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TITLE: *BRCA1* promoter methylation and clinical outcomes in ovarian cancer: an individual patient data meta-analysis

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ABSTRACT
**Background:** BRCA1 methylation has been associated with homologous recombination deficiency, a biomarker of platinum sensitivity. Studies evaluating BRCA1-methylated tubal/ovarian cancer (OC) do not consistently support improved survival following platinum chemotherapy. We examine the characteristics of BRCA1-methylated OC in a meta-analysis of individual participant data.

**Methods:** 2636 participants’ data across 15 studies was analysed. BRCA1-methylated tumors were defined according to their original study. Associations between BRCA1 methylation and clinico-pathological characteristics were evaluated. The effects of methylation on overall survival (OS) and progression-free survival (PFS) were examined using mixed-effects models. All statistical tests were two-sided.

**Results:** 430 (16.3%) tumors were BRCA1-methylated. BRCA1 methylation was associated with younger age and advanced-stage high-grade serous OC. There were no survival differences between BRCA1-methylated and non-BRCA1-methylated OC (median PFS 20 vs 18.5 months, HR 1.01, 95% CI [0.87–1.16], \( P=0.98 \); median OS 46.6 vs 48 months, HR 1.02, 95% CI [0.87–1.18], \( P=0.96 \)). Where BRCA1/2 mutations were evaluated (n=1248), BRCA1 methylation displayed no survival advantage over BRCA1/2 intact (BRCA1/2 wild type non-BRCA1-methylated) OC. Studies used different methods to define BRCA1 methylation. Where BRCA1 methylation was determined using methylation-specific PCR and gel electrophoresis (n=834), it was associated with improved survival (PFS: HR 0.80, 95% CI [0.66–0.97], \( P=0.02 \); OS: HR 0.80, 95% CI [0.63–1.00], \( P=0.05 \)) on mixed-effects modelling.

**Conclusion:** BRCA1-methylated OC displays similar clinico-pathological features to BRCA1-mutated OC, but does not impact survival. Heterogeneity within BRCA1 methylation assays influences survival associations. Refining these assays may better identify cases with silenced BRCA1 function and improved patient outcomes.
INTRODUCTION

Epithelial tubal, primary peritoneal and ovarian cancer, hereafter referred to as ovarian cancer (OC), is an aggressive disease with poor patient outcomes. High-grade serous cancer (HGSC) is the most common and lethal form of OC. Targeting homologous recombination deficiency (HRD), a molecular hallmark in approximately 50% of HGSC, could improve outcomes for a significant number of women with OC. BRCA1/2 germline and somatic mutations are observed in 15-20% of OC and account for ~1/3 of HRD tumors. These mutations are predictors of platinum and poly-ADP-ribose polymerase inhibitors (PARPi) response and are prognostic for improved outcomes in OC. Identifying other mechanisms producing HRD could expand the number of women with OC benefiting from PARPi. Another possible though less well characterised mechanism of HRD is BRCA1 promoter methylation, occurring in approximately 10-15% of HGSC, although reported rates vary between 5-89.9%. By virtue of epigenetic silencing of BRCA1, BRCA1-methylated OC is postulated to compare to BRCA1-mutated OC in terms of HRD, platinum chemotherapy and PARPi sensitivity, clinical characteristics and survival outcomes.

Cell line models of BRCA1-methylated OC display specific sensitivity to platinum chemotherapy and PARPi. Analysis of BRCA1-methylated OC specimens, albeit in small cohorts, consistently display low BRCA1 protein and mRNA expression. In the clinical setting, few retrospective studies have addressed the implication of BRCA1 methylation on clinical characteristics and patient outcomes after platinum chemotherapy in OC, with inconsistent results. Recent large studies utilizing genome wide methylation arrays (GWMA) correlated to BRCA1 mRNA expression to detect BRCA1 methylation demonstrate no prognostic impact on survival. One study, however, shows similar hazard ratios for overall survival (OS) for both BRCA1-methylated (HR 0.74, 95% CI [0.49 – 1.14]) and BRCA1-mutated OC (HR 0.75, 95% CI [0.46 – 1.22]) as compared to BRCA1/2 intact disease, though neither was statistically
significant. In contrast, smaller studies \((n=27 \text{ to } 332)\) utilizing methylation sensitive or methylation specific PCR (MSP) as a diagnostic assay have conflicting findings with regards to associations with platinum sensitivity and survival. While some report statistically significant improvements in survival\(^{16,17}\), as compared to non-\(BRCA1\)-methylated OC, others observe trends towards a worse outcome\(^{18}\). A comprehensive study of the clinical implications of \(BRCA1\)-methylated OC is required.

**METHODS**

**Search strategy and study selection**

The conduct of this meta-analysis followed the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines. Original investigations were sought in PUBMED/Medline through 1 April 2018, with no restrictions on publication date or language. The search strategy followed the syntax: (BRCA* [Title/Abstract] OR BRCA1* [Title/Abstract]) AND methylat* [Title/Abstract] AND ovar* [Title/Abstract], and was performed independently by two investigators (R.K. and B.S.), who independently reviewed abstracts for eligibility. Additionally, abstracts from the 2009-2018 ASCO, ESMO and SGO annual meetings were searched. Eligible articles’ reference lists were reviewed for further potential studies. The inclusion of published and unpublished studies regardless of publication language or date attempts to minimize publication bias.

Eligible studies needed to: 1) assess \(BRCA1\) methylation in fallopian tube, primary peritoneal or ovarian cancer specimens 2) report on participant and disease characteristics 3) report on progression-free survival (PFS) and/or OS 4) provide suitable methodology on their \(BRCA1\) methylation assay. Clinical trials involving PARPi were not eligible. There were no restrictions on the \(BRCA1\) methylation assay used. As \(BRCA2\) methylation seldom occurs in ovarian
cancer, we did not investigate its role in OC. Studies reporting solely on *BRCA2* methylation in OC were therefore excluded.

**Data acquisition**

The following anonymized individual participant data (IPD) was requested from eligible studies’ authors, using a pre-specified template and coding: 1) *BRCA1* methylation assay details, 2) participant and disease characteristics (age, histology, FIGO stage, grade, HRD score, *BRCA1* methylation, *BRCA1/2* mutation), 3) treatment details (receipt of adjuvant/neoadjuvant platinum chemotherapy, degree of surgical cytoreduction, platinum sensitivity) and 4) survival outcomes (platinum-free interval (PFI), PFS and OS). Tumor HRD score is the unweighted sum of loss of heterozygosity (LOH), telomeric allelic imbalance, and large-scale state transitions. A score ≥ 42 defines a tumor as HR deficient.

**Data integrity and risk of bias assessment**

IPD was checked for errors, missing data, and consistency with study publications. Study authors were contacted to resolve discrepancies or obtain missing data. Studies with partially missing data were analysed on the basis of available data.

Bias assessment for studies’ internal validity was performed using the ROBINS-I tool recommended by the Cochrane collaboration for non-randomized studies.

**Statistical analysis**

This meta-analysis aims to clinically characterize *BRCA1*-methylated OC. Its primary and secondary objectives were to determine the clinico-pathological characteristics associated with *BRCA1* methylation and ascertain the prognostic impact of *BRCA1* methylation on PFS and OS, respectively, in OC. A tumor was considered *BRCA1*-methylated if it was defined as such within its original study. OS was the time from diagnosis until death; participants were censored at the
last known survival date. PFS was the time from diagnosis until CA125 and/or RECIST disease progression, or death, whichever occurred first; participants were censored at the last known progression-free date.

Descriptive statistics were calculated for the combined IPD to summarize participants and disease characteristics for BRCA1-methylated OC versus non-BRCA1-methylated OC. The characteristics of interest were age, histotype, stage, grade, residual disease after surgical cytoreduction and platinum sensitivity. Comparisons between BRCA1-methylated and non-BRCA1-methylated OC were made using the generalized Cochran-Mantel-Haenzel test for repeated tests of independence with continuity correction to facilitate combining the multiple cohorts. Where available, HRD scores of BRCA1-methylated OC were compared to BRCA1/2 intact, BRCA1-mutated and BRCA2-mutated OC using unpaired t-tests. The Kaplan Meier method (logrank test) was used to generate survival plots via the R package survival. Forest plots were generated using the R package survcomp. Univariate and multivariate analysis of PFS and OS were performed using Cox proportional hazards regression models, which estimated hazard ratios (HR) and 95% confidence intervals (CI) for each individual datasets. A mixed effects cox model was then used to perform univariate and multivariate analysis of the combined dataset, comparing BRCA1-methylated OC to non-BRCA1 methylated OC. These models were generated using the R packages survival and coxme respectively. Multivariate models were adjusted for the following clinical variables: age, grade, stage, residual disease after surgical cytoreduction. For cohorts with available germline and/or somatic BRCA1/2 mutation data, the above analyses were repeated comparing BRCA1-methylated to BRCA1/2 intact (BRCA1/2-wild type non-BRCA1-methylated) OC, in order to eliminate the potential survival bias attributed by BRCA1/2-mutated OC in the non-BRCA1-methylated population. P-values were adjusted for multiple testing (Benjamini-Hochberg method). Heterogeneity was quantified using the I² statistic, which provides a numerical value ranging between 0% - 100%.
This value and its 95% confidence interval were interpreted according to ranges described in the Cochrane Handbook for Systematic Review of Interventions, with values between 0 – 40%, 30 – 60%, 50 – 90% and 75 – 100% suggesting low, moderate, considerable and substantial heterogeneity, respectively²⁸. For all analyses, P values less than 0.05 (two-tailed) were considered statistically significant. All calculations were performed in the R statistical environment (https://www.r-project.org/).

RESULTS

Study selection
The literature search retrieved 159 records (Figure 1). Three were duplicated datasets, 8 were conference abstracts identified in the Pubmed search, 90 were irrelevant, 21 were reviews, 13 had no survival data, 1 had unsatisfactory methodology details, 1 concerned a PARPi clinical trial and 22 were eligible. Following contact with authors of eligible studies, 5 did not participate and 4 could not access or share the data (Table S1)¹⁴,¹⁷,¹⁸,²⁹-³⁴. In addition, we accessed 2 unpublished cohorts. One author provided data on additional patients not included in the original publication. Overall, 15 observational studies (430 cases; 2206 controls) were included³,¹³,¹⁵,¹⁶,³⁵-⁴³(Tables S2A-S2C).

Quality assessment
Individual study assessments were made on the basis of raw IPD, study manuscript and any necessary clarifications with study authors (Table S3). Studies’ internal validity was deemed overall good, with a likely low risk of bias on the meta-analysis results. Of the 15 studies, 13 had an overall moderate risk of bias, mainly owing to the potential for confounding baseline factors that were nevertheless adjusted for appropriately. In addition, 4 studies had a moderate risk in patient selection with regards to determining the clinicopathological characteristics of BRCA1-
methylated OC, as only HGSC were included. A moderate risk was also found for 5 studies where intended BRCA1 methylation assessment failed due to inadequate tumor tissue/DNA. Two studies had an overall serious risk of bias, owing to missing data. One did not collect tumor grade and OS data for their entire cohort (n=35)\textsuperscript{39}. The other provided IPD for 61.0% (n=147/241) of study participants due to time constraints in data collection and provision\textsuperscript{36}. Neither of these two studies collected data pertaining to BRCA1/2 mutation status or HRD score. The exclusion of these two studies from the entire meta-analysis cohort did not alter the results of the meta-analysis for any of the endpoints measured (data not shown). It was therefore deemed reasonable to include these two studies in this meta-analysis of individual patient data.

**Participant characteristics**

Data was obtained on 2645 participants. 9 participants with dual aberrations (BRCA1 methylation and/or BRCA1/2 mutations) were excluded (Table S2), leaving 2636 participants within the analysis. Amongst participants with known BRCA1/2 mutation status (n=1257), BRCA1 methylation and BRCA1 mutation were mutually exclusive (odds ratio: 0.18, P=0.003), as were BRCA1 methylation and BRCA2 mutation (odds ratio 0.33, P=0.04). In the entire cohort, the median age was 59 years old. Fallopian tube and primary peritoneal cancers comprised 0.35% (n=7/2022) and 1.3% (n=26/2022) of the entire cohort, respectively. 85.4% (n=2247/2630) participants presented with advanced stage disease (FIGO Stage III/IV). 95.6% (n=2396/2506) participants received (neo)adjuvant platinum-based therapy. 79.7% (n=2065/2592) were HGSC. Stage III/IV HGSC comprised 73.9% participants (n=1904/2576). Surgical cytoreduction below 1 cm residual disease was achieved in 71.9% (n=1757/2444) participants (Table S4).

**Association of BRCA1 methylation with participants, disease and molecular characteristics**
The \textit{BRCA1} methylation rate varied in studies from 6.2\% to 73.7\% (Table S2A), with a pooled rate of 16.3\% \((n=430/2636)\). \textit{BRCA1}-methylated OC was significantly associated with younger age \((P=0.005)\), and high grade disease \((P=0.03)\) (Table 1). No other statistically significant clinico-pathological correlations were observed.

Germline and/or somatic \textit{BRCA1/2} mutation status was available for 1248 participants from 7/15 included studies. Of these, 10.6\% \((n=132/1248)\) were \textit{BRCA1}-mutated, 6.5\% \((n=81/1248)\) were \textit{BRCA2}-mutated and 10.3\% \((n=128/1248)\) were \textit{BRCA1}-methylated. Within this cohort with known germline and/or somatic \textit{BRCA1/2} mutation status, \textit{BRCA1} methylation was also associated with younger age \((P=0.007)\) and high grade disease \((P=0.005)\), when compared to patients with \textit{BRCA1/2} intact OC. Furthermore, \textit{BRCA1}-methylated OC was associated with advanced stage \((P=0.01)\) and serous histology \((P=0.009)\), compared to \textit{BRCA1/2} intact OC. \textit{BRCA1} mutation was associated with younger age \((P<0.001)\), high grade \((P=0.006)\), serous histology \((P=0.005)\), advanced stage \((P=0.02)\) and platinum sensitivity \((P=0.008)\), when compared to \textit{BRCA1/2} intact disease. The clinico-pathological profile of \textit{BRCA1}-methylated OC did not differ significantly from that of \textit{BRCA1}-mutated OC (Table S5).

Tumor HRD score was available for 447 participants (MDACC 2010 and TCGA 2011 cohorts). HRD scores were statistically significantly higher in \textit{BRCA1}-methylated OC (median 68, interquartile range (IQR) 62- 74), compared to \textit{BRCA1/2} intact disease (median 26, IQR 18-38.8), \textit{BRCA1}-mutated (median 63, IQR 56- 70) and \textit{BRCA2}-mutated (median 56, IQR 44.5-65.5) disease (Figure 2).

\textbf{Association of \textit{BRCA1} methylation with survival}
The median follow-up time was 2.8 years (range 0-18.3; IQR 1.5-4.8). There was a statistically significant constituent study effect on the cox regression model first used to associate BRCA1 methylation with PFS and OS ($P=1.17 \times 10^{-13}$ and $P=2.33 \times 10^{-4}$, respectively). Statistically significant moderate to substantial heterogeneity was observed between studies for PFS ($I^2 = 62\%$ (CI 26%-86%), $Q= 36.4$, df=14, $P<0.001$). While not statistically significant, we note low heterogeneity between studies for the assessment of OS ($I^2 = 37\%$ (CI 0%-75%), $Q=20.7$, df=13, $P=0.08$), though the 95% confidence interval is wide. Clinically, heterogeneity is expected given observed differences between cohorts with regards to patient/disease clinical characteristics and study characteristics. A mixed-effect model was therefore employed to adjust for study heterogeneity. In order to justify our acceptance of the null hypothesis of this meta-analysis, we performed a power calculation utilizing observed information (study heterogeneity) and assumptions (predicted effect size). Using this information, we calculated that we have an 84% power to detect a modest effect size (cohan’s d of 0.2), across the 15 studies with an average BRCA1 methylation rate of 29 samples per study and an average non-BRCA1 methylation rate of 147 samples per study with an alpha value of 0.05 and a moderate level of heterogeneity. In the combined population, there was no statistically significant difference in PFS and OS between BRCA1-methylated and non-BRCA1-methylated OC (median PFS 20 vs 18.5 months, HR 1.01, 95% CI[0.87-1.16], $P=0.98$; median OS 46.6 vs 48 months, HR 1.02, 95% CI[0.87-1.18], $P=0.96$, respectively). This lack of association persisted in a multivariate model adjusted for age, stage, grade and cytoreduction (Figures 3-4A).

Within the subgroup with known BRCA1/2 mutation status, BRCA1-methylated OC was associated with a worse PFS than BRCA1/2 intact OC on univariate analysis (median PFS 15.7 vs 18 months, HR 1.26, 95% CI[1.02-1.56], $P=0.03$), though this significance was lost on multivariate analysis. There was no OS difference on univariate or multivariate analyses between the BRCA1-methylated and the BRCA1/2 intact groups (median OS 43.5 vs 47.5
months, univariate HR 1.05, 95% CI[0.83-1.32], \( P=0.70 \). \( BRCA1 \)-mutated OC was only associated with a statistically significant improvement in PFS and OS, as compared to \( BRCA1/2 \) intact OC, on multivariate analysis (PFS: median 17.3, univariate HR 0.94, 95% CI[0.75-1.17], \( P=0.57 \); multivariate HR 0.78, 95% CI[0.62-0.99], \( P=0.04 \); OS: median 47.4 months, univariate HR 0.81, 95% CI[0.64-1.03], \( P=0.09 \); multivariate HR 0.76, 95% CI[0.58-0.98], \( P=0.03 \)). \( BRCA2 \) mutation conferred a clear PFS and OS benefit compared to \( BRCA1/2 \) intact OC on univariate and multivariate analyses (median PFS: 28.6 months, univariate HR 0.58, 95% CI [0.44-0.78], \( P<0.001 \); median OS 87 months, univariate HR 0.55, 95% CI [0.40-0.77], \( P<0.001 \)) (Figure 4B, Table S5).

**Exploratory analysis of methylation methodology on survival**

We explored PFS and OS comparing \( BRCA1 \)-methylated OC to non-\( BRCA1 \)-methylated OC within 3 subgroups of the meta-analysis’ entire cohort divided according to methylation assay type: those studies utilising methylation-specific PCR (MSP) with gel electrophoresis (GE) (7 studies, \( n=765 \)), those utilising quantitative analysis of MSP or methylation sensitive restriction endonuclease digestion (MRED; 5 studies, \( n=828 \)), and those utilising genome wide methylation arrays (GWMA; 3 studies, \( n=1043 \)). Methylation assays are further detailed in Table S2A and Figure S2S1. In the combined cohorts utilising MSP-GE, \( BRCA1 \)-methylated OC was associated with an improved PFS and OS (univariate HR 0.80, 95% CI[0.66-0.97], \( P=0.02 \); univariate HR 0.80, 95% CI[0.63-1.00], \( P=0.05 \), respectively) as compared to non-\( BRCA1 \)-methylated OC, though statistical significance was lost for OS on multivariate analysis (\( P=0.08 \)). Amongst cohorts utilising MSP/MRED with quantitative analysis, \( BRCA1 \)-methylated OC was associated with a worse PFS (HR 1.47, 95%CI [1.10-1.96], \( P=0.008 \)) and OS (HR 1.45, 95%CI[1.05-2.00], \( P=0.02 \)), when compared to non-\( BRCA1 \)-methylated OC. This which persisted on multivariate analysis. There were no survival differences observed between
DISCUSSION

Dysfunctional BRCA1 and BRCA2 proteins as a result of BRCA1/2 mutations render OC particularly susceptible to therapies targeting the homologous recombination DNA repair pathway. While the association of BRCA1/2-mutated OC with improved survival resulting from sensitivity to (neo)adjuvant platinum-based chemotherapy is well established, the clinical and therapeutic implications of other BRCA1 dysfunction mechanisms is less understood. In a recent meta-analysis, loss of BRCA1 expression by immunohistochemistry was also associated with a statistically significant improved survival44, although the mechanisms behind absent BRCA1 protein expression were not specified. Gene silencing through promoter methylation is one such mechanism, though other potential indirect or post translational mechanisms leading to BRCA1 inactivation or reduced expression require investigation. Whilst BRCA1 methylation is a recognized event in OC, promoter methylation of BRCA2 appears to be a rare occurrence in OC, if at all. Amongst 6 studies investigating BRCA2 methylation in OC15,45-49, only 2 BRCA2-methylated cases were identified amongst 612 cases tested. This meta-analysis was therefore limited to studies investigating BRCA1 methylation. Reports on BRCA1-methylated OC have been conflicting in terms of clinico-pathological associations, with smaller cohorts observing associations with FIGO stage I/II disease31 or a lack of association with any histotype18. Other cohorts limited their assessment of BRCA1 methylation to homogeneous HGSC cohorts, thereby precluding the detection of histopathological associations. Our study population comprised heterogeneous OC subtypes, though dominated by advanced stage HGSC. We
show that features of BRCA1-methylated OC mirror that of BRCA1-mutated disease in terms of advanced stage, high grade, serous disease, and a younger age at diagnosis.

The expectation is therefore that BRCA1-methylated disease will be sensitive to platinum and PARPi by virtue of HRD. We provide evidence of HRD in BRCA1-methylated OC, as defined by the HRD score, albeit in a limited subgroup of participants derived from 2 studies. Cell line and patient-derived xenograft models of BRCA1-methylated OC demonstrate clear sensitivity to platinum and/or PARPi therapy. Recent data from the ARIEL2 phase II clinical trial demonstrate an encouraging 63% (n=12/19) RECIST response rate amongst BRCA1-methylated recurrent HGSC to the PARPi rucaparib, as compared to response rates of 79% (n=23/29) and 13.5% (n=7/52) observed in BRCA1-mutated and BRCA1 intact/low genome-wide LOH recurrent HGSC, respectively. However, large clinical cohorts (e.g. TCGA) show no difference in PFI by BRCA1 methylation status. Within this meta-analysis, PFI data was unavailable for 56.9% (n=1539/2636) of participants, thereby precluding a representative assessment. Nevertheless, we found no association between BRCA1-methylated OC and platinum sensitivity. Moreover, we observed no PFS difference between BRCA1-methylated and non-BRCA1-methylated OC in the entire cohort. When evaluated against the more appropriate comparator population that is BRCA1/2 intact OC, albeit within a smaller cohort of 1248 patients, BRCA1-methylated OC once again did not display improved survival. In contrast, both BRCA1 and BRCA2 mutations were prognostic of improved PFS/OS on multivariate analysis, as compared to BRCA1/2 intact OC, despite the limited cohort size of 1248 patients. Reasons for the discrepancy in survival between BRCA1-mutated OC and BRCA1-methylated OC, relative to BRCA1/2 intact OC, are unclear and warrant further investigation. Compared to BRCA2 mutations, the survival benefit conferred by BRCA1 mutations is of a lesser magnitude, echoing recent reports evaluating survival in BRCA1/2-mutated OC. Some studies find no survival difference between BRCA1-mutated and BRCA1/2 wild type cancers. A pooled
cohort (>6500 participants) demonstrated the expected positive prognostic effect of \textit{BRCA1} mutation on OS, which was to a markedly lesser degree than observed with \textit{BRCA2} mutation (\textit{BRCA1} mutation HR 0.83, 95% CI 0.74-0.93, \textit{P}<0.001; \textit{BRCA2} mutation HR 0.55, 95% CI 0.47-0.65, \textit{P}=0.002)\textsuperscript{53}. It is likely the survival benefit is diluted by heterogeneity within \textit{BRCA1}-mutated disease, whereby some cases assigned a \textit{BRCA1}-mutated status actually behave in HR proficient manner. Similarly, the difference in survival patterns between \textit{BRCA1} mutation and \textit{BRCA1} methylation, despite sharing similar clinicopathological features, could be explained in part by heterogeneity within \textit{BRCA1}-methylated OC, as discussed further below. Moreover, methylation as a rule is a more dynamic mechanism relative to mutation, and subject to change depending on specific characteristics of the tumor microenvironment. The survival benefit observed in \textit{BRCA1/2}-mutated OC results from their marked sensitivity to platinum-based chemotherapy, used a standard of care in the (neo)adjuvant treatment of OC. Potentially, chemotherapy induces changes affecting the methylation levels of the \textit{BRCA1} promoter, causing earlier and perhaps more frequent resistance to platinum-based chemotherapy than is observed in \textit{BRCA1}-mutated OC. This may manifest as methylation loss in relapsed \textit{BRCA1}-methylated OC, observed in 16.7% - 80% of small mostly retrospective cohorts of 6 to 13 paired primary/recurrent \textit{BRCA1}-methylated HGSC\textsuperscript{43,52,54,55}.

The pooled \textit{BRCA1} methylation rate was 16.3%. While this reflects most reports of \textit{BRCA1} methylation frequency, there is marked variability in the reported occurrence of \textit{BRCA1} methylation in OC (5%-89.9\%)\textsuperscript{9,39}. Amongst studies included in this meta-analysis, \textit{BRCA1} methylation frequency ranged from 6.2% to 73.6%, with an interquartile range of 10.1% - 18.9%. To some extent, this could be explained by cohort sampling bias, in terms of size, populations and histological subtypes included, with slightly higher rates often observed when cohorts are restricted to HGSC (Table S2A). In breast cancer, \textit{BRCA1} methylation is more frequent amongst Asians, as compared to Caucasians\textsuperscript{56}, however ethnicity data was not available in this
meta-analysis. Moreover, tissue sampling size and content introduces bias owing to variations in sampling sites (ovary versus metastatic), neoplastic cell content and intratumor heterogeneity. Four studies report particularly high BRCA1 methylation rates: 21.4%\textsuperscript{35}, 33.6%\textsuperscript{16}, 42%\textsuperscript{39} and 73.7%\textsuperscript{43}. This latter cohort, which confirmed all its MSP-GE determined BRCA1-methylated OC cases with Sanger sequencing, consisted solely of relapsed cases, which may account to some extent for this variation. We also observed that these 4 studies determined BRCA1 methylation using MSP-GE, whilst studies utilising quantitative methodologies or high throughput microarrays reported rates varying between 8.2% and 16%. In a meta-analysis evaluating BRCA1 promoter methylation as a risk for the development of breast cancer, studies utilising MSP were significantly more likely to report higher frequencies of BRCA1 methylation\textsuperscript{56}. Non-specific primer binding or incomplete bisulfite conversion have linked MSP with false positive results, and may account for this observation to some extent\textsuperscript{57}.

The marked variation in the methodology used to determine BRCA1 promoter methylation is also likely to contribute to differences in reported BRCA1 methylation rates (Figure S1). This epigenetic phenomenon is characterized by the methylation of CpG dinucleotides within a \(\sim 2.7\) kB 5’CpG island containing 96 CpG dinucleotides and involving the bidirectional BRCA1 promoter and its adjacent alternative first exons (exon 1a/1b)\textsuperscript{58,59}. An essential regulatory area 202 bp downstream and 20 bp upstream of the BRCA1 transcription start site at exon 1a (according to GenBank U37574) contains sequence specific transcription factor binding sites that prevent transcription when methylated\textsuperscript{60}. However, the individual contribution of CpG dinucleotides within this area (or elsewhere in the 2.7 kb CpG island) to the regulation of BRCA1 transcription has yet to be comprehensively evaluated in OC. Commonly used assays include MRED, MSP, methylation sensitive multiplex ligation probe amplification (MS-MLPA), bisulfite sequencing and, more recently, GWMA. In contrast to the other methodologies, the latter correlates BRCA1 mRNA expression to CpG dinucleotides’ methylation status (9-46
assessed), to enable the selection of relevant CpG probes\textsuperscript{3,15}. Despite this common rationale, 2 studies\textsuperscript{15,42} using the same GWMA assay (Illumina Infinium HumanMethylation 450k Beadchip) selected 8 and 21 CpG probes to determine \textit{BRCA1} methylation status within their samples. Were both assays applied to the same samples, different results may ensue depending on the level of methylation in tumor samples at these CpG sites. PCR-based assays use different primers which assess often overlapping though different regions of the \textit{BRCA1} promoter, resulting in different CpG dinucleotides being interrogated between assays (Table S2A, Figure S12). One study using 2 sets of primers, each targeting a different region within the \textit{BRCA1} promoter, reported different methylation levels within the same OC sample: 17.5% (region 1) and 3.3% (region 2)\textsuperscript{61}. Using an arbitrary cut-off of 10% to define methylation, and had only one set of primers been used, this sample may or may not be defined as \textit{BRCA1}-methylated. Moreover, methylation of individual CpG dinucleotides, which may or may not be involved in regulation of \textit{BRCA1} transcription, cannot be distinguished within a PCR-based assay. The importance of CpG site selection to determine \textit{BRCA1} methylation status was underscored in a study demonstrating a correlation between high methylation levels at the \textit{BRCA1} promoter and the triple negative breast cancer subtype, as determined by pyrosequencing. However the level of methylation was variable across the 11 CpG sites evaluated, with 4 CpG sites displaying low/lack of methylation\textsuperscript{62}. Differences also exist with regards to assay interpretation, which includes GE (used in 8 of the included studies) or quantitative analyses. The latter use varying methodologies to 1) determine non-methylated reads and 2) quantify the percentage methylated reads within a sample. Quantitative analyses arbitrarily define low thresholds (4-10%) to define methylation, without critically evaluating the threshold required for \textit{BRCA1} inactivation. Arguably, a low threshold is selected to account for potential dilution of methylated fragments in specimens with low neoplastic cell content or tumor heterogeneity, though may result in labelling non-\textit{BRCA1}-methylated samples with high neoplastic cell content/homogeneous tumor as \textit{BRCA1}-methylated Finally, whilst technically valid, these assays have not been compared to
one another in the same OC dataset. A study compared methylation status of 4 genes using MSP-GE, quantitative MS-MLPA and quantitative multiplex MSP in 40 breast cancers, and found high discrepancies between MSP-GE and the quantitative assays’ results. Methylation assay heterogeneity was evident between studies included in this meta-analysis. In an exploratory analysis, we identified a survival benefit within a subset of BRCA1-methylated OC identified with MSP-GE, as compared to non-BRCA1-methylated OC. This subset was particularly homogeneous as 5 of the 7 cohorts included evaluated the same 7 CpG sites, whilst 4 CpG sites were common to 6 cohorts (CpG details for 1 cohort were unavailable). Our findings may give credence to these particular CpG sites in terms of their essential role on BRCA1 transcription. However, only 2 of these 7 cohorts individually reported an improved survival for BRCA1-methylated OC, compared to non-BRCA1-methylated OC, as did 1 of 5 eligible studies using identical PCR primers excluded from this analysis (Table S1). While this observation could be related to individual studies’ small sample size, our findings are exploratory and should be interpreted with caution.

Factors other than diagnostic methylation assays may contribute to heterogeneity within BRCA1-methylated OC, as is observed in BRCA1-mutated disease. Indeed, studies suggest resistance to platinum and/or PARPi therapy with BRCA1 mutations occurring within the BRCA1 RING domain. Moreover, mono-allelic BRCA1 mutations do not display an HR-deficient phenotype when BRCA1-locus LOH is absent, as evaluated in a cohort of 52 BRCA1-mutated OC. In this study, 7% of BRCA1-mutated OC had absent BRCA1-locus LOH and a worse survival, compared to BRCA1-mutated OC with BRCA1-locus LOH (P=0.02). Similarly, the discrepancy between preclinical findings and our analysis results with regards to platinum sensitivity and survival may lie in heterogeneity within BRCA1-methylated OC. This has been illustrated in BRCA1-methylated HGSC patient-derived xenograft models, whereby one model was cisplatin sensitive, and the other cisplatin resistant, using the same MSP assay.
Potentially, mono-allelic \textit{BRCA1} methylation with absent \textit{BRCA1}-locus LOH would result in transcription of an intact \textit{BRCA1} on the non-methylated allele, resulting in a functioning \textit{BRCA1} protein. By pooling studies evaluating LOH in \textit{BRCA1}-methylated OC, we found that 19.6% (n=18/92) of \textit{BRCA1}-methylated OC have absent LOH, as determined by analyzing microsatellites near \textit{BRCA1} (Table S6). Current methylation assays do not routinely examine \textit{BRCA1} locus LOH, nor do they differentiate between mono/bi-allelic methylation. In quantitative assays, greater than 50% methylation at the \textit{BRCA1} promoter may assume bi-allelic methylation. A recent study estimated percentage \textit{BRCA1} promoter methylation (adjusted for \textit{BRCA1} locus LOH, neoplastic cellularity and \textit{BRCA1} copy number) to differentiate homozygous (>50% methylation) and heterozygous (<50% methylation) \textit{BRCA1} methylation in 21 \textit{BRCA1}-methylated tumors from the ARIEL2 phase II clinical trial. Encouragingly, homozygous \textit{BRCA1}-methylated OC (n=6) was associated with a longer PFS than \textit{BRCA1/2} intact OC (n=143), though this was not statistically significant (median PFS 14.5 months (95% CI 4.8-18.3 months) vs 5.5 months (95% CI 5-6.2 months), \textit{P}=0.06)\textsuperscript{66}. The development of allele-specific methylation methodologies that include LOH assessment, confirmed by absent/low \textit{BRCA1} mRNA expression, and ideally using specimens with 100% neoplastic cell content should minimize heterogeneity and permit a more accurate determination of \textit{BRCA1} epigenetically silenced tumors, or ‘true’ \textit{BRCA1}-methylated OC.

This is the most extensive meta-analysis to date evaluating the clinical characteristics of \textit{BRCA1}-methylation in OC. The inclusion of published and unpublished studies without publication language restrictions, along with the use of IPD from studies assessed to be of overall good quality further strengthens our results. Limitations include 1) incomplete inclusion of all eligible studies identified through our search strategy, 2) statistically significant heterogeneity between cohorts in the assessment of PFS and OS, which was mitigated by the use of a mixed effect model, 3) heterogeneity in the methylation assays utilised between
included studies, 4) availability of BRCA1/2 mutation status, which enables a more thorough assessment of the prognostic effect of BRCA1 methylation on survival by using BRCA1/2 intact OC as a comparator, was limited to n=1248/2636 patients.

CONCLUSION

In the largest meta-analysis on this topic, we show that BRCA1-methylated OC has a clinico-pathological profile similar to that of BRCA1-mutated OC, presenting at a younger age as advanced stage HGSC. However, BRCA1 methylation does not predict for platinum sensitivity nor is it prognostic of survival. While early and/or frequent platinum resistance mechanisms may account for this observation, there is significant heterogeneity between methylation assays used to detect BRCA1-methylated OC, in terms of the exact CpG sites assessed and the interpretation of the observed result. Moreover, these assays are not allele-specific and do not account for BRCA1-locus LOH. Potentially, a comprehensive assay that examines CpG dinucleotides critical to BRCA1 transcription in OC specimens in an allele-specific manner, combined with assessment of BRCA1-locus LOH may permit a better selection of ‘true’ BRCA1-methylated OC. Defined as such, BRCA1-methylated OC may represent a smaller subset, yet permit a more accurate selection of patients with OC that would derive clear benefit from PARPi and other novel therapies targeting HR-deficient OC.

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NOTES
The study funders had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

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As outlined on the TCGA website (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/citing-tcga), authors are permitted to use data from TCGA once the TCGA Research Network is acknowledged, as above, in their work.

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**DISCLOSURES**
RK has received conference travel reimbursement from Astra Zeneca, outside the submitted work. EB has received travel and advisory board fees from Roche Pharma, Tesaro, Incyte; travel and congress fees from Astra Zeneca; advisory board fees from Clovis, Seattle Genetics, Amgen; and institution funding from Roche Pharma, all outside the submitted work. JS has received honoraria, consulting/advisory fees, travel fees and institutional research funding from Astra Zeneca, Clovis, PharmaMar, Pfizer, TESARO, MSD, as well as institutional research funding from Merck, Bayer; consulting/advisory fees from Lilly, Roche; honoraria from Eisai, Olympus, Johnson and Johnson, TEVA; travel fees from Roche. GM has had a consultant role or participated on scientific advisory boards of Astra Zeneca, Chrysalis, ImmunoMET, Ionis, Mills Institute for Personalized Care (MIPCC), PDX Pharma, Signalchem Lifesciences, Symphogen, Tarveda. GM has stock or options in Catena Pharmaceuticals, ImmunoMet, SignalChem, Spindletop Ventures, Tarveda, and has received research funding from Astra Zeneca, Ionis, Karus Therapeutics, Nanostring, Pfizer, Takeda/Millenium Pharmaceuticals. KT is an employee of and may have stocks in Myriad Genetics. DB has received research funding and clinical trial support from Genentech/Roche, Astra Zeneca, and Beigene. LW has received research support, honoraria or travel reimbursement from TESARO, medac oncol, GSK, MSD, Jenapharm, Roche, Medupdate GmbH. The other authors have declared no conflicts of interest.

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Table 1. Association between BRCA1 methylation status and clinico-pathological factors
(Cochran-Mantel-Haenzel test with p value adjusted for cohort; percentages reflects percentage of total non-missing data)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BRCA1-methylated (n=430)</th>
<th>Non-BRCA1 methylated (n=2206)</th>
<th>Adjusted p-value</th>
</tr>
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<tbody>
<tr>
<td>Age, No. (%)</td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>&lt;59</td>
<td>245 (57%)</td>
<td>1090 (49.6%)</td>
<td></td>
</tr>
<tr>
<td>≥59</td>
<td>185 (43%)</td>
<td>1109 (50.4%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Grade, No (%)</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Low</td>
<td>25 (6%)</td>
<td>198 (9.1%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>389 (94%)</td>
<td>1979 (90.9%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>16</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>FIGO stage, No (%)</td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>I/II</td>
<td>48 (11.2%)</td>
<td>339 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>III/IV</td>
<td>381 (88.8%)</td>
<td>1862 (84.6%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Histology, No (%)</td>
<td></td>
<td></td>
<td>0.97</td>
</tr>
<tr>
<td>Serous</td>
<td>354 (82.3%)</td>
<td>1799 (81.6%)</td>
<td></td>
</tr>
<tr>
<td>Non-serous</td>
<td>76 (17.7%)</td>
<td>407 (18.4%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Residual disease post cytoreduction, No (%)</td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>Macro &lt;1cm</td>
<td>306 (73.6%)</td>
<td>1451 (71.5%)</td>
<td></td>
</tr>
<tr>
<td>Macro ≥1cm</td>
<td>110 (26.4%)</td>
<td>577 (28.5%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>14</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>Platinum sensitivity, No (%)</td>
<td></td>
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<td>0.34</td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>66 (19.4%)</td>
<td>463 (28.2%)</td>
<td></td>
</tr>
<tr>
<td>≥6 months</td>
<td>275 (80.6%)</td>
<td>1177 (71.8%)</td>
<td></td>
</tr>
<tr>
<td>No platinum</td>
<td>17</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>72</td>
<td>460</td>
<td></td>
</tr>
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</table>
Table 2. Univariate and multivariate analysis for PFS and OS according to BRCA1/2 aberrations
(Using a mixed effects model adjusting for cohort)

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFS</td>
<td>OS</td>
</tr>
<tr>
<td><strong>Entire cohort</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=2636)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual disease</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 methylation</td>
<td>1.01 (0.87 – 1.16);</td>
<td>1.02 (0.87 – 1.18);</td>
</tr>
<tr>
<td></td>
<td>p=0.98</td>
<td>p=0.96</td>
</tr>
<tr>
<td><strong>Cohort with known</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BRCA1/2</strong> mutations**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=1248)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>-</td>
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<tr>
<td>Stage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual disease</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 methylation</td>
<td>1.26 (1.02-1.56);</td>
<td>1.11 (0.88-1.41);</td>
</tr>
<tr>
<td></td>
<td>p=0.03</td>
<td>p= 0.35</td>
</tr>
<tr>
<td>BRCA1 mutation</td>
<td>0.99 (0.80-1.23);</td>
<td>0.87 (0.69-1.10);</td>
</tr>
<tr>
<td></td>
<td>p=0.91</td>
<td>p= 0.24</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td>0.58 (0.43-0.77);</td>
<td>0.57 (0.41-0.80);</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

*All clinical variables within the multivariate model are binary, as follows: Age: 0 for <60 (median age) and 1 for ≥60; Grade: 0 for low grade, 1 for high grade; 0 for Stage I/II, 1 for Stage III/IV; Residual disease: 0 for < 1cm, 1 for ≥1cm.
Table 3. Association of BRCA1 methylation with PFS and OS according to methylation assay subgroup

MRED: methylation-sensitive restriction endonuclease digest; MSP: methylation-specific PCR; PFS: progression-free survival; OS: overall survival

<table>
<thead>
<tr>
<th>BRCA1 methylation Hazard Ratio (95%CI); p value (methylated relative to non-methylated)</th>
<th>Univariable PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome wide methylation array correlated to BRCA1 mRNA expression</td>
<td>1.12 (0.89 – 1.41), p=0.33</td>
<td>1.09 (0.85 – 1.39), p =0.51</td>
</tr>
<tr>
<td>(n=976, 767 events)</td>
<td>(n=1043, 690 events)</td>
<td></td>
</tr>
<tr>
<td>MRED or MSP with quantitative analysis</td>
<td>1.47 (1.10 – 1.96), p =0.008</td>
<td>1.45 (1.05 – 2.00), p =0.02</td>
</tr>
<tr>
<td>(n=781, 590 events)</td>
<td>(n=826, 382 events)</td>
<td></td>
</tr>
<tr>
<td>MSP with gel electrophoresis</td>
<td>0.80 (0.66 – 0.97), p =0.02</td>
<td>0.80 (0.63 – 1.00), p =0.05</td>
</tr>
<tr>
<td>(n=706, 518 events)</td>
<td>(n=730, 411 events)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BRCA1 methylation Hazard Ratio (95%CI); p value (methylated relative to non-methylated)</th>
<th>Multivariable* PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome wide methylation array correlated to BRCA1 mRNA expression</td>
<td>1.11 (0.87 – 1.42), p =0.40</td>
<td>1.04 (0.80 – 1.35), p =0.78</td>
</tr>
<tr>
<td>(n=838, 657 events)</td>
<td>(n=896, 591 events)</td>
<td></td>
</tr>
<tr>
<td>MRED or MSP with quantitative analysis</td>
<td>1.36 (1.00 – 1.84), p =0.04</td>
<td>1.40 (1.01 – 1.94), p =0.04</td>
</tr>
<tr>
<td>(n=733, 556 events)</td>
<td>(n=774, 354 events)</td>
<td></td>
</tr>
<tr>
<td>MSP with gel electrophoresis</td>
<td>0.82 (0.68 – 1.00), p =0.05</td>
<td>0.81 (0.64 – 1.03), p =0.08</td>
</tr>
<tr>
<td>(n=655, 482 events)</td>
<td>(n=724, 407 events)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for binary clinical variables as follows: Age: 0 for <60 (median age) and 1 for ≥60; Grade: 0 for low grade, 1 for high grade; Stage : 0 for Stage I/II, 1 for Stage III/IV; Residual disease: 0 for <1cm, 1 for ≥1cm.
Figure 1. Flow chart of study

Figure 2. HRD score as assessed on 447 tumor samples obtained from the TCGA 2011 and MDACC 2010 cohorts, according to underlying BRCA1/2 aberration

Box plots depict the median and 95% confidence intervals of the HRD scores according to the underlying BRCA1/2 aberration in the TCGA 2011 and MDACC 2010 cohorts. The dotted line represents the threshold value of 42 above which samples are considered to be homologous recombination deficient as per the HRD score assay. Stars depict the level of statistical significance between sets of groups (unpaired t-tests). Numbers in each subgroup are as follows: Wild type – n=286; BRCA1 methylation – n=65; BRCA1 mutation – n=57; BRCA2 mutation – n=39.

Figure 3. Univariate and multivariate analyses of BRCA1 methylation on progression-free survival (PFS) and overall survival (OS) by study and for the combined dataset

A: Univariate analyses; B: Multivariate analyses whereby hazard ratios (HR) are derived from a cox proportional hazards model adjusted for binary clinical variables as follows: age: 0 for <60 (median age) and 1 for ≥60; Grade: 0 for low grade, 1 for high grade; 0 for Stage I/II, 1 for Stage III/IV; Residual disease: 0 for <1cm, 1 for ≥1cm. Pooled analyses performed using a mixed effect model adjusting for cohort. Numbers in 'non-BRCA1-methylated' and 'BRCA1-methylated' columns represent number of events/total numbers. CI = confidence interval.

Figure 4. Survival in the entire pooled dataset and a pooled subset with known BRCA1/2 mutation status

Kaplan Meier curves depicting progression-free and overall survival in (A) the entire pooled cohort (n=2636) and (B) a pooled subset of the entire cohort with known BRCA1/2 mutation status (n=1248). (A) BRCA1 methylation (orange) and no BRCA1 methylation (grey) curves are depicted. Univariate hazard ratios (HR), 95% confidence interval (CI) and p value (Log rank test) for BRCA1 methylation vs no BRCA1 methylation, adjusted for study, are given (B) BRCA1 methylation (orange), BRCA1 mutation (dark blue), BRCA2 mutation (light blue) and BRCA1/2 intact (non-BRCA1-methylated BRCA1/2 wild type) (grey) curves are depicted. Univariate hazard ratios, 95% confidence interval (CI) and p value (Log rank test) for BRCA1 methylation vs BRCA1/2 intact, adjusted for study, are given.

Figure 5. Survival analyses in subgroups combined according to methylation assay
Kaplan Meier curves depicting progression-free and overall survival in subgroups combined according to methylation assay. Univariate hazard ratios (HR) and 95% confidence interval (CI) and p value (Log rank test) for BRCA1 methylation (orange), as compared to no BRCA1-methylation (grey), adjusted for cohort, are given.

MRED = methylation-sensitive restriction endonuclease digestion, MSP = methylation specific PCR; CI = confidence interval; BRCA1 meth = BRCA1 methylation; non-BRCA1-meth=no BRCA1 methylation