

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. *N Engl J Med* 2009;361:123-34. DOI: 10.1056/NEJMoa0900212.

SUPPLEMENTARY DATA

FIGURE LEGENDS

Supplementary Figure 1: Synthetic lethality induced by PARP inhibition in a *BRCA*-deficient genetic background. (A) PARP plays a key role in the repair of DNA single-strand breaks that occur all the time as a consequence of normal cell function. (B) When PARP is inhibited, unrepaired single-strand breaks are converted into DNA double-strand breaks during DNA replication. In normal or *BRCA* heterozygous cells (*BRCA*^{+/-}) the double-strand breaks are effectively repaired by homologous recombination DNA repair. PARP inhibition is therefore not toxic to cells with wild-type *BRCA1* or *BRCA2*. Tumor-specific defects in *BRCA1* and *BRCA2* lead to a defect in DNA repair by homologous recombination and are, due to this synthetic lethal interaction, exquisitely sensitive to PARP inhibition.¹³

Supplementary Figure 2: The structure of olaparib (4-[(3-{{4-cyclopropylcarbonyl}piperazin-1-yl}carbonyl)-4-fluorophenyl)methyl]phthalazin-1(2H)-one, also known as AZD2281 and KU-0059436.²⁰

Supplementary Figure 3: Hair follicle pharmacodynamic biomarker assay. Analysis of hair follicle cells for induction of γ H2AX foci as a downstream pharmacodynamic endpoint of the predicted mechanism of action of olaparib. The top diagram illustrates how in the setting of loss of PARP function an unrepaired DNA single-strand break (SSB) progresses to a double-strand break (DSB) upon encountering a replication fork; γ H2AX foci are formed in response to the DSB and can be detected by

immunofluorescence labeling as red foci within the blue-stained nucleus in cells treated with a PARP inhibitor (PARPi, lower left).¹¹ The lower right diagram illustrates how each hair follicle is analyzed on 6 planes and up to 100 nuclei optically assessed by an observer blinded to patient olaparib dose. A cell is scored as positive if ≥ 10 small or >3 large foci per nucleus were seen. An immunofluorescence-labeled proliferation marker Ki67 (green) indicates the proliferating nature of the hair follicle surrogate tissue.

Supplementary Figure 4: Pharmacokinetic studies. Geometric mean plasma concentration-time profiles following single oral dosing of olaparib to cancer patients. The drug administration schedule was adapted, based on acquired PK-PD data, from once daily (10 mg; 20 mg; 40 mg; 80 mg) to twice daily (60 mg to 600 mg) explaining the altered PK acquisition timepoints.

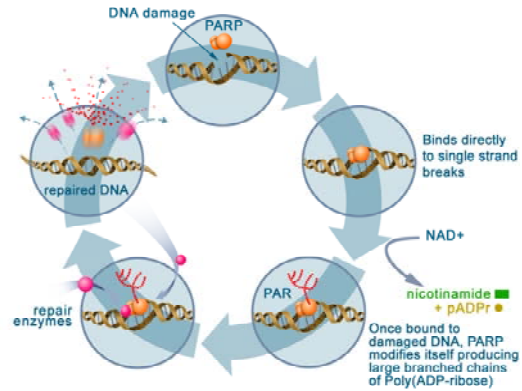
Supplementary Figure 5: Diffusion-weighted magnetic resonance imaging (MRI) demonstrating disease regression in a patient with prostate cancer and *BRCA2* mutation associated with a $>50\%$ decline in PSA.

A 63-year-old man with *BRCA2* mutation and castration resistant prostate cancer. T1-weighted MR images at the level of the right acetabulum in the pelvis obtained (a) prior to, and (c) three months after, initiating treatment showed no substantial change in a 13 mm low-signal intensity (dark) metastasis in the right acetabulum (arrows). Apparent diffusion coefficient (ADC) maps obtained by diffusion-weighted MRI (b) pre-treatment and (d) at three months after treatment showed ADC values increasing from 1.03×10^{-3}

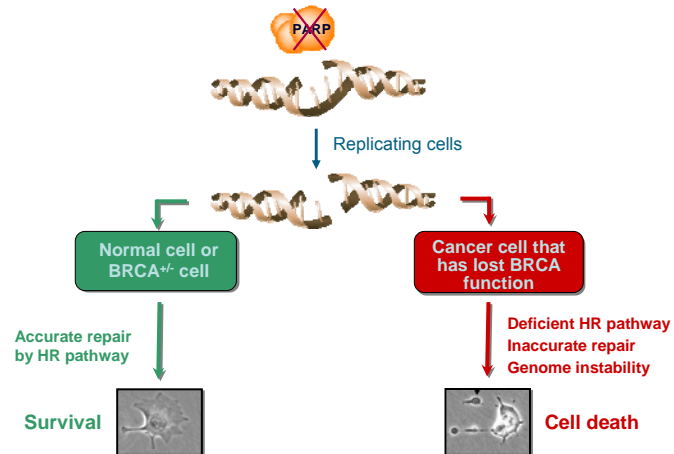
mm²/s to 1.39×10^{-3} mm²/s (>30% increase) at the site of metastatic disease (circled) consistent with disease regression. (e) T1-weighted image at one year after treatment showed resolution of disease (arrow). *[Images courtesy of Dr Dow-Mu Koh, Royal Marsden Hospital, Sutton, UK].*

FIGURES
Supplementary Figure 1:

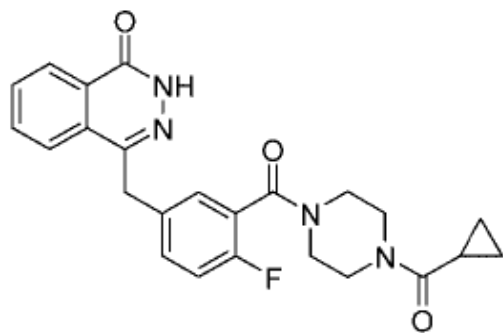
(A) Repair of DNA single strand breaks by PARP



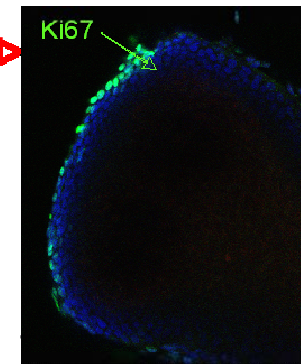
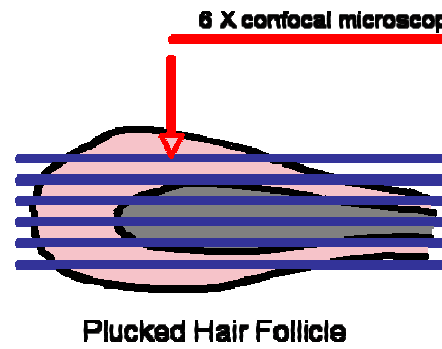
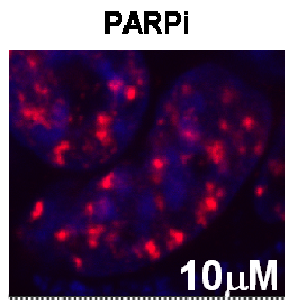
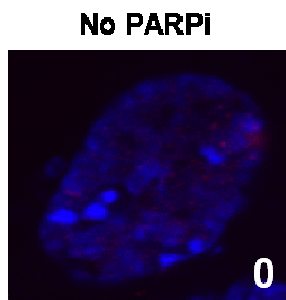
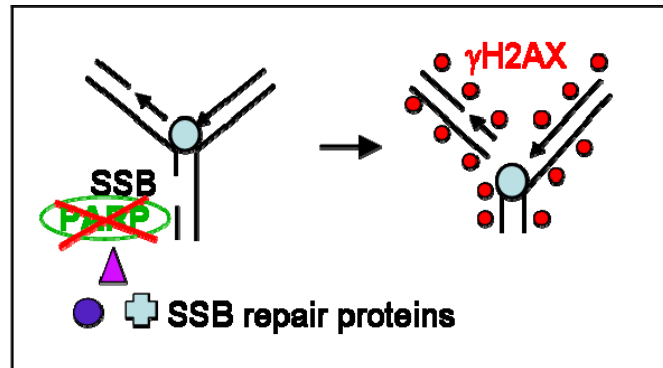
(B) Tumour specific killing by olaparib



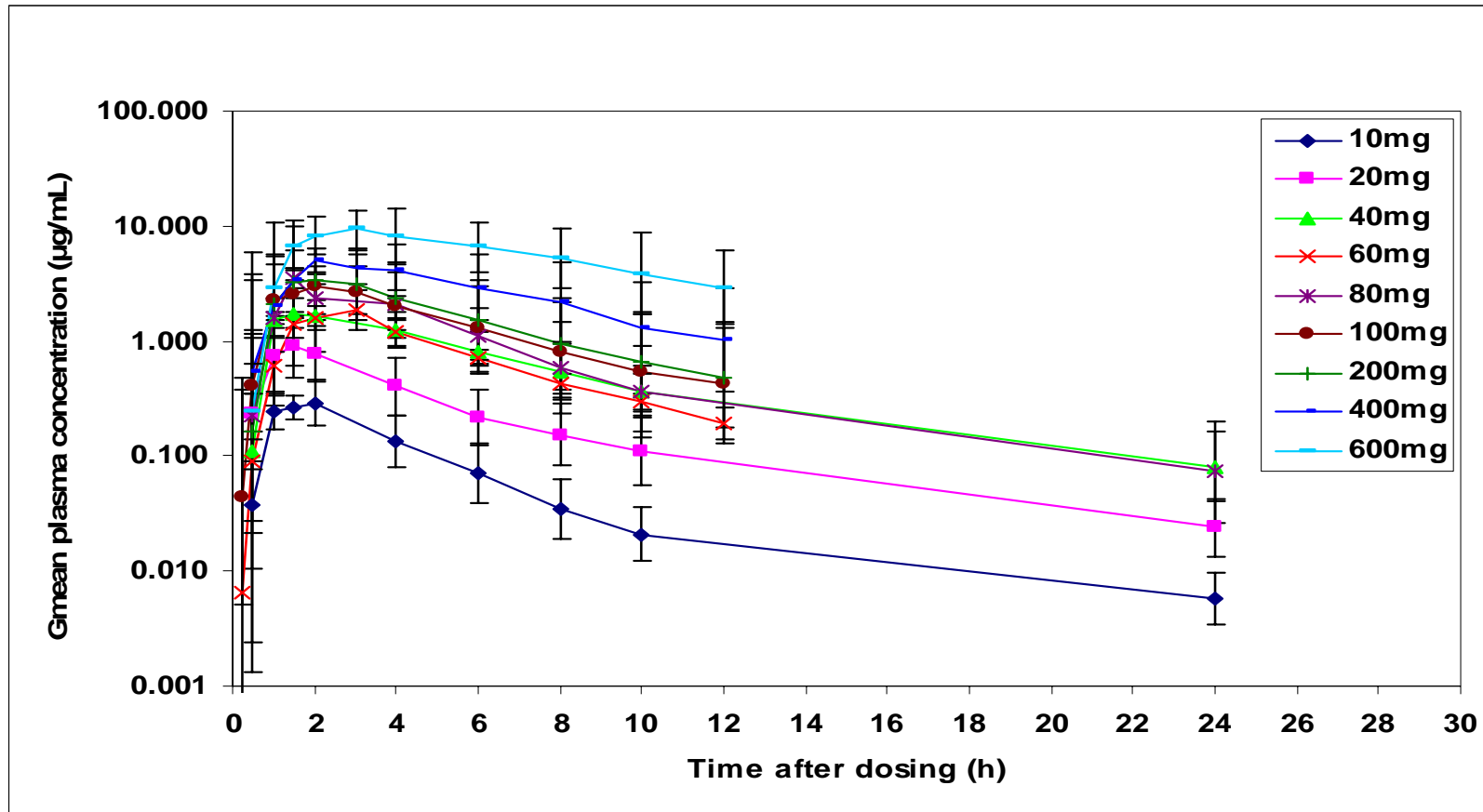
Supplementary Figure 2:



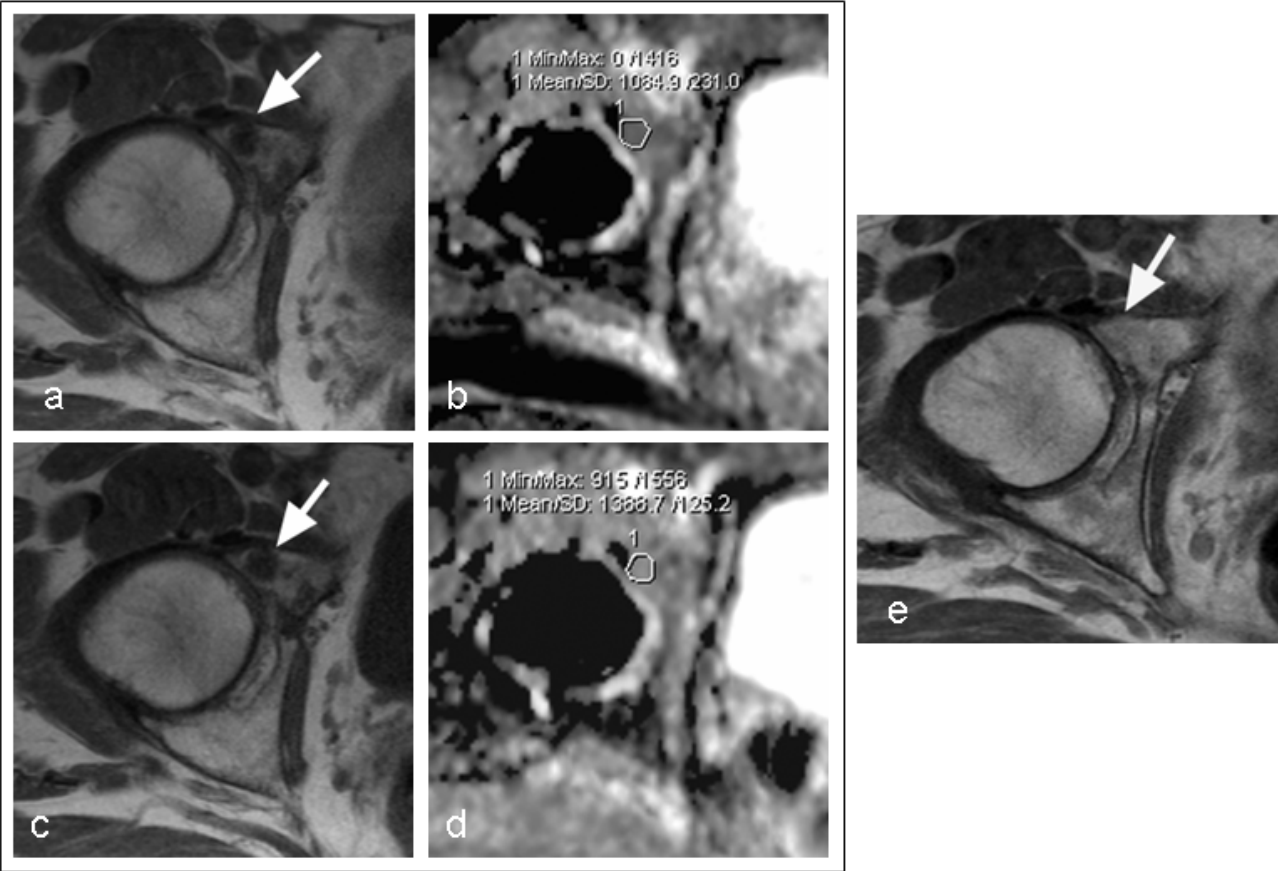
Supplementary Figure 3:



Supplementary Figure 4:



Supplementary Figure 5:



TABLES

Supplementary Table 1:

Dose-escalation scheme; dosing schedule was adapted based on the acquired pharmacokinetic-pharmacodynamic data.

Dose level and schedule	Number of patients	Total number of cycles	Median number of cycles	Number of dose-limiting toxicities in cycle 1 (%)
10 mg daily, 2 out of 3 weeks	3	12	2	0 (0)
20 mg daily, 2 out of 3 weeks	3	5	2	0 (0)
40 mg daily, 2 out of 3 weeks	5	15	2	0 (0)
80 mg daily, 2 out of 3 weeks	3	9	4	0 (0)
60 mg bid, 2 out of 3 weeks	4	10	2	0 (0)
100 mg bid, 2 out of 3 weeks	4	15	3	0 (0)
100 mg bid continuously	5	8	2	0 (0)
200 mg bid continuously	20	94	4	0 (0)
400 mg bid continuously	8	41	2.5	1 (12.5)
600 mg bid continuously	5	13	2	2 (40)

Supplementary Table 2: Supplementary pharmacokinetic data.

Table 2A: Derived pharmacokinetic parameters following administration of a single dose of olaparib at a range of dose levels.

Parameter	Statistic	Dose (mg)								
		10 (n = 3)	20 (n = 3)	40 (n = 5)	60 (n = 4)	80 (n = 3)	100 (n = 8)	200 (n = 20)	400 (n = 8)	600 (n = 5)
C _{max} (µg/ml)	Gmean (CV)	0.38 (36)	1.04 (81)	1.94 (22)	2.55 (33)	3.49 (20)	3.32 (46)	4.22 (50)	6.08 (22)	10.5 (38)
	Range	0.25 – 0.48	0.46 – 1.66	1.50 – 2.68	1.95 – 3.77	3.02 – 4.35	1.68 – 6.77	1.69 – 7.88	4.04 – 7.47	5.90 – 14.5
t _{max} (h)	Median	2.0	1.5	1.5	2.25	1.5	1.0	1.5	1.75	3.0
	Range	0.5 – 2.0	1.0 – 1.5	1.0 – 4.0	0.5 – 3.0	1.5 – 1.5	1.0 – 3.0	1.0 – 4.0	1.5 – 8.0	2.0 – 4.0
AUC ₀₋₁₂ (µg.h/ml)	Gmean (CV)	1.26* (37)	3.74* (52)	9.80*(23)	9.82 (22)	13.2* (43)	19.7 (60)	20.9**(60)	33.5 (47)	68.7 (53)
	Range	0.92 – 1.86	2.29 – 6.07	7.12 – 13.3	7.47 – 12.2	9.25 – 20.8	8.95 – 54.9	9.71 – 45.8	15.8 – 59.5	31.4 – 108.9
AUC ₀₋₂₄ (µg.h/ml)	Gmean (CV)	1.45 (40)	4.80 (46)	13.1 (30)	NC	16.5 (56)	NC	NC	NC	NC
	Range	1.05 – 2.21	3.35 – 7.79	9.44 – 20.6		10.6 – 29.4				
AUC (µg.h/ml)	Gmean (CV)	1.50 (40)	5.04 (44)	13.9 (32)	NC	17.1 (59)	NC	NC	NC	NC
	Range	1.10 – 2.31	3.62 – 8.11	9.97 – 22.9		10.8 – 31.2				
t _½ (h)	Amean (SD)	6.67 (0.29)	6.10 (0.48)	6.14 (0.90)	NC	5.48 (0.31)	NC	NC	NC	NC
	Range	6.41 – 6.99	5.67 – 6.62	5.16 – 7.19		5.12 – 5.66				
CL/F (L/h)	Amean (SD)	6.97 (2.43)	4.20 (1.57)	2.98 (0.85)	NC	5.11 (2.44)	NC	NC	NC	NC
	Range	4.33 – 9.11	2.46 – 5.52	1.75 – 4.01		2.56 – 7.43				
V/F (L)	Amean (SD)	67.6 (26.0)	37.6 (16.4)	25.8 (6.67)	NC	39.8 (17.3)	NC	NC	NC	NC
	Range	40.0– 91.8	20.2 – 52.7	18.2 – 36.0		20.9 – 54.8				

Gmean = geometric mean; CV = coefficient of variation; Amean = arithmetic mean; SD = standard deviation;

NC = not calculated (sampling stopped at 12 hours after dosing)

* value reported is actually AUC₀₋₁₀ because no 12 hour sample was collected

** n = 17

Table 2B: Derived pharmacokinetic parameters on Day 14 of multiple oral dosing of olaparib at a range of dose levels.

Parameter	Statistic	Dose (mg)								
		10 (n = 3)	20 (n = 2)	40 (n = 4)	60 (n = 3)	80 (n = 3)	100 (n = 8)	200 (n = 17)	400 (n = 6)	600 (n = 5)
C _{max} (µg/ml)	Gmean (CV)	0.49 (43)	NC	1.78 (7)	2.08 (12)	4.82 (14)	3.67 (31)	5.62 (50)	7.65 (27)	11.5 (42)
	Range	0.38 – 0.79	1.42 – 1.94	1.66 – 1.93	1.90 – 2.37	4.13 – 5.46	2.81 – 5.86	2.83 – 17.1	5.28 – 10.5	6.49 – 17.6
t _{max} (h)	Median	1.0	NC	1.75	3.0	1.5	1.5	1.5	2.0	1.5
	Range	0.5 – 2.0	1.0 – 1.0	1.0 – 4.0	1.0 – 6.0	1.0 – 2.0	1.0 – 4.0	1.0 – 6.0	1.5 – 3.0	1.0 – 3.0
C _{min} (µg/ml)	Gmean	0.008 (76)	NC	0.12 (37)	0.26 (238)	0.14 (152)	0.45 (90)	0.96** (133)	1.29 (76)	2.18 (177)
	(CV)	0.005–	0.029 – 0.054	0.086 –	0.064 –	0.060 –	0.12 – 0.98	0.21 – 2.95	0.66 – 3.89	0.39 – 5.95
	Range	0.018		0.190	1.00	0.47				
AUC ₀₋₁₂ (µg.h/ml)	Gmean	1.67* (36)	NC	9.20* (19)	11.5 (54)	19.0* (30)	20.5 (52)	33.3** (75)	44.9 (39)	86.8† (42)
	(CV)	1.31 – 2.50	4.48 – 6.51	7.04 – 10.5	7.46 – 20.0	13.9 – 25.1	10.7 – 48.9	11.2 – 162.4	27.7 – 78.7	55.4 – 119.4
	Range									
AUC ₀₋₂₄ (µg.h/ml)	Gmean	1.94 (40)	NC	13.0 (21)	NC*	24.5 (40)	NC*	NC*	NC*	NC*
	(CV)	1.48 – 3.03	5.38 – 8.06	9.54 – 15.0		16.8 – 36.5				
	Range									
CL _{ss} /F (L/h)	Amean	5.39 (1.84)	NC	3.13 (0.72)	5.67 (2.54)	3.43 (1.29)	5.38 (2.39)	7.63** (4.40)	9.44 (3.35)	7.31† (1.38)
	(SD)	3.30 – 6.76	2.48 – 3.72	2.66 – 4.19	2.99 – 8.04	2.19 – 4.76	2.05 – 9.30	1.23 – 17.9	5.08 – 14.4	5.03 – 10.8
	Range									
t _{1/2} (h)	Amean	NC	NC	9.52 (1.64)	NC	8.05 (0.70)	NC	NC	NC	NC
	(SD)	8.26 – 12.2	9.34 – 12.0	7.95 – 11.0		7.27 – 8.62				
	Range									

Gmean = geometric mean; CV = coefficient of variation; Amean = arithmetic mean; SD = standard deviation;

NC = not calculated (n = 2); NC* = NC (sampling stopped at 12 hours after dosing)

* value reported is actually AUC₀₋₁₀ because no 12 hour sample was collected

** n = 14; † n=3

Supplementary Table 3: Pharmacogenomics of PARP inhibitors; radiological and biochemical responses for ovarian, breast and prostate cancer patients according to *BRCA1* or *BRCA2* mutation sequence found (where available)

Tumor type	Gene	Mutation	Clinical benefit from olaparib [§]	Dose
Ovary	<i>BRCA1</i>	5382insC	No	40 mg qd (2 out of 3 weeks)
Ovary	–	Not tested*	Yes	100 mg bid (2 out of 3 weeks)
Peritoneum	<i>BRCA1</i>	3875delGTCT	No	100 mg bid (2 out of 3 weeks)
Ovary	<i>BRCA1</i>	4184del4	No	100 mg bid
Ovary	<i>BRCA1</i>	185delAG	Not evaluable	400 mg bid
Ovary	<i>BRCA1</i>	185delAG	Yes	400 mg bid
Ovary	<i>BRCA1</i>	185delAG	Yes	400 mg bid
Fallopian tube	<i>BRCA1</i>	4184delTCAA	Yes	400 mg bid
Ovary	<i>BRCA1</i>	185delAG	Yes	400 mg bid
Ovary	<i>BRCA1</i>	C.4357+1delG	Not evaluable	400 mg bid
Ovary	<i>BRCA1</i>	Unavailable	No	600 mg bid
Ovary	<i>BRCA1</i>	4693delAA	Yes	200 mg bid
Ovary	<i>BRCA2</i>	8206T>G	Yes	200 mg bid
Breast	<i>BRCA2</i>	3715delG	Yes	200 mg bid
Prostate	<i>BRCA2</i>	6174del T	Yes	200 mg bid
Breast	<i>BRCA2</i>	902delC	Yes	200 mg bid
Breast	<i>BRCA2</i>	4684del4	No	200 mg bid
Ovary	<i>BRCA1</i>	Deletion exon 15–17	No	200 mg bid
Ovary	<i>BRCA1</i>	6kb ins exon 13	No	200 mg bid

Ovary	<i>BRCA1</i>	5396+1 G>A	Yes	200 mg bid
Ovary	<i>BRCA1</i>	1185C>T (Q356X)	Yes	200 mg bid

*Patient with a strong family history of *BRCA*-mutated cancers, but who declined *BRCA* mutation testing

§ Clinical benefit was defined as response by RECIST or accepted tumor marker criteria or disease stabilization ≥4 months.

Patient was treated off-trial due to incidental brain metastases and subsequently responded systemically to olaparib.

Grayed-out cells denote the 3 most common Ashkenazi founder mutations.