The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 9, 2009 VOL. 361 NO. 2

Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from *BRCA* Mutation Carriers

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ABSTRACT

BACKGROUND

The inhibition of poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) is a potential synthetic lethal therapeutic strategy for the treatment of cancers with specific DNA-repair defects, including those arising in carriers of a *BRCA1* or *BRCA2* mutation. We conducted a clinical evaluation in humans of olaparib (AZD2281), a novel, potent, orally active PARP inhibitor.

METHODS

This was a phase 1 trial that included the analysis of pharmacokinetic and pharmacodynamic characteristics of olaparib. Selection was aimed at having a study population enriched in carriers of a *BRCA1* or *BRCA2* mutation.

RESULTS

We enrolled and treated 60 patients; 22 were carriers of a *BRCA1* or *BRCA2* mutation and 1 had a strong family history of *BRCA*-associated cancer but declined to undergo mutational testing. The olaparib dose and schedule were increased from 10 mg daily for 2 of every 3 weeks to 600 mg twice daily continuously. Reversible dose-limiting toxicity was seen in one of eight patients receiving 400 mg twice daily (grade 3 mood alteration and fatigue) and two of five patients receiving 600 mg twice daily (grade 4 thrombocytopenia and grade 3 somnolence). This led us to enroll another cohort, consisting only of carriers of a *BRCA1* or *BRCA2* mutation, to receive olaparib at a dose of 200 mg twice daily. Other adverse effects included mild gastrointestinal symptoms. There was no obvious increase in adverse effects seen in the mutation carriers. Pharmacokinetic data indicated rapid absorption and elimination; pharmacodynamic studies confirmed PARP inhibition in surrogate samples (of peripheral-blood mononuclear cells and plucked eyebrow-hair follicles) and tumor tissue. Objective antitumor activity was reported only in mutation carriers, all of whom had ovarian, breast, or prostate cancer and had received multiple treatment regimens.

CONCLUSIONS

Olaparib has few of the adverse effects of conventional chemotherapy, inhibits PARP, and has antitumor activity in cancer associated with the *BRCA1* or *BRCA2* mutation. (ClinicalTrials.gov number, NCT00516373.)

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This article (10.1056/NEJMoa0900212) was published on June 24, 2009, at NEJM.org.

N Engl J Med 2009;361:123-34.

Cellular DNA is continually subject to damage, which coordinated pathways act to repair, thereby maintaining genomic integrity and cell survival.[1-3] The poly(adenosine diphosphate [ADP]–ribose) polymerases (PARPs) are a large family of multifunctional enzymes, the most abundant of which is PARP1. It plays a key role in the repair of DNA single-strand breaks through the repair of base excisions.[4,5] The inhibition of PARPs leads to the accumulation of DNA single-strand breaks, which can lead to DNA double-strand breaks at replication forks. Normally, these breaks are repaired by means of the error-free homologous-recombination double-stranded DNA repair pathway,[6] key components of which are the tumor-suppressor proteins BRCA1 and BRCA2.[7]

A germ-line mutation in one *BRCA1* or *BRCA2* allele is associated with a high risk of the development of a number of cancers, including breast, ovarian, and prostate cancer.[8-10] Cells carrying heterozygous loss-of-function *BRCA* mutations can lose the remaining wild-type allele, resulting in deficient homologous-recombination DNA repair, which causes genetic aberrations that drive carcinogenesis; the inactivation of the wild-type allele in the tumor is thought to be an obligate step in this process. It leads to the emergence of a tumor that carries a DNA-repair defect that is not shared by the normal tissues of the patient. This tumor-specific defect can be exploited by using PARP inhibitors to induce selective tumor cytotoxicity, sparing normal cells. PARP inhibition in these tumor cells with deficient homologous-recombination repair generates unrepaired DNA single-strand breaks that are likely to cause the accumulation of DNA double-strand breaks and collapsed replication forks.[11-13] Conversely, the normal tissue compartment consists of cells that are heterozygous for *BRCA* mutations and that therefore retain homologous-recombination function and have a sensitivity to PARP inhibitors similar to that of wild-type cells, predicting a high therapeutic index for PARP inhibition in *BRCA* carriers.[14,15]

Such "synthetic lethality" occurs when there is a potent and lethal synergy between two otherwise nonlethal events: in this case, a highly specific PARP inhibitor induces a DNA lesion and a tumor-restricted genetic loss of function for the DNA repair pathway required to repair it (homologous recombination)[13] (Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). We have shown that inhibiting a DNA repair enzyme in the absence of an exogenous DNA-damaging agent to selectively kill tumor cells is a novel approach to cancer therapy.[11] In vitro, *BRCA1*-deficient and *BRCA2*-deficient cells were up to 1000-fold more sensitive to PARP inhibition than wild-type cells, and tumor growth inhibition was also demonstrated in *BRCA2*-deficient xenografts.[11,12,16] Here, we describe a clinical evaluation of the novel, potent, orally active PARP inhibitor olaparib (4-[(3-{[4-cyclopropylcarbonyl)piperazin-1-yl]carbonyl}-4-fluorophenyl)methyl]phthalazin-1(2H)-one; also known as AZD2281 and previously known as KU-0059436)[17] (Fig. 2 in the Supplementary Appendix), with a focus on *BRCA*-mutation carriers.

METHODS

PATIENTS

This study was performed at the Royal Marsden National Health Service (NHS) Foundation Trust (United Kingdom) and the Netherlands Cancer Institute (the Netherlands). Eligibility criteria were an age of 18 years or older, written informed consent, disease that was refractory to standard therapies or for which there were no suitable effective standard treatments, an Eastern Cooperative Oncology Group performance status of 2 or less (on a scale of 0 to 5, with higher scores indicating greater impairment), a washout period of 4 weeks or more after previous anticancer therapy, and adequate bone marrow, hepatic, and renal function. It was not initially required for eligibility that patients be carriers of *BRCA1* or *BRCA2* mutations, although provisions were made in the protocol to permit enrichment of the study population with a substantial proportion of such carriers. Subsequently, in the expansion phase, only carriers of *BRCA1* or *BRCA2* mutations were enrolled. The study was approved by institutional review boards and ethics committees and commenced in June 2005.

STUDY DESIGN

Olaparib was initially given at a dose of 10 mg, once daily, for 2 of every 3 weeks, but this dose was subsequently increased to 60 mg or more, twice daily, given continuously in 4-week cycles (Table 1 in the Supplementary Appendix). Dose escalation was performed on the basis of a modified accelerated-titration design.[18] Briefly, this involved treating at least three patients per dose for one cycle (initially 3 weeks and subsequently 4 weeks), with a doubling of the dose in the ab-

sence of adverse effects of grade 2 or higher during that cycle. Up to six patients were treated if one dose-limiting toxicity was observed at a given dose, and a dose was considered the maximum administered dose if two manifestations of dose-limiting toxicity were observed at that dose during the first treatment cycle. A drug-related adverse effect of grade 3 or 4 occurring in the first cycle was considered a manifestation of dose-limiting toxicity.

Since this was a phase 1 trial, the objectives were to determine safety, the adverse-event profile, the dose-limiting toxicity, the maximum tolerated dose, the dose at which PARP is maximally inhibited, and the pharmacokinetic and pharmacodynamic profiles in both surrogate samples (of peripheral-blood mononuclear cells and plucked eyebrow-hair follicles) and tumor tissue. Once these had been established, a key aim was to test the hypothesis that patients with cancer associated with *BRCA1* or *BRCA2* mutations would show an objective antitumor response to single-agent olaparib treatment.

The study was designed by academic investigators at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research and representatives of KuDOS Pharmaceuticals, the sponsor. Data were collected and analyzed by Theradex under the supervision of the academic investigators. Descriptive statistics were provided by Theradex, with additional analyses performed at the Institute of Cancer Research. Three academic authors wrote the first draft of the manuscript, which was finalized by the coauthors. The principal academic investigator vouches for the completeness and accuracy of the results.

STUDY ASSESSMENTS

Safety evaluations were conducted at baseline and at weekly visits thereafter. Each evaluation consisted of a history taking and physical examination; laboratory panels, including a complete blood count, levels of clotting factors and electrolytes, and liver- and renal-function tests; and an electrocardiographic tracing. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 3.0).[19]

Pharmacokinetic and pharmacodynamic studies were performed at baseline and during the first and second cycles of treatment. Plasma samples were analyzed for the olaparib concentration with the use of solid-phase extraction followed by high-performance liquid chromatography, with detection by means of mass spectrometry. The plasma concentration–time data were analyzed with the use of noncompartmental analysis (WinNonLin, version 4.1; Pharsight) to derive pharmacokinetic parameters after the first dose (single-dose parameters) and after the dose on day 14 (multiple-dose parameters). PARP inhibition was evaluated in pharmacodynamic studies by means of a functional assay (Mesoscale Discovery) involving the analysis of poly(ADP-ribose) (PAR) formation from peripheral-blood mononuclear cells and tumor-tissue cell lysates, all normalized to the amount of PARP1 protein present.[17] The formation of foci of γH2AX, the phosphorylated form of histone H2A histone family member X (H2AX) at serine 139, a marker of DNA double-strand breaks, was evaluated in patients receiving doses of 100 mg or more of olaparib twice daily. This evaluation was performed before treatment, and at multiple time points after treatment, on plucked eyebrow-hair follicles (Fig. 3 in the Supplementary Appendix).[20]

Radiologic assessments by means of computed tomography or magnetic resonance imaging were carried out every two cycles and graded according to the Response Evaluation Criteria in Solid Tumors (RECIST).[21] As appropriate, we carried out additional disease evaluations involving serum tumor markers, including cancer antigen 125 (CA-125) and prostate-specific antigen (PSA), assessed according to Gynecologic Cancer Intergroup (GCIG)[22] and Prostate-Specific Antigen Working Group (PSAWG)[23] criteria, respectively. A tumor-marker response in ovarian and prostate cancers was defined as a decline in the tumor-marker level of more than 50% that was sustained for at least 4 weeks. A radiologic response was defined as a complete or partial response on radiologic assessment, according to RECIST, and the rate of clinical benefit was defined as the number of patients with a radiologic or tumor-marker response or stabilization of disease for 4 months or more.

RESULTS

STUDY PATIENTS

Sixty patients with histologically or cytologically confirmed advanced solid tumors were enrolled. Their baseline characteristics are presented in Table 1; and their initial doses are given in Table 2.

Table 1. Baseline Characteristics of the 60 Study Patients.

Characteristic	Value
Sex — no. (%)	
Male	20 (33)
Female	40 (67)
Age — yr	
Mean	54.8
Range	19–82
Tumor type — no. (%)*	
Ovarian	21 (35)
Breast	9 (15)
Colorectal	8 (13)
Melanoma	4 (7)
Sarcoma	4 (7)
Prostate	3 (5)
Other	11 (18)
ECOG performance status — no. (%)†	
0	18 (30)
1	37 (62)
2	5 (8)
No. of previous treatment regimens — no. (%)	
1	6 (10)
2	11 (18)
3	11 (18)
≥4	32 (53)

* Of the 21 patients with ovarian cancer, 1 had primary peritoneal cancer and 1 had fallopian-tube cancer; 15 had a *BRCA1* mutation and 1 had a *BRCA2* mutation. Of the nine patients with breast cancer, three had a *BRCA2* mutation. Of the three patients with prostate cancer, one had a *BRCA2* mutation. Of the 11 patients with other cancers, 3 had uterine or vaginal cancer, 3 had lung cancer, 2 had pancreatic cancer, 2 had mesothelioma, and 1 had kidney cancer.

† For the Eastern Cooperative Oncology Group (ECOG) performance status, higher scores indicate greater impairment.

Descriptions of the evaluated olaparib doses in 10 separate cohorts are provided in Table 1 in the Supplementary Appendix.

DOSE-LIMITING TOXICITY AND MAXIMUM ADMINISTERED DOSE

Three manifestations of dose-limiting toxicity in the first cycle were observed among patients receiving 400 or 600 mg of olaparib twice daily. A 47-year-old patient with advanced ovarian cancer had grade 3 mood alteration and fatigue on the first day of treatment with 400 mg of olaparib twice daily. These symptoms resolved within 24 hours after discontinuation of olaparib but recurred after reinitiation at 200 mg twice daily, resulting in discontinuation of treatment. A 59-year-old patient with mesothelioma, who had just completed chemotherapy with mitomycin, vinblastine, and carboplatin that had resulted in prolonged myelosuppression, had grade 4 thrombocytopenia during the first month of treatment with 600 mg of olaparib twice daily. The thrombocytopenia resolved within 2 weeks after discontinuation of the drug. The third manifestation of dose-limiting toxicity was observed in a 47-year-old patient with metastatic breast cancer who was receiving 600 mg of olaparib twice daily; on day 8 of treatment, she had grade 3 somnolence that resolved completely within 24 hours after discontinuation of the drug; grade 1 somnolence occurred on readministration of olaparib at 400 mg twice daily. These manifestations of dose-limiting toxicity led to the establishment of the maximum administered dose as 600 mg of olaparib twice daily and the maximum tolerated dose as 400 mg of olaparib twice daily.

SAFETY

Adverse effects that were at least possibly related to olaparib were largely of grade 1 or 2 and included nausea (19 patients [32%]), fatigue (18 patients [30%]), vomiting (12 patients [20%]), taste alteration (8 patients [13%]), and anorexia (7 patients [12%]) (Table 3). A low incidence of myelosuppression was reported: three patients (5%) had anemia, and grade 4 thrombocytopenia developed in two patients (3%).

One patient with advanced non–small-cell lung cancer and a history of recurrent lower respiratory tract infections died from respiratory failure after receiving olaparib for 4 months. Another patient with ovarian cancer died from gram-negative septicemia after receiving olaparib for 1 month, in the absence of neutropenia; she had inguinal disease with cutaneous involvement, with the skin colonized by organisms similar to those causing the septicemia. Both cases were deemed unlikely to be related to olaparib. No obvious increase in the frequency or grade of adverse effects was observed in comparing known *BRCA1* or *BRCA2* mutation carriers with noncarriers.

PHARMACOKINETIC STUDIES

Results of pharmacokinetic studies indicated that olaparib absorption is rapid, with the peak plasma

Table 2. Doses of Olaparib at Baseline in the Study Patients.

Subgroup	<100 mg, Daily or Twice Daily, 2 of Every 3 Wk	100 mg, Twice Daily, 2 of Every 3 Wk	100 mg, Twice Daily, Continuously	200 mg, Twice Daily, Continuously	400 mg, Twice Daily, Continuously	600 mg, Twice Daily, Continuously	All
				number of patients			
All patients							
No. of patients	18	4	5	20	8	5	60
BRCA1	1	1	1	7	6	1	17
BRCA2	0	0	0	5	0	0	5
Wild-type *BRCA* or *BRCA* status unknown	17	3	4	8	2	4	38
Ovarian-cancer subgroup							
No. of patients	4	2	1	7	6	1	21
BRCA1	1	1	1	5	6	1	15
BRCA2	0	0	0	1	0	0	1
Wild-type *BRCA* or *BRCA* status unknown	3	1*	0	1	0	0	5

* Although one patient with ovarian cancer who was receiving olaparib at a dose of 100 mg, twice daily, every 2 of 3 weeks was classified as having wild-type *BRCA* or unknown *BRCA* status, she was included in the *BRCA1* or *BRCA2* subgroup because she had a strong family history of *BRCA*-associated cancer but declined to undergo *BRCA*-mutation testing. Olaparib treatment was continued in all patients as long as they derived clinical benefit.

concentration observed between 1 and 3 hours after dosing (Fig. 4 in the Supplementary Appendix). Thereafter, plasma concentrations declined biphasically, with a terminal-elimination half-life of approximately 5 to 7 hours (Table 2 in the Supplementary Appendix). Exposure to olaparib increased with increasing doses, up to 100 mg, but increased less proportionally as the dose was increased further (Fig. 1A and 1B). The mean volume of distribution was 40.3 liters, and the mean plasma clearance rate was 4.6 liters per hour. After the daily administration of 10, 20, 40, or 80 mg of olaparib for 14 days, drug exposure was not increased markedly over that with a single dose: the area under the curve for olaparib exposure over a 24-hour period increased by approximately 26%. After twice-daily dosing with 60, 100, 200, 400, or 600 mg of olaparib for 14 days, exposure increased by an average of 49%; there was no marked time dependency in the pharmacokinetics of olaparib.

EVIDENCE OF PARP INHIBITION

Figure 1C depicts the average percentage of PARP inhibition in mononuclear cells in association with increasing doses of olaparib, plotted against the steady-state exposure to olaparib. Inhibition of PARP by more than 90%, as compared with the value at baseline, was observed in cells from patients treated with 60 mg or more of olaparib twice daily. Immunoblotting of cell extracts prepared from tumor-biopsy specimens collected before olaparib administration and after 8 days of treatment with olaparib are shown in Figure 1D. PARP inhibition was evidenced by the loss of signal from PAR (a biomarker for PARP activity) after treatment. Pharmacodynamic analysis was also carried out on samples of plucked eyebrow-hair follicles to measure the formation of γH2AX foci after treatment.[24] Induction of γH2AX foci 6 hours after treatment with olaparib (Fig. 1E) indicated that PARP inhibition was rapidly associated with downstream induction of collapsed DNA replication forks and DNA double-strand breaks, as predicted by preclinical models.[11] The induction of γH2AX foci was sustained at all later time points. There was no significant increase in foci induction at doses above 100 mg of olaparib twice daily, which was the lowest dose represented in these analyses.

ANTITUMOR ACTIVITY AS EVIDENCE OF SYNTHETIC LETHALITY

Durable objective antitumor activity was observed only in confirmed carriers of a *BRCA1* or *BRCA2* mutation, apart from one patient with a strong family history of *BRCA* mutation who declined mutational testing but was deemed likely to be a *BRCA* carrier (Table 4 and Fig. 2). Overall, 23 patients who

Table 3. Olaparib-Related Adverse Events Found in at Least 5% of the Safety Population, According to Olaparib Dose.*

Adverse Event	<100 mg, Daily or Twice Daily, 2 of Every 3 Wk (N=18)	100 mg, Twice Daily, 2 of Every 3 Wk (N=4)	100 mg, Twice Daily, Continuously (N=5)	200 mg Twice Daily, Continuously (N=20)	400 mg Twice Daily, Continuously (N=8)	600 mg Twice Daily, Continuously (N=5)	Total (N=60)
	number of patients/total number (percent)						
Anemia							
Grade 1–2	1 (6)	0	0	0	0	1 (20)	2 (3)
Grade 3–4	0	0	0	1 (5)	0	0	1 (2)
Lymphopenia							
Grade 1–2	0	0	0	0	0	0	0
Grade 3–4	0	0	0	2 (10)	1 (12)	0	3 (5)
Diarrhea							
Grade 1–2	0	0	0	2 (10)	1 (12)	0	3 (5)
Grade 3–4	0	0	0	0	0	0	0
Dyspepsia							
Grade 1–2	0	0	0	1 (5)	1 (12)	2 (40)	4 (7)
Grade 3–4	0	0	0	0	0	0	0
Nausea							
Grade 1–2	6 (33)	1 (25)	0	7 (35)	0	3 (60)	17 (28)
Grade 3–4	0	0	0	0	1 (12)	1 (20)	2 (3)
Stomatitis							
Grade 1–2	0	0	0	3 (15)	0	0	3 (5)
Grade 3–4	0	0	0	0	0	0	0
Vomiting							
Grade 1–2	2 (11)	1 (25)	0	5 (25)	0	3 (60)	11 (18)
Grade 3–4	0	0	0	0	1 (12)	0	1 (2)
Anorexia							
Grade 1–2	3 (17)	0	0	2 (10)	0	2 (40)	7 (12)
Grade 3–4	0	0	0	0	0	0	0
Dysgeusia							
Grade 1–2	0	2 (50)	0	2 (10)	1 (12)	3 (60)	8 (13)
Grade 3–4	0	0	0	0	0	0	0
Fatigue							
Grade 1–2	3 (17)	0	1 (20)	4 (20)	5 (62)	4 (80)	17 (28)
Grade 3–4	0	0	0	1 (5)	0	0	1 (2)
Dizziness							
Grade 1–2	0	0	0	1 (5)	0	1 (20)	2 (3)
Grade 3–4	0	0	0	0	1 (12)	0	1 (2)

* The listed adverse events were classified as being possibly, probably, or definitely related to olaparib in the safety population. No grade 5 adverse events related to olaparib were reported at the time of the analysis. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 3.0).

were *BRCA* mutation carriers were treated. Two of these patients could not be evaluated with regard to antitumor response: one received only two doses of olaparib, because of dose-limiting toxicity, and the other had ovarian cancer–associated fatal septicemia from tumor erosion after having received olaparib for 4 weeks, with a decreasing CA-125 level. Of the remaining 21 carriers, 2 had tumors not typically associated with *BRCA*-carrier status: 1 with small-cell lung cancer and 1 with vaginal adenocarcinoma. Both patients were receiving 200 mg of olaparib twice daily, and their disease progressed rapidly within 2 and 7 weeks after the start of treatment, respectively. The remaining 19 *BRCA* carriers had ovarian, breast, or prostate cancers; 12 of the 19 (63%) had a clinical benefit from treatment with olaparib, with radiologic or tumor-marker responses or meaningful disease stabilization (stable disease for a period of 4 months or more). Nine *BRCA* carriers had a response according to RECIST, with the response sustained for more than 76 weeks in one patient (Fig. 2C and Table 4). Further details on the specific *BRCA1* and *BRCA2* mutations and responses are provided in Table 3 in the Supplementary Appendix. No objective antitumor responses were observed in patients without known *BRCA* mutations.

Overall, eight patients with advanced ovarian cancer had a partial response on radiology, according to RECIST (Table 4 and Fig. 2A). On the basis of GCIG criteria for assessing the response of the CA-125 level to olaparib in patients with ovarian cancer, six patients with a *BRCA* mutation had a decline of more than 50% (Table 4 and Fig. 2B). Of the three patients with *BRCA2* breast cancer, one had a complete remission, according to RECIST, and another had stable disease for 7 months; both had a corresponding decline in serum levels of tumor markers (Fig. 2C). The patient with *BRCA2* breast cancer had a complete remission lasting for more than 60 weeks. She had pulmonary and lymph-node metastases and had previously had disease progression while receiving anthracycline-based chemotherapy. A patient with breast cancer (with no family history) who did not undergo *BRCA* testing had regression of cutaneous disease and of multiple subcentimeter brain metastases (not meeting RECIST) that had not previously been treated with radiation or corticosteroids and a decline of more than 50% in serum levels of carcinoembryonic antigen and cancer antigen 15-3.

A patient with castration-resistant prostate cancer who was a *BRCA2* mutation carrier had more than a 50% reduction in the PSA level and resolution of bone metastases. He had been participating in the study for more than 58 weeks at the time of the cutoff date (and has participated for more than 2 years since that date) (Fig. 2C, and Fig. 5 in the Supplementary Appendix).

DISCUSSION

This phase 1 trial of olaparib, an oral PARP inhibitor, showed that the drug has an acceptable side-effect profile and did not have the toxic effects commonly associated with conventional chemotherapy. It has satisfactory pharmacokinetic and pharmacodynamic characteristics. Patients who were carriers of *BRCA1* or *BRCA2* mutations did not appear to have an increased risk of adverse effects, a finding that supports those of our preclinical studies.[11] Of special interest is the antitumor activity in patients with *BRCA* mutation–associated cancer.

These data indicate that using PARP inhibition to target a specific DNA-repair pathway has the necessary selectivity profile and a wide therapeutic window for *BRCA*-deficient cells, supporting the clinical relevance of the hypothesis that *BRCA* mutation–associated cancers are susceptible to a synthetic lethal therapeutic approach.[13,25] Predictive biomarkers of homologous-recombination DNA-repair deficiency in tumor cells should be used to evaluate the broader usefulness of this promising therapeutic strategy.[6] Defects in homologous-recombination repair can also be caused by loss of function of proteins other than BRCA1 and BRCA2, including the RecA homologue RAD51, ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), and checkpoint kinase 1 and 2 homologue (CHK1 and CHK2) proteins, as well as components of the Fanconi's anemia repair pathway.[26] Loss of these proteins also sensitizes cells to PARP inhibition.[6] Such defects in homologous-recombination repair may be relatively common in some sporadic cancers, including breast cancer[27] and ovarian cancer,[28] potentially making this therapeutic strategy more widely useful as an anticancer treatment.

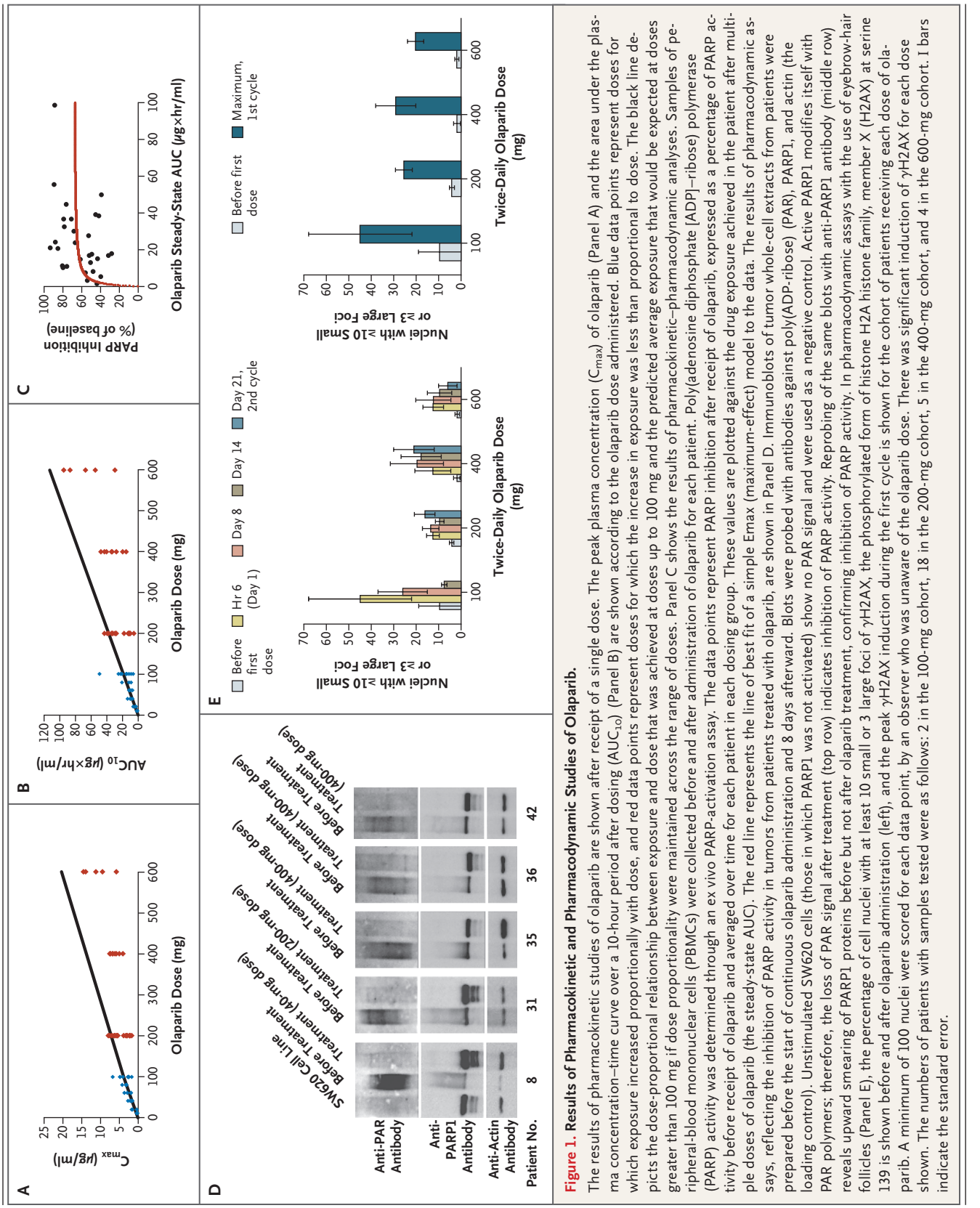


Figure 1. Results of Pharmacokinetic and Pharmacodynamic Studies of Olaparib.

The results of pharmacokinetic studies of olaparib are shown after receipt of a single dose. The peak plasma concentration (C_{max}) of olaparib (Panel A) and the area under the plasma concentration–time curve over a 10-hour period after dosing (AUC_{10}) (Panel B) are shown according to the olaparib dose administered. Blue data points represent doses for which exposure increased proportionally with dose, and red data points represent doses for which the increase in exposure was less than proportional to dose. The black line depicts the dose-proportional relationship between exposure and dose that was achieved at doses up to 100 mg and the predicted average exposure that would be expected at doses greater than 100 mg if dose proportionality were maintained across the range of doses. Panel C shows the results of pharmacokinetic–pharmacodynamic analyses. Samples of peripheral-blood mononuclear cells (PBMCs) were collected before and after administration of olaparib for each patient. Poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) activity was determined through an ex vivo PARP-activation assay. The data points represent PARP inhibition after receipt of olaparib, expressed as a percentage of PARP activity before receipt of olaparib and averaged over time for each patient in each dosing group. These values are plotted against the drug exposure achieved in the patient after multiple doses of olaparib (the steady-state AUC). The red line represents the line of best fit of a simple Emax (maximum-effect) model to the data. The results of pharmacodynamic assays, reflecting the inhibition of PARP activity in tumors from patients treated with olaparib, are shown in Panel D. Immunoblots of tumor whole-cell extracts from patients were prepared before the start of continuous olaparib administration and 8 days afterward. Blots were probed with antibodies against poly(ADP-ribose) (PAR), PARP1, and actin (the loading control). Unstimulated SW620 cells (those in which PARP1 was not activated) show no PAR signal and were used as a negative control. Active PARP1 modifies itself with PAR polymers; therefore, the loss of PAR signal after treatment (top row) indicates inhibition of PARP activity. Reprobing of the same blots with anti-PARP1 antibody (middle row) reveals upward smearing of PARP1 proteins before but not after olaparib treatment, confirming inhibition of PARP activity. In pharmacodynamic assays with the use of eyebrow-hair follicles (Panel E), the percentage of cell nuclei with at least 10 small or 3 large foci of γH2AX, the phosphorylated form of histone H2A histone family, member X (H2AX) at serine 139 is shown before and after olaparib administration (left), and the peak γH2AX induction during the first cycle is shown for the cohort of patients receiving each dose of olaparib. A minimum of 100 nuclei were scored for each data point, by an observer who was unaware of the olaparib dose. There was significant induction of γH2AX for each dose shown. The numbers of patients with samples tested were as follows: 2 in the 100-mg cohort, 18 in the 200-mg cohort, 5 in the 400-mg cohort, and 4 in the 600-mg cohort. I bars indicate the standard error.

Table 4. Clinical Responses in Study Patients for Whom the Response Could Be Evaluated.*

Subgroup and Dose	Total No. of Patients	Partial or Complete Radiologic Response	Radiologically Stable Disease	Tumor-Marker Response	Radiologic or Tumor-Marker Response	Radiologic or Tumor-Marker Response or Stable Disease
				number of patients		
All patients	60	9	7†	7	10	17
Patients with *BRCA1* or *BRCA2* ovarian, breast, or prostate cancer‡	19	9 (8 with ovarian cancer, 1 with breast cancer)	2 (1 with ovarian cancer, 1 with breast cancer)	7 (6 with ovarian cancer, 1 with prostate cancer)	10 (8 with ovarian cancer, 1 with breast cancer, 1 with prostate cancer)	12 (9 with ovarian cancer, 2 with breast cancer, 1 with prostate cancer)
<100 mg twice daily, continuously	1	0	0	0	0	0
100 mg twice daily, 2 of every 3 weeks§	2	1	0	1	1	1
100 mg twice daily, continuously	1	0	0	0	0	0
200 mg twice daily, continuously	10	4	2 (actual duration, 6 and 7 mo)	3	5	7
400 mg twice daily, continuously¶	4	4	0	3	4	4
600 mg twice daily, continuously	1	0	0	0	0	0
Patients with *BRCA1* or *BRCA2* ovarian cancer‡	15	8	1	6	8	9
<100 mg twice daily, continuously	1	0	0	0	0	0
100 mg twice daily, 2 of every 3 weeks§	2	1	0	1	1	1
100 mg twice daily, continuously	1	0	0	0	0	0
200 mg twice daily, continuously	6	3	1 (actual duration, 6 mo)	2	3	4
400 mg twice daily, continuously¶	4	4	0	3	4	4
600 mg twice daily, continuously	1	0	0	0	0	0

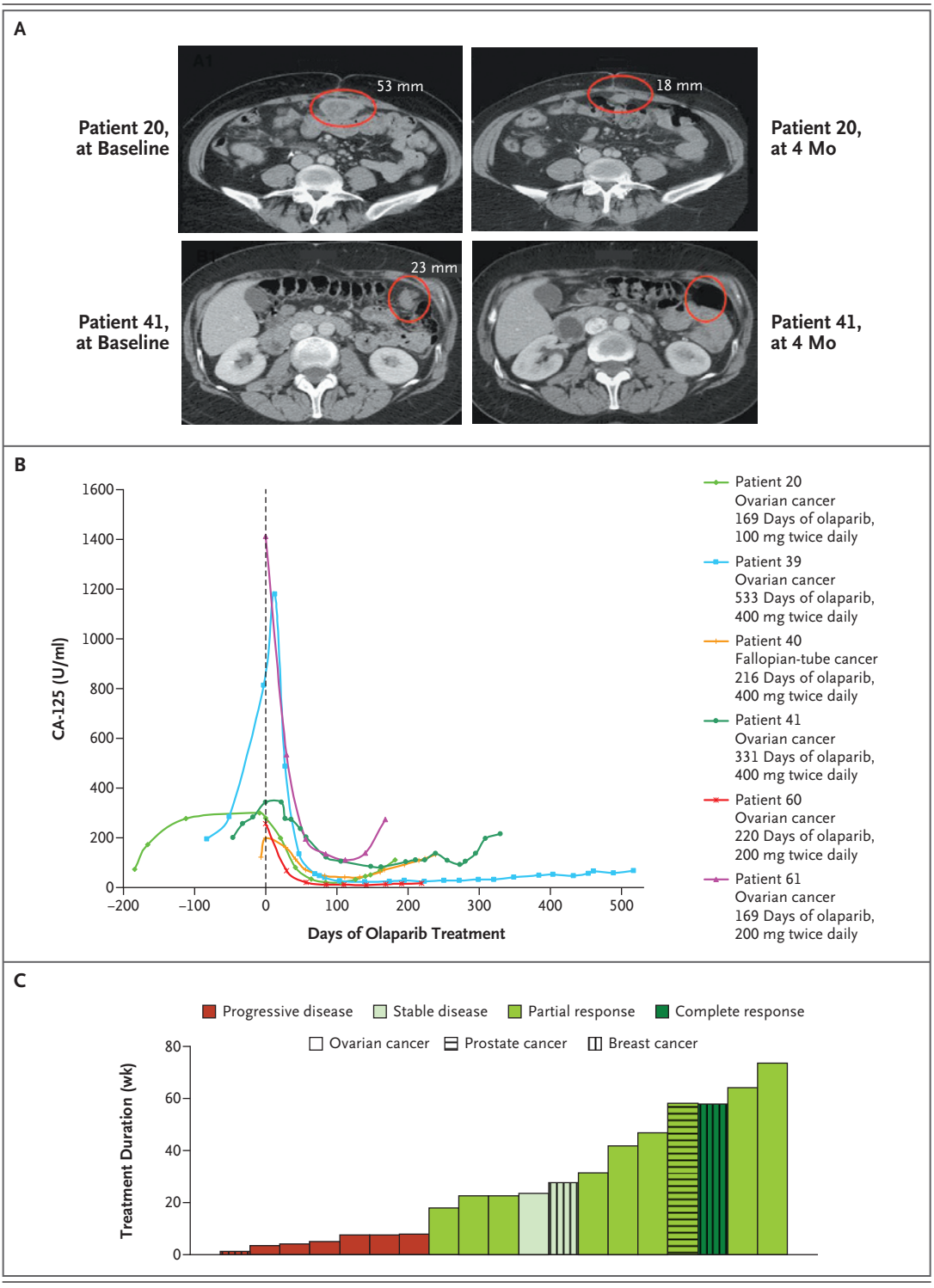
* The radiologic response was graded on the basis of Response Evaluation Criteria in Solid Tumors (RECIST). A tumor-marker response was defined as a decline of more than 50% that was sustained for at least 4 weeks, as assessed according to Gynecologic Cancer Intergroup and Prostate-Specific Antigen (PSA) Working Group criteria.

† Of these seven patients, one had *BRCA2* breast cancer, one had *BRCA2* ovarian cancer, two had non-*BRCA* breast cancer, one had sarcoma, one had renal-cell carcinoma, and one had non–small-cell lung cancer.

‡ Two patients could not be evaluated with regard to tumor response; one stopped olaparib, after having received only two doses, because of dose-limiting toxicity, and one died from sepsis unrelated to olaparib after receiving one cycle of the drug (and having a decline in the cancer antigen 125 level).

§ These patients included one with a strong family history of *BRCA*-mutated cancers, but who declined *BRCA*-mutation testing.

¶ One patient with *BRCA1* fallopian-tube cancer was treated outside the trial owing to an incidental brain metastasis found on day 14 of cycle 1 of olaparib therapy; she subsequently had a systemic response to olaparib.



Not all *BRCA1* or *BRCA2* carriers had a response to olaparib. Various *BRCA1* or *BRCA2* mutations may have resulted in differing homologous-recombination defects and sensitivities to PARP inhibition. Differences in response could also have resulted from preexisting genetic resistance; we and others have shown previously that secondary *BRCA2* mutations may restore *BRCA* function and therefore homologous recombination, causing resistance to PARP inhibitors and platinum com-

Figure 2 (facing page). Radiologic Evidence of Tumor Response to Olaparib.

Computed tomographic (CT) scans of the abdomen in a patient with advanced ovarian cancer (Patient 20), who had a very strong family history suggestive of *BRCA* deficiency but who declined to undergo *BRCA* testing, show a reduction in the size of a peritoneal tumor nodule (encircled in red) by 66% over a 4-month treatment period (top right), as compared with baseline (top left). She received olaparib at a dose of 100 mg, twice daily, for 2 of every 3 weeks. CT scans of the abdomen in another patient with advanced ovarian cancer (Patient 41), who had a *BRCA1* mutation (4693delAA), show complete regression of a peritoneal tumor nodule over a 4-month treatment period (bottom right), as compared with baseline (bottom left). Patient 41 received olaparib (200 mg, twice daily) for a year. Panel B shows biochemical evidence of antitumor activity, measured as cancer antigen 125 (CA-125) levels over time for six patients with advanced ovarian or fallopian-tube cancer who had a response to olaparib therapy according to Gynecologic Cancer Intergroup criteria. The maximum decline in the CA-125 level was 98%, in Patient 39 (from 1180 U per millimeter at baseline to a normal value of 22 U per milliliter). All patients also had a partial response, according to Response Evaluation Criteria in Solid Tumors (RECIST), as evaluated on CT. Panel C shows the duration of treatment and the best response seen in the 19 *BRCA* mutation carriers with ovarian, breast, or prostate cancer who could be evaluated for tumor response. Objective antitumor response was defined as the number of patients with a complete or partial response on radiologic assessment, according to RECIST, whereas the rate of clinical benefit was defined as the number of patients with a radiologic or tumor-marker response or stable disease, for 4 or more months. Tumor-marker response was defined as a decline of more than 50% in tumor-marker levels, sustained for at least 4 weeks.

pounds.[29,30] Assays of homologous-recombination proficiency will be vital to the study of primary or acquired resistance to PARP inhibitors, as well as for identifying sporadic tumors that have defective homologous recombination. Molecular studies of ovarian cancer have, for example, suggested that up to half of high-grade serous cancers may lose *BRCA1* or *BRCA2* function through genetic or epigenetic events.[28] Some sporadic tumors appear to be phenocopies of *BRCA1*- or *BRCA2*-deficient tumors without actually bearing germline mutations in either the *BRCA1* or *BRCA2* gene, a phenomenon that has been described as "BRCAness."[31]

In conclusion, this study raises the possibility that for some anticancer drugs, the traditional processes of clinical development and registration need to be altered. Due consideration must now be given to developing rationally designed, molecularly targeted therapies for patients whose tumors have the same molecular defect but different origins, such as the ovary, breast, or prostate. Such a radical change in drug evaluation and registration may be key to accelerating the development of anticancer drugs.

Supported by KuDOS Pharmaceuticals, which is a wholly owned subsidiary of AstraZeneca. The Drug Development Unit of the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research is supported in part by a program grant from Cancer Research U.K. Support was also provided by the Experimental Cancer Medicine Centre (to the Institute of Cancer Research) and the National Institute for Health Research Biomedical Research Centre (jointly to the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research). Laboratory work was supported in part by Breakthrough Breast Cancer. Olaparib (AZD2281), previously known as KU-0059436, began to be manufactured by AstraZeneca after the company acquired KuDOS Pharmaceuticals.

Drs. Tutt and Ashworth report that they may benefit financially from the development of PARP inhibitors through patents held jointly with KuDOS–AstraZeneca through the Institute of Cancer Research "rewards to inventors" scheme; Drs. Mortimer, Lau, O'Connor, and Carmichael report being employees of KuDOS Pharmaceuticals; Mrs. Swaisland reports being an employee of AstraZeneca; Mrs. Swaisland and Dr. Carmichael report owning equity or stock options in AstraZeneca; Dr. Kaye reports receiving fees from KuDOS and AstraZeneca advisory boards; and Dr. O'Connor reports holding a patent relevant to this study. No other potential conflict of interest relevant to this article was reported.

We thank Dr. Christina Messiou for the computed tomographic scans, Dr. Dow-Mu Koh for the diffusion-weighted magnetic resonance imaging scans, and Dr. Sue Shanley for assistance with *BRCA* mutation screening (all at Royal Marsden NHS Foundation Trust) and Ms. Sarah Jane Mason (Mudskipper Bioscience) for editorial assistance, funded by AstraZeneca, on a previous draft of this article.

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