Published OnlineFirst May 9, 2014; DOI: 10.1158/1940-6207.CAPR-14-0047

Research Article

Cancer Prevention Research

The PARP Inhibitors, Veliparib and Olaparib, Are Effective Chemopreventive Agents for Delaying Mammary Tumor Development in BRCA1-deficient Mice

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Abstract

Poly-ADP ribose polymerase (PARP) inhibitors are effective for the treatment of BRCA-deficient tumors. Women with these mutations have an increased risk of developing breast cancer and would benefit from effective chemoprevention. This study examines whether the PARP inhibitors, veliparib and olaparib, delay mammary gland tumor development in a BRCA1-deficient ($BRCA1^{Co/Co}$;MMTV-Cre;$p53^{+/-}$) mouse model. In dose de-escalation studies, mice were fed with control, veliparib (100 mg/kg diet), or olaparib (200, 100, 50, or 25 mg/kg diet) continuously for up to 43 weeks. For intermittent dosing studies, mice cycled through olaparib (200 mg/kg diet) for 2 weeks followed by a 4-week rest period on control diet. To examine biomarkers, mice were fed with olaparib using the intermittent dosing regimen and mammary glands were evaluated by immunohistochemistry. In mice treated with veliparib or olaparib (200 mg/kg diet), the average age of the first detectable tumor was delayed by 2.4 and 6.5 weeks, respectively, compared with controls. Olaparib also increased the average lifespan of mice by 7 weeks. In dose de-escalation studies, lower concentrations of olaparib delayed tumor development but were less effective than the highest dose. When fed intermittently, olaparib delayed the onset of the first palpable tumor by 5.7 weeks and significantly reduced proliferation and induced apoptosis in hyperplastic mammary glands. In summary, veliparib and olaparib are effective for delaying tumor development and extending the lifespan of BRCA1-deficient mice, and intermittent dosing with olaparib was as effective as continuous dosing. These results suggest that the use of PARP inhibitors is a promising chemopreventive option. *Cancer Prev Res; 7(7); 698–707. ©2014 AACR.*

Introduction

Mutations in the tumor-suppressor *BRCA* genes are the most common cause of hereditary breast cancer, and women with these alterations have a 50% to 80% risk of developing breast cancer by age 70 (1). The currently available options for these women are diligent surveillance or bilateral prophylactic mastectomy, both of which are psychologically difficult, life-altering strategies (2–6). Several FDA-approved antiestrogenic agents exist for breast cancer prevention; however, their efficacy may be limited for BRCA1 mutation carriers. For instance, the SERMs (selective estrogen receptor modulators), tamoxifen and raloxifene, are effective clinically for the prevention of estrogen receptor (ER)–positive breast cancer but not ER-negative breast cancer (7–11), and about 75% of BRCA1-associated breast cancer manifests into triple-negative breast cancer, a subtype associated with poor prognosis (12). Moreover, their benefits in patients with BRCA mutations remain unclear (11, 13–15). Similarly, the effect of the aromatase inhibitor, exemestane, is promising in reducing breast cancer incidence but only in postmenopausal women with high risk for ER-positive breast cancer (16, 17). Hence, an effective and safe chemopreventive option is still lacking for the high-risk population with BRCA1 deficiency.

Recently, poly ADP-ribose polymerase (PARP) inhibitors have emerged as promising agents for the treatment of cancers with *BRCA1* mutations *via* synthetic lethality (18–21). The BRCA1 protein is involved in many fundamental cellular processes such as cell-cycle regulation, transcription, epigenetic modification, and DNA repair (22–24). Normally, BRCA1 is required for homologous recombination repair (HRR), a high-fidelity DNA repair process, to maintain genomic integrity in the cell (25). In BRCA-1 mutation carriers, normal cells still have one copy of the wild-type *BRCA1* gene that allows for efficient DNA repair. However, the loss of both *BRCA1* genes by loss of heterozygosity, which is often observed in tumor cells, forces cells to rely on base excision repair (BER) as a default DNA repair mechanism, a process that requires the enzyme PARP1 for survival. Therefore, the inhibition of PARP1 in

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BRCA1-deficient cells inhibits the BER machinery that facilitates DNA repair and induces these cells to undergo apoptosis. As such, studies have shown that BRCA1-deficient cells are highly sensitive to PARP inhibitors and consequently, they undergo apoptosis because of increased genomic instability (26–28). Several PARP inhibitors have been developed and are being tested in the clinic (29–43). Veliparib (ABT-888) and olaparib (AZD 2281) are two well-tolerated PARP inhibitors that have shown favorable results for the treatment of BRCA1-associated breast cancer in phase I and II clinical trials (34, 36, 43–45); however, their role in chemoprevention has not been elucidated.

In the present studies, we investigated whether olaparib and veliparib are effective chemopreventive compounds in the well-characterized BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mouse model (46). This model was created by crossing a mutant mouse with a conditional knockout of the *BRCA1* gene with a transgenic mouse carrying the MMTV-Cre promoter to specifically delete BRCA1 in mammary epithelial cells. Because BRCA1-associated cancers often have a mutation in *p53*, a tumor-suppressor gene involved in maintaining genomic stability (47), the BRCA1$^{Co/Co}$;MMTV-Cre mouse was crossed with a mouse with a targeted heterozygous *p53* mutation to produce the BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mouse model that we used in our work (46, 48). Because the prolonged use of any compound may result in undesirable side effects and the development of drug resistance, we also tested the efficacy of olaparib using various dosing regimens, including intermittent administration of this drug, and examined various biomarkers to assess the activity of PARP inhibition in the mammary gland.

Materials and Methods

In vivo experiments

Veliparib (ABT-888) and olaparib (AZD 2281) were synthesized (49, 50) by J-Star Research Inc. with purity greater than 95% for both agents. Breeding pairs for the BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice (46) were generously provided by Dr. Chuxia Deng (NIH, Bethesda, MD). All animal studies were done in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Dartmouth College. PARP inhibitors were first dissolved in ethanol and Neobee oil (1:3 ratio), and then mixed into standard 5004 rodent meal powder (Purina) using methods previously described (51). For the continuous dosing and dose de-escalation studies, female mice were fed with control diet or diet containing veliparib (100 mg/kg of diet) or olaparib (200, 100, 50, or 25 mg/kg of diet), starting at 10 weeks of age. For the intermittent, high-dose prevention studies, female mice were fed with control diet or diet containing olaparib (200 mg/kg of diet) intermittently for 2 weeks followed by a 4-week period on control diet. Mice were weighed and palpated weekly for tumors. In accordance with IACUC guidelines, mice were sacrificed if they had a tumor greater than 1.5 cm in diameter, if total tumor burden was greater than 10% of body weight, or if a tumor ulcerated or interfered with mobility.

Biomarker studies

For biomarker studies, mice were fed with olaparib (200 mg/kg) intermittently in diet, as described above, and sacrificed at 18, 24, or 30 weeks of age. Mammary glands were harvested for immunohistochemistry (IHC). To measure proliferation, mice were injected intraperitoneally with bromodeoxyuridine (BrdUrd; BD Pharmingen) in sterile saline (1 mg/mouse) 2 hours before sacrifice. Tissues were then fixed in 10% phosphate-buffered formalin for at least 48 hours before they were embedded in paraffin blocks and sectioned. BrdUrd-positive cells were stained using the BrdUrd *In Situ* Detection Kit (BD Pharmingen). To identify cells undergoing apoptosis, TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling) staining was performed on tissue sections using the TdT2-TACS *In Situ* Apoptosis Detection Kit (Trevigen). Six mice per group were used in the 24-week biomarker studies, whereas 5 mice per group were used in the 30-week biomarker studies. To quantitate the number of BrdUrd-positive cells, 11 to 26 hyperplastic areas as well as 56 to 130 ductal areas from each group were counted. To assess apoptosis, similar numbers of hyperplastic areas and ducts were evaluated. PARP activity in mammary glands from these same mice was also measured using the HT Universal Colorimetric PARP Assay Kit with Histone-coated Strip Wells (Trevigen).

Drug levels in tissues

BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice were fed with veliparib (100-mg/kg diet) or olaparib (200-mg/kg diet) for 2 weeks or intermittently as described above until 30 weeks of age. Blood was collected in heparinized tubes and centrifuged at 5,000 rpm for 5 minutes to separate the plasma. Plasma was extracted with acetonitrile, whereas mammary glands and livers were homogenized in PBS and then extracted with acetonitrile. Samples were separated by reverse-phase high-pressure liquid chromatography (Waters 2695 HPLC) and analyzed by mass spectrometry (Waters single quadrupole MS). Standard curves were generated by spiking tissue homogenates and plasma from the control group with known concentrations of olaparib or veliparib. Waters MassLynx 4.1 software was used to determine drug levels in the tissues of the treatment groups.

Statistical analysis

Results are expressed as mean ± SEM and were analyzed using the *t* test, χ^2 test, or one-way ANOVA on ranks (Wilcoxon signed rank test) using Prism5 or SigmaStat3.5 software. All *P* values are two-sided, and a *P* value of <0.05 was considered statistically significant.

Results

Olaparib and veliparib are effective chemopreventive agents in BRCA1-deficient mice

Transgenic mice with a conditional knockout of *BRCA1* (co) coupled with a mutation in *p53* develop ER-negative tumors as early as 21 weeks of age (52, 53), making them an ideal model to evaluate new chemopreventive drugs for women with BRCA1 mutations. Before testing any drug in

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Table 1. Drug concentrations of veliparib and olaparib in BRCA$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice

	Veliparib (100 mg/kg diet)	Olaparib (200 mg/kg diet)
Plasma, nmol/L	96 ± 12	36 ± 11
Mammary gland, nmol/kg of body weight	96 ± 15	16 ± 3
Liver, nmol/kg of body weight	437 ± 123	636 ± 264

NOTE: Plasma, mammary gland, and liver from mice fed with 100 mg/kg of veliparib or 200 mg/kg of olaparib diet for 2 weeks were harvested. Tissue concentrations of the PARP inhibitors were measured by LC/MS; $n = 3$ mice per group.

long-term *in vivo* experiments, we first did pharmacokinetic studies to determine whether it could be administered in diet. In a pilot pharmacokinetic study, BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice were fed with veliparib (100 mg/kg of diet) or olaparib (200 mg/kg of diet) for 2 weeks. Starting doses were extrapolated from previous studies testing the efficacy of PARP inhibitors *in vivo* (27, 44). As shown in Table 1, tissue levels averaged 96 and 36 nmol/L in plasma, 96 and 16 nmol/kg in the mammary glands, and 437 and 636 nmol/kg in the liver for veliparib and olaparib, respectively; these potent drugs inhibit PARP enzyme activity *in vitro* at concentrations between 5 to 10 nmol/L. When started in diet at 10 weeks of age and fed continuously, both drugs significantly ($P < 0.05$ and $P < 0.001$, respectively) delayed tumor development (Fig. 1A and B). In these studies, 50% of mice fed with control diet had developed tumors by 27.5 weeks of age (Fig. 1A and B). However, 50% tumor incidence was delayed until 30 weeks in mice fed with veliparib (Fig. 1A) and until 35 weeks in mice fed with olaparib (Fig. 1B). As shown in Table 2, the average age that a tumor was first detected in mice fed with olaparib (34.9 ± 1.1 weeks) was 6.5 weeks later than in its control group (28.4 ± 0.9; $P < 0.001$). This change was much greater than in mice fed with veliparib, in which the average age of the first palpable tumor was delayed by 2.4 weeks, compared with its control group ($P < 0.004$). Although veliparib significantly ($P < 0.05$) increased the average lifespan of mice in the treatment group by 2.4 weeks (Table 2), olaparib prolonged survival by 7 weeks ($P < 0.05$). Furthermore, the average tumor burden per mouse was also significantly ($P < 0.05$) reduced from 4.6 ± 0.5 g in the control group to 3.2 ± 0.3 g in the olaparib group; no change was observed between mice fed with veliparib or control (data not shown). These results clearly demonstrated that both PARP inhibitors delayed tumor development in BRCA1-deficient mice. Even though higher drug levels of veliparib could be detected in the mammary glands, olaparib was very effective in this model and was chosen for additional studies.

Dose de-escalation studies with olaparib

An effective chemopreventive drug should reduce the risk of developing cancer without causing significant toxicity. Because olaparib displayed superior efficacy in delaying tumor development and prolonging lifespan over veliparib, we performed a dose de-escalation study to determine the lowest dose that was effective for delaying tumor development. BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice were fed with control diet or diet containing olaparib (100 mg/kg diet). This lower dose of olaparib also significantly ($P < 0.05$) delayed tumor development (Fig. 2A). As shown in Table 2, the average age in which the first palpable tumor was observed increased from 29.5 ± 1.0 weeks in the control group to 32.7 ± 0.8 weeks in mice fed with 100 mg olaparib/kg

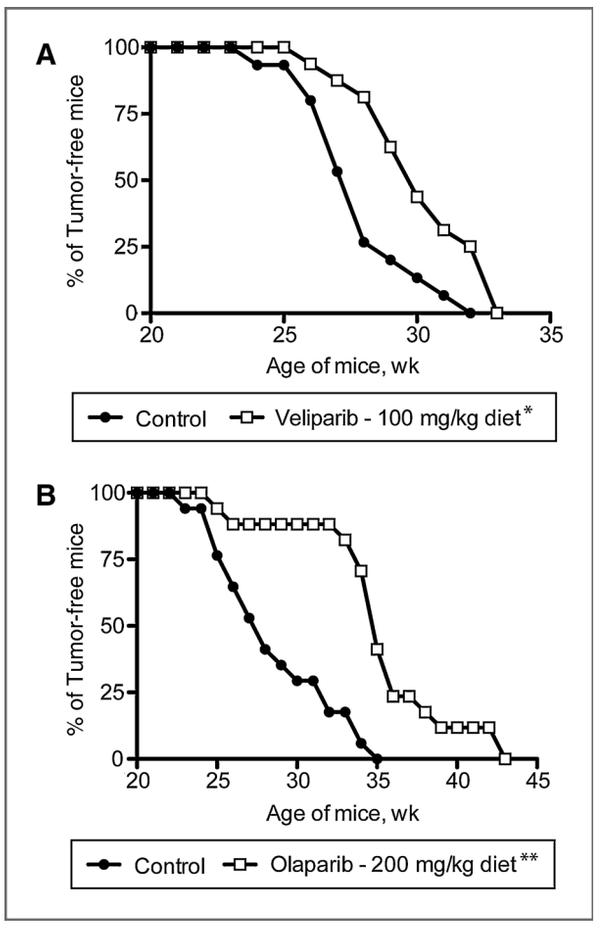

Figure 1. Continuous feeding of veliparib and olaparib diets significantly delayed tumor development in BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice. Mice were fed with either control diet, 100 mg/kg veliparib in diet A, or 200 mg/kg of olaparib in diet B, starting when mice were 10 weeks old, and tumor development was assessed by weekly palpation. No tumors were found before the mice were 20 weeks of age; $n = 15$, 16 in the control and veliparib groups, respectively, in A; $n = 17$ for both olaparib and its respective control group in B; *, $P < 0.05$ versus the control group in A; and **, $P < 0.001$ versus the control group in B.

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Table 2. Continuous treatment with veliparib and olaparib as well as intermittent treatment with olaparib delayed tumor development and extended lifespan of BRCA1-deficient mice

	Veliparib		Olaparib	
Continuous treatment, mg/kg diet	**0 ($n = 15$)**	**100 ($n = 16$)**	**0 ($n = 17$)**	**200 ($n = 17$)**
Average age of first palpable tumor, wk	27.6 ± 0.5	30.0 ± 0.6[b]	28.4 ± 0.9	34.9 ± 1.1[c]
Average lifespan, wk	30.8 ± 0.7	33.2 ± 0.6[a]	31.6 ± 1.0	38.6 ± 1.4[a]

	Olaparib					
Continuous treatment, mg/kg diet	**0 ($n = 14$)**	**100 ($n = 14$)**	**0 ($n = 17$)**	**50 ($n = 14$)**	**0 ($n = 19$)**	**25 ($n = 20$)**
Average age of first palpable tumor, wk	29.5 ± 1.0	32.7 ± 0.8[a]	27.9 ± 1.1	31.4 ± 1.0[a]	28.1 ± 0.7	29.7 ± 0.7
Average lifespan, wk	33.7 ± 0.8	38.0 ± 0.8[b]	32.1 ± 1.3	35.1 ± 1.0	32.5 ± 0.7	34.0 ± 0.8

	Olaparib	
Intermittent treatment, mg/kg diet	**0 ($n = 19$)**	**200 ($n = 18$)**
Average age of first palpable tumor, wk	29.9 ± 1.1	35.6 ± 1.0[c]
Average lifespan, wk	35.5 ± 1.1	39.4 ± 1.1[a]

NOTE: In the continuous feeding and dose de-escalation studies, mice were fed with control, veliparib (100 mg/kg) or olaparib (200, 100, 50, and 25 mg/kg) diet starting when mice were 10 weeks old. In the intermittent study, mice were fed with control diet or cycled through 2 weeks of 200 mg olaparib/kg diet followed by 4 weeks of control diet. All mice were palpated for tumors weekly for tumor development. Litter-matched controls were used for each of the studies.

[a]$P < 0.05$ versus control.

[b]$P < 0.004$ versus control.

[c]$P < 0.001$ versus control.

diet ($P < 0.05$), and average lifespan also increased by 4.3 weeks ($P < 0.05$). Doses of olaparib were subsequently lowered to 50 and then 25 mg/kg diet (Fig. 2B and C). Although all of these doses were able to significantly delay tumor development (Fig. 2A–C), there was clearly a dose-dependent effect. Specifically, there was a significant ($P < 0.05$) delay in the onset of the first tumor by 3.5 weeks in mice that were fed with 50 mg/kg of olaparib diet compared with their control groups (Table 2), but this delay was lost at the 25 mg/kg dose. Taking into consideration the results obtained from all of the continuous feeding studies, 200 mg/kg olaparib diet was the most effective dose, but promising efficacy was still observed at 50 to 100 mg/kg diet with this drug. Notably, olaparib and veliparib were well tolerated in these studies, with only minor hair loss in a few of the mice fed with the highest dose of olarparib and veliparib. Alopecia was not apparent in any of the mice fed with olaparib continuously at 25 to 100 mg/kg diet. No differences in weight were observed in any of the groups fed with the various olaparib diets compared with the control groups (data not shown).

Intermittent dosing with olaparib is as effective as continuous dosing with olaparib for delaying tumor development

Continuous, long-term exposure to PARP inhibitors could lead to adverse drug reactions, acquired drug resistance, and poor patient compliance. To avoid these complications, an intermittent dosing regimen, in which drugs are given for short periods to induce apoptosis in premalignant cells, followed by rest periods to reduce toxicity from the drug, offers an attractive alternative. As such, intermittent paradigms have been used successfully for both the prevention and treatment of cancer (54–58). Because continuous treatment with 200 mg/kg olaparib diet was highly effective in our mouse model, we examined whether this same dose, when given intermittently, would have the same effect. Briefly, mice were fed with olaparib (200 mg/kg diet) for 2 weeks followed by a 4-week rest period of control diet (Fig. 3A). No side effects or weight loss were observed using this paradigm. Notably, 50% of the mice were tumor-free for 35 weeks when mice were treated intermittently with olaparib (Fig. 3B) compared with 28 weeks in the control group. In addition, the average age for the first palpable tumor was 5.7 weeks later in mice fed with intermittent olaparib diet than in the control group (Table 2). Mice fed with olaparib in diets either continuously or intermittently both lived to approximately 39 weeks. Therefore, these results indicated that intermittent dosing with olaparib (Fig. 3B) was as effective at delaying tumor development and extending the lifespan of mice as when continuous dosing was used (Fig. 1B).

Biomarker studies revealed that intermittent treatment with olaparib decreases proliferation and induces apoptosis in mammary glands of BRCA1-deficient mice

PARP inhibitors were developed to induce apoptosis in BRCA-deficient cells through synthetic lethality. To investigate the molecular events that could lead to the observed

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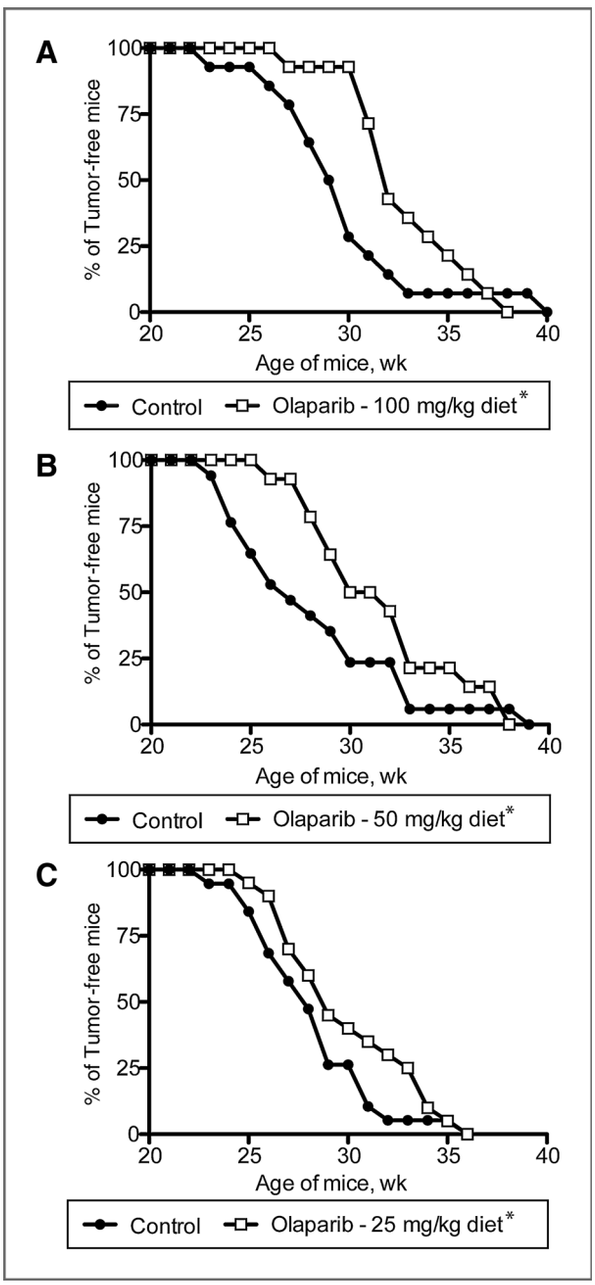

Figure 2. Dose de-escalation studies with BRCA1-deficient mice fed continuously with olaparib diets. Mice were fed with control, 100 mg/kg A, 50 mg/kg B, or 25 mg/kg C, of olaparib diets starting when mice were 10 weeks old and palpated for tumors weekly; $n = 14$ to 19 mice in the control groups; $n = 14$ to 20 mice for the olaparib groups; *, $P < 0.05$ versus the control group.

delay in tumor development, we first tested whether PARP activity could be measured *in vivo* using an enzyme kit that measures the incorporation of biotinylated PAR (poly ADP-ribose) into histone proteins. Unfortunately, no change in PARP enzyme activity was detected in the mammary glands of mice fed with olaparib using this assay (data not shown). We next examined the effects of olaparib on cell proliferation and apoptosis at early stages of tumor development. $BRCA1^{Co/Co}$;MMTV-Cre;$p53^{+/-}$ mice were fed with 200 mg/kg olaparib diet intermittently as previously described until they reached 18, 24, or 30 weeks of age (Fig. 3A). At these time points, the mice had received 2, 3, or 4 cycles of the drug, respectively. The drug concentrations averaged 16, 11, and 159 nmol/L in plasma, mammary glands, and the liver, respectively, in these mice after 30 weeks of intermittent exposure to olaparib, which are similar to the values detected in our pilot pharmacokinetic studies. Cell proliferation was assessed by BrdUrd staining (Fig. 4A and B), and TUNEL staining (Fig. 5A and B) was used to detect cells undergoing cell death. There was almost no pathology in mammary glands of control mice at 18 weeks of age (data not shown). Hence, we pursued the biomarker studies in mice that were 24 and 30 weeks old, in which palpable tumors and pathologic changes on sections from mammary glands were apparent in both age groups. As expected, counterstaining with hematoxylin (Fig. 4A) and methyl green (Fig. 5A) revealed that mammary glands from mice that were 30 weeks old displayed more hyperplasia than those that were 24 weeks of age. There were also more palpable tumors and hyperplastic areas in both control groups compared with groups treated intermittently with olaparib for 24 and 30 weeks. Specifically, at 24 weeks, 2 of 7 mice in the control group had developed palpable tumors versus no mice in the olaparib group ($P = 0.44$). At 30 weeks, 5 of 9 mice in the control group had developed palpable tumors, whereas only 2 of 9 mice fed with olaparib diet had palpable tumors ($P = 0.33$). All mammary glands with palpable tumors were excluded from our immunohistochemical analysis as there were insufficient numbers of these tissues in the olaparib groups to allow statistically meaningful analysis. Quantitation of BrdUrd-positive cells showed that intermittent treatment with olaparib significantly reduced proliferation in both hyperplastic and ductal areas of the mammary gland ($9.9 \pm 0.9\%$ and $4.5 \pm 1.0\%$, respectively; $P < 0.05$) at 24 weeks compared with their controls ($13.5 \pm 1.4\%$ and $9.5 \pm 1.4\%$, respectively; Fig. 4B, left). We observed similar results at 30 weeks, as olaparib significantly decreased the percentage of proliferative cells in hyperplastic and ductal area ($9.0 \pm 0.7\%$ and $8.9 \pm 1.2\%$, respectively; $P < 0.05$), compared with their controls ($15.9\% \pm 1.9\%$ and $17.6 \pm 3.8\%$, respectively; Fig. 4B, right). However, TUNEL staining indicated that olaparib significantly induced apoptosis in hyperplastic areas of mammary gland at 30 weeks (Fig. 5B, right) but not at 24 weeks (Fig. 5B, left). Specifically, the percentage of TUNEL-positive cells in the control group was $8.4 \pm 0.9\%$ and increased to $12.3 \pm 1.5\%$ with intermittent treatment of olaparib at 30 weeks (Fig. 5B; $P < 0.05$, right).

Discussion

In this report, we showed that both veliparib and olaparib significantly delayed tumor development in a mouse model of BRCA1 deficiency. Moreover, dose de-escalation studies revealed that olaparib could significantly delay tumor

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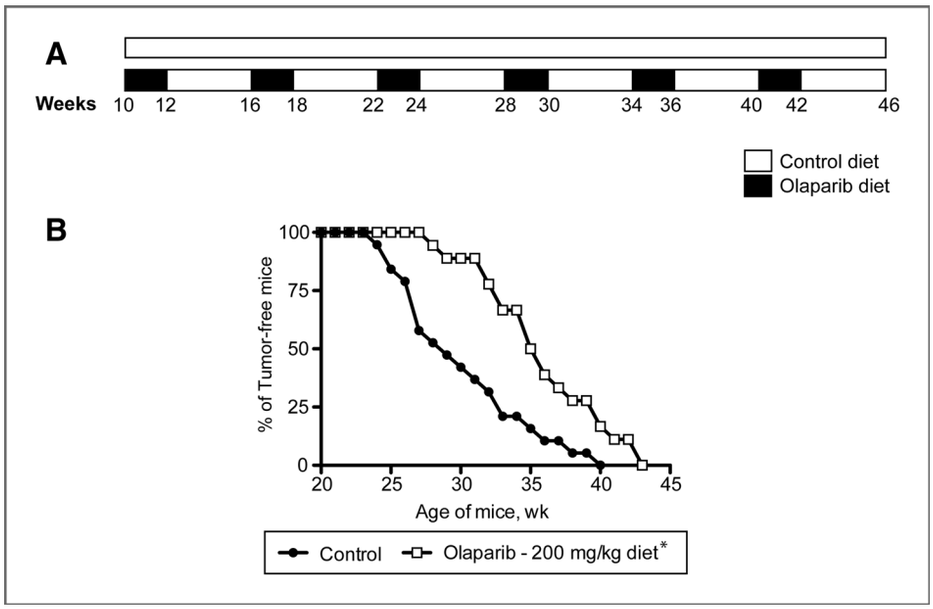

Figure 3. Intermittent treatment with olaparib delayed tumor development in BRCA1$^{Co/Co}$; MMTV-Cre;p53$^{+/-}$ mice. A, intermittent dosing schedule for olaparib. All mice were fed with either control or olaparib diet starting at 10 weeks of age. The control group was fed with control diet throughout the study. Mice in the intermittent dosing regimen underwent cycles of 2 weeks on olaparib diet followed by 4 weeks on control diet for up to 43 weeks in the chemoprevention study and for 18 to 30 weeks in the biomarker studies. B, mice fed using the dosing schedule described in A were palpated weekly to assess tumor development; $n = 19$, 18 for the control and olaparib treatment groups, respectively; *, $P < 0.001$ versus the control group.

development even when mice were fed with very low doses of the PARP inhibitor in diet. Notably, an intermittent regimen with a high dose of olaparib in diet showed similar efficacy in delaying tumor development as when the same dose was given continuously. Our biomarker studies also indicated that intermittent dosing with olaparib significantly reduced cell proliferation and induced apoptosis in the mammary gland of these mice.

Novel scientific findings over the last few decades have increased our understanding of the consequences of heritable mutations in the *BRCA1* gene and allowed for the development of promising agents for breast cancer treatment. However, the need to examine the role of these new compounds for chemoprevention has not been addressed. Our studies showed, for the first time, that the efficacy of PARP inhibitors is not limited to treatment but can also be extended to prevention of BRCA1-associated breast cancer. Importantly, olaparib was well tolerated in these studies, making it a promising drug candidate that should be tested in clinical trials for prevention. In addition, the fact that the intermittent dosing schedule of olaparib was nearly as effective as the continuous dosing olaparib regimen in

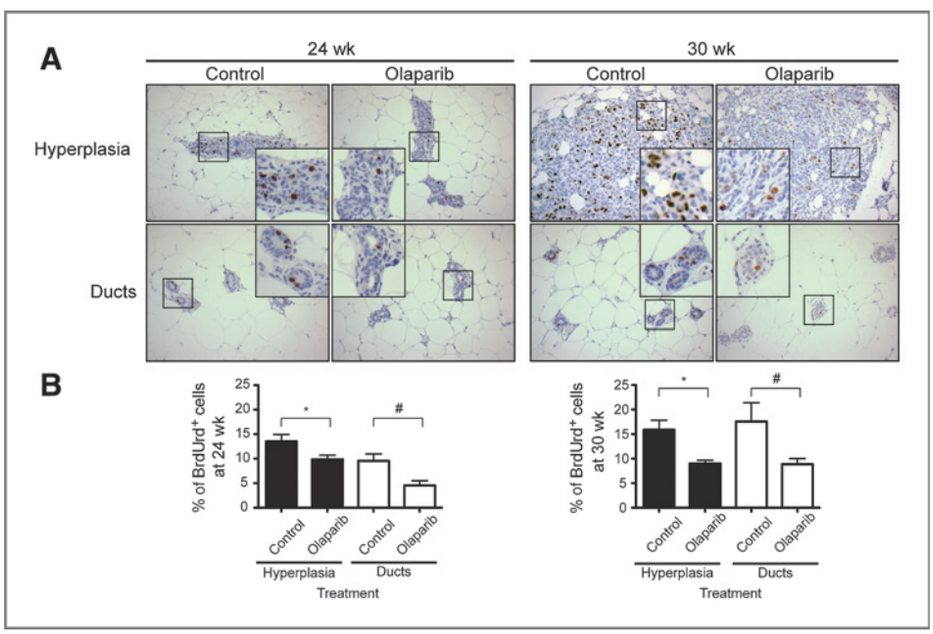

Figure 4. Intermittent treatment with olaparib for 24 to 30 weeks reduced cell proliferation in mammary glands of BRCA1-deficient mice. A, mice on either control or olaparib (200 mg/kg diet) fed intermittently for 24 or 30 weeks were injected with BrdUrd and analyzed by IHC to measure cell proliferation (magnification, ×200; insets ×500). B, BrdUrd-positive cells (stained brown with diaminobenzidine) in hyperplastic and ductal areas were quantitated and expressed as a percentage of BrdUrd-positive cells over total number of cells (stained purple with hematoxylin) counted; $n = 6$ per group at 24 weeks and $n = 5$ mice per group at 30 weeks; more than 3,500 and 1,000 cells from hyperplastic areas and ducts were counted in both groups at each time point; *, $P < 0.05$ versus control in hyperplastic areas; #, $P < 0.05$ versus control in ducts.

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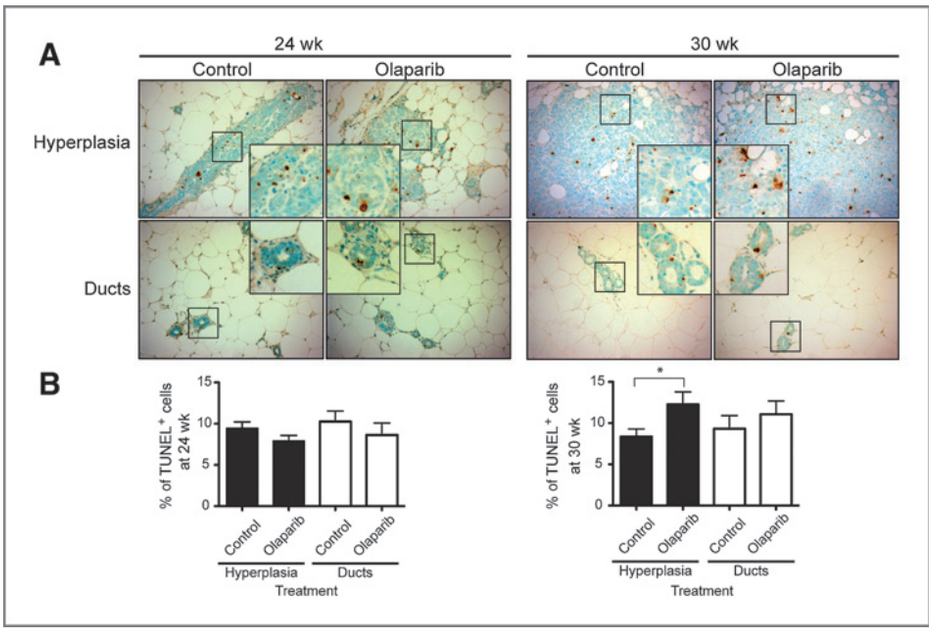

Figure 5. Intermittent treatment with olaparib for 30 weeks induced apoptosis in hyperplastic areas. A, mammary tissues from BRCA1$^{Co/Co}$;MMTV-Cre;p53$^{+/-}$ mice fed with either control or olaparib intermittently in diet (200 mg/kg) for 24 or 30 weeks were harvested and processed for TUNEL staining to measure apoptosis. TUNEL-positive cells in hyperplastic and ductal areas were visualized with diaminobenzidine (brown), followed by counterstaining with methyl green (green; magnification, ×200; insets ×500). B, TUNEL-positive cells were quantitated and expressed as a percentage of TUNEL-positive cells at 24 or 30 weeks over total number of cells counted; $n = 5$ to 6 mice per group at each time point; more than 1,800 cells in hyperplastic areas and 950 cells in ducts were counted in both groups.*, $P < 0.05$ versus control.

delaying tumor development is highly relevant. Although PARP inhibitors exhibit potent antitumor and chemopreventive properties, resistance can develop over prolonged exposure. Cancer cells can acquire secondary mutations that modulate other molecular pathways, leading to the restoration of *BRCA1* gene function, and rendering them resistant to PARP inhibitors. Other modes of drug resistance include changes in PARP expression and decreasing intracellular availability of PARP inhibitors by altering the activity of p-glycoproteins, which affect the efflux of drugs (59, 60). For these reasons, intermittent therapy with PARP inhibitors could be a viable approach for chemoprevention and its potential benefits, if translated into clinical practice, could be marked for high-risk patients with BRCA1 mutations. Moreover, intermittent dosing could also potentially circumvent one of the major challenges in chemoprevention regimens, namely poor adherence because of cost, unfavorable side effects, and the duration of drug exposure (61, 62).

Biomarkers play an integral part in helping us understand the pharmacologic response to a drug of interest. As such, we used clinically relevant biomarkers to examine the effect of intermittent olaparib treatment on the mouse mammary gland. Although PARP activity was examined in the mammary glands of BRCA1-deficient mice, no change in activity was detected in mice fed with olaparib compared with the control diet. Because we examined PARP enzyme activity at the early stages of tumor development, the assay lacked the sensitivity to detect any changes. Moreover, the assay measures the PARP activity of the tissue as a whole, which may not accurately reflect what is happening in individual cells. Additional biomarkers studies demonstrated that olaparib significantly reduced the proliferation of hyperplastic and ductal areas of mammary gland when the mice were only 24 weeks of age. These mice had only received three rounds of high-dose olaparib diet (200 mg/kg) for 2 weeks, but the drug was still able to alter critical molecular events and contribute to the delay in tumor development. Interestingly, we found that intermittent olaparib treatment induced more apoptosis than in the control group only in hyperplastic areas of the mammary glands when the mice were 30 weeks of age but had no effect on the percentage of apoptotic cells in normal ducts. The lack of effect at 24 weeks could be explained by the fact that while DNA fragmentation is a critical part of programmed cell death, apoptosis is a transient cascade involving multiple steps and not enough cells were present in the olaparib-treated tissues to measure differences between the groups. Our present study used TUNEL staining, which specifically labeled fragmented DNA; therefore, it may not be sensitive enough to detect cells that may also be undergoing apoptosis but are at different steps of the pathway. Although more effort is needed to develop additional biomarkers for PARP inhibitors, changes in proliferation and apoptosis could be readily detected by IHC in the earliest stages of tumor development in mice treated with olaparib.

Finally, it is important to note that the benefits that could be gained from olaparib may not be limited to the high-risk population of patients with BRCA1 mutations. The synthetic lethality paradigm observed with PARP1 inhibition is based on the deficiency of the HRR pathway because of a *BRCA1* gene mutation (26). Therefore, this same concept can be and has been applied to alterations in gene products involved in HRR. As such, defects in NBS1, ATR, ATM, CHK1, CHK2 (63), and MRE11 (64), all of which are proteins involved in HRR and are drug targets in cancer, are sensitive to PARP inhibition. In addition, a mutation in the tumor-suppressor gene, *PTEN*, can cause HRR

Published OnlineFirst May 9, 2014; DOI: 10.1158/1940-6207.CAPR-14-0047

deficiency, and consequently, cells with a PTEN mutation undergo apoptosis in the presence of PARP inhibitors through synthetic lethality (65). Interestingly, the activation of the PI3K pathway was evident in BRCA1-related cancer and triple-negative breast cancer and a synergistic effect was observed when the PI3K inhibitor, NVP-BKM120, was used in combination with PARP inhibitors for treatment in the BRCA1-deficient mouse model and patient-derived xenografts (66, 67). Moreover, the synthetic triterpenoids, a class of multifunctional drugs that could target not only the PI3K pathway but also other signaling networks related to DNA-damage response (as reviewed in ref. 68), have shown potentiating effects when used in combination with other chemotherapeutic agents (51, 69, 70). Ongoing *in vivo* studies are currently underway to test their effects on tumor development when used with the PARP inhibitor, olaparib. Thus, these examples not only highlight the potential of PARP inhibitors in combination therapy for chemotherapy (31, 35, 37, 40, 41) but also support the importance of combination therapy for chemoprevention. In summary, the concept of synthetic lethality may be a useful strategy for developing chemopreventive regimens for a variety of cancers. For women with BRCA1 mutations, our results emphasize the therapeutic potential of PARP inhibitors for prevention and may eventually provide an alternative to watchful waiting or prophylactic mastectomy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M.B. Sporn, K.T. Liby
Development of methodology: E.-H. Kim, K.T. Liby
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. To, E.-H. Kim, K.T. Liby
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. To, K.T. Liby
Writing, review, and/or revision of the manuscript: C. To, M.B. Sporn, K.T. Liby
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. To, D.B. Royce, C.R. Williams, R. Risingsong
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Acknowledgments

The authors thank Eric B. York for his expertise in preparing the slides for histology studies.

Grant Support

These studies were supported by grants from Susan G. Komen for the Cure (promise grant to K.T. Liby), the Breast Cancer Research Foundation (BCRF), and the National Foundation for Cancer Research (NFCR; to K.T. Liby and M.B. Sporn).

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Received February 4, 2014; revised April 2, 2014; accepted April 21, 2014; published OnlineFirst May 9, 2014.

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Cancer Prevention Research

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Cancer Prev Res 2014;7:698-707. Published OnlineFirst May 9, 2014.