), the frequency of BRCA1/2 variants in our cohort (14%) is at the lower limit11,28,29. As expected, the majority (78%) of BRCA1/2 variants were detected in HGSOC tumors. Only two out of fifteen CCOC tumors, one out of fourteen EOC and one out of eleven MMMT had a BRCA1/2 variant. Due to relatively small numbers of rare histotypes in our study and in literature, it is hard to compare our findings with those of others. Overall, our prevalence of BRCA1/2 variants appears to be somewhat lower, except for the CCOC histotype. Similar to other reports, no BRCA1/2 variants were found in MOC tumors30. Another difference is the higher occurrence of somatic versus germline variants in our cohort. A recent review reported that 13%-21% of epithelial OC patients have germline BRCA1/2 variants and only 6% harbor somatic BRCA1/2 variants31. Historically, BRCA1/2 testing was biased towards patients with a family history of breast/ovarian cancer and focused on patients with early-onset disease, which may explain higher mutation detection rates in other studies11. However, we cannot exclude a bias in referral for our cohort, as tumors from patients with known germline variants may not have been referred for tumor testing, especially before 2019.

As the majority of the BRCA1/2 variants are of somatic origin, the mutation spectrum is more diverse than previously described for germline variants in the Belgian OC population. In 2004, Claes et al. reported six major recurrent BRCA1/2 variants accounting for 60% of all identified variants in a Belgian cohort. Only two of these recurrent germline variants (BRCA2 c.6275_6276del & c.8904del) were found in two patients of our cohort32.
Figure 3. Frequencies of actionable genetic variants per gene across ovarian histotypes. Drug-target matches were annotated based on their clinical actionability according to ESCAT and patients were assigned to the highest level of actionability. The oncplot shows a summary of the genes affected (rows) in individual patients (columns). Genes are grouped by pathways and patients are grouped according to histotype.
About 50% of all somatic BRCA1/2 variants, 50% of germline BRCA1 and 25% of germline BRCA2 variants were detected in the OCCRs, which indicates only a small enrichment of variants in these regions. Although, the RING-domain is associated with a lower OC risk compared to breast cancer, still one germline and six somatic BRCA1 variants were detected in this region. This illustrates that genotype-phenotype correlations are not strong enough to counsel individual patients. The location of BRCA1/2 mutations is not only associated with risks for specific cancers, but has also been suggested to influence the magnitude of benefit from treatment with platinum salts and/or olaparib (plus bevacizumab). An exploratory analysis of the PAOLA-1 trial suggested that patients with mutations in the DBD region of BRCA2 might be extremely sensitive to platinum, whereas those with mutations in the DBD region of BRCA1 might be less sensitive to platinum but extremely sensitive to olaparib plus bevacizumab. Currently, the biological mechanisms underlying domain-related sensitivity to PARPi are unknown. However, if this intriguing finding could be confirmed in independent studies, treatments may not only be guided by the mutated gene but also by the location of the variant.

About 50% of all somatic BRCA1/2 variants, 50% of BRCA1 and 25% of BRCA2 germline variants were detected in the OCCRs, displaying only a small enrichment of variants in the OC cluster regions. Although, the RING-domain is associated with a lower OC risk compared to breast cancer, still one germline and six somatic BRCA1 variants were detected in this region. An exploratory analysis of the PAOLA-1 trial suggested that the location of mutation in BRCA could influence the magnitude of benefit from platinum salts and/or olaparib (plus bevacizumab): patients with mutations in the DBD region of BRCA2 might be extremely sensitive to platinum, whereas those with mutations in the DBD region of BRCA1 might be less sensitive to platinum but extremely sensitive to olaparib plus bevacizumab. Currently, the biological mechanisms underlying domain-related sensitivity to PARPi are unknown. However, if this intriguing finding could be confirmed in independent studies, treatments may not only be guided by the mutated gene but also by the location of the variant.

The second objective of this study was to evaluate the prevalence of additional actionable genetic variants in epithelial OC beyond BRCA1/2. In 157/168 OCs that underwent multi-gene panel testing, 242 (likely) pathogenic alterations were detected. These variants were ranked according to ESCAT into six levels of evidence ranging from ‘ready for routine use’ (tier-I) to ‘lack of evidence’ (tier-X). Tier-I variants (BRCA1 and BRCA2) were found in nearly 17% of the OC tumors. Several randomized clinical trials have demonstrated improved oncological outcomes with PARPi maintenance treatment over standard of care in newly diagnosed OC patients with BRCA1/2 genetic variants, leading EMA and FDA approvals. OC patients harboring tier-IIB variants can be enrolled in several ongoing clinical studies that test the efficacy of a drug matched to genetic variants, without data being available on overall or disease-free survival. Genes in this category include: TP53, ATM, AKT1, PIK3CA, PIK3R1, PTEN, ERBB2, KRAS, NRAS and BRAF. One notable example is the BOUQUET trial (NCT04931342) that evaluates the efficacy and safety of biomarker-driven therapy in patients with platinum-resistant OC of a rare (non-HGSOC) histotype. In BOUQUET, PIK3CA, AKT1, PTEN, ERBB2, KRAS, NRAS and BRAF, are being used as biomarkers to plan individualized study treatment. Other large ongoing studies on the effectiveness of targeted anti-cancer drugs and immunotherapy, in patients where the tumor is known to have specific genetic variants are NCI-MATCH (NCT02465060), DRUP (NCT02925234) and TAPUR (NCT02693535). Concerning tier-IIIa, OC tumors with FGFR1/2/3 pathogenic variants could be treated with FGFR-inhibitors. These are classified as tier-IA in other tumor types (like urothelial carcinoma and biliary tract cancer) and the ongoing NCI-MATCH trial is investigating, amongst others, the efficacy of FGFRi in additional tumor types. However, to date no supportive clinical data is yet available in OC. ERBB2 amplified tumors are matched to anti-HER2 antibody, trastuzumab, treatment and are classified as tier I-A in breast cancer and are currently investigated in multiple clinical trials in OC patients (e.g. DESTINY: phase 2. NCT04482309). MET inhibitors have shown significant benefit for treatment in MET amplified lung cancers (tier-II), however, data for OC is currently still lacking. BAP1 was categorized as tier-IIIB, as this is a protein that interacts with BRCA1/2 to mediate homologous recombination during DNA repair. Testing PARPi in patients with a deficient BAP1 protein would be conceptually reasonable. Several clinical trials targeting BAP1 alterations in multiple cancer types (not OC) are ongoing (e.g. NCT03207347, NCT04666740, NCT03654833, NCT02925234). Two groups investigated whether silencing of Cyclin D1 (CCND1) could lead to a BRCA-m like phenotype and thus improve the efficacy of PARPi. Micro-RNA and short hairpin-RNA were used in vitro and in vivo in mice to downregulate CCND1 resulting in a substantial benefit in OC management.
Other genes like, POLE, RB1, TERT, GNAS, CTNNB1, RNF43 and SPOP have prognostic values or are frequently altered/upregulated in certain histologic subtypes, but are currently not considered as targets matched to specific treatments (tier-X)\textsuperscript{44-50}.

To our knowledge, the present study is the first and most comprehensive study classifying alterations in a large epithelial OC cohort using ESCAT. Previously, Lapke et al. reported actionable variants in a smaller group of OCs (n=85). In their study, eligibility for targeted therapy was determined by a pathway-based approach; however, no classification according to degrees of clinical evidence was applied\textsuperscript{51}. ESCAT classification has been applied in other cancer types, including head and neck cancer, breast cancer and tumor-agnostic studies; these reported that implementation of ESCAT for clinical-decision making is feasible and beneficial for personalized treatment\textsuperscript{14-17,52-56}. ESCAT scoring in our cohort only identified a limited number of patients (17\%) with tier-I genetic variants; however, tier-II actionable variants were detected in 75\% of the patients. This implies that only a small proportion of reported genetic variants have a direct impact on treatment decisions. Nevertheless, many patients are potentially eligible to specific clinical trials tailored to their cancer's genome and could benefit from drugs targeting a tier-II variant. We hypothesize that one reason why OC is lagging behind other cancer types towards targeted treatments is conventional trial design. Trials in which all patients with a specific cancer type receive the same treatment limit the possible benefits of precision oncology. From our data it is clear that rare histologic subtypes have distinct molecular features that are possibly actionable. Since the most common histotype is HGSOC, it is often the only subtype included in clinical trials. Recently, clinical research has evolved from using cancer type-centered to biomarker-directed and histology-specific trials. These ‘master protocol’ designs are tailored to molecular profiles of the patients. To improve the concept of personalized treatment, the strategy should be implemented earlier in the course of the disease and tumors should have comprehensive tumor profiling at each stage of the disease.\textsuperscript{57,58} Another major challenge for oncologists remains how to integrate patients’ biomarker profiles into therapeutic decision-making as literature is complex, rapidly evolving, and voluminous. Next, local drug access is also critical for clinicians seeking therapeutic options for their patients. To aid clinicians, several knowledge bases have been developed. Recently, a curated version specific to the Australian healthcare setting was developed: \textit{TOPOGRAPH}—Therapy-oriented precision oncology guidelines for recommendation of anticancer pharmaceuticals\textsuperscript{59}. But utility studies on such an electronic compendium with respect to treatment recommendations are warranted.

Strengths of our study include a relatively large sample size that covered a large spectrum of histotypes in OC, participation of a single expert center with uniform management procedures, a small proportion of patients excluded for analysis, full access to all relevant pathology specimens for rigorous review, and the extensive literature search for assessing strength of evidence using ESCAT. There are several limitations to be considered when interpreting the results of this study. First, it was conducted at a single academic center, and the retrospective and anonymized design precluded the collection of additional data. Therefore, we could neither assess patients’ survival time, nor determine whether or not patients received matched drugs to genetic variants. Second, some concerns may be raised that genomic instability evaluating the consequence of HRD beyond \textit{BRCAm} (e.g., loss of heterozygosity, telomeric allelic imbalance and/or large-scale state transitions) was not assessed in our study. Although our panel did contain two genes involved in HRD beyond \textit{BRCA1/2} (i.e., \textit{BAP1} and \textit{ATM}), it has been demonstrated that mutations in non-\textit{BRCA} homologous recombination repair genes (HRRm) did not predict benefit from olaparib (a PARPi) plus bevacizumab in PAOLA-1; hence, non-\textit{BRCA} HRRm in multigene panels do not substitute for HRD determined by genomic instability testing (and \textit{BRCA} mutation status)\textsuperscript{60}. Third, exon-spanning deletions or duplications, gene fusions, mutations in regulatory sequences or deep-intronic mutations could not be detected by the applied molecular analysis. Bearing this in mind, some highly druggable targets are missed (e.g. \textit{NTRK} fusions)\textsuperscript{61,62}. Similarly (fourth), our methods did not yet assess microsatellite instability and tumor mutational burden (too small panel size around 100kb), although both have been shown to be predictive biomarkers of immunotherapy in several solid tumors (albeit with limited evidence in OC). Fifth, the ESCAT is a validated and well-respected instrument; however, tumor heterogeneity (i.e., spatial differences) and evolutionary dynamics of the cancer genome (i.e., temporal differences) may hinder the more extensive use of molecular profiling-based strategies. Sixth, as our cohort comprised mainly white women, validations in other ethnicities are warranted.
CONCLUSIONS
In conclusion, we unveiled a diverse landscape of potentially actionable genetic variants in this histologically and genetically well-defined cohort of OC patients. Unfortunately, directly actionable (ESCAT tier-I) variants accounted only for a small proportion. Multiple biomarker-driven clinical trials recruiting OC patients are currently ongoing; these trials generate information that could ultimately lead to the dawn precision oncology in OC patients.

DATA AVAILABILITY
All data analyzed during this study are included in this published article and its supplementary information files.

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CONTRIBUTIONS
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ETHICS DECLARATION
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