



Prevalence of tumor *BRCA1* and *BRCA2* dysfunction in unselected patients with ovarian cancer

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Objective

The therapeutic benefits of poly(ADP-ribose) polymerase inhibitors highlight the need to evaluate *BRCA1/2* defects in tubal/ovarian cancer (OC). We sought to determine the pattern and disease characteristics associated with tumor *BRCA1/2* mutations and *BRCA1* methylation in women with OC.

Methods

We obtained 111 OC specimens from 2 university hospitals and assessed *BRCA1/2* mutations and *BRCA1* methylation in tumor DNA. The frequency and pattern of *BRCA1/2* defects were examined. Associations between patient/disease characteristics and *BRCA1/2* defects were ascertained (Fisher's exact test). Platinum-free interval (PFI), progression-free survival (PFS), and overall survival (OS) based on the underlying *BRCA1/2* defect were determined (Kaplan-Meier analysis [log-rank test]).

Results

We observed a *BRCA1/2* dysfunction rate of 40% (28/70) in high-grade serous tubal/ovarian cancer (HGSC), including 14.3% *BRCA1* methylation (n=10), 7.1% *BRCA1* mutation (n=5), and 18.6% *BRCA2* mutation (n=13). Defects in *BRCA1/2* genes were associated with stage III/IV HGSC (*BRCA1* methylation: $P=0.005$ [stage III/IV] and $P=0.004$ [HGSC]; *BRCA1/2* mutation: $P=0.03$ [stage III/IV] and $P<0.001$ [HGSC]). Patients with *BRCA1/2*-mutated cancers showed improved OS (hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.43–0.99; $P=0.045$) and a trend toward improved PFI (HR, 0.48; 95% CI, 0.22–1.06; $P=0.07$) and PFS (HR, 0.72; 95% CI, 0.51–1.03; $P=0.07$). No survival differences were observed between *BRCA1*-methylated and *BRCA1/2* wild-type non-*BRCA1*-methylated cancers.

Conclusion

We observed a high tumor *BRCA1/2* dysfunction rate in HGSC with a unique predominance of *BRCA2* over *BRCA1* mutations. While *BRCA1/2* mutations conferred survival benefits in OC, no such association was observed with *BRCA1* methylation.

Keywords: Ovarian cancer; *BRCA1* methylation; *BRCA1* mutation; *BRCA2* mutation

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Introduction

Poly (ADP-ribose) polymerase inhibitors (PARPi) exhibit potent activity in germline *BRCA1/2*-mutated platinum-sensitive relapsed high-grade serous tubal/ovarian cancer (HGSC). In phase III clinical trials, maintenance therapy with PARPi was associated with a 73% reduction in the risk for disease progression or death as compared to placebo [1]. PARPi target the homologous recombination DNA repair defect (HRD) conferred by *BRCA1/2* mutations, leading to tumor genomic instability and cell death. While germline *BRCA1/2* mutations are detected in 15% HGSCs, genomic and functional data suggest the presence of HRD in approximately 50% HGSC [2]. The identification and validation of other HRD-associated biomarkers in sporadic tubal/ovarian cancer (OC) (hereafter referred to as OC) are crucial to potentially expand the number of women with OC who could benefit from DNA repair-targeting agents such as PARPi.

Somatic *BRCA1/2* mutations have been identified in 4–6.4% of HGSCs, wherein they account for 14.2% of HRD cases [2,3]. Evidence suggests that the clinical benefit from PARPi in patients with somatic *BRCA1/2*-mutated HGSC is similar to that observed in those with germline *BRCA1/2*-mutated disease. In the phase III NOVA clinical trial, 19.7% ($n=40$) of *BRCA1/2* mutations were classified as somatic [1]. The median progression-free survival (PFS) associated with niraparib as compared to that with placebo (20.9 vs. 11 months, hazard ratio [HR], 0.27; 95% confidence interval [CI], 0.08–0.9; $P=0.02$) in this subgroup was consistent with the value reported for patients with germline *BRCA1/2*-mutated disease (21 vs. 5.5 months, HR, 0.27; 95% CI, 0.17–0.41; $P<0.001$) [1].

BRCA1 promoter methylation has been identified as a potential biomarker of response to the PARPi rucaparib [4]. *BRCA1*-methylated tumors are negative for *BRCA1* gene and protein expression, suggestive of a resultant HRD phenotype [5,6]. In addition, *BRCA1*-mutated and *BRCA1*-methylated OCs display similar gene signatures, as detected using gene expression and copy number analyses [7]. In the phase II open label ARIEL-2 study, 12/19 (63%) relapsed platinum-sensitive *BRCA1*-methylated OC patients responded to rucaparib as compared to an 80% response rate reported in *BRCA1/2*-mutated OC patients [4]. This early data suggest the potential role of *BRCA1* methylation as a biomarker of response to PARPi.

Considering the benefit of PARPi in *BRCA1/2* dysfunctional OC and the ongoing development of other agents targeting DNA repair, the knowledge of the prevalence and pattern of *BRCA1/2* gene aberrations within an OC population is imperative. The use of tumor tissues offers the advantage of identifying additional potential somatic biomarkers of response to PARPi as compared to germline mutation testing alone. This information may serve as a guide to drug approval strategies for novel DNA repair targeting drugs at a national level, as the distribution of *BRCA1/2* mutations varies between populations [8]. In Ireland, the frequency of *BRCA1/2* gene aberrations in OC is yet to be examined. At the time of this study, genetic testing for *BRCA1/2* mutations in OC in Ireland was carried out on the basis of clinical risk algorithms in a clinician-dependent manner.

Here, we sought to assess the *BRCA1/2* gene profile in a cohort of Irish women with OC by determining the frequencies of *BRCA1/2* mutations and *BRCA1* methylation in tumors and their association with clinical characteristics and survival.

Materials and methods

1. Sample and data collection

We selected 111 patients with OC treated at 2 university teaching hospitals (including a national tertiary referral gynecologic oncology unit) between 2005 and 2013. All histological subtypes, stages, and grades were included to allow accurate assessment of *BRCA1/2*-mutated and *BRCA1*-methylated profiles. Borderline tumors were excluded. In total, 100 patients were retrospectively included from a prospective clinically annotated Discovery bioresource (St. James's Hospital) after receiving ethical approval for this study (reference 2009/29/01). Patients provided written informed consents prior to specimen collection. Within this bioresource, all patients with epithelial OC with available and adequate tumor tissues (>30% neoplastic cell content [NCC]) were included. Eleven samples were obtained from the Beaumont Hospital Pathology Department after receiving approval from the hospital's ethics committee (REC reference 12/02). Clinical data for these patients were retrospectively obtained through medical records. Patients recruited through both bioresources presented either to the outpatient department or as direct inpatient referrals. All survival data were updated to February 15, 2017. A pathologist specializing in gynecological cancers

reviewed fresh-frozen paraffin-embedded (FFPE) tumor specimens for histology and NCC (as per the 2014 World Health Organization Classification). All specimens were obtained prior to chemotherapy (either at primary debulking surgery or peritoneal biopsy). Specimens with less than 30% NCC (n=20) were macrodissected prior to DNA extraction. The majority of the specimens had over 60% NCC.

2. Assessment of tumor *BRCA1/2* defects

The DNA was extracted from FFPE tumor samples using the QIAamp DNA FFPE Tissue kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol, and quantified using the dsDNA BR assay kit (Qubit, London, UK) as per the manufacturer's instructions.

BRCA1 methylation status was assessed using the Methyl-Profiler DNA Methylation polymerase chain reaction (PCR) Array System (SABiosciences, Valencia, CA, USA) following the manufacturer's protocol. In brief, DNA methylation-sensitive and methylation-dependent restriction enzymes were used to selectively digest non-methylated or methylated genomic DNA, respectively. After digestion, DNA samples were subjected to real-time PCR using primers flanking the regions of interest. The relative concentrations of differentially methylated DNA were determined by comparing the amount of each digest with that of a mock digest. A cutoff value of 10% methylation was used to define the methylation status of samples.

BRCA1 and *BRCA2* genes were sequenced using the Tumor BRACAnalysis CDx assay (Myriad Genetics, Munich, Germany and Salt Lake City, UT, USA), as previously described [9]. Only deleterious or suspected deleterious mutations were included in analyses (as per the previously defined criteria [10]). Germline or somatic mutation status was not assessed, owing to the restrictions imposed by patients' informed consent.

3. Statistical analysis

Statistical analysis was performed using SPSS® version 21.0 software. *BRCA1/2* mutations and *BRCA1* methylation were associated to the following variables: patient age, histology, International Federation of Gynecology and Obstetrics (FIGO) stage, degree of surgical cytoreduction, and platinum sensitivity using the Fisher's exact test. Survival analyses were carried out for platinum-free interval (PFI), PFS, and overall survival (OS) to compare patients with *BRCA1/2*-mutated disease or *BRCA1*-methylated disease with patients carry-

ing *BRCA1/2* wild-type non-*BRCA1*-methylated (hereafter referred to as *BRCA1/2*-intact) tumors. PFI was defined as the interval between completion of chemotherapy and disease recurrence (as defined by the CA125/RECIST criteria), death, or date of last follow-up, whichever occurred first. PFS was defined as the interval between first surgical debulking or diagnostic biopsy (for patients receiving adjuvant or neoadjuvant chemotherapy, respectively) and disease recurrence

Table 1. Patient and disease characteristics

| Parameter | No. of patients (%) |
|-----------------------------------|---------------------|
| Age at diagnosis, median (range) | 59 (23–86) |
| FIGO stage | |
| I | 27 (24.8) |
| II | 13 (11.9) |
| III | 56 (51.4) |
| IV | 13 (11.9) |
| Pathology | |
| Serous | |
| High grade | 70 (64.2) |
| Low grade | 0 |
| Endometrioid | |
| Grade 3 | 3 (2.8) |
| Grade 2 | 11 (10.1) |
| Grade 1 | 3 (2.8) |
| Clear cell | 17 (15.6) |
| Mucinous, grade 1 | 3 (2.8) |
| Other | 2 (1.8) |
| Cytoreduction | |
| Microscopic | 53 (66.2) |
| 0–1 cm | 13 (16.3) |
| ≥1 cm | 14 (17.5) |
| Missing | 29 |
| Platinum sensitivity | |
| Resistant ^{a)} | 25 (23.1) |
| Partially sensitive ^{b)} | 12 (11.1) |
| Sensitive ^{c)} | 55 (50.9) |
| No platinum chemotherapy | 16 (14.8) |
| Missing | 1 |

Percentages reflect percentage of total non-missing data.

FIGO, International Federation of Gynecology and Obstetrics; PFI, platinum-free interval.

^{a)}Resistant: PFI less than 6 months; ^{b)}Partially sensitive: PFI between 6–12 months; ^{c)}Sensitive: PFI greater than 12 months.

(as defined by the CA125/RECIST criteria), death, or date of last follow-up, whichever occurred first. OS was defined as the interval between first surgical debulking or diagnostic biopsy (for patients receiving adjuvant or neoadjuvant chemotherapy, respectively) and death from any cause or date of last follow-up, whichever occurred first. All survival estimates were determined using Kaplan-Meier analysis (log-rank test). For all tests, a value of $P < 0.05$ was considered statistically significant. Univariate and multivariate analyses of PFI, PFS, and OS were performed using the Cox proportional hazard regression model, which estimated HR and 95% CI.

Results

1. Patient and disease characteristics

Patient and disease characteristics are listed in Table 1. Two patients were excluded from the analysis, one owing to insufficient tumor DNA and the other who carried both *BRCA2* mutation and *BRCA1* methylation, leaving a total cohort of 109 evaluable patients. The median age of patients at diag-

nosis was 59 years, and 63.3% (n=69) presented with advanced stage disease (FIGO stage III/IV). In total, 64.2% patients (n=70) had HGSC; stage III/IV HGSC comprised 53.2% (n=58) of the cohort, and 78.9% (n=86) and 5.5% (n=6) of patients received adjuvant and neo-adjuvant platinum-based therapy, respectively. None of the patients received PARPi therapy during the course of illness. The first PARPi therapy in Ireland was approved after the end of the follow-up period. Reasons for no primary chemotherapy included stage IA/IB disease (6%, n=7), peri-operative death (3%, n=3), age greater than 80 years old (3%, n=3), and other (3%, n=3). Microscopic surgical debulking (R0) was achieved in 66.2% (n=53/80) of patients with available data (data were missing for 26.6% [n=29] patients).

2. Frequency of *BRCA1/2* aberrations

Methylation analysis revealed 10 tumors with at least 10% *BRCA1* promoter methylation (median, 49.86%; range, 18.11–69.23%). All *BRCA1*-methylated tumors were stage III HGSC, totaling a *BRCA1* methylation rate to 14.3% (n=10/70) in HGSC. Tumor *BRCA1/2* gene sequencing re-

Table 2. Details of *BRCA1/2* mutations identified in the Irish cohort

| Gene | Age | Stage | Exon | Mutation (HGVS cDNA) | Protein (HGVS protein) | Mutation type |
|--------------|-----|-------|------|----------------------|------------------------|-----------------------------|
| <i>BRCA1</i> | 50 | 3 | 10 | c.1808C>A | p.Ser603* | Nonsense |
| | 37 | 3 | 11 | c.2418del | p.Ala807Hisfs*8 | Frameshift |
| | 40 | 3 | 11 | c.962G>A | p.Trp321* | Nonsense |
| | 65 | 3 | 3 | del exon 3 | | Large genomic rearrangement |
| | 49 | 4 | 2 | c.68_69del | p.Glu23Valfs*17 | Frameshift |
| <i>BRCA2</i> | 57 | 3 | 10 | c.1310_1313del | p.Lys437Ilefs*22 | Frameshift |
| | 57 | 3 | 11 | c.3570del | p.Lys1191Serfs*6 | Frameshift |
| | 55 | 3 | 11 | c.3717del | p.Lys1239Asnfs*20 | Frameshift |
| | 61 | 3 | 11 | c.4638del | p.Phe1546Leufs*22 | Frameshift |
| | 66 | 2 | 11 | c.4712_4713del | p.Glu1571Glyfs*3 | Frameshift |
| | 59 | 3 | 11 | c.5073dupA | p.Trp1692Metfs*3 | Frameshift |
| | 71 | 1 | 11 | c.5101C>T | p.Gln1701* | Nonsense |
| | 49 | 1 | 11 | c.6486_6489del | p.Lys2162Asnfs*5 | Frameshift |
| | 74 | 3 | 11 | c.6486_6489del | p.Lys2162Asnfs*5 | Frameshift |
| | 53 | 3 | 11 | c.6486_6489del | p.Lys2162Asnfs*5 | Frameshift |
| | 43 | 3 | 11 | c.6486_6489del | p.Lys2162Asnfs*5 | Frameshift |
| | 54 | 3 | 2 | c.19G>T | p.Glut7* | Nonsense |
| | 55 | 4 | 7 | c.631+1G>A | Unknown | Splice variant |

HGVS, Human Genome Variation Society.

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vealed 18 pathogenic mutations (5 *BRCA1* and 13 *BRCA2*) with an overall tumor *BRCA1/2* mutation rate of 16.5% (n=18/109). No individuals were identified to carry more than a single tumor mutation, giving credence to each reported deleterious or suspected deleterious mutation. All mutations were identified in HGSC, and the combined germline and somatic *BRCA1/2* mutation rate in HGSC was 25.7% (18/70). *BRCA1* mutations were only observed in stage III/IV disease, while 3 *BRCA2* mutations occurred in stage I/II cancers. In

total, 16 of 18 mutations were classified as pathogenic as per the CLINVAR database [11], and 15 of 18 were curated as per the ENIGMA consortium [12]. *BRCA1* mutations comprised one large genomic rearrangement, 2 frameshift, and 2 nonsense mutations, of which the c.1808C>A mutation has not been previously reported. The common *BRCA1* Ashkenazi Jewish founder mutation c68_69del (also been reported as a separate British founder mutation [13,14]) was identified in one patient of unknown ethnicity. Most *BRCA2*

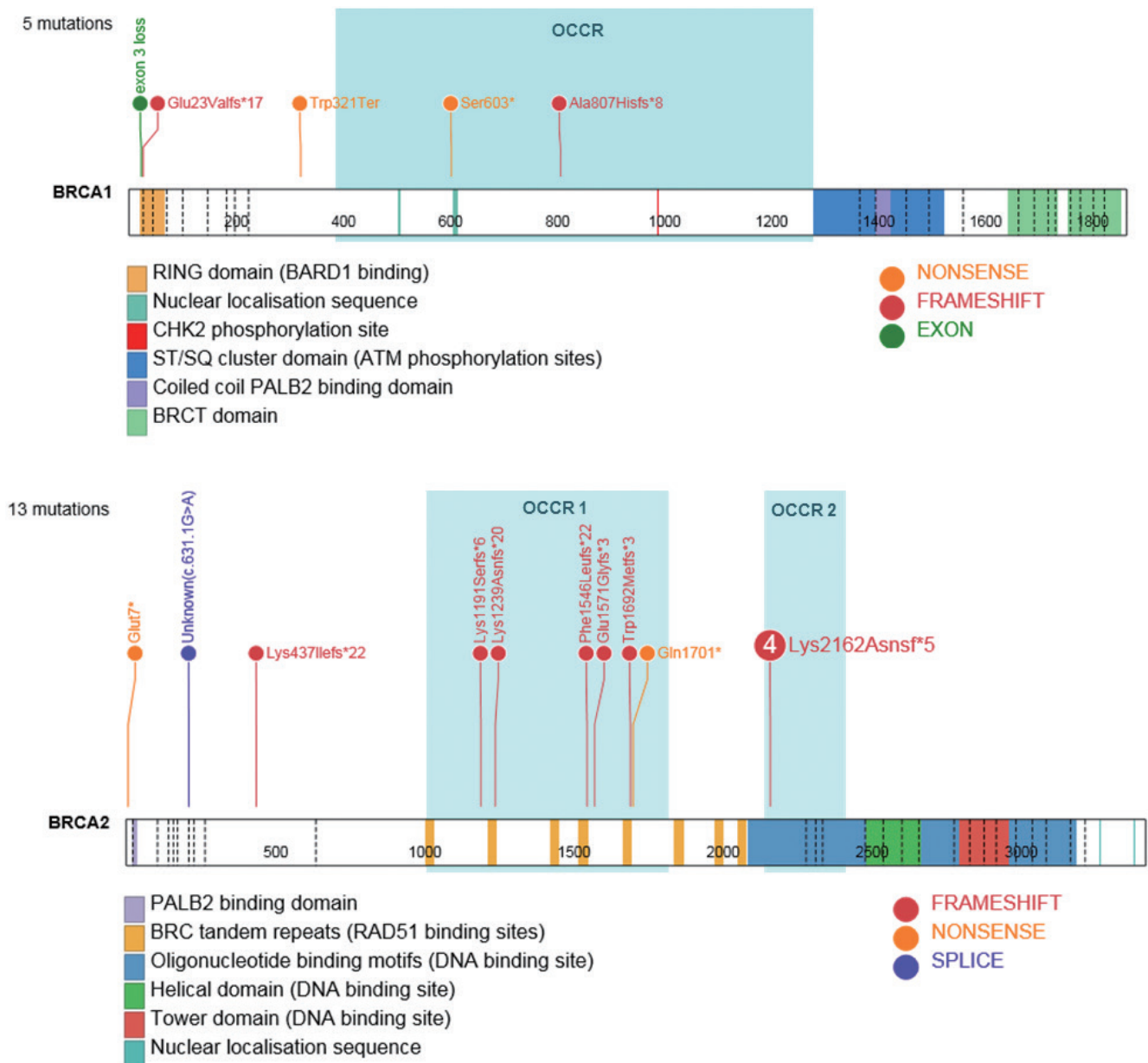


Fig. 1. Localization of the identified *BRCA1/2* mutations in *BRCA1/2* proteins. Numbers on the protein graph correspond to amino acid locations; dashed lines delineate exons. Figure created using ProteinPaint software [16]. OCCR, ovarian cancer cluster region.

mutations were frameshift mutations except for a previously unreported nonsense mutation, c.19G>T, and one splice variant, c.631+1G>A, which were thought to result in abnormal mRNA splicing. Biochemical analysis revealed a similar mutation at this splice donor site that was found to be deleterious by Myriad Genetics laboratories. The *BRCA2* mutation c.6486_6489del was identified in 4 samples from unrelated patients, thereby accounting for 31% of all *BRCA2* mutations. This mutation has been previously reported as a germline variant in hereditary breast OC syndrome and observed in multiple ethnicities. Overall, 76.9% (10/13) of *BRCA2* mutations were located in the RAD51-binding domain (exon 11), which is essential for homologous recombination DNA repair [15]. Three variants of unknown significance (2.7%) were identified in 2 patients (Table 2; Fig. 1 and [16]).

3. Association of patient and disease characteristics with *BRCA1/2* gene aberrations

Using the Fisher's exact test, *BRCA1/2* mutations were found to be significantly associated with stage III/IV disease ($P=0.03$)

and HGSC ($P<0.001$). We failed to identify any association with younger age or platinum sensitivity. This is potentially owing to the small number and unknown germline/somatic status of *BRCA1/2* mutations in our cohort. *BRCA1* methylation also significantly differed between HGSC and non-HGSC ($P=0.004$). It was observed in 22.7% of HGSC but not detected among other OC subtypes, which comprised 34% of the entire cohort. Moreover, *BRCA1*-methylated OC was associated with FIGO stage III/IV disease ($P=0.005$). No significant correlation was identified between *BRCA1* methylation and platinum sensitivity or other clinical variables (Table 3).

4. Survival analyses

BRCA1/2 aberrations were identified in FIGO stage III/IV HGSCs, with the exception of 3 *BRCA2* mutations (FIGO stage I/II disease). Survival analyses were restricted to FIGO stage III/IV HGSC (n=58) to minimize the bias of low stage and grade in the *BRCA1/2* intact arm, thus allowing a more accurate assessment of the survival impact of *BRCA1/2* aberrations. After a median follow-up of 3.8 (range, 0–11.5) years, pa-

Table 3. Correlation between tumour *BRCA1/2* defects and clinico-pathological factors

| Parameter | Non mut/meth (n=81) | <i>BRCA1</i> meth (n=10) | <i>BRCA1/2</i> mut (n=18) | P-value | |
|-----------------------|------------------------|-----------------------------|------------------------------|---------------------------------------|--|
| | | | | <i>BRCA1</i> meth vs. non mut/meth | <i>BRCA1/2</i> mut vs. non mut/meth |
| Age | | | | | |
| <59 | 33 (40.7) | 6 (60) | 12 (66.7) | 0.320 | 0.070 |
| ≥59 | 48 (58.6) | 4 (40) | 6 (33.3) | | |
| FIGO stage | | | | 0.005 | 0.030 |
| I-II | 37 (45.7) | 0 (0) | 3 (16.7) | | |
| III-IV | 44 (54.3) | 10 (100) | 15 (83.3) | | |
| Pathology | | | | 0.004 | <0.001 |
| High grade serous | 42 (51.9) | 10 (100) | 18 (100) | | |
| Non-high grade serous | 39 (47.6) | 0 (0) | 0 (0) | | |
| Cytoreduction | | | | 1.000 | 0.440 |
| Macro <1 cm | 47 (58) | 7 (70) | 12 (66.7) | | |
| Macro ≥1 cm | 11 (13.6) | 2 (20) | 1 (5.5) | | |
| Missing | 23 (28.4) | 1 (10) | 5 (27.8) | | |
| Platinum sensitivity | | | | 0.150 | 0.750 |
| PFI <6 mon | 17 (21) | 5 (50) | 3 (16.7) | | |
| PFI ≥6 mon | 48 (59.3) | 5 (50) | 13 (72.2) | | |
| No chemo/missing | 16 (19.8) | 0 (0) | 2 (11.1) | | |

FIGO, International Federation of Gynecology and Obstetrics; Non mut/meth, *BRCA1/2* wild type non-*BRCA1*-methylated; *BRCA1* meth, *BRCA1*-methylated; *BRCA1/2* mut, *BRCA1/2*-mutated; Macro, macroscopic residual disease; PFI, platinum-free interval.

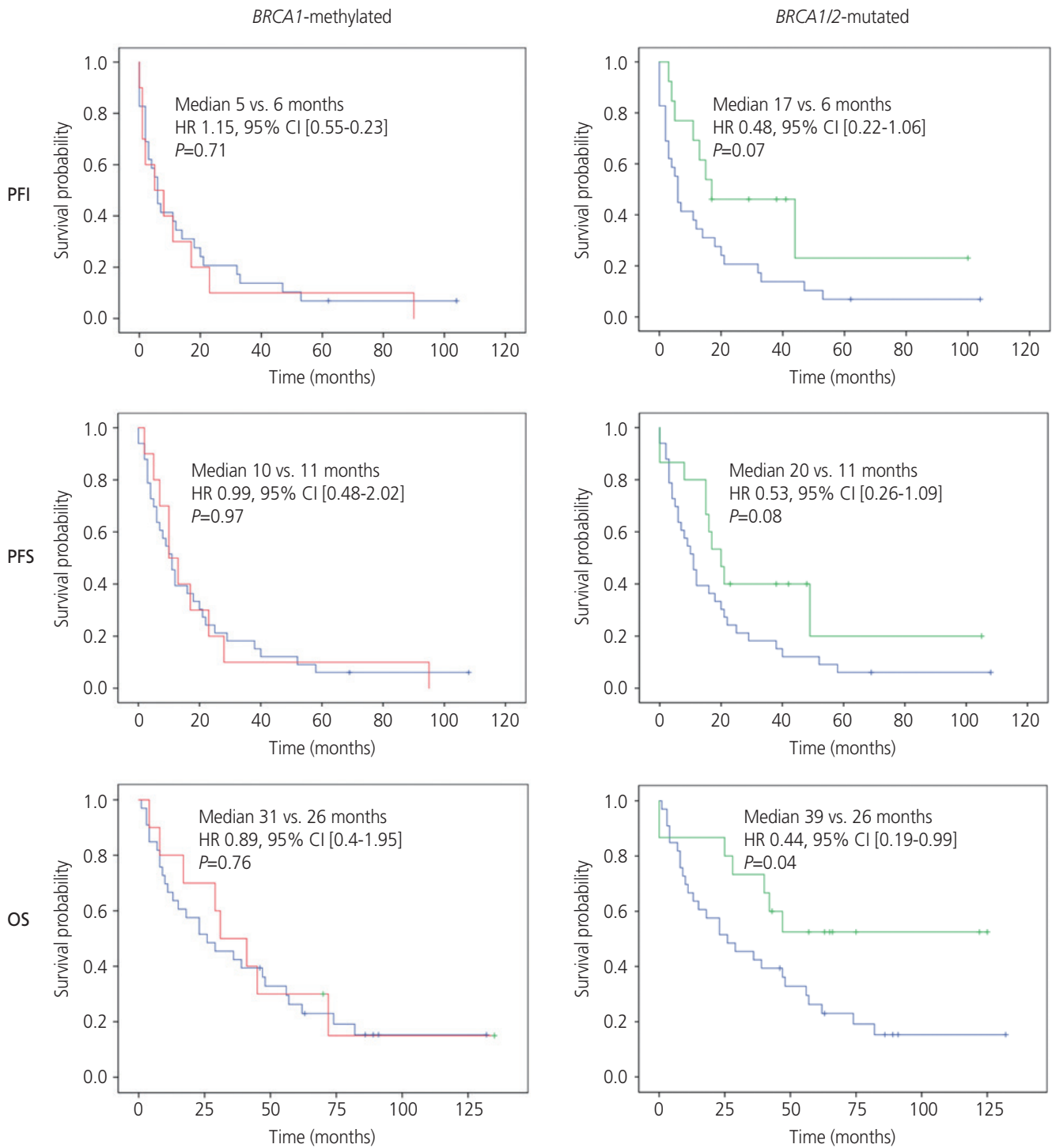


Fig. 2. Survival analyses according to tumor-specific *BRCA1/2* defect. Platinum-free interval (PFI), progression-free survival (PFS), and overall survival (OS) of patients with International Federation of Gynecology and Obstetrics (FIGO) stage III and IV high-grade serous tubal/ovarian cancer (HGSC). Comparison of patients with *BRCA1*-methylated HGSC and those with *BRCA1/2*-mutated HGSC to patients with *BRCA1/2* wild-type non-*BRCA1*-methylated HGSC. In all graphs, blue curves indicate non-*BRCA1*-methylated; red curves indicate *BRCA1*-methylated; and green curves indicate *BRCA1/2*-mutated. HR, hazard ratio; CI, confidence interval.

tients with *BRCA1/2*-mutated tumors showed a trend toward improved PFI and PFS as compared to those with *BRCA1/2*-intact tumors (median survival: 17 vs. 6 months [HR, 0.48; 95% CI, 0.22–1.06; $P=0.07$] and 20 vs. 11 months [HR, 0.53; 95% CI, 0.26–1.09; $P=0.08$], respectively). The lack of expected statistical significance likely relates to the small sample size. OS significantly improved in patients with *BRCA1/2*-mutated tumors (median survival of 39 months) as compared to that in patients with *BRCA1/2*-intact disease (median survival of 26 months) (HR, 0.44; 95% CI, 0.19–0.99; $P=0.045$). No difference in survival was identified between the *BRCA1*-methylated and *BRCA1/2*-intact groups, as evident from the estimated median survivals of 5 vs. 6 months (HR, 1.15; 95% CI, 0.55–2.38; $P=0.71$), 10 vs. 11 months (HR, 0.99; 95% CI, 0.48–2.02; $P=0.97$), and 31 vs. 26 months (HR, 0.89; 95% CI, 0.40–1.95; $P=0.76$) for PFI, PFS, and OS, respectively (Fig. 2). After adjustment for residual disease in the multivariate analysis, *BRCA1/2*-mutated OC lost statistical significance with respect to improved OS (though the trend was similar), while the associations between *BRCA1* methylation and PFI, PFS, and OS failed to show any significant change (Table 4).

Discussion

This is the first study to assess the prevalence of *BRCA1/2* aberrations in Irish patients with OC. We found an overall *BRCA1/2* dysfunction rate of 25.7% (9.2% *BRCA1*-methylated and 16.5% *BRCA1/2*-mutated tumors). All cases were observed in HGSC, which comprised 64.2% of the study popu-

lation. Within this subgroup, 14.3% of tumors were *BRCA1*-methylated and 25.7% were *BRCA1/2*-mutated, making an overall *BRCA1/2* dysfunction rate of 40% in HGSC. Our findings are in line with those of other large studies, which reported germline/somatic *BRCA1/2* mutation and *BRCA1* methylation rates in the range of 19–27% and 10.5–14%, respectively, in HGSC [2,3,9,17]. Considering the therapeutic benefits of PARPi in *BRCA1/2*-mutated HGSC, and possibly in *BRCA1*-methylated HGSC [4], this degree of *BRCA1/2* dysfunction within the most aggressive and lethal subtype of OC reinforces the crucial need outlined in the recent international guidelines to routinely test germline *BRCA1/2* mutation status in patients with non-mucinous OC [18]. Testing FFPE tumor specimens for *BRCA1/2* mutations using next-generation sequencing (NGS) may allow rapid analysis using low concentrations of DNA samples, making it a cost-effective approach. Tumor DNA sequencing differs from germline DNA sequencing owing to tumor heterogeneity and the risk of nucleic acid degradation during paraffin embedding process. As a result, concerns exist in using tumor *BRCA1/2* mutation testing followed by germline testing of mutation-positive cases to comprehensively detect germline *BRCA1/2* mutations. Our study was restricted in terms of testing the germline/somatic status of the identified mutations from tumor DNA. However, the tumor BRACAnalysis CDx test used in this study has been validated in different cohorts of HGSC FFPE specimens with matched blood samples. Upon application to FFPE specimens corresponding to each blood sample, this test correctly identifies all cases of germline-mutated *BRCA1/2* HGSC in addition to 8.7% cases of so-

Table 4. Univariate and multivariate analyses for platinum-free interval (PFI), progression-free survival (PFS) and overall survival (OS) according to *BRCA1/2* aberrations amongst advanced stage high grade serous ovarian cancers

| Variable | PFI | | PFS | | OS | |
|-----------------------|------------------|---------|------------------|---------|-------------------|---------|
| | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Univariate analyses | | | | | | |
| <i>BRCA1/2</i> mut | 0.48 (0.22–1.06) | 0.070 | 0.53 (0.26–1.09) | 0.080 | 0.44 (0.19–0.99) | 0.050 |
| <i>BRCA1</i> meth | 1.15 (0.55–2.38) | 0.710 | 0.99 (0.48–2.02) | 0.970 | 0.89 (0.40–1.95) | 0.760 |
| Multivariate analyses | | | | | | |
| <i>BRCA1/2</i> mut | 0.42 (0.00–0.97) | 0.040 | 0.52 (0.25–1.09) | 0.080 | 0.55 (0.24–1.29) | 0.170 |
| <i>BRCA1</i> meth | 1.10 (0.52–2.35) | 0.810 | 1.00 (0.47–2.11) | 1.000 | 0.89 (0.39–2.05) | 0.790 |
| Residual disease | 2.75 (1.13–6.70) | 0.030 | 2.57 (1.15–5.75) | 0.020 | 4.31 (1.83–10.20) | 0.001 |

The residual disease variable within the model is binary as follows: 0: <1 cm residual disease at surgical cytoreduction, 1: ≥1 cm residual disease at surgical cytoreduction.

BRCA1/2 mut, *BRCA1/2*-mutated; *BRCA1* meth, *BRCA1*-methylated.

matic *BRCA1/2* mutations [19]. Other reports using different *BRCA1/2* panels and NGS platforms, where both germline and tumor tissues were available for analysis, have shown a discordance rate of $\leq 3\%$ between tumor and blood-based testing for *BRCA1/2* germline mutation [20]. Moreover, upfront tumor *BRCA1/2* mutation followed by reflex germline mutation testing in mutation-positive patients may serve as a more cost-effective strategy than upfront germline mutation testing followed by subsequent tumor mutation testing in germline mutation-negative cases. Finally, the availability of tumor DNA allows *BRCA1* methylation testing, further reinforcing the potential greater utility of tumor tissues in detecting therapeutic targets beyond germline *BRCA1/2* mutations in a single test. However, further studies are warranted to determine the potential of *BRCA1* methylation, in contrast to germline/somatic *BRCA1/2* mutations, as a plausible therapeutic target.

In our study, *BRCA1* methylation, like *BRCA1/2* mutation, was associated with advanced stage HGSC. We failed to observe any association with younger age at diagnosis, contradicting the previous reports [17]. *BRCA1* methylation decreased *BRCA1* mRNA and protein expression in OC [5,6], suggestive of the sensitivity of HRD to platinum chemotherapy and PARPi. *In vitro*, *BRCA1*-methylated breast/OC cell lines demonstrate high sensitivity to cisplatin and olaparib as compared to *BRCA1/2*-intact cell lines [21,22]. We failed to translate these findings in the clinic, consistent with no survival difference between *BRCA1*-methylated OC and *BRCA1/2*-intact OC. Several large studies corroborate our observations [2,17], while others report a negative prognostic effect of *BRCA1* methylation on survival [23]. Nevertheless, a few small studies have reported the superior platinum response and improved PFS amongst *BRCA1*-methylated tumors [24,25]. A larger study involving 213 patients with OC demonstrated similar values of HR for OS in germline *BRCA1*-mutated and *BRCA1*-methylated disease (HR, 0.88; 95% CI, 0.64–1.24 and HR, 0.89; 95% CI, 0.60–1.30, respectively; each group was compared to a *BRCA1/2*-intact population) [26]. Data regarding clinical responses of *BRCA1*-methylated OC to PARPi are limited to the ARIEL-2 study results, which reported a promising response rate of 63% in *BRCA1*-methylated tumors (n=12/19) [4]. These conflicting results are likely related to sample size and heterogeneity within *BRCA1*-methylated OC, as observed with *BRCA1*-mutated OC. While *BRCA2*-mutated OC consistently shows

significant survival benefits, some reports revealed no survival difference between *BRCA1*-mutated OC and *BRCA1/2* wild-type OC [27]. The survival benefit conferred by *BRCA1* mutations may be potentially of lesser magnitude or diluted by the heterogeneous effect of different *BRCA1* mutations [28] and mono- or biallelic *BRCA1* mutations [29] on homologous recombination, thereby necessitating large cohorts to confirm this benefit [30]. Further, a significantly larger cohort of *BRCA1*-methylated OC would be necessary to detect survival benefits, if any.

The clinicopathological associations of *BRCA1/2*-mutated disease observed in the present study are similar to those previously reported. *BRCA1/2* mutations were solely detected in HGSC, were associated with improved OS, and showed a trend toward significantly better PFI and PFS. The small sample size of our study limits the strength of survival analyses. A single *BRCA2* mutation, c6486_6489del, accounted for 22% of all mutations detected. This is a known pathogenic germline mutation associated with hereditary breast OC syndrome. No tumor carried the *BRCA1* c.2681_2682delAA variant, a founder mutation originating from Irish/West Scottish Celts [31]. We observed the predominance of *BRCA2* mutations, with a *BRCA2:BRCA1* mutation ratio of 2.6:1. In Caucasian HGSC cohorts, germline *BRCA1* mutations were found to be 1 to 3 times more frequent than *BRCA2* mutations [2,3,32]. *BRCA1* mutations have higher penetrance and confer a 36–53% lifetime OC risk as compared to an estimated 11–25% lifetime risk with *BRCA2* mutations [33]. The small sample size of our study may possibly lead to biased results. However, the heterozygote population distribution of *BRCA1/2* mutations varies worldwide. An analysis of the Exome Aggregation Consortium and Exome Variant Server databases demonstrates a high frequency heterozygote *BRCA2* germline mutations in some populations, with some populations having a very low rate of *BRCA1* mutation carriers [8]. A very low frequency of *BRCA1* heterozygote population could potentially explain our findings, though this cannot be verified in the absence of the frequency of *BRCA1/2* mutation in Irish population. Two publications had reported a relatively higher *BRCA2:BRCA1* mutation ratio [34,35], including a 2.7:1 ratio of *BRCA2:BRCA1* mutations amongst 120 patients with non-mucinous OC undergoing routine *BRCA1/2* germline mutation testing. Interestingly, the reports originate from Scotland/Northern Ireland and West Scotland. These populations share a common Celt genetic

ancestry with the Irish population, thereby reinforcing the likelihood that our findings could be representative of the distribution of *BRCA1/2* mutations amongst Irish patients with HGSC. Drawing such conclusions is however limited in our study by ethical constraints to perform germline testing on the identified tumor mutations to determine their germline/somatic status. As these reported mutations occurred singly in individuals and were classified as deleterious or suspected deleterious, a predominance of *BRCA2* mutations has therapeutic and prognostic implications for patients because 76.9% of *BRCA2* mutations identified were located in the RAD51-binding domain of the gene. These mutations are associated with improved PFS and OS in contrast to those located outside this domain [15].

In conclusion, we observed a *BRCA1/2* dysfunction rate of 40% within the Irish HGSC population, owing to *BRCA1/2* mutations and *BRCA1* promoter methylation in tumors; we noted a unique predominance of *BRCA2* over *BRCA1* mutations. This observation reinforces the need for routine *BRCA1/2* germline and somatic testing to facilitate therapy selection (PARPi and other forthcoming DNA repair targeting agents) and cancer prevention for germline mutation carriers and their relatives. A better understanding of the clinical and therapeutic relevance of *BRCA1* methylation in OC is needed, given its potential to expand the therapeutic benefits of DNA repair targeted agents to a larger number of women with OC.

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Conflict of interest

Roshni D. Kalachand has received conference travel fees from Astra Zeneca. Kirsten M. Timms is an employee of and may hold shares in Myriad Genetics Inc. The other authors have no conflicts of interest to declare.

Ethical approval

Samples and patient data obtained from the Discovery biore-source (St James's Hospital) received approval from the hospital's ethics committee (reference 2009/29/01).

Samples and patient data obtained from the Beaumont Hospital Pathology Department received approval from the hospital's ethics committee (REC reference 12/02).

Patient consent

The patients provided written informed consent for the research carried out within this publication.

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