Deleterious somatic variants in 473 consecutive individuals with ovarian cancer: results of the observational AGO-TR1 study (NCT02222883)

Jan Hauke,1 Eric Hahnen,1 Stephanie Schneider,2 Alexander Reuss,3 Lisa Richters,1 Stefan Komposs,4 André Heimbach,5 Frederik Marmé,6 Sandra Schmidt,1 Katharina Prieske,7 Heidrun Gevensleben,8 Alexander Burges,9 Julika Borde,10 Nikolaus De Gregorio,11 Peter Nürnberg,12,13 Ahmed El-Balat,14 Holger Thiele,12,15 Felix Hilpert,16,17 Janine Altmüller,12,15 Werner Meier,18 Dimo Dietrich,19 Rainer Kimmig,20 Birgid Schoemig-Markieffka,21 Karin Kast,22,23 Elena Braicu,24 Klaus Baumann,25,26 Christian Jackisch,27 Tjouong-Won Park-Simon,28 Corinna Ernst,1 Lars Hanker,29 Jacobus Pfisterer,30 Andreas Schnelzer,31,32 Andreas du Bois,2 Rita K Schmutzler,1 Philipp Harter2

ABSTRACT

Background For individuals with ovarian cancer (OC), therapy options mainly depend on BRCA1/2 germline status. What is the prevalence of deleterious somatic variants, that is, does genetic tumour testing identify subgroups of individuals who also might benefit from targeted therapy?

Methods Paired analysis of tumour-derived versus blood-derived DNA to determine the prevalence of deleterious somatic variants in OC predisposition genes (ATM, BRCA1/2, BRIP1, MSH2/6, PALB2, RAD51C/D and TP53) and the PIK3CA and PTEN genes in individuals with OC (AGO-TR1 study, NCT02222883). Results were complemented by BRCA1, PALB2 and RAD51C promoter methylation analyses and stratified by histological subtype; 473 individuals were included.

Results The combined analyses revealed that deleterious germline variants in established OC predisposition genes (all: 125/473, 26.4%; BRCA1/2: 97/473, 20.5%) and deleterious somatic variants in established OC predisposition genes excluding TP53 (all: 39/473, 8.2%; BRCA1/2: 30/473, 6.3%) and promoter methylation (all: 67/473, 14.2%; BRCA1: 57/473, 12.1%; RAD51C: 10/473, 2.1%; PALB2: 0/473) were mutually exclusive, with a few exceptions. The same holds true for deleterious somatic PIK3CA and/or PTEN variants (33/473, 7.0%) found to be enriched in endometrioid and clear cell OC (16/35, 45.7%); 84.3% of the deleterious single-nucleotide/indel germline variants in established OC predisposition genes showed significantly higher variant fractions (VFs) in the tumour-derived versus blood-derived DNA, indicating a loss of the wild-type alleles.

Conclusion Tumour sequencing of the BRCA1, BRCA2, PIK3CA and PTEN genes along with BRCA1 and RAD51C promoter methylation analyses identified large subgroups of germline mutation-negative individuals who may be addressed in interventional studies using PARP or PI3K/AKT/TOR inhibitors.

Trial registration number NCT02222883

INTRODUCTION

According to the Global Burden of Disease Cancer Collaboration,1 worldwide, approximately 254 000 women are diagnosed with ovarian cancer (OC), and 163 000 women die from the disease every year. Despite improvements in therapy, the prognosis for OC is still poor, with an average 5-year survival rate of 47.4% (National Cancer Institute, Cancer Stat Facts: Ovarian Cancer, https://www.cancer.gov/about-cancer/cancer-facts благодарственые removes for clinical decision making.2 3 Additional OC predisposition genes (ATM, BRIP1, MSH2, MSH6, PALB2, RAD51C, RAD51D and TP53) were validated by, for example, Norquist et al and Lilyquist et al in cohorts sufficiently large to make reliable statements.8 9 Disease associations for these genes have been clinically validated using the ClinGen clinical validity framework.10 While associations for ATM and PALB2 are not firmly established and penetrances for genes like RAD51C/D and BRIP1 still have to be investigated in different populations, we defined these eight genes (in addition to BRCA1/2) as ‘established OC predisposition genes’ for this investigation. Data on the prevalence of deleterious
somatic alterations in additional OC predisposition genes are still sparse, and the clinical relevance of these findings is a matter of debate.14–15

It is suggested that the heterozygous germline inactivation of OC predisposition genes may be accompanied by a somatic inactivation of the second allele by another deleterious variant, loss of heterozygosity (LOH) or promoter methylation, resulting in an HR deficiency and limited DNA repair capacity of the tumour cells. However, it has recently been proposed that BRCA1 promoter hypermethylation rarely occurs in individuals with an underlying deleterious BRCA1/2 germline variant, although this phenomenon has not been studied extensively, and the evidence from individual studies is limited.16 Therefore, this investigation was initiated to determine whether additional tumour testing identifies a sufficiently large subgroup of individuals with deleterious somatic variants who may be addressed in upcoming interventional studies. We conducted a paired next-generation sequencing (NGS) analysis of blood-derived and tumour-derived DNA from formalin-fixed paraffin-embedded (FFPE) tumour samples obtained from 473 OC individuals enrolled in the observational AGO-TR1 study.17 18 This investigation was complemented by promoter methylation analyses of the BRCA1, PALB2 and RAD51C genes, which were the genes with the most frequent deleterious germline variants in the AGO-TR1 study sample. The analysis of BRCA2 promoter methylation was omitted based on previous study results.19–21 In addition, we investigated the occurrence of deleterious somatic PIK3CA and PTEN variants and stratified our findings by histological subtypes.

STUDY SAMPLE AND METHODS

Study sample

A total of 523 consecutive individuals with invasive epithelial OC were recruited and counselled in 20 centers of the Arzbeigemeinschaft Gynaekologische Onkologie (AGO), Germany. All individuals were older than 18 years and provided written informed consent prior to enrolment in the trial. Demographic data, disease characteristics, family history and medical history were documented as described previously.17 FFPE tumour samples were available from 496 of 523 individuals enrolled in the AGO-TR1 study.

Next-generation sequencing

All DNA samples were centrally analysed (Center for Hereditary Breast and Ovarian Cancer, University Hospital Cologne, Germany) by targeted NGS covering the entire coding regions and exon-flanking sequences (±15 nt) of 27 genes (ATM, NM_000051.3; BARD1, NM_000465.3; BRCA1, NM_007294.3; BRCA2, NM_000059.3; BRIP1, NM_032043.2; BUB1B, NM_001211.3; CDH1, NM_004640.4; CHEK1, NM_001330427.1; CHEK2, NM_007914.3; FAM17A, NM_13907.62; FANCM, NM_020937.3; MAP3K1, NM_005291.1; MLH1, NM_00249.3; MRE11A, NM_005991.3; MSH2, NM_000251.2; MSH6, NM_00179.2; NBN, NM_002485.4; PALB2, NM_024675.3; PIK3CA, NM_006213.8; PMS2, NM_00035.6; PTEN, NM_000314.6; RAD50, NM_005732.3; RAD51C, NM_058216.2; RAD51D, NM_002878.3; STK11, NM_000455.4; TP53, NM_000465.5; and XRC6, NM_005341.1). The methodologies and the results of the germline analysis of 25 genes (excluding PIK3CA and MAP3K1) were described previously.17 The hybridisation capture-based NGS method (Agilent, Santa Clara, California, USA) was suitable for the analysis of DNA derived from either blood or FFPE tumour samples (Agilent SureSelect XT protocol optimised for 200 ng of genomic DNA). For the isolation of DNA from FFPE tumour samples, H&E-stained 3 µm tissue sections were centrally investigated (Institute of Pathology, University Hospital Bonn, Germany); that is, tumour areas containing ≥80% tumour nuclei were chosen for DNA isolation. DNA isolation from FFPE tumour samples was conducted using standard procedures (see supplementary Materials and Methods). Sequencing was performed on MiSeq or HiSeq4000 devices (Illumina, San Diego, California, USA). Of the 496 tumour samples, 488 samples were successfully analysed by targeted NGS with a mean read coverage of at least 100×. For these samples, the mean read coverage was 570× (range 110×–1802×). Bioinformatic analyses were carried out using the SOPHiA DDM platform (Sophia Genetics, Saint-Sulpice, Switzerland) with a minimum cut-off value of 5% for VFs.

Detection of CNVs

The germline analyses covered the detection of CNVs. All 523 blood-derived DNA samples were screened for CNVs in the BRCA1/2 genes by Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA probemixes P002 (BRCA1) and P045 (BRCA2) (MRC-Holland, Amsterdam, The Netherlands). In addition, NGS data were used to identify CNVs in non-BRCA1/2 genes using an in silico CNV detection tool incorporated in the SOPHiA DDM platform. Neither CNV calling using the in silico CNV detection tool of the SOPHiA DDM platform nor MLPA-analysis were suitable for FFPE-derived DNA samples. Thus, CNV detection in tumour samples was not included in this investigation.

Variant classification

Variants were considered somatic if they were not identified in a paired germline analysis of the corresponding blood sample. Variant classification was performed as previously described.18–21 All genetic variants were classified using a five-tier variant classification system as proposed by the International Agency for Research on Cancer Unclassified Genetic Variants Working Group, namely, deleterious=class 5, likely deleterious=class 4, variant of uncertain significance=class 3, likely benign=class 2 and benign=class 1. Variants reported to occur in large outbred control reference groups at an allele frequency of ≥1% were generally considered benign. Class 4/5 variants were subsequently defined as ‘deleterious variants’.

Quantitative methylation analysis

Bisulfite conversion of 2 ìg of DNA was performed using the innuCONVERT Bisulfite Basic Kit (Analytik Jena, Jena, Germany). Quantitative methylation-specific PCR assays were designed to allow specific amplification of the bisulfite-converted methylated gene promoter sequences of BRCA1, PALB2 and RAD51C. The assays were located in regions previously described to be methylated in OC.23–25 (online Supplementary Figure 1). The assays and the oligonucleotide sequences are described in the online supplementary table 1. PCR was performed in 20 µL reaction volumes with PCR buffer26; per cent methylation was calculated using the 2–ΔΔCT method.27 Triplicate measurements were carried out for each sample, and median methylation levels were computed with values ≥5% considered positive. Of the 496 tumour samples, methylation
analyses for all three target genes were successfully performed for 473 samples.

**Statistical analyses**
Statistical analyses were conducted using SPSS Statistics V.25. Fisher’s exact test and Student’s t-test were used to calculate levels of significance, with p values < 0.05 considered significant.

**RESULTS**
**Prevalence of deleterious germline variants in 473 consecutive individuals with OC**
We focused on 473 individuals enrolled in the AGO-TR1 study for which NGS data for the tumour samples along with promoter methylation analyses of the **BRCA1**, **PALB2** and **RAD51C** genes were available (CONSORT-like diagram, online Supplementary Figure 2). In this subgroup, comprising 90.4% of the overall AGO-TR1 study sample (473/523), the prevalence of deleterious germline variants was similar to that observed in the overall group, that is, deleterious germline variants in established OC genes (ATM, **BRCA1**, **BRCA2**, **BRI1**, **MSH2**, **MSH6**, **PALB2**, **RAD51C**, **RAD51D** and **TP53**). The vast majority of the deleterious single-nucleotide/indel variants affecting the established OC predisposition genes (97/115; 84.3%) showed significantly higher VFs in the tumour-derived versus blood-derived DNA (p < 0.05, Fisher’s exact Test; online Supplementary Table 2), indicating a loss of the wild-type (WT) alleles in the neoplastic tissue. The remaining 14 deleterious single-nucleotide/indel variants were observed in nine of the additional genes, and significantly increased VFs in the tumour tissue were observed for only five variants (35.7%; NBN [2/2], **BUB1B** [1/1], **FAM175A** [1/1], **XRCC2** [1/1], **FANCM** [0/3], **CHEK2** [0/2], **MRE11A** [0/2], **CHEK1** [0/1] and **RAD50** [0/1]).

Deleterious somatic variants in 473 consecutive individuals with OC
Deleterious somatic **TP53** variants (386 variants) were identified in 383 of 473 individuals (81.0%, table 1, figure 1). The majority of the deleterious somatic **TP53** variants were missense variants (238/386; 61.7%; figure 1). Of note, deleterious somatic **TP53** variants were most prevalent in individuals with high-grade serous OC (88.5%) and serous/papillary OC (94.7%). Excluding **TP53**, deleterious somatic variants predominantly affected the **BRCA1** (18 variants; 18 of 473 individuals, 3.8%; table 1) and **BRCA2** genes (12 variants; 12 of 473 individuals, 2.5%; table 1). Deleterious somatic variants in all other established OC predisposition genes were rare (14 variants; 12 of 473 individuals, 2.5%; 4 × **ATM**, 1 × **BRI1**, 1 × **MSH2** and 3 × **MSH6**, 1 × **PALB2**, 1 × **RAD51D**; table 1). Among the additional genes analysed, deleterious somatic variants were most prevalent in the **PIK3CA** gene (26 variants; 24 of 473 individuals, 5.1%), mostly missense variants described to be activating (online Supplementary Table S3, figure 1). Deleterious somatic **PTEN** variants (24 variants) were identified in 16 of 473 individuals (3.4%). Deleterious somatic variants in **PIK3CA** and/or **PTEN** were most prevalent in individuals with high-grade endometrioid, low-grade endometrioid or clear cell OC (16 of 35 individuals, 45.7%; table 1). Notably, we observed the co-occurrence of deleterious somatic **PIK3CA** and **PTEN** variants in 5 of 22 individuals (22.7%) with high-grade endometrioid OC (online Supplementary Table 3).

### Table 1: Study sample stratified by histological subtype, mutation and methylation status. Information on histological subtype was not available for six tumours (‘missing’).

<table>
<thead>
<tr>
<th>Histological subtype</th>
<th>Individuals</th>
<th>gBRCA1 (%)</th>
<th>gBRCA2 (%)</th>
<th>gOCgenes (%)</th>
<th>sTP53 (%)</th>
<th>sBRCA1 (%)</th>
<th>sBRCA2 (%)</th>
<th>sOCgenes (%)</th>
<th>sPIK3CA (%)</th>
<th>sPTEN (%)</th>
<th>mBRCA1 (%)</th>
<th>mRAD51C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-grade serous</td>
<td>373</td>
<td>66 (17.7)</td>
<td>21 (5.6)</td>
<td>23 (6.2)</td>
<td>330 (88.5)</td>
<td>14 (3.8)</td>
<td>10 (2.7)</td>
<td>9 (2.4)</td>
<td>7 (1.9)</td>
<td>2 (0.5)</td>
<td>50 (13.4)</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>High-grade endometrioid</td>
<td>22</td>
<td>3 (13.6)</td>
<td>0 (0)</td>
<td>2 (9.1)</td>
<td>9 (40.9)</td>
<td>2 (9.1)</td>
<td>1 (4.5)</td>
<td>2 (9.1)</td>
<td>5 (22.7)</td>
<td>6 (27.3)</td>
<td>2 (9.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serous/papillary grade unknown</td>
<td>19</td>
<td>2 (10.5)</td>
<td>0 (0)</td>
<td>1 (5.3)</td>
<td>18 (94.7)</td>
<td>1 (5.3)</td>
<td>0 (0)</td>
<td>1 (5.3)</td>
<td>0 (0)</td>
<td>2 (10.5)</td>
<td>1 (5.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Low-grade serous</td>
<td>16</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
<td>2 (12.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (12.5)</td>
<td>1 (6.3)</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Low-grade endometrioid</td>
<td>4</td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (54.2)</td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>1 (14.3)</td>
<td>2 (52.6)</td>
<td>4 (50.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>6</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (66.7)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>6</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other/unspecified</td>
<td>18</td>
<td>2 (11.1)</td>
<td>0 (0)</td>
<td>1 (5.6)</td>
<td>15 (83.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (16.7)</td>
<td>1 (5.6)</td>
<td>2 (11.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>6 (100)</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>473</td>
<td>75 (15.9)</td>
<td>23 (4.9)</td>
<td>28 (5.9)</td>
<td>383 (81.0)</td>
<td>18 (3.8)</td>
<td>12 (2.5)</td>
<td>12 (2.5)</td>
<td>24 (5.1)</td>
<td>16 (3.4)</td>
<td>57 (12.1)</td>
<td>10 (2.1)</td>
</tr>
</tbody>
</table>

A total of 18 tumours were summarised as ‘other/unspecified’ of which 11 tumours showed a mixed histopathology (defined by two or more distinct histological subtypes present based on routine histopathological assessment), 4 tumours were undifferentiated, 2 were adenocarcinomas and 1 was a pleomorphic cellular tumour. In the group of individuals with high-grade serous carcinoma, the mean age at first diagnosis was significantly younger in carriers of deleterious germline **BRCA1/2** variants (52.4 years, range 30–77; p = 0.0001, Student’s t-test) and in individuals with **BRCA1** promoter methylation (55.5 years, range 32–78; p = 0.0205, Student’s t-test) compared with all individuals with high grade serous carcinoma (59.2 years, range 21–93).

g, germline; m**BRCA1**, m**BRCA2** promoter methylation ≥5%; m**RAD51C**, m**RAD51D** promoter methylation ≥5%; s, somatic.
Deleterious somatic variants in all additionally tested genes were rare. Deleterious somatic MAP3K1 variants (five variants) were identified in 5 of 473 individuals (1.1%; figure 1). All other genes affect less than 1% of all individuals for each gene (figure 1; 1 × BARD1, 2 × CHEK1, 4 × FANCM, 1 × PMS2).

Promoter methylation analyses of the BRCA1, PALB2 and RAD51C genes
Promoter methylation analyses were carried out for the BRCA1, PALB2 and RAD51C genes in the 473 tumour samples. PALB2 gene promoter methylation was not detected in any of our tumour samples (online Supplementary Table S3). PALB2 promoter methylation was previously described in OC with a clear cell subtype 23 (representing only 1.3% of our study sample; table 1) but not in high-grade serous carcinoma. 28 Methylation of the BRCA1 gene promoter was identified in 57 of 473 individuals (12.1%; table 1). Methylation of the RAD51C gene promoter was identified in 10 of 473 individuals (2.1%; table 1).

Combined analysis of the study results
In summary, 125 of 473 individuals (26.4%) carried deleterious germline variants in the established OC predisposition genes, with LOH representing the second hit necessary for tumour development in a large proportion (84.3%) of these samples (figure 2). Deleterious somatic variants in established OC predisposition genes (excluding TP53) were identified in...
Cancer genetics

39 of 473 individuals (8.2%, figure 2). Promoter methylation of the BRCA1 and RAD51C genes was observed in 57 of 473 individuals (12.1%) and 10 of 473 individuals (2.1%), respectively (figure 2). With a few exceptions, these somatic single-nucleotide/indel variants and methylation of BRCA1 and RAD51C and deleterious germline variants were mutually exclusive. The same holds true for the subgroup of 33 individuals (7.0%) carrying deleterious somatic PIK3CA and/or PTEN gene variants (figure 2).

DISCUSSION

It is generally assumed that the heterozygous germline inactivation of cancer predisposition genes may be accompanied by a somatic inactivation of the second allele by deleterious variant, LOH or promoter methylation in the neoplastic tissue. In this large investigation of 473 individuals with OC, we demonstrated that the majority of the deleterious germline single-nucleotide/indel variants affecting established OC predisposition genes (97/115; 84.3%) showed significantly higher VFs in tumour-derived versus blood-derived DNA samples (online Supplementary Table 2), indicating the loss of the WT alleles by either large deletions (ie, LOH with copy number losses) or copy number neutral LOH.\(^{30}\)

Regarding individuals with deleterious BRCA1/2 germline variants, only 1 of 97 individuals (1.0%, figure 2) carried a deleterious germline variant plus a different deleterious somatic variant in the BRCA2 gene (individual #92; online Supplementary Table 3).

Regarding other established OC predisposition genes, only 1 of 28 individuals (3.6%, figure 2) carried a deleterious germline variant plus a different deleterious somatic variant in the MSH6 gene (individual #119; online Supplementary Table S3). The BRCA1/RAD51C promoter methylation was not found in individuals carrying a deleterious germline or somatic variant in the same gene. Thus, deleterious somatic single-nucleotide/indel variants or promoter methylation rarely represent the second hit in the development of hereditary OC.

Deleterious somatic variants in OC predisposition genes (excluding TP53) were predominantly found in the BRCA1 (18 variants; 18 of 473 individuals, 3.8%) and BRCA2 genes (12 variants; 12 of 473 individuals, 2.5%). The prevalence of deleterious somatic BRCA1/2 variants is comparable with those reported in previous studies.\(^{11,13,30}\) Deleterious somatic variants in other established OC predisposition genes (excluding TP53) were generally rare, affecting less than 1% of all individuals for each gene (figure 1). Consequently, our analyses demonstrate that the vast majority of deleterious variants found in the tumour were of germline origin. Regarding BRCA1/2, 128 deleterious variants were found in the tumour, of which 98 (76.6%) were proven germline variants. For all genes significantly associated with OC (excluding TP53), 168 deleterious variants were found in the tumour, of which 126 (75.0%) were proven germline variants. Thus, tumour testing without prior germline testing may require genetic counselling due to its potential implications for the stratification of the risk of the individual for other malignancies (ie, breast cancer) and, most importantly, the stratification of the cancer risks of family members who may benefit from predictive testing and prophylactic measures. Moreover, genetic tumour testing alone may miss deleterious germline CNVs in OC predisposition genes.\(^{31}\)

Due to the high prevalence of deleterious germline variants in OC predisposition genes, germline testing is generally recommended in individuals with OC. We suggest that germline mutation analysis of established or proposed OC predisposition genes should be complemented with genetic tumour testing in patients without deleterious germline variants in these genes. Genetic tumour testing of OC predisposition genes, the PIK3CA and PTEN genes and BRCA1 and RAD51C promoter methylation analyses identified a large subgroup of germline mutation-negative individuals (125/348, 35.9%), which may be addressed in future interventional studies using poly ADP ribose polymerase (PARP) or phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) inhibitors.\(^{32}\)

Matulonis et al\(^{11}\) already described the activity of olaparib in combination with a PI3K-inhibitor. However, they focused only on high-grade serous OC. Our data suggest that treatment options with PI3K inhibitors could also be further investigated in other histological subtypes. In our series, the highest rates of deleterious somatic PIK3CA and/or PTEN variants were shown in the subgroup of individuals with endometrioid and clear cell tumours (table 1).

In summary, germline, somatic or epigenetic alterations that likely impair OC predisposition gene activity were identified in 224 of 473 individuals (47.4%, figure 2), with an even higher prevalence of (epi)genetic alterations in the subgroup of individuals with high-grade serous OC (figure 2B).

Regarding other established OC predisposition genes, germline testing is generally recommended with regard to progression-free survival among women with newly diagnosed advanced OC and a deleterious BRCA1/2 variant, with a 70% lower risk of disease progression or death with olaparib compared with placebo.\(^{34}\) The question emerges, however, whether the presentation of BRCA1/RAD51C promoter methylation or a deleterious somatic BRCA1/2 variant represents a robust predictive marker for therapy response, especially regarding PARP inhibitor therapy. The response to PARP inhibition was found to depend on HR deficiency and is not restricted to deleterious BRCA1/2 germline variants,\(^{16}\) and in vitro analyses suggested that BRCA1 hypermethylation and BRCA1 deleterious variants conferred the same degree of sensitivity to PARP inhibition.\(^{3}\) Further prospective studies will help clarify the clinical significance of these somatic (epi)mutations as biomarkers to direct targeted therapy.

Limitations of this study

Due to the mean read coverage of 570×, we used a VF cut-off value of at least 5%. Therefore, we cannot exclude the existence of low-abundance variants in the tumour samples investigated in this study.

Author affiliations

1Center for Hereditary Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University Hospital Cologne, Cologne, Germany
2Department of Gynecology and Gynecologic Oncology, Kliniken Essen-Mitte Evangelische Huyssens-Stiftung/Knappschaft GmbH, Essen, Germany
3Coordinating Center for Clinical Trials, Philippus-University of Marburg, Marburg, Germany
4Department of Women’s Health, University Hospital Tuebingen, Tuebingen, Germany
5Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany
6National Center for Tumor Disease, Department of Gynecology, University of Heidelberg, Heidelberg, Germany
7Department of Gynecology and Gynecologic Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
8Institute of Pathology, University Hospital Bonn, Bonn, Germany
9Department of Gynecology, University Hospital Munich-Großhadern, Munich, Germany
10Center for Hereditary Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University Hospital Cologne, Cologne, Germany
11Department of Gynecology and Obstetrics, University Hospital, Universität Ulm, Ulm, Germany
Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer reviewed.
Cancer genetics