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EVOLVE: A Multicenter Open-Label Single-Arm Clinical and Translational Phase II Trial of Cediranib Plus Olaparib for Ovarian Cancer after PARP Inhibition Progression

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ABSTRACT

Background: PARP inhibitors (PARPi) are standard-of-care therapy for high-grade serous ovarian cancer (HGSOC). We investigated combining cediranib (antiangiogenic) with olaparib (PARPi) at emergence of PARPi resistance.

Methods: The proof-of-concept EVOLVE study (NCT-02681237) assessed cediranib–olaparib combination therapy after progression on a PARPi. Women with HGSOC and radiographic evidence of disease progression were enrolled into one of three cohorts: platinum sensitive after PARPi; platinum resistant after PARPi; or progression on standard chemotherapy after progression on PARPi (exploratory cohort). Patients received olaparib tablets 300 mg twice daily with cediranib 20 mg once daily until progression or unacceptable toxicity. The coprimary endpoints were objective response rate (RECIST v1.1) and progression-free survival (PFS) at 16 weeks. Archival tissue (PARPi-naïve) and baseline biopsy (post-PARPi) samples were mandatory. Genomic mechanisms of resistance were assessed by whole-exome and RNA sequencing.

Results: Among 34 heavily pretreated patients, objective responses were observed in 0 of 11 (0%) platinum-sensitive patients, 2 of 10 (20%) platinum-resistant patients, and 1 of 13 (8%) in the exploratory cohort. Sixteen-week PFS rates were 55%, 50%, and 39%, respectively. The most common grade 3 toxicities were diarrhea (12%) and anemia (9%). Acquired genomic alterations at PARPi progression were reversion mutations in *BRCA1, BRCA2*, or *RAD51B* (19%); *CCNE1* amplification (16%); *ABCB1* upregulation (15%); and *SLFN11* downregulation (7%). Patients with reversion mutations in homologous recombination genes and/or *ABCB1* upregulation had poor outcomes.

Conclusions: This is currently the largest post-PARPi study identifying genomic mechanisms of resistance to PARPis. In this setting, the activity of cediranib–olaparib varied according to the PARPi resistance mechanism.

Introduction

Ovarian cancer is the second leading cause of gynecologic cancer death worldwide (1). Disease initially responds well to platinum and taxane chemotherapy, but recurs in most women diagnosed with stage III/IV ovarian cancer; 5-year survival rates are 42% and 29%, respectively (2). High-grade serous ovarian cancer (HGSOC), the most common type (2), is characterized by severe genomic instability, nearly universal *TP53* mutations leading to dysfunctional p53, and defects in homologous recombination DNA repair pathways in half of the cases (3). The discovery that inhibiting PARP enzyme function in *BRCA1/2*-mutated cancers causes synthetic lethality (4) heralded precision targeted therapy in HGSOC. Three PARP inhibitors (PARPi; olaparib, niraparib, and rucaparib) are approved treatments for recurrent ovarian cancer (5–8), and recently olaparib was approved as first-line maintenance therapy for *BRCA1/2*-mutated disease (9). Clinical adoption of PARPis has significantly influenced HGSOC treatment, extending progression-free survival (PFS) while maintaining quality of life (10, 11). Contemporary investigations are moving PARPi usage earlier in the treatment paradigm, but currently there is no evidence that PARPi re-exposure is effective. The emergence of resistance raises the question of treatment options after progression on a PARPi. Several mechanisms of resistance have been described in the preclinical setting (12), but their clinical relevance has not been evaluated systematically.

Given the importance of angiogenesis in ovarian cancer (13), targeted combination treatments leveraging microenvironment modulation provide attractive opportunities to extend the benefit of PARPis. Cediranib, an oral inhibitor of vascular endothelial growth factor receptors 1–3 and c-kit tyrosine kinases, induces hypoxia and reduces angiogenesis (14). Hypoxia leads to aberrant DNA damage and repair signaling, which in turn causes genetic instability (15). Cediranib also suppresses the homologous recombination DNA repair pathway through downregulation of *BRCA1/2* and *RAD51* gene expression (16). In a randomized phase II trial, patients with

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Translational Relevance

As PARP inhibitors (PARPi) move earlier in the treatment paradigm, identifying mechanisms of acquired resistance to guide treatment post PARPi is an urgent unmet need. In this pilot phase II study, simultaneously targeting angiogenesis and homologous recombination deficiency (HRD) by combining cediranib and olaparib was tolerable and active in patients with high-grade serous ovarian cancer that had progressed on a PARPi. Comprehensive interrogation of paired biopsies revealed resistance mechanisms including reversions in HRD genes (*BRCA1/2, RAD51*), upregulation of multidrug efflux pump gene *ABCB1*, amplification of *CCNE1*, and downregulation of *SLFN11*. Patients with reversion mutations in HRD genes and overexpression of *ABCB1* had worse outcomes than patients without reversions or with intact *BRCA1/2* genes. Cediranib–olaparib combination therapy showed some activity after progression on a PARPi. Interestingly, translational findings hint that patients with reversion mutations in *BRCA1/2* or *RAD51* genes, or upregulated *ABCB1*, may not be candidates for cediranib–olaparib; further study is required to understand whether these patients should receive alternative treatment options.

measurable platinum-sensitive relapsed high-grade serous or endometrioid disease or with deleterious germline *BRCA1/2* mutations were randomized to receive either olaparib capsules 400 mg twice a day or olaparib 200 mg twice a day combined with cediranib 30 mg daily. The combination regimen demonstrated significantly improved PFS, which was more pronounced in patients with no known *BRCA* mutation (17). The same effect was seen for overall survival (OS; ref. 18). However, initial reports of the randomized phase III GY004 trial (NCT02446600) indicate that the olaparib–cediranib combination therapy failed to improve PFS (primary endpoint) compared with platinum-based chemotherapy in platinum-sensitive recurrent ovarian cancer (19).

We hypothesized that adding cediranib to olaparib may overcome resistance after progression on a PARPi. EVOLVE is an investigator-initiated phase II study assessing the efficacy and safety of cediranib–olaparib in HGSOC (regardless of *BRCA* status) that has progressed on a PARPi. As this was designed as a hypothesis-generating study, paired (pre- and post-PARPi) tumor samples were mandatory to identify mechanisms of PARPi resistance.

Materials and Methods

Study design and participants

This phase II study (NCT02681237), performed at two centers in Canada and Spain, enrolled women with a histologically confirmed diagnosis of recurrent ovarian, fallopian tube, or primary peritoneal cancer with high-grade serous or high-grade endometrioid histology and radiographically documented disease progression on any PARPi. Patients were required to have disease evaluable by RECIST v1.1 and amenable to a baseline biopsy. Key exclusion criteria included: active bowel obstruction, untreated or unstable central nervous system (CNS) metastases, any active significant cardiovascular event, >1+ proteinuria, and myelodysplastic syndrome/acute myeloid leukemia. Women requiring maximal doses of calcium channel blockers to stabilize blood pressure and those who were unable to discontinue CYP3A4 inhibitors and inducers were excluded. There was no limit to the number of prior treatment lines.

The study was approved by each participating site's institutional review board or ethics committee. All patients provided written informed consent according to the Declaration of Helsinki and Good Clinical Practice.

Procedures

Patients were enrolled into one of three cohorts according to platinum sensitivity status as defined by the Gynecologic Cancer InterGroup (11): platinum sensitive, platinum resistant, or an exploratory cohort including patients whose disease had progressed on a PARPi and progressed again on subsequent standard chemotherapy, regardless of platinum sensitivity. Oral olaparib 300 mg tablets were administered twice a day and oral cediranib 20 mg once daily, repeated every 28 days. The cediranib dose of 20 mg has been used in other trials, such as the randomized phase II BAROCCO trial (20), and was chosen in the present study based on the safety profile, particularly at the time of PARPi rechallenge. One dose reduction of cediranib to 15 mg was suggested in the event of intolerable cediranib-related toxicities. The olaparib dose could be reduced to 250 mg twice a day, with a second dose reduction to 200 mg twice a day for the management of olaparib-related toxicities. Treatment was continued until disease progression, unacceptable toxicity, or withdrawal of informed consent, whichever occurred first.

Tumors were assessed according to RECIST v1.1 every 8 weeks until radiologic progression. Toxicity was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. Provision of archival tissue (initial diagnostic tissue, before PARPi) and baseline biopsy (after PARPi) samples was mandatory; biopsy at the time of disease progression was optional.

Outcomes

The primary objective was to determine the efficacy of cediranib–olaparib in women whose ovarian cancer had progressed on prior PARPi therapy, assessed by objective response rate (RECIST v1.1) at 8 weeks and PFS rate at 16 weeks. We aimed to understand whether the addition of cediranib to olaparib overcomes resistance to PARPi and allows repeated response to PARPi. Secondary objectives were to evaluate disease control rate, safety, and mechanisms of resistance to PARPis.

Genomics methodology

DNA and RNA were coisolated using QIAGEN All-Prep DNA/RNA/miRNA Universal Co-Isolation kit from formalin-fixed paraffin-embedded tissues from archival (initial diagnostic sample before PARPi), baseline (at study entry after initial PARPi), and progression (on study after cediranib–olaparib) samples. Whole-exome sequencing, total RNA sequencing, and shallow whole-genome sequencing (s-WGS) libraries were constructed at the Princess Margaret Genomics Centre (www.pmgenomics.ca) using the methodology previously described by Lheureux and colleagues (21).

Sequencing reads were aligned to human genome reference (build hg38) using Burrows–Wheeler aligner (BWA-MEM) software (22). Somatic single-nucleotide variants were called using MuTect (version 1.1.5; ref. 23); insertions and deletions were called using VarScan2 (version 2.4.2; ref. 24). Variants were filtered to retain only nonsynonymous exonic changes present with a minor allele frequency >1% in 1000 Genomes phase 3 (release version 5.20130502), coverage >20×, and variant allele frequency >5%. Sequenza (version 2.1.0) and ichorCNA (github repo cloned May 21, 2019; https://github.com/broadinstitute/ichorCNA) were used for copy-number calling for exome and s-WGS data, respectively (25, 26). RNA sequencing reads

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were aligned using STAR (version 2.4.2a; ref. 27), and fusions were detected using STAR-Fusion (https://www.biorxiv.org/content/10.1101/120295v1). Gene expression analysis was performed using RSEM (version 1.3.0), and FPKM+0.01 values were $\log_2$ transformed and quantile normalized. "Overexpression" was defined as a ≥4-fold increase in expression between archival and baseline samples.

Statistical analysis

This proof-of-concept study aimed to detect an initial efficacy signal from the cediranib–olaparib combination after PARPi failure. The target clinical benefit rate (complete response, partial response, or stable disease for ≥16 weeks) was 30% in the platinum-sensitive cohort and 10% in the platinum-resistant and exploratory cohorts, based on expectations with standard chemotherapy. We analyzed efficacy in the intention-to-treat population and separately by cohort (three cohorts of ≥10 patients with evaluable tissue samples). The 16-week PFS and 1-year OS rates were estimated using Kaplan–Meier methodology with corresponding 95% confidence intervals (CI). All statistical analyses were performed in R version 3.5.2 (28).

Results

Between June 2016 and October 2018, 34 women were enrolled (platinum-sensitive cohort $n = 11$; platinum-resistant cohort $n = 10$; exploratory cohort $n = 13$). All patients had HGSOC, and 62% harbored somatic and/or germline *BRCA1/2* mutations (**Table 1**). Patients had received a median of five prior treatment lines. Six patients (18%) had received two lines of the same prior PARPi (olaparib $n = 4$; niraparib $n = 2$), and 18% had received prior antiangiogenics (bevacizumab $n = 5$, cediranib $n = 1$).

At the data cutoff (June 24, 2019), the median duration of follow-up was 11.6 months; 31 patients had completed therapy and 3 remained on treatment (**Fig. 1**). The most common reason for study discontinuation was disease progression ($n = 27$); 5 patients were in follow-up at the data cutoff and withdrew consent for follow-up [one was being treated as standard of care outside the study site and did not want to travel for follow-up; the other withdrew during cycle 1 as she found the toxicities intolerable, although most adverse events (AE) were considered unrelated to study medications]. Objective response rates were 0% in the platinum-sensitive cohort,

Table 1. Baseline patient characteristics.

Characteristic	Cohort 1: platinum sensitive ($n = 11$)	Cohort 2: platinum resistant ($n = 10$)	Cohort 3: exploratory ($n = 13$)
Age, years	56 (51–66)	57 (51–64)	59 (55–70)
ECOG PS			
0	1 (9)	3 (30)	3 (23)
1	10 (91)	7 (70)	10 (77)
High-grade serous histology	11 (100)	10 (100)	13 (100)
Cancer origin			
Ovarian	11 (100)	7 (70)	13 (100)
Primary peritoneal	0	2 (20)	0
Fallopian tube	0	1 (10)	0
BRCA mutation			
Germline	*BRCA1*: 6 (55) *BRCA2*: 3 (27)	*BRCA1*: 5 (50) *BRCA2*: 1 (10)	*BRCA1*: 3 (23) *BRCA2*: 1 (8)
Somatic	*BRCA1*: 1 (9)	—	*BRCA1*: 1 (8)
FIGO stage			
II	0	1 (10)	0
III	6 (55)	5 (50)	9 (69)
IV	0	0	2 (15)
Unknown	5 (45)	4 (40)	2 (15)
Platinum sensitivity			
Sensitive	11 (100)	0	2 (15)
Resistant	0	10 (100)	11 (85)
Median number of prior regimens	3 (27)	5 (50)	6 (46)
Prior PARPi (first PARPi)			
Maintenance	8 (73)	1 (10)	4 (31)
Treatment	3 (27)	8 (80)	8 (62)
Both	0	1 (10)	1 (8)
Type of prior PARPi (first PARPi)			
Olaparib	7 (64)	2 (20)	5 (38)
Niraparib	0	2 (20)	2 (15)
Rucaparib	4 (36)	5 (50)	6 (46)
Veliparib	0	1 (10)	0
Number of prior PARPis			
1	10 (91)	10 (100)	8 (62)
2	1 (9)	0	5 (38)
Prior antiangiogenic	0	2 (20)	4 (31)

Note: All data are number (%) or median (interquartile range).
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; FIGO, International Federation of Obstetrics and Gynecology.

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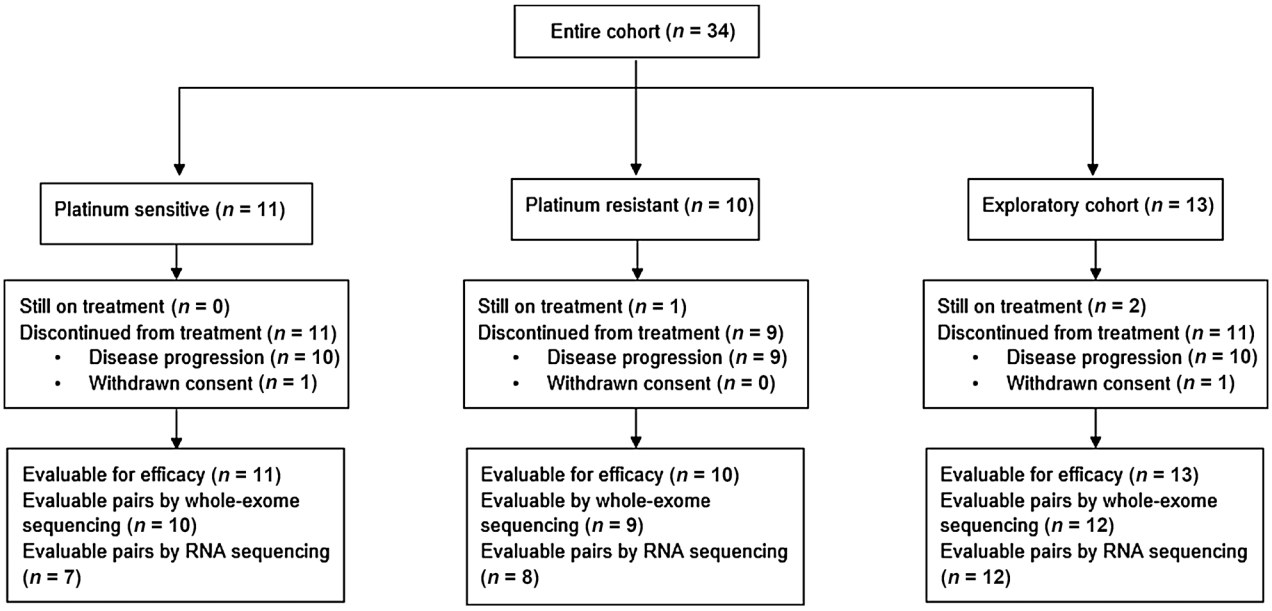

Figure 1.
EVOLVE trial consort diagram showing subject flow through each cohort and stage of phase II trial.

20% in the platinum-resistant cohort, and 8% in the exploratory cohort (**Table 2**). Corresponding 16-week PFS rates were 55%, 50%, and 39%, respectively.

Treatment was well tolerated; AEs were as expected and manageable. The most common all-grade AEs were diarrhea (68%), nausea (53%), fatigue (44%), and vomiting (44%), typically grade 1/2 (**Table 3**). Overall, 38% of patients experienced grade 3 AEs, most commonly diarrhea (12%) and anemia (9%). Grade 3 thrombocytopenia and neutropenia were both absent. There was one grade 4 AE (myelodysplastic syndrome in a patient with germline *BRCA1* mutation; prior treatments included two lines of platinum-based chemotherapy and maintenance olaparib for 21 months) and no grade 5 AEs. Supplementary Table S1 shows cediranib-related AEs.

Cediranib dose reductions and interruptions were implemented in 12% and 53% of patients, respectively. Corresponding percentages for olaparib were 15% and 47%. The median duration of dose interruption was 14.5 days for cediranib and 21 days for olaparib. There were no toxicity-related treatment discontinuations.

Exome data were generated from archival samples (before PARPi) for 33 patients, baseline samples (after progression on PARPi) for 32 patients, and from paired archival and baseline samples for 31 patients. This paired-sample cohort was used to interrogate acquired resistance mechanisms. Paired archival and baseline RNA sequencing data ($n = 27$) were used to examine changes in gene expression.

TP53 variants were detected in 30 of 33 archival samples (29/31 paired; **Fig. 2**), consistent with HGSOC histology. Pathological review by gynecologic pathology experts confirmed high-grade serous morphology for the *TP53*-negative cases. The *TP53* locus was also reviewed manually to rule out false negatives. *BRCA1/2* alterations were present in 21 patients (**Table 1**); of these, 20 harbored truncating *BRCA1/2* variants and 5 (two platinum-sensitive, three platinum-resistant) had secondary reversion mutations in matched progression tumors (**Fig. 2**; Supplementary Fig. S1). Across a set of 17 homologous

Table 2. Treatment responses in the intention-to-treat population.

Parameter	All patients ($n = 34$)	According to study cohort			According to platinum sensitivity	
		Cohort 1 ($n = 11$)	Cohort 2 ($n = 10$)	Cohort 3 ($n = 13$)	Sensitive ($n = 13$)	Resistant ($n = 21$)
Objective response	3 (9)	0	2 (20)	1 (8)	0	3 (14)
Complete response	0	0	0	0	0	0
Partial response	3 (9)	0	2 (20)	1 (8)	0	3 (14)
Disease control	23 (68)	9 (82)	6 (60)	8 (62)	11 (85)	12 (57)
Stable disease	20 (59)	9 (82)	4 (40)	7 (54)	11 (85)	9 (43)
Progressive disease	8 (24)	2 (18)	4 (40)	2 (15)	2 (15)	6 (29)
Unevaluable	3 (9)	0	0	3 (23)	0	3 (14)
CA-125 response	5 (15)	2 (18)	1 (10)	2 (15)	2 (15)	3 (14)
16-Week PFS	47 (33–67)	55 (32–94)	50 (27–93)	39 (19–77)	54 (33–89)	43 (26–70)
1-Year OS	—	82 (62–100)	69 (45–100)	40 (18–92)	83 (65–100)	52 (33–82)

Note: All data are number (%) or % (95% CI).
Abbreviations: CA-125, cancer antigen-125.

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Table 3. Patients with AEs considered possibly, probably, or definitely treatment related (intention-to-treat population, $n = 34$).

	Grade				
AE	**1**	**2**	**3**	**4**	**Any**
Diarrhea	16 (47)	3 (9)	4 (12)	0	23 (68)
Nausea	15 (44)	3 (9)	0	0	18 (53)
Fatigue	11 (32)	3 (9)	1 (3)	0	15 (44)
Vomiting	9 (26)	4 (12)	2 (6)	0	15 (44)
Hypertension	1 (3)	5 (15)	2 (6)	0	8 (24)
Anemia	1 (3)	2 (6)	3 (9)	0	6 (18)
Generalized muscle weakness	2 (6)	3 (9)	1 (3)	0	6 (18)
Neutrophil count decreased	5 (15)	1 (3)	0	0	6 (18)
Anorexia	3 (9)	2 (6)	0	0	5 (15)
Creatinine increased	5 (15)	0	0	0	5 (15)
QTc interval prolonged	3 (9)	2 (6)	0	0	5 (15)
Hoarseness	5 (15)	0	0	0	5 (15)
Mucositis oral	3 (9)	2 (6)	0	0	5 (15)
Proteinuria	3 (9)	2 (6)	0	0	5 (15)
Constipation	3 (9)	1 (3)	0	0	4 (12)
Headache	4 (12)	0	0	0	4 (12)
White blood cell decreased	1 (3)	3 (9)	0	0	4 (12)
Alanine aminotransferase increased	2 (6)	0	1 (3)	0	3 (9)
Aspartate aminotransferase increased	1 (3)	1 (3)	1 (3)	0	3 (9)
Myalgia	1 (3)	0	1 (3)	0	2 (6)
Peripheral sensory neuropathy	1 (3)	0	1 (3)	0	2 (6)
Dehydration	0	0	1 (3)	0	1 (3)
Ejection fraction decreased	0	0	1 (3)	0	1 (3)
Myelodysplastic syndrome	0	0	0	1 (3)	1 (3)
Thromboembolic event	0	0	1 (3)	0	1 (3)

Note: All data are number (%). Grade 1/2 events that occurred in ≥10% of the patients and any grade 3/4 events are included.

recombination deficiency genes covered by exome sequencing (*ATM, ATR, EMSY, BRIP1, CHEK1, CHEK2, RAD50, RAD51C, RAD51D, BRIP1, BARD1, RAD50, FAM175A, NBN, PALB2, PTEN*, and *MRE11*), we observed a single *RAD51B* mutation reversion whereby an 18 bp in-frame deletion detected in the archival specimen was absent in the baseline sample. Overall, patients with homologous recombination gene (*BRCA1/2* or *RAD51B*) reversions showed markedly worse PFS (median 1.9 months) than those with mutated or wild-type homologous recombination genes (4.8 and 6.4 months, respectively; **Fig. 3**). Patients with *BRCA1/2* reversions invariably stopped treatment early (within 2 months from initiation) because of disease progression (**Fig. 4**). We also observed *BRCA1/2* amplification or overexpression in three baseline samples, suggesting functional restoration of homologous recombination deficiency; this was not associated with treatment response (**Fig. 4**). Furthermore, expression of other homologous recombination genes changed over time (Supplementary Fig. S2). Examination of other known intrinsic or acquired mechanisms of PARPi resistance (31–34) revealed *CCNE1* amplification/overexpression in 11 of 31 paired cases (three in both archival and baseline, seven in baseline alone, one in archival alone; **Fig. 2**; Supplementary Fig. S3). We also reviewed loss of *TP53BP1* function in *BRCA1/2*-mutant cases and perturbations in *ABCB1, SLFN11*, and *DYNLL1* expression in baseline versus archival samples. Although no *TP53BP1* and *DYNLL1* loss was detected, *ABCB1* was overexpressed in four baseline samples and amplified in one, whereas *SLFN11* was significantly downregulated in two baseline samples.

Previously described or proposed mechanisms of PARPi resistance were detected in 55% of evaluable progression tumors in this study. Mechanisms were similarly distributed across all three cohorts (**Fig. 2**). Upregulation of multidrug efflux pump gene *ABCB1* was indicative of worse PFS and OS (**Fig. 3**). Baseline samples were enriched with gene fusions versus archival samples (Supplementary Fig. S4), consistent with increased genome instability following treatment cycles administered before enrollment into EVOLVE.

To supplement the gene-centric approach, we used pathway enrichment methods to identify change over time in expression patterns. We focused on DNA repair and angiogenesis gene sets using gene set variation analysis to calculate an enrichment score for each sample. Comparison of archival and baseline samples revealed a significant increase in the angiogenic pathway during treatment, except in patients previously treated with antiangiogenic therapy (Supplementary Fig. S5). Progression biopsies from 2 patients showed upregulation of the angiogenesis pathway compared with the corresponding archival specimen.

Discussion

Cediranib–olaparib combination therapy showed some activity after progression on prior PARPi in all three cohorts, despite heavy pretreatment in most patients. The combination was well tolerated, and the safety profile was consistent with previous findings (18). AEs were manageable with early intervention, using loperamide and antihypertensive therapy as required. Only 4 patients required a cediranib dose reduction and 5 required an olaparib dose reduction; no patients discontinued treatment because of toxicity. The observed tolerability of the combination may be related to the cediranib starting dose of 20 mg. In the phase II trial of cediranib–olaparib in PARPi-naïve ovarian cancer, patients received a lower olaparib dose (200 mg twice daily capsules) but a higher cediranib dose (30 mg once daily) than in our study (18). However, at this dose, the drug-related AEs

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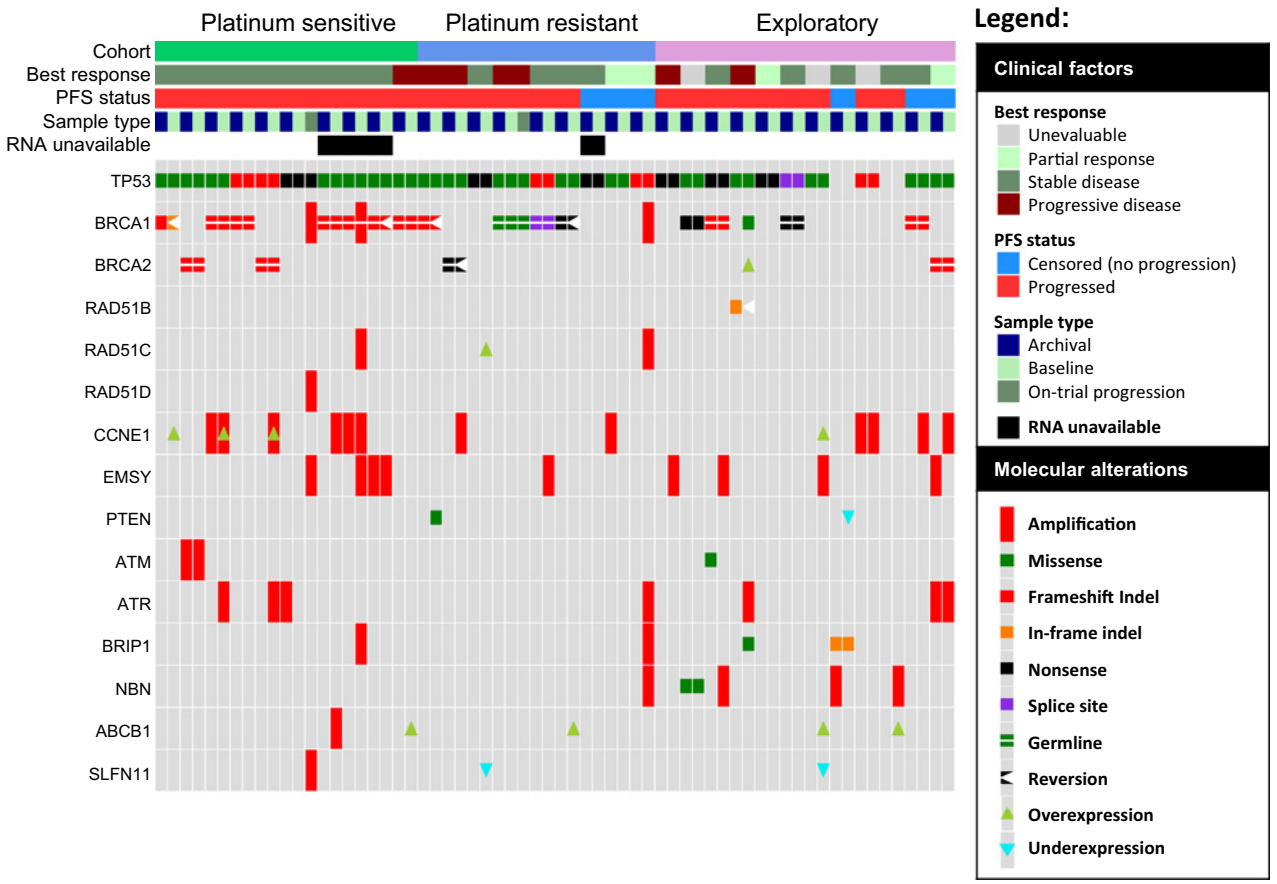

Figure 2.

Acquired mechanisms of PARPi resistance. Overexpression and underexpression are defined as >4-fold change up or down from archival sample levels, respectively. Genomic data visualizations were generated using the ComplexHeatmap package (version 1.99.4) in R (version 3.5.1; ref. 29). Indel, insertion–deletion.

were more common in the cediranib–olaparib arm than with olaparib alone; 70% of patients experienced a grade ≥3 AE, particularly diarrhea and fatigue (18). In addition, 77% of patients in the cediranib–olaparib treatment group required dose reductions compared with 24% receiving olaparib alone. In our trial evaluating PARPi rechallenge, we were cautious about potential AEs and used a cediranib dose of 20 mg, which demonstrated some activity in the recently reported BAROCCO trial (20).

One described mechanism of drug resistance involves reversion of the original truncating mutations in *BRCA1/2* genes, restoring functional protein expression and the ability of cancer cells to repair DNA by homologous recombination (35, 36). Reversion mutations in *BRCA1/2* and *RAD51C/D* are associated with primary and acquired resistance to the PARPi rucaparib (37, 38). In our study, 5 patients had *BRCA1/2* reversion mutations at the time of progression on a PARPi, which correlated with poor outcome. However, based on our study design, we cannot discern whether this resistance mechanism was acquired during platinum treatment or *de novo* because of PARPi as the pre-PARPi sample was the archival tissue. In the ARIEL2 study, secondary somatic mutations restoring *RAD51C* and *RAD51D* were associated with acquired resistance to the PARPi rucaparib (37), but this was not detected in EVOLVE. Instead, we observed *RAD51B* reversion mutation in one post-PARPi progression sample. We hypothesize that during PARPi and/or platinum treatment, a *RAD51B* wild-type clone emerged selectively. Interestingly, *RAD51C* was significantly overexpressed in the baseline biopsy sample after progression on a PARPi (Supplementary Fig. S2). Preclinical evidence shows that *RAD51C*-deficient cancer cells are sensitive to PARPi (39), and loss of *RAD51C* promoter hypermethylation may lead to PARPi resistance (40). Recently, a study showed that homozygous or hemizygous BRCA1 methylation predicts rucaparib clinical response, and that loss of *BRCA1* methylation after exposure to chemotherapy disables a therapeutic mechanism of response (41). Therefore, the *RAD51C* overexpression seen in our study may contribute to acquired PARPi resistance.

These mechanisms of acquired resistance in HGSOC with reversion mutations in the homologous recombination genes *BRCA1/2* and *RAD51* effectively restore homologous recombination DNA repair and confer clinical resistance to PARPis (37, 38, 42) and platinum (43). These mechanisms can be shared between platinum and PARPi resistance. Our study showed that these homologous recombination gene reversion mutations confer poor prognosis and cannot be overcome by adding cediranib to olaparib. This finding leads us to suggest incorporating detection of reversion mutations in homologous recombination DNA repair genes for patient selection or as a stratification factor for future post-PARPi clinical trials. With the

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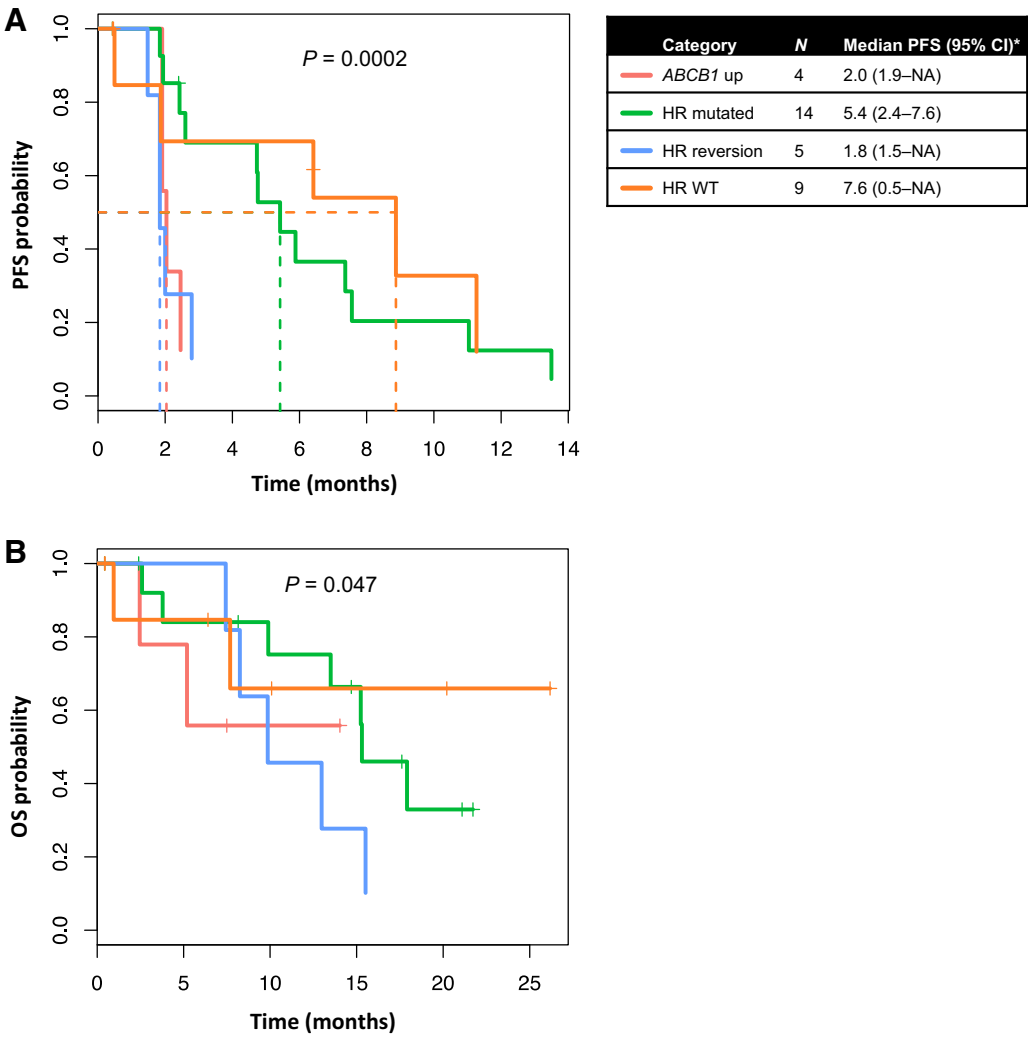

Category	N	Median PFS (95% CI)*
ABCB1 up	4	2.0 (1.9–NA)
HR mutated	14	5.4 (2.4–7.6)
HR reversion	5	1.8 (1.5–NA)
HR WT	9	7.6 (0.5–NA)

Figure 3.

Efficacy in patients according to mutation status [*ABCB1* upregulated vs. homologous recombination mutated vs. mutations in homologous recombination pathway genes (*BRCA1, BRCA2, RAD51B*) vs. wild-type homologous recombination pathway genes]. One patient with a *BRCA1* reversion and *ABCB1* upregulation is included in the subgroup *ABCB1* upregulated. **A,** PFS. **B,** OS. *The upper bound of some 95% CIs for median PFS could not be accurately estimated due to small sample number. HR, homologous recombination; NA, not applicable; WT, wild-type.

successful incorporation of liquid biopsies into clinical practice, circulating tumor DNA analysis of reversion mutations and potentially promoter methylation of homologous recombination deficiency genes should be a valuable tool for disease monitoring and early management of emerging resistant clones.

Another acquired mechanism of resistance to PARPis involves upregulation of drug efflux pumps encoded by genes such as *ABCB1* [multidrug resistance 1 (MDR1)]. Overexpression of MDR1 often leads to cross-resistance to structurally unrelated drugs (32). In our study, we observed four cases of *ABCB1* overexpression (15% of 27 evaluable for RNA sequencing) and one case of DNA amplification that did not lead to transcript upregulation. With our technical methodology, we were unable to determine whether *ABCB1* upregulation was a consequence of structural rearrangements, a recently described mechanism of *ABCB1* upregulation in HGSOC (44, 45). Interestingly, 2 patients with *ABCB1* upregulation also had other mechanisms of PARPi resistance, suggesting that multiple cellular pathways act in parallel to confer clinical drug resistance at progression on PARPis.

Our study also shows an increased burden of gene-fusion transcripts in the post-PARPi setting, suggesting enhanced genomic instability in these tumors. This preliminary observation warrants further investigation in a larger dataset as targeting these changes may present a new therapeutic opportunity. *CCNE1* amplification has been observed at progression on PARPis, and although not considered as a drug resistance mechanism *per se* (32), given trends toward mutual exclusivity with *BRCA1/2* alterations due to synthetic lethality (46), it is commonly considered a surrogate biomarker of poor response to platinum (32, 44). However, we observed more *CCNE1* amplifications in *BRCA1/2*-mutated than *BRCA1/2* wild-type tumors (33% vs. 15%, respectively), and in platinum-sensitive than platinum-resistant patients (36% vs. 20%, respectively). The most likely explanation for these discrepancies versus previous reports is the extensive pretreatment and selection criteria in our study. *CCNE1* amplifications were

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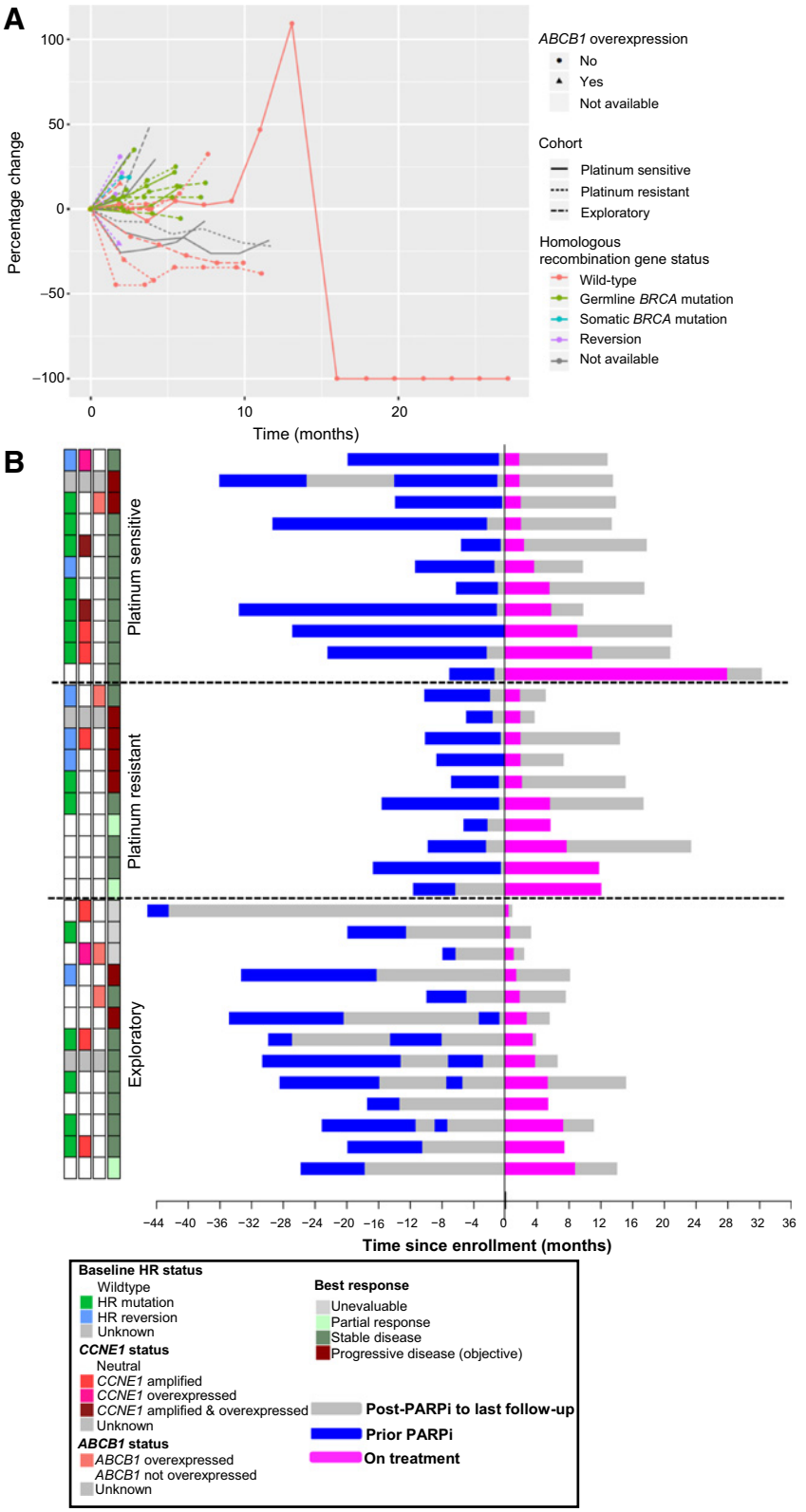

Figure 4.

Mechanisms of PARPi resistance mapped to clinical outcome. Three *CCNE1* patients were still on treatment at the last date of follow-up. **A,** Change in tumor size over time according to *ABCB1* overexpression, homologous recombination gene status, and cohort. **B,** Each bar represents 1 patient in the study from first PARPi treatment to last date of follow-up. Time zero represents study entry; duration of prior PARP inhibitor and on trial are in blue and pink, respectively. Identified resistance mechanisms were used to annotate the spider plot (30), showing the longitudinal percentage change from baseline in tumor size, and a bar chart showing time to progression (27). HR, homologous recombination.

detected in only 12% of archival (treatment-naïve) samples, probably because eligible patients had previously responded to platinum therapy, as required for access to prior PARPi. Intriguingly, when baseline samples were examined, *BRCA1/2*-deficient patients lacking a subsequent reversion mutation but who also had *CCNE1* amplifications experienced a significant improvement in PFS versus all other patients (median, 7.6 vs. 2.5 months, respectively; Supplementary Fig. S3). This suggests that these patients have at least partial activity of this synthetic lethality phenotype, despite their long treatment history. Resistance to PARPis can also be driven by *SLFN11* inactivation, which may open specific treatment options, as suggested in a preclinical model where resistance to PARPis was overcome by ATR inhibition (47).

We observed increased enrichment of the angiogenesis pathway in samples taken after progression on a PARPi, whereas patients who had received prior antiangiogenic therapy showed the opposite effect, with post-PARPi samples showing decreased pathway enrichment (Supplementary Fig. S4). Although we observed no significant relationship between angiogenesis pathway enrichment and on-trial response, this could be due to potential confounders and small sample sizes. Thus, findings from this study would benefit from a randomized design and larger sample size in the postcombination setting to understand better how the various involved mechanisms affect response or benefit from this combination.

One of the main limitations of our study is the small sample size, albeit this is, to the best of our knowledge, the largest prospective translational study with paired archival and baseline biopsies characterizing resistance mechanisms after PARPi. These results should be considered as hypothesis generating and merit further exploration in a larger prospective trial. Translational findings from this study may also be affected by tumor heterogeneity. As described by Patch and colleagues (44), there is considerable tumor heterogeneity in HGSOC within and between metastatic sites. The site for biopsy post PARPi was the progressive lesion, but this was not necessarily the same site as the archival (pre-PARPi) tissue.

In conclusion, our study suggests that the cediranib–olaparib combination is tolerable with some activity in women with ovarian cancer following PARPi failure. Our data also hint that patients with reversion mutations in homologous recombination genes, and/or upregulated *ABCB1*, should perhaps be considered for other options to overcome their aggressive disease transformation. This observation in a small study warrants further evaluation. Several additional mechanisms of PARPi resistance have been observed, leading to potential therapeutic opportunities targeting these acquired new vulnerabilities.

Disclosure of Potential Conflicts of Interest

S. Lheureux is a paid consultant for AstraZeneca and GlaxoSmithKline and reports receiving speakers bureau honoraria from Roche and Merck. A. Oaknin reports receiving speakers bureau honoraria from Roche, AstraZeneca, PharmaMar, Clovis Oncology, Immunogen, and Genmab. J.P. Bruce is an unpaid consultant/advisory board member for Bowhead Health. N.C. Dhani reports receiving speakers bureau honoraria from AstraZeneca. V. Bowering reports receiving speakers bureau honoraria from AstraZeneca. L. Fariñas-Madrid reports receiving speakers bureau honoraria from MSD, Roche, and AstraZeneca, and is an unpaid consultant/advisory board member for Tesaro. A.M. Oza is an unpaid consultant/advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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EVOLVE: A Multicenter Open-Label Single-Arm Clinical and Translational Phase II Trial of Cediranib Plus Olaparib for Ovarian Cancer after PARP Inhibition Progression

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