Phase 1 Combination Study of the CHK1 Inhibitor Prexasertib and the PARP Inhibitor Olaparib in High-grade Serous Ovarian Cancer and Other Solid Tumors

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ABSTRACT

Purpose: Checkpoint kinase 1 (CHK1) plays a central role in the response to replication stress through modulation of cell-cycle checkpoints and homologous recombination (HR) repair. In BRCA-deficient cancers with *de novo* or acquired PARP inhibitor resistance, the addition of the CHK1 inhibitor prexasertib to the PARP inhibitor olaparib compromises replication fork stability, as well as HR proficiency, allowing for sensitization to PARP inhibition.

Patients and Methods: This study followed a 3+3 design with a 7-day lead-in of olaparib alone, followed by 28-day cycles with prexasertib administered on days 1 and 15 in combination with an attenuated dose of olaparib on days 1–5 and 15–19. Pharmacokinetic blood samples were collected after olaparib alone and following combination therapy. Patients enrolled to the expansion phase of the study underwent paired tumor biopsies for pharmacodynamic (PD) assessments.

Results: Twenty-nine patients were treated. DLTs included grade 3 neutropenia and grade 3 febrile neutropenia. The MTD/recommended phase 2 dose (RP2D) was prexasertib at 70 mg/m^2 i.v. with olaparib at 100 mg by mouth twice daily. Most common treatment-related adverse events included leukopenia (83%), neutropenia (86%), thrombocytopenia (66%), and anemia (72%). Four of 18 patients with *BRCA1*-mutant, PARP inhibitor–resistant, high-grade serous ovarian cancer (HGSOC) achieved partial responses. Paired tumor biopsies demonstrated reduction in RAD51 foci and increased expression of γ-H2AX, pKAP1, and pRPA after combination exposure.

Conclusions: Prexasertib combined with olaparib has preliminary clinical activity in *BRCA*-mutant patients with HGSOC who have previously progressed on a PARP inhibitor. PD analyses show that prexasertib compromises HR with evidence of induction of DNA damage and replication stress.

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Introduction

Cell-cycle checkpoints serve to halt the progression of the cell cycle in response to DNA damage, allowing time for DNA repair and maintenance of genomic integrity. Checkpoint kinase 1 (CHK1) is a key regulator of DNA replication and cell-cycle progression (1). Activation of CHK1 in response to DNA damage results in cell-cycle arrest at the S and G_2 checkpoints, due to its role in regulation of CDK2 and CDK1 activities, respectively, allowing time for DNA repair. Phosphorylated CDK1 arrests the cell cycle in G_2–M, whereas phosphorylated CDK2 blocks initiation of DNA origins of replication during S phase. In addition to its roles in DNA replication and checkpoint control of the cell cycle, CHK1 has been shown to activate proteins involved in homologous recombination (HR) repair (e.g., BRCA2, RAD51, FANCE, and FANCD2; refs. 2–4) and is also involved in replication fork progression (5), as well as in the cellular response to replication stress (6).

Prexasertib is a selective small-molecule inhibitor of CHK1 (IC_{50} 1 nmol/L) and CHK2 (IC_{50} 8 nmol/L) that has been shown to induce DNA damage and replication catastrophe through CHK1-dependent mechanisms in preclinical models (7). Early Phase 1 clinical studies established the recommended phase 2 monotherapy dose (RP2D) at 105 mg/m^2 administered intravenously on a biweekly schedule, with preliminary antitumor activity seen at the 80 mg/m^2 dose (8). Additional activity has been reported in *BRCA* wild-type high-grade serous ovarian cancers (HGSOC) at the RP2D (9).

PARP inhibitors have established activity in *BRCA*-mutated and other HR repair–deficient cancers, acting through synthetic lethality (10). With the approval of PARP inhibitors and widespread use in the maintenance setting, resistance to PARP inhibitors has emerged, leading to treatment failure. Mechanisms of resistance include restoration of HR repair or stabilization of replication forks (11, 12). Emerging data show that both restored HR and replication fork stabilization can be overcome by cell-cycle checkpoint inhibition, including inhibition of ATR, CHK1, and WEE1 kinases (13). We previously demonstrated that prexasertib-mediated CHK1 inhibition sensitizes PARP inhibitor–resistant HGSOC cell lines and patient-derived xenograft models to PARP inhibition by these mechanisms (14). This therapeutic strategy of combining CHK1 inhibition with PARP inhibition represents a novel approach to enhance

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Resistance to PARP inhibition has emerged as a therapeutic barrier in the treatment of high-grade serous ovarian cancer (HGSOC). Mechanisms of resistance, including restoration of homologous recombination (HR) repair or stabilization of replication forks, can be overcome by checkpoint kinase 1 (CHK1) inhibition, which disrupts restored HR and destabilizes replication forks. Here, we report the safety and recommended phase 2 dose of the CHK1 inhibitor prexasertib combined with olaparib. Analysis of paired biopsies after combination treatment provided pharmacodynamic proof-of-mechanism, demonstrating CHK1-mediated modulation of HR repair via reduction in RAD51 focus formation and induction of replicative stress with increased pRPA staining. These effects were associated with increased DNA damage, assessed by γ-H2AX and pKAP1 expression. The prexasertib/olaparib combination demonstrated preliminary antitumor activity in patients with platinum- and PARP inhibitor–resistant HGSOC. This experience will inform further development of combination strategies directed at reversing PARP inhibitor resistance to enhance antitumor activity of these classes of agents.

antitumor activity of these classes of agents. On the basis of these preclinical data, we conducted a Phase 1 study of the combination of prexasertib and olaparib in HGSOCs and other solid tumors.

Patients and Methods

Ethics approval and consent to participate

The Dana-Farber Cancer Institute institutional review board approved the study, and participants provided written informed consent before study entry. The study was monitored by the Data Safety Monitoring Committee at Dana-Farber/Harvard Cancer Center and conducted in accordance with the ethical guidelines in accordance of the Declaration of Helsinki.

Study population

Patients with advanced solid tumors without approved curative therapy or effective palliative therapy, ≥18 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status 0–1, and measurable disease per RECIST version 1.1 (15) were eligible for the study. Patients enrolled to the expansion cohort had HGSOC or fallopian tube cancer with documented *BRCA1* or *BRCA2* mutation. All patients were required to have adequate organ function defined by: white blood cell count $\geq 3.0 \times 10^9$/L, absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, hemoglobin ≥10 g/dL, total bilirubin ≤1.5 × institutional upper limit of reference range (ULRR), aspartate aminotransferase and alanine aminotransferase ≤2.5 × ULRR (≤5 × ULRR in the setting of liver metastases), creatinine ≤1.5 × institutional ULRR or creatinine clearance ≥60 mL/min by Crockcroft–Gault formula, and QTc ≤ 470 msec on screening ECG. Subjects were required to have completed previous cancer treatment at least 3 weeks before study entry (6 weeks for nitrosureas or mitomycin C). Prior treatment with a PARP inhibitor was permitted; patients enrolled to the expansion phase of the study were required to have received at least 6 months of prior PARP inhibitor and derived clinical benefit. Intervening treatment between prior PARP inhibitor exposure and initiation of this trial was permitted. Prior CHK1 inhibitor treatment was excluded. Exclusion criteria included gastrointestinal processes that would interfere with olaparib absorption, untreated brain metastases or carcinomatous meningitis, pregnancy or lactation, active infection, active Hepatitis B or C, or HIV status requiring combination antiretroviral therapy due to potential pharmacokinetic (PK) interactions. In addition, evidence of pneumonitis on screening baseline imaging or prior history of myelodysplastic syndrome (MDS) or acute myelogenous leukemia were excluded on the basis of known toxicities associated with olaparib.

Study design and treatment administration

This was a single-institution investigator-initiated study. The primary objective was to determine the safety and tolerability of the combination. Secondary objectives were to assess preliminary antitumor activity and explore pharmacodynamics (PD) markers of target engagement and combinatorial proof-of-mechanism in paired tumor biopsies in a cohort of *BRCA*-mutant ovarian cancers with prior progression on a PARP inhibitor. This study followed a 3 + 3 dose-escalation design with a 7-day lead-in of olaparib alone (cycle 0), followed by prexasertib administered intravenously on days 1 and 15 of a 28-day cycle in combination with olaparib administered orally twice daily at the same dose as the lead-in dose. The 7-day lead-in was incorporated to accommodate PK and PD evaluation of olaparib in the absence and presence of prexasertib. On the basis of expected neutropenia and anemia for olaparib and published hematologic toxicities of prexasertib monotherapy where 73.3% of patients experienced grade 4 neutropenia (8), we chose to start at doses below the monotherapy RP2D for the individual agents in anticipation of overlapping hematologic toxicities. The first dose level evaluated prexasertib at 80 mg/m^2 and continuous dosing of olaparib at 200 mg twice daily (Supplementary Fig. S1). On the basis of prolonged grade 4 neutropenia lasting more than 7 days experienced by all participants enrolled to this administration schedule, accrual was temporarily halted, and the protocol was revised to accommodate an intermittent dosing schedule of olaparib on days 1–5 and 15–19 in combination with prexasertib on days 1 and 15 of a 28-day cycle. This strategy of attenuated olaparib dosing was previously used during development in combination with carboplatin due to overlapping hematologic toxicities (16). Filgrastim was permitted in subsequent cycles if cycle 1 absolute neutrophil count was less than 1.5×10^9/L, necessitating a treatment delay.

Dose-limiting toxicity definitions and study assessments

Safety was assessed via monitoring of toxicities during the lead-in cycle 0 + cycle 1 according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4.03. Dose-limiting toxicities (DLT) were defined as previously described (17), including grade 4 neutropenia >5 days despite growth factor support or febrile neutropenia, grade 4 thrombocytopenia, and any grade 3–4 non-hematologic toxicities related to study drug and occurring during the lead-in and/or cycle 1. Grade ≥3 nausea, vomiting, diarrhea, or rise in creatinine were considered dose limiting if refractory to management and not improved to grade 1 within 48 hours. Inability to tolerate 80% of olaparib and prexasertib during the lead-in and cycle 1 was considered dose limiting. After review of tolerability for each cohort dose schedule by the Safety Review Committee in conjunction with both drug manufacturers, a single-dose schedule was selected as the RP2D for further planned enrollment. An expansion cohort of up to 10 patients was planned at the RP2D to further evaluate safety, tolerability, and PD endpoints. During enrollment to the expansion cohort, the study was amended to remove the lead-in of olaparib to avoid unnecessary exposure of patients with PARP inhibitor–resistant

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Table 1. Patient demographics.

Patient demographics	
Patients enrolled/treated	29
Sex	
Male	2
Female	27
Median age in years (range)	62 (32–80)
Mean number of prior therapies (range)	6 (2–10)
Prior PARP inhibitor	19
Mean duration on first-line PARP inhibitor (range in months)	10 (2–24)
Mean duration on second-line PARP inhibitor (range in months); $n = 7$	1.8 (1–4)
Average time from previous PARP exposure (range in months)	17 (1–60)
Diagnoses:	
Ovarian/fallopian tube carcinoma	23
Soft tissue sarcoma (including leiomyosarcoma)	3
Colorectal carcinoma	1
Prostate carcinoma	1
Estrogen receptor–positive breast carcinoma	1

tumors to monotherapy. The removal of the lead-in did not affect the ability to assess effects of prexasertib on DNA repair, DNA damage, and replication stress processes.

A physical examination and assessment of vital signs were performed at screening, start of treatment on C0D1, and on days 1 and 15 starting with cycle 1. Hematology and chemistry assessments were performed at screening, weekly during the first two cycles, and on days 1 and 15 starting with cycle 3. Electrocardiograms were performed at the start of each cycle. Radiologic assessments by CT or MRI were performed at screening and every 2 months during the first 6 cycles, and every 3 cycles thereafter if patients continued to receive treatment. For patients enrolled to the expansion cohort, tumor biopsies were performed on C0D2 after olaparib alone and on C1D16 after the combination of prexasertib and olaparib administration for the first 5 patients; after removal of the monotherapy lead-in, biopsies were performed pre-treatment and on C1D16 for the last 5 patients.

Pharmacokinetic assessments

Blood samples were collected in 4-mL plastic Vacutainer tubes with spray-coated sodium heparin tubes during cycle 0 and spray-coated sodium heparin and spray-dried EDTA tubes on cycles 1 and 2 at designated timepoints. Blood collection tubes were centrifuged to harvest the plasma that was stored in cryovials at $-80°C$. Prexasertib and olaparib plasma concentrations were quantified using a validated high-pressure liquid chromatography–mass spectrometry/mass spectrometry method. PK data were analyzed using population PK analyses.

PD assessments

Tumor biopsies were obtained on C0D2 and C1D16 in 5 patients and pre-treatment and C1D16 in 5 patients. Serial sections of formalin-fixed paraffin-embedded biopsies were stained for RAD51 foci as a marker of target engagement and surrogate marker for HR repair, along with geminin as a marker of $S–G_2$ transition to confirm decreases in RAD51 were not due to cycling of cells out of S phase (18). Tumor cells with greater than 5 foci in each nucleus were considered RAD51-foci positive based on prior validation studies for this assay (19). Depending on the tumor content of the specimen, 100–600 tumor cells were counted to determine the percentage of RAD51-foci–positive cells. Biopsies were also stained for γ-H2AX, pKAP1, and pRPA as markers of downstream DNA damage and replication stress, as previously described (14, 19, 20). To determine changes in γ-H2AX, the H-score was estimated on the basis of the formula: $[1 \times (\%\text{cells } 1+) + 2 \times (\%\text{cells } 2+) + 3 \times (\%\text{cells } 3+)]$. Cells with strong pan-nuclear stain were scored 3+, weak pan-nuclear stain and visible foci were scored 2+, visible foci (>5/nucleus) without pan-nuclear stain were scored 1+. Staining for pKAP1 and pRPA was qualitatively assessed.

Statistical analysis

No formal hypothesis testing was performed. Descriptive statistics (mean, median, standard deviation, minimum, maximum) were used to summarize continuous variables. Frequencies and percentages were used to summarize categorical variables. Statistical outputs were produced using SAS, version 9.4.

Results

Patient disposition and characteristics

A total of 29 patients were enrolled between April 11, 2017 and February 13, 2020, including a 10-patient expansion cohort of *BRCA1/2*-mutant patients with HGSOC at the MTD for evaluation of PD endpoints in paired tumor biopsies (**Table 1**). A total of 19 patients, including the 10-patient expansion cohort, had tumors harboring *BRCA1/2* mutations. One patient had colorectal cancer with germline *BRCA1* mutation; the remaining 18 patients had HGSOC. All but 2 of these patients with HGSOC had progressed within 6 months of first-line platinum-based therapy. Nineteen patients had received prior PARP, with an average of 17 months since the last dose of a PARP inhibitor, before enrolling on study. All patients who received at least one treatment were evaluable for safety, and 25 patients were evaluable for response by RECIST v1.1 (Supplementary Fig. S2). Three patients enrolled to the initial phase of the study with continuous dosing of olaparib were removed from the study during the first month of treatment at the treating physician's discretion due to prolonged myelosuppression lasting more than 7 days and requiring growth factor support. Four patients enrolled to the dose-escalation phase of the study with intermittent olaparib experienced clinical disease progression during the first cycle and were replaced for purposes of dose-declaration decisions. Two patients enrolled to the expansion phase of the study withdrew from further treatment after 4 and 2

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Table 2. Summary of AEs attributed to study treatment.

	Schedule 1 Prexasertib 80 mg/m^2 D1–15 Olaparib 200 mg BID continuous[b] (n = 3)				Schedule 1A Prexasertib 80 mg/m^2 D1–15 Olaparib 200 mg BID D1–5, 15–19 (n = 7)					Dose cohort Schedule -1A Prexasertib 70 mg/m^2 D1–15 Olaparib 100 mg BID D1–5, 15–19 (n = 19)					
Adverse events[a]	Gr 1	Gr 2	Gr 3	Gr 4	Gr 1	Gr 2	Gr 3	Gr 4	%	Gr 1	Gr 2	Gr 3	Gr 4	%	Avg. Total N (%)
Blood and lymphatic system disorders															
White blood cell count ↓	—	—	—	2	—	1	2	4	100	—	2	4	9	79	24 (83%)
Neutrophil count ↓	—	—	—	2	—	1	—	6	100	—	1	3	12	84	25 (86%)
Lymphocyte count ↓	1	1	1	—	2	2	3	—	100	3	7	3	1	74	24 (83%)
Platelet count ↓	1	—	1	—	1	2	1	—	57	9	2	1	1	68	19 (66%)
Anemia	—	1	—	—	2	4	1	—	100	1	7	5	—	68	21 (72%)
Gastrointestinal disorders															
Abdominal pain	—	—	—	—	—	1	—	—	14	1	1	—	—	11	3 (10%)
Diarrhea	—	—	—	—	—	—	—	—	0	4	3	—	—	37	7 (24%)
Dyspepsia	—	1	—	—	—	—	—	—	0	—	2	—	—	11	3 (10%)
Oral mucositis	—	—	—	—	—	—	—	—	0	3	—	—	—	16	3 (10%)
Nausea	2	—	—	—	1	1	—	—	29	4	—	—	—	21	8 (28%)
Vomiting	1	—	—	—	1	—	1	—	29	1	1	—	—	11	5 (17%)
Metabolism and nutrition															
Anorexia	—	—	—	—	2	—	—	—	29	2	—	—	—	11	4 (14%)
Hyponatremia	—	—	—	—	1	—	—	—	14	2	—	—	—	11	3 (10%)
Investigations															
AST ↑	—	—	—	—	—	—	—	—	0	3	—	—	—	16	3 (10%)
Creatinine ↑	—	—	—	—	1	—	—	—	14	2	—	—	—	11	3 (10%)
General/administration site															
Fatigue	2	—	—	—	4	1	—	—	71	8	—	—	—	42	15 (52%)
Nervous system															
Headache	1	—	—	—	2	—	—	—	29	2	—	—	—	11	5 (17%)
Musculoskeletal/connective tissue															
Arthralgia	—	—	—	—	—	2	—	—	29	1	—	—	—	5	3 (10%)

Abbreviations: AE, adverse event; AST, aspartate aminotransferase; BID, twice daily; Gr, grade.
[a]All AEs represent the number of patients experiencing the AE felt to be related to study treatment, reported as worst grade for each patient.
[b]Patients enrolled to the continuous olaparib dosing cohort had incomplete toxicity data due to removal from the study before completion of cycle 1.

cycles, respectively, due to hardship of travel; one was an international patient. All patients were off study at the time of data cutoff for this publication (December 31, 2020).

Adverse events

The most common adverse events (AE) attributed to treatment and occurring in ≥10% of patients are summarized in **Table 2**. A total of 1,306 AEs of any grade and any causality were captured for the trial. Of these AEs, 790 were attributed to treatment, and 624 (79%) of these events were grade 1/2. Most common treatment-related AEs included decreased white blood cell count (83%; n = 24), decreased neutrophil count (86%; n = 25), anemia (72%; n = 21), decreased platelet count (66%; n = 19), and fatigue (52%; n = 15). All three patients enrolled to the continuous dosing schedule for olaparib experienced DLTs with prolonged neutropenia lasting more than 7 days and required growth factor support. There were two DLTs on the intermittent dosing schedule at the dose level of olaparib 200 mg BID on days 1–5 and 15–19 in combination with prexasertib at 80 mg/m^2, with one event of febrile neutropenia and a second event of neutropenia precluding the patient from receiving 80% of the planned dose during cycle 1. On the basis of these events, the MTD/RP2D was considered to be prexasertib at 70 mg/m^2 i.v. with olaparib at 100 mg by mouth twice daily on the intermittent schedule.

PK analyses

Mean prexasertib exposures for patients enrolled to the 70 and 80 mg/m^2 dose levels were 717.4 ng/mL (range, 310–1,070 ng/mL) and 721.1 ng/mL (range, 327–1,090 ng/mL) at 60 minutes after dosing, respectively, within range of previously published data (Supplementary Fig. S3A; ref. 8). Steady-state PK parameters for olaparib alone versus combination therapy with prexasertib showed no statistical difference (Supplementary Fig. S3B). C_{max} for olaparib was 5,260 ng/mL in combination with prexasertib, consistent with previous reports based on 5 days of dosing (21).

PD analyses

Four of 10 patients enrolled to the expansion phase of the study had appropriate tumor content in paired tumor biopsies for analysis. Two had tumor biopsies obtained on C0D2 and C1D16 and two had tumor biopsies obtained pre-treatment and on C1D16. Notably, baseline levels of RAD51 foci, γ-H2AX, and pKAP1 staining were similar between the patients who had baseline biopsies pre-treatment compared with those who had baseline biopsies on C0D2 after initiation of the olaparib lead-in (**Fig. 1**). Two additional patients had unsuccessful on-treatment biopsies due to lack of tumor content within the sample, consistent with treatment response. Three patients were unable to undergo on-treatment biopsies due to thrombocytopenia, precluding our ability to pursue

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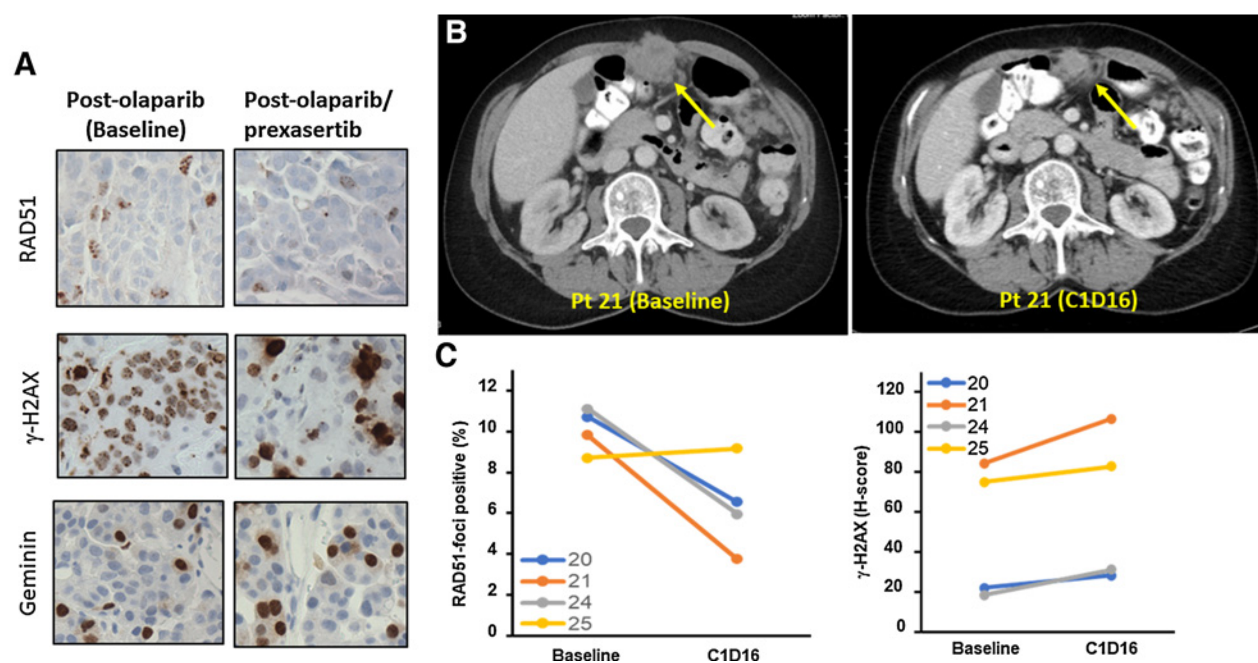

Figure 1.

Prexasertib suppresses DNA repair by HR in *BRCA1*-mutant patients with HGSOC. Shown are immunohistochemical staining profiles (**A**) with corresponding Coherence Tomography (CT) image of biopsied lesion (**B**). The addition of prexasertib shows reduction in RAD51 foci, as a surrogate marker of HR repair, with concurrent increase in γ-H2AX staining as a marker of DNA damage. Comparisons of RAD51 foci and γ-H2AX in four *BRCA*-mutant patients with HGSOC are shown in **C**.

safe sample collection. One patient experienced bradycardia as a complication of anesthesia during the scheduled pre-treatment biopsy and declined a repeat attempt of the procedure.

Sample images for IHC-staining of RAD51 foci, γ-H2AX, and geminin with corresponding radiologic response of the biopsied lesion in a *BRCA1*-mutant patient with HGSOC are shown in **Fig. 1A** and **B**. RAD51 foci decrease with the addition of prexasertib, consistent with compromise of HR, with concurrent increase in DNA damage as shown by increases in γ-H2AX. Geminin staining shows no significant changes in distribution of cells in S-phase. Increases in pKAP1 and pS3/4-RPA32 staining were also seen with the combination of prexasertib and olaparib (Supplementary Fig. S4). Of the 4 paired samples with appropriate tumor content, 3 had decreases in RAD51 foci with the addition of prexasertib; all 4 had increases in γ-H2AX (**Fig. 1C**).

Antitumor activity

Twenty-five of 29 patients (86%) had measurable disease and were evaluable for best response to therapy by RECIST v1.1. One patient with leiomyosarcoma harboring ATRX and DAXX alteration maintained a best response of stable disease (SD) and remained on study for 11 cycles. Twelve patients (41%) had disease progression during the first two cycles, including 5 of 10 patients with SD by tumor metrics but with clinical progression in non-measurable sites.

Ten of the 18 patients (56%) with *BRCA*-mutant, PARP inhibitor–resistant HGSOC remained on study for 4+ cycles. Four patients (22%) achieved a confirmed PR (**Fig. 2**). Of these, 3 maintained a PR for 9, 10, and 12 cycles; a 4th patient achieved a confirmed PR at 6 cycles but subsequently progressed after a treatment break and dose reduction.

Discussion

In this study, we show that prexasertib can be given safely in combination with an attenuated course of olaparib, and report preliminary antitumor activity with PD confirmation of target engagement and proof-of-principle of CHK1-mediated modulation of HR via reduction in RAD51 focus formation, along with induction of DNA damage.

Strategies aimed at reversing PARP inhibitor resistance through co-targeting of DNA repair mechanisms have been hindered by overlapping hematologic toxicities, requiring alternative dosing schedules to maintain efficacy (22). In this study, mechanism-based myelosuppression did require us to pursue an attenuated course of olaparib in combination with prexasertib. Although overlapping hematologic toxicities limited our ability to achieve the monotherapy RP2D of either olaparib or prexasertib, we were able to achieve biologically active combinatorial dosing and were able to demonstrate PD evidence of target engagement and antitumor activity at these doses. PK studies additionally confirmed no evidence of accumulation of prexasertib nor alteration of PKs when combined with an attenuated course of olaparib, when compared with historical controls, suggesting that PK interactions did not play a role in the observed heightened hematologic toxicities.

One AE of special interest observed in this study included a case of MDS occurring in a patient during the 90-day follow-up off-study period. This patient continued to experience prolonged anemia and thrombocytopenia off-study. Further workup and bone marrow assessment and karyotype confirmed del 5q MDS. This patient had previous exposure to olaparib for 24 months and was 16 months from her last olaparib dose before study entry. A retrospective review of the World Health Organization pharmacovigilance database estimates the

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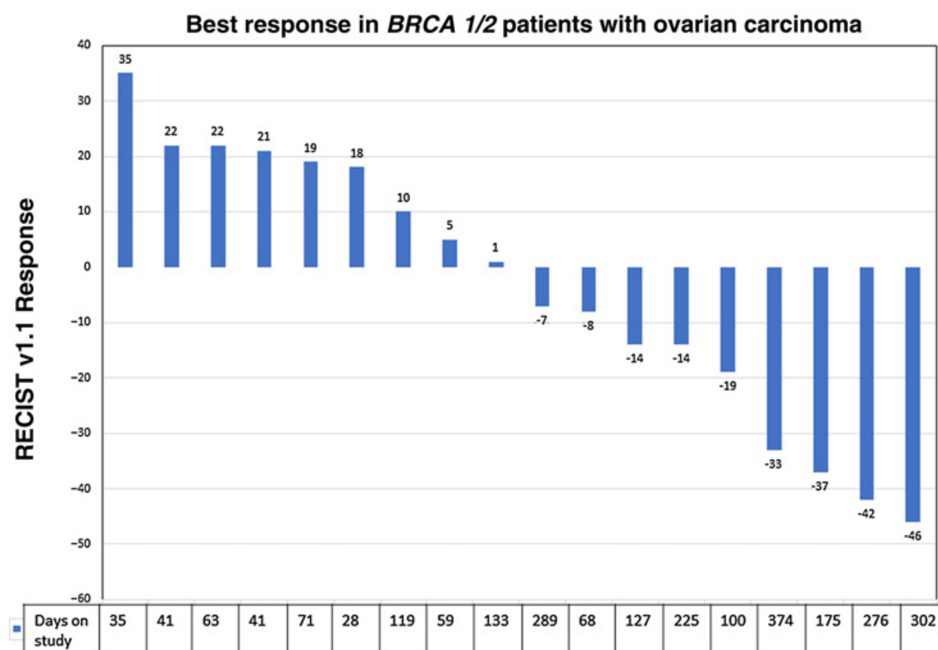

Figure 2.
Clinical response in *BRCA1/2*-mutant patients with ovarian cancer. Shown are the best responses by RECIST v1.1 for the 18 *BRCA*-mutant patients with ovarian cancer enrolled. Four patients achieved a confirmed partial response (PR). Ten of 18 patients remained on study past 4 cycles.

incidence of MDS/AML in patients treated with PARP inhibitors at 0.73% with a mean latency period from first exposure of PARP inhibitor of 17.8 months (23). Although latency and attribution to prior PARP inhibitor exposure is consistent with published reports, contribution from a second challenge of PARP inhibitor, additional exposure to a second DNA repair inhibitor, added with inherited risk factors such as germline *BRCA1* mutation could not be ruled out (24, 25).

Noteworthy were the responses seen in this study in heavily pretreated *BRCA1/2*-mutant patients with ovarian cancer who had previously progressed on PARP inhibitors; all but 2 of these patients had progressed within 6 months on first-line platinum-based therapy. In the four patients who had sufficient tumor content in biopsies for PD analysis, all four samples had >5% RAD51 foci at baseline, consistent with restoration of HR as a mechanism of resistance to PARP inhibition. In line with our previously published data (14), the addition of prexasertib resulted in compromised RAD51 focus formation and enhanced DNA damage as shown by increase in γH2AX and pKAP1 (**Fig. 1A**; Supplementary Fig. S4). Of these 4 patients, 2 had best response of −14% and a third had stabilization of disease with +5% in index lesions. A fourth patient had clinical progression with +19% increase in index lesions after 2 cycles. Unfortunately, of the patients who achieved PR, 2 had unsuccessful on-treatment biopsies consistent with treatment response and 2 were enrolled during the dose-optimization phase of the study where tumor biopsies were not mandated. The clinical responses seen in our study were not attributed to prexasertib activity alone, as previously published data for prexasertib monotherapy showed responses only in *BRCA* wild-type patients with HGSOC (9).

Additional responses of interest include a patient with leiomyosarcoma harboring ATRX/DAXX alteration who had progressed through 6 prior lines of chemotherapy during a 2-year period before study entry and maintained SD for 11 months on study. Loss of ATRX has been associated with alternative lengthening of telomeres in sarcoma (26) and has previously demonstrated sensitivity to ATR inhibitors in preclinical models (27). The ATRX/DAXX histone chaperone complex more recently has been identified as a key interactor with FANCD2 in HR repair and replication fork recovery (28). This is the first report of activity of CHK1 inhibition and PARP inhibition in a soft tissue sarcoma harboring ATRX/DAXX alteration. Further evaluation in defective DNA damage repair settings is warranted.

The results of this study highlight the challenges of development of combinatorial strategies and overlapping toxicities. The therapeutic strategy of combining CHK1 inhibition with an attenuated course of PARP inhibition represents a novel approach to enhance antitumor activity of these classes of agents while limiting toxicity to patients. The responses seen in this study support further evaluation in *BRCA*-mutant patients with ovarian cancer comparing PARP inhibitor–sensitive and PARP inhibitor–resistant settings, and other DNA damage repair alterations such as *ATRX* mutations.

Authors' Disclosures

K.T. Do reports grants from Dana-Farber Cancer Institute during the conduct of the study, as well as reports employment with Moderna Therapeutics at the time of this submission. B. Kochupurakkal reports grants from Eli Lilly during the conduct of the study. K. Parmar reports grants from Eli Lilly and Co. during the conduct of the study. J.-B. Lazaro reports grants from Eli Lilly and Co. during the conduct of the study. A.D. D'Andrea reports grants from Lilly during the conduct of the study. A.D. D'Andrea also reports personal fees from Cyteir, Cedilla, and Ideaya, as well as grants from EMD Serono outside the submitted work. G.I. Shapiro reports grants and personal fees from Lilly during the conduct of the study. G.I. Shapiro also reports grants and personal fees from Merck KGaA/EMD Serono and Sierra Oncology, grants from Merck & Co., and personal fees from Pfizer, G1 Therapeutics, Roche, Bicycle Therapeutics, Fusion Pharmaceuticals, Cybrexa Therapeutics, Astex, Almac, Ipsen, Bayer, Angiex, Daiichi Sankyo, Seattle Genetics, Boehringer Ingelheim, ImmunoMet, Asana, Artios, Atrin, Concarlo Holdings, Syros, Zentalis, CytomX Therapeutics, and Blueprint Medicines outside the submitted work; in addition, G.I. Shapiro has a patent for Dosage Regimen for Sapacitabine and Seliciclib issued to Cyclacel Pharmaceuticals and a patent for Compositions and Methods for Predicting Response and Resistance to CDK4/6 Inhibition pending to Liam Cornell. No disclosures were reported by the other authors.

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Authors' Contributions

K.T. Do: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, methodology, writing–original draft, writing–review and editing. **B. Kochupurakkal:** Data curation, formal analysis, writing–review and editing. **S. Kelland:** Data curation, project administration, writing–review and editing. **A. de Jonge:** Project administration, writing–review and editing. **J. Hedglin:** Data curation, validation, writing–review and editing. **A. Powers:** Data curation, writing–review and editing. **N. Quinn:** Data curation, writing–review and editing. **C. Gannon:** Data curation, writing–review and editing. **L. Vuong:** Data curation. **K. Parmar:** Data curation, writing–review and editing. **J.-B. Lazaro:** Data curation, writing–review and editing. **A.D. D'Andrea:** Validation, writing–review and editing. **G.I. Shapiro:** Conceptualization, supervision, writing–review and editing.

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