Clinical Epigenetics

RESEARCH

Open Access

BRCA loss of function including *BRCA1* DNA-methylation, but not *BRCA*-unrelated homologous recombination deficiency, is associated with platinum hypersensitivity in high-grade ovarian cancer

Heidelinde Fiegl[1*†], Simon Schnaiter[2†], Daniel U. Reimer[1], Katharina Leitner[1], Petra Nardelli[1], Irina Tsibulak[1], Verena Wieser[1], Katharina Wimmer[2], Esther Schamschula[2], Christian Marth[1] and Alain G. Zeimet[1*]

Abstract

Background In high-grade ovarian cancer (HGOC), determination of homologous recombination deficiency (HRD) status is commonly used in routine practice to predict response to platinum-based therapy or poly (ADP-ribose) polymerase inhibitors (PARPi). Here we tested the hypothesis that BRCA loss of function (LOF) due to epigenetic or genetic aberrations is a better predictor for the clinical outcome than HRD. One hundred thirty-one HGOC tissues were tested for *BRCA* DNA-methylation, *BRCA* mutations, HRD and *BRCA1* mRNA expression, followed by a comprehensive survival analysis.

Results *BRCA1*-methylation was detected in 11% of the tumors, exclusively in *BRCA1*-wild-type (wt) HGOCs. *BRCA1*-methylated tumors (*BRCA1*-meth) had HRD-scores similar to those of *BRCA*-mutated (mut) tumors, and higher compared to unmethylated-*BRCA*-wt tumors (*BRCA*-wt-unmeth; $P < 0.001$). Platinum-refractory or -resistant HGOCs at first recurrence were all *BRCA*-unmeth cancers. Only one of the *BRCA*-mut cancers had a platinum-resistant recurrence. Thus, 99% of relapses in cancers with epigenetic or genetic *BRCA*-alterations were platinum-sensitive. Multivariate analysis confirmed BRCA-LOF as an independent predictor of progression-free survival (PFS) and overall survival (OS), whereas HRD-status had no predictive value for PFS and OS. Patients with *BRCA*-wt-unmeth cancers had the worst outcome compared to patients with cancers harboring epigenetic or genetic *BRCA*-alterations (PFS: $P = 0.007$; OS: $P = 0.022$). Most importantly, the *BRCA*-wt-unmeth subfraction of HRD-positive HGOCs exhibited the same poor survival as the entire HRD-negative cohort.

Conclusion In HGOC *BRCA* mutational status together with *BRCA1*-methylation exhibit the best predictive power for favorable clinical outcome and thus high sensitivity to platinum-based therapy, whereas *BRCA*-unrelated HRD positivity was not associated with improved platinum sensitivity.

†Heidelinde Fiegl and Simon Schnaiter contributed equally.

*Correspondence:
Heidelinde Fiegl
Heidelinde.Fiegl@i-med.ac.at
Alain G. Zeimet
Alain.Zeimet@i-med.ac.at
Full list of author information is available at the end of the article

Keywords Ovarian cancer, *BRCA1*, *BRCA2*, DNA-methylation, Homologous recombination deficiency (HRD), Platinum sensitivity, PARP inhibitor therapy

Background

Epithelial ovarian cancer (EOC) is the eighth most common cancer in women and is the leading cause of death from gynecologic cancers [1, 2]. Most EOCs are diagnosed at an advanced stage (FIGO-stage III–IV), of which approximately 85% are HGOC.

Treatment options for EOC have evolved significantly in recent years with the use of targeted therapies, such as antiangiogenic agents and PARPi [3–6]. Homologous recombination repair (HRR) deficiency (HRD) is considered to be a predictive biomarker for platinum and PARPi therapy response in HGOC [7, 8]. A well-recognized intrinsic biomarker of PARPi response in EOC is platinum sensitivity, thus leading to the approval of olaparib, niraparib and rucaparib monotherapy as maintenance therapy in platinum-sensitive recurrent EOC [9–11]. HRD occurs in about half of HGOC as shown by pathway analyses or clinical trials [12, 13]. The main causes of HRD are germline or somatic mutations in the *BRCA1* and *BRCA2* genes (13–20%), but also other genes of the HRR-pathway (e.g. *PALB2, RAD51C/D*) and a number of currently unknown factors [12, 14–18]. Identification of additional mechanisms leading to HRD may increase the number of patients who might benefit from PARPi-therapy. *BRCA1* DNA-methylation, another mechanism of *BRCA1*-inactivation, has been reported in 5–12% in OCs [12, 19–23]. To date, *BRCA*-methylation determination is not separately performed in routine analysis. Kalachand et al. recently described in a meta-analysis including data from 2636 OC patients across 15 studies that *BRCA1*-methylated (*BRCA1*-meth) OC showed similar clinicopathological features as *BRCA1*-mut OC in terms of high grade and young age at diagnosis, but unlike *BRCA*-mutated (*BRCA*-mut) cancers, *BRCA1*-meth OC was not associated with improved survival [24]. Recently, we described that *BRCA1/2* mRNA expression has been shown to be of clinical significance in OC [25]. Here, we investigated BRCA loss of function (LOF) via epigenetic or genetic aberrations in relation to HRD and *BRCA1* mRNA expression to gain a better understanding of the clinical significance of BRCA-LOF emphasizing also *BRCA1* promotor methylation for platinum sensitivity and its impact on the prognosis of OC patients.

Methods

Patients and samples

EOC tissue samples from 131 patients obtained during primary debulking ($n=123$) or biopsy prior to neoadjuvant chemotherapy ($n=8$) were collected and processed at the Department of Obstetrics and Gynecology, Medical University of Innsbruck (MUI) between 1989 and 2021. Staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) classification system. Only patients who received platinum-based first-line chemotherapy were eligible for this study; a total of 54 patients received bevacizumab maintenance therapy; PARPi were not yet approved for routine use at that time. All patients were monitored within the outpatient follow-up program of our department. Clinical, pathological and follow-up data were stored in a database according to the privacy policy of our hospital. The study was approved by the Ethics Committee of the MUI (reference numbers: AN2015-0038 346/4.17; 1054/2019) and conducted following the Declaration of Helsinki. An overview of the molecular analyses performed on the patient samples and the corresponding case numbers is shown in Supplementary Fig. 1.

DNA-isolation

Genomic DNA was isolated from pulverized, quick-frozen specimens ($n=131$) using the DNeasy Tissue Kit (Qiagen, Hilden, Germany).

BRCA-methylation analysis

Bisulfite-modification of all 131 tumor DNA samples was performed using the EZ DNA-Methylation-Gold-Kit (Zymo Research, CA, USA) according to the manufacturer's instructions. Primers and probes for *BRCA1*- and *BRCA2* promoter-methylation analysis were used as previously described [26]. MethyLight-analysis was performed and the percentage of methylated reference (PMR) values was calculated, as previously reported [25], using a *BRCA1* PMR > 10 as the cut-off for promoter-hypermethylation, as described by Weisenberger et al. [26]. PMR-values were adjusted for the proportion of tumor cells (this proportion was calculated within each sample based on the variant allele frequencies (VAF) of SNPs in regions of LOH in neoplastic cells).

BRCA-mutation analysis

Targeted NGS was conducted for all 131 tumor samples using the TruSight Cancer Sequencing Panel (Illumina, San Diego, USA) on the Illumina MiSeq®- and the NextSeq-system (Illumina, CA, USA). Mutation analysis was performed using SeqNext® Software (JSI medical systems GmbH, Ettenheim, Germany). Variants with a VAF of at least 10% were classified according to the consensus recommendations of the American College of Medical Genetics [27].

Array analysis

The DNA from 120 tumor samples was analyzed using the Global Screening Array (Illumina) according to the manufacturer's protocol. To determine the tumor cell proportion, data were analyzed with Illumina GenomeStudio 2.0 and NxClinical (Bionano, San Diego, CA, USA, SNP-FASST2-Segmentation Algorithms). For the determination of tumor cell proportion, regions of copy number neutral loss of heterozygosity (LOH) were selected and the tumor cell proportion (aberrant cell fraction) was calculated based on the variant allele frequency of the SNPs. For the determination of the HRD-score, regions of LOH, allelic imbalance and copy number variations were quantified.

HRD-assessment

HRD-data were available for 120 patients analyzed in this study. For HRD-quantification, a LOH-score and an Aneuploidy-Normalized-Telomeric-Imbalance (ANTI)-Score were used, based on the LOH-score published by Frampton et al. and using a modification of the Telomeric-Allelic-Imbalance (TAI)-score by Birkbak et al. [28, 29]. Both variables were equally weighted and integrated into a common HRD-score with a diagnostic cut-off value of ≥ 1.77 to discriminate between HRD-positive (HR-deficient) and HRD-negative (HR-proficient) tumors [30]. To determine genome-wide copy number variation and allelic variation, the DNA was analyzed by the Global Screening Array (Illumina) according to the manufacturer's protocol, and data analysis was performed using Illumina GenomeStudio 2.0 and NxClinical (Bionano, SNP-FASST2-Segmentation Algorithms) software. This HRD-test has been validated by paired analyses using Myriad MyChoice DX (Myriad Genetics). A correlation-coefficient of 0.86 was revealed between the respective scores, with 100% agreement in the classification of samples as HRD-positive and HRD-negative [30].

BRCA1 mRNA expression analysis

RNA-isolation, reverse-transcription and quantitative real-time PCR were performed as previously described [25]. Primers and probe for *BRCA1* were purchased from Applied Biosystems (Foster City, CA, USA, Applied Biosystems Assay ID: Hs01556193_m1). *BRCA1*-mRNA expression was adjusted to tumor cell proportion ($n=63$).

Statistics

Comparisons between two continuous variables were made using the Mann–Whitney test and between more continuous variables using the Kruskal–Wallis test. The Chi-square test was used to test for differences in categorical variables. Survival analysis was performed using Kaplan–Meier curves and the log-rank test. Cox-regression analysis was used for multivariate survival analysis (all variables from in the univariate analysis which revealed P values <0.2 were included in the multivariate analysis). For analyses of *BRCA1* DNA methylation in relation to platinum sensitivity, progression-free patients who were lost to follow-up within 6 months after the last platinum treatment ($n=9$) were excluded. All statistical analyses were performed using SPSS (version 29.0; SPSS Inc., Chicago, IL, USA).

Results

BRCA1 methylation status in relation to BRCA mutations and clinical pathological characteristics

Analysis of *BRCA1*- and *BRCA2*-methylation in 131 HGOC tissue samples revealed *BRCA1*-methylation in 11% (14/131) of the tumors. No *BRCA2*-methylation was observed in any of the samples. Furthermore, in none of the *BRCA1*-mut cancers a *BRCA1* promotor methylation was identified, suggesting that epigenetic silencing of *BRCA1* and *BRCA1*-mutations may be mutually exclusive. *BRCA1*-methylation was almost exclusively found in 19% of *BRCA*-wild-type (wt) tumors (13/70), whereas only one *BRCA2*-mut tumor was *BRCA1*-meth (Table 1). Furthermore, 83% (10/12) of *BRCA1*-meth tumors were positively associated with HRD-positivity, whereas *BRCA*-wt-unmethylated (*BRCA*-wt-unmeth) tumors were predominantly HRD-negative (73%; 41/56) ($P<0.001$). For completeness, in general, HRD-positive scores were detected in 63% of the samples analyzed (76/120) and in 98% of *BRCA*-mut tumors (51/52). The respective HRD-scores of the different molecular subgroups are depicted in Fig. 1A. Notably, the HRD-scores did not differ between *BRCA1*-meth (median HRD-score = 2.65) and *BRCA1*-mut tumors (median HRD-score = 2.73). Furthermore, lower *BRCA1* mRNA expression was observed in *BRCA1*-meth or *BRCA1*-mut tumors in comparison to *BRCA1*–wt tumors. (Fig. 1B; $P=0.005$, $P=0.031$).

BRCA-LOF by epigenetic or genetic aberrations was associated with younger age ($P=0.007$), but with no

Table 1 Associations of *BRCA* DNA-methylation and clinicopathological and genetic features

Variable			*BRCA*-methylation[§]			BRCA-LOF by epigenetic or genetic aberrations		
		Total	No	Yes	*P* value	No	Yes	*P* value
Age	≤ 59.8 years	66	57	9	0.271	21	45	**0.007**
	> 59.8 years	65	60	5		36	29	
FIGO stage	I/II	15	14	1	0.592	5	10	0.398
	III/IV	116	103	13		52	64	
Tumor grade	2	54	50	4	0,309	25	29	0.590
	3	77	67	10		32	45	
Residual disease	Macroscopically tumor-free or < 1 cm	91	83	8	0.244	44	47	0.080
	Any tumor residual (> 1 cm)	38	32	6		12	26	
	n.a	2	–	–		–	–	
Histology	HGSOC	114	102	12	0.877	50	64	0.835
	HGEOC	17	15	2		7	10	
BRCA genetic aberrations	Wild-type	70	57	13	**0.002**	57	13	**< 0.001**
	Mutation (*BRCA1; BRCA2*)	61 *(45; 16)*	60 *(45; 15)*	1 *(0; 1)*		0	61 *(45;16)*	
HRD	Low (< 1.77)	44	42	2	0.092	41	3	**< 0.001**
	High (> 1.77)	76	65	11		15	61	

The significance level (P) was determined by Chi Square analysis

Bold values indicate *P*-values < 0.05; the numbers in italics represent the specific data for *BRCA1* and *BRCA2* mutations

[§] *BRCA1 DNA methylation; BRCA2 DNA methylation was not detected*

FIGO International Federation of Gynecology and Obstetrics; *HGEOC* high-grade endometrioid ovarian cancer; *HGSOC* high-grade serous ovarian cancer; *HRD* homologous recombination deficiency; *LOF* loss of function; *n.a.* not available

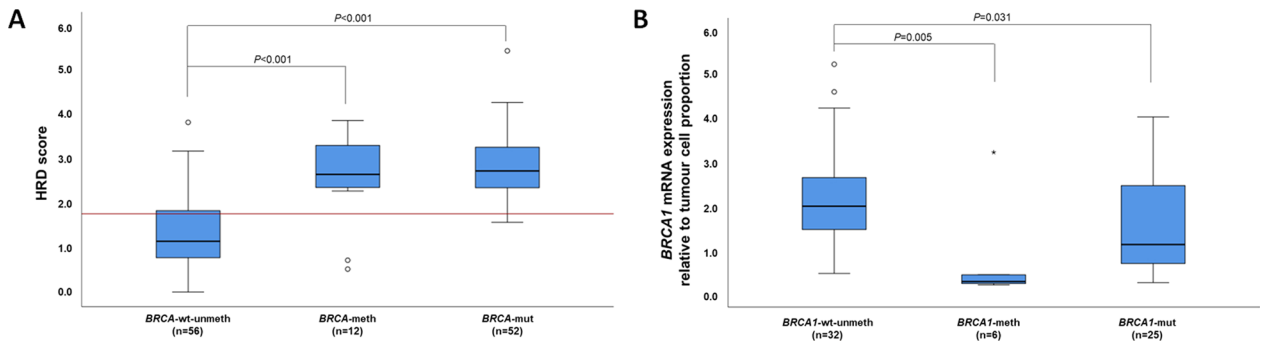

Fig. 1 *BRCA1* DNA-methylation in relation to *BRCA*-mutations, HRD and *BRCA1* mRNA expression. (**A**) HRD-scores in different OC subgroups according to *BRCA* epigenetic or genetic aberrations ($n = 120$). HRD-score threshold was defined as ≥ 1.77 (red line). The HRD-scores above this threshold were considered HRD-positive (HR-deficient) and values below the threshold were considered HRD-negative (HR-proficient). *Note: The BRCA2-mut-meth tumor is included in the BRCA-mut group.* (**B**) *BRCA1* mRNA expression in relation to *BRCA1* epigenetic or genetic aberrations ($n = 63$) *Note: BRCA2-mut tumors are included in the BRCA1-wt group, the BRCA2-mut-meth tumor is included in the BRCA1-meth group*

other classic clinicopathological features as shown in Table 1.

BRCA-LOF by epigenetic or genetic aberrations and platinum sensitivity

Platinum sensitivity was defined by the interval between the last platinum administration and the first recurrence: disease progression within six months was considered platinum-resistant and after six months platinum-sensitive. Referring selectively to the *BRCA*-methylation and -mutational status, 71 of 72 cancers with epigenetic ($n = 12$) or genetic ($n = 59$) *BRCA*-aberrations were found to be platinum-sensitive (positive predictive value (PPV) = 99%; $P < 0.001$), and 92% (11/12) of HGOC patients classified as platinum-resistant had *BRCA*-wt-unmeth tumors, whereby it should be noted that none of

the platinum-resistant cancers showed *BRCA1*-methylation. We also analyzed this separately in HGOC-patients with residual disease after primary surgery ($n=35$) in order to obtain better comparability and a more precise estimation of platinum-responsiveness. Again, 24 out of 25 tumors with epigenetic ($n=5$) or genetic ($n=19$) *BRCA*-aberrations were classified as platinum-sensitive (PPV = 96%; $P=0.001$) and 83% (5/6) of HGOC patients with assumed platinum-resistant cancers had *BRCA*-wt-unmeth tumors.

However, based on the common diagnostic approach combining the *BRCA*-mutational and HRD-status, 80 of 84 *BRCA*-mut and/or HRD-positive tumors were platinum-sensitive (PPV = 95%; $P=0.011$) and only 64% (7/11) of patients considered as platinum-resistant had *BRCA*-wt, HRD-negative tumors. In patients with residual disease, 25 of 27 *BRCA*-mut and/or HRD-positive tumors were platinum-sensitive (PPV = 93%; $P=0.005$) and 67% (4/6) of cancers defined as platinum-resistant were *BRCA*-wt, HRD-negative. When considering HRD-status alone, the PPV for platinum-sensitive recurrent disease was 91% (20/22) in cases with residual disease.

BRCA-LOF by epigenetic or genetic aberrations and survival

Univariate survival analysis revealed that patients with tumors harboring BRCA-LOF had a favorable progression-free survival (PFS) and overall survival (OS) (median-PFS: 2.7 years, 95% CI 1.5–3.9; median-OS: 7.8 years, 95% CI 6.0–9.6) compared to patients with *BRCA*-wt-unmeth cancer (median-PFS: 1.8 years, 95% CI 1.1–2.4; median-OS: 5.4 years, 95% CI 3.5–7.3) as shown in Table 2 and Figs. 2A and 2B (PFS: $P=0.011$; OS: $P=0.047$). This was also confirmed in the multivariate analysis (Table 3A; PFS: HR 0.5, 95% CI 0.3–0.8, $P=0.007$ and OS: HR 0.6, 95% CI 0.3–0.9, $P=0.022$).

Interestingly, the subfraction of patients with HRD-positive tumors which are unrelated to BRCA-LOF (HRD-pos. *BRCA*-wt-unmeth) representing 20% (15/76) of all HRD-positive tumors; with a median-PFS of 1.8 years, (95% CI 1.4–2.2) and a median-OS of 6.6 years, (95% CI 0.2–13.1) did not differ in survival from patients with HRD-negative *BRCA*-wt-unmeth tumors (median-PFS: 1.8 years, 95% CI 0.5–3.0; median-OS: 5.4 years, 95% CI 3.2–7.6) (Table 2; Fig. 2C, D), nor did they differ from patients of the entire HRD-negative cohort (median-PFS: 2.1 years, 95% CI 1.0–3.3, $P=0.533$; median-OS: 5.4 years, 95% CI 3.3–7.5, $P=0.743$; Fig. 2E, F). Survival analysis of these patients with HRD-positive tumors unrelated to BRCA-LOF in relation to BRCA-LOF tumors, revealed a significantly worse PFS (Fig. 2C; $P=0.048$), but no difference in OS (Fig. 2D; $P=0.131$).

Neither HRD- nor *BRCA* mutational status as usually determined in routine practice had any predictive value in our cohort (Table 2; Table 3B).

A subgroup analysis revealed that superior survival in patients with tumors harboring BRCA-LOF was only observed in patients without the addition of bevacizumab to chemotherapy as a maintenance-therapy (median-PFS: 2.5 years, 95% CI 0.5–4.5, median-OS: 7.8 years, 95% CI 5.8–9.7) compared to patients with *BRCA*-wt-unmeth HGOC (median-PFS: 1.3 years, 95% CI 0.8–1.9; median-OS: 3.9 years, 95% CI 0.0–8.5) (Table 2; PFS and OS each $P=0.030$). This survival advantage disappeared in patients treated with bevacizumab (Table 2). These findings were confirmed in the multivariate analyses shown in Table 3C for PFS (HR 0.4, 95% CI 0.2–0.8, $P=0.007$) and OS (HR 0.5, 95% CI 0.3–0.9, $P=0.012$).

Discussion

Based on data from The Cancer Genome Atlas (TCGA), Yang et al. showed loss of *BRCA1* mRNA expression by *BRCA1*-methylation in 10% (33/316) of HGSOC and no promoter-hypermethylation of *BRCA2* [23]. This was confirmed by Abkevich et al. [22] and is also consistent with our results. According to our data, *BRCA1*-methylation appears to affect the DNA HRR-mechanism to the same extent as pathogenic mutations of both *BRCA* -genes, substantiated by an almost identical extent of genome-wide scarring (HRD-score) in both cancer subgroups. This is in agreement with the findings of Kalachand et al. [24].

In recent years, the prognostic and predictive significance of *BRCA1*-methylation in OC has been the subject of intense debate. A decade ago, TCGA-data on OC-patients treated with platinum-based chemotherapy showed a significant OS superiority in *BRCA*-mut compared to *BRCA*-wt cancers, but this was not the case for *BRCA1*-wt-meth cancers, which behaved like *BRCA*-wt cancers [12].

A recent meta-analysis with data from 2,636 OC patients showed that *BRCA1*-methylation was not associated with increased survival [24]. However, in our work we focus on BRCA-LOF due to epigenetic or genetic aberrations and compared this combined assessment with clinicopathological features and the determination of HRD-status to predict clinical outcome. BRCA-LOF was detected predominantly in tumors from younger patients, but there was no association between *BRCA1* methylation and age. It would appear that the status of the *BRCA* mutation is the primary driver of this effect, with germline *BRCA* mutations potentially associated with this finding. In terms of survival, it is noteworthy that the subfraction of patients with tumors exhibiting HRD-positivity unrelated to BRCA-LOF had the same

Table 2 Univariate survival analysis in 131 HGOC-patients

Variable		Progression-free survival			Overall survival		
		events/total	Median, years (95% CI)	*P* value	events/total	Median, years (95% CI)	*P*-value
Age	< 60.0 years	46//66	2.4 (1.6–3.2)	0.682	34/66	6.9 (5.0–8.8)	0.487
	> 60.0 years	39/65	2.2 (1.3–3.1)		32/65	5.8 (2.8–8.8)	
FIGO stage	I/II	5/15	n.r	**0.005**	5/15	9.8 (4.9–14.6)	0.075
	III/IV	80/116	2.0 (1.5–2.4)		61/116	6.4 (4.6–8.1)	
Tumor grade	2	35/54	1.9 (1.2–2.6)	0.562	32/54	6.4 (2.9–9.9)	0.556
	3	50/77	2.5 (1.7–3.3)		34/77	6.5 (5.1–8.0)	
Residual disease	Macroscopically tumor-free or < 1 cm	53/91	2.7 (2.2–3.2)	**< 0.001**	33/91	7.8 (5.5–10.1)	**< 0.001**
	Any tumor residual (> 1 cm)	31/38	1.5 (1.1–1.9)		32/38	3.0 (1.6–4.3)	
Histology	HGSOC	74/114	2.0 (1.4–2.5)	0.222	56/114	6.3 (4.8–7.8)	0.133
	HGEOC	11/17	3.1 (2.2–4.1)		10/17	8.9 (7.0–10.9)	
BRCA genetic aberrations	No	45/70	1.8 (0.9–2.8)	0.085	35/70	5.7 (3.9–7.6)	0.113
	Yes	40/61	2.5 (1.5–3.5)		31/61	7.8 (5.6–9.9)	
HRD status	Negative (HR-proficient)	29/44	2.1 (1.0–3.3)	0.273	23/44	5.4 (3.3–7.5)	0.250
	Positive (HR-deficient)	49/76	2.4 (1.6–3.2)		36/76	6.9 (5.2–8.5)	
Subgroup BRCA-unmeth	*Negative (HR-proficient)*	*27/42*	*2.1 (0.9–3.4)*	*0.372*	*21/42*	*5.4 (3.1–7.7)*	*0.328*
	Positive (HR-deficient)	*44/65*	*2.2 (1.5–2.9)*		*32/65*	*6.9 (5.0–8.7)*	
Subgroup BRCA-meth	*Negative (HR-proficient)*	*2/2*	*1.3 (n.r.)*	*0.639*	*2/2*	*4.9 (n.r.)*	*0.999*
	Positive (HR-deficient)	*5/11*	*3.1 (0.0–6.7)*		*4/11*	*3.4 (n.r.)*	
Subgroup BRCA-wt-unmeth	*Negative (HR-proficient)*	*27/41*	*1.8 (0.5–3.0)*	*0.680*	*21/41*	*5.4 (3.2–7.6)*	*0.879*
	Positive (HR-deficient)	*11/15*	*1.8 (1.4–2.2)*		*8/15*	*6.6 (0.2–13.1)*	
BRCA-LOF by epigenetic or genetic aberrations	No (wt, unmeth)	39/57	1.8 (1.1–2.4)	**0.011**	30/57	5.4 (3.5–7.3)	**0.047**
	Yes (mut or meth)	46/74	2.7 (1.5–3.9)		36/74	7.8 (6.0–9.6)	
Subgroup HRD negative (HR-proficient)	*No (wt, unmeth)*	*27/41*	*1.8 (0.5–3.0)*	*0.332*	*21/41*	*5.4 (3.2–7.6)*	*0.436*
	Yes (mut or meth)	*2/3*	*5.0 (0.0–11.0)*		*2/3*	*7.8 (3.2–12.4)*	
Subgroup HRD positive (HR-deficient)	*No (wt, unmeth)*	*11/15*	*1.8 (1.4–2.2)*	*0.098*	*8/15*	*6.6 (0.2–13.1)*	*0.159*
	Yes (mut or meth)	*38/61*	*2.5 (1.4–3.6)*		*28/61*	*7.8 (5.8–9.8)*	
Subgroup no maintenance therapy with bevacizumab	*No (wt, unmeth)*	*21/31*	*1.3 (0.8–1.9)*	***0.030***	*24/31*	*3.9 (0.0–8.5)*	***0.030***
	Yes (mut or meth)	*29/46*	*2.5 (0.5–4.5)*		*29/46*	*7.8 (5.8–9.7)*	
Subgroup maintenance therapy with bevacizumab	*No (wt, unmeth)*	*18/26*	*2.5 (1.9–3.0)*	*0.057*	*6/26*	*6.6 (n.r.)*	*0.615*
	Yes (mut or meth)	*17/28*	*3.0 (1.6–4.4)*		*7/28*	*6.4 (1.5–11.3)*	

The significance level (*P*) was determined by log-rank test

Bold values indicate *P* values < 0.05; subgroup analyses are shown in italics

CI confidence interval; *FIGO* International Federation of Gynecology and Obstetrics; *HGEOC* high-grade endometrioid ovarian cancer; *HGSOC* high-grade serous ovarian cancer; *HR* homologous recombination; *HRD* homologous recombination deficiency; *LOF* loss of function; *n.r.* not reached; *wt* wild-type

poor survival as patients with HRD-negative cancers. Overall, our data show that from the generally expected 50% HRD-positive HGOCs, only the group of BRCA-LOF cancers, consisting of *BRCA*-mut or *BRCA1*-meth cancers represent those with exceptionally high sensitivity to platinum-based chemotherapy.

Interestingly the proportion of patients with platinum-resistant recurrence among *BRCA1/2* mutant cases in our cohort is very low compared to the literature -Alsop et al. report a percentage of 14.9% of platinum-resistant patients with *BRCA1/2* mutations [31]. Multifactorial causes such as *BRCA* mutation type and prevalence, surgical radicality, the interval between surgery and the start of chemotherapy, chemotherapy dose density, the use of combination chemotherapy or chemo-monotherapy could explain this difference.

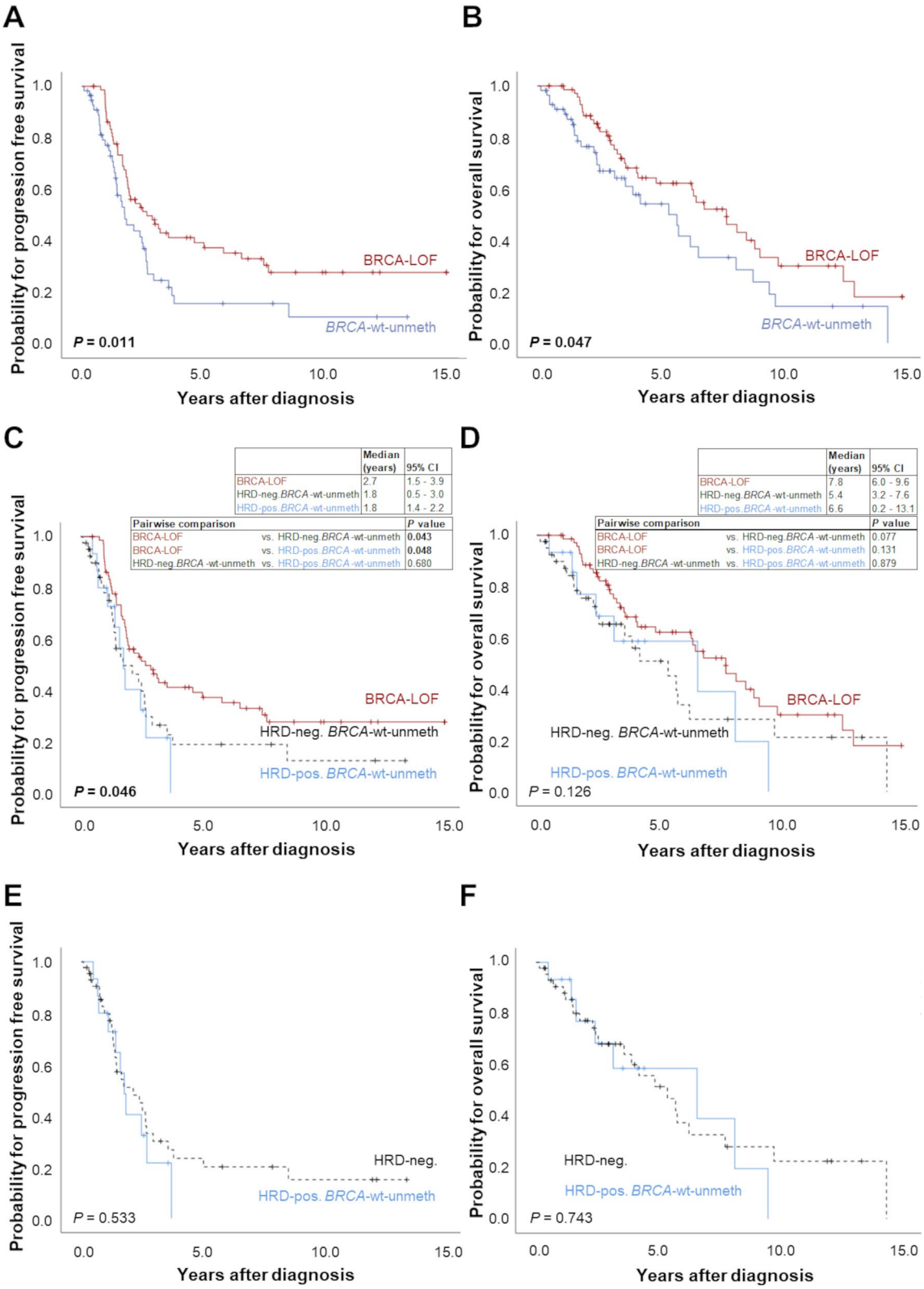

Fig. 2 BRCA-LOF by epigenetic or genetic aberrations and survival. (**A**) Analysis of progression-free survival (PFS) and (**B**) overall survival (OS). Subgroup analyses of HRD-positive *BRCA*-wt-unmeth HGOC for (**C**) PFS and (**D**) OS compared with HRD-negative *BRCA*-wt-unmeth and with BRCA-LOF HGOC and for (**E**) PFS and (**F**) OS compared with the entire HRD-negative cohort

Table 3 Multivariate analysis in HGOC patients

Variable		Progression-free survival		Overall survival	
		HR of progression (95% CI)	*P*-value	HR of death (95% CI)	*P* value
A					
FIGO stage	I/II versus III/IV	2.5 (1.0–6.3)	**0.048**	1.6 (0.6–4.1)	0.310
Residual disease	no versus yes	2.2 (1.4–3.5)	**< 0.001**	2.5 (1.5–4.2)	**< 0.001**
Histology	HGSOC versus HGEOC	–	–	0.7 (0.3–1.3)	0.257
BRCA-LOF	no versus yes	0.5 (0.3–0.8)	**0.007**	0.6 (0.3–0.9)	**0.022**
B					
FIGO stage	I/II versus III/IV	2.6 (1.0–6.6)	**0.041**	**1.6 (0.6–4.1)**	**0.320**
Residual disease	no versus yes	2.0 (1.3–3.2)	**0.002**	**2.3 (1.4–3.9)**	**0.001**
Histology	HGSOC versus HGEOC	–	–	**0.7 (0.3–1.4)**	0.301
BRCA genetic aberrations	no versus yes	0.7 (0.4–1.1)	0.099	**0.7 (0.4–1.1)**	0.152
C					
FIGO stage	I/II versus III/IV	2.1 (0.8–6.1)	0.154	1.5 (0.6–4.0)	0.374
Residual disease	no versus yes	2.3 (1.3–4.2)	**0.007**	2.1 (1.2–3.7)	**0.012**
Histology	HGSOC versus HGEOC	–	–	0.6 (0.3–1.2)	0.149
BRCA-LOF	no versus yes	0.4 (0.2–0.8)	**0.007**	0.5 (0.3–0.9)	**0.012**

Clinicopathological features and (A) BRCA-LOF by epigenetic or genetic aberrations and (B) *BRCA* mutational status in 131 patients. (C) Clinicopathological features and BRCA-LOF in the subgroup of 77 patients without maintenance therapy with bevacizumab

The significance level was determined by Cox regression analysis

Bold values indicate *P* values < 0.05

CI confidence interval; *FIGO* International Federation of Gynecology and Obstetrics; *HGEOC* high-grade endometrioid ovarian cancer; *HGSOC* high-grade serous ovarian cancer; *HR* hazard ratio; *HRD* homologous recombination deficiency; *LOF* loss of function

Platinum-sensitivity of cancers is closely related to their sensitivity to PARPi and is therefore considered to be an intrinsic biomarker for PARPi-efficacy. Overlapping gene dependencies for carboplatin- and PARPi-response have been previously described by Coehlo et al. [32]. Therefore, we are tempted to speculate that the combined assessment of epigenetic or genetic *BRCA*-inactivation may also be an important surrogate to reliably predict PARPi response. However, this hypothesis must be evaluated in separate clinical trials of PARPi therapy.

In our survival analyses, the BRCA-LOF due to epigenetic or genetic *BRCA*-inactivation was a more powerful predictor of HGOC-outcome than HRD-testing. Patients with *BRCA*-wt-unmeth tumors had the most unfavorable clinical outcome, regardless of HRD-status, presumably due to impaired platinum-sensitivity. The fact that none of platinum-resistant cancers in our investigations showed *BRCA1*-methylation supports this hypothesis.

Interestingly, we also observed that in the group of patients receiving bevacizumab maintenance-therapy, the survival disadvantage in patients with *BRCA*-wt-unmeth tumors disappeared. It may be assumed that this group of patients with less platinum sensitive tumors will derive the greatest benefit from antiangiogenic therapy.

In a recent post-hoc exploratory biomarker analysis of pre- and post-platinum samples from ARIEL2-study (a single-arm, open-label, phase-2 trial testing efficacy of the PARPi rucaparib in recurrent HGOC), Swisher et al. described that high-level methylation of the *BRCA1*-promoter measured by quantitative methylation-sensitive digital droplet polymerase chain reaction (MS-ddPCR) was a strong biomarker of rucaparib-sensitivity, with similar power as *BRCA* -mutations [33]. Also Kondrashova et al. identified homozygous or hemizygous *BRCA1* methylation as a predictor for rucaparib clinical response using HGSOC patient-derived xenografts (PDX) and archival tumor and pre-treatment biopsy samples from 23 patients from ARIEL2-study [34]. Blanc-Durand et al. recently showed that OCs with high *BRCA1*-hypermethylation were very likely to exhibit high genomic instability scores as an indicator of HRD which is in line with our observations [35]. They identified *BRCA1*-methylation in 19% of *BRCA*-wt tumors and concluded that these patients would therefore be good candidates for PARPi-treatment. They proposed to differentiate between biallelic *BRCA1*-promoter methylation (high-methylation; ≥ 70%) leading to a homozygous gene silencing and a monoallelic methylation with incomplete silencing of *BRCA1* (low-methylation; 30–69%). Despite this distinction, these authors did not find a statistically significant difference in PFS.

In our analysis, we did not use a cutoff to dichotomize our small cohort of 14 methylated cancers into high and low *BRCA1* DNA methylation categories. But the median adjusted PMR-value among these *BRCA1* methylated specimens was quite high at 70.6, with a minimum PMR-value of 32.7 (data not shown). However, we could show in our cohort that *BRCA1*-methylation lead to a significant loss of *BRCA1* mRNA-expression and consequently to a BRCA-LOF, the primary end-point of our study.

It should be noted that the number of cases included in our study represents a limitation together with the fact that none of the included patients was treated with PARPi. Therefore, further validation in a larger cohort, preferably in patients with HGOC treated with first-line PARPi maintenance, would be beneficial.

Conclusion

There is a high risk that parts of the clinically relevant subgroup of *BRCA1*-meth OC will remain unrecognized if only HRD-testing is performed in addition to *BRCA* mutation analysis. Distinguishing this subgroup is particularly important because completely *BRCA*-unrelated HRD-positive cancers have been shown to lack high sensitivity to platinum-based chemotherapy and probably also to PARPi treatment. Therefore, we strongly advocate the additional determination of *BRCA1* methylation status to *BRCA* mutation status and HRD status in HGOC for a more accurate prediction of sensitivity to platinum and, presumably, to PARPi.

Further retrospective validation of a joint determination of *BRCA* loss by epigenetic or genetic aberrations in HGOC patients treated with first-line PARPi maintenance therapy is urgently warranted. This could validate our further hypothesis that the beneficial effect of PARPi maintenance therapy in HRD-positive *BRCA*-wt HGOCs shown in large randomized phase III clinical trials is predominantly due to the *BRCA1*-meth subfraction of the tumors.

Abbreviations

ANTI	Aneuploidy-normalized-telomeric-imbalance
LOF	Loss of function
BRCA1-meth	*BRCA1*-methylated
BRCA-mut	*BRCA*-mutated
DNA	Deoxyribonucleic acid
EOC	Epithelial ovarian cancer
HGOC	High-grade ovarian cancer
HRD	Homologous recombination deficiency
HRR	Homologous recombination repair
FIGO	International Federation of Gynecology and Obstetrics
LOH	Loss of heterozygosity
OS	Overall survival
PMR	Percentage of methylated reference
PARPi	Poly (ADP-ribose) polymerase inhibitors
PFS	Progression-free survival
TAI	Telomeric-allelic-imbalance
TCGA	The Cancer Genome Atlas
BRCA-wt-unmeth	Unmethylated-*BRCA*-wt
VAF	Variant allele frequencies
wt	Wild-type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-024-01781-0.

Supplementary Material 1: Figure 1. Overview of the molecular analyses performed on the patient samples and the corresponding case numbers.

Acknowledgments

We thank Kathrin Außerlechner for her excellent technical assistance. The introduction and discussion section have been proofread using DeepL Write, after using this service the authors have reviewed and edited the content as needed and take full responsibility for the content of the publication.

Author contributions

Conceptualization: HF, KW, CM, AGZ; Methodology: HF, SS, DUR, ES; Project administration: HF; Investigation: HF, SS, DUR, KL, PN, IT, VW, KW, ES; Supervision: CM, AGZ; Writing - Original Draft: HF, SS, KL, PN, IT, VW, AGZ; Writing - Review & Editing: HF, SS, DUR, KL, PB, IT VW, KW, ES, CM, AGZ; All authors read and approved the final manuscript.

Funding

This work was supported by the Verein zur Krebsforschung in der Frauenheilkunde. Financial supporter neither had any role in the design of this study, its execution, analyses or interpretation of the data, nor in the decision to submit results for publication.

Availability of data and materials

All data generated or analyzed during this study are included in this article. Raw data from this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Innsbruck Medical University (reference numbers: AN2015-0038 346/4.17; 1054/2019) and conducted in accordance with the Declaration of Helsinki Principles

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. However, the following authors receive financial support for the specified activities: KL reports travel expenses from GSK, Roche, Eisai and MSD. IT reports honoraria from Roche and Astra Zeneca; travel expenses from Eisai, GSK, PharmaMar, Roche, Astra Zeneca, Pfizer; participation on advisory boards from Astra Zeneca. VW reports honoraria from Roche, Novartis; travel expenses from Roche; participation on advisory boards from Novartis. CM reports consulting fees from Roche, Novartis, Amgen, MSD, PharmaMar, Astra Zeneca, GSK, Seagen; honoraria from Roche, Novartis, Amgen, MSD, PharmaMar, Astra Zeneca, GSK, Seagen; travel expenses from Roche, Astra Zeneca; participation on advisory boards from Roche, Novartis, Amgen, MSD, Astra Zeneca, Pfizer, PharmaMar, GSK, Seagen. AGZ reports consulting fees from Amgen, Astra Zeneca, GSK, MSD, Novartis, PharmaMar, Roche-Diagnostics, Seagen; honoraria from Amgen, Astra Zeneca, GSK, MSD, Novartis, PharmaMar, Roche, Seagen; travel expenses from Astra Zeneca, Gilead, Roche; participation on advisory boards from Amgen, Astra Zeneca, GSK, MSD, Novartis, Pfizer, PharmaMar, Roche, Seagen.

Author details

[1]Department of Obstetrics and Gynecology, Medical University of Innsbruck, Innsbruck, Austria. [2]Institute of Human Genetics, Medical University of Innsbruck, Innsbruck, Austria.

Received: 2 August 2024 Accepted: 11 November 2024
Published online: 27 November 2024

References

1. International WCRF. Cancer Trends. 2020. Available from: https://www.wcrf.org/cancer-trends/ovarian-cancer-statistics/.
2. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet. 2014;384(9951):1376–88.
3. Aghajanian C, Goff B, Nycum LR, Wang YV, Husain A, Blank SV. Final overall survival and safety analysis of OCEANS, a phase 3 trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent ovarian cancer. Gynecol Oncol. 2015;139(1):10–6.
4. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26):2473–83.
5. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmana J, et al. Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation. J Clin Oncol. 2015;33(3):244–50.
6. Moore KN, Secord AA, Geller MA, Miller DS, Cloven N, Fleming GF, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol. 2019;20(5):636–48.
7. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. Science. 2017;355(6330):1152–8.
8. Wen H, Feng Z, Ma Y, Liu R, Ou Q, Guo Q, et al. Homologous recombination deficiency in diverse cancer types and its correlation with platinum chemotherapy efficiency in ovarian cancer. BMC Cancer. 2022;22(1):550.
9. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154–64.
10. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949–61.
11. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92.
12. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609–15.
13. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzalez-Martin A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381(25):2416–28.
14. Pal T, Permuth-Wey J, Betts JA, Krischer JP, Fiorica J, Arango H, et al. *BRCA1* and *BRCA2* mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104(12):2807–16.
15. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, et al. *BRCA* reversion mutations in circulating tumor DNA Predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. Cancer Discov. 2019;9(2):210–9.
16. Ngoi NYL, Tan DSP. The role of homologous recombination deficiency testing in ovarian cancer and its clinical implications: Do we need it? ESMO Open. 2021;6(3):100144.
17. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer. Cancer Discov. 2015;5(11):1137–54.
18. Uzilov AV, Ding W, Fink MY, Antipin Y, Brohl AS, Davis C, et al. Development and clinical application of an integrative genomic approach to personalized cancer therapy. Genome Med. 2016;8(1):62.
19. Catteau A, Harris WH, Xu CF, Solomon E. Methylation of the *BRCA1* promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. Oncogene. 1999;18(11):1957–65.
20. Geisler JP, Hatterman-Zogg MA, Rathe JA, Buller RE. Frequency of *BRCA1* dysfunction in ovarian cancer. J Natl Cancer Inst. 2002;94(1):61–7.
21. Baldwin RL, Nemeth E, Tran H, Shvartsman H, Cass I, Narod S, et al. *BRCA1* promoter region hypermethylation in ovarian carcinoma: a population-based study. Cancer Res. 2000;60(19):5329–33.
22. Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer. 2012;107(10):1776–82.
23. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, et al. Association of *BRCA1* and *BRCA2* mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA. 2011;306(14):1557–65.
24. Kalachand RD, Stordal B, Madden S, Chandler B, Cunningham J, Goode EL, et al. *BRCA1* Promoter methylation and clinical outcomes in ovarian cancer: an individual patient data meta-analysis. J Natl Cancer Inst. 2020;112(12):1190–203.
25. Tsibulak I, Wieser V, Degasper C, Shivalingaiah G, Wenzel S, Sprung S, et al. *BRCA1* and *BRCA2* mRNA-expression prove to be of clinical impact in ovarian cancer. Br J Cancer. 2018;119(6):683–92.
26. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet. 2006;38(7):787–93.
27. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
28. Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer Discov. 2012;2(4):366–75.
29. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. 2013;31(11):1023–31.
30. Schnaiter S, Schamschula E, Laschtowiczka J, Fiegl H, Zeimet AG, Wimmer K, Reimer D. Stratification of homologous recombination deficiency (HRD) negative high grade ovarian cancer by the type of peritoneal spread into two groups with distinct survival outcome. Cancers. 2024;16(11):2129.
31. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, et al. *BRCA* mutation frequency and patterns of treatment response in *BRCA* mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol. 2012;30(21):2654–63.
32. Coelho R, Tozzi A, Disler M, Lombardo F, Fedier A, Lopez MN, et al. Overlapping gene dependencies for PARP inhibitors and carboplatin response identified by functional CRISPR-*Cas9* screening in ovarian cancer. Cell Death Dis. 2022;13(10):909.
33. Swisher EM, Kwan TT, Oza AM, Tinker AV, Ray-Coquard I, Oaknin A, et al. Molecular and clinical determinants of response and resistance to rucaparib for recurrent ovarian cancer treatment in ARIEL2 (parts 1 and 2). Nat Commun. 2021;12(1):2487.
34. Kondrashova O, Topp M, Nesic K, Lieschke E, Ho GY, Harrell MI, et al. Methylation of all *BRCA1* copies predicts response to the PARP inhibitor rucaparib in ovarian carcinoma. Nat Commun. 2018;9(1):3970.
35. Blanc-Durand F, Tang R, Pommier M, Nashvi M, Cotteret S, Genestie C, et al. Clinical relevance of *BRCA1* promoter methylation testing in patients with ovarian cancer. Clin Cancer Res. 2023;29(16):3124–9.

Publisher's Note