Clinical Cancer Research

# Efficacy of Chemotherapy in *BRCA1/2* Mutation Carrier Ovarian Cancer in the Setting of PARP Inhibitor Resistance: A Multi-Institutional Study

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## Abstract

**Purpose:** Preclinical data suggest that exposure to PARP inhibitors (PARPi) may compromise benefit to subsequent chemotherapy, particularly platinum-based regimens, in patients with *BRCA1/2* mutation carrier ovarian cancer (PBMCOC), possibly through the acquisition of secondary *BRCA1/2* mutations. The efficacy of chemotherapy in the PARPi-resistant setting was therefore investigated.

**Experimental Design:** We conducted a retrospective review of PBMCOC who received chemotherapy following disease progression on olaparib, administered at ≥200 mg twice daily for one month or more. Tumor samples were obtained in the post-olaparib setting where feasible and analyzed by massively parallel sequencing.

**Results:** Data were collected from 89 patients who received a median of 3 (range 1–11) lines of pre-olaparib chemotherapy. The overall objective response rate (ORR) to post-olaparib chemotherapy was 36% (24 of 67 patients) by Response Evaluation Criteria in Solid Tumors (RECIST) and 45% (35 of 78) by RECIST and/or Gynecologic Cancer InterGroup (GCIG) CA125 criteria with median progression-free survival (PFS) and overall survival (OS) of 17 weeks [95% confidence interval (CI), 13–21] and 34 weeks (95% CI, 26–42), respectively. For patients receiving platinum-based chemotherapy, ORRs were 40% (19 of 48) and 49% (26/53), respectively, with a median PFS of 22 weeks (95% CI, 15–29) and OS of 45 weeks (95% CI, 15–75). An increased platinum-to-platinum interval was associated with an increased OS and likelihood of response following post-olaparib platinum. No evidence of secondary *BRCA1/2* mutation was detected in tumor samples of six PARPi-resistant patients [estimated frequency of such mutations adjusted for sample size: 0.125 (95%-CI: 0–0.375)].

**Conclusions:** Heavily pretreated PBMCOC who are PARPi-resistant retain the potential to respond to subsequent chemotherapy, including platinum-based agents. These data support the further development of PARPi in PBMCOC. *Clin Cancer Res; 19(19); 5485–93. ©2013 AACR.*

## Introduction

The observation that *BRCA1/2*-deficient cells were exquisitely sensitive to PARP inhibitors (PARPi; ref. 1, 2) led to the clinical testing of this synthetic lethal approach. The proof-of-concept antitumor strategy of using single-agent PARP inhibition in *BRCA1* and *BRCA2 (BRCA1/2)*-mutated tumors has now been validated by emerging safety and efficacy data from phase I–II clinical trials (3–6). Olaparib (AZD2281, KU-0059436) is a selective and potent inhibitor of PARP1/2 with *in vitro* potency in the nanomolar

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## Translational Relevance

This study provides support for the lack of significant clinical cross-resistance between PARP inhibitors (PARPi), a promising class of novel targeted agents, and platinum-based chemotherapy, the current standard-of-care, in patients with ovarian cancer with germline *BRCA1/2* mutations. First, high-response rates to chemotherapy in the PARPi-resistant setting were elicited in heavily pretreated patients. Second, the platinum-free interval remained prognostic of overall survival and predictive of response to platinum-based regimens in the PARPi-resistant setting despite the interim use of olaparib. Secondary *BRCA1/2* mutations have been proposed as a common mechanism of resistance to PARPi; however, these were not detected in a series of tumor samples in this study using massively parallel sequencing. This indicates that other resistance mechanisms are likely to be important and merit further investigations. These novel findings provide support for the continued development of PARPi in patients with *BRCA1/2*-associated ovarian cancer.

range and was the first oral PARPi to be used in this context. The efficacy of olaparib was confirmed in a randomized trial in *BRCA1/2* mutation-positive patients with platinum-resistant/partially sensitive relapsed epithelial ovarian cancer (EOC; ref. 7). This study also showed an unexpectedly high level of efficacy for pegylated liposomal doxorubicin (PLD) in these patients (7).

The incidence of *BRCA1/2* mutations in high-grade serous EOC is estimated at 15% to 18% irrespective of family history. Furthermore, up to 50% of patients with this commonest EOC histologic subtype may have other genetic changes that also lead to homologous recombination deficiency (8–10). The potential for these other mutations to phenocopy the effects of *BRCA1/2* mutations has led to the assessment of PARPi monotherapy in patients with sporadic high-grade serous EOC (negative for germline *BRCA1/2* mutations). In this patient group, clinical responses to single-agent olaparib have been confirmed in patients with platinum-sensitive relapsed disease (11). A placebo-controlled trial conducted in patients with platinum-sensitive, relapsed high-grade serous EOC (germline *BRCA1/2* mutation status unknown in 64%, positive in 22%, and negative in 14% of patients) subsequently assessed the use of single-agent olaparib as maintenance therapy. Treatment with olaparib significantly improved progression free survival (PFS) compared with placebo (8.4 months versus 4.8 months) with a striking HR of 0.35 (95%-CI, 0.25–0.49). However, this effect on PFS did not translate into an overall survival (OS) difference in an interim analysis (12). Possible contributory factors to this lack of OS difference include the genetic admixture of patients with or without germline *BRCA1/2* mutations and the reduction of the likelihood of response to subsequent chemotherapy following olaparib treatment. The latter would be consistent with data from preclinical models, which have predicted the development of cross-resistance between PARPi and platinum-based therapies through the acquisition of secondary mutations that restore the *BRCA1/2* open reading frame and functional protein expression (13–15). Secondary *BRCA1/2* mutations have been associated with clinical platinum resistance and their incidence is reportedly high at more than 46% in patients with platinum-resistant EOC (16). Our previous data additionally suggest a correlation between prior platinum sensitivity and antitumor response to PARPi in patients with *BRCA1/2* deficiencies (5). However, there are as yet no published clinical data about the use of chemotherapy, particularly platinum-based regimens, in the PARPi-resistant setting.

The primary objective of this study was therefore to provide the first clinical evaluation of chemotherapy in patients with *BRCA1/2* mutation-associated EOC following the development of disease progression on olaparib treatment. In addition to assessing objective response rates (ORR), associations between baseline clinical factors with OS and response to treatment with platinum-based chemotherapy in the post-olaparib setting were analyzed. To investigate the hypothesis that PARPi-resistant patients may harbor secondary *BRCA1/2* mutations, tumor samples were obtained wherever feasible in the post-olaparib (PARPi-resistant) setting before chemotherapy and were examined using massively parallel sequencing.

## Materials and Methods

This is a retrospective study of patients with EOC with germline *BRCA1/2* mutations who received chemotherapy following objective evidence of disease progression on olaparib administered at doses 200 mg or more twice daily for more than a month in eight hospitals worldwide between April 2006 and May 2012. The minimum dose of 200 mg twice a day was selected because this was a pharmacologically active dose showed to inhibit both PARP target and pathway where antitumor responses were observed. This was also the dose used in the phase I clinical trial expansion cohort of patients with *BRCA1/2*-mutated EOC (5). Demonstration of objective evidence of disease progression on olaparib by response evaluation criteria in solid tumors (RECIST) or Gynecologic Cancer InterGroup (GCIG) CA125 criteria was mandatory in these patients and was confirmed by all investigators by site-specific review. Patients without evidence of radiologically measurable disease (using RECIST) were excluded, unless the disease was evaluable using GCIG CA125 criteria (17, 18). Patients who were initially only evaluable using GCIG-CA125 criteria at the start of treatment but assessed using a different CA125 assay during the course of treatment or received intervention to their pleura and/or peritoneum within 28 days before response assessments were excluded from response rate calculations. However, all patients with evaluable disease (by either criterion) were included in the survival analyses. Demographic and

clinicopathologic factors, treatment details, and antitumor responses were recorded. All patients had previously undergone *BRCA1/2* mutation testing following appropriate genetic counseling and informed consent. The study protocol was approved by the Institutional Review Board or independent ethics committee at each site.

Patients were defined as platinum-sensitive, partially sensitive, and resistant if the treatment-free interval was $\geq$12, $\geq$6 and <12, and <6 months, respectively, after their last pre-olaparib platinum-based chemotherapy (19). An additional time interval was calculated, that is, platinum-to-platinum (PTP) interval, which describes the interval from the end of the last pre-olaparib platinum treatment to the start of the first post-olaparib platinum-based chemotherapy. During chemotherapy in the post-olaparib setting, patients had safety evaluations following each treatment cycle and tumor response assessments after every two cycles, as assessed by RECIST criteria, and/or after every cycle with serum CA125 levels using GCIG criteria (17, 18).

### Tumor tissue and DNA extraction

Following patient consent, formalin-fixed paraffin-embedded or fresh-frozen tumor tissue was obtained at surgery for relapsed disease where clinically indicated. Sample anonymization, tissue access, and recording of clinical data were undertaken in accordance with the Human Tissue Act and Multicentre Research Ethics Committee Guidelines. Microdissection was conducted as previously described (20). In two additional cases, tumor cells were isolated from ascites by Dynal bead separation. DNA was extracted using the DNeasy Kit (Qiagen).

### Massively parallel sequencing of *BRCA1*, *BRCA2*, *PTEN*, and *TP53*

Two micrograms of DNA was fragmented to 200 bp fragments using a Covaris E-Series instrument and paired-end libraries were prepared using the SureSelect Target Enrichment System for Illumina Paired-End Sequencing Library Kit (Agilent). The libraries were then hybridized to a custom RNA bait library targeting *BRCA1*, *BRCA2*, *TP53*, and *PTEN* (eArray; Agilent) and quantified using a Bioanalyzer DNA chip (Agilent) and quantitative PCR (qPCR). Final libraries were run on an Illumina HiSeq using a 2 $\times$ 76 basepair paired-end strategy. After quality controls, reads were aligned to the human reference genome (GRCh37) using BWA (21). PCR duplicates and off-target reads were subsequently filtered and variants were called using GATK (22, 23). Bam files were visualized using the Integrated Genome Viewer (IGV, Broad).

### Statistical analysis

Descriptive and inferential statistics were applied to baseline clinical and laboratory data. To account for the limited sample size, the Laplace and adjusted Wald methods were used to derive the conservative point estimate and 95% confidence interval (95% CI; ref. 24), respectively, of the frequency of secondary *BRCA1/2* mutations. The Kaplan–Meier method was used to estimate intervals between milestones along a timeline. PFS and OS following post-PARPi chemotherapy were assessed from first administration of drug. Differences between defined groups of patients were assessed using log-rank (Mantel–Cox) test, multinomial logistic and Cox regression, where appropriate. In the univariate analysis, a $P$ value of 0.10 was adopted as the limit for inclusion in the multivariate analysis; in the latter, $P$ values less than 0.05 were considered significant. All $P$ values presented were two-sided. The SPSS program (Version 16.0) was used for all statistical analysis.

## Results

### Patient, tumor, and treatment characteristics

A total of 89 *BRCA1/2* mutation carriers with EOC were included (Table 1). Sixty-six (74%) patients had germline *BRCA1* mutations, whereas 23 (26%) carried *BRCA2* mutations. These patients had received a median of three lines of chemotherapy before olaparib treatment. The percentages of patients with platinum-resistant, partially sensitive, and sensitive relapses before olaparib exposure were 40%, 43%, and 17%, respectively. The median interval from initial diagnosis to the start of post-olaparib chemotherapy was 47.2 months (95% CI, 38.1–56.3) and that between the commencement of olaparib and subsequent line of systemic chemotherapy was 7.4 months (95% CI, 6.5–8.3).

### Efficacy of chemotherapy in the setting of PARPi resistance

Of 89 patients, 78 had disease measurable by RECIST and/or GCIG CA125 criteria. Chemotherapy regimens used are detailed in Tables 2 and 3; the majority comprised either a platinum and/or taxane chemotherapy and these were administered through weekly, three-weekly, or monthly schedules. Of note, single-agent paclitaxel chemotherapy was administered weekly. The overall ORR with the use of any first-line chemotherapy following olaparib treatment was 36% (24 of 67 patients) by RECIST and 45% (35 of 78 patients) by RECIST and/or GCIG criteria. After a median follow-up of 113 weeks (26.3 months), the overall median PFS was 17 weeks (95% CI, 13–21) and median OS was 34 weeks (95% CI, 26–42). In comparison, the use of platinum-based regimens resulted in ORRs of 40% (19 of 48 patients) by RECIST criteria and 49% (26 of 53 patients) by RECIST and/or GCIG criteria with a median PFS of 22 weeks (95% CI, 15–29) and a median OS of 45 weeks (95%-CI: 15–75). Objective responses to chemotherapy in the post-PARPi setting were observed regardless of pre-PARPi platinum sensitivity (Table 3). Responses were also observed with the use of other chemotherapeutic regimens; the use of taxane and PLD monotherapies were associated with ORRs by RECIST and/or GCIG criteria of 50% (5 of 10 patients) and 30% (3 of 10 patients), respectively (Table 2). The median overall interval between first diagnosis and death was 66.3 months (95% CI, 53.4–79.2)

**Table 1.** Patient, tumor, and treatment characteristics

| Characteristics | Values |
|---|---|
| Total no. of patients | 89 |
| Age (y), median (range) | 51 (31–77) |
| Stage at diagnosis—no. (%) | |
| 1 | 5 (6) |
| 2 | 7 (8) |
| 3 | 63 (71) |
| 4 | 8 (9) |
| Not known | 6 (7) |
| Histology grade—no. (%) | |
| 2 | 13 (15) |
| 3 | 66 (74) |
| Not known | 10 (11) |
| Histology—no. (%) | |
| Serous papillary | 67 (75) |
| Endometrioid | 7 (8) |
| Adenocarcinoma not otherwise specified | 15 (17) |
| Prior no. of lines of systemic chemotherapy (excluding olaparib)—no. (%) | |
| 1 | 13 (15) |
| 2 | 30 (34) |
| 3 | 23 (26) |
| ≥4 | 21 (24) |
| Not known | 2 (2) |
| *BRCA* mutation status—no. (%) | |
| *BRCA1* | 66 (74) |
| *BRCA2* | 23 (26) |
| Optimally debulked—no. (%) | |
| Yes | 48 (54) |
| No | 23 (26) |
| Not known | 18 (20) |
| Previous breast cancer—no. (%) | |
| Yes | 29 (33) |
| Chemotherapy for breast cancer | 11 (38) |
| No | 60 (67) |
| Sensitivity to last pre-PARPi platinum chemotherapy—no. (%) | |
| Sensitive (TFI >12 m) | 15 (17) |
| Partially sensitive (TFI >6 and ≤12 m) | 38 (43) |
| Resistant (TFI <6 m) | 36 (40) |
| Overall response rate to olaparib—no. of responses/no. evaluable (%) | |
| RECIST | 34/86 (40) |
| RECIST and/or CA125 | 41/87 (47) |

with a median interval between first diagnosis and the start of olaparib of 35.8 months (95% CI, 32–40).

### Factors associated with OS following post-olaparib platinum-based chemotherapy

In the univariate analysis (Table 4), factors significantly associated with OS following post-olaparib platinum-based chemotherapy included best response to olaparib by RECIST and/or GCIG criteria [overall $P = 0.031$; stable disease (SD) versus progressive disease (PD); $P = 0.026$; HR = 0.299 (95% CI, 0.103–0.868); partial response (PR)/complete response (CR) versus PD; $P = 0.009$; HR = 0.257 (95% CI, 0.093–0.709) and the PTP interval [$P = 0.018$; HR = 0.960 (95%-CI: 0.928–0.993)]. Interestingly, pre-PARPi platinum-sensitivity [measured by the treatment-free interval (TFI) following last pre-olaparib platinum] was not significantly associated with OS (overall $P = 0.332$).

In the multivariate analysis, the PTP interval remained significant [$P = 0.036$; HR = 0.962 (95%-CI, 0.928–0.997)], whereas the best response (RECIST and/or GCIG) to olaparib showed a trend to significance [overall $P = 0.056$; SD versus PD, $P = 0.027$; HR = 0.301 (95%-CI: 0.104–0.875); PR/CR versus PD, $P = 0.022$; HR = 0.298 (95%-CI: 0.106–0.838)]. These results are summarized in Table 4.

### Predictors of response to post-olaparib platinum-based chemotherapy

The association between post-PARPi platinum-based chemotherapy response (RECIST and/or GCIG) and baseline factors was assessed; these baseline factors included pre-PARPi platinum sensitivity (TFI following last pre-PARPi platinum), best response to olaparib (RECIST and/or GCIG), and the PTP interval (Table 5). The PTP interval was significantly associated with objective response to post-PARPi platinum-based chemotherapy [PR/CR versus PD, $P = 0.045$, OR = 1.12 (95% CI, 1.01–1.26)]. A trend to significance was observed between the PTP interval and the comparison of SD with PD on post-PARPi platinum-based chemotherapy [$P = 0.061$, OR = 1.12 (95% CI, 1.11–1.25)]. By contrast, the interval between last pre-PARPi platinum and start of post-PARPi chemotherapy was not significantly associated with the response to non-platinum regimens (CR/PR versus PD, $P = 0.57$).

### DNA sequencing of clinical samples

A massively parallel sequencing strategy that focused on four genes, *BRCA1*, *BRCA2*, *TP53*, and *PTEN*, was used as described previously (25). For each sample, the 0.1 Mb targeted region was sequenced to a median depth of more than 200 times, and more than 99.9% of target regions were covered sufficiently for confident variant calling (depth >10 reads). In 6 patients with objective evidence of disease progression on treatment with olaparib (Supplementary Table S1), secondary *BRCA1/2* mutations were not observed [conservative estimated frequency adjusted for sample size: 0.125 (95% CI, 0–0.375)]. Coding mutations in *PTEN* were not detected; the coding mutations found in *BRCA1*, *BRCA2*, and *TP53* are summarized in Table 6.

## Discussion

This is the first study to systematically describe the use of chemotherapy in patients with *BRCA1/2*-mutated EOC in the PARPi-resistant setting. Our data indicate that

**Table 2.** Types and efficacies of chemotherapies used in the post-olaparib first-line setting

| | RECIST | | RECIST and/or GCIG | |
|---|---|---|---|---|
| **Regimens** | **Evaluable** | **Responses (%)** | **Evaluable** | **Responses (%)** |
| Platinum-based chemotherapies | 48 | 19 (40) | 53 | 26 (49) |
| Platinum single-agent | 6 | 3 (50) | 6 | 3 (50) |
| Platinum-taxane | 19 | 8 (42) | 22 | 14 (64) |
| Platinum-PLD | 10 | 4 (40) | 11 | 5 (45) |
| Platinum-others | 13 | 4 (31) | 14 | 4 (29) |
| Taxane single-agent | 10 | 4 (40) | 10 | 5 (50) |
| PLD single-agent | 5 | 0 (0) | 10 | 3 (30) |
| Others | 4 | 1 (25) | 5 | 1 (20) |
| **Total** | **67** | **24 (36)** | **78** | **35 (45)** |

patients continue to have the potential to respond to further chemotherapy; the overall ORRs of first-line chemotherapy following PARPi were 36% by RECIST criteria and 45% by RECIST and/or GCIG criteria. For platinum-based regimens, the ORRs were 40% and 49% according to RECIST criteria and RECIST and/or GCIG criteria, respectively. It is noteworthy that similar levels of ORRs were also observed with the use of taxanes and PLD as monotherapies (Tables 2 and 3). Although published data on ORRs are scarce in patients with EOC carrying germline *BRCA1/2* mutations in the third-line (and later) settings, the ORRs observed in this study seem to be in keeping with other phase II data for the same regimens in the general EOC population (26). Overall, these data point to significant differences in the mechanisms of resistance between PARPi and chemotherapy including platinum-based agents.

The exploratory analyses involving predictors of response and OS with the use of post-PARPi platinum-based chemotherapy indicate that both endpoints were significantly associated with the PTP interval despite the use of the putatively cross-resistant olaparib during this time interval. If resistance mechanisms to olaparib and platinum-based agents had been significantly overlapping, we would not have expected to observe either a high ORR to platinum-based regimens (approximately 50% in heavily pretreated patients in the fifth-line setting) or a PTP interval which retained prognostic and predictive values despite the interim use of olaparib. In this context, we note that the use of non-cross–resistant treatments to prolong the platinum-free interval seems to be associated with an increased likelihood of response to later rechallenge with platinum in previous studies (27). These data may also prove useful in planning future clinical trials and in the selection of therapy, that is, platinum or non-platinum–based treatments, for patients previously treated with PARPi. For instance, previously platinum-resistant patients who had a prolonged remission with olaparib might be expected to benefit when rechallenged with a platinum-based regimen in the post-PARPi setting. Although our data might suggest that the use of PARP inhibitors could possibly restore platinum sensitivity, there are currently no other preclinical or clinical data to support this hypothesis; further work is certainly warranted in this regard.

**Table 3.** Breakdown of objective responses to different chemotherapeutic regimens used in the post-PARPi setting according to pre-PARPi platinum sensitivity

| | Response by RECIST and/or GCIG criteria (events/evaluable; %) | | |
|---|---|---|---|
| **Chemotherapeutic regimen used** | **Platinum-resistant** | **Platinum-partially sensitive** | **Platinum-sensitive** |
| Platinum-based chemotherapies | 5/14 (36) | 16/26 (62) | 5/13 (38) |
| Platinum single-agent | 1/3 (33) | 1/2 (50) | 1/1 (100) |
| Platinum-taxane | 3/5 (60) | 9/10 (90) | 2/7 (29) |
| Platinum-PLD | 1/4 (25) | 3/5 (60) | 1/2 (50) |
| Platinum-others | 0/2 (0) | 3/9 (33) | 1/3 (33) |
| Taxane single-agent | 3/8 (38) | 2/2 (100) | 0/0 (0) |
| PLD single-agent | 2/6 (33) | 1/3 (33) | 0/1 (0) |
| Others | 1/2 (50) | 0/3 (0) | 0/0 (0) |
| Total | 11/30 (37) | 19/34 (56) | 5/14 (36) |

**Table 4.** Table summarizing the association of baseline factors with OS in patients treated with platinum-based chemotherapy in the post-olaparib setting

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| **Variables** | **HR** | ***P*** | **HR** | ***P*** |
| Age (y) | 0.981 (0.948–1.015) | 0.267 | - | - |
| Grade (3 vs. 2) | 1.066 (0.325–3.493) | 0.916 | - | - |
| Stage at diagnosis (III or IV vs. I or II) | 1.072 (0.442–2.599) | 0.877 | - | - |
| Serous histology | 0.834 (0.293–2.380) | 0.735 | - | - |
| Optimally debulked (<1 cm) | 0.598 (0.291–1.229) | 0.162 | - | - |
| *BRCA2* vs. *BRCA1* | 0.970 (0.461–2.042) | 0.936 | - | - |
| Previous breast cancer | 0.661 (0.341–1.281) | 0.220 | - | - |
| Residual disease after first-line treatment | 1.665 (0.773–3.588) | 0.193 | - | - |
| Olaparib RECIST and/or GCIG response | | 0.031 | | 0.056 |
| SD vs. PD | 0.299 (0.103–0.868) | 0.026 | 0.301 (0.104–0.875) | 0.027 |
| PR/CR vs. PD | 0.257 (0.093–0.709) | 0.009 | 0.298 (0.106–0.838) | 0.022 |
| Last pre-PARPi platinum sensitivity | | 0.332 | | |
| Partial sensitive vs. resistant | 0.902 (0.448–1.815) | 0.773 | - | - |
| Sensitive vs. resistant | 0.522 (0.214–1.276) | 0.154 | | |
| PTP interval (mo) | 0.960 (0.928–0.993) | 0.018 | 0.962 (0.928–0.997) | 0.036 |

Secondary *BRCA1/2* mutations leading to restoration of BRCA1/2 function have been associated with platinum resistance and proposed as a common mechanism linking platinum and PARPi resistance. However, in this study, secondary *BRCA1/2* mutations were not detected by massively parallel sequencing of tumor samples collected from PARPi-resistant patients despite having achieved sufficient coverage and depth for confident variant calling. The conservative estimated frequency of such mutations in this PARPi-resistant cohort of patients is 0.125 (95% CI, 0–0.375). Although this estimate is adjusted for the limited size of our sample population, we recognize the limitations of this analysis and also that a significant proportion of patients treated with platinum-based regimens did not achieve objective responses. On the basis of the published data, we would have expected to observe a higher frequency of secondary *BRCA1/2* mutations and it would clearly be desirable to study a larger patient population to address definitively their clinical relevance (16). There is currently one published case in the literature (reported by our group) of an patient with EOC with *BRCA1/2* germline mutation who developed a PARPi-resistant lesion with a secondary *BRCA1/2* mutation (which restored the *BRCA2* open reading frame; ref. 25); she was not included in the current study as she did not receive post-olaparib chemotherapy. In this study, the failure to observe secondary *BRCA1/2* mutations in the PARPi-resistant patients suggests other mechanisms may play a role in mediating PARPi resistance. These might include changes in the activity of the ABC

**Table 5.** Associations between objective response to first-line post-olaparib platinum-based chemotherapy and (i) olaparib response, (ii) pre-olaparib platinum sensitivity (measured by TFI), and (iii) interval between last pre-olaparib and first post-olaparib platinum-based chemotherapies (PTP interval)

| | | Post-olaparib platinum chemotherapy response by RECIST and/or GCIG criteria | | | |
|---|---|---|---|---|---|
| | | SD vs. PD | | PR/CR vs. PD | |
| | | ***P*** | **OR (95% CI)** | ***P*** | **OR (95% CI)** |
| Olaparib response by RECIST and/or GCIG | SD vs. PD | 0.105 | 14 (0.58–338.78) | 0.184 | 7.0 (0.40–123.35) |
| | PR/CR vs. PD | 0.341 | 3.6 (0.26–50.33) | 0.275 | 3.4 (0.38—30.66) |
| TFI after last pre-PARPi platinum (months) | | 0.874 | 1.008 (0.915–1.110) | 0.921 | 1.005 (0.916–1.102) |
| PTP interval (months) | | 0.061 | 1.117 (0.995–1.254) | 0.045 | 1.124 (1.003–1.264) |

**Table 6.** Results of massively parallel sequencing of tumors obtained from the six patients in this study.

| | | | | *BRCA1* mutation reads (%) | | | *BRCA2* mutation reads (%) | | | *TP53* mutation reads (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Time of tumor biopsy | Biopsy site | *BRCA1* mutation (rs number) | Wild-type alleles | Mutant alleles | *BRCA2* mutation (rs number) | Wild-type alleles | Mutant alleles | *TP53* mutation | Wild-type alleles | Mutant alleles |
| 1 | Diagnosis | Right ovary | c.3005delA/p.N1002fs (80357846) | 257 (58) | 183 (42) | c.5946delT p.2003 (80359550) | 17 (89) | 2 (11) | c.949C>T/p.Q317* | 169 (76) | 52 (24) |
| | Diagnosis | Left ovary | c.3005delA/p.N1002fs (80357846) | 79 (24) | 244 (76) | – | – | – | c.949C>T/p.Q317* | 179 (79) | 48 (21) |
| | Diagnosis | LN | c.3005delA/p.N1002fs (80357846) | 112 (21) | 426 (79) | – | – | – | c.949C>T/p.Q317* | 178 (77) | 52 (23) |
| | Post-PARPi | LN 1 | c.3005delA/p.N1002fs (80357846) | 102 (18) | 453 (82) | – | – | – | c.949C>T/p.Q317* | 164 (71) | 66 (29) |
| | Post-PARPi | LN 2 | c.3005delA/p.N1002fs (80357846) | 88 (17) | 435 (83) | – | – | – | c.949C>T/p.Q317* | 160 (73) | 59 (27) |
| 2 | Diagnosis | Right ovary | c.3760_3761insT/p.K1254fs (80357986) | 87 (42) | 120 (58) | – | – | – | c.659A>G/p.Y220C | 15 (42) | 21 (58) |
| | Post-PARPi | Pelvic sidewall tumor | c.3760_3761insT/p.K1254fs (80357986) | 130 (51) | 126 (49) | | | | c.659A>G/p.Y220C | 1 (6) | 16 (94) |
| 3 | Diagnosis | Left ovary | c.66_67delAG/p.E23fs (80357713) | 28 (17) | 132 (83) | — | — | — | – | – | – |
| | Post-PARPi | Peritoneal tumor | c.66_67delAG/p.E23fs (80357713) | 24 (27) | 66 (73) | | | | | | |
| 4 | Post first-line | Vaginal vault lesion | c.3450_3453delCAAG/p.Q1111fs (80357903) | 50 (12) | 382 (88) | — | — | — | – | – | – |
| | Post-PARPi | Para-aortic LN | c.3450_3453delCAAG/p.Q1111fs (80357903) | 74 (20) | 298 (80) | | | | | | |
| 5 | Post-PARPi | Ascites | – | — | — | c.8975_9100del/p.Pro2992_Gln3034 (80359736) | 167 (61) | 104 (38) | c.794A>G/p.L265P | 60 (79) | 16 (21) |
| 6 | Post-PARPi | Ascites | c.1961_1962insA/p.K654fs (80357853) | 311 (57) | 232 (43) | — | — | — | c.489insTGCTTG/pS166* | 243 (98) | 5 (2) |

Abbreviation: LN, lymph node.

transporter family (these would not be relevant to carboplatin resistance) and/or the loss of 53BP1 function leading to restoration of homologous recombination (28). Such events would have been undetected using our strategy and further evaluation of these mechanisms is certainly warranted in light of our findings.

A potential benefit for the use of PARPi therapy in patients with EOC with germline *BRCA1/2* mutations lies in its favorable toxicity profile and route of administration compared with chemotherapy (7), leading to a longer chemotherapy-free interval and associated improvements in quality-of-life (12). Another relevant question is whether PARPi therapy for *BRCA1/2* mutation-positive EOC adds a meaningful OS benefit to the current standard of care, i.e., platinum-based chemotherapy. In this series, the relatively short median OS of 34 weeks following post-olaparib chemotherapy is likely to reflect the extent of prior therapy for most patients (3 median lines of pre-olaparib chemotherapy). Moreover, patients in this series could only access olaparib through clinical trials mainly (83%) involving resistant/partially sensitive disease, that is, patients with anticipated shorter OS. The median OS from initial diagnosis of 66.3 months for our patients lies within the range previously reported in observational studies of *BRCA1/2*-mutated patients with EOC before PARPi became available (29, 30) and randomized trials will be required to properly assess the impact of olaparib on OS in *BRCA1/2* mutation-associated EOC patients. In this context, a recently reported subgroup analysis of OS in the olaparib maintenance trial does suggest an OS benefit (HR of 0.74) for patients positive for germline and/or somatic BRCA1/2 mutations which was not evident in the overall population (HR of 0.88; ref 12, 31).

A strength of this study is that it represents the combined experience from several comprehensive cancer centers and data are relatively mature. We were therefore able to collect data from 89 patients despite the strict inclusion of only germline *BRCA1/2*-carrier EOC patients who were exposed to an adequate dose and duration of olaparib treatment. However, it has the limitations inherent in a retrospective study and central review of responses was not conducted. Nevertheless, all data were reviewed and confirmed by experienced investigators at each site.

In summary, our data indicate that chemotherapy, including platinum, taxane, and anthracycline-based regimens, retains efficacy in heavily pretreated EOC patients with germline *BRCA1/2* mutations who have progressed on olaparib. The analyses of prognostic and predictive clinical variables also point to PARPi and platinum-based regimens lacking significant clinical cross-resistance. In addition, the failure to observe secondary *BRCA1/2* mutations in this study indicates that other mechanisms of PARPi resistance are likely to be important. Together with evidence from several published studies showing a high level of patient acceptability to single-agent PARPi treatment, these data provide strong support for the further evaluation of single-agent PARPi in patients with EOC with *BRCA1/2* mutations.

## Disclosure of Potential Conflicts of Interest

C. Gourley has commercial research grant from AstraZeneca. J.S. de Bono is employed as a Professor at the Institute of Cancer Research and is a consultant/advisory board member of and has commercial research grant from AstraZeneca. A. Ashworth may benefit financially from the development of PARP inhibitors through patents held jointly with AstraZeneca through the Institute of Cancer Research and rewards to inventors scheme. S. B. Kaye is a consultant/advisory board member of AstraZeneca Advisory Board.

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