Clinical Cancer Research

Long-Term Responders on Olaparib Maintenance in High-Grade Serous Ovarian Cancer: Clinical and Molecular Characterization

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Abstract

Purpose: Maintenance therapy with olaparib has improved progression-free survival in women with high-grade serous ovarian cancer (HGSOC), particularly those harboring *BRCA1/2* mutations. The objective of this study was to characterize long-term (LT) versus short-term (ST) responders to olaparib.

Experimental Design: A comparative molecular analysis of Study 19 (NCT00753545), a randomized phase II trial assessing olaparib maintenance after response to platinum-based chemotherapy in HGSOC, was conducted. LT response was defined as response to olaparib/placebo >2 years, ST as <3 months. Molecular analyses included germline *BRCA1/2* status, three-biomarker homologous recombination deficiency (HRD) score, *BRCA1* methylation, and mutational profiling. Another olaparib maintenance study (Study 41; NCT01081951) was used as an additional cohort.

Results: Thirty-seven LT (32 olaparib) and 61 ST (21 olaparib) patients were identified. Treatment was significantly associated with outcome ($P < 0.0001$), with more LT patients on olaparib (60.4%) than placebo (11.1%). LT sensitivity to olaparib correlated with complete response to chemotherapy ($P < 0.05$). In the olaparib LT group, 244 genetic alterations were detected, with *TP53*, *BRCA1*, and *BRCA2* mutations being most common (90%, 25%, and 35%, respectively). *BRCA2* mutations were enriched among the LT responders. *BRCA* methylation was not associated with response duration. High myriad HRD score (>42) and/or *BRCA1/2* mutation was associated with LT response to olaparib. Study 41 confirmed the correlation of LT response with olaparib and *BRCA1/2* mutation.

Conclusions: Findings show that LT response to olaparib may be multifactorial and related to homologous recombination repair deficiency, particularly *BRCA1/2* defects. The type of *BRCA1/2* mutation warrants further investigation. *Clin Cancer Res; 23(15); 4086–94. ©2017 AACR.*

Introduction

Epithelial ovarian cancer remains the leading cause of death from gynecologic malignancies, with high-grade serous ovarian cancer (HGSOC) the most common histologic subtype (1). Following cytoreductive surgery and platinum-based chemotherapy, approximately 70% of patients with HGSOC relapse despite an initial response to therapy (2). Given the practical certainty of recurrence in relapsed HGSOC, a maintenance approach was proposed to delay subsequent progression via continuation of treatment beyond cytotoxic chemotherapy. The PARP inhibitor (PARPi) olaparib (Lynparza) is the first validated molecularly targeted agent for HGSOC. Ledermann and colleagues demonstrated the benefit of maintenance olaparib after response to platinum-based chemotherapy in women with HGSOC recurrence (NCT00753545; ref. 3). Patients were randomized to placebo or olaparib, introduced within 8 weeks of completion of the last dose of platinum-based chemotherapy and maintained until progression. Olaparib maintenance was associated with significantly longer progression-free survival [PFS; median, 8.4 vs. 4.8 months; HR, 0.35; 95% confidence interval (CI), 0.25–0.49; $P < 0.001$; data maturity 58%]. Patients with a *BRCA1* mutation (*BRCA1*m) or a *BRCA2* mutation (*BRCA2*m) had significantly

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Translational Relevance

This is the first study to report long-term responders to a PARP inhibitor, with response durations of >2 years, in the context of platinum-sensitive, high-grade serous ovarian cancer. Based on extensive molecular profiling, the durable long-term responses were multifactorial and driven by germline and somatic *BRCA1/2* mutations. The majority of patients in the long-term responders group harbored homologous recombination repair deficiency, with enrichment for mutations in *BRCA2*, compared with short-term responders. This pilot study also highlights potential non–*BRCA1/2*-mutated patients who demonstrated durable clinical benefit with the PARP inhibitor olaparib and, together with an ongoing larger cohort study (NCT02489058), seeks to identify additional molecular characteristics that can predict susceptibility to olaparib. Further investigation may allow outreach to more patients for treatment with olaparib.

greater PFS benefit with olaparib maintenance than those receiving placebo (median, 11.2 vs. 4.3 months; HR, 0.18; 95% CI, 0.10–0.31; $P < 0.0001$; data maturity 52%; ref. 4). Overall survival improved by 3 months, which was not significant, possibly because of posttrial cross-over, whereby 22.6% of placebo patients received PARPi in subsequent clinical trials following progression on placebo. Olaparib is now approved in Europe, Australia, and Canada for the maintenance treatment of women with relapsed, *BRCA1/2*m-positive (germline or somatic) HGSOC who have had complete or partial response to platinum-based chemotherapy (5). Olaparib is also approved in the United States for monotherapy in patients with germline *BRCA1/2*m advanced HGSOC who have been treated with ≥3 prior lines of chemotherapy (6). Proteins encoded by the *BRCA1/2* genes are crucial effectors of double-strand break DNA repair (7); as a result, *BRCA1/2*m carriers are known to be highly responsive to DNA-damaging agents, including platinum-based chemotherapies (8, 9) and PARPi (8), although mechanisms of action and resistance to PARPi are not fully understood (10). Other than deleterious *BRCA1*m or *BRCA2*m, there are no predictive biomarkers for sensitivity to olaparib. Data from The Cancer Genome Atlas (TCGA) and functional studies indicate that approximately half of HGSOC are homologous recombination repair (HR) defective (11–13). PARP is involved in the repair of single-strand DNA breaks, and its inhibition can result in replication-associated double-strand breaks. Such HR-deficient cells as those found in *BRCA1/2*-mutated tumors, whether repaired via error-prone pathways or persistent without repair, cause further genetic instability and can lead to cell death (14).

We hypothesize that studying patients with HGSOC with prolonged response to olaparib may identify additional biomarkers of response beyond *BRCA1/2*m. The objective of this study was to identify and characterize long-term (LT) responders to olaparib maintenance in comparison with short-term (ST) responders in terms of clinical and molecular profile to pinpoint additional markers of response and explore potential resistance factors.

Materials and Methods

Patient population

We investigated the molecular and clinical characteristics of LT and ST responders randomized to maintenance olaparib or placebo in the phase II, randomized, double-blind study of olaparib in patients with platinum-sensitive relapsed HGSOC following treatment with ≥2 lines of platinum-based chemotherapy (NCT00753545; Study 19; ref. 3). This trial enrolled 265 patients, with 136 patients assigned to olaparib and 129 to placebo. Given that the greatest PFS benefit was at 11.2 months, we defined LT responders, whatever their *BRCA1/2* status, as having a double PFS benefit, that is, of >2 years. ST responders were defined as having PFS of <3 months, given the PFS observed in the placebo group of 4.8 months.

A second comparison/confirmation cohort from the open-label, randomized, phase II study of olaparib/carboplatin/paclitaxel with olaparib maintenance versus carboplatin/paclitaxel/observation in patients with platinum-sensitive recurrent HGSOC (NCT01081951; Study 41) was evaluated (15). In the combination phase, 156 patients were treated (81 in the olaparib-plus-chemotherapy group and 75 in the chemotherapy-alone group), and 121 continued to maintenance or no further treatment (66 in the olaparib-plus-chemotherapy group and 55 in the chemotherapy-alone group). Given that patients in this study received

Table 1. Characteristics of patients on olaparib capsule and placebo maintenance therapy—Study 19

	Clinical status			*BRCA* status			
Treatment duration	**Number of lines of prior chemotherapy [median (range)]**	**Initial FIGO state (n pts)**	**RECIST at baseline (*n*)**	**Platinum sensitivity status (*n*)**	***BRCA*m (*n* = 74)**	***BRCA* WT (*n* = 57)**	***BRCA* missing (*n* = 5)**
Olaparib arm (*n* = 136)							
ST responders:							
<3 months	2.8 (2–5)	1II/19III/1IV	PR: 16	6–12 months: 9	10 (14%)	9 (16%)	2
21 pts (15%)			CR: 5	12 months: 12	7 *BRCA1*		
LT responders:							
>2 years	2.9 (2–8)	3I/II25III/3IV	PR: 14	6–12 months: 11	22 (30%)	10 (18%)	0
32 pts (24%)		Missing: 1	CR: 18	>12 months: 21	10 *BRCA1*		
Placebo arm (*n* = 129)							
ST responders:							
<3 months	2.8 (2–8)	3I/II27III/8IV	PR: 25	6–12 months: 22	19 (31%)	18 (30%)	3
40 pts (31%)		Missing: 2	CR: 15	>12 months: 18	16 *BRCA1*		
LT responders:							
>2 years	2 (2)	5 III	PR: 1	6–12 months: 1	5 (8%)	0	0
5 pts (4%)			CR: 4	>12 months: 4	4 *BRCA1*		

Abbreviations: CR, complete response; FIGO, International Federation of Gynecology and Obstetrics; PR, partial response; pts, patients; WT, wild-type

olaparib with chemotherapy, the ST responders were defined as patients with PFS of <6 months, taking into account the time over which olaparib was administered with chemotherapy to assess the maintenance period.

Data collection

Clinical trial data were prospectively obtained for all treated patients (Table 1) and archival tumor specimens, predominantly taken at the time of initial diagnosis, from patients who consented to further use of their specimens. Previously performed germline *BRCA1/2* testing (Myriad Genetics) results were obtained.

Molecular investigations

Assessment of somatic *BRCA1/2* mutations (Myriad tumor testing and Foundation Medicine), determination of the three-biomarker HR deficiency (HRD) score (myChoice HRD test, Myriad Genetics; ref. 16), and *BRCA1* methylation (Myriad tumor testing) were conducted (17). HRD scores were determined using an assay that calculates scores for whole-genome tumor LOH, telomeric allelic imbalance and large-scale state transition. The HRD score is the sum of the three scores, where $\geq$42 is a predefined threshold validated as a high HRD score (16). The Methyl-Profiler DNA Methylation PCR Assay System (SABiosciences) was used to quantify methylation levels, with a used quantitative cut-off of promoter methylation of >10%. HRD status was determined on the basis of the combination of the dichotomized HRD score using the predefined HRD threshold and tumor *BRCA1/2* status (scored as mutated if deleterious or suspected deleterious mutations in germline or somatic *BRCA1/2* were present). Therefore, HRD was defined as a high HRD score ($\geq$42) and/or *BRCA1/2*-mutated tumor (16).

Mutational profile determined by the Foundation Medicine T5 panel (entire coding sequence of 287 cancer-related genes plus select introns from 27 genes) and other genetic alterations (deletions and functional rearrangements) were assessed on archival tumor tissue (18). Classification of *TP53* mutations (*TP53*m) was determined using the WHO International Agency for Research on Cancer Database.

Pathology review

Pathology review for tumor cell content was performed for all patients with molecular analyses, whereas pathology review of histologic subtype by an expert pathologist in gynecologic cancer, blinded to cohort or outcome, was only performed for patients with remaining tissue.

Statistical analysis

Descriptive analyses from all LT and ST responders to olaparib/placebo were assessed for statistical significance. Fisher exact or χ^2 tests were used to test for associations between individual explanatory variables and response duration (LT vs. ST) as appropriate. SPSS and Excel were used for all analyses. Given the exploratory data analysis, no type 1 error correction was performed.

Results

Clinical data

Data were collected from 265 total patients on study for the patients with LT and ST responses to olaparib therapy or placebo as part of Study 19. Thirty-seven patients were identified as LT responders, of whom 32 (86.5%) had received olaparib. Of the 61 ST patients identified, 21 (34.4%) had received olaparib. The

Table 2. Molecular profiling of patients on olaparib and placebo—Study 19

	Olaparib arm													
	ST responders						LT responders							
	All pts (n = 21)	HRD score $\geq$42 (n = 9)	HRD score <42 (n = 4)	HRD score missing (n = 8)	*TP53* mutations (n = 12)	*TP53* WT (n = 4)	*TP53* missing (n = 5)	All pts (n = 32)	HRD score $\geq$42 (n = 21)	HRD score <42 (n = 3)	HRD score missing (n = 8)	*TP53* mutations (n = 27)	*TP53* WT (n = 1)	*TP53* missing (n = 4)
*BRCA*m (n = 32)	10 (48)	6 (67)	0	4 (50)	7 (58)	1 (25)	2 (40)	22 (69)	18 (86)	2 (67)	2 (25)	20 (74)	0	2 (50)
BRCA WT (n = 19)	9 (43)	2 (22)	3 (75)	4 (50)	5 (42)	3 (75)	1 (20)	10 (31)	3 (14)	1 (33)	6 (75)	7 (26)	1 (100)	2 (50)
BRCA missing (n = 2)	2 (9)	1 (11)	1 (25)	-	-	-	2 (40)	-	-	-	-	-	-	-
	Placebo arm													
	ST responders							LT responders						
	All pts (n = 40)	HRD score $\geq$42 (n = 18)	HRD score <42 (n = 11)	HRD score missing (n = 11)	*TP53* mutations (n = 29)	*TP53* WT (n = 1)	*TP53* missing (n = 10)	All pts (n = 5)	HRD score $\geq$42 (n = 4)	HRD score <42 (n = 0)	HRD score missing (n = 1)	*TP53* mutations (n = 4)	*TP53* WT (n = 1)	*TP53* missing (n = 0)
*BRCA*m (n = 24)	19 (48)	13 (72)	1 (9)	5 (46)	16 (55)	0	3 (30)	5 (100)	4 (100)	-	1 (100)	4 (100)	1 (100)	-
BRCA WT (n = 18)	18 (45)	5 (28)	10 (91)	3 (27)	13 (45)	1 (100)	4 (40)	0	0	-	0	0	0	-
BRCA missing (n = 3)	3 (7)	0	0	3 (27)	0	0	3 (30)	0	-	-	-	-	-	-

NOTE: Data are number (percentage).
Abbreviations: Pts, patients; WT, wild-type.

main characteristics are summarized in Tables 1 and 2. LT responders on olaparib (32 patients) had a median of three prior lines of therapy, with one-third having relapsed within 6 to 12 months of their penultimate platinum-based chemotherapy. Of the LT responders on olaparib, 44% (14/32) had partial responses to their most recent platinum-based chemotherapy, with residual disease evident at the time of olaparib maintenance (Table 1). The other 18 of 32 (56%) LT responders on olaparib had complete response to the most recent platinum-based chemotherapy, in comparison with only five of 21 (24%) olaparib ST responders (Table 1). Complete response to platinum-based chemotherapy at the time of olaparib maintenance was associated with LT response to olaparib ($P = 0.026$, univariate analysis). The treatment-free interval following the penultimate platinum-based chemotherapy did not correlate with LT response (Supplementary Table S1). Sixty-five of 98 archival tumor samples (37 LT and 61 ST) were from primary tumors, and nine of 98 were from metastases (this information was unavailable for the other 24 patients).

Receipt of olaparib over placebo was significantly associated with LT response (32/53, 60.4% vs. 5/45, 11.1%; $P < 0.0001$). More patients treated with olaparib were LT than ST responders ($P = 0.052$).

Molecular analysis

***BRCA1/2* status (germline and somatic).** From the LT responders, 27 of 37 (73%) had loss-of-function mutations in *BRCA1/2*. Among the 32 LT olaparib responders, *BRCA1*m and *BRCA2*m were found in 10 and 13 patients, respectively, with one patient showing deleterious mutations in both *BRCA1* and *BRCA2* (Tables 2 and 3). All five LT responders receiving placebo were *BRCA1/2*m positive (Table 2). A greater number of *BRCA1/2*m was found in LT responders compared with ST responders (Table 3). In contrast, among the 21 olaparib ST responders, 10 were found to carry a deleterious *BRCA1/2*m (seven of 10 were in *BRCA1*; Tables 2 and 3).

We further analyzed the type and location of *BRCA1/2*m in the olaparib cohort between LT and ST responders (Fig. 1 and Supplementary Fig. S1). Of the 17 patients on olaparib who had *BRCA1*m (LT and ST), nine had founder mutations (E23fs* or Q1756fs*), whereas only one patient had a founder *BRCA2*m (S1982fs*; ST responder group). Interestingly, among patients on olaparib, mutations in the DNA-binding domains of *BRCA1* ($n = 1$) or *BRCA2* ($n = 4$) were only seen in the LT responder group.

BRCA1 methylation status was available for 27 of 37 (73%) LT responders, all of whom were negative. In contrast, *BRCA1* methylation status was available for 42 of 61 (69%) ST responders, eight of whom (19%) had *BRCA1* methylation. Methylation of *BRCA1* was not associated with LT olaparib response.

Homologous recombination repair deficiency. No significant difference was seen between LT and ST responders according to the HRD score (Table 3). Most of the patients enrolled in the study were characterized by HR-deficient status (81% of the LT and ST on olaparib). Among data available for 26 of the 32 (81%) LT responders on olaparib, 25 patients (96%) had HRD, in contrast to 76% of the ST olaparib responders (Table 3). Of the 21 patients with high HRD score in the LT responder group, three were *BRCA1/2* wild-type. Of the nine ST patients with a high HRD score, two (22%) were *BRCA1/2* negative in the ST responder group. However, a significant number of HRD scores are missing for the *BRCA1/2* wild-type group (Table 2).

Taking together high HRD score (≥42) and/or *BRCA1/2*-mutated tumor, there was a trend toward HRD in LT versus ST responders, with 96% of LT responders and only 76% of ST responders showing HRD ($P = 0.07$).

Next-generation sequencing analysis. Next-generation sequencing was performed on archival tumor DNA from 44 patients [28 (87%) LT and 16 (76%) ST responders on olaparib]. From 44

Table 3. Analysis of *BRCA1/BRCA2* mutational frequency and HRD in the olaparib arm, including total *BRCA1/2* mutations and stratification by somatic mutations—Study 19

Number of patients with each mutation shown	LT responders $N = 32$	ST responders $N = 21$	*P* value (Fisher exact test)
***BRCA* status available**	$N = 32$	$N = 19$	
BRCA mutation	22[a] (69%)	10 (53%)	0.2179[b]
BRCA1 mutation	10 (45%)	7 (70%)	1.0000[b]
BRCA2 mutation	13 (59%)	3 (30%)	0.0631[b]
BRCA WT	10 (31%)	9 (47%)	
Unknown/missing (excluded from analysis)	–	2 missing	
Somatic *BRCA* mutations available	$N = 32$	$N = 19$	
Olaparib treated ($n = 53$)	6	3	
BRCA mutation	6[a] (20%)	3 (16%)	1.0000
BRCA1 mutation	2	1	1.0000
BRCA2 mutation	5	2	0.6909
Placebo treated ($n = 45$)	1	3	
***BRCA1* methylation status available**	$N = 23$	$N = 14$	
Methylated	0	2 (14%)	
Unmethylated	23 (100%)	12 (86%)	
Unknown/missing (excluded from analysis)	9 missing	7 missing	
HRD score available	$N = 24$	$N = 13$	
HRD score (≥42)	21 (88%)	9 (69%)	0.2128
HRD score (<42)	3 (12%)	4 (31%)	
HRD status available	$N = 26$	$N = 17$	
HR deficient	25 (96%)	13 (76%)	0.0707
HR nondeficient	1 (4%)	4 (24%)	

NOTE: HRD scores are given for LT responders and ST responders, and HRD status is defined as a high HRD score and/or presence of a *BRCA1/2* mutation.
[a]One LT responder had both *BRCA1* and *BRCA2* mutations.
[b]*P* value between groups compared with WT.

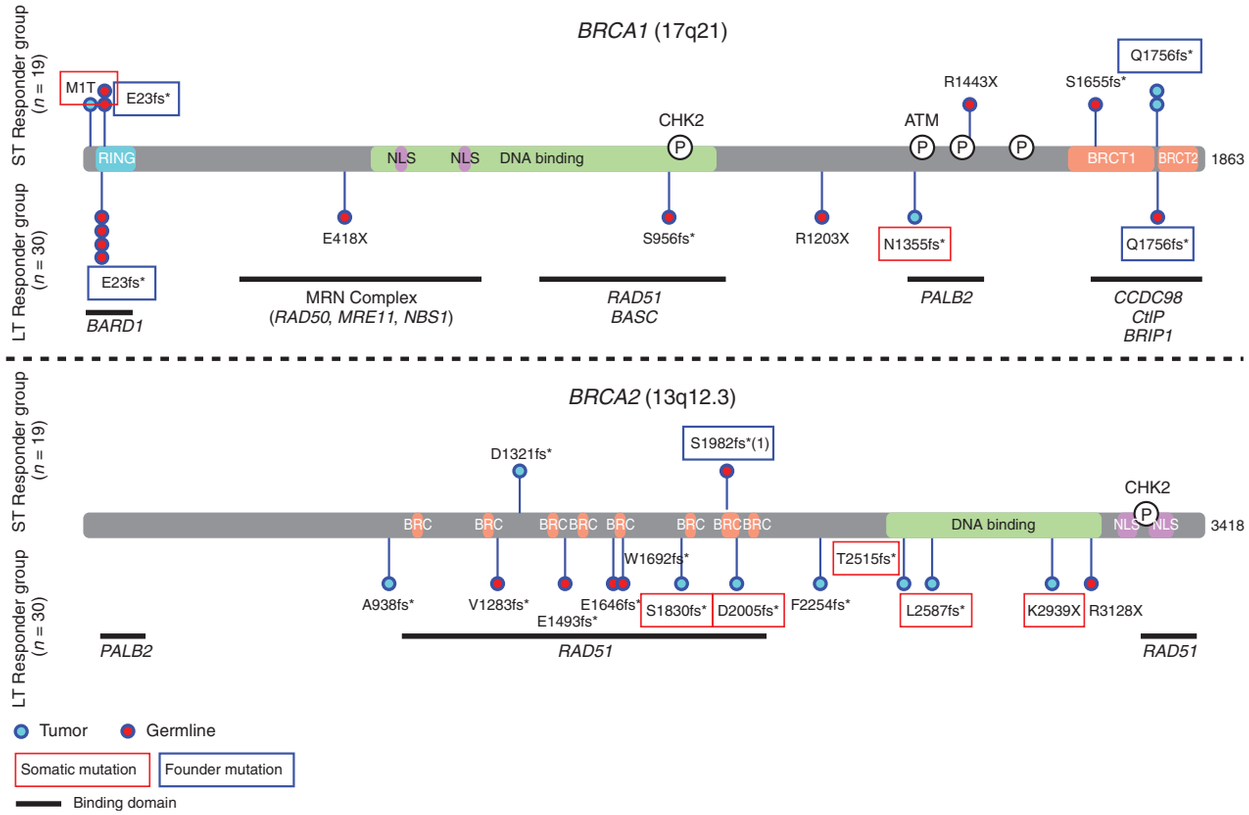

Figure 1.

Location of the *BRCA1/2* mutation on the *BRCA1/2* gene for patients on olaparib—Study 19. Somatic deletion exon 17 (not shown) found in *BRCA1* in LT responder group. Large insertion (somatic, not shown) and deletion (not shown) found in *BRCA2* in ST and LT responder group, respectively. VUS and SNP variants not shown.

patients, over 600 identified alterations were classified by type and likely functional significance by Foundation Medicine (Supplementary Table S2). A total of 242 likely functional generic alterations in 99 genes were found, with *TP53*, *BRCA1*, and *BRCA2* mutations being the most common (89%, 34%, and 36% of patients, respectively; Fig. 2 and Supplementary Table S2).

TP53 signaling was considered aberrant in most cases (90%), as expected for the HGSOC histology subtype (27 of 28 patients with available data analyzed in the LT responder group, 12 of 16 in the ST responder group; Supplementary Fig. S1). The patient with no *TP53*m in the LT responder group had pathology reviewed and HGSOC confirmed. Of the four patients with no *TP53*m in the ST responder group, two had confirmed HGSOC. Other types of mutations and gene amplifications were observed, particularly in genes encoding proteins involved in DNA repair and damage response, regulation of cell cycle, apoptosis, and MAPK/PI3K signaling (Fig. 2 and Supplementary Table S2). Furthermore, four patients had alterations in *PTEN* (two homozygous deletions, one somatic variant, and one functional intergenic truncation), all of whom were LT responders on olaparib. These *PTEN* alterations co-occurred with *BRCA1/2* mutation for three of the patients, only 1 LT patient had a *PTEN* mutation in the absence of a *BRCA1/2* mutation. In contrast, no *PTEN* alterations were seen in the ST responder group (Supplementary Table S3). Interestingly, three patients randomized to the placebo arm had *PTEN* alterations and were ST responders (Supplementary Table S3).

Validation cohort

LT and ST responders from Study 41 were identified and analyzed (Fig. 3 and Supplementary Table S4). In total, 19 LT responders were identified, and all were in the olaparib arm. Olaparib maintenance was also significantly associated with LT responders ($P < 0.0001$). *BRCA1/2*m was statistically correlated with LT response to olaparib (Supplementary Table S4). *BRCA1*m and *BRCA2*m were observed in six and five LT responders, respectively. The 11 ST patients were all *BRCA1/2* wild-type.

Discussion

There are limited data describing patients with ovarian cancer who experience prolonged benefit from PARP inhibition, other than evidence for the role played by deleterious mutations in *BRCA1/2*. Defective DNA repair via HR repair deficiency is a fundamental vulnerability in HGSOC and can be exploited with PARPi, such as olaparib, by induction of cancer-specific synthetic lethality (19). Examination of broader clinical and molecular data of extreme responders may uncover potential biomarkers of response (20). We identified LT and ST responders to PARPi from

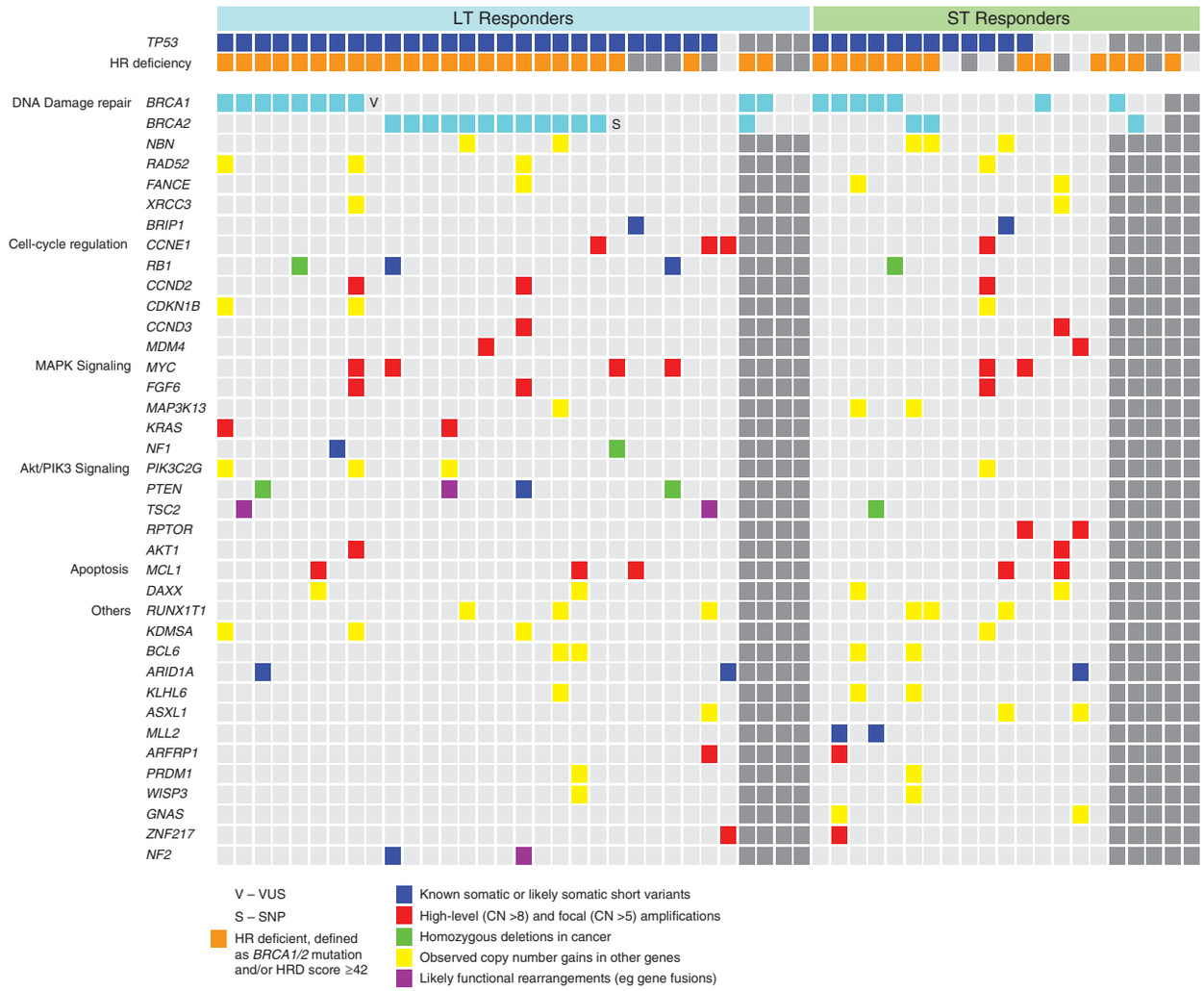

Figure 2.

Mutations and other gene alterations in LT and ST responders to olaparib—Study 19. Only events that occurred in at least two patients are shown. Columns represent patients. Dark gray columns are samples that failed sequencing or analysis. A total of 51 patients were analyzed (32 LT responders, 19 ST responders), but seven failed sequencing; therefore, the molecular profiles of 44 patients are shown. Novel rearrangements of unknown significance, short variants of unknown significance, and nonfocal lower-level [copy number (CN) $\leq$ 8] amplifications of genes known to be recurrently amplified in cancer are excluded.

women with HGSOC entered into two olaparib maintenance trials, Studies 19 and 41, and have molecularly characterized these exceptional responders. We found that 32 and 19 patients with recurrent platinum-sensitive HGSOC achieved LT (defined as >2 years) response to olaparib maintenance as part of the studies, respectively. Germline and somatic *BRCA1/2*m were observed to be associated with LT response to olaparib, with an enrichment of *BRCA2*m in the LT olaparib responders, compared with the frequency of *BRCA1*m and *BRCA2*m in HGSOC detected at the start of the trial and the *BRCA1/2* ratio observed in the general population (21). Our finding is in agreement with data suggesting differences in outcome and response to therapy between *BRCA1* and *BRCA2* genotypes (22). Previous studies have shown that *BRCA2*m is associated with prolonged survival in invasive epithelial ovarian cancer (23). In 2012, Liu and colleagues showed that the presence of a *BRCA2*m was associated with longer survival and better therapy response than a *BRCA1*m in HGSOC (24). Many LT olaparib responders had a *BRCA2*m in the *RAD51*-binding domain, described as a frequent site of *BRCA2*m by TCGA (11), but also in DNA-binding sites (Fig. 3). As such, mutations in the *RAD51* region are expected to attenuate or abolish interactions with *RAD51*, resulting in failure to load *RAD51* to DNA-damage sites (24). Our data also suggest that silencing of *BRCA1* through promoter methylation does not result in improvement in response to platinum-based chemotherapy or to sequential chemotherapy and maintenance olaparib therapy, as previously suggested by TCGA and other publications showing a lack of survival benefit and correlation with platinum sensitivity (11, 25).

However, our study did not identify a potential mechanism involved in the small group of *BRCA1/2* wild-type patients who

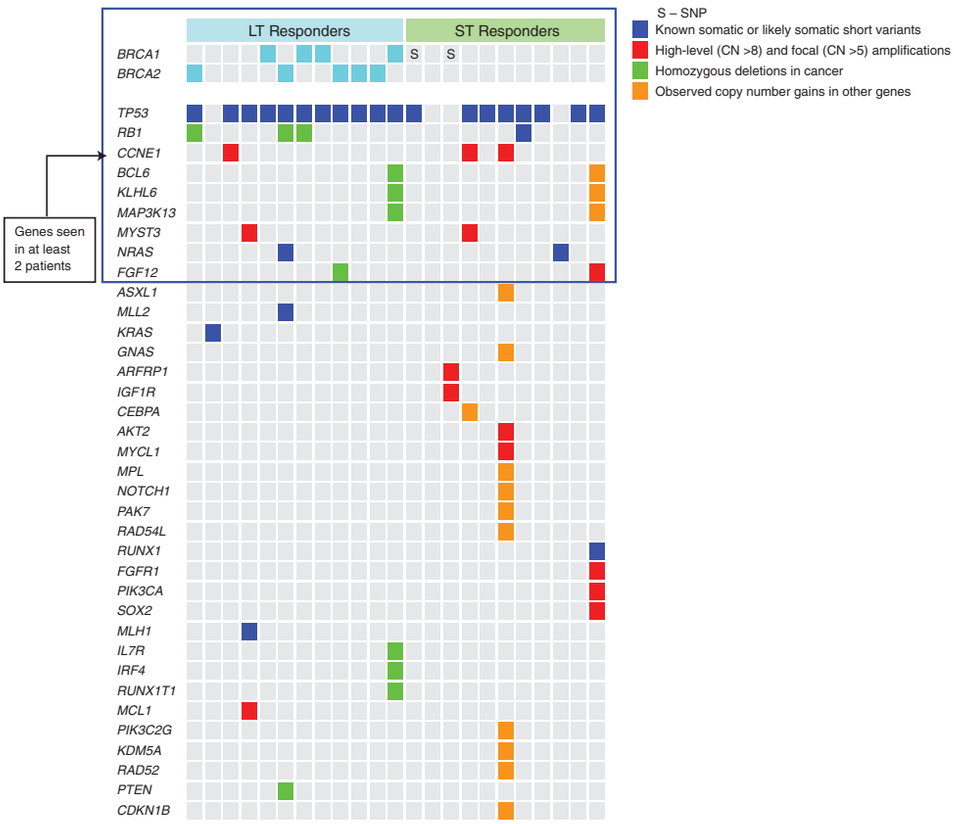

Figure 3.
Study 41 data showing LT and ST responders to olaparib and placebo, with mutations and other gene alterations given for LT and ST responders. Novel rearrangements of unknown significance, short variants of unknown significance, and nonfocal lower-level (CN $\leq$ 8) amplifications of genes known to be recurrently amplified in cancer are excluded.

had a LT benefit to olaparib maintenance, currently not eligible for olaparib in clinical practice. Beyond *BRCA1/2*m, there have been a number of mechanisms of HRD described that may correlate with platinum and PARP response (11), and newly developed homologous recombination repair panels have assessed several additional novel genes, including *NBN*, *MRE11*, *RAD50*, *RAD51C*, *PALB2*, *BARD1*, and *BRIP1* (19, 26, 27). Our results show that the majority of patients enrolled in the study were HR deficient, a potential enrichment due to the selection of patients with HGSOC based on platinum-sensitivity recurrence and objective response to platinum. The phase II study ARIEL2 investigating rucaparib (another PARPi) monotherapy in patients with recurrent platinum-sensitive HGSOC has confirmed *BRCA1/2*m as a biomarker of response, as well as genomic LOH, a potential predictive surrogate marker for HRD (28). It was hypothesized that the inability of the cell to perform HR repair leads to genomic scarring and LOH, thus enabling the use of high LOH as a signature of HRD. However, no data are currently available for LT responders and PARPi progression. Recently, the maintenance phase III study of niraparib, another PARPi, showed increased PFS in all patients—germline *BRCA1/2* patients (the group with greatest benefit), and non-*BRCA1/2* carriers (comprising of both HRD-positive and HRD-negative tumors; ref. 29). Eligible patients had to have achieved response following four to six cycles of platinum-based chemotherapy with a CA-125 in the normal range or reduced by 90% for at least 7 days, an absence of measurable disease greater than 2 cm at study commencement. Consistent with our findings, the PFS benefit to PARPi is driven by minimal residual disease (complete response prior maintenance), *BRCA1/2* mutation, and HRD-positive tumors, though not exclusively. The current HRD assays available did not completely identify biomarkers involved in response or resistance to PARPi.

Interestingly, four genetic alterations in the *PTEN* gene were observed, of which three were associated with *BRCA1/2*m in the LT responders on olaparib but none in the ST responder group. Moreover, in the placebo arm, three patients with *PTEN* alterations had disease progression within three months (Supplementary Table S3). The significance of this finding is not clear but warrants further investigation. Although many LT responders to olaparib harbor a *BRCA1/2*m, our data show the occurrence of ST responders to olaparib in patients with *BRCA1/2*m. This finding highlights the limitation of the analysis on archival rather than tumor tissue at the time of disease progression. There is evidence that in cells deficient in DNA-damage repair, such as those with *BRCA1/2*m, additional mutations can restore function and allow effective DNA repair (30). Reversion mutations in *BRCA1/2* have been described and associated with olaparib and platinum resistance (31), although this effect was supposed to be minimized by the selection of patients with response to platinum-based chemotherapy. Understanding therapeutic resistance requires comprehensive disease assessment at the specific time of therapeutic intervention; timing and treatment strategy are imperative to efficacy. Several mechanisms of resistance have been described related to the HR pathway (30). Although tumors harboring a *BRCA1/2*m lack the HR repair pathway required for error-free repair of DNA double-strand breaks (32), other DNA-repair pathways exist that can become engaged, ultimately leading

to olaparib resistance but platinum sensitivity, as with non-homologous end-rejoining alterations (33, 34). As such, determining the disease- and therapy-specific HRD signature is important. Reports on this signature show varying gene lists, and these differences are likely attributed to variances in methodologies. Peng and colleagues identified a HR defect gene signature that can be used to functionally assess HR status and predict clinical outcomes (27). Pennington and colleagues reported that germline and somatic mutations in 13 HR genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas (26). These signatures need prospective validation.

Conclusion

This is the first study contrasting LT with ST responders to PARPi in terms of clinical and molecular data. Our results show that LT response to olaparib has been observed in platinum-sensitive recurrent HGSOC. This durable response may be multifactorial and driven by germline and somatic *BRCA1*/2m. This pilot study warrants a larger cohort to characterize LT responders. A study is ongoing to identify LT and ST responders to olaparib and allows for additional tumor tissue collection for analysis (NCT02489058).

Disclosure of Potential Conflicts of Interest

D. Hodgson, J.D. Robertson, and J.C. Barrett hold ownership interest (including patents) in AstraZeneca. K.M. Timms and J.S. Lanchbury hold ownership interest (including patents) in Myriad Genetics Inc. S.B. Kaye and J. Ledermann are consultant/advisory board members for AstraZeneca. C. Gourley reports receiving commercial research grants from AstraZeneca and Novartis; speakers bureau honoraria from AstraZeneca and Roche; and is a consultant/advisory board member for AstraZeneca and Clovis Oncology. C.L. Scott reports receiving speakers bureau honoraria from Prime Oncology, and is a consultant/advisory board member for AstraZeneca and Clovis Oncology. No potential conflicts of interest were disclosed by the other authors.

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References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11–30.
2. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet 2014;384:1376–88.
3. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 2012;366:1382–92.
4. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: A preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014;15:852–61.
5. Lheureux S, Bowering V, Karakasis K, Oza AM. Safety evaluation of olaparib for treating ovarian cancer. Expert Opin Drug Saf 2015;14:1305–16.
6. Kim G, Ison G, McKee AE, Zhang H, Tang S, Gwise T, et al. FDA approval summary: Olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. Clin Cancer Res 2015;21:4257–61.
7. Li ML, Greenberg RA. Links between genome integrity and BRCA1 tumor suppression. Trends Biochem Sci 2012;37:418–24.
8. Tan DS, Kaye SB. Chemotherapy for patients with *BRCA1* and *BRCA2*-mutated ovarian cancer: Same or different? Am Soc Clin Oncol Educ Book 2015;35:114–21.
9. Gorodnova TV, Sokolenko AP, Ivantsov AO, Iyevleva AG, Suspitsin EN, Aleksakhina SN, et al. High response rates to neoadjuvant platinum-based therapy in ovarian cancer patients carrying germ-line BRCA mutation. Cancer Lett 2015;369:363–7.
10. Scott CL, Swisher EM, Kaufmann SH. Poly (ADP-ribose) polymerase inhibitors: Recent advances and future development. J Clin Oncol 2015;33:1397–406.
11. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609–15.
12. Middleton FK, Patterson MJ, Elstob CJ, Fordham S, Herriott A, Wade MA, et al. Common cancer-associated imbalances in the DNA damage response confer sensitivity to single agent ATR inhibition. Oncotarget 2015;6:32396–409.
13. O'Donnell RL, Kaufmann A, Woodhouse L, McCormick A, Cross PA, Edmondson RJ, et al. Advanced ovarian cancer displays functional intratumor heterogeneity that correlates to ex vivo drug sensitivity. Int J Gynecol Cancer 2016;26:1004–11.
14. Javle M, Curtin NJ. The role of PARP in DNA repair and its therapeutic exploitation. Br J Cancer 2011;105:1114–22.
15. Oza AM, Cibula D, Benzaquen AO, Poole C, Mathijssen RH, Sonke GS, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: A randomised phase 2 trial. Lancet Oncol 2015;16:87–97.
16. Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. Breast Cancer Res 2014;16:475.
17. Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer 2012;107:1776–82.

18. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31: 1023–31.
19. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous recombination deficiency: Exploiting the fundamental vulnerability of ovarian cancer. Cancer Discov 2015;5:1137–54.
20. Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, et al. Genome sequencing identifies a basis for everolimus sensitivity. Science 2012;338:221.
21. Pancino G, Mortada MH, Charpin C, Osinaga E, de Cremoux P, Betaille B, et al. Two monoclonal antibodies identify antigens preferentially expressed on normal human breast cells versus breast cancer cells. Hybridoma 1991; 10:241–53.
22. Liu J, Cristea MC, Frankel P, Neuhausen SL, Steele L, Engelstaedter V, et al. Clinical characteristics and outcomes of BRCA-associated ovarian cancer: Genotype and survival. Cancer Genet 2012;205:34–41.
23. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA 2011;306:1557–65.
24. Liu G, Yang D, Sun Y, Shmulevich I, Xue F, Sood AK, et al. Differing clinical impact of BRCA1 and BRCA2 mutations in serous ovarian cancer. Pharmacogenomics 2012;13:1523–35.
25. Chiang JW, Karlan BY, Cass L, Baldwin RL. BRCA1 promoter methylation predicts adverse ovarian cancer prognosis. Gynecol Oncol 2006;101:403–10.
26. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res 2014;20:764–75.
27. Peng G, Chun-Jen LC, Mo W, Dai H, Park YY, Kim SM, et al. Genome-wide transcriptome profiling of homologous recombination DNA repair. Nat Commun 2014;5:3361.
28. McNeish IA, Oza AM, Coleman RL, Scott CL, Konecny GE, Tinker A, et al. Results of ARIEL2: A Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis. J Clin Oncol 2015;33(Suppl):abst 5508.
29. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med 2016;375:2154–64.
30. Bouwman P, Jonkers J. Molecular pathways: How can BRCA-mutated tumors become resistant to PARP inhibitors? Clin Cancer Res 2014;20:540–7.
31. Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature 2015;521:489–94.
32. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21.
33. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MI, et al. Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. Nature 2015;518:258–62.
34. Ceccaldi R, O'Connor KW, Mouw KW, Li AY, Matulonis UA, D'Andrea AD, et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. Cancer Res 2015;75: 628–34.