

6 Constitutional *BRCA1* Promoter Methylation in Patients With Ovarian Cancer: Results of the Observational AGO-TR1 Study

Mohamad Kayali, BSc¹; Eric Hahnen, PhD¹ ; Alexander Burges, MD² ; Alexander Reuss, PhD³ ; Julia Caro-Valenzuela, MSc¹ ; Nikolaus de Gregorio, MD⁴; Florian Heitz, MD⁵ ; Sandra Schmidt¹; Frederik Marmé, MD⁶ ; Felix Hilpert, MD^{7,8} ; Pauline Wimberger, MD⁹ ; Stefan Kommoss, MD¹⁰; Katharina Prieske, MD^{8,11}; Christoph Engel, MD^{12,13} ; Paul Buderath, MD¹⁴; Dimo Dietrich, MD¹⁵ ; Rita Schmutzler, MD¹; Jalid Sehoul, MD¹⁶ ; Kerstin Rhiem, MD¹; Philipp Harter, MD⁵ ; and Jan Hauke, PhD¹ 

DOI <https://doi.org/10.1200/PO-25-01230>

ABSTRACT

PURPOSE Constitutional epimutations arise early in development and are present across normal tissues, including peripheral blood. Constitutional *BRCA1* promoter methylation has emerged as a risk factor for *BRCA1*-associated cancers, such as ovarian cancer (OC), and may serve as a biomarker for OC risk. This study retrospectively evaluated the clinical relevance of constitutional *BRCA1* promoter methylation in 473 patients with OC enrolled in the observational AGO-TR1 study (ClinicalTrials.gov identifier: [NCT02222883](https://clinicaltrials.gov/ct2/show/study?term=NCT02222883)).

MATERIALS AND METHODS *BRCA1* promoter methylation was quantified by the methylation-specific real-time polymerase chain reaction using whole blood-derived DNA from 476 female controls and 473 patients with OC along with 473 corresponding tumor-derived DNA samples. Methylation levels $\geq 1.0\%$ were considered methylation-positive.

RESULTS *BRCA1* promoter methylation in blood-derived DNA was detected in 42 of 473 patients with OC and in 26 of 476 controls (8.9% v 5.5%; odds ratio [OR], 1.69 [95% CI, 1.02 to 2.80], $P = .0432$), with the strongest association observed with methylation levels $\geq 10\%$ (OR, 6.17 [95% CI, 1.37 to 27.72], $P = .018$). Patients with *BRCA1* promoter methylation in blood-derived DNA were diagnosed at a younger median age than those without (54.0 v 60.0 years, $P = .018$). Constitutional *BRCA1* promoter methylation was less frequent in patients carrying pathogenic germline variants in OC predisposition genes than in noncarriers (4.1% v 10.5%; OR, 0.37 [95% CI, 0.14 to 0.96], $P = .04$) and showed no association with a family history of cancer or platinum-based chemotherapy before blood draw. *BRCA1* promoter methylation in blood-derived DNA was correlated with tumor *BRCA1* promoter methylation ($P < .001$). Tumor *BRCA1* promoter methylation was observed in 64 of 473 samples (13.5%), half (32 of 64) of which were attributable to constitutional *BRCA1* promoter methylation also detectable in the blood.

CONCLUSION Constitutional *BRCA1* promoter methylation accounts for a substantial proportion of OCs and represents a robust biomarker for individual OC risk.

ACCOMPANYING CONTENT

 [Data Sharing Statement](#)

Accepted May 14, 2026

Published June 12, 2026

JCO Precis Oncol 10:e2501230

© 2026 by American Society of Clinical Oncology

Creative Commons Attribution
Non-Commercial No Derivatives
4.0 License

INTRODUCTION

Women carrying pathogenic germline variants (PGVs) in *BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C*, and *RAD51D* are at an increased risk for ovarian cancer (OC) compared with the general female population.¹⁻³ In addition, OC is part of the tumor spectrum of the Lynch syndrome, and women with PGVs in *MLH1*, *MSH2*, and *MSH6* are at an elevated OC risk.⁴ Up to 20% of patients with OC carry heterozygous PGVs in *BRCA1/2*, and up to 5% carry PGVs in further OC

predisposition genes.^{5,6} A proportion of OCs may additionally be explained by unfavorable OC-specific polygenic risk scores (PRSs).^{3,7} However, the majority of unselected OCs are not caused by known heritable risk factors such as PGVs in established OC predisposition genes or PRSs. Pathogenic somatic variants (PSVs) in OC predisposition genes and *BRCA1* promoter methylation observed in the tumor tissue are usually attributed to sporadic OC, not associated with a positive family history of cancer as shown for PGVs.⁸ PGVs and PSVs in *BRCA1/2* and *BRCA1* promoter methylation

CONTEXT

Key Objective

Does constitutional *BRCA1* promoter methylation detectable in blood-derived DNA contribute to ovarian cancer (OC) risk, and how does it relate to tumor methylation, germline pathogenic variants, and other clinical variables?

Knowledge Generated

BRCA1 methylation in blood-derived DNA was detected in 8.9% of patients with OC and 5.5% of controls (odds ratio [OR] 1.69), with the strongest association at methylation levels $\geq 10\%$ (OR, 6.17). Patients with constitutional *BRCA1* methylation were diagnosed at a younger median age. Constitutional *BRCA1* methylation was less frequent in carriers of pathogenic germline variants (4.1% v 10.5%) and not associated with cancer family history or chemotherapy before blood draw. *BRCA1* methylation in blood-derived DNA correlated with tumor *BRCA1* methylation. Tumor *BRCA1* methylation was observed in 64 of 473 samples (13.5%), half of which were attributable to constitutional *BRCA1* methylation.

Relevance

Assessment of constitutional *BRCA1* methylation could support OC risk stratification. Prospective studies are required to quantify age-dependent OC risks.

observed in the tumor tissue are associated with a homologous recombination-deficient tumor phenotype, predicting favorable response to treatment with DNA-damaging agents and DNA repair inhibition, such as platinum or poly(ADP-ribose) polymerase (PARP) inhibitors.^{9–11} Notably, PGVs and PSVs in *BRCA1/2* and *BRCA1* promoter methylation observed in the tumor tissue are mutually exclusive, with only a few exceptions described.⁸

In addition, constitutional *BRCA1* promoter methylation has emerged as a risk factor for *BRCA1*-associated cancers such as OC and breast cancer (BC).¹² Constitutional epimutations such as constitutional *BRCA1* promoter methylation occur prenatally, affecting all three germ layers, and are thus detectable in normal tissues such as blood. Constitutional *BRCA1* promoter methylation has been detected in 4%–10% of female newborns, suggesting a common and early event during life.^{13,14} However, it remains unclear whether constitutional *BRCA1* promoter methylation observed in blood-derived DNA is affected by factors such as individual disease status, age at blood draw, and/or chemotherapy before blood draw, among others. In a nested case-control study, Lønning et al analyzed blood-derived DNA samples of 3,493 women who were cancer-free at blood draw, including 511 women who developed incident high-grade serous OC, a *BRCA1*-associated histologic tumor phenotype. In this study, *BRCA1* promoter methylation in blood-derived DNA was significantly associated with the risk of incident high-grade serous OC (hazard ratio [HR], 1.93 [95% CI, 1.36 to 2.73]; $P < .001$).¹² Thus, constitutional *BRCA1* promoter methylation detectable in blood-derived DNA may be a valuable biomarker to identify individuals prone to developing *BRCA1*-associated tumors, such as OC, and to improve the clinical management of diseased individuals. These data prompted us to retrospectively assess the role of constitutional *BRCA1*

promoter methylation in 473 patients with OC enrolled in the observational AGO-TR1 study (ClinicalTrials.gov identifier: [NCT02222883](https://clinicaltrials.gov/ct2/show/study?term=NCT02222883)).

MATERIALS AND METHODS

AGO-TR1 Study Patients

Between March 2015 and July 2015, the AGO-TR1 study recruited 523 consecutive patients with invasive epithelial OC at 20 German study centers of the German Gynecological Oncology Study Group (AGO Study Group); 281 patients were included with a primary OC diagnosis, and 242 patients with platinum-sensitive recurrence of OC. All participants were older than 18 years at study inclusion and provided written informed consent before trial enrollment. The study protocol was approved by the ethics committee of the Landesärztekammer Nordrhein (Nr. 2014340) and registered (ClinicalTrials.gov identifier: [NCT02222883](https://clinicaltrials.gov/ct2/show/study?term=NCT02222883)). Demographic data, disease characteristics, family history, and medical history were documented as previously described.⁶ Venous blood samples were available from all 523 patients, and corresponding formalin-fixed paraffin-embedded (FFPE) tumor samples were available from 473 patients.^{6,8} A detailed description of the 473 study patients with available blood and tumor samples is provided in [Table 1](#).

Control Individuals

Female control individuals were recruited from the LIFE-Adult-Study, a population-based cohort study focusing on the prevalence, early onset markers, genetic predispositions, and the role of lifestyle factors of major civilization diseases.¹⁵ We selected 476 cancer-free female control individuals age between 59 and 68 years at the time of blood draw, with a median age at blood draw of 64 years.

TABLE 1. Patient Characteristics

Variable	All (n = 473)
Age at diagnosis, years, median (range)	59 (16-93)
Age at blood draw, years, median (range)	61 (18-93)
Time point of study inclusion, %	
Primary diagnosis of OC	257 (54.3)
Platinum-sensitive recurrence of OC	216 (45.7)
Time point of blood draw, %	
Before chemotherapy start, enrolled at primary diagnosis	110 (23.3)
After chemotherapy start, enrolled at primary diagnosis (median 112 days, range 1-983)	132 (27.9)
After chemotherapy start, enrolled at recurrence (median 1,098 days, range, 321-5,869)	212 (44.8)
Unknown/missing	19 (4.0)
Family history of cancer, % ^a	
Positive	198 (41.9)
Negative	266 (56.2)
Unknown/missing	9 (1.9)
Histologic subtype of OC, %	
High-grade serous	373 (78.9)
High-grade endometrial	22 (4.7)
Serous/papillary, grade unknown	19 (4.0)
Low-grade serous	16 (3.4)
Low-grade endometrial	7 (1.5)
Clear cell	6 (1.3)
Mucinous	6 (1.3)
Others/unspecified	18 (3.8)
Unknown/missing	6 (1.3)
Germline mutation status, % ^b	
With PGVs in <i>BRCA1/2</i>	97 (20.5)
With PGVs in non- <i>BRCA1/2</i> OC predisposition genes	24 (5.1)
With PGVs in any OC predisposition gene	121 (25.6)
Without PGVs in any OC predisposition gene	352 (74.4)

Abbreviations: BC, breast cancer; OC, ovarian cancer.

^aA positive family history was defined as at least one relative with BC or OC or BC in personal history.

^b*BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C*, and *RAD51D* and the Lynch syndrome genes in *MLH1*, *MSH2*, and *MSH6* were considered established OC predisposition genes.

DNA Isolation

Genomic DNA was isolated from blood samples and FFPE tumor samples using standard methods, as described in detail previously.^{6,8} Briefly, for the isolation of DNA from FFPE tumor samples, hematoxylin and eosin–stained 3- μ m tissue sections were centrally investigated (Institute of Pathology, University Hospital Bonn, Bonn, Germany), and tumor areas containing >80% tumor nuclei were chosen for DNA isolation.

Quantitative *BRCA1* Promoter Methylation Analysis

Quantitative *BRCA1* gene promoter methylation analysis was performed as described previously,⁸ and the genomic *BRCA1* gene promoter target region (hg38, Chr17:43125354–43125465) was shown to be affected by DNA methylation.^{8,16} We used a quantitative methylation-specific polymerase chain reaction (PCR; qMSP) assay to amplify the bisulfite-converted methylated *BRCA1* gene promoter (forward primer: gaagtttagcgcagtttacgtc, reverse primer: ctaaaaaactactacttaacgataac, hydrolysis probe: 6-FAM-ttcgcgtttttcgtgttacgga-BBQ-650). A quantitative PCR assay targeting the *ACTB* gene locus (hg38, Chr7:5532118–5532223) was used to quantify the total amount of bisulfite-converted template DNA (forward primer: cccttaaaattacaaaaaccacaa, reverse primer: ggagga ggttagtaagtgttttg, hydrolysis probe: Atto-647N-accaccacc-caacacacaataacaaacaca-BHQ-2). The *ACTB* target region does not contain any CpG sites; therefore, its amplification is not methylation-sensitive. Briefly, 2 μ g of DNA was bisulfite-converted using an innuCONVERT Bisulfite Basic Kit (Analytik Jena, Jena, Germany). For qMSP, 25 ng of bisulfite-converted DNA per PCR replicate was used as the template. Three nanograms of bisulfite-converted template DNA prepared from artificially methylated DNA (CpGenome Universal Methylated DNA, Millipore, Billerica, MA), as previously described, was used as the reference standard (calibrator). PCR oligonucleotide concentrations were 0.1 μ M (*ACTB*) and 0.3 μ M (*BRCA1*), respectively. PCR was performed in 20- μ l reaction volumes using an AB 7500 Fast Real-Time PCR System (Life Technologies Corporation, Carlsbad, CA) with an initial denaturation/heat activation step at 95°C for 20 minutes, followed by 50 cycles of 2 seconds at 62°C, 45 seconds at 56°C, and 15 seconds at 95°C. The *BRCA1* qMSP assay was performed in duplicate using a quantitative *ACTB* assay. Measurements were performed in triplicate for each DNA sample, and the median Δ CT values were computed. The percent methylation levels relative to the calibrator were calculated using the $\Delta\Delta$ CT method.¹⁷ Samples with *BRCA1* promoter methylation levels <1.0% were considered unmethylated.

Statistical Analyses

Odds ratios (ORs) were calculated using the OR calculator (MedCalc Software Ltd, Version 23.2.1).¹⁸ ORs, 95% CIs, and *P* values are given in brackets. Independent, nonparametric variables were compared using the Mann-Whitney U test, with exact *P* values given in brackets. Dependent nonparametric variables were compared using the Wilcoxon signed-rank test. Correlations were assessed using Spearman's correlation test. The latter three statistical tests were performed using the DATAtab Online Statistics Calculator (DATAtab e.U. Graz, Austria).¹⁹ Statistical significance was set at *P* values <.05.

RESULTS

BRCA1 promoter methylation in blood-derived DNA was observed in 42 of 473 patients with OC (8.9%), with a median

BRCA1 promoter methylation level of 4.0% (range, 1.1%–56.1%) in *BRCA1* methylation–positive samples (Fig 1A). Patients with OC and *BRCA1* promoter methylation in blood-derived DNA had an earlier age at diagnosis (AAD) than patients without *BRCA1* promoter methylation. The median AAD was 54.0 years in patients with *BRCA1* promoter methylation versus 60.0 years in patients without *BRCA1* promoter methylation (exact $P = .018$). In addition to 473 patients with OC, we analyzed 476 female control individuals

who were cancer-free at the time of blood draw. *BRCA1* promoter methylation in blood-derived DNA was observed in 26 of the 476 control individuals (5.5%), with a median *BRCA1* promoter methylation level of 3.7% (range, 1.1%–16.6%) in *BRCA1* methylation–positive samples (Fig 1A). The median age at blood draw was 64 years (range, 60–67) in control individuals with *BRCA1* promoter methylation versus 65 years (range, 59–68) in patients without *BRCA1* promoter methylation (exact $P = .289$).

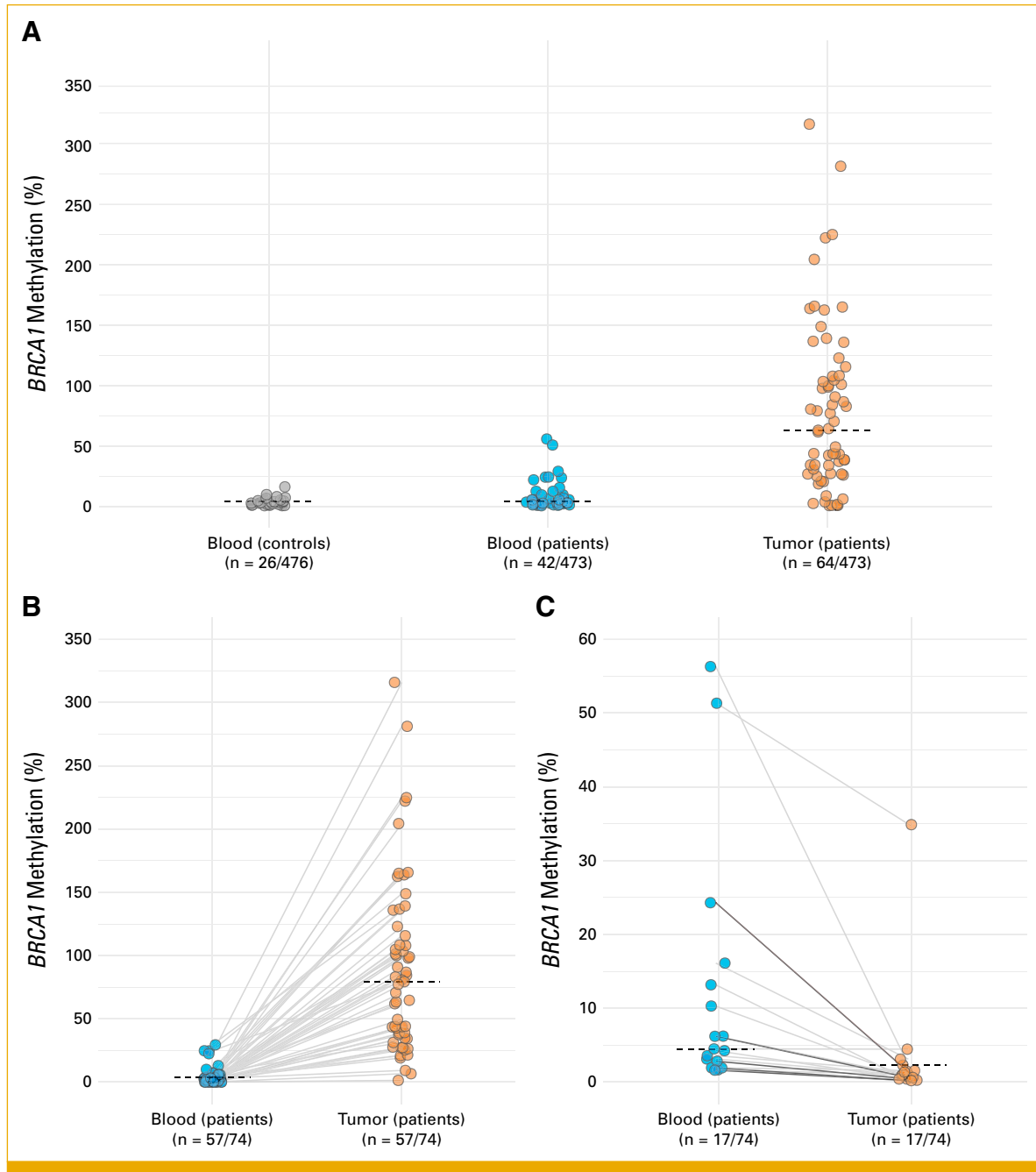


FIG 1. (A) *BRCA1* promoter methylation levels in blood-derived DNA of 476 female control individuals, 473 patients with OC, and *BRCA1* promoter methylation levels in tumor-derived DNA of 473 corresponding tumor samples; (B) *BRCA1* methylation levels in 57 patients with higher *BRCA1* promoter methylation levels observed in tumor- versus blood-derived DNA; (C) *BRCA1* methylation levels in 17 patients with lower *BRCA1* promoter methylation levels observed in tumor- versus blood-derived DNA; median *BRCA1* promoter methylation levels are shown as dashed lines. OC, ovarian cancer.

The overall prevalence of *BRCA1* promoter methylation–positive samples was significantly higher in patients with OC than in control individuals (8.9% v 5.5%, OR, 1.69 [95% CI, 1.02 to 2.80], $P = .0432$). We explored whether higher *BRCA1* promoter methylation levels are similarly associated with OC (Table 2). *BRCA1* promoter methylation levels of 5.0% or above were also significantly associated with OC in our study sample (4.0% v 1.5%, OR, 2.80 [95% CI, 1.17 to 6.73], $P = .021$), with the highest association with OC observed in the subgroup of individuals with *BRCA1* methylation levels of 10% or above (OR, 6.17 [95% CI, 1.37 to 27.72], $P = .018$).

In tumor-derived DNA, *BRCA1* promoter methylation was detected in 64 of 473 patients with OC (13.5%), with a median *BRCA1* promoter methylation level of 64.0% (range, 1.2%–316%) in *BRCA1* methylation–positive samples (Fig 1A). We observed a correlation between *BRCA1* promoter methylation levels in tumor-derived DNA and AAD, as described previously.⁸ The median AAD in patients with *BRCA1* promoter methylation in tumor-derived DNA was 56.0 years (range, 32–78) versus 60.0 years (range, 16–93) in patients without *BRCA1* promoter methylation (exact $P = .041$).

A positive correlation between *BRCA1* DNA methylation levels in blood- and in tumor-derived DNA samples was observed in the overall study sample ($r = 0.57$, $P < .001$, Spearman correlation test). Of the 42 patients with *BRCA1* promoter methylation in blood-derived DNA, 76.2% (32 of 42) also showed *BRCA1* promoter methylation in tumor-derived DNA. Of the 431 patients without *BRCA1* promoter methylation in blood-derived DNA, 7.4% (32 of 431) showed *BRCA1* promoter methylation in tumor-derived DNA. Thus, patients with *BRCA1* promoter methylation detected in blood-derived DNA had a significantly higher probability of *BRCA1* promoter methylation in tumor-derived DNA than patients without *BRCA1* promoter methylation in blood-derived DNA (OR, 39.9 [95% CI, 18.0 to 88.5], $P < .001$).

A prevalence of *BRCA1/2* PGVs of 20.5% (97 of 473) was detected in the AGO-TR1 study sample of 473 patients with OC, and another 5.1% (24 of 473) carried PGVs in additional OC predisposition genes, as previously described.^{6,20} Patients with PGVs in any established OC predisposition gene (121 of 473, *BRCA1*, *BRCA2*, *BRIP1*, *MSH2*, *MSH6*, *PALB2*, *RAD51C*, *RAD51D*) show a significantly younger median AAD

(53.0 years, range, 28–77) than patients without PGVs (61.0 years, range, 16–93; exact $P < .001$). As expected, a positive PGV status was significantly associated with a positive family history of cancer (71 of 121 PGV carriers v 127 of 352 noncarriers with a positive family history of cancer; OR, 2.52 [95% CI, 1.65 to 3.84], $P < .0001$) and a high-grade serous tumor phenotype (106 of 121 PGV carriers v 267 of 352 noncarriers with a high-grade serous tumor phenotype; OR, 2.25 [95% CI, 1.24 to 4.08], $P = .0074$). *BRCA1* promoter methylation in blood-derived DNA was significantly less frequent in patients with PGVs in any OC predisposition gene (5 of 121, 4.1%) than in patients without PGVs (37 of 352, 10.5% [OR, 0.37 [95% CI, 0.14 to 0.96], $P = .04$]). In addition, *BRCA1* promoter methylation in tumor-derived DNA was significantly less frequent in patients with PGVs in any OC predisposition gene (1 of 121, 0.8%) than in patients without PGVs (63 of 352, 17.9%; OR, 0.04 [95% CI, 0.005 to 0.28], $P = .0013$).

Regarding *BRCA1* promoter methylation levels, 74 patients showed *BRCA1* promoter methylation in blood- or tumor-derived DNA or both. We observed generally higher methylation levels in tumor-derived DNA (Fig 1A, $P < .001$, Wilcoxon signed-rank test). Of the 74 patients, 57 showed higher *BRCA1* promoter methylation levels in the tumor than in blood-derived DNA (including 32 patients without *BRCA1* promoter methylation levels in blood-derived DNA, Fig 1B). The remaining 17 patients showed lower *BRCA1* promoter methylation levels in tumor- than in blood-derived DNA (including 10 without *BRCA1* promoter methylation detected in tumor-derived DNA, Fig 1C). Notably, no PGV carriers were observed in the group of 57 patients with increased *BRCA1* promoter methylation in tumor-derived DNA, whereas 5 PGV carriers were observed in the group of 17 patients with decreased *BRCA1* promoter methylation in tumor-derived DNA (Fig 1C, thicker lines).

Regarding further clinical variables, we identified no association between *BRCA1* promoter methylation and a family history of cancer. Of the 42 patients with *BRCA1* promoter methylation in blood-derived DNA, 19 reported a positive family history of cancer (49.8%) versus 41.5% (179 of 431) of the methylation-negative patients (OR, 1.15 [95% CI, 0.62 to 2.20], $P = .6423$). Of the 64 patients with *BRCA1* promoter methylation in tumor-derived DNA, 26 reported a positive

TABLE 2. Prevalence of Constitutional *BRCA1* Promoter Methylation in Blood-Derived DNA in Patients With OC ($n = 473$) and in Female Control Individuals ($n = 476$) According to Minimal *BRCA1* Promoter Methylation Cutoff Levels

Minimal <i>BRCA1</i> Promoter Methylation Cutoff (%)	No. of OC Patients With <i>BRCA1</i> Promoter Methylation in Blood (%)	No. of Controls With <i>BRCA1</i> Promoter Methylation in Blood (%)	OR (95% CI, P)
1.0	42/473 (8.9)	26/476 (5.5)	1.69 (1.02 to 2.80, .043)
2.5	32/473 (6.8)	17/476 (3.6)	1.96 (1.07 to 3.58, .029)
5.0	19/473 (4.0)	7/476 (1.5)	2.80 (1.17 to 6.73, .021)
10.0	12/473 (2.5)	2/476 (0.4)	6.17 (1.37 to 27.72, .018)

Abbreviations: OC, ovarian cancer; OR, odds ratio.

family history of cancer (40.6%) versus 42.1% (172 of 409) of the methylation-negative patients (OR, 0.94 [95% CI, 0.55 to 1.61], $P = .83$). The same holds true when restricting a positive family history definition to patients with first-degree relatives with BC and/or OC only. Of the 42 patients with *BRCA1* promoter methylation in blood-derived DNA, five reported a positive family history of cancer (11.9%) versus 13.5% (58 of 431) of the methylation-negative patients (OR, 0.87 [95% CI, 0.33 to 2.30], $P = .7776$). Of the 64 patients with *BRCA1* promoter methylation in tumor-derived DNA, six reported a positive family history of cancer (9.4%) versus 13.9% (57 of 409) of the methylation-negative patients (OR, 0.64 [95% CI, 0.26 to 1.55], $P = .32$). In addition, no association was observed when restricting positive family history definition to relatives with OC only (blood: 5 of 42, 11.9%, v 54 of 431, 12.5%; OR, 0.94 [95% CI, 0.36 to 2.50], $P = .91$; tumor: 7 of 64, 10.9%, v 52 of 409, 12.7%; OR, 0.84 [95% CI, 0.37 to 1.95], $P = .69$).

In addition, *BRCA1* promoter methylation in blood-derived DNA was not associated with the high-grade serous tumor phenotype (33 of 42, 78.6% v 340 of 431 without promoter methylation, 78.9%; OR, 0.98 [95% CI, 0.45 to 2.12], $P = .962$). Patients with *BRCA1* promoter methylation in tumor-derived DNA tended to have a high-grade serous tumor phenotype, not reaching significance in our study sample (57 of 64, with promoter methylation, 89.1% v 340 of 431 without promoter methylation, 78.9%; OR, 2.18 [95% CI, 0.96 to 4.94], $P = .0621$).

A total of 242 patients were enrolled at primary diagnosis of OC, with the time point of blood draw known (Table 1). In 110 of these patients, blood was drawn before starting platinum-based chemotherapy, and in 132 patients, blood was drawn after chemotherapy start, the latter with a median time difference between the start of chemotherapy and blood draw of 112 days (Table 1, range 1–983 days). Of the first mentioned 110 patients, 10 (9.1%) showed *BRCA1* promoter methylation in blood-derived DNA versus 14 of 132 (10.6%) in the latter group of patients (OR, 0.84 [95% CI, 0.36 to 1.98], $P = .694$). In methylation-positive samples, methylation levels did not differ between both groups (median 5.87%, range, 1.72–24.64 v median 5.94%, range, 1.05–51.13, exact $P = .841$). Another 212 patients were enrolled at platinum-sensitive recurrence of OC and, by definition, had undergone chemotherapy before blood draw (Table 1, median 1,098 days, range, 321–5,869 days). Of these 212 patients, 17 (8.02%) showed *BRCA1* promoter methylation in blood-derived DNA (median methylation levels of 2.93%, range, 1.72–56.1). No significant differences were observed when comparing the number of methylation-positive patients enrolled at platinum-sensitive recurrence of OC (17 of 212) versus patients enrolled at primary diagnosis of OC (24 of 242, 9.92%, OR, 0.79 [95% CI, 0.41 to 1.52], $P = .482$). Moreover, promoter methylation levels did not differ between patients enrolled at platinum-sensitive recurrence of OC versus patients enrolled at primary diagnosis of OC (exact $P = .323$). Thus, exposure to platinum-based chemotherapy

before blood draw did not significantly affect *BRCA1* promoter methylation levels in blood-derived DNA in our analyses.

DISCUSSION

In this retrospective analysis of an observational AGO-TR1 study, we confirmed a positive association between constitutional *BRCA1* promoter methylation and OC (Table 2). A case-control analysis (934 patients, 1,698 controls) by Lønning et al published in 2018 revealed a positive association between constitutional *BRCA1* promoter methylation and overall OC (OR, 1.83 [95% CI, 1.27 to 2.63]), with an elevated OR observed for high-grade serous OC (2.91 [95% CI, 1.85 to 4.56]).²¹ A larger, nested case-control study by Lønning et al¹² published in 2022 (3,493 women who were cancer-free at the time of blood draw, including 511 women who developed incident high-grade serous OC) confirmed an association between constitutional *BRCA1* promoter methylation and the risk of incident high-grade serous OC (HR, 1.93 [95% CI, 1.36 to 2.73]). In our case-control analysis, we demonstrated that higher *BRCA1* promoter methylation levels were associated with higher ORs of up to 6.17 (95% CI, 1.37 to 27.72, $P = .018$, Table 2), suggesting that methylation levels may be considered for OC risk stratification. However, this finding was less pronounced in the more complex next-generation sequencing-based *BRCA1* methylation analysis performed by Lønning et al.¹² In line with previous studies, we demonstrated (1) that constitutional *BRCA1* methylation and PGVs in OC predisposition genes are mutually exclusive, with a few exceptions only, indicating that PGVs and epimutations are independent risk factors, (2) that constitutional *BRCA1* methylation is not associated with the family history of cancer, and (3) that constitutional *BRCA1* methylation observed in controls does not correlate with the age at blood draw.^{12,22} In contrast to previous studies, however, we observed a significantly younger median AAD in patients with constitutional *BRCA1* methylation than in patients without constitutional *BRCA1* methylation (mean 56.0 v 60.0 years, respectively). This difference becomes more apparent when also considering the PGV status. In patients with PGVs in OC predisposition genes, the median AAD was 53.0 years (range, 28–77 years) versus 61.0 years (range, 16–93 years) in patients without ($P < .001$). In patients without PGVs and with constitutional *BRCA1* promoter methylation, the median AAD was 54.0 years (range, 25–78 years). In patients without both risk factors, the mean AAD was 62.0 years (range, 16–93 years). Thus, we suggest that constitutional *BRCA1* promoter methylation is associated with the risk of OC at an earlier age.

It is a matter of debate whether constitutional *BRCA1* promoter methylation is affected by the individual disease status or chemotherapy before blood draw.²² In our analyses, we demonstrated that exposure to platinum-based chemotherapy before blood draw did not significantly affect *BRCA1* promoter methylation levels in blood-derived DNA.

BRCA1 promoter methylation in tumor-derived DNA is a frequent finding observed in 9% to 20% of all patients with OC.^{23–26} In the AGO-TR1 study, we observed *BRCA1* promoter methylation in tumor-derived DNA in 13.5% (64 of 473) of all patients with OC, which is in line with previous studies. For OC, however, it is still unclear to what extent constitutional *BRCA1* promoter methylation detected in blood-derived DNA correlates with *BRCA1* promoter methylation in tumor tissue. We demonstrated that half (32 of 64) of all OCs with *BRCA1* promoter methylation in the tumor tissue were due to constitutional *BRCA1* promoter methylation, whereas the remaining half (32 of 64) appeared to be tumor-specific.

The cause of constitutional *BRCA1* promoter methylation remains unclear. In rare cases, constitutional *BRCA1* promoter methylation may be triggered by germline variants (secondary constitutional epimutations [SCEs]).²² In two families with BC and OC, Evans et al identified the c.-107A>T variant in the *BRCA1* 5'UTR to cause monoallelic *BRCA1* promoter hypermethylation.²⁷ In our study sample of 473 patients with OC, the c.-107A>T variant was absent, as shown by Sanger sequencing (data not shown). However, we cannot rule out SCEs because of yet unknown *BRCA1* germline variants that trigger SCEs affecting the *BRCA1* promoter.

With a median age at blood draw of 61.0 years in patients with OC and 64.0 years in female control individuals, patients and control individuals were not perfectly age-

matched. However, we observed no significant correlation between *BRCA1* promoter methylation and age at blood draw in control individuals, consistent with previous findings. The number of OC patients with histologic tumor phenotypes other than high-grade serous OC was low ($n = 88$, Table 1) and probably too low to identify a negative association between *BRCA1* promoter methylation in blood-derived DNA and non-high-grade serous OC subtypes described by others. Several samples showed high *BRCA1* promoter methylation levels in tumor-derived DNA of up to 316% (Fig 1A). This may be due to (or a combination of) tumor-specific amplification of the methylated *BRCA1* promoter, loss of the reference *ACTB* allele, or incomplete methylation of the artificially methylated DNA used as a reference standard to calculate methylation levels. We refrained from arbitrarily setting these values to 100%.

In conclusion, constitutional *BRCA1* promoter methylation detectable in blood-derived DNA appears to be a reliable biomarker for OC risk stratification not significantly affected by age at blood draw, individual disease status, or platinum-based chemotherapy before blood draw. We suggest the further development of elevated *BRCA1* promoter methylation as a biomarker for the assessment of individual risks of OC and also other *BRCA1*-associated tumors, especially triple-negative BC. Prospective studies are required to quantify age-dependent cancer risks associated with constitutional *BRCA1* promoter methylation to offer risk-adjusted preventive measures.

AFFILIATIONS

¹Center for Familial Breast and Ovarian Cancer, Institute for Hereditary Cancers, Center for Integrated Oncology (CIO), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

²Department of Gynecology, University Hospital Munich-Großhadern, Munich, Germany

³Coordinating Center for Clinical Trials, Philipps-University of Marburg, Marburg, Germany

⁴Department of Gynecology, Klinikum am Gesundbrunnen, SLK-Kliniken Heilbronn GmbH, Heilbronn, Germany

⁵Department of Gynecology and Gynecologic Oncology, Kliniken Essen-Mitte, Essen, Germany

⁶Department of Gynecology, University Mannheim, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany

⁷Department of Gynecology, University of Kiel, Kiel, Germany

⁸Onkologisches Therapiezentrum, Krankenhaus Jerusalem, Hamburg, Germany

⁹Department of Carl-Gustav-Carus University, Technische Universität Dresden and National Cancer Center (NCT Dresden), Dresden, Germany

¹⁰Department of Women's Health, University Hospital Tübingen, Tübingen, Germany

¹¹Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

¹²Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany

¹³LIFE - Leipzig Research Centre for Civilization Diseases, University of Leipzig, Leipzig, Germany

¹⁴Department of Gynecology, University of Essen, Essen, Germany

¹⁵Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Bonn, Bonn, Germany

¹⁶Department of Gynecology and Gynecological Oncology, Charité, Campus Virchow, Berlin, Germany

CORRESPONDING AUTHOR

Eric Hahnen, PhD; e-mail: eric.hahnen@uk-koeln.de.

DISCLAIMER

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

EQUAL CONTRIBUTION

P.H. and J.H. contributed equally as senior authors.

PRIOR PRESENTATION

Presented in part at the GfH Annual Meeting, Munich, Germany, March 4-6, 2026.

SUPPORT

Supported by AstraZeneca, Germany (observational AGO-TR1 study), European Regional Development Fund and Free State of Saxony, project numbers 713-241 202, 713-241 202, 14505/2470, and 14575/2470 (LIFE, Leipzig Research Center for Civilization Diseases), and by the Imhoff-

Stiftung (18/2024), Faculty of Medicine, University of Cologne (BRCA1 promoter methylation analyses).

DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI <https://doi.org/10.1200/PO-25-01230>.

AUTHOR CONTRIBUTIONS

Conception and design: Mohamad Kayali, Eric Hahnen, Alexander Burges, Alexander Reuss, Felix Hilpert, Pauline Wimberger, Dimo Dietrich, Rita Schmutzler, Philipp Harter, Jan Hauke

Financial support: Mohamad Kayali, Rita Schmutzler, Philipp Harter

Administrative support: Mohamad Kayali, Felix Hilpert, Rita Schmutzler, Jalid Sehoul, Jan Hauke

Provision of study materials or patients: Mohamad Kayali, Eric Hahnen, Nikolaus de Gregorio, Florian Heitz, Frederik Marmé, Felix Hilpert, Katharina Prieske, Christoph Engel, Rita Schmutzler, Jalid Sehoul, Philipp Harter

Collection and assembly of data: Mohamad Kayali, Eric Hahnen, Alexander Burges, Alexander Reuss, Nikolaus de Gregorio, Florian Heitz, Sandra Schmidt, Frederik Marmé, Felix Hilpert, Stefan Kommoss, Katharina Prieske, Christoph Engel, Dimo Dietrich, Jalid Sehoul, Philipp Harter, Jan Hauke

Data analysis and interpretation: Mohamad Kayali, Eric Hahnen, Alexander Burges, Alexander Reuss, Julia Caro-Valenzuela, Florian Heitz, Frederik Marmé, Felix Hilpert, Stefan Kommoss, Paul Buderath, Dimo Dietrich, Kerstin Rhiem, Philipp Harter, Jan Hauke

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians ([Open Payments](http://OpenPayments)).

Eric Hahnen

Patents, Royalties, Other Intellectual Property: Dr Hahnen is co-inventor of a patent "Method for assessing homologous recombination deficiency in ovarian cancer cells" (EP4141127B1, WO/2023/031121) issued outside the submitted work

Alexander Burges

Honoraria: Roche Pharma AG, Tesaro, AstraZeneca

Consulting or Advisory Role: Tesaro

Travel, Accommodations, Expenses: Tesaro

Nikolaus de Gregorio

Consulting or Advisory Role: MSD, Roche, Eisai Germany, AstraZeneca, GlaxoSmithKline, Myriad Genetics, Novartis, Clovis Oncology, Gilead Sciences

Travel, Accommodations, Expenses: AstraZeneca

Florian Heitz

Honoraria: Roche, AstraZeneca, Tesaro/GSK, Immunogen, Seagen, AbbVie, Pharma

Consulting or Advisory Role: Roche, AstraZeneca, GlaxoSmithKline, Novocure, PharmaMar, Zai Lab, AbbVie, Pharma

Research Funding: AstraZeneca (Inst), Amedes (Inst)

Frederik Marmé

Honoraria: Roche/Genentech, Novartis, Pfizer, AstraZeneca, Clovis Oncology, Eisai, Genomic Health, MSD Oncology, Immunomedics (Inst), Seagen, Myriad Genetics, Pierre Fabre, GlaxoSmithKline, AGENDIA, Lilly, Gilead Sciences, Daiichi Sankyo, Menarini Group, Böhlinger-Ingelheim, Novocure, Pharma&, NIONTECH, Gilead Sciences, Immunogen

Consulting or Advisory Role: AstraZeneca, Pfizer, Roche (Inst), Genomic Health, CureVac, Amgen, Vaccibody (Inst), Immunomedics (Inst), Eisai, GlaxoSmithKline, Gilead Sciences, Seagen, Clovis Oncology, Biontech, Böhlinger-Ingelheim, INCYTE (Inst), Gilead Sciences, German Breast Group, Nerviano Medical Sciences, Pharma, AstraZeneca (Inst), Stemline Menarini, Gilead Sciences (Inst), Myriad Genetics, Myriad Genetics (Inst), GlaxoSmithKline (Inst)

Research Funding: Roche/Genentech (Inst), Novartis (Inst), AstraZeneca (Inst), Tesaro (Inst), Clovis Oncology (Inst), MSD Oncology (Inst), Vaccibody (Inst), Gilead Sciences (Inst), GlaxoSmithKline (Inst)

Travel, Accommodations, Expenses: Roche, Pfizer, AstraZeneca, Gilead Sciences, Menarini Group

Felix Hilpert

Honoraria: AstraZeneca, MSD, Novartis, GlaxoSmithKline, AbbVie

Consulting or Advisory Role: AstraZeneca, Roche, AbbVie, MSD

Travel, Accommodations, Expenses: AbbVie

Pauline Wimberger

Honoraria: AstraZeneca, MSD Oncology, Eisai, Novartis/Pfizer, Roche Pharma AG, Amgen, Pfizer, GlaxoSmithKline, Clovis Oncology GmbH, Teva, Lilly, AbbVie, Gilead Sciences, Daiichi Sankyo

Consulting or Advisory Role: Amgen, AstraZeneca, MSD Oncology, Novartis, Roche Pharma AG, Tesaro, PharmaMar, GlaxoSmithKline, Pfizer, Clovis Oncology GmbH, Teva, Eisai Germany, Eisai Germany, Lilly, AbbVie, Gilead Sciences, Daiichi Sankyo

Research Funding: Amgen, AbbVie, AstraZeneca, MSD Oncology, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma AG, Lilly, Clovis Oncology GmbH

Travel, Accommodations, Expenses: Roche Pharma AG

Stefan Kommoss

Honoraria: AstraZeneca, GlaxoSmithKline, MSD, Eisai Germany

Consulting or Advisory Role: GlaxoSmithKline, MSD, AstraZeneca, Novartis, Eisai

Research Funding: GlaxoSmithKline (Inst)

Travel, Accommodations, Expenses: Eisai, AstraZeneca, AbbVie, Hologic

Katharina Prieske

Other Relationship: Medac

Paul Buderath

Honoraria: Roche, AstraZeneca/Daiichi Sankyo, GlaxoSmithKline

Consulting or Advisory Role: Roche

Dimo Dietrich

Stock and Other Ownership Interests: Bicycle Therapeutics

Honoraria: Bicycle Therapeutics

Patents, Royalties, Other Intellectual Property: Patents (licensed to Qiagen)

Travel, Accommodations, Expenses: Bicycle Therapeutics

Rita Schmutzler

Honoraria: MSD/AstraZeneca

Consulting or Advisory Role: MSD/AstraZeneca

Jalid Sehoul

Honoraria: AstraZeneca, Eisai, Johnson & Johnson, PharmaMar, Pfizer, MSD Oncology, GlaxoSmithKline, Bayer, Clovis Oncology, Roche, Vifor Pharma, Hexal, Novartis, Esteve Pharmaceuticals, Incyte, Phytolife Nutrition, Jenapharm, Kyowa Kirin International, Oncoinvent, Daiichi, Medtronic Covidien, Amgen, AbbVie, Corcept Therapeutics, Gilead Sciences, Myriad Pharmaceuticals, AbbVie, Tubulis GmbH

Consulting or Advisory Role: AstraZeneca, Clovis Oncology, PharmaMar, Merck, Pfizer, MSD Oncology, Lilly, Novocure, Johnson & Johnson, Roche, Ingress Health, Sobi, GlaxoSmithKline, Alkermes, Eisai, Oncinvent, Intuitive Surgical, Seagen, Bayer/Vital, Mundipharma, Sanofi Aventis GmbH, Immunogen, Tubulis GmbH, Daiichi Sankyo, Bristol Myers Squibb, Karyopharm Therapeutics, Corcept Therapeutics, AbbVie, Aristo, Genmab, Gilead Sciences, Tubulis GmbH

Research Funding: AstraZeneca (Inst), Clovis Oncology (Inst), Merck (Inst), Bayer (Inst), PharmaMar (Inst), Pfizer (Inst), MSD Oncology (Inst), Roche (Inst), GlaxoSmithKline (Inst), Lilly (Inst), IQvia (Inst), Mural (Inst), MSD (Inst), AbbVie, Aristo, Genmab, Gilead Sciences, Tubulis GmbH

Travel, Accommodations, Expenses: AstraZeneca, Clovis Oncology, PharmaMar, Roche Pharma AG, MSD Oncology, Olympus

Kerstin Rhiem

Honoraria: AstraZeneca, Novartis, Streamedup!

Travel, Accommodations, Expenses: AstraZeneca, Novartis, Streamedup!

Philipp Harter

Honoraria: AstraZeneca, Roche, Clovis Oncology, Stryker, MSD Oncology, Zai Lab, Lilly, Sotio, Eisai, GlaxoSmithKline, Amgen, Mersana, Exscientia, Daiichi Sankyo, Karyopharm Therapeutics, AbbVie, MSD Oncology

Consulting or Advisory Role: AstraZeneca, Roche, Tesaro, Merck, GlaxoSmithKline, Clovis Oncology, Immunogen, Mersana, Exscientia, Novartis, Eisai, Miltenyi Biomedicine, MSD Oncology, Corcept Therapeutics, BioNTech SE, AbbVie, Zymeworks

Research Funding: AstraZeneca (Inst), Roche (Inst), Genmab (Inst), GlaxoSmithKline (Inst), Immunogen (Inst), Clovis Oncology (Inst), Seagen (Inst), Novartis (Inst), Immatics (Inst), AbbVie (Inst), MSD Oncology (Inst)

Travel, Accommodations, Expenses: AstraZeneca

Jan Hauke

Honoraria: AstraZeneca

Research Funding: AGO Research GmbH (Inst), GBG Forschungs (Inst)

No other potential conflicts of interest were reported.

REFERENCES

- Kuchenbaecker KB, Hopper JL, Barnes DR, et al: Risks of breast, ovarian, and contralateral breast cancer for *BRCA1* and *BRCA2* mutation carriers. *Jama* 317:2402-2416, 2017
- Lee A, Yang X, Tyrer J, et al: Comprehensive epithelial tubo-ovarian cancer risk prediction model incorporating genetic and epidemiological risk factors. *J Med Genet* 59(7):632-643, 2022
- Yang X, Leslie G, Doroszk A, et al: Cancer risks associated with germline *PALB2* pathogenic variants: An international study of 524 families. *J Clin Oncol* 38:674-685, 2020
- Dominguez-Valentin M, Sampson JR, Seppälä TT, et al: Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the Prospective Lynch Syndrome Database. *Genet Med* 22:15-25, 2020
- Alsop K, Fereday S, Meldrum C, 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* 30:2654-2663, 2012
- Harter P, Hauke J, Heitz F, et al: Prevalence of deleterious germline variants in risk genes including *BRCA1/2* in consecutive ovarian cancer patients (AGO-TR-1). *PLoS One* 12:e0186043, 2017
- Dareng EO, Tyrer JP, Barnes DR, et al: Polygenic risk modeling for prediction of epithelial ovarian cancer risk. *Eur J Hum Genet* 30:349-362, 2022
- Hauke J, Hahnen E, Schneider S, et al: Deleterious somatic variants in 473 consecutive individuals with ovarian cancer: Results of the observational AGO-TR1 study (NCT02222883). *J Med Genet* 56:574-580, 2019
- Schouten PC, Richters L, Vis DJ, et al: Ovarian cancer-specific *BRCA*-like copy-number aberration classifiers detect mutations associated with homologous recombination deficiency in the AGO-TR1 trial. *Clin Cancer Res* 27:6559-6569, 2021
- Stefansson OA, Villanueva A, Vidal A, et al: *BRCA1* epigenetic inactivation predicts sensitivity to platinum-based chemotherapy in breast and ovarian cancer. *Epigenetics* 7:1225-1229, 2012
- Kondrashova O, Topp M, Nesic K, et al: Methylation of all *BRCA1* copies predicts response to the PARP inhibitor rucaparib in ovarian carcinoma. *Nat Commun* 9:3970, 2018
- Lønning PE, Nikolaenko O, Pan K, et al: Constitutional *BRCA1* methylation and risk of incident triple-negative breast cancer and high-grade serous ovarian cancer. *JAMA Oncol* 8:1579-1587, 2022
- Lønning PE, Eikesdal HP, Løes IM, et al: Constitutional Mosaic Epimutations—A hidden cause of cancer? *Cell Stress* 3:118-135, 2019
- Al-Moghrabi N, Al-Showimi M, Al-Yousef N, et al: Methylation of *BRCA1* and *MGMT* genes in white blood cells are transmitted from mothers to daughters. *Clin Epigenetics* 10:99, 2018
- Loeffler M, Engel C, Ahnert P, et al: The LIFE-Adult-Study: Objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. *BMC Public Health* 15:691, 2015
- Ibragimova I, Cairns P: Assays for hypermethylation of the *BRCA1* gene promoter in tumor cells to predict sensitivity to PARP-inhibitor therapy. *Methods Mol Biol* 780:277-291, 2011
- Jung M, Kristiansen G, Dietrich D: DNA methylation analysis of free-circulating DNA in body fluids. *Methods Mol Biol* 1708:621-641, 2018
- MedCalc Software. https://www.medcalc.org/calc/odds_ratio.php
- numiqo: DATAtab Online Statistics Calculator. <https://datatab.de>
- Hauke J, Hahnen E, Schneider S, et al: Deleterious somatic variants in 473 consecutive individuals with ovarian cancer: Results of the observational AGO-TR1 study (NCT02222883). *J Med Genet* 56:574-580, 2019
- Lønning PE, Berge EO, Bjørnslett M, et al: White blood cell *BRCA1* promoter methylation status and ovarian cancer risk. *Ann Intern Med* 168:326-334, 2018
- Lønning PE, Nikolaenko O, Knappskog S: Constitutional epimutations: From rare events toward major cancer risk factors? *JCO Precis Oncol* 10:1200/PO-24-00746
- Baldwin RL, Nemeth E, Tran H, et al: *BRCA1* promoter region hypermethylation in ovarian carcinoma: A population-based study. *Cancer Res* 60:5329-5333, 2000
- Cunningham JM, Cicek MS, Larson NB, et al: Clinical characteristics of ovarian cancer classified by *BRCA1*, *BRCA2*, and *RAD51C* status. *Sci Rep* 4:4026, 2014
- Geisler JP, Hatterman-Zogg MA, Rathe JA, et al: Frequency of *BRCA1* dysfunction in ovarian cancer. *J Natl Cancer Inst* 94:61-67, 2002
- Sahnane N, Carnevali I, Formenti G, et al: *BRCA* methylation testing identifies a subset of ovarian carcinomas without germline variants that can benefit from PARP inhibitor. *Int J Mol Sci* 21:9708, 2020
- Evans DGR, van Veen EM, Byers HJ, et al: A dominantly inherited 5' UTR variant causing methylation-associated silencing of *BRCA1* as a cause of breast and ovarian cancer. *Am J Hum Genet* 103:213-220, 2018